NEMB

NanoEngineering for Medicine and Biology Conference

Bringing together the relevant players and key stakeholders to discuss the integration of engineering, materials science and nanotechnology.

CONFERENCE
Feb 21 - 24, 2016

EXHIBITION
Feb 22 - 23, 2016

JW Marriott Houston, Houston, TX, USA

Program

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The American Society of Mechanical Engineers (ASME)

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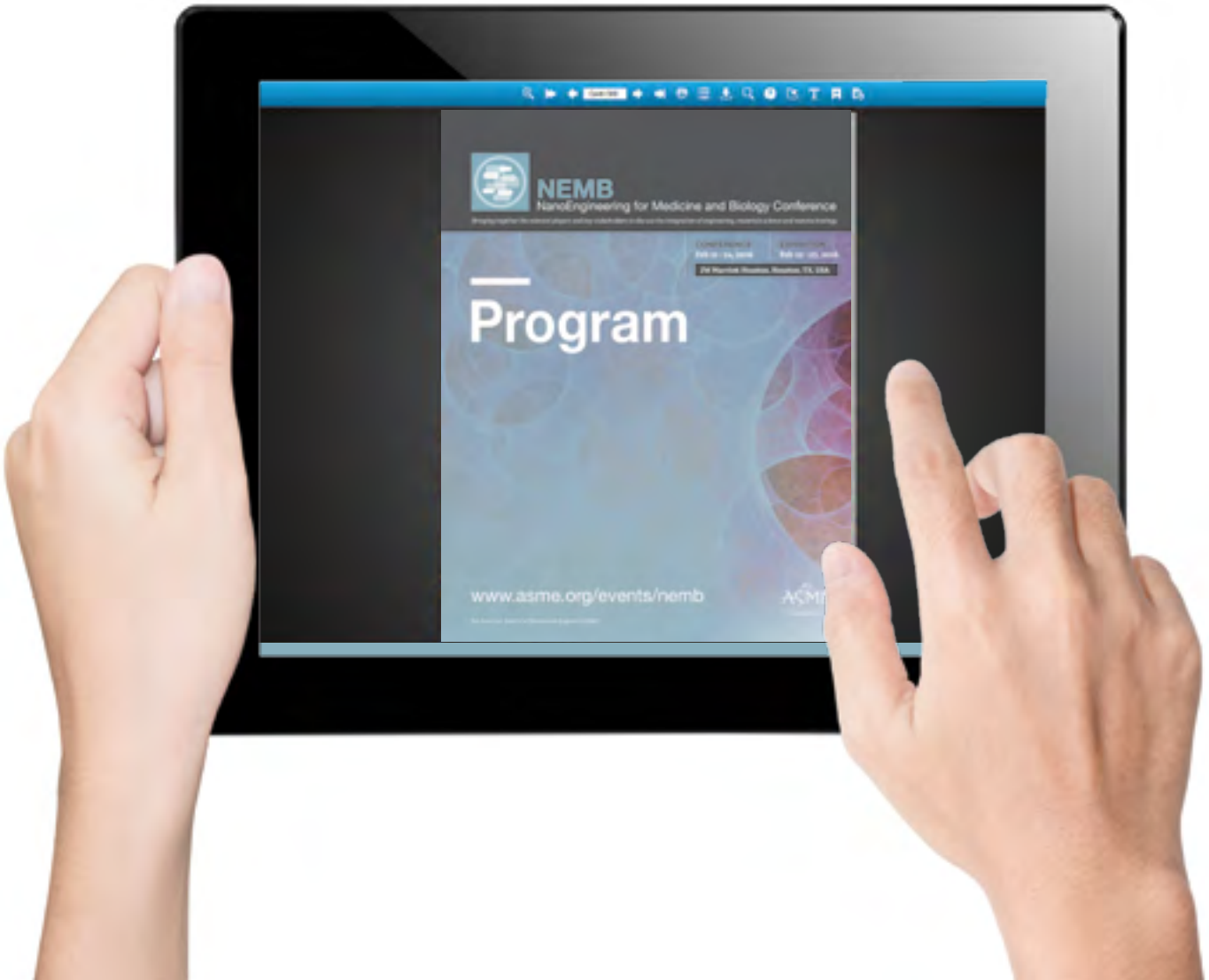

Welcome

Dear Attendee of the NEMB2016:

On behalf of the organizers, we would like to welcome you to Houston to the 5th American Society for Mechanical Engineers (ASME) NanoEngineering in Medicine and Biology Conference (NEMB 2016). At this meeting, you will have the opportunity to discuss the most recent advances in nanobiotechnology and nanomedicine with luminaries and pioneers of the field. Whether it's a tutorial, plenary, keynote and theme presentation, a poster presentation, or a social event, we believe that you will enjoy these exciting elements of the conference. We hope you will benefit from the academic and industrial breadth of the meeting.

Applications of nanoengineering for medicine and biology are having a dramatic impact on a myriad of healthcare needs, product development and biomedical research. These include nanoparticle-based imaging probes and drug/gene carriers for new and improved disease diagnostics and therapeutics, bio-inspired nanomaterials and nanostructures, and novel nanoengineering tools for biological and medical applications. These frontiers of engineering, biology and medicine at the micro- and nano-scale will be highlighted at NEMB 2016. In addition, work contributing to completely new fundamental understanding of biological processes and phenomenon at the nanoscale will also be presented. Together these fundamental and applied aspects of the meeting provide the basis for solving grand challenge problems in cancer, infectious disease, cardiovascular, neurological diseases and global health. Nanoengineers and nanoscientists are indeed changing the world and are having a profound impact on medicine and biology!

We would like to acknowledge support for the conference from the National Science Foundation, Rice University, and many generous funders acknowledged on our website and in our program. We would also like to acknowledge and highlight the many volunteers who helped assemble the scientific program for this conference especially our Steering Committee (Professors John Bischof, Michael Deem, Guy Genin, Robert Griffin and Sulin Zhang), the Tutorial Speakers and Participants, Track Chairs, and Poster Awards committee (led by Rafael Davalos, Emily Day and David Juncker). You will see that amongst the Plenary and Keynote Speakers, Track Chairs and other attendees many are leading figures in the field. In addition to stimulating scientific and technical discussions, there will be ample opportunities for networking, especially for young researchers to mingle with senior investigators. Showcasing cutting edge research in nanobioengineering with broad participation constitutes the heart of this meeting, as evident by how the talks, sessions, and tracks are constructed in the technical program. Finally, working hard behind the scenes is a devoted staff of technical assistants from ASME. The latter includes lead organizer extraordinaire Christine Reilley, meetings manager Jimmy Le, and Program Manager, Norma Johnston.

We wish you a productive and enjoyable stay in Houston. Please stay tuned for information about the next NEMB conference!

Sincerely,

Gang Bao
Conference Chair

Warren Chan
Technical Program Chair

Contents

PLEASE JOIN US AT THE

OPENING RECEPTION

SUNDAY, FEBRUARY 21

5:00 - 8:00 PM

GRAND BALLROOM

JW MARRIOTT HOUSTON

PLEASE JOIN US FOR AN EVENING OF ENGAGING CONVERSATIONS, FUN DISCUSSIONS, AND EXCITING NETWORKING. THERE WILL BE PLENTY OF FOOD AND DRINK!

AUDIOVISUAL EQUIPMENT IN SESSION ROOMS

All technical sessions are equipped with one LCD projector and one screen. Laptops will NOT be provided in the sessions. Presenters MUST bring their own or arrange in advance to share.

BADGE REQUIRED FOR ADMISSION

All conference attendees must wear the official ASME 2016 NEMB badge at all times in order to gain admission to technical sessions, exhibits and conference receptions. Without a badge, you will NOT be allowed to attend any conference activities.

CONFERENCE CLOSING LUNCH

Join us on Wednesday, February 24, from 11:30am - 1:00pm for a closing lunch where all the hardworking program volunteers will be acknowledged, the sponsors and exhibitors will be thanked, and the award winners will be announced. Don't miss it!

CONFERENCE EVENT CONNECT APP

Download the new ASME Event Connect App and hold the entire program in the palm at your hand! The new ASME Event Connect App allows you to easily look up sessions, search for papers or people, message with other attendees, post to various social media platforms, and create your own schedule.

The ASME Event Connect App is available at the App Store, Google Play, and Windows Market.

CONFERENCE REFRESHMENT BREAKS

Three morning coffee breaks and two afternoon breaks will be provided. Please join your fellow attendees for a few minutes of discussion of the sessions that you attended or catch up on what you may have missed earlier in the day.

REGISTRATION

Location:
Bexar Travis Foyer (2nd floor)

Hours:
Sunday, February 21
11:00 AM – 7:00 PM

Monday, February 22
7:00 AM – 6:00 PM

Tuesday, February 23
7:00 AM – 6:00 PM

Wednesday, February 24
7:00 AM – 11:00 AM

JOIN THE FUN ON TWITTER #NEMB2016

The **#NEMB2016** Twitter stream will be displayed prominently around the meeting space. We encourage you to participate by using the hashtag **#NEMB2016** to tweet about anything that's on your mind with regards to the 2016 NEMB conference.

EXHIBITS INFORMATION

Location:
Lamar Room (2nd floor)

Hours:
Monday, February 22
10:30 AM – 4:30 PM

Tuesday, February 23
10:30 AM – 4:30 PM

MEMBERSHIP TO ASME (ONE-YEAR FREE)

Registrants who paid the non-member conference registration fees will receive a complimentary one-year ASME Membership. ASME will automatically activate this complimentary membership for qualified attendees. Please allow approximately four weeks after the conclusion of the conference for your membership to become active. Visit www.asme.org/membership for more information about the benefits of ASME Membership.

PRESENTER ATTENDANCE POLICY

According to ASME's Presenter Attendance Policy, if a paper is not presented at the conference, the paper will not be published in the official Archival Proceedings, which are registered with the Library of Congress and are abstracted and indexed. The paper also will not be published in the ASME Digital Collection and may not be cited as a published paper.

PROGRAM AT-A-GLANCE

SUNDAY, FEBRUARY 21					
	7:00 AM	8:00 AM	9:00 AM	10:00 AM	
Bexar/Travis Foyer					
Harris					
Hidalgo					
Navarro					
Sam Houston					
Bexar/Travis					
Grand Ballroom					

MONDAY, FEBRUARY 22					
	7:00 AM	8:00 AM	9:00 AM	10:00 AM	
Bexar/Travis Foyer	Conference Registration (7:00 AM - 6:00 PM)				
Harris			5-1 Micro- and Nano-materials design for controlle releases (9:30 - 11:00 AM)		
Hidalgo			3-1: Flow and Transport Devices I (9:30 - 11:00 AM)		
Navarro			4-2: Neural Processes (9:30 - 11:00 AM)		
Sam Houston			1-1: Translational Nanoparticles (9:30 - 11:00 AM)		
Bexar/Travis			4-1: Physical Processes and Mechanics (9:30 - 11:00 AM)		
Lamar				Conference Exhibition (10:30 AM - 4:30 PM)	
Grand Ballroom	Conference Breakfast (7:30 - 8:30 AM)	**Plenary 7-3 John C. Bischof: Thermal Contrast and Nanowarming: How Nanoparticle Heaters Are Improving Diagnostics and Regenerative Medicine** (8:30 - 9:15 AM)			

	11:00 AM	12:00 PM	01:00 PM	02:00 PM	03:00 PM	04:00 PM	05:00 PM	06:00 PM
	Conference Registration (11:00 AM - 7:00 PM)							
				8-1: Cancer Immunotherapy (2:15 - 3:45 PM)				
		8-2: Precision Genome Editing (12:30 - 2:00 PM)						
				8-3: Biomanufacturing (2:15 - 3:45 PM)				
				8-4: Entrepreneurship (2:15 - 3:45 PM)				
		8-5: Technology Transfer (12:30 - 2:00 PM)						
						Plenary 7-5 Nicholas A. Peppas: Nano-engineering Intelligent and Diagnostic Therapeutic Systems (4:00 - 4:45 PM)	Poster Session and Opening Reception (5:00 - 8:00 PM)	

	11:00 AM	12:00 PM	01:00 PM	02:00 PM	03:00 PM	04:00 PM	05:00 PM	06:00 PM
	4-3: Tissue Mechanics (11:30 AM - 1:00 PM)					4-4: Self-Assembly (4:00 - 5:30 PM)		
	3-2: Flow and Transport Devices II (11:30 AM - 1:00 PM)					3-5: Vascular and Bone Engineering (4:00 - 5:40 PM)		
	3-3: Detection Systems (11:30 AM - 1:00 PM)					3-4: Tissue Engineering (4:00 - 5:40 PM)		
	1-2: MRI, Multi-Modal, and Theranostic Nanoparticles (11:30 AM - 1:00 PM)					1-3: Advanced Optical Nanoparticles and Imaging Techniques (4:00 - 5:30 PM)		
	2-1: Emerging nanotherapeutics (11:30 AM - 1:00 PM)					2-2: Nanoparticle-biological interactions (4:00 - 5:50 PM)		
				Plenary 7-4 Dennis Discher: Nano Aspects in the Physics of Cancer: From Nuclear Remodeling to Self Recognition for Gene/Drug Delivery (2:30 - 3:15 PM)				

PROGRAM AT-A-GLANCE

TUESDAY, FEBRUARY 23					
	7:00 AM	8:00 AM	9:00 AM	10:00 AM	
Bexar/Travis Foyer	Conference Registration (7:00 AM - 6:00 PM)				
Harris			3-6: Biosensors (9:30 - 11:00 AM)		
Hidalgo			4-5: Nano-design concepts (9:30 - 11:00 AM)		
Navarro			3-7: Extracellular Matrix (9:30 - 11:10 AM)		
Sam Houston			1-4: Novel Nanoparticles for Imaging, Diagnostics, and Therapy (9:30 - 11:00 AM)		
Bexar/Travis			2-3: Cancer nanomedicine (9:30 - 11:00 AM)		
Lamar				Conference Exhibition (10:30 AM - 4:30 PM)	
Grand Ballroom	Conference Breakfast (7:30 - 8:30 AM)	**Plenary 7-1 Kathy Ferrara: Image-Guided Nanotherapy in the Treatment of Cancer and Cardiovascular Disease** (8:30 - 9:15 AM)			

WEDNESDAY, FEBRUARY 24					
	7:00 AM	8:00 AM	9:00 AM	10:00 AM	
Bexar/Travis Foyer	Conference Registration (7:00 - 11:00 AM)				
Harris			4-8: Cellular Engineering (9:30 - 11:00 AM)		
Hidalgo			5-6 Nanoparticle synthesis (9:30 - 11:00 AM)		
Navarro			3-9: Toward Point-of-Care Diagnostics (9:30 - 11:00 AM)		
Sam Houston			2-6: Temperature-sensitive nanomedicine (9:30 - 11:00 AM)		
Bexar/Travis			2-7: Modeling nanoparticle transport (9:30 - 11:00 AM)		
Grand Ballroom	Conference Breakfast (7:30 - 8:30 AM)	**Plenary 7-6 Rebecca Richards-Kortum: Point-of-Care Diagnostics for Low-Resource Settings** (8:30 - 9:15 AM)			

	11:00 AM	12:00 PM	01:00 PM	02:00 PM	03:00 PM	04:00 PM	05:00 PM	06:00 PM
	5-2 Nanomaterial properties and sensing applications (11:30 AM - 1:10 PM)					5-5 Assembled materials (4:00 - 5:40 PM)		
	5-3 Self-powered or low energy powered biodevices (11:30 AM - 1:00 PM)					4-7: Molecular Interactions and Simulations (4:00 - 5:40 PM)		
	5-4 Novel printing and synthesis techniques for biomaterial scaffolds (11:30 AM - 1:00 PM)					8-6: Organ Preservation (4:00 - 5:00 PM)	8-7: Organ Preservation (Technical Session) (5:00 - 6:30 PM)	
	2-4: Nanoparticles for immunoengineering (11:30 AM - 1:00 PM)					2-5: Nanoparticle engineering (4:00 - 5:30 PM)		
	4-6: Cellular Interactions (11:30 AM - 1:10 PM)					3-8: Tissue Models (4:00 - 5:40 PM)		
				Plenary 7-2 Kam W. Leong: Direct Cell Reprogramming via Genome Editing (2:30 - 3:15 PM)				

	11:00 AM	12:00 PM	01:00 PM	02:00 PM	03:00 PM	04:00 PM	05:00 PM	06:00 PM
	Closing Banquet Lunch (11:30 am - 1:00 pm)							

Conference Tutorials

TUTORIAL 2: PRECISION GENOME EDITING

Presenter: Gang Bao, Rice University
Date/Time: Sunday, February 21, 12:30 – 2:00 PM
Location: Hidalgo

Description: The ability to precisely modify genomes will revolutionize many areas in life, including healthcare, agriculture, energy and the environment. With recent advent of engineered nucleases such as clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins, we now have very efficient molecular scissors that can cut genomic DNA in cells at pre-selected locations and introduce controlled gene modifications according to the needs. This has led to the development of precision genome editing, a new field in life sciences and engineering focusing on precisely modifying genomes using engineered nucleases.

In this tutorial I will discuss the basic features of engineered nucleases such as zinc-finger nucleases (ZFNs), transcription activator-like (Tal) effector nucleases (TALENs) and CRISPR/Cas9 systems, and review some web-based tools developed for target selection and off-target site prediction in designing engineered nucleases, and experimental tools for quantifying nuclease activity and specificity. I will then illustrate the application of genome editing for treating single-gene disorders such as sickle cell disease. The challenges in developing genome editing strategies for treating human diseases are discussed.

TUTORIAL 5: TECHNOLOGY TRANSFER

Presenter: Andy Castillo, Rice University
Date/Time: Sunday, February 21, 12:30 – 2:00 PM
Location: Bexar/Travis

Description: Tremendous resources are invested in academic research at universities and research institutes across the world. Research, innovation, and intellectual property (IP) are all valuable commodities resulting from this vast investment and academic technology transfer offices play a crucial role in the protection and promotion of these commodities. This tutorial aims to provide a general overview of technology transfer from an academic perspective. The tutorial will begin with an introduction to intellectual property and the basics of IP protection. Discussions will also cover IP obligations for federally funded research (Bayh-Dole Act), and understanding inventorship and IP ownership. The tutorial will wrap up with patenting strategies and an overview of licensing and commercialization of IP. Those wishing to gain a general knowledge of protecting and commercializing their inventions and intellectual property are encouraged to attend.

TUTORIAL 1: CANCER IMMUNOTHERAPY

Presenter: Xian C. Li, MD, PhD, Houston Methodist Hospital and Cornell University Medical College
Date/Time: Sunday, February 21, 2:15 – 3:45 PM
Location: Harris

Description: Cancer is emerging as a great threat to public health and becomes a leading cause of death in the US. Consequently, our strategies to treat cancer patients are pressing and also rapidly evolving over time, and mobilizing the immune cells to eradicate cancer cells has generated tremendous excitement nowadays. Cancer immunotherapy is widely regarded as a “paradigm shift” in the field.

In this tutorial, I will highlight the basic function of the immune system, the cell types involved, the principles of T cell immune responses, as well as regulatory mechanisms and key checkpoints in the system to avoid overstimulation of the immune cells. I will then discuss how tumors evade and the immune system for their growth and metastasis, and the unique attributes of tumor microenvironment, followed by discussion of current emerging strategies in cancer immunotherapies, including advantages and limitations of such strategies, as well as ongoing research in the field in developing much improved therapies for cancer patients. Finally, I will provide a broad perspective on cancer immunotherapy in the context of other therapeutic strategies and where this area is heading. The goal of this tutorial is to provide the audience with a broad introduction on the immune system and ways to harness the power of the immune system to treat cancer patients.

TUTORIAL 3: BIOMANUFACTURING WORKSHOP

Presenter: Kaiming Ye, Binghamton University, State University of New York
Date/Time: Sunday, February 21, 2:15 – 3:45 PM
Location: Navarro

Description: Creation of highly organized multicellular constructs, including tissues and organoids, will revolutionize tissue engineering and regenerative medicine. The development of these technologies will enable the production of individualized organs for patient-tailored organ transplantation or individualized tissues for cell-based therapy. These lab-produced high order tissues and organs can also serve as disease models for pathophysiological study and drug screening. The manufacturing of these organs and tissues requires the assembly of multiple types of cells into a high-order tissue structure that imitates in vivo embryo developmental environments. The development of a multicellular system for directed stem cell differentiation has been proposed and explored for decades. Unlike single cellular differentiation system, multicellular system provides tissue niches that mimic in vivo microenvironment where surrounding cells offer instructive niches such as essential cell-cell and cell-extracellular matrix (ECM) interactions to guide cell differentiation and maturation. Cell co-cultures might be the first multicellular systems that have been successfully used for directing stem cell differentiation. In light of their success in directing stem cell differentiation, they have been limited to several special cases. Most time, they failed to produce satisfied results due to their limitation in mimicking 3D tissue structures where multiple types of cells are arranged in a high order. It becomes clear that a complete stem cell differentiation and maturation system requires the capture of complexity and hierarchical structure of tissues in order to achieve a strict temporal and spatial control of the process. Several approaches including soft lithographical approach that allows patterning cells in the hydrogel layer-by-layer have been attempted, leading to only modest success due to its use of polymer master molds for patterning. Recently, a new technology, i.e. additive manufacturing also called 3D printing has emerged and has been widely used for printing machines, goods and even bioproducts including implants. The advantage of a 3D printing is its capability of arranging a complicated structure of an object by layer-by-layer printing based on pre-determined patterns designed through computer-assisted-design (CAD). This lecture is to review the stat-of-the-art of these technologies and to discuss the future direction of the field.

TUTORIAL 4: ENTREPRENEURSHIP

Presenter: Ken Diller, UT Austin
Date/Time: Sunday, February 21, 2:15 – 3:45 PM
Location: Sam Houston

Description: This tutorial will present a combination of guidelines and personal experiences for academic scientists in pursuing entrepreneurial

development of their intellectual property. Topics that will be addressed include: opportunities and limitations on faculty in becoming entrepreneurs; juggling the simultaneous roles of academic and entrepreneur; what does it take to become successful; expectations for time, effort, and other resources required; how to learn at least some of the skills that contribute to the entrepreneurial processes; and personal anecdotes by the presenter.

TUTORIAL 6: ORGAN PRESERVATION

Presenter: Sebastian Giwa, The Organ Preservation Alliance
Date/Time: Tuesday, February 23, 4:00 - 5:00 PM
Location: Navarro

Description: Organ transplantation has been one of the most significant medical advances in the past 50 years and remains in many cases the only effective therapy for end-stage organ failure. The process of organ transplantation involves the removal of an organ from a donor body, storing this organ for transportation, and allowing it to be transplanted into another body, all while preserving the organ without significant damage. This has only been possible by amassing a good understanding of the potential effects of hypoxic injury on donated organs, and how to prevent these by applying organ preservation. In this tutorial, you will gain a better understanding of organ preservation, how applications of hypothermia have become central to the process, and what advances in nanotechnology, tissue engineering, cryobiology, and heat transfer are informing new innovations.

ORGAN PRESERVATION (TECHNICAL SESSION)

Date/Time: Tuesday, February 23, 5:00 – 6:30 PM
Location: Navarro

NEMB2016-6168: Despite its massive public health impact, organ transplantation still faces great challenges after 50 years; Organ Preservation is a key solution. (Keynote)
A. O. Gaber, Houston Methodist Research Institute

NEMB2016-6169: Nanoscale Solutions for Organ Banking Challenges (Keynote)
John Bischof, University of Minnesota

NEMB2016-6170: Applying the “Materials Genome Initiative” to Design Next Generation Cryo Protectants (Technical Presentation)
Gloria Elliott, University of North Carolina at Charlotte

Abstract: Recognizing that discovery and deployment of advanced materials is crucial to achieving global economic competitiveness, the White House recently launched a new program called the ‘Materials Genome Initiative’ (MGI) with the vision of deploying materials innovations “twice as fast, at a fraction of the cost”. Embodying the same principles that enabled rapid advances in the mapping of the human genome, this multi-agency program is strategically mobilizing resources and infrastructure in order to support the rapid development of materials that address challenges in clean energy, national security, and human welfare. This program emphasizes the use of advanced computational tools to speed materials development, including machine learning, visual analytics, data-mining, and software tools that can impact all stages of discovery, testing, manufacture, and deployment. This approach can be applied to the development of preservation compositions that are designed specifically for large organ banking.

In order to make progress with the vitrification of complex organs, it is clear that further advances in cryoprotectant compositions will be necessary. Systematic solution optimization using empirical methods has enabled the identification of superior combinations of cryoprotectants for various cell and tissue types, yet the range of chemicals used as protectants is still quite narrow and it is oftentimes not well understood why some compositions work better than others. Formulations that have been optimized for use in small tissues have toxicity profiles that limit their use in larger systems, where long incubation times are needed to allow diffusion of protectants into the interior of thick tissues. Compositions that have reduced toxicities yet form glasses at modest cooling rates, resist cracking, and do not crystallize during rewarming are needed for large volume vitrification applications. Implementing computational methods into the design of vitrification solutions can reduce experimental time and provide valuable insights for new materials. For example, many important glassy state properties, including glass transition temperature, fragility, and coefficient of thermal expansion lend themselves to prediction by molecular dynamics computer simulations. Mathematical methods can also be applied to predict injury caused by osmotic stress. These and other examples that demonstrate an MGI approach to the development of next generation cryoprotectant compositions will be presented.

NEMB2016-6171: DoD Health Objectives and Current/Future Support for Organ Banking (Keynote)
Luis Alvarez, United States Military Academy

NEMB2016-6175: The Grand Challenges of Organ Banking: How the World Is Beginning to Align to Meet Them
Sebastian Giwa, Organ Preservation Alliance

Abstract: In the last year considerable momentum has developed around the grand scientific challenge of banking organs and other complex tissues. Organ impairment is responsible for more deaths than cancer every year in the U.S., making it the nation’s #1 killer. Yet organ impairment, major strategies for cancer treatment and research, and many other approaches in other areas of biomedicine are critically bottlenecked by limitations in our ability to bank living organs and other complex tissues. Breakthroughs in organ and complex tissue banking have the potential to affect millions of patients each year worldwide. The 1st Global Organ Banking Summit in 2015, with events at Stanford, NASA, and Lawrence Berkeley National Lab, brought together leading experts in mechanical engineering, cell biology, cryobiology, organ transplantation and other areas to identify the key remaining challenges of organ and complex tissue banking. Some of these ‘organ banking sub-challenges’ are amenable to nanoscale solutions, creating a fertile space for the application of existing nanotechnology approaches and the development of new ones, that is receiving increasing interest from U.S. science agencies, the research community and the general public. The Organ Banking Session at NEMB is part of a growing conversation around organ banking that in 2015 included the Global Organ Banking Summit, along with an NSF-sponsored Technology Roadmapping Process, Washington, D.C. organ banking workshop, White House Roundtable and West Point workshop involving multiple NIH institutes, DARPA, NSF, NASA, the Dept. of Defense, the White House, and other governmental bodies as well as industry leaders and academic subject matter experts in a number of disciplines surrounding the organ banking challenge. The Department of Defense alone has released six new government grant pipelines targeting the preservation and banking of complex tissues. At the NEMB organ banking session, we will outline the public health impact and growing interest of the organ banking challenge, as well as the key scientific sub-challenges and many of the promise approaches being used to meet these challenges. Discussions will focus on the recent nanotechnology breakthroughs that show promise for organ banking and on untapped opportunities to apply nanoscale solutions to make breakthroughs in organ and tissue banking.

Plenary Sessions

PLENARY TITLE: NICHOLAS A. PEPPAS: NANO-ENGINEERING INTELLIGENT AND DIAGNOSTIC THERAPEUTIC SYSTEMS

Date/Time: Sunday, February 21, 04:00-04:45 PM

Location: Grand Ballroom

Presenter: **Nicholas A. Peppas**, University of Texas, Austin

Session Description: Engineering the molecular design of intelligent biomaterials by controlling recognition and specificity is the first step in coordinating and duplicating complex biological and physiological processes. Recent developments in protein delivery have been directed towards the preparation of targeted formulations for protein delivery to specific sites, use of environmentally-responsive polymers to achieve pH- or temperature-triggered delivery, usually in modulated mode, and improvement of the behavior of their mucoadhesive behavior and cell recognition. We address design and synthesis characteristics of novel crosslinked networks capable of protein release as well as artificial molecular structures capable of specific molecular recognition of biological molecules. Molecular imprinting and microimprinting techniques, which create stereo-specific three-dimensional binding cavities based on a biological compound of interest can lead to preparation of biomimetic materials for intelligent drug delivery, drug targeting, and tissue engineering. We have been successful in synthesizing novel glucose- and protein-binding molecules based on non-covalent directed interactions formed via molecular imprinting techniques within aqueous media.

Bio: Nicholas A. Peppas is the Cockrell Family Regents Chaired Professor in the Departments of Chemical, Biomedical Engineering and Pharmacy and Director of the Institute of Biomaterials, Drug Delivery and Regenerative Medicine of the University of Texas at Austin. From 2009 to 2015 he was also the Chairman of the Department of Biomedical Engineering . His work in biomaterials, polymer physics, drug delivery and bionanotechnology follows a multidisciplinary approach by blending modern molecular and cellular biology with engineering principles to design the next-generation of medical systems and devices for patient treatment. Over the past 40 years he has set the fundamentals and rational design of drug delivery systems and developed models of drug and protein diffusion in controlled release devices and biological tissues. In 2012 he received the Founders Award of the National Academy of Engineering (NAE), the highest recognition of the Academy, for these contributions to the field. Peppas is a member of the NAE, National Academy of Medicine (NAM), National Academy of Inventors (NAI), the National Academy of France, the Royal Academy of Spain, the Academy of Athens and the Academy of Texas. He has been recognized with awards from AIChE (Founders Award, William Walker Award, Institute Lecture, Jay Bailey Award, Bioengineering Award, Materials Award), the Biomedical Engineering Society (Distinguished Scientist Award), the American Institute of Medical and Biological Engineering (Galletti Award), the Society for Biomaterials (Founders, Clemson and Hall Awards), the Controlled Release Society (Founders, Heller and Eurand Awards) and other societies. In 2008, AIChE named him on of the One Hundred Chemical Engineers of the Modern Era. He is President of the International Union of Societies of Biomaterials Science and Engineering, Past-Chair of the Engineering Section of the American Association for the Advancement of Science, and Past-Chair of the Council of BME Chairs. Previously, he served as President of SFB and the Controlled Release Society. He is a fellow of AAAS, AIChE, APS, ACS, MRS, SFB, BMES, AIMBE, CRS, AAPS, and ASEE. He has supervised the research of 100 PhDs and about 180 postdocs and graduate students. Peppas holds a Dipl. Eng. from the NTU of Athens (1971), a Sc.D. from MIT (1973), and honorary doctorates from the Universities of Ghent, Parma, Athens, Ljubljana, Patras and Sichuan.

PLENARY TITLE: THERMAL CONTRAST AND NANOWARMING: HOW NANOPARTICLE HEATERS ARE IMPROVING DIAGNOSTICS AND REGENERATIVE MEDICINE

Date/Time: Monday, February 22, 08:30-09:15 AM

Location: Grand Ballroom

Presenter: **John C. Bischof**, University of Minnesota

Session Description: Gold and magnetic nanoparticles have unique and tunable properties that allow transduction of optical (light), or radiofrequency (RF) electromagnetic fields to affect heating of biomaterials at multiple scales. This talk will explore the underlying physics and relative advantages of each form of nanoparticle heating and then introduce several applications. First, laser heating of gold nanoparticles is used to achieve an order of magnitude improvement in sensitivity for common point-of-care (POC) diagnostic assays (i.e. a lateral flow immunoassay or LFA) through “thermal” vs. visual contrast. This increase in sensitivity addresses the main weakness of the LFA, increasing opportunities for use in POC settings and avoiding the cost, time and labor of laboratory tests. Second, RF heating of magnetic nanoparticles deployed within biomaterials can be used for improved cancer hyperthermia and regenerative medicine. For instance, in regenerative medicine, “nanowarming” by this method can heat vitrified biomaterials at a sufficiently rapid and uniform rates to avoid crystallization and cracking, thereby addressing an important technology bottleneck. In summary, this talk demonstrates the growing opportunities for nanoparticle heating in biomedical applications.

Bio: Bischof’s area of research is in thermal bioengineering with a focus on biopreservation, thermal therapy and nanomedicine. Awards and recognition for his work include: the ASME Van Mow Medal; several “Career,” “First” and “Young Investigator” Awards from the NSF, NIH and Whitaker Foundation; and Fellowships in AIMBE, ASME, Society for Cryobiology, Alexander von Humboldt, and the Japan Society for the Promotion of Science (JSPS). He has served as the President of the Society for Cryobiology and Chair of the Bioengineering Division of the ASME.

Bischof obtained a B.S. in Bioengineering from U.C. Berkeley (UCB) in 1987, an M.S. from UCB and U.C. San Francisco in 1989 and a Ph.D. in Mechanical Engineering from UCB in 1992 under Boris Rubinsky. After a Post-doctoral Fellowship at Harvard in the Center for Engineering in Medicine under Mehmet Toner he joined the University of Minnesota in 1993. Bischof is now a Distinguished McKnight University Professor in the Departments of Mechanical and Biomedical Engineering, the inaugural Carl and Janet Kuhrmeyer Chair in Mechanical Engineering and the Associate Head of the Institute for Engineering in Medicine at the University of Minnesota.

PLENARY TITLE: NANO ASPECTS IN THE PHYSICS OF CANCER: FROM NUCLEAR REMODELING TO SELF-RECOGNITION FOR GENE/DRUG DELIVERY

Date/Time: Monday, February 22, 02:30 -03:15 PM
Location: Grand Ballroom

Presenter: **Dennis Discher**, University of Pennsylvania

Session Description: Tissue cells, implants, and particles of any type interact with the innate immune system, especially phagocytes that try to 'eat' everything. However, 'Self' cells are spared due to a polypeptide found on all cells that marks cells (as well as engineered viruses and particles) as 'Self', limiting their phagocytic clearance in vitro and in vivo. The phagocyte's cytoskeleton drives the decision downstream of adhesion. If an injected cell is recognized as 'Self' and if it has stem-like properties, then further interactions with the surrounding tissue can influence its differentiation. Matrix elasticity is one physical feature that directs stem cell fate and reflects the fact that tissues can be very soft like fat and brain, or increasingly stiff like striated muscle and rigid like bone. Stem and progenitor cells use myosin-II to feel and respond to such elasticity differences, with physical signals propagating all the way into the nucleus, which feeds back on gene expression. What unifies these mechanisms of immune or matrix recognition is a convergence of decision-making pathways on cytoskeletal force generation.

Bio: Dennis E. Discher is the Robert D. Bent Chair Professor and Director of the NCI-funded Physical Sciences Oncology Center at the University of Pennsylvania. He received a Ph.D. jointly from the University of California, Berkeley and San Francisco in 1993 for biophysical studies of cell membranes, and was a U.S. National Science Foundation International Fellow in computational biophysics at the University of British Columbia until 1996. He has coauthored more than 200 publications with >30,000 citations that range in topic from matrix effects on stem cells and physical properties of the cell nucleus to mechanisms of 'self' recognition by macrophages and self-assembling polymers applied to disease, with papers appearing in Science, Cell, and various Nature journals. ISI lists 'Matrix elasticity directs stem cell lineage specification' (Cell 2006) as the 3rd most cited experimental research paper in the field of Molecular Biology & Genetics since 2004. Additional honors and service include: election to the U.S. National Academy of Engineering, a Presidential Early Career Award for Scientists and Engineers from the U.S. National Science Foundation, the Friedrich Wilhelm Bessel Award from the Humboldt Foundation of Germany, a LabEx Visiting Fellow at the Institut Jacques Monod – Paris, and membership on the Editorial Board for Science.

PLENARY TITLE: IMAGE-GUIDED NANOTHERAPY IN THE TREATMENT OF CANCER AND CARDIOVASCULAR DISEASE

Date/Time: Tuesday, February 23, 08:30-09:15 AM
Location: Grand Ballroom

Presenter: **Katherine Whittaker Ferrara**, University of California, Davis

Session Description: In vivo imaging can greatly enhance the optimization of nanodelivery methods. In our laboratory, we combine the use of positron emission tomography (PET) and optical imaging to quantify delivery, the use of magnetic resonance imaging (MRI) for anatomic and functional imaging and to assess local temperature and the use of ultrasound to enhance delivery. Using this combination of techniques, we have developed activatable particles for the delivery of chemotherapeutics in cancer and targeted nanovehicles for the delivery of miRNA in cardiovascular disease. Our cancer focus has involved identifying nanodelivery strategies that are curative for local disease and strategies that synergize with immunotherapy for the treatment of systemic disease. We have developed methods to load activatable particles with a metal-drug complex and to release this complex by increasing the local temperature using ultrasound. Full release of the chemotherapeutic occurs only in the region of elevated temperature and in the presence of a reduced pH. We find that such a strategy enhances efficacy while reducing systemic toxicity. We will describe both the nanotherapeutic strategies and the imaging systems that facilitate this approach. In addition, we have recently explored the use of targeted, coated cationic particles to facilitate the delivery of biologic therapeutics to endothelial cells and will demonstrate the imaging approaches required for the optimization of such a design and the resulting efficacy in cardiovascular disease.

Bio: Katherine Whittaker Ferrara is a Distinguished Professor of Biomedical Engineering at UC Davis and the Director of theCenter for Content Rich Evaluation of Therapeutic Efficacy (cCRETE). She is a member of the National Academy of Engineering and a fellow of the IEEE, American Association for the Advancement of Science, the Biomedical Engineering Society, the Acoustical Society of America and the American Institute of Medical and Biological Engineering. She currently chairs the Clinical Molecular Imaging Probes (CMIP) NIH study section. Dr. Ferrara received her Ph.D. in 1989 from the University of California, Davis. Prior to her PhD, Dr. Ferrara was a project engineer for General Electric Medical Systems, involved in the development of early magnetic resonance imaging and ultrasound systems. Following an appointment as an Associate Professor in the Department of Biomedical Engineering at the University of Virginia, Charlottesville, Dr. Ferrara served as the founding chair of the Department of Biomedical Engineering at UC Davis. Her laboratory is known for early work in aspects of ultrasonics and has more recently expanded their focus to broadly investigate molecular imaging and drug delivery. Dr. Ferrara's laboratory has received numerous awards including the Achievement Award from the IEEE Ultrasonics, Ferroelectrics and Frequency Control Society, which is the top honor of this society.

PLENARY TITLE: DIRECT CELL REPROGRAMMING VIA GENOME EDITING

Date/Time: Tuesday, February 23, 02:30-03:15 PM
Location: Grand Ballroom

Presenter: **Kam W. Leong**, Columbia University

Bio: Kam W. Leong is the Samuel Y. Sheng Professor of Biomedical Engineering at Columbia University. His lab works on nanoparticle-mediated nonviral gene delivery and immunotherapy—from design and synthesis of new carriers to applications for hemophilia and infectious diseases. The lab also works on the application of nanostructured biomaterials for regenerative medicine, particularly on understanding cell-topography interactions and on the application of

nonviral vectors for direct cellular reprogramming. Kam has published more than 300 peer-reviewed research manuscripts with over 26,000 citations and holds more than 50 issued patents. His work has been recognized by a Young Investigator Research Achievement Award of the Controlled Release Society, Distinguished Scientist Award of the International Journal of Nanomedicine, and Clemson Award for Applied Research of the Society for Biomaterials. Kam is the Editor-in-Chief of Biomaterials, a member of the National Academy of Inventors, and a member of the USA National Academy of Engineering. He received his PhD in Chemical Engineering from the University of Pennsylvania.

PLENARY TITLE: REBECCA RICHARDS-KORTUM: POINT-OF-CARE DIAGNOSTICS FOR LOW-RESOURCE SETTINGS

Date/Time: Wednesday, February 24, 08:30-09:15 AM

Location: Grand Ballroom

Present: **Rebecca Richards-Kortum**, Rice University

Session Description: There are many barriers to successful development and dissemination of new technologies to improve health care in low-resource settings. These include a lack of infrastructure (e.g., reliable electrical power, clean water), lack of trained personnel, limited financial resources for health care that consequently limits availability of healthcare equipment and consumables, and lack of functional health systems to track results and patients for effective follow-up care. To be useful, new technologies must be affordable, robust, simple to use, and capable of functioning in a setting with limited infrastructure. Advances in optical technologies, molecular recognition, and low power sensors now offer the ability to design low-cost platforms for point-of-care (POC) diagnostics. Efforts to integrate molecular imaging together with miniature microscopes are now yielding new POC diagnostics for both infectious and chronic diseases. Driven by advances in consumer electronics, high resolution imaging can be obtained with low cost devices; advances in digital signal processing provide the ability to automate analysis. This talk will highlight strategies that have proven to be effective for design of high performance, affordable diagnostic technologies with promise to improve health care in low-resources settings. We are using these advances to improve early detection of cervical, esophageal, colon, breast, and oral cancer; clinical trials are underway in Houston, Brazil, and China. Finally, we will discuss the potential to combine point-of-care detection and treatment of early disease in low-resource settings and opportunities to use these approaches in both low- and high-resource settings.

Bio: Rebecca Richards-Kortum is the Stanley C. Moore Professor of Bioengineering at Rice University. Previously, she held the Cockrell Family Chair in Engineering #10 and was a Professor of Biomedical Engineering at the University of Texas at Austin, where she was also a Distinguished Teaching Professor. Guided by the belief that all of the world's people deserve access to health innovation, Rebecca's research and teaching focus on developing low-cost, high-performance technology for low-resource settings. She is known for providing vulnerable populations in the developing world access to life-saving health technology, focusing on diseases and conditions that cause high morbidity and mortality. Rebecca's work in appropriate point-of-care screening technologies has earned her induction into the National Academy of Engineering, the National Academy of Sciences, the National Academy of Inventors, and the American Academy of Arts and Sciences. After receiving a B.S. in Physics and Mathematics from the University of Nebraska-Lincoln in 1985, Rebecca continued her graduate work at the Massachusetts Institute of Technology, where she received an M.S. in Physics in 1987 and a PhD in Medical Physics in 1990.

TRACK 1 NANOIMAGING

MONDAY, FEBRUARY, 22

1-1
TRANSLATIONAL NANOPARTICLES

Sam Houston 9:30am - 11:00am

Session Organizer: **Weibo Cai**, *University of Wisconsin-Madison, Madison, WI, United States*

9:30am Translational Ultrasmall Particle Imaging Tools for Molecular Cancer Imaging and Intraoperative Treatment

Keynote. NEMB2016-5920

***Michelle Bradbury**, Memorial Sloan Kettering Cancer Center, New York, NY, United States*

Despite recent advances in imaging probe development for biomedicine, the translation of targeted diagnostic platforms remains challenging. Nanomaterials platforms currently under evaluation in oncology clinical trials are largely non-targeted drug delivery vehicles or devices to thermally treat tissue; these are typically not surface modified for targeted detection by clinical imaging tools. New tumor-selective platforms need to satisfy critical safety benchmarks, in addition to assaying targeted interactions with the microenvironment and their effects on biological systems. Coupled with metabolic imaging and analysis tools, such as PET, complete and quantitatively accurate data sets for whole body distributions, targeting kinetics, and clearance profiles of new diagnostic platforms undergoing preclinical testing or transitioning into early-phase clinical trials can be acquired.

In the operating theatre, there is also an urgent need for implementing new image-directed visualization tools that can enhance surgical vision, facilitate minimally invasive surgical procedures, and dramatically alter surgical outcomes of oncological patients. The lack of clear surgical vision impacts the ability of the operating surgeon to accurately and specifically identify the extent of malignancy, microscopic tumor burden, or remnant disease. Collectively, these factors affect therapeutic outcome, prognosis, and treatment management. Newer molecular imaging probe designs coupled with state-of-the-art device technologies, may enhance cancer care, provide real-time imaging guidance, and lead to new, more efficient approaches for early-stage detection and treatment.

Advances in nanotechnology have also fueled a paradigm shift in targeting and safely delivering drugs in conjunction with image-directed approaches. The size, architecture, and chemical composition of particle-based drug delivery vehicles can be fine-tuned to achieve properties optimal for loading and controlled release of therapeutic agents, patient safety/compliance, favorable kinetic profiles, and reducing unwanted side effects. By combining therapeutic particle tracer preparations with quantitative bioimaging approaches, drug delivery, lesion localization, and the extraction of key tumor biologic properties can be achieved for individualizing treatment planning. In turn, dosage regimens needed to achieve therapeutic efficacy might be estimated based on knowledge of drug specific activity and dose, uptake kinetics, and IC50 values.

The ability to flexibly adapt the formulation of clinically-promising drugs to improve their physicochemical and/or biological properties, in combination with metabolic imaging tools, will be important to quantify and establish suitable clinical trial endpoints. Issues relating to solubility, transport, barrier penetration, time-dependent changes in drug uptake, and intratumoral distribution are additional considerations. These properties are often not generally evaluated in the context of drug delivery due to the complexity of the biological systems involved and the inability to serially monitor this process non-invasively in the absence of drug labeling. The future success of molecular medicine will, in part, rest upon our ability to offer improved clinical trial designs addressing the foregoing issues. In conclusion, the adoption of such an approach for image-directed drug delivery in clinical settings will have far-reaching implications for personalizing cancer care in terms of treatment planning, stratification to appropriate trial arms, and response monitoring.

10:00am Intrinsically Radiolabeled Nanoparticles: An Emerging Paradigm

Technical Presentation. NEMB2016-5904

***Weibo Cai**, University of Wisconsin-Madison, Madison, WI, United States*

With the rapid growing interests in using radioisotopes for nanooncology, a broad spectrum of radiotracers has been generated for positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging in different diseases. To date, different radioisotopes have been labeled to carriers, such as antibodies, peptides, nanoparticles, etc., for in vivo biomarker expression level imaging, early tumor detection, drug biodistribution pattern studies, and so on. The most widely used radiolabeling strategy involves the use of exogenous chelators which could coordinate with certain radioisotopes to form stable complexes. Well-established chelators, such as 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), p-isothiocyanatobenzyl-desferrioxamine (Df-Bz-NCS) and diethylene triamine pentaacetic acid (DTPA), etc., have been employed for radiolabeling of copper-64 (^{64}Cu, $t_{1/2}$=12.7 h), zirconium-89 (^{89}Zr, t1/2=78.4 h), and indium-111 (^{111}In, $t_{1/2}$=2.8 d) for imaging in preclinical studies.

Different isotopes vary significantly in their coordination chemistry, making selection of the right chelator for a specific isotope vital. However, this could be tricky and even impossible to achieve in some cases. For example, it is still a major challenge to radiolabel certain isotopes such as arsenic-72 (^{72}As, $t_{1/2}$=26 h) and germanium-69 (^{69}Ge, $t_{1/2}$=39.1 h). In addition, although most of the radiolabeling could be done under mild conditions, in some cases successful radiolabeling might require very harsh conditions (e.g. high reaction temperature with prolonged incubation time) which limit their use.

The other concerns of using traditional radiolabeling strategies include the possible alteration of pharmacokinetics of carriers and potential detachment of radioisotopes, which could lead to problems such as off-targeting and false positives. Since the carrier itself is not labeled and PET (or SPECT) only detects signals from the radioisotope, the integrity (or stability) of the radiolabeled system in the complicated physiological environment should always be thoroughly investigated before in vivo imaging applications. Detachment of the radioisotopes from the carriers can also lead to potential transchelation to proteins, causing erroneous interpretation of the results. Therefore, successful chelator-based radiolabeling requires in-depth knowledge of the coordination chemistry and selection of the best chelator for every radioisotope.

To address these concerns, recent research has been focusing on developing more reliable chelator-free radiolabeling techniques, which could fully take advantage of the unique physical and chemical properties of well-selected inorganic or organic nanoparticles for radiolabeling, and more importantly, offer an easier, faster, and more specific radiolabeling possibility. Therefore, intrinsically radiolabeled nanoparticles have becoming an increasingly more important research topic, which could be achieved mainly via four different methods. The four major categories for intrinsically radiolabeled nanoparticles include: 1) hot-plus-cold precursors, 2) specific trapping, 3) cation exchange, and 4) proton beam activation.

Representative examples of each category will be briefly illustrated. The main focus of this talk will be on our own recent work that involves the radiolabeling of a variety of nanomaterials via “specific trapping”, and the nanomaterials investigated in our laboratory include iron oxide nanoparticles, micelles, silica-based nanoparticles, multifunctional/multimodal hybrid nanomaterials, among others. Although still in the early stages, design and

synthesis of intrinsically radiolabeled nanoparticles have shown its attractive potential in offering an easier, faster, more stable, and more specific radiolabeling for the next generation of molecular imaging.

10:20am Renal Clearable Luminescent Metal Nanoparticles for Biomedical Imaging

Technical Presentation. NEMB2016-5932

***Jie Zheng**, The University of Texas at Dallas, Richardson, TX, United States*

While inorganic nanoparticles with size-dependent material properties open up unprecedented opportunities for novel biomedical technologies, translation of these nanoparticles into clinical practices has been hampered by the potential toxicity resulted from their long-term nonspecific accumulation in healthy tissues. Emergence of renal clearable inorganic nanoparticles makes it possible to address this long-term challenge. In the past few years, we used glutathione, a tri-amino-acid peptide to stabilize 2-3nm gold nanoparticles, which can give different colored luminescence upon their valence states of gold atoms (1). These glutathione coated gold nanoparticles (GS-AuNPs) have little interactions with serum proteins; and more impressively, they can be cleared from the body through kidneys with an efficiency of 10-100 times better than the same sized AuNPs (2) and exhibit unique molecular-like pharmacokinetics (3). By further modifying the surface chemistry, we found that these NPs can be successfully tuned to avidly target cancer cell membrane under mild acidic conditions (6.5 - 5.3) even in the presence of serum proteins (4). More recently, we found that they can passively target the MCF-7 breast cancer through enhanced permeability and retention (EPR) effect (5), which can be further enhanced through PEGylation (6). No limited to cancer imaging, noninvasive fluorescence kidney functional imaging can also be achieved with these renal clearable luminescent metal NPs (7). This new class of renal clearable AuNPs holds great promise to address challenges in biomedical imaging (8,9).

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10:40am Frozen Naphthalocyanine Micelles for Multimodal Intestinal Imaging

Technical Presentation. NEMB2016-5951

***Yumiao Zhang**, University at buffalo, buffalo, NY, United States, **Mansik Jeon**, Pohang Univ of Science and Technology, Gyeongsangbuk-do, Korea (Republic), **Laurie Rich**, s, Roswell Park Cancer Institute, buffalo, NY, United States, **Hao Hong**, University of Michigan, Ann Arbo, MI, United States, **Jumin Geng, Paschalis Alexandridis**, University at Buffalo, Buffalo, NY, United States, **Jan Huizinga**, McMaster University, Hamilton, ON, Canada, **Mukund Seshadri**, Roswell Park Cancer Institute, buffalo, NY, United States, **Weibo Cai**, University of Wisconsin-Madison, Madison, WI, United States, **Chulhong Kim**, Pohang University of Science and Technology, Pohang, Korea (Republic), **Jonathan Lovell**, University at Buffalo, Buffalo, NY, United States*

There is a need for safer and improved methods for non-invasive imaging of the gastrointestinal tract. Here, we report the development of a family of nanoparticles that can withstand the harsh conditions of the stomach and intestine, avoid systemic absorption, and provide good optical contrast for photoacoustic imaging. The hydrophobicity of naphthalocyanine dyes was exploited to generate frozen micelles (nanonaps), with tunable near-infrared absorption. Nanonaps, following oral administration in mice, passed safely through the gastrointestinal tract. Non-invasive, non-ionizing photoacoustic techniques were used to visualize nanonap intestinal distribution with low background and remarkable resolution, and enabled real-time intestinal functional imaging with ultrasound co-registration. Positron emission tomography following seamless nanonap radiolabelling allowed complementary whole-body imaging.

1-2
MRI, MULTI-MODAL, AND THERANOSTIC NANOPARTICLES

Sam Houston 11:30 AM - 1:00 PM

Session Organizer: **Jonathan Lovell**, *University at Buffalo, Buffalo, NY, United States*

11:30am ^{19}Fluorine Magnetic Resonance Imaging with Perfluorocarbon Nanoparticles

Keynote. NEMB2016-5929

***Gregory Lanza**, Washington University School of Medicine, St. Louis, MO, United States*

^{19}Fluorine (^{19}F) magnetic resonance imaging (MRI) has its research roots dating back over 35 years. Over that time span, ^{1}H imaging flourished and was adopted throughout the world with an endless array of applications and imaging approaches, making MR an indispensible pillar of biomedical diagnostic imaging. For many years during this timeframe, ^{19}F imaging research continued at a slow pace as the various attributes of the technique were explored. However, over the last decade and particularly the last several years, the pace and clinical relevance of ^{19}F imaging has exploded. In part this is due to advances in MRI instrumentation, ^{19}F/^{1}H coil designs, and ultrafast pulse sequence development for both preclinical and clinical scanners. These achievements coupled with the interest in molecular imaging of anatomy and physiology combined with a cadre of innovative agents, has brought the concept of ^{19}F to the brink of clinical use. Indeed, ^{19}F/^{1}H molecular imaging may address the severe lack of clinical noninvasive imaging capability for noncancer lung disease, such as asthma, emphysema, interstitial lung diseases, pulmonary hypertension and more.

12:00pm A Multi-Functional Nanoplatform For The Enhancement And Prediction Of Therapeutic Response To External Beam Radiation Therapy

Technical Presentation. NEMB2016-5971

***Ajlan Al Zaki**, **Casey McQuade, Gary Kao, Jay Dorsey**, **Andrew Tsourkas**, University of Pennsylvania, Philadelphia, PA, United States*

Gold nanoparticles have garnered interest as both radiosensitzers and computed tomography (CT) contrast agents. However, the extremely high concentrations of gold required to generate CT contrast is far beyond that needed for meaningful radiosensitization, which limits their use as combined therapeutic-diagnostic - theranostic - agents. To establish a theranostic

nanoplatform with well-aligned radiotherapeutic and diagnostic properties for better integration into standard radiation therapy practice, we developed a gold- and superparamagnetic iron oxide nanoparticle (SPION)-loaded micelle (GSM). Intravenous injection of GSMs into tumor-bearing mice led to selective, tumoral accumulation, enabling magnetic resonance (MR) imaging of tumor margins. Subsequent irradiation led to a 90-day survival of 71% in GSM-treated mice, compared with 29% for irradiation-only mice. Furthermore, measurements of the GSM-enhanced MR contrast were highly predictive of tumor response. Therefore, incorporating GSMs with radiation therapy could augment cancer treatment by facilitating imaging, increasing the efficacy of therapy, and helping to predict response. Moreover, since GSMs are prepared using a highly modular synthetic pathway, additional components, including standard and alternative therapeutics could readily be incorporated into the micelle's core while targeting moieties (e.g. tumor-specific antibodies or Fab's) can be coupled onto the unobstructed micelle surface, further broadening the range and types of tumors that can be effectively treated. The extravasation properties of these particles may also make them useful in treating diseases localized to regional lymph nodes, such as Hodgkin's lymphoma. Therefore, it is envisioned that translation of GSM to oncology could have far reaching implications.

Despite the perceived biocompatibility of gold nanoparticles, the protracted elimination and long-term persistence of gold within many organ systems remains a concern for clinical translation. To improve the excretion of gold in our nanoplatform, we prepared micelles with 0.9 nm or 5 nm gold nanoparticles tightly packed within the hydrophobic core. The use of many small gold nanoparticles was expected to allow for improved excretion of gold, compared with single large gold nanoparticles, owing to the smaller size and larger surface-to-volume ratio of the individual gold nanoparticles within the micelle. Following intravenous administration of micelles, organs were harvested and examined for gold content using inductively coupled plasma optical emission spectrometry (ICP-OES) for up to 3 months post-injection. The micelles containing 0.9 nm gold nanoparticles showed a 72% and 67% reduction in gold content in the liver and spleen, respectively, between 1 day and 3 months post-injection, compared with a 38% and 35% reduction in mice receiving micelles with 5 nm gold nanoparticles. Furthermore, feces and urine analysis revealed approximately 7.5 and 100 times more gold, respectively, in mice that received micelles with 0.9 nm gold nanoparticles one day after injection. These findings suggest that the excretion profile of inorganic nanomaterials may be improved if clusters of small inorganic materials are used in favor of single solid particles.

12:20pm Magnet-Optical nanoparticles for Magnetomotive Photoacoustic Imaging

Technical Presentation. NEMB2016-6145

Xiaohu Gao, Junwei Li, *University of Washington, Seattle, WA, United States*

Photoacoustic imaging has emerged as a highly promising tool to diagnose tumor lesion site with deep tissue penetration. However, image contrast under in vivo conditions is far from optimal due to background signals from tissue. We have previously developed the new concept of magnetomotive photoacoustic (mmPA) imaging, which is capable of dramatically reducing the influence of background signals and producing high-contrast images. Here we addressed two significant advances toward clinical translation of this technology. First, we prepared a new class of compact, uniform, magneto-optically coupled core-shell nanoparticles through localized copolymerization of conducting polymers on an iron oxide nanoparticle surface. The resulting multifunctional nanoparticles solve the photo-instability and small-scale synthesis problems previously encountered by the gold coating approach. In parallel, we developed a new generation of mmPA imaging featuring cyclic magnetic motion and ultras! ound speckle tracking (USST), whose imaging capture frame rate is several hundred times faster than the photoacoustic speckle tracking (PAST) method we demonstrated previously. These advances enable robust artifact elimination caused by physiologic motions and first application of the mmPA technology in vivo for sensitive solid tumor imaging.

12:40pm Albumin-Biomineralized Gd:CuS Theranostic Nanoprobes for In Vivo Photoacoustic /MR Imaging-Guided Tumor Photothermal Therapy

Technical Presentation. NEMB2016-6096

Weitao Yang, Jin Chang, *Tianjin University, Tianjin, China,* ***Bingbo Zhang***, *Tongji University, Shanghai, China*

Photothermal therapy (PTT) has attracted increased interests due to its noninvasiveness and low systemic adverse effects. There is, however, still a strong desire for biocompatible and versatile PTT agents with accurate imaging guidance on its therapy efficiency. In this study, bovine serum albumin (BSA), as a biotemplate was employed for biomimetic synthesis of Gd-integrated CuS hybrid nanoprobes (Gd: CuS CuS CuS CuS CuS CuS@BSA promising for imaging guidance on potent tumor ablation with high spatial resolution and deep tissue penetration.

1-3
ADVANCED OPTICAL NANOPARTICLES AND IMAGING TECHNIQUES

Sam Houston 4:00pm - 5:30pm

Session Organizer: **Andrew Tsourkas**, *University of Pennsylvania, Philadelphia, PA, United States*

4:00pm Plasmonic Nanoparticle Vesicles for Cancer Imaging and Therapy

Keynote. NEMB2016-5930

Shawn Chen, *National Institute of Biomedical Imaging and Bioengineering, Bethesda, MD, United States*

Vesicular structures can have tailored structural properties and built-in releasing mechanisms, controlled by stimuli-responsive polymer building blocks towards cancer diagnosis and therapy. In this talk, examples of how gold nanoparticles are assembled to have enhanced optical properties (scattering, photothermal conversion, and SERS) for Raman detection, photoacoutsic imaging and photothermal treatment of cancer will be illustrated. The interparticle coupling and disintegration of the plasmonic vesicles can be tailored by altering structural parameters and conformational changes of the covalently bound polymer brushes.

4:30pm Deep and high-resolution three-dimensional tracking of single particles using nonlinear and multiplexed illumination

Technical Presentation. NEMB2016-6003

Evan Perillo, Yen-Liang Liu, Cong Liu, Andrew K Dunn, Tim Yeh, *University of Texas at Austin, Austin, TX, United States*

Molecular trafficking within engineered three-dimensional multicellular models is critical to the understanding of the development and treatment of various diseases including cancer. However, current tracking methods are either confined to two dimensions or limited to an interrogation depth of 15 µm. Here we present a new 3D tracking method capable of quantifying rapid molecular transport dynamics in highly scattering environments at depths up to 200 µm. The system has a response time of 1 ms with a temporal resolution down to 50 µs in high signal-to-noise conditions, and a spatial localization precision as good as 35 nm. Built upon spatiotemporally multiplexed two-photon excitation, this approach requires only one detector for 3D particle tracking and allows for two-photon, multi-color imaging. 3D tracking of epidermal growth factor receptor (EGFR) complexes at a depth of 100 µm in tumor spheroids is demon-

strated. Our 3D tracking microscope is built upon spatiotemporally multiplexed two-photon excitation and uses time-gated analysis via a photon counting histogram to discern the molecular 3D position. Feedback control then steers the excitation to lock-on to the single molecule as it travels at a high speed. The molecular trajectories are reconstructed from the recorded actuator positions from the feedback control loop operating at 1-5 ms. Dynamics down to 50 µs can be inferred from analysis of the photon counting histogram. In our method, the first PMT channel is used for particle tracking while the second and the third PMT channels can be used for two-photon scanning microscopy, colocalization analysis, and energy transfer studies. We have coined this technique TSUNAMI (Tracking Single particles Using Nonlinear And Multiplexed Illumination).

Reference: E. Perillo et al., "Deep and high-resolution three-dimensional tracking of single particles using nonlinear and multiplexed illumination," Nature Communications, 2015.

4:50pm Eddy current microscopy for biomedical applications

Technical Presentation. NEMB2016-6004

***Josh Javor**, **Vish Subramaniam**, **Joseph West, Travis Jones**, The Ohio State University, Columbus, OH, United States*

The non-invasive, real-time detection and imaging of microscopic morphological artifacts in biological tissue is imperative to the diagnosis and subsequent treatment of various diseases. Optical microscopy is the standard in biological and biomedical applications and is invaluable in medical diagnosis. By its nature, it is capable of discriminating between different morphological structures, tissue types, and cells only after fixing (e.g. with formaldehyde or formalin) and staining (e.g. for hematoxylin and eosin) procedures are applied. There is a need therefore to explore non-invasive and real-time approaches that might complement optical microscopy, but yet yield different information regarding electromagnetic properties. For example, when cancer cells or small solid tumors are present in otherwise normal tissue, there are changes in electromagnetic characteristics in addition to morphological changes in the surrounding normal tissue that can be detected without the fixing and staining processes required for optical microscopy. Eddy-current detection is a technique that has been used to detect microscopic features, such as carbon nanotubes, beneath the surface in conductive specimens and can be extended to detect physical features in heterogeneous biological samples with varying electrical conductivities and morphological structures. The purpose of this study is to develop a scalable eddy current detection tool to image morphological structures on the order of the dimensions of the immediate environment around individual cells (~ 10 µm-100 µm). A detection scheme using a dual axis coil is used, in which the inner coil produces a temporally varying magnetic field resulting in eddy currents in the specimen whose magnetic fields are detected by the outer coil. Lock-in amplification is used to amplify small changes (nV) in the magnitude of the detector coil waveform arising from eddy currents in the sample. First, a fabrication technique is developed to manufacture multi-turn, multi-layer (25 turns, 2 layers) coils with the smallest diameter of commercially available magnet wire (25µm) around a 300 µm core. A circuit element model is then developed and its governing equations are solved in MATLAB to predict and compare with experimental measurements. Images and measurements are generated using eddy current microscopy on metal ring phantoms, animal tissue, plant leaves, and superficial skin samples from mouse models for squamous cell carcinoma. The results were analyzed in a contour plot using MATLAB and compared to macroscopic (mm) eddy current detection and observable artifacts in the samples. Improving the resolution of eddy current detection in biological samples using smaller probes (80µm) is also discussed.

5:10pm Direct, Multiplexed Molecular Profiling Using Fluorescence Lifetime Imaging

Technical Presentation. NEMB2016-6031

***Maha Rahim**, **Rajesh Kota, Enrico Gratton, Jered Haun**, University of California, Irvine, Irvine, CA, United States*

Cancer is complex and heterogeneous. Successful diagnostic techniques will require molecular characterization of cancer to interrogate the diverse cell types and functional states within tumors. Next generation sequencing and mass spectrometry offer multiplexed molecular analysis. However, genomic methods are destructive, and transcript levels do not always reflect functional levels. These strategies also require interrogation of a population of cells in bulk, sacrificing spatial resolution. Advancements enabling single-cell transcriptional analysis still lack the ability to study protein in its cellular context, and complex regulatory processes and signaling pathways cannot be elucidated from the genome alone. In this study, we have developed a nanoparticle targeting platform using fluorescence lifetime imaging (FLIM) capable of direct detection of functional molecules and extreme multiplexing while maintaining spatial resolution.

We performed FLIM on nanoprobes in solution and analyzed lifetime using the phasor approach. The phasor approach to FLIM, pioneered by Dr. Enrico Gratton and the Laboratory of Fluorescence Dynamics, greatly simplifies lifetime analysis. Instead of fitting complicated exponential functions, the phasor approach transforms histograms of time delays at each image pixel, replacing intricate decay curves to a simple graphical representation. We confirmed distinct phasor locations for 4 nanomaterial probes: a PDA liposome, organic fluorophore rhodamine, fluorophore-loaded nanoparticle, and cadmium selenide/cadmium sulfide quantum dot. Next, we conjugated these probes to a monoclonal antibody targeting HER2. Binding assays of immunoconjugates revealed optimal labeling concentrations between 1-30 nM. FLIM of HER2-targeted nanoprobes labeled onto SK-BR-3 cells demonstrated that phasor locations for each probe closely matched the solution measurements. Simultaneous targeting of EpCAM and HER2 with pairs of nanoprobes resulted in combination phasor results that correlated with expression levels. Targeting performed on a panel of cell lines with varying biomarker expression levels of HER2, EpCAM, transferrin receptor, and folate receptor, also resulted in combined phasor results that correlated well with expression levels measured by flow cytometry. To our knowledge, this is the first demonstration of resolving 4 probes based on fluorescence lifetime. To fully maximize the potential of this technology, a diverse set of nanomaterial probes with sufficient phasor resolution is needed. Thus, future work will focus on creating a library of probes with distinct lifetimes and extending detection into other spectral windows. We can achieve this by encapsulating fluorescent nanoprobes within a silica shell, thereby modulating lifetime through increased non-radiative decay. The extensive level of molecular information offered by our technology would make it possible to richly characterize heterogeneous tumor specimens to improve detection and enable host-cell subtyping, and rare cell detection.

5:30pm Comparison of Continuous-Wave and Frequency-Domain Fluorescence Tomography in a Commercial Hybrid Small Animal Scanner

Technical Presentation. NEMB2016-6163

***Eva Sevick-Muraca**, Center for Molecular Imaging, The Brown Foundation Institute of Molecular Medicine, The University of Texas Health Science Center, Houston, TX, United States*

Development of nanoimaging probes for detection of cancer metastases requires sensitive imaging devices for colocalization of imaging probes with metastatic lesions in clinically relevant animal models. With the advent of far-red fluorescent protein gene reporters, we and others have shown the ability to non-invasively track metastases as a function of time to tumor-draining lymph node basins, but have lacked a tomography approach sensitive enough for detection. Recently, we designed and installed a miniaturized near-infrared and far-red sensitive, gain-modulated intensified CCD camera system into the CT gantry of a Siemens Inveon PET/CT system to compare and demonstrate fluorescence tomography on phantoms and animals. By gain modulating the intensifier at a radio-frequency of 100MHz, we were able to conduct time-dependent frequency-domain measurements. By driving the intensifier with a constant voltage, we were able to conduct continuous wave measurements. Assessment of quantitative recovery of fluorescence absorption cross section was performed using a fully parallel, regularization-free, linear reconstruction algorithm with diffusion approxima-

tion and higher order simplified spherical harmonics approximation to the radiative transport equation (RTE). Regardless of reconstruction approach, the results show that while frequency-domain measurements may result in superior image reconstructions over CW measurements, data acquisition times were significantly longer, limiting utility for small animal imaging. Herein we demonstrate the adaptation of recently available CMOS detectors that can mitigate acquisition rates while maintaining signal to noise ratios that are needed for robust image reconstruction for far-red and near-infrared fluorescence tomography in small animals. Our next steps are to track cancer metastases in 3D with far-red fluorescent gene reporters and with near-infrared nano- and antibody-based probes that target cancer.

TUESDAY, FEBRUARY, 23

1-4
NOVEL NANOPARTICLES FOR IMAGING, DIAGNOSTICS, AND THERAPY

Sam Houston 9:30am - 11:00am

Session Organizer: **Jie Zheng**, *The University of Texas at Dallas, Richardson, TX, United States*

9:30am Nanohybrid Liposomal Cerasome for Imaging Guided Cancer Therapy

Keynote. NEMB2016-6079

***Zhifei Dai**, Peking University, Beijing, China*

Both liposomes and silica nanoparticles (NPs) have been widely investigated in the field of medicine. However, the serious drawbacks of silica NPs are their inherently non-biodegradability, high rigidity and low biocompatibility. Despite the excellent biocompatibility, liposomes still have not attained their full potential as drug and gene delivery vehicles due to the insufficient morphological stability. Therefore, we developed a cerasome with a liposomal bilayer structure and an atomic layer of polyorganosiloxane networks on its surface by molecularly designed lipidic organoalkoxysilane. Such unique structure gives wide applicability to cerasomes in roles as drug and gene delivery systems.

Cerasome combines the advantages of both conventional liposomes and silica NPs: (1) The siloxane surface can facilitate the stabilization of cerasomes in an environment with a slightly alkaline pH or a significant salt concentration; (2) The presence of a liposomal bilayer structure reduces the overall rigidity and density of cerasomes greatly compared to silica NPs; (3) Cerasomes can be loaded with hydrophilic, hydrophobic as well as amphiphilic drugs and imaging agents without destroying their morphological stability; (5) The silanol groups on the surface of cerasome can be functionalized to allow the easy bioconjugation of biomolecules with silane-coupler chemistry.

Various therapeutic agents (doxorubicin, paclitaxel, siRNA and radionuclides etc.) and imaging agents (radionuclides, quantum dots, gold and Fe_3O_4 etc.) were loaded into cersomes. Moreover, their surface was outfitted with ligands for targeting delivery to the tumor sites. Each component would operate a different function, such as molecular targeting, contrast enhanced imaging (fluorescence, PET, MRI, CT, photoacoustic and ultrasound etc.) and therapy (chemotherapy, radiotherapy, photothermal therapy, photodynamic therapy, gene therapy or combined therapy). Therefore, the cerasomes serve as a theranostic nanomedicine to be capable of noninvasive imaging and remote-controlled therapy.

References
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10:00am Leukocyte-based Biomimetic Nanovesicles for the Imaging of Inflamed Vasculature

Technical Presentation. NEMB2016-6049

***Jonathan Martinez, Roberto Molinaro, Naama Toledano Furman, Michael Evangelopoulos, Enrica De Rosa**, Houston Methodist Research Institute, Houston, TX, United States, **Roman Sukhovershin**, Methodist Research Institute, Houston, TX, United States, **Kelly A Hartman, John P. Cooke**, Houston Methodist Research Institute, Houston, TX, United States, **Ennio Tasciotti**, The Methodist Hospital Research Institute, Houston, TX, United States*

Uncontrolled inflammation plays a significant role in the establishment and progression of the cancer lesion. Specific probes that can provide detailed information on the location, extent and duration of the inflammatory process hold promise to improve the treatment of these disorders. Targeted nanoparticles offer great advantages for diagnostic imaging, however their sequestration by the mononuclear phagocyte system and inability to adequately negotiate other biological barriers has hindered their targeting, increased their toxicity, and limited their clinical success. Here we present a new biomimetic approach able to address these limitations. We developed Cell-derived Biomimetic Nanovesicles (CBN) composed by plasma membrane proteins (derived from leukocytes), and incorporated into a liposomal bilayer. This method allowed the transfer of 200 distinct membrane associated proteins to the surface of the CBN. The CBN showed the traditional physicochemical features of liposomes, and their ability to load diverse payloads (e.g., hydrophilic, amphiphilic, and hydrophobic). In addition, the CBN exhibited preferential docking and firm adhesion onto TNF-α activated (i.e., inflamed) endothelia. Furthermore, when systemically administrated, the incorporation of leukocyte self tolerance membrane proteins (CD47, CD45), enabled the CBN to exhibit longer circulation time and delayed the sequestration by the mononuclear phagocyte system. The CBN represents a platform that successfully combined the unique features of liposomes with leukocyte-like characteristics to develop a novel delivery platform that fuses nanotechnology with membrane biology.

The ability of CBN to target inflamed vasculature in vivo was tested using three distinct models of localized tissue inflammation, cancer lesion, and atherosclerotic plaque. We used high-speed intravital microscopy (IVM) to evaluate flow dynamics, biodistribution, targeting and tissue accumulation. We established a local inflammatory site using a single injection of lipopolysaccharide in the ears of the mice. IVM analysis revealed that CBN targeted inflamed vessels within the ear at a 10-fold higher concentration than liposomes. Using syngeneic models of breast cancer and melanoma, we observed CBN targeting and adhering to cancer vessels within the first 30 minutes after systemic administration, followed by substantial extravasation in the perivascular region by 24 hours. In addition, we observed minimal accumulation in the liver and spleen of CBN whereas liposomes displayed negligible tumor accumulation and were predominately sequestered in the liver and spleen. Atherosclerosis was induced in apolipoprotein E-deficient (apoE-/-) mice fed on a high-fat diet for 4 months and then injected intravenously with CBN. After 1 hour, CBN were surrounding each atherosclerotic plaque of the aorta. In addition to using optical imaging to evaluate the location of the inflammatory lesion, the versatility of CBN permits the loading of contrast agents to enable magnetic resonance imaging, ultrasound, computed tomography, and radioisotope imaging. As a matter of fact after incorporation with gadolinium, CBN exhibited r2 relaxivities >>40 $mM^{-1}s^{-1}$, a significant increase compared to gadolinium-based contrast agents in the

clinic. In summary, CBN represent a promising solution for the identification of inflamed vasculature through a variety of imaging modalities.

10:20am Graphene: Tumor Targeting and Chelator-Free Radiolabeling

Technical Presentation. NEMB2016-5964

***Sixiang Shi, Cheng Xu**, University of Wisconsin-Madison, Madison, WI, United States, **Kai Yang**, Soochow University, Suzhou, Jiangsu, China, **Robert J. Nickles**, University of Wisconsin-Madison, Madison, WI, United States, **Zhuang Liu**, Soochow University, Suzhou, Jiangsu, China, **Weibo Cai**, University of Wisconsin-Madison, Madison, WI, United States*

Objectives: Graphene, an emerging nanomaterial with single-layered carbon atoms in two dimensions has attracted tremendous interest, due to its unique electronic, optical, mechanical and chemical properties, and has been applied as a versatile platform for cancer imaging and therapy. Our goal was to employ graphene oxide (GO) and reduced graphene oxide (RGO) for in vivo tumor vasculature targeting via conjugating different targeting ligands, and to quantitatively evaluate the tumor targeting efficacy with positron emission tomography (PET). In addition, novel chelator-free radiolabeling was also investigated to directly label 64Cu onto the graphene surface based on transition metal-pi interactions. By eliminating the influences of chelator, chelator-free radiolabeling can maintain the native properties (e.g. size, structure, drug loading capacity and pharmacokinetics) of nanoparticles, which enables a more precise control over their in vivo fate.

Methods: RGO and GO nanosheets, with amino group-terminated PEG chains on the surface, were conjugated to NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) for ^{64}Cu ($t_{1/2}$ = 12. 7 h) labeling and TRC105 (an antibody that binds to CD105, a receptor overexpressed on tumor vasculature) or VEGF121 (a naturally occurring protein that bind to VEGFR, another vascular marker of tumor angiogenesis) for tumor vasculature targeting. FACS analysis, size measurements, and serum stability studies were performed to characterize the RGO and GO conjugates. In vivo serial PET imaging and ex vivo biodistribution studies were carried out to evaluate tumor targeting efficacy and pharmacokinetics of the nanoconjugates. Chelator-free radiolabeling of RGO and GO nanosheets was conducted by directly mixing the nanosheets with 64Cu at different concentrations and temperatures. The labeling stability and imaging capacity of ^{64}Cu-RGO-PEG was confirmed by serum stability studies and in vivo PET imaging. Anti-cancer drug doxorubicin (DOX)-loaded RGO-PEG was also investigated to understand the influence of drug loading on the labeling efficiency.

Results: RGO and GO nanosheets had sizes of ~20-100 nm and superb stability after PEGylation. Serial PET imaging showed rapid and persistent uptake of ^{64}Cu-NOTA-RGO-TRC105 (5.0±0.6, 5.6±0.2, 5.7±0.2, 4.5±0.4, and 4.0±0.5 %ID/g at 0.5, 3, 6, 24, and 48 h post-injection; n = 4) in 4T1 tumors and ^{64}Cu-NOTA-GO-VEGF121 (6.5±1.7, 8.2±1.4, 7.7±1.4, 5.7±0.8, and 4.7±0.7 %ID/g at 0.5, 3, 6, 16, and 24 post-injection; n=4) in U87MG tumors, demonstrating excellent tumor contrast and was several fold higher than the non-targeted RGO and GO conjugates. Various in vivo (e.g. blocking with TRC105 or VEGF121), in vitro (e.g. flow cytometry), and ex vivo (e.g. histology) studies further confirmed the targeting specificity. By avoiding the use of NOTA, 40-80% ^{64}Cu was intrinsically labeled onto RGO-PEG at different concentrations and temperatures, while the labeling yield of GO-PEG was only 5-20% due to incomplete graphene structure with less pi-bonds. The labeling stability of ^{64}Cu-RGO-PEG (>> 75% after 24 h incubation) was comparable to ^{64}Cu-NOTA-RGO-PEG as indicated by the serums stability studies. Prominent tumor uptake was achieved via passive targeting alone, suggesting excellent imaging capacity of 64Cu-RGO-PEG. After loading DOX, the labeling yield of RGO(DOX)-PEG was slightly decreased (>> 30 %) due to the competition between DOX and 64Cu for pi-bonds on the surface of RGO, further validating the existence of Cu-pi interactions.

Conclusions: We reported the vasculature targeting of RGO and GO conjugates, with enhanced tumor uptake and excellent specificity, providing the first examples of active targeting of graphene-based nanomaterials. In addition, we reported the first chelator-free radiolabeling of RGO nanosheets with ^{64}Cu, which provided important guidance for the future research on the radiolabeling and in vivo applications of graphene-based nanomaterials.

10:40am Improving the Diagnostic Sensitivity for Infectious Pathogens by Combinatorial Quantum Dot Barcoding

Technical Presentation. NEMB2016-5912

***Jisung Kim**, University of Toronto, Toronto, ON, Canada, **Mia Biondi, Jordan Feld**, University Health Network, Toronto, ON, Canada, **Warren Chan**, University of Toronto, Toronto, ON, Canada*

Detection of asymptomatic infections requires high diagnostic accuracy in order to promptly quarantine infected individuals, prevent further spread of the disease, and deliver effective treatment to those with clinical disease. While sensitivity is one of the important measures of diagnostic accuracy, many studies have focused on improving the analytical sensitivity, the minimum amount of detectable analyte, to lower the limit of detection (LOD). However, strategies to improve the clinical sensitivity, the probability of a test to correctly identify diseased patients as positive, have only been explored to a limited extent. These two parameters relate to each other as the level of clinical sensitivity may be reduced by poor analytical sensitivity because some diseases, especially at the early stage of infection, present insufficient level of biomarker and can lead to false-negative test results. Apart from the poor analytical sensitivity, viruses exist as quasi-species and present sequence variations accumulated from high mutation rates, representing a major challenge to the development of nucleic acid-based diagnostic tests.

Here, we combined recent advances in Quantum Dot (QD) barcoding technology with Recombinase Polymerase Amplification (RPA) to demonstrate enhancement in diagnostic sensitivity with Hepatitis B Virus (HBV) infected patient samples. RPA was used to improve analytical sensitivity by enriching the amount of target DNA, while multiple regions within the genome were detected via QD barcode-based multiplexed assay to further improve clinical sensitivity. As opposed to polymerase chain reaction (PCR) that requires expensive thermocycling steps, RPA operates at a low constant temperature, which will be advantageous in resource-limited settings. Furthermore, QD barcodes offer a promising diagnostic platform for simultaneous detection of multiple amplification regions within HBV genome due to their unique optical signatures.

A total of 72 clinical samples with a diverse background were used to represent various phases of HBV infection and disease course. The viral DNA was first extracted from patient serum using magnetic microbeads, four conserved regions of the extracted genome were amplified by RPA, amplified products were detected by multiplexed QD barcode assay, and finally fluorescence signals were measured via flow cytometry. In a blinded experiment, clinical sensitivity was compared between single-plexed and multiplexed detection schemes, and our combinatorial analysis demonstrates a systematic increase in clinical sensitivity from 54.9-66.7% to 80.4-90.5% with multiplexed detection for diagnostic purposes. We also demonstrate development of Receiver Operating Characteristic (ROC) curves, which identified cutoff intensity levels to achieve 100% specificity for all four regions. Thus, these results suggest that we can achieve near perfect clinical diagnosis of patients infected with HBV by using multiple QD barcodes in the detection process.

TRACK 2 NANOPARTICLE-BASED DELIVERY

MONDAY, FEBRUARY, 22

2-1
EMERGING NANOTHERAPEUTICS

Bexar/Travis 11:30 AM - 1:00 PM

Session Organizer: **Nicole Levi-Polyachenko**, *Wake Forest School of Medicine, Winston-Salem, NC, United States*
Session Co-Organizer: **Jacob Berlin**, *City of Hope, Beckman Research Inst., Duarte, CA, United States*

11:30am Delivery of siRNA with Nanoparticles Based on PEG-PLA Block Polymer

Keynote. NEMB2016-6154

***Jun Wang**, University of Science and Technology of China, Hefei, Anhui, China*

Clinical application of RNA interference is limited by the delivery of siRNA in vivo. We have developed nanoparticle system with stealthy property for efficient siRNA encapsulation and delivery, which is fabricated with poly(ethylene glycol)-b-poly(d,l-lactide) (PEG-PLA), siRNA and a cationic lipid, using a double emulsion-solvent evaporation technique. Such formulation exhibited high encapsulation efficiency of siRNA, significant down-regulation of gene expression in vivo, and was used for anti-tumor treatments. To more efficiently down-regulate gene expression in solid tumor, such nanoparticles should be more effectively internalized by tumor cells, escaped from the endosome/lysosome and released the cargo in cells, and we further improved its efficacy via integrating different strategies using block copolymer of PEG and PLA. We synthesized block copolymer of PEG and PLA bridged with a linkage responsive to the tumor acidity, and fabricated nanoparticles, which deshielded the PEG layer at the slightly acidic tumor extracellular microenvironment and facilitated the delivery of siRNA to tumor cells after accumulation at the tumor site. In addition, by incorporating highly membrane-active cationic helical polypeptides to the internal aqueous phase during the nanoparticle preparation process, we significant improved the endosomal escape of nanoparticles. Moreover, we observed that replacing PLA block with poly(lactide-co-glycolide) (PLGA) block promoted intracellular siRNA release. Our results demonstrated that integrating these strategies significantly improved the down-regulation of oncogene expression with specific siRNA in tumor, and substantially reduced the dose of injected siRNA to achieve similar efficacy in tumor growth inhibition when compared with our previous nanoparticle system.

12:00pm Nanoparticle Targeting to Cartilage: Effects of Surface Charge on Nanoparticle Interactions with Joint Tissues

Technical Presentation. NEMB2016-6064

***Shannon Brown, Blanka Sharma**, University of Florida, Gainesville, FL, United States*

Introduction: Osteoarthritis (OA) is a debilitating disease characterized by the degeneration of cartilage in articulating joints, and is presently without a cure. Chondrogenic growth factors and small molecule drugs are currently under investigation as OA therapeutics, but lack targeting modalities and localization to the cartilage, resulting in poor joint retention and low efficacy after direct injection into the joint. The overall goal of this research is to advance our understanding of how nanoparticle (NP)-based delivery systems can be engineered for localizing therapeutics to OA cartilage. Towards this goal, this study investigates the impact of NP zeta potential on cartilage and synovial fluid (SF) interactions using ex vivo models. We hypothesize that cationic NPs will exhibit greater retention within the cartilage due to electrostatic interactions with the anionic extracellular matrix components. However, the affect of SF on NPs is unknown, and may impact those interactions.

Materials and Methods: NPs were formulated using FDA approved poly (lactic-co-glycolic acid) (PLGA) for its demonstrated biocompatibility in joints and controllable physicohemical properties. The zeta potential of the NPs was controlled by emulsification of the organic PLGA solution in 1% polyvinyl alcohol (PVA NPs) or 1% PVA plus 0.2% didodecyldimethylammonium bromide (DMAB NPs) to yield negatve and positive zeta potentials respectively. The NPs were loaded with nile red dye for ex vivo imaging and quantification. Hydrodynamic diameter, zeta potential, and polydispersity, before and after incubation with SF were analyzed with ZetaPALS Dynamic Light Scattering. Analysis of NP retention within cartilage was conducted on viable bovine cartilage explants with intact superfical zone. NPs were constituted in either phosphate buffered saline (PBS) or bovine SF, incubated on the cartilage surface for 30 min, washed, and imaged with a Zeiss Axio fluorescence microscope. To quantify NP retention, explants were homogenized and dye was extracted using methanol which was measured using a fluorescent plate reader (BioTek Syngery HT) against known standards prepared in a similar manner.

Results and Discussion: Mean NP characteristics (n = 3) varied with emulsifier composition - PVA NPs were 218 nm in diameter with a zeta potential of -19.7mV, and DMAB NPs were 202 nm with a zeta potential of 17.1 mV. In PBS, dye loading efficiency was 27.3% ± 4.3% and 26.4% ± 4.1% (n=3) for PVA and DMAB particles respectively. Size and zeta potential changed upon incubation with SF, indicating adsorption of macromolecules from the SF onto the NP surface. Specifically, the size of the PVA and DMAB NPs increased to 300nm and 250nm, respectively; the DMAB NPs also experienced a reversal in zeta potential at -20mV. Overall, the cationic DMAB NPs demonstrated greater surface interaction and penetration with cartilage compared to the anionic PVA NPs, however, both NPs experienced a significance drop in retention when incubated with SF. This was more pronounced with DMAB NPs, where the decreased retention may be attributed to adsorption of negatively charged hyaluronic acid or serum albumins onto the cationic NP surface, thereby diminshing the electrostatic interactions between the cationic NPs and the negatively charged gycosaminoglycans in the cartilage.

Conclusions: Results indicate that surface properties, particularly zeta potential, play an important role in NP interactions with articular cartilage, and SF can significantly influence NP-cartilage interactions. The use of cationic surfactants, such as DMAB, can facilitate cartilage interactions important for localizing NP delivery systems to the tissue. This study represents a step towards understanding how physicochemical properties of NPs impact tissue interactions wthin the joint. Ongoing studies investigate the impact of NP size, presence of OA, off-target tissue binding, and conjugation of targeting peptides.

12:20pm Ultrarapid Rewarming of Vitrified Biomaterials at 1000 °C/min

Technical Presentation. NEMB2016-6142

***Navid Manuchehrabadi**, University of Minnesota, Minneapolis, MN, United States, **Meng Shi**, University of Xi'an Jiaotong, China, China, China, **Aiden Carley Clopton**, University of Minnesota, Minneapolis, MN, United States, **Feng Xu, Jinbin Qiu, Tian Jian Lu**, University of Xi'an Jiaotong, China, China, **John Bischof**, Univ Of Minnesota, Minneapolis, MN, United States*

Vitrification is an attractive technology to bank and store tissues in a glassy vs. crystalline state for eventual warming and use in regenerative medicine. For example, tissues have been successfully vitrified by loading high molarity (6 – 8.4 M) cryoprotective agents (CPA) such as VS55, DP6 and even glycerol at critical cooling rates of 2.5, 40 and 85 °C/min respectively. However, successful warming from the vitrified state often cannot be achieved by standard methods as it requires critical warming rates of 55, 185 and 32000

°C/min to avoid devitrifiation. Furthermore, these rates must be sufficiently uniform to avoid thermal stresses in excess of the yield stress (typically 2 MPa) which can crack the tissue. By achieving faster yet sufficiently uniform rates of cooling and warming, the concentration of the CPA can also be reduced thereby reducing chemical (i.e. CPA) toxicity on the tissue. Here we present a new technology that allows ultra-rapid warming rates (~1000 °C/min), which may allow dramatic improvements in preservation of vitrified biomaterials.

Acknowledgements. Funding from NSF CBET 1336659, the MN Futures grant and the Kuhrmeyer Chair to JCB and NM are gratefully acknowledged. Manuchehrabadi and Shi contributed equally to the work and are co-first authors. ACC worked as a volunteer high school student researcher in BHMT lab UM during summer 2015. Lu and Bischof are joint corresponding authors.

12:40pm Engineering the Next Generation of Drug-loaded Functionalized Electrospun Poly(caprolactone) Scaffolds for Cancer Treatment

Technical Presentation. NEMB2016-6099

Manisha Jassal, Vijay Boominathan, Tracie Ferreira, Sukalyan Sengupta, Sankha Bhowmick, *Univ Of Massachusetts-Dartmouth, Dartmouth, MA, United States*

Controlled drug delivery is required to improve the therapeutic efficacy of the drug and to reduce the potential toxic effects by delivering the drug at a rate governed by the physiological need of the site of action. Developing new scaffolds to deliver drug in a controlled manner to a tumor is an important task and translating the performance of these scaffolds to an in-vivo model is the next logical step. The tumor environment is different than the normal healthy tissue in terms of blood flow, oxygen and nutrient supply, tissue oxygen and pH distribution. Similarly, the interstitial fluid flow (fluid found in stroma of tissue) is also different in a tumor than in a healthy tissue. In cancer, vasculature irregularities lead to increased flow rates through the tissue. The drug delivery from a scaffold implanted near the tumor would depend on this fluid flow, if the drug is physically/ionically bound to the scaffold. In the current study, poly(caprolactone) (PCL) fibers were fabricated by electrospinning, followed by hydrolysis to introduce functional groups on the fiber surface. These functional groups (-COOH) were then utilized to ionically bind doxorubicin hydrochloride (DOX), an FDA approved anticancer drug. The scaffolds exhibit differences in release behavior between a very narrow window of pH 6.0 and pH 7.2 that would have a wide variety of applications due to acidic extracellular pH of most tumors.

The purpose of this study is to determine the usefulness of these DOX-loaded electrospun PCL scaffolds in an in-vivo situation. In here, a continuous flow system was designed using a syringe pump and flow rates that correspond to interstitial fluid flow rates experienced in tumors. The drug release from scaffolds was studied with the continuous flow system with media maintained at different pH and a differential drug release was obtained under these conditions. Then, a composite scaffold system consisting of drug-loaded electrospun scaffolds and hydrogels that release acid were subjected to the same continuous flow system in order to establish a control on the drug release profile by utilizing the pH-dependent DOX release from functionalized PCL fibers. Further, the effect of DOX being released from the electrospun scaffolds was studied on human embryonic kidney cells under different pH environment. The results corroborate the pH-dependent release of electrostatically bound DOX that can be exploited for site-specific targeted controlled DOX delivery. In the end, the drug-loaded scaffolds were implanted in an actual animal model (zebrafish) to study the drug release and determine the applicability of these scaffolds in real-life scenario.

2-2
NANOPARTICLE-BIOLOGICAL INTERACTIONS
Bexar/Travis 4:00pm - 5:30pm

Session Organizer: **Jun Wang**, *University of Science and Technology of China, Hefei, Anhui, China*

4:00pm Stem Cell/Nanoparticle Conjugates For Targeted Cancer Therapy

Keynote. NEMB2016-6088

Jacob Berlin, *City of Hope, Beckman Research Inst., Duarte, CA, United States*

Targeted drug delivery is a long-standing goal for cancer therapy. Nanoparticles have shown promise as platforms for targeted drug delivery, but major challenges remain for controlling the distribution of nanoparticles within tumors. Neural Stem Cells (NSCs) are appealing candidates for use as carriers for nanoparticles in order to overcome these biodistribution challenges. NSCs have demonstrated inherent tumor tropic properties in invasive and metastatic tumor models, migrating selectively to invasive tumor foci, penetrating hypoxic tumor regions, and even traversing through the blood-brain barrier to access intracranial tumor foci following intravenous administration. NSCs do not intrinsically have any anti-tumor efficacy; they must be modified in some way to exploit their tumor targeting abilities. My collaborator, Dr. Karen Aboody, is a pioneer in genetically altering NSCs to express an enzyme that will convert a prodrug into the active compound. This approach was recently evaluated in a first-in-human safety/feasibility clinical trial using modified NSCs to treat recurrent gliomas.

As NSC-based therapy moves into the clinic, there is an opportunity to develop complementary techniques to enable NSCs to destroy tumors. The combination of NSCs and nanoparticles offers the potential of a general drug targeting system. We have demonstrated that NSCs can either be modified to bear nanoparticles on their surface or can internalize them. The nanoparticles can release drugs or used for photothermal ablation. In all cases, the NSCs remained viable and targeted the delivery of the nanoparticles to tumors in vivo, enhancing the therapeutic efficacy of the nanoparticles.

4:30pm Magnetic enhancement of vascular permeability for targeted drug delivery

Technical Presentation. NEMB2016-5974

Sheng Tong, *Rice University, Houston, TX, United States,* ***Yongzhi Qiu***, *Georgia Institute of Technology and Emory University, Atlanta, GA, United States,* ***Linlin Zhang, Gang Bao***, *Rice University, Houston, TX, United States*

Vascular endothelium presents a major transport barrier to therapeutic agents administrated systemically. In normal vasculature, endothelial cells form a monolayer via cell-cell junctions that only allow selective extravasation of solutes, ions and small molecules, while others have to pass through vascular endothelium via less effective transcytosis. Current drug delivery strategies often involve conjugation or encapsulation of therapeutic agents with macromolecules or nanoparticles. Transvascular transport of these drug carriers thus relies on leaky vessels arising from pathological angiogenesis, e.g. tumor angiogenesis. However, drug delivery through leaky vasculature is rarely effective due to the heterogeneity in tumor vasculature. Developing proactive delivery strategies independent of pathophysiological conditions are paramount for adequate and uniform intratumoral distribution of therapeutic agents. Here we present an engineering approach of controlling vascular permeability via magnetic iron oxide nanoparticles (MNPs) and a well-designed magnetic field for enhanced targeted drug delivery.

Over the past few years, MNPs have emerged as promising drug carriers for their potentials in image-guided drug delivery, magnetic drug targeting and hyperthermia. As nano-sized magnets, MNPs experience a force along the gradient of magnetic field. We have shown that magnetic force could enhance the endocytosis of MNPs by human umbilical vein endothelial cells (HUVECs) without affecting cell viability. Importantly, internalized MNPs

provide leverage for applying forces directly to intracellular organelles. In HUVECs cultured statically, an external magnetic field could induce redistribution of lysosomes containing MNPs and the formation of actin stress fibers along the field gradient. Here we further examined whether vascular permeability could be controlled using intracellular magnetic force. It is known that in endothelial cells, a portion of actin filaments are connected to VE-cadherin, a key component of adherens junctions that mediate the paracellular transport pathway of vascular endothelium. Reorganization of actin filaments can disrupt endothelial adherens junctions and increase vascular permeability. In light of these evidences, we designed magnetic field through numerical simulations, and investigated magnetic force induced the structural and functional changes of vascular endothelium in vitro in endothelialized microfluidic channels and in vivo in a mouse model.

Our studies demonstrated that in endothelialized microfluidic channels cultured with flow, the uptake of MNPs by vascular endothelium could be enhanced with a magnetic field. Subsequently, an intracellular magnetic force perpendicular to the flow direction temporarily disrupted actin stress fibers and endothelial adherens junctions formed under the physiological flow condition. Furthermore, in an in vivo study, we showed that MNPs could be targeted to local vasculature using an external magnetic field. After that, the changes in local vascular permeability was examined using ICG angiography. ICG injected intravenously showed increased accumulation in tissues treated magnetically.

In summary, this study offers a promising approach for remote control of vascular permeability by disrupting endothelial adherens junctions with intracellular magnetic force. It can be used to overcome the intrinsic heterogeneity in pathological angiogenesis and provide entry for drug molecules in diseases that do not involves changes in local vascular permeability or up regulation of disease markers. At last, this approach extends targeted delivery to a broad range of therapeutic agents that can not be conjugated or encapsulated using current fabrication techniques.

4:50pm A Proteolytic Surface Modification Enhances Nanoparticles Diffusion In Cancer Stroma

Technical Presentation. NEMB2016-6057

***Alessandro Parodi**, Houston Methodist Research Institute, Houston, TX, United States, **Francesca Taraballi**, Houston Methodist Research Inst., Houston, TX, United States, **Claudia Corbo, Jonhatan Otto Martinez**, Houston Methodist Research Institute, Houston, TX, United States, **Ennio Tasciotti**, The Methodist Hospital Research Institute, Houston, TX, United States*

Tumor extracellular matrix (ECM) represents a major biological barrier affecting the proper diffusion of many therapeutics and drug delivery systems in the cancer parenchyma. This biological barrier limits the efficacy of promising therapeutic approaches including the delivery of siRNA or agents intended for thermoablation. After extravasation typical nanotherapeutics are stucked in the sub-endothelial space and are unable to reach the poorly vascularized and anoxic regions deep within the cancer lesion. Tumor ECM is a well-characterized net of proteins and biomolecules exploited by the cells to sustain their growth and movement. The remodeling of tumor ECM is well-known to be naturally driven by the action of proteases that affect its integrity through specific proteolytic enzymatic action. Bromelain is a proteolipid complex that can be easily purified from the stem the of pineapple plant and is commercialized as dietary supplement, and approved by FDA to mitigate the inflammatory processes that accompany post-orthopedic surgery. Its role in cancer disease was recently tested in pre-clinical studies and literature showed that Bromelain can contribute in favoring cancer cell apoptosis. Here we investigated the benefits of Bromelain surface modification to increase the diffusion of synthetic particles in the tumor ECM by exploiting its proteolytic bioactivity.

Bromelain was conjugated to the surface of mesoporous silica nanoparticles through an APTES chemical linker. The system was characterized in vitro and in vivo for its ability to be internalized by different cells of the tumor microenvironment and for its cytotoxicity. The data showed that Bromelain increased the affinity of the particles for the cellular membrane as well as for tumor extracellular matrix. Further experiments were focused on determining the activity of Bromelain modified particles in digesting commercial tumor extracellular matrix matrigel, showing that at T and pH different from physiological conditions the bioactivity of this surface modification is affected. Compared to uncoated nanoparticles, Bromelain modified particles increased particles diffusion in vivo in a murine model of syngeneic orthotopic breast cancer where the modified particle penetrated deeper in the tumor parenchyma and increased their residence time in the lesion. In conclusion, we showed that Bromelain efficiently increased the penetration of synthetic nanoparticles in the tumor parenchyma. Further studies will be performed to understand the impact of this procedure on tumor biology. In fact, while our work support the hypothesis that the use of proteolytic agents can favor the diffusion of nanoparticles in tumor parenchyma, the loss of tumor ECM can favor cancer cells mobility and metastatic progression, therefore more investigation are currently undergoing.

5:10pm Cancer Targeting Nanoparticle Exhibit Minimal Interaction With Cancer Cells In Vivo

Technical Presentation. NEMB2016-5914

***Qin Dai, Ding Ding, Stefan Wilhelm, Warren C.W. Chan**, University of Toronto, Toronto, ON, Canada*

Tumor targeting using nanoparticles is an exciting and emerging field. In particular, cellular targeting using nanoparticles garnered interest due to its proposed ability to recognize overexpressed receptors on malignant cancer cell surfaces. The purpose behind this approach is to increase specific destruction of cancer cell to enhance therapeutic effect, while reducing non-specific interactions that could damper its intended purpose. This is no easy task, since only <1% of the injected nanoparticles reach the tumor and even less are likely to interact with the cells. Therefore there is a dire need to provide systematic evidences that quantify the efficiency of nanoparticle targeting to malignant cancer cells, as well as nanoparticle interactions with its surrounding microenvironment. Here we show that >>90% of the ErbB2 (epidermal growth factor receptor 2) cancer targeting nanoparticles within the tumor microenvironment are extracellular matrix associated and unable to interact with cells. By using flow cytometry analysis on tumor single cell suspension, we showed that tumor associated macrophage exhibit 3 fold difference in interaction with 50nm ErbB2 cancer targeting nanoparticles compared to SKOV-3 cancer cells, suggesting that the nanoparticles are not targeting cancer cells as intended. Furthermore, nanoparticle per tumor associated macrophage is significantly higher than that per cancer cell. In comparison, 15 and 100nm nanoparticles exhibited 5 fold less interaction with cells in the tumor. These data collectively suggest that there's a size dependence on nanoparticle interaction with cells in vivo, however this interaction is mainly dominated by tumor associated macrophage nanoparticle interaction despite the presence of cancer targeting surface ligands. This study provides a systematic understanding and quantitative outlook at in vivo nanoparticle-cell interaction within the tumor microenvironment, and calls for action for future quantification in cancer-nanoparticle investigations. Tumor associated macrophage targeting nanoparticles should be investigated in detail as an alternative cancer treatment strategy.

5:30pm MRI-Guided Laser Ablation

Technical Presentation. NEMB2016-6164

***R. Jason Stafford**, The University of Texas MD Anderson Cancer Center, Department of Physics, Houston, TX, United States*

Interstitial laser ablation systems aim to coagulate a localized tissue volume. Modern clinical systems utilize compact, high power, solid state laser sources which incorporate cooled applicators in order treat larger volumes of tissue. Additionally, systems operate in the near infrared part of the spectrum and therefore are synergistic emerging nanophotonic applications which

provide a wealth of opportunity to provide more conformal and tumor specific targeted approaches to therapy.

MRI is a unique modality for providing guidance of these rapid, high-temperature approaches to laser ablation owing to its inherent temperature sensitivity integrated with exquisite soft tissue anatomy and functional imaging capabilities making it useful for planning, targeting, monitoring and verifying therapy delivery in a "closed-loop" fashion. MR temperature imaging data can be integrated with physical models of bioheat transfer and biological models of tissue damage so that it can play a significant role in increasing the safety and efficacy of these rapid ablation procedures by offering a mechanism monitor and control both high tissue temperatures at the probe interface and nearby critical structures, as well as aid in prediction of lesion development, particle activation or drug delivery.

Here we provide an overview of MRI guidance of laser ablation procedures with examples provided in brain, prostate, liver and bone as well as a brief discussion of some of the current challenges associated with application of this technique in various anatomical locations as well as the potentially complimentary role of high performance computing and simulation may have on planning, monitoring and verification when this unique image-feedback modality is incorporated into treatment.

TUESDAY, FEBRUARY, 23

2-3
CANCER NANOMEDICINE

Bexar/Travis 9:30am - 11:00am

Session Organizer: **Chris Jewell**, *University of Maryland, College Park, MD, United States*

9:30am Chemophototherapy Using Long-Circulating Liposomes Conferred with Light-Triggered Cargo Release

Keynote. NEMB2016-6155

***Jonathan Lovell**, University at Buffalo, Buffalo, NY, United States*

Stealth liposomes can be used to extend the blood circulation time of encapsulated therapeutics. Inclusion of 2 mol% porphyrin-phospholipid (PoP) imparted optimal near infrared (NIR) light-triggered release of doxorubicin (Dox) from conventional sterically stabilized stealth liposomes. The type and amount of PoP affected drug loading, serum stability and drug release induced by NIR light. Cholesterol and PEGylation were required for Dox loading, but slowed light-triggered release. Dox in stealth PoP liposomes had a long circulation half-life in mice of 21.9 h and was stable in storage for months. Following intravenous injection and NIR irradiation, Dox deposition increased ~7 fold in treated subcutaneous human pancreatic xenografts. Phototreatment induced mild tumor heating and complex tumor hemodynamics. A single chemophototherapy treatment with Dox-loaded stealth PoP liposomes (at 5-7 mg/kg Dox) eradicated tumors while corresponding chemo- or photodynamic therapies were ineffective. A low dose 3 mg/kg Dox phototreatment with stealth PoP liposomes was more effective than a maximum tolerated dose of free (7 mg/kg) or conventional long-circulating liposomal Dox (21 mg/kg). To our knowledge, Dox-loaded stealth PoP liposomes represent the first reported long-circulating nanoparticle capable of light-triggered drug release.

10:00am Inhibiting Chemoresistant Cancer Stem Cells and Improving the Safety Profile of Chemotherapy through Nanoparticle-Mediated Drug Delivery

Technical Presentation. NEMB2016-5975

***John Perry**, Stowers Institute for Medical Research, Kansas City, MO, United States, **Laura González-Fajardo, Lalit Mahajan, Dennis Ndaya, Derek Hargrove, Rajeswari M. Kasi**, University of Connecticut, Storrs, CT, United States, **Linheng Li**, Stowers Institute for Medical Research, Kansas City, MO, United States, **Xiuling Lu**, University of Connecticut, Storrs, CT, United States*

Chemoresistance and recurrence of tumors remain among the primary challenges in developing effective cancer treatments. While high-dose chemotherapy, which is the hallmark of current therapeutic strategies, can sometimes be effective in eliminating the majority of cancer cells, it often fails to eliminate cancer stem cells (CSCs), a subset of cells that are frequently resistant to standard chemotherapy. In addition, high-dose chemotherapy frequently causes severe side effects and leads to the evolution of resistant clones with subsequent relapse and rapid disease progression. We previously reported the development of an amphiphilic brush-like block copolymer composed of polynorbonene-cholesterol/polyethylene glycol that self-assembles in aqueous media to form long circulating nanostructures capable of encapsulating doxorubicin (DOX-NPs). In this study, we assessed the efficacy and toxicity of that nanoparticle formulation in tumor-bearing mouse models and compared its effects with the non-encapsulated form (free DOX) and the commercial liposomal doxorubicin Doxil®.

DOX-NPs at a dose of 5 mg/kg significantly reduced the growth of subcutaneous tumors after treating severe combined immunodeficiency (SCID) mice bearing subcutaneous human lung A549 cancer once per week up to 8 weeks without any observed side effects. On the contrary, significant weight loss, early toxic cardiomyopathy, acute toxic hepatopathy and reduced hematopoiesis were prevalent in mice treated with free DOX at a dose of 1 mg/kg, although it showed comparable tumor inhibition to DOX-NPs.

In a leukemia mouse model, we developed a new treatment strategy to combine DOX at a lower dose with a conventional chemotherapeutic drug Nelarabine. Leukemic mice were treated with 5 daily injections of free DOX at 0.5 or 0.15 mg/kg with and without Nelarabine. Alternatively, a single injection on day 1 of 0.8 or 2.5 mg/kg of DOX-NPs, or Doxil® was administered with and without Nelarabine. At 10 days post-treatment, bone marrow was analyzed by flow cytometry to determine frequency of leukemic stem cells (LSCs) and normal hematopoietic stem and progenitor cells (HSPC). While cytotoxic chemotherapy induced LSC expansion, low-dose doxorubicin administered daily for five days prevented this expansion and even facilitated recovery of HSPCs. DOX-NPs allowed for further reduction of LSCs compared to free doxorubicin and effectively eliminated this population. This effect was obtained through only a single low-dose injection at 0.8 mg/kg. Doxil® was not as effective at preventing the LSC expansion induced by chemotherapy or at facilitating HSPC recovery as DOX-NPs.

It was identified that DOX can target LSCs via inhibition of Akt and β-catenin driven self-renewal. Low but effective doses of DOX offer a new opportunity to target drug-resistant CSCs while reducing systemic toxicity. DOX-NPs exhibited slow but steady release of DOX from NPs which provides an optimal sustainable drug concentration for LSC inhibition. Using precisely designed copolymer nanoparticles to target tumorigenic cells discretely from their bulk progeny and preferentially over normal stem/progenitor cells will substantially improve patient outcomes, reduce relapse, and provide a new paradigm for effective and safe cancer treatment.

10:20am A combined chemical and magneto-mechanical induction of cancer cell death by the use of functionalized magnetic iron nanowires

Technical Presentation. NEMB2016-5968

***Aldo I. Martinez Banderas**, King Abdullah University of Science and Technology, Thuwal Jeddah, Jeddah,Saudi Arabia, **Antonio Aires**, Instituto Madrileno de Estudios Avanzados Nanociencia, Madrid, Madrid,Spain, **Jose Perez**, **Nouf Alsharif**, King Abdullah University of Science & Technology, Thuwal, Makkah,Saudi Arabia, **Francisco Teran, Jael Fernandez Canderas**, Instituto Madrileno*

de Estudios Avanzados Nanociencia, Madrid, Madrid,Spain, ***Jurgen Kosel****, King Abdullah University of Science & Technology, Thuwal, Select State/Province,Saudi Arabia,* ***Aitziber Lopez Cortajarena****, Instituto Madrileno de Estudios Avanzados Nanociencia, Madrid, Madrid,Spain*

Cancer prevails as one of the most devastating diseases being at the top of death causes for adults despite continuous development and innovation in cancer therapy. Nanotechnology may be used to achieve therapeutic dosing, establish sustained-release drug profiles, and increase the half-life of drugs avoiding efflux or degradation. In this context, magnetic nanowires (NWs) have shown a good biocompatibility and cellular internalization with a low cytotoxic effect. In this study, we induced cancer cell death by combining the chemotherapeutic effect of doxorubicin (DOXO)-functionalized iron NWs with mechanical disturbance under a low frequency alternating magnetic field. Two different agents, APTES and BSA were separately used for coating NWs permitting further functionalization with DOXO. Internalization was qualitatively and quantitatively assessed for both formulations by confocal reflection microscopy and inductively coupled plasma-mass spectrometry. From confocal reflection analysis, BSA formulations demonstrate to have a higher internalization degree and a broader distribution within the cells, as well as less agglomeration in comparison to APTES formulations. The functionalized NWs generated a comparable cytotoxic effect in MDA-MB-231 breast cancer cells in a DOXO concentration-dependent manner, (~60% at the highest concentration tested) that was significantly different from the effect produced by the free DOXO (~95% at the same concentration) and non-functionalized NWs formulations (~10% at the same NWs concentration). A synergistic cytotoxic effect is obtained when a low frequency magnetic field (1 mT, 10 Hz) is applied to cells treated with DOXO-functionalized BSA or APTES-coated NWs that is again comparable (~70% at the highest concentration). Furthermore, the cytotoxic effect of both groups of coated NWs in absence of the drug increased notoriously when the field is applied in an NW concentration-dependent manner (~25% at the highest concentration tested). In summary, a combined method for cancer cell destruction was developed by the conjugation of the magneto-mechanical properties of the iron NWs coupled with the chemotoxic effect of an anticancer drug. This combination yielded better results than the individual effects. Both APTES and BSA coatings were efficient for the functionalization of the NWs and demonstrated to be highly biocompatible.

10:40am In Vitro Characterization of Pegylated Liposomal Doxorubicin Containing Various Phosphatidylcholines with Different Solid to Gel Phase Transition Temperatures

Technical Presentation. NEMB2016-6159

Hamidreza Farzaneh*, Mashhad University of Medical Sciences, Mashhad, Iran*

The slow release of Pegylated liposomal doxorubicin (Doxil®) at tumor site might be an obstacle towards efficient cancerous tumor treatment. Therefore, maybe modification of liposomes to lower solid-to-gel phase transition temperature (Tm) increase drug efficacy. In this investigation, we prepared four liposomal variants of Doxil® formulation, in which the main phospholipid, Hydrogenated phosphatidylcholine (HSPC) were substituted with Egg derived PC, Dipalmitoeyl PC, and Dimeristoyl PC , termed HSPC -L, EPC -L and DPPC -L and DMPC -L, respectively. The liposomes were prepared by thin film hydration and ammonium sulphate gradient loading technique. The doxorubicin concentration and phospholipid content were determined by respective spectrofluorimetry (Ex: 500; Em: 583) and according to Bartlet phosphate method. The physicochemical properties of the liposomes, including size distribution profile, drug to lipid ratio, and long-term drug retention indicated that all liposomes are stable enough to retain similar drug phospholipid ratios (1 mg doxorubicin/ 10 µmole phosphate), zeta average and polydispersity indexes. The doxorubicin release follow-up in RPMI: FCS culture media (70:30 v/v) showed that liposomes did not release any drug within 48 h. However, in long-term follow-ups, until one week, liposomes-serum protein aggregation occurred and the liposomes lost their drug contents, correlated to the Tm of main phospholipid (74% DMPC -L >> 63% EPC -L >> 42% DPPC -L >> 16 % HSPC -L). Although DPPC has Tm above 37 °C, they showed a remarkable liposome-serum precipitates in the media, denoting the importance of high Tm inclusion of HSPC in liposomal formulation. The in vitro cytotoxicity impact showed that DMPC- L and EPC -L were more toxic than HSPC- L in C26 colon carcinoma, B16F0 melanoma, and NIH 3T3 fibrobalst cells, using MTT assay. Such toxicity could be related to increasing drug release of these liposomes confirmed by exposing cells with culture media containing liposomes at 37 °C within 3 h. Although fast delivering liposomes are more efficacious in eradicating cancerous cells, the toxic and serum-related aggregation may cause significant side effects in animals. Interaction of liposomes with serum proteins is a matter of consideration as these liposomes (EPC -L and DMPC -L) are more predisposed to become phagocytosed and accumulated in macrophages. As a result, there might be myelotoxicity and more hepatotoxicity as these liposomes might gathered more in these organs than those of Doxil®. These issues merit further investigation in animal models.

2-4
NANOPARTICLES FOR IMMUNOENGINEERING

Sam Houston 11:30am - 1:00pm

Session Organizer: **Jonathan Lovell**, *University at Buffalo, Buffalo, NY, United States*

11:30am Harnessing Biomaterials to Study and Improve Therapeutic Vaccines

Keynote. NEMB2016-6156

Chris Jewell*, University of Maryland, College Park, MD, United States*

Vaccines and immunotherapies have generated some of the largest impacts on human health in history, but a fundamental challenge facing the field is how to direct the specific properties of immune responses that are elicited. This idea of tuning immune response is critical in designing more efficacious and specific vaccines and immunotherapies. Toward this goal, we are developing two new strategies to study and exploit the interactions between biomaterials and lymph nodes – key tissues that coordinate adaptive immune response. In one approach we have combined direct lymph node injection with degradable polymer depots. We are using this system to directly study the link between local lymph node function and systemic immunity by probing the roles of signal density, combination, and material properties. We are also applying this idea to directly reprogram the lymph node microenvironment for therapeutic vaccination in the areas of autoimmunity and cancer. In mouse models of multiple sclerosis, depots loaded with regulatory cues and self-antigens permanently reverse paralysis after a single treatment administered at the peak of disease. These effects are systemic but antigen-specific, and result from local changes in the lymph node microenvironment. The second focus area is the design of new modular materials we have created using polyionic immune signals to form stable vaccine capsules. These immune polyelectrolyte multilayers (iPEMs) are self-assembled entirely from antigens and adjuvants to allow selective activation of pro-inflammatory signaling pathways without requiring other carrier components such as polymers or lipids. In mice, peripheral injection of iPEMs enhances the function of dendritic cells in draining lymph nodes, potently expands antigen-specific T cells against the antigens used to build iPEMs, and provides protection during tumor challenge. Ultimately, these strategies could contribute to better understanding of the interactions between biomaterials and the immune system, and improve the rational design of materials that serve not only as carriers, but also as agents that actively direct immune response.

12:00pm Biomimetic Nanovesicles for the Treatment of Inflammatory-Based Diseases

Technical Presentation. NEMB2016-6048

***Roberto Molinaro**, Houston Methodist Research Institute, Houston, TX, United States, **Dickson Kirui**, Naval Medical Research Unit San Antonio, San Antonio, TX, United States, **Jonathan Martinez, Michael Evangelopoulos, Enrica De Rosa, Claudia Corbo, Alessandro Parodi**, Houston Methodist Research Institute, Houston, TX, United States, **Ennio Tasciotti**, The Methodist Hospital Research Institute, Houston, TX, United States*

A primary directive in nanotechnology is to develop drug delivery platforms that effectively reduce systemic toxicity while retaining the activity of the drug, thereby increasing the therapeutic index. The ideal delivery platform should: i) modulate drug bioavailability and pharmacokinetics, ii) protect the payload from degradation/clearance in biological environments, and iii) favor the biodistribution and targeting at the site of interest. These properties are particularly relevant for the delivery of drugs characterized by high toxicity (chemotherapeutics) and of compounds with poor stability in harsh biological environments (siRNA). Several organic (lipids, polymers) and inorganic (silicon, silica, gold and iron oxide) materials were chosen for their biocompatibility and have been manipulated at the micro and nano scale to synthesize a plethora of drug delivery systems. For both types of materials, the addition of proper surface modifications represents a key step for the achievement of high therapeutic efficacy. A majority of currently available nanotherapeutics must be equipped with a coating of hydrophilic polymers, e.g. polyethylene glycol, to prevent surface interaction with opsonizing agents and delay early sequestration by the cells of the mononuclear phagocytic system (MPS). Similarly, to achieve selective delivery to the diseased tissue, it is necessary to functionalize the surface of the carrier with targeting molecules, e.g. aptamers, antibodies, folate, transferrin. In the efforts to engineer such materials, the scientific community was compelled to challenge the extraordinary ability of our body to recognize, label, sequester, and clear foreign objects. In this scenario, bio-inspired approaches have emerged as a one-step solution to simultaneously evade MPS and negotiate the transport across various biological barriers. In particular, the physiology of circulating immune cells provides a valuable example for the development of injectable carriers. Leukocytes freely circulate in the bloodstream and accumulate in the diseased tissue through the selective interaction with inflamed vasculature.

In this study, we investigated how leukocyte membranes could be manipulated and exploited as a proteolipid material to formulate a new generation of biomimetic drug delivery systems. These biomimetic nanovesicles (BVs) are a novel hybrid formulation that combines the leukocyte's ability to escape immune surveillance, target the inflamed vasculature, and cross the endothelial barrier with the liposome's capacity to load, retain and release a cadre of different payloads in a controlled fashion. Particles' ability to escape the MPS and target the inflamed endothelium has been investigated both in vitro and in vivo. The in vitro exposure of BVs to macrophages was characterized by a significant reduction in phagocytosis, confirming the stealth properties of the biomimetic proteolipid material. When systemically injected, BVs exhibited a significant reduction in spleen and liver uptake at 1 hour (10 and 50 fold decrease, respectively), compared to bare liposomes. In addition, blood circulation studies demonstrated a 5-fold increase in prolonged circulation. Using an in vitro flow chamber we tested the adhesion of BVs under physiologically relevant shear stresses. Compared to conventional liposomes, BVs retained the targeting features of leukocytes and preferentially recognized the inflamed endothelium. A similar comparison was evaluated in vivo using intravital microscopy (IVM) on lipopolysaccharide-induced and tumor-associated models of inflammation. In both cases, BVs exhibited an increased adhesion toward tumor-associated vasculature at 1 and 24 h after injection. Lastly, IVM analysis of the adhesion dynamics of BVs to inflamed tumor endothelium revealed that while bare liposomes uniformly distributed across the vessel section, BVs exhibited substantial accumulation at the vessel walls. This behavior remarkably mimics the innate ability of leukocytes to selectively adhere to inflamed vessels prior to trans-endothelial migration. In conclusion, here we showed a proof of concept demonstration of the use of proteolipid materials isolated from living cells to impart biological properties and functions to synthetic liposome-like vesicles. Finally, this approach can be used as a technological platform for diagnostic and therapeutic applications suitable to a broad range of disorders that have low therapeutic alternatives (e.g., rheumatoid arthritis, cancer, inflamed bowel diseases) but share the same inflammatory background.

12:20pm Immunological Impact of Membrane-derived Biomimetic Nanoparticles

Technical Presentation. NEMB2016-6047

***Michael Evangelopoulos, Michael Evangelopoulos, Alessandro Parodi, Claudia Corbo, Jonathan Martinez, Mauro Ferrari**, Houston Methodist Research Institute, Houston, TX, United States, **Ennio Tasciotti**, The Methodist Hospital Research Institute, Houston, TX, United States*

Recent discoveries have demonstrated that biomimicry can be employed to bestow synthetic carriers with surface modifications derived from biological components, thereby providing robust physiological tolerance and biological targeting. With the recent popularity of biomimetic delivery platforms, the evaluation of carrier biocompatibility continues to remain a major component for the successful translation into the clinic. Our group previously demonstrated cellular membranes of circulating leukocytes could be easily isolated and transferred onto the surface of synthetic nanocarriers while maintaining key biological functions. These carriers, referred to as Leuko-like Vectors (LLV) [1], are composed of a loadable, biodegradable [2], and biocompatible [3] multistage nanovector (MSV) [4] core with a proteolipid outer shell purified from leukocyte cell membrane (CM). This outer layer was shown to retain membrane proteins [5] that are critical for increased adhesion towards inflamed endothelium while also providing prolonged circulation time. Despite this, while the functionalization of drug delivery vectors with cellular materials have been demonstrated to provide synthetic particles with unique biological properties, current literature lacks a comprehensive analysis on the biological impact of these new platforms; in particular, for what pertains the systemic inflammation and immune response that may arise following particle administration. Herein, we comparatively analyzed unmodified multistage nanovectors with particles functionalized with murine and human leukocyte cellular membranes and the immunological effects that may arise in vitro and in vivo. Previously, LLV demonstrated an active avoidance of opsonization and phagocytosis, in addition to superior targeting of inflammation and prolonged circulation. In this work, we performed a comprehensive evaluation of the importance of the source of cellular membrane in increasing their systemic tolerance and minimizing an inflammatory response. Time-lapse microscopy revealed LLV developed using a cellular coating derived from a syngeneic murine source resulted in the avoidance of macrophage uptake, decreased liver accumulation and no impact on hepatic function. As biomimicry continues to develop, this work demonstrates the necessity to consider the source of biological material in the development of drug delivery carriers. Our findings indicate the source of membrane is critical in inhibiting cellular internalization and rapid clearance of the particles in vitro and in vivo, respectively, although less so in triggering an acute inflammatory response.

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[2] Martinez JO, Chiappini C, Ziemys A, Faust AM, Kojic M, Liu X, et al. Engineering multi-stage nanovectors for controlled degradation and tunable release kinetics. Biomaterials. 2013;34:8469-77.
[3] Martinez JO, Boada C, Yazdi IK, Evangelopoulos M, Brown BS, Liu X, et al. Short and long term, in vitro and in vivo correlations of cellular and tissue responses to mesoporous silicon nanovectors. Small. 2013;9:1722-33.
[4] Tasciotti E, Liu X, Bhavane R, Plant K, Leonard AD, Price BK, et al. Mesoporous silicon particles as a multistage delivery system for imaging and therapeutic applications. Nature nanotechnology. 2008;3:151-7.
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12:40pm Nanochannel platforms for tightly controlled therapeutic delivery and cell transplantation

Technical Presentation. NEMB2016-5993

***Alessandro Grattoni**, Houston Methodist Research Institute, Houston, TX, United States*

Silicon nanochannel platforms leveraging nano-constrained diffusion for tightly controlled therapeutic release and immunoisolated cell transplantation: Through cutting-edge implementation of fabrication techniques developed in the microelectronics industry, our group is able to create dense arrays of nanochannels ranging from nanometers to millimeters in height with a precision of ±5%. Two device platforms have been invented in order to leverage these capabilities: a silicon nanochannel membrane for drug delivery and a surface-modified polymer system for cell transplantation. The drug delivery system employs adaptable channel sizes down to 2.5 nm to closely constrain molecular transport, linearizing Fickian diffusion to achieve constant administration. Implantable drug delivery devices are fashioned by integrating these nanochannel membranes within bioinert metallic or polymeric capsules. These devices are minimally-invasive, can be implanted subcutaneously, and provide linear (zero-order) release of drugs and biomolecules. Clinically-relevant dosages of testosterone for hormone replacement have been released for more than 6 months at a constant rate with this platform. Further innovations include active, on-board control systems to permit remote manipulation or activation, enabling telemedicine or chronotherapy regimens. The polymeric cell transplantation system was primarily developed for pancreatic islet allografts. This device, the "NanoGland", is used to provide an immunoprotective environment for bioactive allografts by isolating cells from inflammation and rejection mechanisms while permitting interaction with glucose, insulin, nutrients, and waste exchange from the interstitial environment. Combining the NanoGland with the silicon nanochannel membranes has allowed controlled release of immunosuppressive material or factors for cell growth and vascularization following cell transplantation.

2-5
NANOPARTICLE ENGINEERING

Sam Houston 4:00pm - 5:30pm

Session Organizer: **Zhenpeng Qin**, *University of Texas at Dallas, Richardson, TX, United States*

4:00pm Leveraging Physiology for Precision Drug Delivery

Keynote. NEMB2016-6157

***Zhen Gu**, University of North Carolina at Chapel Hill and North Carolina State University, Raleigh, NC, United States*

Spurred by recent advances in materials chemistry, molecular pharmaceutics and nanobiotechnology, stimuli-responsive "smart' systems offer opportunities for delivering drugs in dose-, spatial- and temporal-controlled fashions. In this talk, I will discuss our ongoing efforts in using physiological signals, such as blood sugar level, enzyme activity and ATP gradient for on-demand drug delivery in a programmed manner. I will first present the glucose-responsive synthetic systems for biomimetic delivery of insulin for diabetes treatment. I will further discuss programmable delivery of anticancer therapeutics, the release of which can be activated in the tumor microenvironment or subcellular environment.

4:30pm Lung Specific Gene Delivery through Regulated Membrane Activation

Technical Presentation. NEMB2016-5922

***Jin Wang**, Baylor College of Medicine, Houston, TX, United States*

Cystic fibrosis is a well-studied systemic genetic disorder that affects mostly the lungs. Gene therapy has long been regarded as the ultimate cure for cystic fibrosis. Although clinical development of gene therapy started over two decades ago, there are no gene therapy products approved in the U.S. The current development of gene therapy is mainly hampered by ineffective nucleic acid delivery. Gene therapy can be classified by the gene delivery method as viral and non-viral delivery. About 70% of gene therapy clinical trials performed to date utilized viral delivery. Although viral gene delivery usually can achieve high gene expression, it is also limited by immunogenicity, DNA packaging capacity and difficulty for large-scale vector production. In contrast, non-viral gene delivery has the potential to address many of the limitations associated with viral gene delivery, including lower immunogenicity, use of essentially unlimited size of DNA, and cost-effective scalability. Intravascular injection of naked plasmid DNA in a large volume, known as hydrodynamic delivery, can achieve gene expression in the liver and muscle, but with a relatively low expression level. Both organic and inorganic materials have been used to formulate nanoparticles (NPs) in order to enhance the efficacy of plasmid DNA delivery. Similar to hydrodynamic injection of free plasmids, some of these nanoparticles predominantly express genes in liver hepatocytes, which holds the promise to treat liver related diseases. However, gene delivery to the lung has not been extensively studied. It was reported that linear PEI mediated systemic gene delivery can achieve preferential gene expression in the lung, which was unfortunately attributed to aggregation of NPs in vivo and can cause life-threatening lung embolism.

In this contribution, we will introduce a new strategy-regulated membrane activation (RMA) for gene delivery that is completely different from the previously reported viral and non-viral based strategies. Taking advantage of a precisely engineered biodegradable polymer, we can transiently activate cell membranes to significantly enhance plasmid delivery to the lung in mice. In addition, we demonstrated that this RMA process is safe and reversible in vivo and does not induce appreciable toxicity and organ damage. Therefore, we expect RMA as a novel gene therapy strategy for lung diseases.

4:50pm A Magnetic Switch for Controlling Gene Delivery in Vivo

Technical Presentation. NEMB2016-5987

***Haibao Zhu, Sheng Tong, Gang Bao**, Rice University, Houston, TX, United States*

Targeted delivery of vehicle to specific tissue or organ is a critical quest for gene delivery in clinical therapy. Intravascular administration of vectors is the main approaches to transport of the exogenous gene to the target tissue or organ. However, most of the delivery vehicle will be blocked in liver, lung, and spleen, duo to high speed blood flow and tissue microstructure. Even there are several approaches, such as nano particles vector or optical trigger system, can direct the gene of interest expression in target tissue. Still those systems are hard to match the clinical therapy requirement. To develop efficiency targeted system for gene delivery in vivo still urgent.

Here, we have developed a hybrid system, consists of magnetic nanoparticles associated with baculovirus which is an insect virus with large foreign DNA loading capacity and serum associated inactivation. Duo to magnetic nanopartilces association, the virus can be guided to target tissue by using a magnetic field in a noninvasive manner. Meanwhile the virus fusion into cells process also can be facilitated by magnetic force, so as to avoid serum inactivation. In our study, the magnetic nanoparticles conjugated with TAT peptide, which show positive charge, were firstly synthesized. The positively charge nanoparticels associated with baculovirus was confirmed by TEM. In vitro study, the hybrid system can be directed by external magnetic field to accumulate and mediate EGFP expression in target cells in hydrodynamic and physiology flow condition. Also based on serum inactivation assay, we found the external magnetic field can protect baculovirus from serum inactivation in vitro. Several criteria, such as virus/particles ratio, particles size, magnetic strength, and incubation time, determine the protection efficiency. The viruses also were successfully directed to the target tissue in vivo, such as lung and liver, by external magnetic field, respectively. And based on the in vivo image and qRT-PCR

data, we found only the targeted tissue which treated with magnetic field showed detectable lucferase expression. These findings demonstrated our hybrid system can not only be directed to target tissue or organ, but also be an idea triggerable gene delivery system by external magnetic field in vivo.

Overall, our hybrid system provides a promising way that could be adapted for targeted delivering gene in vivo.

5:10pm Hybrid Polyurethane/Phospholipid Nanoformulations for Anticancer Drug Delivery

Technical Presentation. NEMB2016-5991

***Clara Mattu, Gianluca Ciardelli**, Politecnico di Torino, Torino, Italy*

In this contribution application of polyurethane block copolymers with modulated hydrophilic/hydrophobic balance for the preparation of drug-loaded nanoparticles is described.

Polyurethanes are a versatile group of biocompatible polymers that can be obtained with a wide range of different properties, based on the selection of their building blocks [1].

In this work, the polyurethane soft segment composition has been modulated by varying the ratio between the hydrophobic poly(ε-caprolactone)-diol and the hydrophilic poly(ethylene glycol), (0, 20 and 30% PEG), so as to obtain polymers containing both hydrophilic and lipophilic domains to host both, water-soluble and insoluble drugs. In previous works we have shown that polyurethane nanoparticles are able to circulate can still be detected in blood stream 3 hours post-injection and that the modulation of the soft segment composition influences the hydrophilic/hydrophobic balance, the cellular internalization and the drug-loading capacity of the nanoparticles [2]. Using the nanoprecipitation method followed by self-assembly of phospholipids on the particles surface, phospholipid/PUR hybrid nanoparticles (size < 200 nm), composed by a PUR core of varying hydrophilic/hydrophobic balance and a surface corona of phospholipids.

The effect of the core composition on nanoparticles mean size and size distribution has been investigates. Regardless of the polymer composition small size carriers (80 - 100 nm mean size) have been obtained. At higher PEG content (30%) a significant decrease in particle's mean size and increase in PDI was observed.

Confocal analysis shows internalization of nanoparticles by glioblastoma cancer cell line (U-87) and their localization in the cells cytoplasm.
The PUR matrix, composed of randomly-distributed hydrophilic and hydrophobic domains (as confirmed by TEM images) has been exploited to successfully co-encapsulate hydrophilic and hydrophobic anti-neoplastic drugs (Doxorubicin and Docetaxel). For both drugs, high loading efficiency and sustained release profiles has been obtained and a concentration and time-dependent in vitro cytoxic profile has been determined (using U-87 cancer cells).

As expected, the encapsulation efficiency of the hydrophobic drug DCTXL is strongly dependent on the polymer composition. More hydrophobic polymer (with less PEG content) shows higher affinity with the drug and, thus, higher entrapment efficiency (of about 80 %). Moreover entrapment efficiency appears to be independent from the initial drug input provided (20, 50 and 100 µg). Drug release profile (Fig. 1C) also showed a dependence from the polymer composition with fastest release from hydrophilic carriers.

As for the hydrophilic drug Doxorubicin, loading efficacy was quite high for all polymers (at about 50 %) and release faster, regardless of the soft segment's composition. Both drugs have been loaded in the PUR core without affecting the nanoparticles size. Drug release profiles and in vitro cytotoxicity tests with combined therapy are in progress.

This contribution shows that polyurethanes are suitable candidates for the preparation of drug-loaded nanoparticles and that the modulation of their soft segment composition can be exploited for the co-encapsulation of multiple chemotherapeutics for more efficient chemotherapy.

[1] C. Mattu et al. J. Nanop. Res. 2012; 14:1306.
[2] C. Mattu et al. Eur. J. Pharm. Biopharm. 2013, 85(3); 463.

WEDNESDAY, FEBRUARY, 24

2-6
TEMPERATURE-SENSITIVE NANOMEDICINE

Sam Houston 9:30am - 11:00am

Session Organizer: **Zhenpeng Qin**, *University of Texas at Dallas, Richardson, TX, United States*

9:30am Antibody-Coated Nanoshells for Targeted Photothermal Therapy and Signal Cascade Interference of Triple-Negative Breast Cancer

Keynote. NEMB2016-5927

***Rachel Edelstein, John Gagianas, Emily Day**, University of Delaware, Newark, DE, United States*

Introduction: Triple Negative Breast Cancer (TNBC) is an aggressive disease with higher mortality rates and lower progression-free survival rates than other breast cancer subtypes. TNBC is difficult to treat because it does not express the necessary receptors to be susceptible to standard targeted or hormonal therapies. Recent studies demonstrate that hyperactive Wnt signaling drives TNBC. Therefore, we created a novel nanoparticle platform that can neutralize Wnt signaling in TNBC cells via signal cascade interference and also provide targeted cell ablation via photothermal therapy. Here, we describe our in vitro success using these nanoparticles to attack TNBC.

Our therapy consists of FZD7-NS, which are silica core/gold shell nanoshells (NS) coated with antibodies against Frizzled7 (FZD7), a receptor that plays a critical role in regulating Wnt signaling in TNBC. Briefly, the key mediator of Wnt signaling is beta-catenin. In the absence of wnt ligands, beta-catenin is constitutively degraded. When wnt ligands bind FZD7 receptors that are overexpressed on TNBC cells, beta-catenin is stabilized and enters the nucleus where it activates transcription of several genes. These genes promote cell proliferation, survival, and migration, resulting in treatment resistance, metastasis, and a poor clinical prognosis. Since FZD7 is amplified in 67% of TNBC, it provides an excellent biomarker for targeted therapy. Here, we demonstrate that FZD7-NS can bind TNBC cells to block wnt ligands from binding FZD7, thereby reinstating beta-catenin degradation. This signal cascade interference results in decreased rates of cell migration and proliferation. In addition, we demonstrate that FZD7-NS can selectively destroy TNBC cells by producing heat upon exposure to near infrared light. These data support continued development of FZD7-NS as a multifunctional therapy for treatment of TNBC and other cancers with hyperactive Wnt signaling.

Methods: NS were coated with FZD7 antibodies using OPSS-PEG-NHS linkers. Empty spaces on NS were filled with mPEG-SH. An ELISA quantified antibodies bound. Control NS were coated with only mPEG-SH. We studied treatment effects using MDA-MB-231 TNBC cells that overexpress FZD7 and non-cancerous MCF10A breast cells with low FZD7 expression. Immunohistochemistry confirmed FZD7 expression in these cells. For all treatment studies, cells were exposed to FZD7-NS or PEG-NS for 4 hours then rinsed to remove unbound particles. NS binding to cells was visualized with multiphoton microscopy. To assess photothermal therapy, samples were irradiated with 808nm light (80 W/cm2, 4 minutes) then cell viability was evaluated with calcein AM and ethidium homodimer-1 staining. To assess the impact of treatment on Wnt signaling, we used immunofluorescence and Western

blotting to measure beta-catenin expression. Finally, scratch assays and EdU incorporation assays were performed to elucidate the functional impact of FZD7-NS and PEG-NS on cell migration and proliferation.

Results: The ELISA revealed ~100 antibodies bound per FZD7-NS. Immunohistochemistry confirmed that MDA-MB-231, but not MCF10A, express FZD7. Accordingly, FZD7-NS bound MDA-MB-231 but not MCF10A cells, and PEG-NS did not bind either cell type. The level of FZD7-NS binding to MDA-MB-231 cells was sufficient to enable selective photothermal therapy. In addition, immunofluorescence and Western blotting confirmed that beta-catenin expression decreased in MDA-MB-231 cells exposed to FZD7-NS relative to controls. This resulted in reduced rates of cell migration and proliferation, validating Wnt inhibition as a viable strategy to treat TNBC.

Conclusions: Our results confirm that FZD7-NS can bind TNBC cells to enable selective photothermal therapy and inhibition of Wnt signaling, resulting in decreased cell proliferation and migration. These findings support continued development of FZD7-NS as a multifunctional therapy for treatment of TNBC and other cancers with hyperactive Wnt signaling. In addition, they suggest that nanoparticles coated with other antibodies may be useful as tools to elicit signal cascade interference against a variety of disease-promoting pathways.

10:00am Thermal transport in porous biological tissue due to gold/magnetic nanoparticle heating

Technical Presentation. NEMB2016-6118

Daipayan Sarkar, ***Abdolhosse Haji-Sheikh***, ***Ankur Jain***, *The University of Texas at Arlington, Arlington, TX, United States*

Recent studies in hyperthermia have demonstrated the importance of localized heating of tissue by gold or magnetic nanoparticles. In order to understand the physical phenomenon of thermal transport in biological tissue, the most common transport equation to estimate temperature distribution was developed by Pennes and is popularly known as the Pennes bioheat transfer equation. A generalized Pennes bioheat transfer equation accounts for the effect of various physical phenomena such as conduction, advection, volumetric heat generation due to metabolism and spatial heating. In the scenario of a thermal therapy based on nanoparticle heating, the transport and localized heating of tissue becomes a challenging problem and requires optimization. Gold or magnetic nanoparticles are introduced in the blood vessel and are attracted towards the malignant tissue as the surfaces of these nanoparticles are coated with ligands. Then, a laser beam or an alternating magnetic field is applied externally to the targeted region. In the presence of an external field these nanoparticles produce thermal energy and heat the malignant tissue locally. The size, density of the nanoparticles and the intensity of the external field are some crucial parameters in determining the specific absorption rate (SAR) for efficient design of thermal based therapy for cancer. In this theoretical work, a dual phase lag model for the Pennes bioheat transfer is considered to govern thermal transport in porous biological tissue.

The model considered in this work accounts for the phase lag times, which are expressed in terms of the properties of blood and tissue and the interphase convective heat transfer coefficient and blood perfusion rate. A detailed mathematical analysis for temperature distribution in a tissue is presented which uses the method of separation of variables. The tissue external surface is exposed to flux intensities that are considered uniform and varying with time as two test cases. Results from the analytical model are compared with a Galerkin based integral solution and are found to be in good agreement. Based on the different combinations of heat generating rates and applied external field, a correlation between SAR and rate of temperature rise in tissue is determined. Results from this work may help in proper optimization of therapeutic treatments involving gold/magnetic nanoparticle heating. In addition, a separate example scenario is considered where the tumor region is modeled as an inclusion in the dense tissue matrix, where the thermophysical properties of the tumor are different to the surrounding tissue. For this case, a semi-analytical Galerkin based integral solution is presented that closely predicts the temperature solution in the tissue.

10:20am Sustained Delivery of GC-1 from a Nanochannel Device for Metabolic Syndrome

Technical Presentation. NEMB2016-5962

Carly Filgueira, Eugenia Nicolov, R. Lyle Hood, *Houston Methodist Research Institute, Houston, TX, United States,* ***Jenaro Garcia-Huidobro***, *University of Miami, Miami, FL, United States,* ***Jean Z. Lin***, *Boston University School of Medicine, Boston, MA, United States,* ***Daniel Fraga, Omaima M. Sabek, A. O. Gaber***, *Houston Methodist Research Institute, Houston, TX, United States,* ***Kevin J. Phillips***, *Baylor College of Medicine, Houston, TX, United States,* ***Alessandro Grattoni***, *Houston Methodist Research Institute, Houston, TX, United States*

Rising at alarming rates, obesity affects over 500 million adults and 42 million children worldwide. In mammals, white adipose tissue (WAT) stores excess energy as lipid in fat, while brown adipose tissue (BAT) can uniquely perform adaptive thermogenesis to maintain body temperature. Nuclear receptors (NRs) play central roles in metabolic syndrome, making them attractive drug targets. Specifically, thyroid hormone receptors (TRs) are ligand activated NRs that regulate genes involved in lipid metabolism and homeostasis. Thyroid hormone receptor beta (TRβ) agonists stimulate BAT, releasing energy in the form of heat by uncoupling the respiratory chain through uncoupling protein 1 (UCP1). Deleterious effects of excess agonists signal a need for therapies where beneficial influences on weight loss and insulin sensitivity are enhanced with minimal side effects. Existing technologies to deliver therapies cannot sustain constant release and require frequent dosing adjustments. Nanotechnology based strategies offer novel delivery mediums to overcome these limitations. The nanochannel delivery system (nDS) utilizes customizable nanochannels for constant, sustained release of molecules without actuation or moving components, has demonstrated scalability to 12 months in vivo for various drugs, and is industrially fabricated in FDA compliance. We validate the use of the nDS capable of releasing the TRβ-selective agonist GC-1 (sobetirome). GC-1 is of particular interest as it has the potential to be used as a therapeutic agent for a variety of disorders, such as obesity, hyperlipidaemia, and hypercholesterolaemia. GC-1 as a weight loss inducing drug has recently garnered significant media attention. Two separate Phase 1 studies have shown that when compared to a placebo, GC-1 elicited reduced levels of low-density lipoprotein (LDL) in a healthy volunteer population not enriched for elevated LDL levels and that the drug was generally well tolerated. It is a subtype-selective TR agonist that selectively binds to and activates TRbeta over TRalpha and also offers tissue selective thyromimetic properties.

In our work, diet induced obese (DIO) C57BL/J6 male mice aged 18 weeks were fed a high-fat diet to induce obesity and implanted with capsules loaded with either GC-1 or PBS as a control. Overall, we observed significant reductions in body weight and fat mass for nDS-GC-1 treated mice compared to control nDS-PBS and genes involved in adaptive thermogenesis were generally increased in the WAT of nDS-GC-1 treated mice. Decreases in both cholesterol and glucose further demonstrate the cholesterol-lowering and anti-diabetic actions of nDS-released GC-1. Our drug/device combination offers a new product and is not restricted to TR agonists alone, but applicable for nuclear receptor agonists, antagonists, as well as partial and inverse agonists, and selective nuclear receptor modulators. Most reported studies with TRβ-selective agonists so far involve either oral gavage, i.p. administration, or s.c. injection. The present studies show administration from a novel drug delivery system, the nDS, consisting of an implantable nanofluidic membrane capable of tunable long-term zero-order release of therapeutic agents in ranges relevant for clinical applications. Release of agonists from the nDS allows for sustained, long-term constant release and a personalized molecular drug-delivery system. Our preliminary data from this short term dosing study suggest that TR agonism by nDS-released GC-1 elicits striking anti-obesogenic and anti-diabetic effects. GC-1's ability to decrease fat mass while sparing lean mass, coupled with its sustained release from the nDS, illustrate the potential of this system to treat obesity and metabolic syndrome. These results have implications for understanding the etiology and treatment of diabetes and metabolic disease. We believe there is strong po-

tential that the anti-obesity and anti-diabetic actions observed from nDS-administered GC-1 could be translated to humans.

10:40am Nanowarming of Arteries

Technical Presentation. NEMB2016-6136

Navid Manuchehrabadi, Zhe Gao, Jinjin Zhang, Hattie Ring, Qi Shao, Michael McDermott, Feng Liu, Yung Chung Chen, Alex Fok, Michael Garwood*, university of minnesota, minneapolis, MN, United States,* ***Kelvin G.M. Brockbank****, Department of Bioengineering, Clemson University, Clemson, SC, United States,* ***Christy L. Haynes****, University of Minnesota, Minneapolis, MN, United States,* ***John Bischof****, Univ Of Minnesota, Minneapolis, MN, United States*

There is an ongoing clinical need for long term banking of transplantable tissues such as arteries, veins, skin, heart valves and cartilage. One approach to this is vitrification of tissue in a glassy vs. crystalline state at temperatures below the glass transition. Unfortunately, rewarming these tissues from the vitrified state requires both fast and uniform thawing to avoid crystallization and cracking which have limited the adoption of vitrification in the past. Here we present new physical, chemical, computational and biological data using "nanowarming" to address this limitation. Specifically, we deploy 10 mg Fe/ml biocompatible mesoporous silica-coated iron oxide magnetic nanoparticles (mslONPs) in a cryoprotective agent (VS55) which, when exposed to an appropriate RF field, improves the uniformity and speed of rewarming from the vitrified state. The mslONPs are comprised of a 10 nm Fe_3O_4 core and 25 nm mesoporous silica shell co-modified with PEG and trimethylsilane. As a proof of principle human dermal fibroblasts (HDF) and porcine carotid arteries (inner diameter of ~ 3 mm; wall thickness of ~1 mm and length: ~3-5 mm) were chosen as the systems of study. Protocols were optimized to step load the nanoparticle impregnated VS55 into the HDF and artery systems with negligible toxicity. The systems were then vitrified at roughly 10 °C/min. Micro computed tomography was used as a quality control tool to demonstrate both the loading and vitrification of the VS55 into the arteries. Rewarming was carried out in a low frequency (20KA/m, 360 KHz) RF field achieving warming rates >> 55 °C/min (a critical rate to avoid devitrification of VS55) or by less optimal rates (< 10 °C/min). Thermal measurements and modeling verified the rates of freezing and heating in the systems tested. Further mechanical modeling verified that the thermal stress remained below a 2 MPa yield stress of the tissue during nanowarming and no cracks were identified in histology. The presence and washout of mslONPs in cells and luminal structure of arteries was verified by TEM and sweep imaging with Fourier transform (SWIFT) MRI, respectively. The viability of HDFs and arteries 1 day after nanowarming were assessed by Hoechst-PI assays and Alamar Blue and shown to remain ~ 85% of controls vs. ~ 30% of control when less optimal warming was used. In conclusion, this study provides the first evidence that nanowarming can provide both the uniformity and speed necessary to successfully return cells and tissues from the vitrified state.

Acknowledgements: Funding from NSF CBET 1336659, NIH R43HL123317, NIH P41EB015894, the MN Futures grant (UM), and the Kuhrmeyer Chair to JCB are gratefully acknowledged. We also thank the Visible Heart Lab (Iaizzo) Charles Soule and Tinen Healy for access to porcine arteries and Connie Chung for help with initial cell culture experiments.

2-7
MODELING NANOPARTICLE TRANSPORT

Bexar/Travis 9:30am - 11:00am

Session Organizer: **Zhen Gu**, *University of North Carolina at Chapel Hill and North Carolina State University, Raleigh, NC, United States*

9:30am Modeling cell mechanics from single cells to tissues

Keynote. NEMB2016-6010

Sean Sun*, Johns Hopkins University, Baltimore, MD, United States*

Animal cells are mechanically complex, but enough experimental knowledge have been accumulated for significant quantitative understanding. We discuss active forces and active mass fluxes that are important for determining cell shape and cell volume in a variety of environments. We consider active mechanical response of the cell to external mechanical as well as chemical perturbations, and describe how to include these active processes in a mechanochemical model. From fundamental force balance considerations, we derive a set of mathematical equations to compute cell shape and cell volume for a given biochemical content. The application of this framework for understanding single cell mechanics, tissue cell dynamics and collective cell motility will be discussed. In particular, collective dynamics in confluent cell monolayers will be highlighted.

10:00am The evolution of multivalent nanoparticle adhesion revealed using Nano Adhesive Dynamics Simulations

Technical Presentation. NEMB2016-6044

Mingqiu Wang, Jered Haun*, University of California, Irvine, Irvine, CA, United States*

Targeted delivery of imaging or therapeutic agents holds tremendous potential to transform detection and treatment of diseases such as cancer and atherosclerosis. However, this potential has remained largely untapped clinically because molecularly-targeted agents have failed to provide sufficient delivery yield and/or specificity. Nanomaterial carriers offer numerous advantages as a delivery platform, but targeted nanoparticle agent development has focused primarily on generating specificity and evaluating thermodynamic behavior. But adhesion within the body is a dynamic process, and thus we believe that a kinetic treatment will be far more powerful. In previous work, we developed a framework to study multivalent nanoparticle adhesion from a kinetic standpoint. This work also uncovered that nanoparticle binding stability increases over time, which we captured by developing a time-dependent detachment rate with temporal (β) and magnitude (kD0) components. We have now developed a multi-scale dynamic simulation based on the Adhesive Dynamics simulation framework to study the dynamics and biophysics of multivalent nanoparticle binding to specific molecular targets. Using our Nano Adhesive Dynamics (NAD) simulations to model an antibody-conjugated nanoparticle binding to ICAM-1, we were able to replicate the time-dependent nanoparticle detachment behavior from experiments by tuning the bond mechanical properties, specifically the reactive compliance (γ) and bond spring constant (σ). We observed bonds progressively increasing over time from one to as many as six depending on the density of antibody or ICAM-1 employed. Furthermore, the time-course by which bonds increased precisely matched the rate at which nanoparticles adhesion was stabilizing. Interestingly, experimental results could be matched over a spectrum of γ-σ combinations, and these conditions were linked by similar mechanical work being performed on bonds (equal to the bond chemical energy) and the resultant average bond lifetime (~0.1 s). Since we could not identify a unique solution, we performed optical tweezers experiments at different force loading rates and found γ 0.27 nm. Using this value, we found exquisite fits could be achieved for both the temporal (β) and magnitude (kD0) components of our time-dependent detachment rate across 9 different antibody and ICAM-1 density conditions using σ= 0.8 N/m. Specifically, β ranged from 0.7-0.9, in comparison to 0.75 for experiments, while kD0 deviated by less than 50% at all molecular density conditions. Based on this work, we have correlated the nanoparticle-scale parameters β and kD0 with individual bond information such as the average lifetime and equilibrium number; respectively. Our NAD simulations provide a unique tool for analyzing multivalent nanoparticle adhesion data in a dynamic context and interpreting behavior at at the level of individual bonds. Most importantly, the NAD simulations will be a powerful tool for designing targeted nanoparticle agents and leveraging control over multivalency.

10:20am Modeling Intraarterial Cationic Nanoparticle Delivery

for Glioma Treatments

Technical Presentation. NEMB2016-5902

***Shaolie Hossain**, Texas Heart Institute, Houston, TX, United States, **Shailendra Joshi**, Columbia University, New York, NY, United States*

Although intra-arterial (IA) drug have been extensively investigated in the past, no pharmacokinetic model accurately describes this method of drug delivery. The failure is in part due to the hydrodynamic complexities that superimposed on regional pharmacokinetics. Nanoparticles are considered important vehicles for targeted release of drugs to treat brain cancers and other focal neurological diseases. The regional deposition of nanoparticle is a dynamic balance between the forces of particle attachment and hydrodynamic forces that tend to dislodge them. Few computational models have explored the relationship of regional nanoparticle delivery after IA injections. Optically tagged nanoparticles can provide a valuable insight into the pharmacokinetics of IA drug delivery. In the present work we describe the predicted and observed behavior of nanoparticles after intracarotid injections. In these simulations we sought to determine how the size and charge delivery affects endothelial nanoparticle deposition. Both experimental and observed data supported the improvement in regional tissue deposition with transient cerebral hypoperfusion, cationic charge and larger particle size. This computational model that includes regional blood flow, injection profile and anatomical parameters provides the conceptual framework to understand and improve intraarterial drug delivery to avoid complications and failures observed during IA chemotherapy.

10:40am Rotation-facilitated rapid transport of nanorods in mucosal tissues

Technical Presentation. NEMB2016-5921

***Xinghua Shi**, Institute of Mechanics, CAS, Beijing, Beijing, China*

Mucus is a viscoelastic gel layer that typically protects exposed surfaces of the gastrointestinal (GI) tract, lung airways, and other mucosal tissues. Particles targeted to these tissues can be efficiently trapped and removed by mucus, thereby limiting the effectiveness of such drug delivery systems. In this study, we experimentally and theoretically demonstrated that cylindrical nanoparticles (NPs), such as mesoporous silica nanorods, have superior transport and trafficking capability in mucus compared with spheres of the same chemistry. The higher diffusivity of nanorods leads to deeper mucus penetration and a longer retention time in the GI tract than that of their spherical counterparts. Molecular simulations and stimulated emission of depletion (STED) microscopy revealed that this anomalous phenomenon can be attributed to the rotational dynamics of the NPs facilitated by the mucin fibers and the shear flow. These findings shed new light on the shape design of NP-based drug delivery systems targeted to mucosal and tumor sites that possess a fibrous structure/porous medium.

TRACK 3 NANO AND MICROFLUIDICS

MONDAY, FEBRUARY, 22

3-1
FLOW AND TRANSPORT DEVICES I

Hidalgo **9:30am - 11:00am**

Session Organizer: **Salman R. Khetani**, *University of Illinois at Chicago, Chicago, IL, United States*

9:30am Engineering NanoFluidic Cell Access

Keynote. NEMB2016-5935

***Nicholas Melosh**, Stanford University, Stanford, CA, United States*

The cell's lipid membrane is one of the most vital cell components as the gate-keeper in and out of the cytoplasm and a critical barrier to integrating technology with biological cells. Newly discovered forms cancer therapy and future biological technologies rely upon manipulating chemical or electronic flow across this membrane. Unfortunately, artificially controlling access through the lipid layer is surprisingly difficult; current techniques often involve harsh chemicals or creating holes that cause cell cytotoxicity. Here we investigate how scaling fluidic technology to the nanoscale can provide non-perturbative access through the cell wall and into biological cells, providing a long-term communication channel for chemical or biological signals. We achieved high-efficiency chemical delivery and control by mimicking natural gap junction proteins, creating arrays of "nanostraws". These nanoscale (100-500 nm) diameter straws are formed by templating high-aspect ratio pores, allowing precise control of height, diameter and thickness. The nanostraws deliver a wide variety of materials that could normally not pass through the cell wall, yet do not disturb natural cell function. Models of how these materials penetrate the lipid bilayer show that a simple impaling mechanism is insufficient, but instead rely upon cellular traction forces to drive membrane rupture.

Surprisingly, we discovered that these nanoscale conduits can not only deliver material into cells, but also can extract minute quantities of cellular proteins and small molecules out. Our data shows that intracellular contents from thousands of cells down to even a single cell can be non-destructively sampled and quantitatively analyzed multiple times over the course of one week, a feat which has not been previously possible. This advance enables new studies of how cells temporally evolve within fully interconnected cell monolayers in response to different therapies and lineage drivers. These, and other similar nanofluidic technologies, open a new area for engineered devices to play a leading role in future biological technologies and healthcare.

10:00am A Microfluidic Platform for Transformation of Bacteria with Variable Electric Fields

Technical Presentation. NEMB2016-6131

***Paulo Garcia**, Massachusetts Institute of Technology, Cambridge, MA, United States, **Jeffrey Moran, Zhifei Ge**, MIT, Cambridge, MA, United States, **Cullen Buie**, Massachusetts Institute of Technology, Cambridge, MA, United States*

Electroporation is an established microbiology and biotechnological tool that results from exposure of cells to external electric fields of sufficient strength to disrupt the plasma membrane of microorganisms. The exposure of the microorganisms to the external electric fields induces an increase in the local trans-membrane voltage (TMV). When the local TMV exceeds a critical

threshold value, pores are created on the membrane of the cells, allowing for transport of ions and macromolecules across the membrane. Associated with the disruption of the cell membrane is the increase of permeability of foreign molecules, including foreign DNA, that may be inserted through the defects and into the cells.

Flow-through electroporation methods have been developed by Geng et al. [1] for delivery of genes into cells and by Wang et al. for electrical lysis of bacterial cells [2]. These flow-through electroporation platforms are based on a geometric constriction that has a uniform cross-sectional area. Adjusting the DC voltage applied, the constriction length, and the flow-rate allows cell exposure to a uniform electric field that if selected carefully can successfully transform cells genetically. However, in many cases the optimal conditions for genetic transformation are unknown as for intractable or difficult-to-transfect cells.

We developed a flow-through electroporation platform that removes the electric field as a variable in the genetic transformation process. Specifically, our device employs a constriction with a variable cross-sectional area that results in a linear electric field gradient. In order to increase the throughput of the electroporated sample and increase the potential for transfection efficiency, a computational model to optimize experimental conditions was used. The computational model numerically coupled the fluid flow, electric, and thermal responses to determine the optimum flow rate that is required to expose cells to a subset of the continuous spectrum of the pulsed electric fields while allowing for heat dissipation generated by resistive heating.

Our results demonstrate that a flow rate of about 50 µL/min may be optimal for dissipating the heat entirely from the constriction region before the delivery of the subsequent pulse (t = 100 ms). Specifically, at t = 50 ms there may not be sufficient time for dissipation of the thermal energy deposited within the constriction region. However, numerical results corroborate the initial simulation estimates, demonstrating that 100 ms is sufficient to transport the cells outside the constriction region and remove the heat generated during the electroporation pulse before the onset of the following pulse. The computational models were validated with experimental results in E. coli BL21 in which successful transformation was achieved with plasmid DNA (K176011) encoding for green fluorescent protein (GFP) expression and ampicillin resistance. Future work will further optimize the channel geometry and test bacterial strains that are considered difficult-to-transfect with promising impact in biotechnology and drug discovery.

1. Geng, T., et al., Transfection of cells using flow-through electroporation based on constant voltage. Nat. Protocols, 2011. 6(8): p. 1192-1208.
2. Wang, H.-Y., A.K. Bhunia, and C. Lu, A microfluidic flow-through device for high throughput electrical lysis of bacterial cells based on continuous dc voltage. Biosensors and Bioelectronics, 2006. 22(5): p. 582-588.

10:20am Nanoscale Semipermeable Membrane Design with Topology Optimization for Portable Dialysis Device

Technical Presentation. NEMB2016-5928

Paul Braden, *Air Force, Salt Lake City, UT, United States,* ***Stephen Braden***, *St. Mary's University, San Antonio, TX, United States,* ***Lauren Gainer***, *Texas A&M University Medical School, College Station, TX, United States*

Medical devices are reaching a new level of sophistication through the introduction of nanotechnology. A major possibility for research is hemodialysis for early renal failure patients by means of osmosis through a semipermeable membrane. Currently, such hemofiltration is performed external to the body in large scale, counter-current flow diffusion to maximize efficiency and concentration coefficient according to Darcy-Stoke's Law. The empirical relationship defines the key contributors to osmosis as geometric features such as input and output diameters, pressure gradient, viscosity, permeability of the medium, shear friction, etc. Kidneys perform this function of blood filtration by means of some one million nephrons which are composed of a glomerulus and tubule. As blood enters the glomerulus with smaller fluid molecules and waste, the larger blood molecules and proteins are not allowed to pass through. The collected fluid molecules are passed via the tubule to the bladder and excreted as urine from the body. Given the viscous nature of the blood, its multiple molecular compositions and the varying hydrostatic blood pressure against the interior surface of the blood capillaries, the diffusion is difficult to model. One possible tool for potentially designing a similar optimized nanoscale semipermeable membrane for dialysis is topology optimization. Topology optimization uses sets of boundary conditions and loads to build a material layout that will meet desired performance criteria. Use of such a tool could open the door to the possibility of a portable dialysis device option for small scale hemofiltration. The hope is to create a device much like the insulin pump for diabetics that can be used to replace extracorporeal dialysis devices in the future.

In this example of designing a flow optimized, semipermeable membrane, topology optimization provides a visualization and analysis tool. The boundary conditions consist of the hydrostatic pressure on the walls of the device along with the size and concentrations of the blood molecules and fluid particles in the blood. Other inputs are the size constraints dictated by the size of the renal arteries and influx of fluid, namely the mass flow and velocity. Once the inputs are given, the topology optimization tool can generate a material layout for the filter. Flow is then simulated against this filter to find the flow output and effectiveness of the filter. Efficiency is derived in comparison to kidney filtration rates and the results show that the improved design has potential as an integrated system for diffusion of waste from the blood. It is expected that the topology optimized design could help increase the lifespan of the dialysis device, minimize its size to an acceptable level for portability and perform at a better efficiency than other extracorporeal dialysis devices.

10:40am Enhanced microfluidic mixing via a tricritical spiral vortex instability

Technical Presentation. NEMB2016-5956

Amy Shen, Simon Haward, Noa Burshtein, Kazumi Toda-Peters, *Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan,* ***Rob Poole***, *University of Liverpool, Liverpool, United Kingdom*

The ability of fluids to mix is greatly enhanced by turbulence, which occurs at large values of the Reynolds number Re= UL/mu, where U and L are characteristic velocity and length scales respectively and mu is the kinematic viscosity of the fluid. Small length scales tend to suppress Re, making it difficult to develop turbulent mixing in microfluidic devices. Improved understanding and characterization of stability conditions for flows through intersecting geometries is vital for the optimization of many laboratory microfluidic experiments and also practical lab-on-a-chip designs, including for the specific goal of enhancing the mixing of fluids in channels with small dimensions operating at low Re.

The planar elongational flow field generated by the cross-slot geometry has found applications in many research areas including for studies of macromolecular dynamics, extensional rheometry and elastic instabilities of viscoelastic fluids, hydrodynamic trapping, and imposing controlled deformations to complex biological structures.

In this work, we report the results of detailed experimental studies of the spiral vortex flow instability of Newtonian fluids and dilute polymer solutions in cross-slots with a range of aspect ratios and over a wide range of Re. In contrast to previous studies, we identify appropriate order parameters that characterize the instability as a function of Re in each case. At small Reynolds numbers, Re, the flow is two-dimensional and a sharp symmetric boundary exists between fluid streams entering the cross-slot from opposite directions. Above an a dependent critical value Rec(a) ~ 20-100, the flow bifurcates to an asymmetric state (though remains steady and laminar), and a single three-dimensional spiral vortex structure develops around the central axis of the outflow channel. Image analysis allows an assessment of the mixing quality between the two incoming fluid streams (one stream fluorescently-dyed with rhodamine b), which undergoes a significant increase following the onset of the instability. For Re >> Rec, the mixing parameter grows

according to a sixth-order Landau potential. Fitting parameters indicate the transition is second order at a = 0.5, and passes through a tricritical point, becoming first order for a >> 1. A simple scaling of the fitting parameters with allows full collapse of the experimental data. This instability can be used to drive enhanced mixing at the moderate Re that can be achieved in microfluidic devices and we show that further mixing enhancement can be achieved by patterning the surfaces of the channel walls. The effect of adding a small concentration (~0.01 wt%) of high molecular weight polymer is to reduce the value of Rec in comparison to the Newtonian solvent.

3-2
FLOW AND TRANSPORT DEVICES II

Hidalgo **11:30 AM - 1:00 PM**

Session Organizer: **Horacio Espinosa**, *Northwestern University, Evanston, IL, United States*

11:30am Electrokinetic Flows through Nanopores

Keynote. NEMB2016-5919

***Sandip Ghosal**, Northwestern University, Evanston, IL, United States, **Mao Mao**, COMSOL, Burlington, MA, United States, **John Sherwood**, DAMTP, Cambridge University, Cambridge, Cambridgeshire, United Kingdom*

Transport of small ions or polymers through pores in membranes ranging in diameter from a few to a few hundred nanometers play an important role in the biology of the cell as well as in nanotechnology. Examples of the former include ion channels in membranes that are responsible for nerve impulse propagation, the transport of protein precursors through pores in the mitochondrial membrane, the movement of mRNA out of the nucleus of Eukaryotic cells through pores in the nuclear membrane etc. Important examples of the latter are nanopore based fast DNA sequencing using the Coulter counter principle, insertion of foreign bodies into cells by electroporation and Scanning Ion Conductance Microscopy. In all of these applications electrophoresis of charged particles through small pores is a dominant theme. However, recent work has shown that the hydrodynamic flow generated by electroosmotic effects greatly influence the nature of these transport processes. Such transport problems span a range of scales from the molecular to the continuum: the smallest biological pores (e.g. ion channels) are barely larger than water molecules themselves and can only be understood in terms of a discrete molecular level representation. However, in most of the applications in nanotechnology where pores are in the range of 5-1000 nm, the continuum model of electrokinetics that combine Stoke's Flow with the Nernst-Planck-Poisson model of ion transport is fairly effective, at least to a first approximation. Here we will explore some recent experimental work on observing very small scale flows through nanopores and attempt to understand such effects from fundamental studies using a sequence of models that employ the continuum electrokinetic picture. The first and simplest of such models is that of a single circular hole in an insulating membrane of fixed constant surface charge density. The membrane separates two chambers filled with an electrolyte across which an electric voltage has been applied. Potentials are assumed low compared to the Boltzmann scale so that the Debye-Hückel approximation is permissible. The relevant parameters here are the ratio of the hole radius to the Debye length usually a very large quantity that nevertheless could be of order unity for nanopores, and some measure of the dimensionless surface charge. For this problem we show that one can calculate explicitly the electro-osmotic flow rate per unit applied voltage and this quantity has a nontrivial nonlinear dependence on the ratio of pore radius to Debye length. We then construct an approximate theory for a circular pore in a membrane of finite thickness. For membrane thicknesses that are large compared to pore radius we recover the standard result for flow through infinitely long channels whereas our result reduces to that of the zero thickness membrane considered earlier in the opposite limit of thin pores. Thus, we arrive at a concept quite analogous to that of "access resistance" of pores but in the context of resistance to electroosmotic flow under the influence of an applied voltage. We perform full numerical simulations of the underlying continuum equations to obtain numerical results to check our theoretical deductions. An appropriate generalization to a large number of holes in the membrane should result in a theory of membrane transport based on membrane microstructure. Similarly, consideration of oscillating pressure and electric fields should lead naturally to calculations of dynamic mobility and a fundamental understanding of electroseismic and seismoelectric phenomena that have many novel applications ranging from imaging of biological tissues to remote sensing in hydrogeology. These are some areas of potential future development.

12:00pm Nano Fountain Probe Technology for In Vitro Single Cell Studies

Technical Presentation. NEMB2016-5923

***Horacio Espinosa**, Northwestern University, Evanston, IL, United States, **Ruiguo Yang, Mark Duncan**, iNfinitesimal LLC, Skokie, IL, United States*

We present a broadly-applicable microfluidic technology, the Nanofountain Probe (NFP), for single cell delivery of biomolecules and functional nanoparticles. The NFP is a scanning probe nanodelivery tool that makes use of on-chip fluid reservoirs and integrated microchannels to deliver liquid solutions to sharp-apertured dispensing tips [1,2,3]. The unique tip geometry allows for both sub-100-nm nanopatterning on substrates for subsequent cell culture, as well as direct biomolecular delivery inside cells with minimum invasiveness [4,5]. In this presentation we will articulate the working principles and demonstrate the in vitro single cell transfection of biomolecules (DNA, RNA, plasmids) [5]. Applications including temporal delivery of RNA molecular beacons for single cell live analysis [6] and gene editing with CRISPR/Cas9 will be presented. Likewise, the use of the NFP technology in the generation of cell lines that eliminate limited dilution will be illustrated.

The presentation will close with a discussion of the impact of microfluidic technology in applications such as single cell manipulation and analysis, stem cell research, and drug screening.

[1] N. Moldovan, et al., J. Micromech. Microeng. 16, 10, 2006.
[2] N. Moldovan, et al., JMEMS, 15, 2006.
[3] O. Loh, et al., PNAS, 105, 43, 2008.
[4] O. Loh, et al., Small, 5, 14, 2009.
[5] W. Kang, et al., Nano Letters, 13, 6, 2013.
[6] J.P. Giraldo-Vela, et al., Small, 11, 20, 2015.

12:20pm A Microfluidic Rectifier Enabling Zero Backflow in the Pulsatile Flow Regime

Technical Presentation. NEMB2016-6009

***Vladimir Coltisor**, Texas Tech University Department of Mechanical Engineering, Lubbock, TX, United States, **Lee-Woon Jang**, Department of Mechanical Engineering/Texas Tech University, Lubbock, TX, United States, **Jungkyu Kim**, Texas Tech University, Lubbock, TX, United States*

Many microfluidic pumps use pulsatile flow to deliver discrete volumes to specific target locations. Backflow within pulsatile microfluidic pumps can have an adverse effect on droplet generation and causes unwanted mixing due to breakdown in laminar flow boundaries. A fluidic diode would provide a rectifying effect and restrict backflow allowing for a much more precise flow pattern. Fluidic rectifying structures have been proposed in the past however, many of them work at high Reynolds numbers. Microfluidic rectifiers tend to be for continuous flow and pulsatile flow diodes tend to be mostly lifting gate structures and flap structures. None of these structures eliminate backflow completely. We developed a fluidic rectifier comprised

of active and passive components that not only remove all backflow but also allows for control over dispersed volume.

A passive microfluidic rectifier in the shape of a triangle with a base of 198 micrometers and a height of 210 micrometers and an active microfluidic rectifier along with three consecutive microfluidic valves were fabricated using soft lithography technique. Two polydimethylsiloxane (PDMS) layers, which are pneumatic and fluidic structures, were designed and manufactured with 10:1 PDMS and then these layers were bonded after an oxygen plasma treatment. As a final step, this assembled PDMS structure was bonded on an oxygen plasma treated glass slide. Pneumatic actuation of lifting gate structures was used to create pulsatile flow and a diodic pump with differential voltage control supplied pneumatic actuation to the active rectifier. A flow sensor was used to generate flow profiles of each micropump and rectifier structure.

This microfluidic rectifier was then tested under various pulsatile flow conditions which were generated by the three microfluidic valves. Different pressures which were used to optimize flow patterns and characterization. Outflow profiles from the microfluidic rectifier were then compared with the output profiles which were obtained from the microfluidic channel without the rectifier structure. Flow data that was collected from both was compared after normalization. Decrease in backflow was observed when using the fluidic diode. When flow profiles were generated backflow in the straight channel was 40.00% out of total volumetric flow per cycle. The passive rectifier was able to reduce the backflow to 25.34% out of total volumetric flow, and with the addition of the active microfluidic rectifier there was no backflow on the pulsatile flow profile. Using this microfluidic rectifier, a droplet generation requiring a continuous forward flow was demonstrated and quality of drops were characterized by measuring polydispersity index. By comparison of this index, we found that the index from the microfluidic rectifier show a similar index from the index acquired from syringe pump based droplet generator. This microfluidic rectifier can be used in any fluidic system requiring zero backflow, which can be a substitute for syringe pumps. This zero backflow platform can also be used for a portable droplet generator which would simplify the complexity of current droplet platforms.

12:40pm 3D Integrated Vascularized Tumor, Liver, and Heart Microfluidic Platforms for In Vitro Transport and Toxicity Studies

Technical Presentation. NEMB2016-6126

***Jeehyun Park, Nichole Rylander**, University of Texas at Austin, Austin, TX, United States*

Introduction: Chemotherapeutic drug development typically involves the use of 2D in vitro cell cultures or in vivo animal models. 2D models are insufficient for studying cell response to therapies, as cell cultures in 3D matrices have shown decreased responses to drugs compared to similar 2D dosages, and the incorporation of altered intratumoral flow conditions further decreases uptake and efficacy. Though animal models provide more physiologically accurate environments for toxicity studies, the sheer volume of animal specimens required for validation can quickly become cost-prohibitive. 3D in vitro platforms overcome these issues by recreating physiologically relevant cell microenvironments for cost-effective, high throughput drug screening capability. Chemotherapy development and dose optimization must also include drug effects on and interactions with the endothelium, metabolism by the liver, and toxicity in the liver and the heart. Certain therapies require activation by metabolism in the liver, and combination liver-tumor microfluidic platforms are needed to assess the effects of chemotherapeutic drugs post-liver metabolism.

Methods: The Rylander group has designed and validated a 3D vascularized microfluidic platform mimicking the breast tumor microenvironment using type I collagen from rat tails to study nanoparticle transport and therapeutic efficacy. Each platform was cultured in FEP tubing implementing a subtractive needle method to form vasculature. Endothelial cells were seeded in the resulting channels, and flow protocols optimized by the Rylander group were used to induce formation of a confluent endothelial layer around the channels. We have adapted this tumor platform to create microfluidic vascularized liver and cardiac platforms to assess the interaction of fluorescently labeled nanoparticles and chemotherapies (cisplatin, doxorubicin) with the endothelium in these models as well as the transport and biodistribution of particles through these systems independently and in an integrated, plug-N-play fashion. Using our high-resolution confocal imaging system, we imaged long-term growth of cells in each tissue platform (tumor, liver, heart) and the effects of cell seeding density, cell composition (co-cultures of various cell types), matrix composition, and flow conditions on the growth and remodeling of each tissue platform.

Results: Cell growth and behavior were assessed, using a combination of fluorescently labeled cells and membrane and nuclear staining (DAPI, calceinAM), following perfusion of chemotherapeutic drugs, at clinically relevant dosages, through each collagen platform. Real-time confocal imaging recorded the distribution of fluorescent nanoparticles in each tissue compartment, focusing specifically on the effect of tumor cells on increasing the permeability of the endothelium, and aggregation of particles in the endothelium and surrounding tissues, dependent on the properties of each individual tissue platform. Using our plug-N-play format, we observed the time-lapse biodistribution of labeled nanoparticles flowing through each of the three vascularized tissue compartments in series.

3-3
DETECTION SYSTEMS

Navarro **11:30 AM - 1:00 PM**

Session Organizer: **Gabe Kwong**, *Georgia Tech, Atlanta, GA, United States*

11:30am Microfluidics for Digital Biological Measurements

Keynote. NEMB2016-6138

***Daniel Chiu**, University of Washington, Seattle, WA, United States*

Digital measurements report the presence and activity of the individual building blocks of biological systems, such as individual molecules and single cells. This presentation describes microfluidic devices and instruments we have developed for carrying out digital biological measurements. As one example, this presentation will describe a simple and robust microfluidic device for digitizing samples into a large array of discrete volumes for carrying out digital PCR. An another example, I will discuss a platform we developed for isolated single rare cells from peripheral blood, which we have employed for isolating circulating tumor cells from peripheral blood of cancer patients.

12:00pm Mathematical framework for activity-based biomarkers

Technical Presentation. NEMB2016-6007

***Gabe Kwong**, Georgia Tech, Atlanta, GA, United States, **Jaideep Dudani, Emmanuel Carrodeguas, Eric Mazumdar, Seyedeh M. Zekavat**, MIT, Cambridge, MA, United States, **Sangeeta Bhatia**, Koch Institute/Mit, Cambridge, MA, United States*

Advances in nanomedicine are providing sophisticated functions to precisely control the behavior of nanoscale drugs and diagnostics. Strategies that coopt protease activity as molecular triggers are increasingly important in nanoparticle design, yet the pharmacokinetics of these systems are challenging to understand without a quantitative framework to reveal nonintuitive associations. We describe a multicompartment mathematical model to predict strategies for ultrasensitive detection of cancer using synthetic biomarkers, a class of activity-based probes that amplify cancer-derived signals into urine as a noninvasive diagnostic. Using a model formulation made of a PEG core conjugated with protease-cleavable peptides, we explore a vast design space and identify guidelines for increasing sensitivity that depend

on critical parameters such as enzyme kinetics, dosage, and probe stability. According to this model, synthetic biomarkers that circulate in stealth but then activate at sites of disease have the theoretical capacity to discriminate tumors as small as 5 mm in diameter—a threshold sensitivity that is otherwise challenging for medical imaging and blood biomarkers to achieve. This model may be adapted to describe the behavior of additional activity-based approaches to allow cross-platform comparisons, and to predict allometric scaling across species.

12:20pm Detecting and Trapping of a Single C. elegans Worm in a Microfluidic Chip for Automated Microplate Dispensing

Technical Presentation. NEMB2016-5966

Israel Desta, Abdelrazak Al-Sharif, Nour AlGharibeh, Nahal Mustafa, Nikolas Giakoumidis*, New York University Abu Dhabi, Abu Dhabi, Abu Dhabi, United Arab Emir.,* ***Kris Gunsalus****, New York University, New York, NY, United States,* ***Yong-Ak Song****, New York University Abu Dhabi, Abu Dhabi, Abu Dhabi, United Arab Emir.*

Caenorhabditis elegans worm is one of the small invertebrate animals, about 1 mm in length and 80 um in diameter, that is most studied in the genetic, developmental, and biochemical researches. It is transparent at all stages of life and reproduces asexually making the population genetically invariant. Furthermore, the possibility of observing important signaling pathways that are quite similar to human pathways makes C. elegans an important animal model in high-throughput drug screening. To dispense C. elegans worms into microwell plates for drug screening, sorting system such as COPAS Biosort is currently used that is based on the fluorescence-activated cell sorting (FACS) technology. Both counting and sorting of worms require fluorescence labeling of worms prior to sorting and dispensing, making the process tedious and time-consuming. In addition, because of the high cost of such automatic sorting systems, most of the labs still use a simple volume-based dispensing method with a varying number of worms depending on the concentration of worm suspension. To accurately quantify and compare the effect of drugs between individual wells, there is a need for simple and inexpensive dispensing unit to count and dispense a specific number of worms, starting from a single to multiple worms into each microwell accurately and rapidly.

Due to the comparable size scale, there has been a great deal of efforts in the microfluidic community to find ways of manipulating C. elegans worms using microfluidic systems. As evidenced in the diverse applications of microfluidic systems ranging from laser surgery to phenotypical analysis, microfluidic systems have been proven useful for manipulation of C. elegans worms. However, no microfluidic system has yet been demonstrated for direct dispensing of C. elegans worms into microwell plates. In this paper, we demonstrate a microfluidic system in PDMS, poly(dimethylsiloxane), for label-free detecting, trapping and dispensing of a single C. elegans worm into a microplate. It consisted of two PDMS layers, a flow and a control layer underneath. Using five microfluidic pneumatic valves, a single worm was trapped upon an optical detection with a pair of optical fibers integrated perpendicular to the constriction channel and then dispensed into each microwell using a robotic handling system. To integrate a standard optical fiber of 125 um in diameter to a channel height of 37 um, we applied a two-step PDMS molding process. On the launching side, the optical fiber was connected to a laser light source with a wavelength of 660 nm. On the detection side, the optical fiber was connected to a photodiode and coupled to a LabVIEW program through an Arduino board. Up on a signal received from the photodiode, the pneumatic valves were actuated to trap and dispense a single worm into a microwell with a constant amount of M9 buffer solution. Several parameters were significant in improving the dispensing yield. The most critical ones were the flow rate and the concentration of worms in the suspension. At a concentration of 20-25 worms/10 uL and a flow rate of 10 uL/min, we could dispense a single worm into each microwell rapidly and with high accuracy. Due to its simple design and facile fabrication, we expect that this microfluidic detection and trapping chip can be expanded to a multiplexed dispensation system of C. elegans worms for high-throughput drug screening purposes.

12:40pm Point-of-Care Monitoring of Red Blood Cell Adhesion and Deformability as a Marker of Disease Severity and Treatment Response in Sickle Cell Disease

Technical Presentation. NEMB2016-5994

Yunus Alapan, Anima Adhikari, Ceonne Kim, Evren Gurkan-Cavusoglu, Jane Little, Umut Gurkan*, Case Western Reserve University, Cleveland, OH, United States*

Abnormal adhesion of blood cells to endothelium are pathophysiologically central to Sickle Cell Disease (SCD). Despite recent advances in identifying and targeting cellular adhesion in SCD, knowledge of abnormal cellular adhesion has not been integrated into routine clinical care or trial design, due to a requirement for complicated custom-designed systems, highly trained personnel, and extensive sample manipulation. The SCD Biochip is a novel point-of-care (POC) microfluidic assay that allows rapid, preprocessing-free, and standardized interrogation of red blood cell (RBC) adhesion to endothelium components (fibronectin, FN, or laminin, LN) in whole blood.
After a complete blood count as a standard of care, 15 µL of surplus whole blood was injected, without processing, on to FN- or LN-functionalized Biochips at or above the physiological shear stresses of post-capillary venules (1-5 dyne/cm^2). Serial quantitative and qualitative evaluations of RBC adhesion and deformability, using standardized protocols, were performed on >>100 SCD adult subjects with correlative clinical data.

Greater RBC adherence per unit area to FN or LN was seen in HbSS compared with compound heterozygous SCD. HbSS samples with a low fetal hemoglobin (HbF) displayed greater adhesion to FN or LN than did samples with a high HbF (8% HbF as the cutoff). RBC adhesion was also significantly greater in samples from HbSS patients with higher LDH levels compared to those with lower LDH levels (500 IU/L as the cutoff). Qualitative analyses revealed deformable and non-deformable HbSS-containing RBCs. Deformable RBCs adhere less to FN when exposed to higher shear stresses, and the most tightly adherent cells were non-deformable RBCs. At highest shear stress (50 dyne/cm2), 75±17% of adhered HbSS RBCs were non-deformable (n=9). Overall, there was a significant association between adhered non-deformable RBCs (%) and serum LDH levels (n=21, Pearson correlation coefficient of 0.79, p<0.005).

Longitudinal analyses performed on individual subjects ~ 1 month apart showed stable RBC adhesion to FN or LN being treated for SCD with transfusions or hydroxyurea (stable levels of HbA or HbF). In two patients monitored over 4 months, adhesion to FN or LN dropped >>60% from baseline after episodes of transfusion (elevated levels of HbA) or initiation of hydroxyurea (elevated levels of HbF).

The SCD Biochip evaluates, simply and with small sample volumes, complex adhesion properties, which reflect clinical phenotypes, including hemoglobin composition, hemolysis, and treatment status. Applied serially and under varied clinical scenarios, this adaptable POC technology will yield a more precise characterization of abnormal adhesive events in a given individual and a more accurate assessment of response to therapy overall.

3-4
TISSUE ENGINEERING
Navarro **4:00pm - 5:40pm**

Session Organizer: **Laxman Saggere***, Univ Of Illinois/Chicago, Chicago, IL, United States*

4:00pm Matrix Composition and Biophysical Characteristics Coordinately Influence Liver Progenitor Differentiation

Technical Presentation. NEMB2016-5999

***Andreas Kourouklis**, University of Illinois Urbana-Champaign, Urbana, IL, United States, **Kerim Kaylan**, University of Illinois, Urbana, IL, United States, **Gregory Underhill**, University of Illinois Urbana-Champaign, Urbana, IL, United States*

Recent efforts have utilized microfabricated platforms such as high-throughput cellular microarrays to examine combinations of microenvironmental signals that determine cell functions. To date, the majority of these approaches have focused on the biochemical properties of extracellular matrix (ECM) or soluble growth factors, and have yet to address the vast number of biophysical cues presented by cellular microenvironments. Here, by integrating a cellular microarray platform with defined substrates of modular stiffness, we sought to systematically investigate the combinatorial effects of ECM composition and mechanical stiffness on the differentiation of liver progenitor cells. Although several pathways have been suggested to regulate liver progenitor fate decisions, a potential role for biophysical signals had not previously been explored. Cellular microarrays were fabricated through the seeding of liver progenitor cells onto defined islands of ECM proteins supported by polyacrylamide gel substrates. Independently presented islands of 5 distinct ECM proteins, including their pair combinations, were created by means of a contact microarrayer. To control the biophysical stimuli, the elastic modulus of the polyacrylamide gels was tuned between 4, 13 and 30 kPa by changing the cross-linking density. Progenitor cells were induced to differentiate within the array of protein islands, and at distinct time points, both hepatocyte (e.g. albumin) and biliary (e.g. osteopontin) markers were quantitatively evaluated using immunostaining and a custom imaging analysis pipeline. The results of these microarray studies suggest that substrate stiffness influences liver progenitor fate decisions in a manner dependent on the protein composition of the ECM. In particular, biliary differentiation, was broadly reduced on 4 kPa substrates compared to stiffer 30 kPa substrates. However, our results demonstrate that in contrast to type I collagen and fibronectin, type IV collagen supports biliary differentiation independent of the elastic modulus of the supporting gel substrate. To further examine the physical interactions associated with this combinatorial ECM signaling, we developed an approach for interfacing traction force microscopy with the cellular microarrays. The systematic assessment of cell-derived traction forces illustrated that the degree of cell traction was consistent with the biliary marker expression data, which indicated that liver progenitor differentiation is force-correlated and further modulated by the protein composition of the ECM. By merging traction force microscopy with defined biochemical microenvironments, we have established a unique approach for investigating cell-ECM interactions. Continued efforts focused on the processes guiding liver differentiation would form the foundation for the optimization of stem cell differentiation protocols and the development of cell-based therapies.

4:20pm Demonstration of a Multiport Microfluidic Chip with Independently Addressable Ports towards the Development of a Chemical Retinal Prosthesis

Technical Presentation. NEMB2016-6119

***Corey M Rountree, Ashwin Raghunathan**, University of Illinois at Chicago, Chicago, IL, United States, **John B. Troy**, Northwestern University, Evanston, IL, United States, **Laxman Saggere**, Univ Of Illinois/Chicago, Chicago, IL, United States*

Photoreceptor degeneration diseases cause irreversible loss of vision for millions worldwide and no cure exists to restore vision for those affected. Retinal prostheses based on electrical stimulation are being developed as a restorative aid, but current electrical-based prosthesis technology is limited in its ability to provide high resolution natural vision. To circumvent these limitations, we propose a microfluidic-approach wherein the retina is biomimetically stimulated using native neurotransmitters. We have recently demonstrated the feasibility of this approach by focally injecting 1 mM glutamate into wild-type (WT) rat retinas from both the epiretinal and subretinal sides via a micropipette and eliciting physiological responses from retinal ganglion cells (RGC). To advance this approach further, we are currently exploring 2D spatial stimulation of the retina via simultaneous multisite injections of glutamate. Here, we present, for the first time, the development a multiport microfluidic device with independently addressable ports and in-vitro testing of the device for 2D surface stimulation of explanted rat retinas.

The preliminary multiport microfluidic device features on-chip reservoirs for storing chemicals on one side and a 3x3 array of delivery ports (10 microns dia) on the other, with each port connected to a separate reservoir via a microchannel. The device (1 cm sq. and 1.3 mm thick) is a composite of two layers – a silicon layer containing the delivery ports and microchannels and a glass layer containing the reservoirs – that were independently microfabricated and then anodically bonded. The reservoirs were filled with glutamate (1 mM) and connected to an 8-channel pressure injector that permits independent actuation of each reservoir via tubing. The device was interfaced with an explanted WT rat retina placed over a multi-electrode array (MEA) with the RGC side contacting the electrodes and photoreceptor side contacting the device delivery ports. The RGC responses to the chemical and light stimuli were recorded using the MEA and the recorded data was processed using custom Matlab scripts.

Using short duration (10-30 ms) and low pressure (0.1-3 psi) pulses, nanoliters of glutamate were injected into the retina through either single or multiple ports in various patterns. The glutamate injections stimulated a total of 408 unique RGCs across 9 retinas. Most injections produced purely excitatory RGC responses but a subset (35%) elicited both inhibitory and excitatory responses suggesting differential stimulation of the OFF and ON pathways in the inner retina. Stimulations corresponding to single port injections were spatially localized with a median spread of 155 microns (equivalent to a LogMAR value of 1.5), comparable to those of current generation electrical prostheses and our previous works with micropipettes. Stimulations corresponding to multiple simultaneous injections in simple horizontal/vertical line patterns as well as more complex shapes elicited strongly corresponding RGC responses.

If the chemical-evoked neural patterns could be translated to blind human subjects, the combination of simple patterns such as these might enable text recognition similar to the current generation electrical prostheses. Furthermore, the first ever demonstration of biomimetic differential stimulation of the inner retinal neurons with exogenous glutamate indicates the possibility of achieving more effective and naturalistic means of conveying visual information to the brain with a subretinal chemical-based prosthesis than an electrical prosthesis. These preliminary data supporting localized and patterned chemical stimulation of the retina suggest that not only a microfluidic-based chemical retinal prosthesis is feasible, but also it could be a better alternative to electrical prosthesis for people who are affected by photoreceptor degeneration diseases.

The work was supported by the National Science Foundation NSF-EFRI grant number 0938072.

4:40pm Uniform Electric Field Generation in Circular Dishes for Cell Stimulation by A 3D Designed Polymeric Insert

Technical Presentation. NEMB2016-5946

***Hsieh-Fu Tsai**, Okinawa Institute of Science and Technology Graduate University, Kunigami kun, Okinawa, Japan, **Ji-Yen Cheng**, Academia Sinica, Taipei,Taiwan, **Amy Shen**, Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan*

This work reports a CAD design principle to fabricate a polymeric insert to create uniform electric field (EF) in circular petri dishes or multi-well plates for cell stimulation.

Conventional application of uniform EF in vitro in the physiological range has been achieved in rectangular shaped microfluidic channel. However, existing in vitro electrical stimulation systems have several disadvantages including complicated device preparation procedure, low usage of cell culture area, low cell yield and low cellular product recovery. The effective stimulation area percentage to total area reported to date ranged from 4% to 45.4%. Using only two-dimensional geometry, it is very difficult to create uniform EF in a circular area from two electric potentials due to different

electrical resistances originated from the length difference between the diameter of the circle and the length of any parallel chord of the bottom circular chamber. Marotta et al., reported a direct EF stimulation device with a 6-well plate, but the EF was not uniform and the cells were exposed to electrolysis products.

To address this challenge, we designed a three-dimensional (3D) polymeric insert to apply a uniform EF at the bottom of common tissue cultureware such as a multi-well plate well or a petri dish. By using 3D computer aided design approach to equalize the electric resistance throughout the device, a uniform EF with a coefficient of variance (CV) of 1.2 % can be created with high stimulation area percentage of at least 69.5%, two fold increase compared to current reported electrical stimulation device, in a 35 mm petri dish or a 6-well plate well. The design principle can be adjusted to easily scale up the device for bigger petri dishes and increases the effective stimulation area percentage further. In particular NIH/3T3 mouse embryonic fibroblast cells were used to validate the performance of the 3D designed Poly(methyl methacrylate) insert in a circular-shaped 6-well plate.

In principle, the inserts can be mass-produced by injection molding and easily adapted by common laboratories without microfabrication capability in the future. Taking the advantage of high stimulation area of our device, the cell yield is increased and large amount of cellular products can be recovered which is highly beneficial for downstream biochemical analysis. Our polymeric inserts can be a general tool for cell-EF studies as well as electrical pacing in tissue engineering and electrical stimulation for biotechnology applications.

5:00pm Cells Interact With Graphene Nanopetals Coated On Carbon Fibers

Technical Presentation. NEMB2016-5979

***Soham Ghosh**, Purdue University, Boulder, CO, United States, **Guoping Xiong, Timothy Fisher, Bumsoo Han**, Purdue University, West Lafayette, IN, United States*

Interaction of biological cells with synthetic materials is an important research area in tissue engineering and regenerative medicine. As a promising emerging biomaterial candidate, graphene and its derivatives show wide range of mechanical, electrical and optical properties tunable to a high precision. Specifically, 2D single-layer graphene (SLG), few-layer graphene (FLG) and 3D nanostructures have been the focus of much recent research because of their unique properties. Implementing the capabilities of this versatile material with biological cells and tissues may have potential to advance tissue engineering and regenerative medicine. However the interaction of cells with graphene based materials remains challenging. Limited success has been reported for cell culture in graphene environment specific to neural cells and stem cells. But generally large class of cell types do not adhere or respond to graphene based material easily unless significant chemical modifications are performed, which have their own limitations. As an advancement to circumvent this technical limitation, we report the strong interaction of biological cells with graphene nanopetals (GPs) grown on cylindrical carbon fibers.

Commercial carbon fiber tows (YSH-60) was used as the substrate to grow GPs through microwave plasma chemical vapor deposition (MPCVD). As the model system graphene nanopetals were grown on carbon fibers. As a result, GPs grow approximately 500 to 800 nm out from the carbon fiber surface, with a typical width of a single, unwrinkled 2D petal ranging from 100 nm to 900 nm, and a petal thickness of a few nanometers, exhibiting distinct surface roughness. On contrast, the bare carbon fiber surface is relatively smooth with visible nanoscale grooves. The fibers (bare/ GP) were attached on partially polymerized type 1 collagen gel. After complete polymerization, cells were cultured on this platform.

Cells could be found attached on the GP coated fibers but did not attach on bare fiber (control). Fibroblast, epithelial and endothelial cells show similar behavior. Time lapse imaging revealed that cells attach on the fibers by creating blebs and protrusion, the scale of which is compared to irregularities of graphene nanopetals. Guided cell movement was also observed along the fibers. Terminating the graphene petals with hydrogen and oxygen did not affect the attachment frequency of the cells. This attachment is most likely caused by the ridges on nanopetal, which cells use as mechanical anchors. Further understanding the mechanisms of this interaction and improvising this method can be very useful to design novel tissue engineering and regenerative medicine strategies.

5:20pm Understanding the Effects of Variable Biaxial Stretch on NIH/3T3 Fibroblasts

Technical Presentation. NEMB2016-5998

***Hamed Ghazizadeh**, North Carolina Agricultural and Technical State University, Greensboro, NC, United States, **Soodeh B Ravari, Dennis R LaJeunesse**, University of North Carolina at Greensboro, Greensboro, NC, United States, **Shyam Aravamudhan**, North Carolina Agricultural and Technical State University, Greensboro, NC, United States*

Introduction: Cells, especially those of fat and muscle, are constantly subjected to mechanical stress during various physical activities. Understanding the role of the physical activity and the resultant mechanical stresses are critical for various cellular activities in the body, such as controlling cell growth, migration, differentiation, apoptosis, and wound repair. In order to isolate the effects of different parameters, current studies have considered applying regular (waveform) forces, which may not be able to mimic in vivo conditions. In this study, we demonstrated the effects of 25%, 50%, and 75% variability in amplitude of applied mechanical forces (5-10% strain) on cell response by comparing it with the regular waveform forces to understand whether it is possible to control cell functions through irregular mechanical forces.

Methodology: We report on the cellular and mechanistic response of NIH/3T3 fibroblastic cells cultured on silicone flexible membranes subjected to cyclic biaxial stretch using a custom-built stretching system, as previously described (Karumbaiah et al. 2012). The membrane was plasma-treated and Collagen coated to increase cell adhesion. In this work, the viability and morphological changes at the cell surface were studied in response to cycle-by-cycle variability in amplitude of the applied force. In order to better mimic in vivo, the cell responses were studied at 5%, 7.5% and 10% strain (low stretching magnitudes) while keeping the frequency constantly at 0.05 s-1 and being stretched for 6 hours and 24 hours.

Results: The results indicate that cell proliferation increased slightly about 5-15% while showing no significant change in cell viability (only 1-3% change). However, proliferation decreased slightly for variable amplitude (up to 6%). In both cases (regular and irregular waveforms), the spreading factor increased significantly by about 25-50%, which indicates the role of actin filament response to mechanical forces. Additionally, cells tended to migrate to the center and corners of the membrane due to the shear stress resulting from media movements.

Conclusion: Overall, low stretching magnitudes and low frequencies, which are closer to that of in vivo, have no considerable negative effects on the cells' viability, while cell proliferation increased considerably which makes it possible to control fibroblast's proliferation rate through exposure time and strain percentage. Cells were also found to respond negatively to variable cyclic forces. Lastly, the resultant shear stress in most of the biaxial systems is inevitable and could be the driving force for cell migration.

3-5
VASCULAR AND BONE ENGINEERING

Hidalgo **4:00pm - 5:40pm**

Session Organizer: **Hyunjoon Kong**, *University of Illinois At Urba-*

na-Champaign, Urbana, IL, United States

4:00pm On The Intrinsic Stress Fiber Contractile Forces In Semilunar Heart Valve Interstitial Cells

Technical Presentation. NEMB2016-6139

***Michael Sacks**, **Yusuke Sakamoto**, University of Texas at Austin, Austin, TX, United States*

Heart valve interstitial cells (VICs) play a critical role in the maintenance and pathophysiology of heart valve tissues. Normally quiescent in the adult, VICs can become activated in periods of growth and disease. When activated, VICs exhibit increased levels of cytokines and extracellular matrix (ECM) synthesis, and upregulated expression and strong contraction of α-smooth muscle actin (α-SMA) fibers. However, it remains unknown how expression and contraction of the f-actin augmented α-SMA fibers, which vary among different VIC types, contribute to the VIC mechanical function, including the nucleus and other cytoskeleton contributions. Moreover, our current understanding of VIC intracellular biophysical behavior results mainly from whole cell imaging and whole-cell biomechanical studies.

To being to address these limitations, we recently developed a novel computational model for VIC biomechanical behavior that incorporated 1) the underlying 3D cytoskeletal network, 2) the oriented α-SMA stress fibers with passive elastic and active contractile responses, 3) a finite deformable elastic nucleus. We examined the respective mechanical responses of aortic and pulmonary VICs, which are known to have different levels of α-SMA expression levels and contractile behaviors. To calibrate the model, we simulated the combined mechanical responses of VICs in both micropipette aspiration (MA) and atomic force microscopy (AFM) experiments. These two states were chosen as the VICs were under significantly different mechanical loading conditions and activation states, with the α-SMA fibers inactivated in the MA studies while fully activated in the AFM studies. Our model predicted that the substantial differences found in stiffening of the AVIC compared to the PVICs was due to a 9 to 16 times stronger intrinsic AVIC α-SMA stress fiber contractile force.

In the present study, we extended this model to explore how the expression levels of F-actin and α-SMA change, and how the quantified 3D structure of the stress fibers and their contractile behaviors change and are influenced by the expression levels of F-actin and α-SMAs. Bulk cell mechanical responses were obtained using a 5-micron diameter micro-indenter system. An improved stress fiber contraction model with length-tension relationship to study how the amount of these proteins within the cell can affect the contractile behavior of stress fibers, using activation states controlled by KCl concentration and TGF-b. We also explored the hypothesis that the total contraction strength linearly depends on the expression levels of co-localized F-actin and α-SMA. Model results were verified with traction force microscopy results and with the application of CytoD as a control to remove the stress-fiber contractile function. Our complete VIC computational model revealed treatment of TGF-b and KCl activates the VICs, increasing F-actin and α-SMA expression. Overall, activated VICs become stiffer due to: 1) greater expression of the stiffer F-actin stress fiber phase, 2) stronger net contractility of stress fibers, and 3) the addition of α-SMA fibers. The magnitude of traction in the TFM data was very similar to the cell contraction strength estimated in this study. We are currently extending the model to account for observed loading rate dependence, which suggest the stress fibers are viscoelastic, which is critical to the high strain environment that the VICs reside in the functioning heart valve.

4:30pm Diagnostic Imaging of Vascular Defects Using Nanoclusters with Maximum Theoretical Magnetic Resonance Relaxivity

Technical Presentation. NEMB2016-6106

***Cartney Smith**, Kala Pharmaceuticals, Waltham, MA, United States, **Dawn Ernenwein, Nicholas Clay**, University of Illinois At Urbana-Champaign, Urbana, IL, United States, **Sanjay Misra**, Mayo Clinic, Rochester, MN, United States, **Steven Zimmerman, Hyunjoon Kong**, University of Illinois At Urbana-Champaign, Urbana, IL, United States*

Inflammatory, leaky blood vessels are one of the primary characteristics of vascular diseases, which remain a leading cause of disability and morbidity worldwide. Early detection of pathologic vasculature leads to better therapeutic outcomes, which has stimulated efforts to locate vascular defects using non-invasive imaging modalities such as magnetic resonance imaging (MRI). However, the ability to locate leaky vasculature remains elusive, due in part to the low sensitivity of MRI to imaging probes that could potentially highlight such areas. This study demonstrates that superparamagnetic iron oxide nanoparticle (SPION) clusters assembled to present an MR relaxivity approaching their theoretical maximal possible value can localize in the extravascular space of leaky vessels and mark the region of interest in an MRI scan, even at doses 5-10 fold lower than those typically used in preclinical studies. Such SPION clusters were prepared by packaging nanoparticles with a hyperbranched, amphiphilic polyglycerol inspired by glycogen. We suggest that the results of this study will greatly serve to expedite current efforts to detect vascular diseases during early stages of their progression and ultimately improve quality of patient care.

4:50pm Enhancing Bone Augmentation by Mimicking the Human Osteogenic Niche

Technical Presentation. NEMB2016-6045

***Silvia Minardi**, Department of NanoMedicine, Houston Methodist Research Institute, Houston, TX, USA, Houston, TX, United States, **Francesca Taraballi**, Houston Methodist Research Institute, Houston, TX, United States, **Bruna Corradetti**, Universita' Politecnica delle Marche, Ancona, Ancona, Italy, **Jeffrey Van Eps, Fernando Cabrera, Xin Wang, Bradley K. Weiner, Ennio Tasciotti**, Houston Methodist Research Institute, Houston, TX, United States*

Regenerative osteogenesis represents a major clinical need, as hundreds of thousands of patients are left with insufficient healing of bony defects related to multiple insults, as congenital abnormalities, traumatic injury or surgically-induced deficits. The limitations of the current devices and therapeutic strategies, dictated the development of a plethora of synthetic grafting materials. The main advantages of off-the-shelf synthetic materials include: lower cost, biocompatibility, and biosafety, lower risk of rejection and simplification of the procedure to a single operation. A synthetic material that closely mimics the composition and structure of the human osteogenic niche represents great potential to successfully address this high demand. Our approach to achieve these goals is biomimicry.
In this study, we describe a material obtained through a biologically inspired process, recapitulating bio-mineralization. The resulting magnesium-doped hydroxyapatite/type I collagen scaffold was fully characterized by XRD, FTIR, ICP and TGA, as well as its micro- and nano-structure and nano-mechanical properties, by AFM. We demonstrated mimicking of both the chemical, physical and morphological cues of human bone. The apatite phase nucleated on the collagen fibers presented the same composition and level of crystallinity of that of human trabecular bone. Human bone marrow-derived mesenchymal stem cells were used to test the in vitro capability of the scaffold to promote osteogenic differentiation. Cells promptly adhered onto the material, and were found completely reorganized few days after seeding. After 3 weeks of culture, a significant increase in the expression of osteoblastogenesis-associated markers (Spp1 and Bglap) was found. Finally, to assess its in vivo osteoinductive potential, the scaffold was implanted in an ectopic model in rabbit, where most osteogenic stimuli, such as cytokines, bone cells and mechanotransduction are lacking (or significantly reduced). Remarkably, a striking mass of trabecular bone formed on the volume of the implanted scaffold (approximately 2 cm x 1 cm x 1 cm). This result was achieved in only 2 weeks, which is a third of the time usually necessary for scaffolds to produce bone-like tissue. Furthermore, we showed that the remodeling and maturation of this new bone mass initiated as early as 6 weeks after implantation.

Altogether, these results indicate that a high level of structural mimicry of the scaffold to the composition and structure of the human osteogenic niche can translate in a faster and more efficient osteoinduction in vivo. Our study represents a significant step in the development of acellular off-the-shelf substitutes for applications of bone augmentation, not requiring growth factors, whose use is still highly controversial.

5:10pm A Shear Gradient Activated Microfluidic Device for Real-Time Quantitative Assessment of Blood Hemostasis in vitro and ex vivo

Technical Presentation. NEMB2016-6015

Abhishek Jain, Amanda Graveline, Anna Waterhouse, Andyna Vernet*, Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA, United States,* ***Robert Flaumenhaft****, Beth Israel Deaconess Medical Center at Harvard Medical School, Boston, MA, United States,* ***Donald E. Ingber****, Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA, United States*

Rapid rise in cardiac procedures and use of extra-corporeal devices have increased the need for integrated instruments for more accurate monitoring of whole blood hemostasis and platelet function in real-time. Here, we describe a microfluidic device that is composed of a biomimetic arteriolar blood vessel network including the architecture of stenosis, leading to application of pathophysiological shear rates and shear gradients to flowing whole blood, which act as hemodynamic activators to cause blood clotting inside the device. Imaging of fluorescently-labeled fibrinogen in whole blood perfused through this device revealed that clot formation follows a sigmoidal trend (S-curve) in this device. Building on this observation, we created an analytical model that predicted the temporal dynamics of blood clotting that was based on a simple pressure measurement using an inline pressure sensor. We validated this model at clinically-relevant heparin doses (0.1-2.5 IU/ml) at various shear rate gradients and found that the parametric fitting parameter can be used as a characteristic clotting time (τCT) with sensitivity higher than standard assays. Then, to rapidly form clots (<30 mins) and be able to work with low blood volume (<1 ml), we coated the device with type I fibrillar collagen and then showed that the ?CT is sensitive to antiplatelet drug dose, abciximab in its clinical range (0-10 µg/ml). We have tested the performance of this device in clinical settings in vitro, and found that τCT was increased by ~58% when we perfused blood from patients who were regularly taking antiplatelet medication. Further, blood from patients who have Hermansky-Pudlak syndrome (HPS) congenital bleeding disorder did not clot in this device, thus confirming that this device could also act as a more sensitive diagnostic tool of platelet function. Finally, to test the efficacy of this device as an ex vivo monitor or as direct attachment to extracorporeal systems, we tested this on an endotoxemia porcine model and found that as the coagulopathy progressed in the pig over 8 hours and blood became hypercoagulated, the τCT also reduced and trend correlated well with changes in plasma thrombin-antithrombin time (TAT) and fibrinogen. In a second study, when we injected different concentrations of unfractionated heparin sequentially into a pig, we found that in addition to displaying a linear dose response, the microfluidic clotting times we determined ex vivo using the microfluidic device were also significantly more sensitive than those measured using standard lab tests (aPTT/ACT). Therefore, this bioinspired shear gradient incorporating global hemostasis and platelet function monitoring device, when used in vitro, can be more sensitive and accurate than current gold standard lab tests, and when used ex vivo, can allow real-time surveillance of antithrombotic therapy using native blood.

5:30pm Biomimetic Nanofibrous Composites for Tendon-Bone Interface Regeneration

Technical Presentation. NEMB2016-5924

Ece Bayrak, Burak Ozcan, Cevat Erisken*, TOBB University of Economics and Technology, Ankara, Select State/Province,Turkey*

Injuries associated with tendons are among the most common trauma, with over 250,000 rotator cuff tendon repairs performed annually in the US [1]. The native tendon-bone (TB) interface is comprised of diverse tissues, namely, tendon, fibrocartilage and bone, containing multiple cell phenotypes such as fibroblasts, fibrochondrocytes and osteoblasts in respective zones [2]. Current clinical approach, mechanical fixation, for tendon reconstruction grafts often fail to reestablish this hierarchical transition post-surgery leading to as high as 90% recurrence rates [3]. Therefore, there is a need for new augmentation matrices to improve the biological fixation to obtain a scarless healing at the TB interface.

Our approach to TB integration focuses on the use of biomimetic, nanofibrous scaffolds incorporated with bioactive agents. Growth factors injected into the zone of injury facilitates restoration of the normal function of TB interface [4]. In this regard, transforming growth factor beta3 (TGF-b3) was found to be upregulated during TB insertion development [5]. Similarly, connective tissue growth factor (CTGF) was shown to be sufficient to differentiate mesenchymal stem cells into tendon specific cells [6]. Hydroxyapatite is known as the major mineral-based component of the bone tissue and polycaprolactone (PCL) is a versatile biomaterial utilized for the engineering of multiple tissues including tendon, ligament, cartilage as well as bone [7]. The objectives of this study are to 1) fabricate PCL-based scaffolds containing TGF-b3, CTGF and nano-HA (nHA), where concentrations of CTGF and nHA change in opposite directions, while TGF-b3 is located in the middle portion of the nanofibrous composites [such organization is expected to contribute to generation of tendon (in CTGF rich zone), fibrocartilage (by TGF-b3) and bone (in nHA rich zone) upon positioning the scaffold in proper zones], 2) establish controlled release of TGF-b3 and CTGF from nanofiber scaffolds, and 3) to investigate stem cell behavior on these scaffolds. Such a design is proposed for the first time, represents a significant departure from the conventional stratified approach, and is expected to contribute to scar-free TB interface regeneration.

Our findings show that linearly varying nHA distribution can be accomplished across the scaffold thickness that is also the case in native TB interface[8]. Incorporation of nHA into PCL nanofibers led to increased mean fiber diameter from 361±9nm to 459±21nm, and a decrease in contact angle from 120.01±2.77 ° to 115.24±1.17 °. We have also demonstrated that TGF-b3 can be incorporated into nanofiber scaffolds with electrospinning and released in a sustained manner. We are to investigate the response of stem cells on the proposed scaffolds for interface-related matrix formation (collagen and glycosaminoglycans) and expression of relevant markers such as collagen types I, II, and X.

This study not only reveals the importance of design and use of biomimetic scaffolds in tissue engineering but also yields new insights into the effect of bioactive molecules on interface regeneration by controlling their local availability. These discoveries will serve as the foundation for the development of biomimetic tissue engineering technologies aimed at promoting biological graft fixation.

This research is funded by TUBITAK.
[1]Gulotta+ Am J Sports Med 2009; [2]Benjamin+ J Anat 1986, [3] Galatz+ J Bone Joint Surg 2004; [4]Gulotta+ Clin Sports Med 2009; [5]Galatz+ J Orthop Res 2007; [6]Lee+ J Clin Invest 2010; [7] Woodruff&Hutmacher Prog Polym Sci 2010; [8]Genin+ Biophys J 2009.

TUESDAY, FEBRUARY, 23

3-6
BIOSENSORS

Harris 9:30am - 11:00am

Session Organizer: **Jungkyu Kim**, *Texas Tech University, Lubbock, TX, United States*

9:30am DNA assays leveraging ion concentration shock waves

Keynote. NEMB2016-5918

Juan Santiago, *Stanford University, Stanford, CA, United States*

We use on-chip isotachophoresis (ITP) to create electric-field-driven shock waves of ion concentration. These waves are formed at the interface between a high mobility leading electrolyte (LE) and a low mobility trailing electrolyte (TE). Ionic species with mobilities bracketed by these electrolyte species focus at the LE-to-TE interface. For trace sample concentrations, multiple species mix and co-focus inside a single, order 10 µm wide zone. Multiple reactants can be mixed and then pre-concentrated by more than 50,000x in a few minutes to accelerate chemical reactions. We apply this technique to extract and purify DNA or RNA targets from complex biological samples and to immediately co-focus these with synthetic DNA probes that we design. We can complete in 30 sec chemical reactions which would normally take 4 days. Presented will be recent work on applying this technique to heterogenous reactions between macromolecule targets in solution and synthetic DNA probes immobilized on a surface. Quantitation of the reaction product provides a sequence-specific detection scheme, and so the technique has applications to medical diagnostics and basic biological studies. Current work also includes integrating ITP-based extraction and focusing to fractionate and analyze nuclear versus cytoplasmic RNA from single cells.

10:00am Chemically Patterned Paper-Based Microfluidic Device for Bioassay

Technical Presentation. NEMB2016-6062

TRINH LAM, Jungkyu Kim, *Texas Tech University, Lubbock, TX, United States*

Paper-based microfluidics has been a promising technology that has accomplished many achievements in biological and chemical analysis. This technique offers a number of useful capabilities: the ability to minimize consumption of samples and reagents for various bioassays, cost effective fabrication, and relatively rapid analysis. However, current fabrication methods creating hydrophobic barriers such as photolithography, wax printing, and ink jet printing, still require a complex fabrication technique, expensive equipment, and thermal sensitive property. In this study, we introduce a simple procedure to create hydrophobic barriers using printing cutter and trichlorosilane (TCS) coating through chemical vapor deposition (CVD). Using this Chemically Patterned Paper-based Microfluidic (cPMD) technique, we are able to create a micro scale patterns on various paper matrices which are independent of temperature changes.

To create a hydrophilic fluidic pattern, a part of chromatographic paper was masked by using patterned vinyl tape, and TCS was then vaporized onto the masked chromatographic paper. After a certain settling time inside vacuum for CVD, the vinyl tape was removed to fabricate a paper-based microfluidic device with desired shaped channels. The cPMD method was characterized and optimized by defining duration time of CVD associated with resolution of hydrophobic patterns. From a series of experiments with different sized patterns, cPMD technique enables formation of pattern as low as 500 µm width on both chromatographic and nitrocellulose papers. With this optimized CVD conditions, we demonstrated glucose assay on the patterned chromatographic paper. All paper-based glucose assay results were compared to assay results from a standard 96-well plate. With glucose assay on this paper-based microfluidic device, various concentrations of glucose were prepared and then tested on the paper. With increasing of the glucose concentration, the color of each channel on the paper gets darker. Using iColorMeter app on a smart phone, the images of the assayed papers were obtained and analyzed RGB intensity of each well to determine their glucose concentration. All the results from paper-based glucose assay was then compared to 96 well-plate results. From glucose assay, the detection limit of the device was found to be below 10 µg/mL. Compared to other glucose sensing devices, the paper-based microfluidic device can provide a proper limit of detection. As compared to a commercially available glucose sensor, the paper-based microfluidic device offers a simple, rapid, and cost-effective sensing platform which provides a relevant limit of detection. Generally, this paper-based microfluidic technique could be applied to other biomarkers such as cardiac panel, cytokines, and liver panels, which requires much high sensitivity.

10:20am Counter-Flow DNA Preconcentration Without Applied Electric Fields

Technical Presentation. NEMB2016-6042

Sarah M. Friedrich, *Johns Hopkins University, Baltimore, MD, United States,* ***Jeffrey M. Burke***, *Circulomics, Inc., Baltimore, MD, United States,* ***Kelvin Liu***, *Circulomics Inc., Baltimore, MD, United States,* ***Tza-huei Wang***, *Johns Hopkins University, Baltimore, MD, United States*

We report a method to spontaneously induce DNA migration and concentration without the use of externally applied electric fields. Electric fields are commonly used to manipulate DNA molecules because they have a high negative charge. For example, gel and capillary electrophoresis techniques exploit differences in electrokinetic mobility to separate DNA molecules by size. Similarly, concentration techniques such as large volume sample stacking and isotachophoresis utilize changes in electrokinetic velocity to concentrate DNA molecules at buffer interfaces. However, employment of electric fields in microfluidic devices necessitates careful design and placement of electrodes, additional microfabrication steps, consideration for gas bubble generation and pH shifts, and the use of expensive power supplies. Alternatively, pressure-driven flow can be used to manipulate DNA molecules without the use of electric fields. Indeed, pressure-driven flow through long microchannels has been used to separate DNA molecules by size analogously to capillary electrophoresis, but without a gel matrix or applied electric field. Using Cylindrical Illumination Confocal Spectroscopy (CICS) as the detection method for single molecule hydrodynamic separations (SML-FSHS) increased the limit of detection to tens of molecules, but the small pL injection volume restricts the concentration range of DNA molecules to the mg/mL (or pM) level. In-line preconcentration methods could enable analysis of circulating cell-free DNA (ng/mL), an emerging biomarker for cancer and prenatal testing, without sample amplification or adjustment of the microfluidic handling and injection volume. In this work, we demonstrate that pressure-driven flow can also be used to concentrate DNA without the use of electric fields in a method we call Counter-Flow Concentration (CFC). We also demonstrate the ease with which this concentration method could be integrated with downstream separation and sizing analysis by coupling CFC to Single Molecule Free Solution Hydrodynamic Separation (SML-FSHS). As opposed to current electrokinetic concentration methods, counter-flow concentration (CFC) is very simple to assemble and operate. A high concentration salt solution (25 mM Tris-HCl) is driven via pressure-driven flow through a microcapillary (5 micrometer diameter, commercially purchased from Polymicro Technologies) to empty into a large reservoir containing a low ionic strength solution (water) and dilute DNA molecules. Diffusion of the salt species down the concentration gradient (away from the capillary and into the sample) induces diffusiophoretic migration of DNA in the opposite direction and towards the high ionic strength region. Surprisingly, this migration occurs in the opposite direction of flow and results in DNA molecules spontaneously accumulating in a small picoliter volume immediately adjacent to the capillary outlet. We have investigated the effects of flow rate on the concentration factor and have demonstrated that the use of an optimized counter-flow rate can achieve higher concentration factors than applying no flow. The concentration mechanism is also highly dependent upon the buffer pair and geometry: concentration only occurs when the high ionic strength buffer flows into the low ionic strength sample.

The concentrated bolus can be easily captured by quickly reversing the flow direction and injecting the small concentrated volume into the capillary. Thus, CFC can be coupled to SML-FSHS sizing analysis by individually controlling the pressure at both sides of the capillary with separate valves. This integrated platform is capable of fully automated DNA concentration, separation, and detection. We used this platform to quantify the concentration factor of DNA molecules over a range of sizes (2 to 27 kbp) from a single

sample. With an optimized counter-flow rate, the concentration mechanism is stable for more than 25 minutes and capable of concentrating DNA over 1000 times, extending the concentration sensitivity of SML-FSHS over 3 orders of magnitude to 5 ng/mL (150 fM). Using our integrated method, DNA preconcentration, separation, and sizing can all be accomplished without the requirement of electrodes or applied electric fields.

10:40am Nanoelectronic Sensor for Rapid Detection of DNA

Technical Presentation. NEMB2016-6072

***Darius Saadat-Moghaddam, Jong-Hoon Kim**, Washington State University Vancouver, Vancouver, WA, United States*

The completions of a series of genome projects have dramatically increased the capacity of genome information. The increasing availability of genetic information demands more simple and rapid methodology for nucleic acid testing. To this end, cumbersome procedural steps with long amplification time (>>1hour) for polymerase chain reaction (PCR) have been a major challenge for rapid identification of genetic information. The ideal goal would be the development of a rapid, simple detection methodology without cumbersome sample preparation steps and/or instrumentation. This paper addresses the challenge of rapidly identifying genetic information. Unlike other amplification-based detection methods, a nanoelectronic sensor, single-walled carbon nanotubes (SWCNTs) modified nanostructured needle, is capable of rapidly concentrating and detecting small amounts of DNA, due to a concentration and reaction step controlled by an electric field. The nanoelectronic sensor is fabricated by combination of top-down (for chip structure) and bottom-up (for SWCNTs assembly) fabrication approaches; a) fabricate microscale-needle structure and b) immobilize SWNCTs onto the structure to enhance the charge transduction through DNA. When the nanoelectronic sensor concentrates target DNA to its surface using an electric field, target analytes in a sample are circulated by electrokinetic flow. Subsequently, the concentrated targets are further attracted to the tip by a dipole moment of polarized targets (dielectrophoresis), transporting targets. The selectivity is conferred by capillary force through the withdrawal of the sensor out of the solution. The capillary force excludes molecules and particles that are >>80% of the width of the tip. λ DNA, a model analyte, is used to capture on the sensor surface with applying alternating current (AC) field (20V peak-to-peak, 5MHz). To check the sensitivity, various concentration of λ DNA in TE buffer is tested from 100aM to 1pM with 10-fold increment. For the electrical detection, DI water is utilized to reduce non-Faradaic current, and thus increase signal-to-noise ratio. The nanoelectronic sensor dipped into DI water and an electric detection is performed. Upon presence of DNA on the sensor surface, the current is decreased. To validate the electrical measurement results, the optical measurement is also conducted with fluorescence microscope. In conclusion, the sensitivity is 100 aM (3.2pg/mL) with the assay time of 5 min. Based on the mean value of electrical signal, the nanoneedle shows a potential to directly measure the concentration of DNA in the sample. The nanoelectornic sensor can detect DNA at the concentration of 100 attomolar (aM) in a given sample volume (e.g. 5 µL). Since the nanoelectronic sensor does not require nucleic acid amplification, it is significantly faster and simpler than PCR approaches.

3-7
EXTRACELLULAR MATRIX

Navarro **9:30am - 11:10am**

Session Organizer: **Gregory Underhill**, *University of Illinois Urbana-Champaign, Urbana, IL, United States*

9:30am Cellular microarrays reveal combinatorial effects of Notch ligands, TGF-beta, and extracellular matrix on liver progenitor differentiation

Technical Presentation. NEMB2016-5973

***Kerim Kaylan, Viktoriya Ermilova, Ravi Yada, Gregory Underhill**, University of Illinois Urbana-Champaign, Urbana, IL, United States*

Microenvironmental regulation plays a crucial role in stem and progenitor cell fate/function in a variety of developmental and disease contexts. Regulation can occur simultaneously via multiple modes, including cell-cell, cell-substrate, and cell-soluble factor interactions. Although existing methods provide powerful means of investigating each of these interactions individually, there remains a need for high throughput approaches capable of deconstructing combinatorial regulation by multiple modes. For instance, the bipotential differentiation of liver progenitor cells underlies liver development and bile duct formation as well as liver regeneration and disease. This fate specification process is dependent on the action of multiple modes of microenvironmental regulation in a spatially-dependent and temporally-sequenced manner. Prior work has shown that TGF-beta and Notch signaling as well as extracellular matrix (ECM) composition play key roles in both this specification process and associated tissue morphogenesis. Thus, elucidation of the complete mechanistic details of this process, including interactions between TGF-beta, Notch, and ECM, requires the combinatorial capabilities described above.

Utilizing a series of approaches, including co-cultures and cellular microarrays, we identified distinct contributions of different Notch ligands and ECM proteins in liver progenitor fate decisions. We demonstrated a cooperative influence of JAG1 and TGF-beta-1 on cholangiocytic differentiation. We further established ECM-specific effects using cellular microarrays on polyacrylamide hydrogel substrates, known to exhibit well-defined material and mechanical properties. These ECM arrays contained 32 distinct combinations of collagen I, collagen III, collagen IV, fibronectin, and laminin. In the same format, we observed that presentation of exogenous Fc-recombinant JAG1, DLL1, and DLL4 conjugated with Protein A/G (a means of clustering to improve retention in the hydrogel and cellular recognition) was sufficient to induce cholangiocytic differentiation. The extent of differentiation induced by arrayed Notch ligands remained dependent on ECM composition. Last, by combining these Notch ligand arrays with shRNA-based knockdown of Notch ligands in progenitor cells, we systematically examined the effects of both cell-extrinsic and cell-intrinsic Notch ligand.

Our results highlight the importance of divergent Notch ligand function and combinatorial microenvironmental regulation in liver progenitor fate specification and additionally serve to highlight a cellular microarray platform capable of interrogating multiple modes of regulation simultaneously. Ongoing studies aim to delineate the potential cooperation between gradients of mechanical forces and Notch ligand-mediated progenitor cell differentiation.

9:50am Microscale Modeling of Layered Fibrous Networks Applied to Biomaterials for Tissue Engineering

Technical Presentation. NEMB2016-6140

***Michael Sacks**, The University of Texas At Austin, Austin, TX, United States, **James Carleton, Greg Rodin**, University of Texas at Austin, Austin, TX, United States*

Layered fibrous networks form the primary microstructure of many natural and engineered tissues. Networks of collagen, elastin, and other fibrous proteins make up the extracellular matrix (ECM), which is the primary structural component of cartilage, tendons, blood vessels, heart valves, and other soft tissues. In this work, we introduce a method for modeling materials that have a layered, nano-fibrous network structures. A primary application is in the field of tissue engineering, where high fidelity models of electrospun scaffolds are needed to better understand how the network geometry affects the mechanical and biological function of the tissues that are grown on the scaffolds. Scaffold geometry has a strong influence on the tissue's macroscopic mechanical behavior, cell proliferation and attachment, nutrient and waste flows, and extracellular matrix (ECM) generation.The random walk algorithm used to generate the scaffold geometry mimics the electrospinning

process. The 3-D, layered geometry that it generates is a good representation of the actual scaffold geometry, and the fidelity of this geometric representation represents a significant improvement over current models.

The network fibers are modeled as Cosserat rods, in which director vectors are used to keep track of the orientation of the fiber cross section. This approach allows large, 3-D rotations and axial stretches to be handled accurately. Proper treatment of these deformations is important since the large macroscopic strains experienced by tissues during normal function result in large rotations and stretches of the network fibers at the microscale. The individual fibers were connected using constraints at the points of intersection and at the periodic boundary. Periodic boundary conditions are applied to complete the specification of the network boundary value problem.

We first developed an understanding of basic fibrous network phenomena for initially isotropic network geometries. The effects of periodic box size, number of samples, and number of layers on the uncertainty in geometric and mechanical QOIs was quantified. Predictions were found to be reasonably accurate for moderate box and sample sizes. Multilayer scaffolds were found to be accurately modeled by a single network layer, despite the fact that the monolayer contains one third the number of fiber-to-fiber contacts as the real scaffold. This finding suggests that the fiber interactions are secondary to the effects of fiber volume fraction and ODF. This conclusion is also supported by the finding that the bending energy contribution to the total strain energy in the network is negligible.

The effects of fiber alignment, tortuosity, and material model, both individually and in combination, on the macroscopic stretch vs. stretch behavior were explored. For moderately tortuous networks of linear fibers, increasing fiber alignment significantly increases mechanical anisotropy, and significantly increases nonlinearity under stress-controlled loading. For isotropic networks of linear fibers, increasing tortuosity moderately increases nonlinearity at small strains. A linear fiber was compared with a fiber modeled as an incompressible Yeoh hyperelastic material. Large differences in the nonlinearity of fiber response translate into large differences in the network response. For networks of aligned fibers using a Yeoh model, increasing tortuosity significantly increases material nonlinearity under stress controlled loading. These simulations allow the effects of geometry and fiber material properties on macroscale behavior to be studied systematically in a way that is virtually impossible using current experimental techniques.

Overall, the simulations presented represent an important advancement in the field of tissue engineering. The accurate representation of detailed fibrous network geometry permits a greater understanding of the complex mechanisms underlying the macroscopic behavior unique to these biomaterials. Furthermore, simulations of scaffold fiber networks form the basis for understanding how interactions with cellular and ECM phases contribute to the growth, remodeling, and ultimate mechanical and biological behavior of the entire engineered tissue. Insights gained from such simulations can significantly aid the process of designing scaffold network geometries that result in engineered tissues that function as well as or better than the native tissues they are intended to replace.

10:06am Rapid Generation of Collagen-based Microtissues to Study Cell-matrix Interactions

Technical Presentation. NEMB2016-5972

***Alexandra L Crampton, Marie-Elena Brett, David K Wood**, University of Minnesota, Minneapolis, MN, United States*

Introduction:
2D in vitro models have long been the workhorses of drug discovery; however, 3D culture models are better able to recapitulate the complex 3D environment of tissues in vitro and have thus come to the forefront of tissue engineering. One of the most common material choices for 3D tissue scaffolds is collagen, which is typically cast as relatively large (~mm) bulk gels to study embedded or adhered cellular behavior. But fabrication of bulk gels is labor and time intensive, and the large gels have significant diffusion limitations for nutrients and signaling molecules, and they are difficult and cumbersome to analyze. Collagen-based microtissues could be the ideal 3D culture platform to study 3D cellular behavior, so we designed and optimized a method to rapidly generate uniform collagen gels using straightforward methods that are compatible with high throughput technologies.

Methods:
Microtissues were constructed of collagen, with or without cells encapsulated within the hydrogel and/or adhered to the hydrogel surface. A solution of 2-6 mg/mL collagen and 8 x 10^5 - 2 x 10^6 cells/mL was perfused into a PDMS microfluidic droplet generator at 150 µl/hr, and fluorocarbon oil with 2% EA surfactant was perfused at 1000 µl/hr to overcome the jetting to dripping transition and produce droplets. Droplets were formed at 4°C, and polymerized for 30 minutes in a 37°C incubator. Encapsulated cell (NIH 3T3 fibroblasts or MDA-MB-231eGFP breast cancer cells) behavior and/or adhered cell (HUVECs or SUM 149mCherry cancer cells) behavior was observed in a droplet capture device for 24-72 hours post-polymerization.

Results:
We found that polymerized microtissues had a narrow distribution of size with a coefficient of variance ranging from 8.4% - 13.9%, which is sufficiently low that the size variance does not interfere with subsequent analyses. We also observed that encapsulated cell viability was high (79% - 95%) and remained consistent for the experimental trials (fluctuations were at most 6%). In assessing cell-matrix interactions, we observed that cancer cells spread out and sent projections throughout the collagen matrix, indicating that these cells are able to adhere and interact with the 3D environment of the collagen microtissues. For microtissues with encapsulated fibroblasts, we observed and quantified serum-induced gel compaction (25% in 2 mg/mL collagen, 21% in 4 mg/mL collagen, and no significant contraction in 6 mg/mL collagen), which shows a trend already reported in analogous bulk gel experiments: that less gel compaction is observed at higher collagen concentrations. Finally, we characterized successful co-culture conditions with our microtissues via confocal microscopy.

Discussion:
Our easy-to-use method to miniaturize collagen-based tissue constructs maintains the 3D in vitro environment, while alleviating several obstacles associated with larger avascular tissue constructs. The microtissues formed with our platform are uniform in size and are generated rapidly (>>4500/hour), which facilitates large-scale experiments with large numbers of treatment groups and hundreds of replicates. Our method uses only common laboratory temperatures (4°C and 37°C) and requires no on-chip temperature control or temperature gradients, which means this technology can be easily disseminated and can be integrated within a high throughput pipeline. Outside of the method development, we have also demonstrated the biological potential for this platform via novel patterning of cell co-cultures within and around the microtissues. Finally, we have shown, for the first time to the author's knowledge, quantification of gel compaction for tissues on this small size scale (~200 µm). Overall, this is a highly versatile platform for 3D cell culture that is engineered to facilitate quantitative studies of drugs, cellular interactions, and biological mechanisms in high throughput applications.

10:22am 3D cell culture and osteogenic and chondrogenic differentiation of human mesenchymal stem cells plated onto an electrospun scaffold

Technical Presentation. NEMB2016-6069

***Laura Pandolfi, Naama Toledano**, Houston Methodist Research Institute, Houston, TX, United States, **Francesca Taraballi**, Houston Methodist Research Inst., Houston, TX, United States, **Ennio Tasciotti**, The Methodist Hospital Research Institute, Houston, TX, United States*

Regenerative processes in living tissues draw from reservoirs of stem cells. Among the plethora of stem cells, mesenchymal stem cells (MSC) have been shown to be multipotent progenitors, able to provide an optimal microenvironment at the site of injury. Due to their high plasticity, immunosuppressive potential and immunomodulatory properties, MSC hold promises in a diver-

sity of regenerative medicine applications. Extracellular matrix (ECM) represents an essential player in stem cell niche, since it can directly or indirectly modulate the maintenance, proliferation, self-renewal and differentiation of stem cells. When creating 3D scaffolds for cells, it is preferable to provide cells with an environment which closely resembles their native ECM. Electrospinning has been proved to be an effective technique for the controlled fabrication of micro-fiber meshes for tissue engineering applications, due to its ability to produce biocompatible and biomimetic scaffolds capable of supporting cell growth.

Herein we proposed an electrospun gelatin based scaffold to resemble the ECM topography, for the sustainment of stem cells survival, proliferation and differentiation. We characterized the scaffold using scanning electron microscopy, Fourier transform infrared spectroscopy, and confocal microscopy. We tested the biocompatibility of the electrospun scaffolds using human MSC (hMSC). Early passage hMSC were seeded onto the scaffold to assess cell growth up to one month. The results of the in vitro tests indicated uncompromised cell viability and full cell adhesion to the patch for all the duration of the experiment. Multilineage potential of cells toward osteogenic and chondrogenic differentiation was assessed in vitro for up to 1 month. The differentiation of hMSC was carried out using chondrogenic and osteogenic inducing factors. SEM and confocal microscopy analysis were used to compare the morphology of cells. Cytoskeleton morphology was assessed by fluorescent staining; cells on the patch exhibited spread morphology with abundant cytoskeleton staining. Our data show that when appropriately induced, MSC seeded onto the electrospun scaffold have the ability to differentiate into the osteogenic and chondrogenic lineages; on the other side, hMSC harvested with the untreated culture media maintain their immunosuppressive potential and immunomodulatory properties.

A scaffold which mimics the native ECM while allowing for cell infiltration and proliferation is important for regenerative medicine applications to permit the penetration of host cells and vasculature, augmenting diffusion of nutrients and waste products and enhancing integration into the host tissue. Future application of this versatile scaffold platform to induced pluripotent stem cells for functional tissue repair and regeneration will further expand its potential for regenerative therapies.

10:38am A Suspended and Aligned Nanofiber Network Assay to Study Collective Cell Migration and Gap Closure Dynamics

Technical Presentation. NEMB2016-6125

***Puja Sharma**, Virginia Tech, Blacksburg, VA, United States, **Jerry Lee**, National Cancer Institute, NIH, Bethesda, MD, United States, **Bahareh Behkam, Amrinder Nain**, Virginia Tech, Blacksburg, VA, United States*

Collective cell migration described as coordinated movement of multiple cells with well-maintained cell-cell adhesions is relevant to a myriad of physiological phenomenon including wound healing, morphogenesis and metastasis. In certain physiological conditions involving poorly developed or absent extra-cellular matrix (ECM), collective cell migration is observed in the form of suspended sheets. Our understanding of suspended cell migration is limited, as traditional wound healing scratch tests are conducted on featureless 2D substrates, which do not faithfully recapitulate the behavior of cells in their native fibrous ECM environment. Here, we present a novel gap closure assay system comprised of suspended and aligned arrays (in parallel or crosshatch configurations) of ECM mimicking 500 nm diameter fibers bridging the gap between two fibroblast monolayers cultured on raised platforms separated by a few millimeters. Fibroblasts sense fiber alignment and emerge from the monolayers as either individual leaders showing elastic recoils or as chains or as collective groups with intact cell-cell adhesions. Using time-lapse microscopy, we find that the emerging cells form streams, which advance rapidly (200µm/day) for a few days followed by slow growth (20µm/day). Suspended cell sheets (SCS) formed between the cells streams, advance away from the monolayer in oscillatory patterns, with neighboring sheets demonstrating advancement in out-of-phase mode. SCS advancement is inversely related to its span distance, with advancement severely compromised at span distances exceeding 375µm. Advancing SCS converge to form local gaps which contrary to findings from 2D models, close faster from regions of high curvature. Gap closure kinetics is regulated by gap size and shape, with gaps <85,000 µm2 closing and >> 140,000 µm2 not closing regardless of shape, while shape influencing closure of those in between. The inhibition of actin-myosin interactions through treatment with 10 µM and 20 µM y-27632 compromised the closure of large local gaps (>>40,000µm2). However, small local gaps (<10,000µm2) closed despite exposure to the drug. Overall gap closure was significantly influenced by fiber architecture with crosshatch patterned fibers closing the gaps faster when compared to gaps bridged by parallel array of fibers. We have systematically studied the emergence of cells from monolayers initiating formation of cell streams, and SCS, thus facilitating the overall collective cell migration process. Given the observed influence of size and fiber architecture on gap closure, findings from this study could be utilized to design in vitro scaffolds that serve as a foundation to generate non-closing diabetic wound, and or to study metastatic events in cancer biology.

3-8
TISSUE MODELS

Bexar/Travis 4:00pm - 5:40pm

Session Organizer: **Dan Dongeun Huh**, *Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, United States*

4:00pm Microengineered cell and tissue systems: Evolution of in-vitro liver technologies

Technical Presentation. NEMB2016-5969

***Osman Berk USTA**, Harvard Medical School - Massachusetts General Hospital, Boston, MA, United States*

The liver performs many key functions such as serving as the metabolic hub of the body. For this reason, the liver is the focal point of many investigations aimed at understanding an organism's toxicological response to endogenous and exogenous challenges. We will present a survey and critical comparison of in-vitro liver technologies along a broad spectrum, but focus on the current renewed push to develop "organs-on-a-chip" in our laboratory and elsewhere.

4:20pm Microfabricated Co-Cultures Containing Induced Pluripotent Stem Cell-Derived Liver Cells for Drug Development

Technical Presentation. NEMB2016-6002

***Brenton Ware, Salman R. Khetani**, University of Illinois at Chicago, Chicago, IL, United States*

Introduction: Drug-induced liver injury is a leading cause of drug attrition in the pharmaceutical industry. Significant differences in liver pathways across species now necessitate the use of human-relevant cultures for assessing liver-drug interactions. While primary human hepatocytes (PHHs) isolated from the liver are considered ideal for such purposes, these cells are in limited supply for screening large (millions) libraries of drugs. Furthermore, lack of genetic diversity restricts the use of PHHs for identifying mechanisms underlying inter-individual susceptibility to drugs. Induced pluripotent stem cell-derived human hepatocyte-like cells (iHeps) have the potential to address the aforementioned limitations with PHHs. However, iHeps remain fetal-like (i.e. low adult liver functions) under conventional culture formats that rely exclusively on extracellular matrices and soluble factors. Here, we utilized soft lithography and co-culture with stromal cells to develop and characterize a micropatterned co-culture (iMPCC) platform that can further mature iHeps towards an adult liver phenotype and maintain these functions for at least 4 weeks in vitro. We then tested toxicity of a panel of drugs in this platform and compared results to both PHH cultures and clinical findings.

Methods: SU-8 photoresist spun-coated on a silicon wafer was patterned with desired features (circular holes 500 µm in diameter with 1200 µm center-to-center spacing) using conventional photolithography. Polydimethylsiloxane (PDMS) was then cast over the SU-8 pattern to form positive impressions of the desired features, and circular "buttons" were bore out using an industrial punch. These buttons were then attached one-by-one to the pillars of a larger PDMS structure whose pillars matched the wells of an industry standard 96-well plate. This multi-part PDMS stamp then served as a mask to protect desired circular regions of adsorbed collagen (with dimensions noted above) from ablation by oxygen plasma. The iHeps (Cellular Dynamics International, Madison, WI) selectively attached to the collagen islands and then murine embryonic 3T3-J2 fibroblasts were seeded in the remaining bare areas to create iMPCCs. Live markers in iMPCCs were measured using published protocols. In addition, ~1-week stabilized co-cultures were treated with up to 100×Cmax (maximum plasma concentration) of a drug three times over six days. Liver functions (i.e. albumin, urea) in drug-treated wells were compared to solvent-only control wells. A drug that reduced at least one of the measured functions to below 50% of controls was declared toxic, whereas a drug that did not reduce any of the functions to below 50% of controls was declared non-toxic.

Results and Discussion: Several major liver-specific mRNA transcripts (i.e. HNF-4α, HNF-6) were up-regulated in iMPCCs relative to control cultures. Albumin and urea production by iMPCCs both reached steady-state levels of 5-6 µg/hr/million cells by the first week of culture and remained stable for 3 more weeks. Activities of several cytochrome P450 (CYP) enzymes were also maintained in iMPCCs for 4 weeks, reaching up to 73% of levels observed in stable PHH cultures. Furthermore, iMPCCs were able to detect 21 of 37 hepatotoxic drugs (65% sensitivity) and 10 of 10 non-toxic drugs (100% specificity). These results were remarkably similar to outcomes seen with PHHs using the same set of drugs (70% sensitivity, 100% specificity). On the other hand, conventional cultures of iHeps with their reduced functional capacities showed a 38% reduction in sensitivity. iMPCCs were able to distinguish the relative toxicity of structural drug analogs (toxic/non-toxic drug pairs: troglitazone/rosiglitazone, tolcapone/entacapone) and displayed known bioactivation-mediated mechanisms of acetaminophen toxicity. In conclusion, controlling interactions between iHeps and embryonic fibroblasts using microfabrication tools can significantly mature and sustain liver functions in the iHeps for at least 4 weeks. Proof-of-concept drug toxicity studies show that iMPCCs could be useful for an initial toxicity screen of a large drug library in industry standard 96-well plates. In the future, iMPCCs could be used in human-on-a-chip platforms being designed to assess multi-organ responses to pharmaceuticals. Lastly, the development of a library of patient-specific iHeps could enable personalized drug screening.

4:40pm A human blinking "eye-on-a-chip"

Technical Presentation. NEMB2016-6051

***Jeongyun Seo, Woo Yul Byun, Andrea Frank**, Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, United States, **Giacomina Massaro-Giordano, Vivian Lee, Vatinee Bunya**, Scheie Eye Institute, Department of Ophthalmology, Perelman School of Medicine at the University of, Philadelphia, PA, United States, **Dan Dongeun Huh**, Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, United States*

The ocular surface is a central anatomical and functional unit of the eye that protects the ocular system from external environment. As a principal protective barrier in this unit, the cornea consists of epithelium and a sub-epithelial collagen-rich stromal tissue containing keratocytes. At the circumferential margin of the cornea, the corneal epithelium grades into the conjunctival epithelium lined with goblet cells that are responsible for producing mucus into the tear fluid. The ocular surface is also under the constant influence of dynamic microenvironment created by spontaneous eye blinking-induced eyelid movements and concomitant spreading of the tear fluid that permits hydration and lubrication of the cornea and conjunctiva.

This structural, functional, and environmental complexity of the ocular surface, however, poses major technical challenges to in vitro investigation of its physiology and pathology using traditional cell culture models. As a result, research in this area has relied heavily on expensive and time-consuming ex vivo or in vivo animal studies that often fail to model biological responses in humans. These critical drawbacks of exiting models have greatly limited our fundamental understanding and hampered the development of new therapeutic approaches to ocular surface diseases. To address this serious lack of physiological model systems, we have developed a microengineered organ-on-a-chip model of the human eye that replicates 3D architecture, physiological functionality, and dynamic mechanical microenvironment of the ocular surface in the human eye.

As the first step towards the development of this eye model, we generated the porous 3D shell scaffolds by microengineering planar polystyrene cell culture scaffolds to create a curvature similar to that of the cornea in vivo. Throughout prolonged cell culture, the 3D shell scaffolds retained the original curvature and maintained its structural stability. These scaffolds were then incorporated into our eye-on-a-chip system by sandwiching them between upper and lower PDMS slabs that contained a circular chamber and a microfluidic channel, respectively. To recreate a stromal layer in the cornea, the sandwiched scaffold was impregnated with human primary keratocytes suspended in collagen precursor. We also recapitulated the unique concentric patterns of the corneal and conjunctival epithelia using our novel 3D cell patterning techniques that enabled precise positioning and deposition of human corneal and conjunctival epithelial cells at the central and peripheral regions of the scaffold surface, respectively. Furthermore, simulation of eye blinking was accomplished by integrating a biomimetic hydrogel eyelid with our microengineered cell culture model. The hydrogel eyelid was linked to a computer-controlled miniature motor and electromechanically actuated to slide along the convex scaffold surface to mimic the eyelid movements. We successfully demonstrated spreading of artificial tear fluid and surface wetting of scaffold during the hydrogel movements. The motor was programmed to simulate physiological blink scenarios of the human eye by precisely controlling kinematics and patterns of the eyelid movements.

Our on-going studies focus on leveraging this model system to develop a microengineered human disease model of dry eyes. We aim to demonstrate the capability of our human blinking "eye-on-a-chip" to recapitulate the central disease mechanisms in the dry eye disease, which are the ocular surface tissue damages caused by tear hyperosmolarity as a result of excessive evaporation and/or insufficient secretion of the tear fluid.

We believe that our human blinking eye-on-a-chip system offers the promise to address critical technical barriers to progress ocular biology and clinical ophthalmology by providing a novel experimental platform that enables replication, visualization, and analysis of key biological processes in the human eye. Moreover, this approach may enable the development of human disease models that represent cost-effective and more predictable alternatives to conventional animal models for the identification and development of new therapeutic approaches.

5:00pm Microfluidic Hydrogel System Provides Improved In Vitro Platform for Blood Brain Barrier Studies

Technical Presentation. NEMB2016-6104

***Candice Hovell, Yoshitaka Sei, Cole Weiler**, Georgia Institute of Technology, Atlanta, GA, United States, **Gilda Barabino**, City College of New York, New York, NY, United States, **Lakeshia Taite**, Texas A&M University College of Veterinary Medicine & Biomedical Sciences, College Station, GA, United States, **YongTae Kim**, Georgia Institute of Technology, Atlanta, GA, United States*

The development of new drug candidates is a timely and costly process. Central nervous system (CNS) drugs are particularly challenging to develop because of the presence of the blood brain barrier (BBB). The BBB maintains the homeostatic environment of the brain by restricting the passage of molecules from systemic circulation via a variety of specialized endothelial processes. These specializations arise from exposure to chemical and me-

chanical cues from surrounding cell types as well as physiologically relevant flow and extracellular matrix stiffness. In vitro models aid in the early development and characterization of CNS drug candidates and allow for highly controlled examination of BBB function and dysfunction at the cellular level as it pertains to the pathogenesis of neurobiological diseases. However, current in vitro BBB models available for preliminary drug studies have neglected the inclusion of several critical parameters including (i) controlled shear flow that directs endothelial function in the lumen and (ii) appropriate culture of multiple cellular constituents of the BBB. Several microfluidic models of the BBB exist in the literature, and some incorporate multiple relevant cell types. However, all existing co-culture models necessitate the incorporation of a semipermeable membrane to achieve relevant cellular geometries. Furthermore, existing co-culture models pay little attention to effect of extracellular matrix composition and stiffness on the supplementary cell types. Additionally, traditional characterization of the BBB structure and function in the existing in vitro models has been largely superficial. Currently, most in vitro models are evaluated via a combination of transendothelial electrical resistance (TEER) measurements, histology for relevant proteins (ZO-1, PgP), and conventional permeability assays. While these benchmarks provide useful information, they are far from comprehensive enough to be used to develop truly predictive models. We have addressed these two challenges (the simultaneous incorporation of shear flow and direct cell contact, and the comprehensive characterization of genetic and protein specializations characteristic of brain microvasculature) with our study. Our novel in vitro model of the BBB is composed of microfluidic channels crafted inside a cell laden hydrogel system through the use of sacrificial gelatin elements. The hydrogel system is a tailored blend of extracellular matrix proteins previously demonstrated to facilitate the healthy culture of astrocytes. In addition to the development of our novel model, we have adapted a multilayered microfluidic endothelial culture system previously used in our lab to serve as a dynamic transwell culture of the BBB with shear flow controlled and three cell types cultured. Using this system, we are able to measure TEER establishment and disruption in the presence of relevant cues. This system also allows us a means to compare our novel lumen device to dynamic transwell microfluidic models of the BBB already present in the literature. To evaluate the degree to which our novel model recapitulates relevant in vivo BBB features, we have developed primers pairs for a list of 96 PCR targets (93 BBB specific genes and 3 housekeeping genes) for use in high throughput qPCR analysis (Biomark Fluidigm System) that will be used in addition to traditional characterization techniques, such as TEER and histology. Thus far, we have compared the expression levels of these targets in our model relative to several commonly used platforms for in vitro BBB studies: static transwell cultures, spheroids, and our multilayer microfluidic dynamic transwell system. Preliminary results indicate that the type of culture system drastically influences the degree to which the endothelial cells are specialized. Current work is aimed at further elucidating how individual culture conditions (presence/lack of flow, alterations in ECM stiffness, presence of cellular constituents) contribute to the expression of relevant specializations.

5:20pm Three-dimensional (3D) Invasion of Breast Cancer Cells in a Well-Defined Tumor-Stroma Platform

Technical Presentation. NEMB2016-5967

***Danh Truong, Alison Llave**, Arizona State University, Tempe, AZ, United States, **Julieann Puleo, Ghassan Mouneimne**, The University of Arizona Cancer Center, Tucson, AZ, United States, **Roger Kamm**, Massachusetts Institute of Technology, Cambridge, MA, United States, **Mehdi Nikkhah**, Arizona State University, Tempe, AZ, United States*

Breast cancer is one of the leading causes of mortalities in the United States and will ultimately result in approximately 40,000 deaths by the end of 2015. The majority of those deaths are due to invasion and dissemination of cancer from the primary tumor. While current regimens are able to treat localized diseases, there are still few effective treatments for metastatic disease. Cancer invasion is a fundamental aspect of metastasis that initiates from the complex heterogeneous tumor microenvironment. The tumor stroma is a three-dimensional (3D) dense tissue with a distinct architecture and heterogeneous cellular organization. Moreover, the tumor microenvironment is composed of confounding factors (e.g. spatial organization of cells, 3D biomolecular gradients, etc.) that hinder the understanding of how and why cancer cells invade throughout the surrounding tissues. These complex characteristics must be recapitulated within physiologically relevant in vitro models to fully appreciate the dynamics of cancer invasion. Microengineering technology provides a powerful approach to develop flexible platforms with the ability to precisely control biochemical and biophysical cues with well-defined 3D cellular organization to mimic the native tumor microenvironment. Current models are able to replicate the tumor architecture, however, they were either limited by creating a 3D stroma but with a two-dimensional (2D) tumor region or by incorporating a 3D stroma and tumor but without any capacity for vasculature, cell invasion, and modulation of microenvironmental cues (e.g. biophysical and biochemical signaling). Furthermore, studies of 3D cellular chemoinvasion (i.e. invasion due to a chemoattractant) have been limited and most analyses have been performed in 2D.
Our research focuses on the development of a physiologically relevant 3D breast tumor-stroma model on a chip using microfluidics technology and advanced biomaterials (hydrogels). This platform enables a spatially organized tumor-stroma interaction model for characterization of 3D breast cancer chemoinvasion within a native like microenvironment. The device, fabricated from polydimethylsiloxane (PDMS), contains specific geometric regions that enable spatial compartmentalization of the tumor and stroma. Highly invasive breast cancer cells (SUM-159) were encapsulated within an ECM gel (Matrigel®:collagen I) and injected into a one region of the device to produce the “primary tumor”. This region was then surrounded by an outer collagen ECM gel to create the stromal component. This combination produced a 3D tumor-stroma-on-a-chip model. Our studies revealed that the device was capable of high-resolution time-lapse and immunofluorescent imaging of cancer cell invasion and morphology within a 3D matrix. We modulated biochemical cues to stimulate cancer chemoinvasion throughout the tumor-stroma regions. Cancer cells experienced 3D epidermal growth factor (EGF) gradients emanating from the stromal region, which strongly influenced their phenotype and invasive profile. Particularly, the EGF-stimulated cells demonstrated enhanced proliferation, invasive protrusions, and migratory speed. Using this platform, additional studies are planned to elucidate biomolecular interactions (e.g growth factors or anti-cancer drugs) in the context of the tumor-stroma complex. Moreover, this platform will be used to investigate intracellular pathways that are known to be involved in cells migration and chemoinvasion, such as the PI3K pathway. By spatially organizing the tumor-stroma to better mimic the human breast cancer architecture, we envision that this work will build a foundation for a breast tumor-on-a-chip. We plan to later include further complexities such as heterotypic interactions and a vasculature network to work our way toward a platform that would be suitable for effective drug screening and personalized medicine.

WEDNESDAY, FEBRUARY, 24

3-9
TOWARD POINT-OF-CARE DIAGNOSTICS

Navarro **9:30am - 11:00am**

Session Organizer: **Savas Tasoglu**, *UConn, Storrs, CT, United States*

9:30am Challenges and Opportunities for Ultrasensitive Immunoassays: Large Volume Capillaric Microfluidics and Digital Nanodot Arrays

Keynote. NEMB2016-6160

***David Juncker**, McGill University, Montreal, QC, Canada*

The difficulty of making reliable ultrasensitive assays and the limitations of microfluidics affinity binder and complications of multiplexing will be intro-

duced in the context of an engineering challenge will be discussed. Our recent work on digital immunoassays with single molecule detection and noise rejection by spatial exclusion will be introduced, and results for ultrasensitive assays presented. Strategies for multiplexing will be presented, along with preliminary results. Next, we will present capillaric circuits that are capillary powered microfluidics for autonomous fluidic operations that are ideally suited for point-of-care applications. Newly 3D printed capillaric circuits made by rapid prototyping will be introduced, and the design rules discussed. The application of rapidly prototyped capillarics to bacterial detection and immunoassays by taking advantage of sequential flow operations and large volume flow for rapid and high sensitivity results will be shown.

10:00am Self-Contained Magnetic Levitation Platform for Sickle Cell Disease Diagnostics

Technical Presentation. NEMB2016-5990

***Bekir Yenilmez**, University of Connecticut, Storrs, CT, United States, **Stephanie Knowlton**, University of Connecticut, Mech. Eng., Storrs, CT, United States, **Savas Tasoglu**, UConn, Storrs, CT, United States*

Low-cost and portable point of care technology is critical for enabling rapid, on-site disease diagnostics. A particular need exists for sickle cell disease diagnostics, as the majority of those carrying the disease live in Sub-Saharan Africa, where ubiquitous testing procedures have yet to be implemented. A serious complication of sickle cell disease is stroke and many of these strokes are “silent” strokes which do not present the typical noticeable symptoms and are generally only detectable through imaging, such as with an MRI. 17% of children under the age of 14 and 23% under the age of 18 with sickle cell disease are estimated to suffer from silent strokes.
Here, we present a simple yet powerful platform to perform density-based analysis of micro-particles, such as cells, using magnetic levitation. Under deoxygenated conditions, red blood cells from patients with sickle cell disease have been shown to attain higher densities than those from healthy patients. This is a portable, self-contained device to levitate particles of interest, image them using an embedded low-cost optical system and a camera module, and process the captured images in order to assess the distribution of the particles. All components are contained in a compact, portable 3D-printed casing. The required sample preparation is simple, requiring only a drop of blood from the patient via finger stick. The blood sample is mixed with a paramagnetic gadolinium medium and sodium metabisulfite (which are both low-cost reagents) then loaded into a glass microcapillary which is inserted between two permanent magnets separated 1 mm apart with like poles facing each other. The magnets form a magnetic field which exerts a force on the cells in the direction of the centerline between the magnets with magnitude greatest at the surface of the magnets and approaching zero at the centerline. A second force, the buoyancy force, relies on the density of the particle relative to that of the medium. Particles levitate at a height at which the magnetic force is in equilibrium with the buoyancy force. Levitating blood cells are then imaged and their levitation heights are used to calculate the particle distribution. This value serves as an indicator to diagnose sickle cell disease, as demonstrated by a pilot study of blood samples from patients with and without the disease. Sample preparation, magnetic levitation, and analysis are completed within only 10 minutes.
This device will enable rapid, on-site testing for sickle cell disease in order to allow proper care to be administered and prevent complications early on for children with the disease. The device is user-friendly, label-free, and portable, making it ideas for point-of-care disease diagnostics. The platform has been designed with easily accessible and inexpensive components, allowing widespread use of this method by researchers, clinicians, and patients alike. Future applications to other diseases, including cancer, malaria, and Babessia, can have a tremendous impact on global health, particularly in developing countries where more advanced testing is inaccessible as well as in-home testing and disease monitoring.

10:20am Assessing Hemostasis and Platelet Function using a Microfluidic Device Lined with Fixed Human Endothelium

Technical Presentation. NEMB2016-6037

***Abhishek Jain, Andries D. van der Meer, Anne-Laure Papa, Riccardo Barrile**, Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA, United States, **Angela Lai**, Carnegie Mellon University, Pittsburgh, PA, United States, **Benjamin Schlechter**, Beth Israel Deaconess Medical Center at Harvard Medical School, Boston, MA, United States, **Monicah A. Otieno, Calvert S. Louden**, Janssen Pharmaceutical Research and Development, Pre-Clinical Development and Safety,, Spring House, PA, United States, **Geraldine A. Hamilton**, Emulate Bio Inc., Cambridge, MA, United States, **Alan D. Michelson, Andrew L. Frelinger III**, Boston Children’s Hospital at Harvard Medical School, Boston, MA, United States, **Donald E. Ingber**, Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA, United States*

Mutual signaling between inflamed endothelium and activated platelets is widely recognized as critical for regulation of hemostasis, initiation of platelet aggregation and thrombotic disorders associated with various diseases, including atherosclerosis, sepsis, and diabetes. Yet no reliable and practical diagnostic assays exist that can measure the effects of cross-talk between platelets and inflamed vessel walls in the presence of physiological shear. Over the last decade or so, multiple flow chambers and microfluidic devices that contain microchannels with a physiologically-relevant size have been lined by living endothelium and exposed to flowing blood to study the basic science, but they have not been used in clinical diagnostic settings due to the difficulty in maintaining living endothelial cells in devices. Specifically, because it is extremely difficult to maintain the stability, sterility and storage of living cell cultures for extended times in non-controlled settings, it would be virtually impossible to maintain the robustness, sensitivity and standardization of these assays.

Here, we describe a simple microfluidic device lined by a chemically preserved human endothelium that retains its ability to support thrombus formation and platelet adhesion as blood flows through its channels at an arterial shear rate. We demonstrate the potential clinical value of this chemopreserved endothelialized device by showing that thrombus formation and platelet function can be measured within minutes using a small volume (0.5 mL) of whole blood taken from subjects receiving antiplatelet medications, and demonstrating potentially greater reliability than standard platelet function tests and collagen-coated perfusion chambers.

We first coated the inner surface of a rectangular microfluidic device with type I collagen and then cultured human umbilical vein endothelial cells (HUVECs) on all four walls of the channel to create a rectangular tube lined by a continuous, confluent endothelial cell monolayer. When we activated the endothelium in the device by adding increasing doses of TNF-α (0, 5, and 100 ng/ml) and fixed the cells with 4% paraformaldehyde, we found that the fixed endothelium continued to exhibit a dose-dependent increase in intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1), as well as von Willebrand factor (VWF) and tissue factor (TF). These results confirm that the fixed endothelium retains expression of multiple molecules that mediate adhesion of blood cells and platelets after activation with TNF-α. When blood from a healthy donor was flowed over an endothelium that was fixed without prior treatment with TNF-α, there was virtually no platelet adhesion on the surface, as would be expected for a healthy endothelium. In contrast, when devices were used with endothelium that was treated with increasing doses of TNF-α prior to fixation, a dose-dependent increase in platelet surface adhesion to the endothelial layer was observed. Importantly, we did not observe any significant difference in platelet adhesion when we compared living versus chemopreserved endothelium and the morphology of thrombi appeared similar to that of thrombi formed on living endothelium in vivo. Thus, these findings confirm that the fixed surface of the endothelium retains its pro-thrombotic function after fixation and it is capable of reproducing physiological thrombosis in our microfluidic device.

To establish clinical utility, we also perfused whole blood of patients who were regular users of antiplatelet drugs, including aspirin or both aspirin and clopidogrel. We found that these subjects showed a significant reduction in platelet aggregation when tested using our devices lined by a fixed endo-

thelium compared to healthy donors. In contrast, conventional aggregometry analysis was very time consuming and tedious. Also, on a collagen-coated flow chamber we found no significant difference in platelet coverage between normal controls and samples from subjects on antiplatelet agents.

Taken together, this study using clinical blood samples demonstrates that our microfluidic device containing fixed endothelium could potentially become an accurate, rapid and reliable point-of-care platelet function assay. Because human endothelial cells are chemically preserved by fixation, it can also be stored, shipped and used when required, either in a laboratory setting or in the clinic.

10:40am Design and evaluation of a mobile nucleic acid amplification testing system in a hospital emergency setting

Technical Presentation. NEMB2016-6089

***Dong Jin Shin, Tza-huei Wang, Pornpat Athamanolap, Liben Chen**, Johns Hopkins University, Baltimore, MD, United States*

Integration of sample processing and detection is an essential feature in the delivery of molecular assays under POC settings. However, an often overlooked pitfall of integration is that it increases the complexity of the instrument and disposable components, which gives rise to sophisticated but expensive devices or instruments whose portability and affordability are compromised. Here we present a fully mobile platform for nucleic acid amplification test (NAAT) utilizing a droplet magnetofluidic cartridge. We evaluated our platform for testing urogenital chlamydia infection from patients in the hospital emergency department, with results in agreement with the clinical laboratory-based NAAT.

Our system consists of three components: a droplet microfluidic cartridge, a rechargeable lithium polymer battery-powered mobile instrument for cartridge processing, and a smartphone app for user interface, data acquisition and user training. The microfluidic cartridge design is based on the principle of open-surface magnetofluidic manipulation, which enables bioassays requiring multiple buffer exchanges using magnetic particle-based analyte transport and obviates the need for precise microfabrication or pneumatic controls such as pumps and valves. We developed a novel design and fabrication process for a sealed magnetofluidic cartridge using poly-methylmethacrylate (PMMA) as the base material and polytetrafluoroethylene (PTFE) film for hydrophobic surface generation. The cartridge utilizes the adhesive force of aqueous buffers on hydrophilic surfaces in order to form discrete buffer reservoirs that are required for DNA extraction and amplification, which is traversed by magnetic particles on the smooth PTFE surface at each buffer exchange step of the assay. Each cartridge is less than $2 in material cost using off-the-shelf reagents and materials, which compares favorably with the current cost of NAAT (US$33.48). The mobile instrument is an integrated electromechanical device that performs magnetic bead manipulation, thermal incubation, optical signal processing and wireless communication to the smartphone interface. Microcontroller-mediated control of an RC servomotor along a pre-programmed routine facilitates bead washing and transport. Alternating the top and bottom magnet on the same position of the cartridge causes the magnetic particles to traverse the two surfaces, facilitating efficient particle mixing. Thermal incubation is facilitated by a PID-controlled thermoelectric element driven using microcontroller-mediated output from an H-bridge amplifier. Passive optical components facilitate routing of fluorescence signal from amplification reaction to a smartphone CMOS sensor for detection. Bluetooth-based communication facilitates integration of the peripheral sensor-actuator modules into the smartphone user interface. The entire workflow takes approximately 1 hour to complete.

Urogenital chlamydia was selected as the focus of this study based on a needs assessment for POC tests among clinicians and opinion leaders. Fluorescent LAMP assay incorporating bead-based DNA extraction was initially developed and characterized, yielding analytical sensitivity of 102-103 genomic copies per test and no cross-reactivity with normal vaginal flora. Afterwards, platform validation was performed using an archived set of 20 vaginal swabs with results in agreement with the standard NAAT. The platform was subsequently evaluated in the Emergency Department at Johns Hopkins Hospital in Baltimore, USA. Volunteers for the study were recruited from a set of patients visiting for pelvic examination in the emergency room. On-site staff using the instrument for the first time was trained using the tutorial resource accessed via the smartphone application. Samples from each patient were tested in parallel by the platform and the gold standard NAAT assay for verification. We correctly identified 2 positives among 30 patients, demonstrating comparable performance to the gold standard for the samples tested. Evaluation by the on-site staff using a subset of the samples also yielded results in agreement with the standard.

Comparative effectiveness studies attest to the importance of cost and sensitivity as decisive parameters for patients to seek POC over standard NAAT. In particular, POC tests offer important strategies to address the Chlamydia epidemic, since diagnosis and immediate treatment can prevent transmission to sexual partners. By reducing turnaround for NAAT from days to an hour, our work demonstrates a paradigm-changing approach for making affordable and sensitive diagnostic tests more readily accessible outside centralized diagnostic workflow.

TRACK 4 NANO-PHENOMENA IN LIVING SYSTEMS

MONDAY, FEBRUARY, 22

4-1
PHYSICAL PROCESSES AND MECHANICS

Bexar/Travis 9:30am - 11:00am

Session Organizer: **Sulin Zhang**, *Penn State Univ, University Park, PA, United States*

9:30am Fluctuations in in-vitro experiments of cells on substrates

Keynote. NEMB2016-5950

***Vikram Deshpande**, Cambridge Univ, Cambridge Cb21pz, United Kingdom*

Complex bio-chemical processes attempt to maintain constant time-averaged concentrations of a range of proteins within cells in a process commonly referred to as cellular homeostasis. These chemical processes typically cause fluctuations of the state of cells that depends on the extra-cellular environment. We present a statistical mechanics view to model these fluctuations. First the probability of observing a cell in a particular state is estimated in terms of the free-energy of that state using the basic idea of Gibbs entropy. Next a model is presented to estimate this free-energy. The model includes stress-fiber reorganisation and the associated contractility by considering the energetics of the actin/myosin functional units that constitute the stress-fibers. This model then used to elucidate the range of states over which the cell can fluctuate in a particular environment and the probability of observing each of those states. Finally predictions are presented for a range of experimentally observed phenomena using this approach. This includes: (i) the spreading of cells as a function of the stiffness and the substrate; (ii) durotaxis whereby cells tends to migrate guided by rigidity gradients on substrates and (iii) differentiation of stem cells guided by the stiffness of substrates.

10:00am Coarse-Grained Modeling of Cell Responses to Active Rotational Nanoparticle

Technical Presentation. NEMB2016-5931

***Xianqiao Wang, Liuyang Zhang**, University of Georgia, Athens, GA, United States*

Recent years have witnessed the explosive growth of interest in nanoparticles (NPs) with a wealth of biomedical applications since they are widely used as carriers to translocate drug molecules and useful materials into cell interiors. A number of simulation suggested that the membrane translocation of anisotropic NPs is often accompanied by spontaneous and continuous rotation of the NPs. Take the spontaneous rotation for example, the ligand coated NPs with anisotropic patterns rotated to preferred orientation while penetrating through the membrane. Similar behavior was observed for the translocation of graphene sheets across the membrane. In the endocytosis process of anisotropic NPs, the anisotropic NPs generally undergo a transient rotation during the wrapping process to minimize the free energy. Different from the limited effect of spontaneous rotation of anisotropic NPs, the promoting effect of continuous NP rotation is ascribed to the enhanced membrane monolayer protrusion as well as exerts a shearing force to rupture the membrane. However, the fundamental mechanism of how a vesicle responds to the active RNP remains poorly understood.
On one hand, here we perform dissipative particle dynamics simulations to analyze the rotation frequencies, size, and coating patterns of the RNTs as they interact with the vesicle so as to provide novel designs of drug delivery applications. Results have revealed that the RNTs are capable to trigger local disturbance around the vesicle and therefore promote the vesicle translocation toward the RNTs. By investigating the translocation time and driving forces required for RNTs to enter inside of the vesicle under various rotation frequencies as well as the interaction energy between coated RNTs and the vesicle, we have tuned the coating pattern of the ligands on the surface of RNTs to open a specified channel in the vesicle for promoting the drug delivery. Our findings can provide useful guidelines for the molecular design of patterned RNTs for controllable bio/inorganic interfaces and help establish qualitative rules for the organization and optimization of ligands on the surface of desired drug delivery carriers.
On the other hand, cell-to-cell communications via the tunneling nanotubes or gap junction channels are vital for the development and maintenance of multicellular organisms. Instead of these intrinsic communication pathways, how to design artificial communication channels between cells remains a challenging but interesting problem. Here we perform dissipative particle dynamics simulations to analyze the interaction between rotational nanotubes (RNTs) and vesicles so as to provide a novel design mechanism for cell-to-cell communication. Simulation results have demonstrated that the RNTs are capable of generating local disturbance and promote vesicle translocation toward the RNTs. Through ligand pattern designing on the RNTs, we can find a suitable nanotube candidate with a specific ligand coating pattern for forming the RNT-vesicle network. The results also show that a RNT can act as a bridged channel between vesicles which facilitates substance transfer. Our findings provide useful guidelines for the molecular design of patterned RNTs for creating a synthetic channel between cells.

10:20am Biophysical insights into the architecture of the nuclear envelope

Technical Presentation. NEMB2016-6076

***Mehdi Torbati**, University of Houston, Houston, TX, United States, **Tanmay Lele**, University of Florida, Gainesville, FL, United States, **Ashutosh Agrawal**, University of Houston, Houston, TX, United States*

The nuclear envelope is a barrier that surrounds the nucleus and consists of the inner nuclear membrane (INM) and the outer nuclear membrane (ONM). These two bilayers are uniformly spaced and fuse at the sites of nuclear pore complexes (NPC). How the spacing between the two bilayers and the distance between nuclear pores are maintained, are however, not yet understood. Furthermore, the mechanism of pore formation and distribution of NPCs in nuclear envelope has not yet been investigated.

In this paper we study the effects of different mechanisms, including distributed loads on the membrane due to LINC complexes, protein-induced spontaneous curvature, and the interplay between equilibrium in-plane tension and transmembrane pressure, on the architecture of the nuclear envelope. Among all these possible mechanisms, our study reveals that the resting tension of the membrane and the distributed load acting over the ONM due to cytoskeleton are the main mechanical factors responsible for maintenance of the nuclear envelope architecture. Our data also suggests a simple mechanism for formation of new pores regulated by the interplay between the resting tension and the external pressure of ~10-100 Pa on the ONM.

10:40am Mechanical stress directs metastatic-like dispersion and malignant transformation in cancerous microtissues

Technical Presentation. NEMB2016-6018

***Sulin Zhang**, Penn State Univ, University Park, PA, United States, **Yao Zhang**, Pennsylvania State University, University Park, PA, United States*

Metastasis, the spread of cancer cells from the primary tumor to invade distant tissues and organs, is the leading cause of cancer mortality. Yet the biophysical mechanisms regulating cancer metastasis have remained poorly

understood. Here we reveal that cell traction force exerted on extracellular matrix directs and signals metastatic-like dispersion and malignant transformation of cancerous HCT-8 cell colonies in vitro. Our cell culture studies on gels evidence that the initiation of metastatic-like dispersion of HCT-8 colonies depends on both gel stiffness and colony size: dispersion occurs for cell colonies on stiff gels but not on soft ones; on stiff gels smaller colonies disperse statistically earlier than larger ones. Traction force microscopy studies show that the traction force is inherent to the gel stiffness and colony size, indicating that there exists a traction force threshold at the pre-dispersion stage above which the cell colonies disperse into individual malignant cells and below which the cell colonies remain cohesive. The finding underscores the importance of cellular forces for regulating cancer metastasis and progression of malignancy of cells, and opens up a unique avenue for the development of anti-metastasis therapies.

4-3: TISSUE MECHANICS

Harris 11:30am - 1:00pm

Session Organizer: **Xianqiao Wang**, *University of Georgia, Athens, GA, United States*

11:30am Non-linear elasticity and relaxation in polymer networks and soft tissues

Keynote. NEMB2016-5939

***Paul Janmey**, Univ Of Pennsylvania, Philadelphia, PA, United States*

The stiffness of tissues in which cells are embedded has effects on cell structure and function that can act independently of or override chemical stimuli. Most measurements of tissue stiffness report elastic moduli measured at a single frequency and at a low strain, but tissues and the cells within them are subjected strains that often exceed the range of linear viscoelasticity. Rheologic measurements of liver, brain, and adipose tissues over a range of shear, compressive, and elongational strains show that the viscoelastic response of these tissues differs from that of synthetic hydrogels that have similar elastic moduli when measured in the linear range. The shear moduli of soft tissues generally decrease with increasing shear or elongational strain, but they strongly increase under uniaxial compression. This response requires contributions from both cells and the extracellular matrix. In contrast, networks of ECM constructs such as crosslinked collagen or fibrin soften under compression, but strongly increase shear modulus when deformed in extension. The mechanisms leading to the unusual strain-dependent rheology of soft tissues and fibrous networks are not explained by current models of polymer mechanics, but appear to relate to local and global volume conservation within the networks and tissues.

12:00pm The Role Of Fibrin Cross-Linking On Forces Among Endothelial Cells In Vasculogenesis

Technical Presentation. NEMB2016-6133

***Andrea Malandrino**, Massachusetts Institute of Technology / Institute for Bioengineering of Catalonia, Cambrige, MA, United States, **Michael Mak, Roger D. Kamm**, Massachusetts Institute of Technology, Cambridge, MA, United States*

Mechanics of soft fibrous matrices fundamentally relates to cell behavior in development and disease. Such matrices have important properties, such as strain-stiffening, or viscoelasticity, that emerge from their composition, dynamics, and structural organization. One of the key components in the structural organization is the covalent cross-linking of the fiber mesh. In the present work, we study how the cross-link density within a fibrin gel affects the 3D force exchanges and distant mechano-communication among endothelial cells. We use an in vitro model of vasculogenesis previously characterized in our lab (Whisler et al., 2014) and we run experiments with cells encapsulated in the fibrin matrix at two densities: (i) a cell density that facilitates vascular network formation, and (ii) a much lower density where vascular networks do no form. To systematically vary the cross-link density, we polymerize fibrin in the presence of different concentration of factor XIII, a well-known cross-linker for human fibrin gel. Such cross-linker variations encompass a physiologically relevant level and three progressively decreasing values. Fluorescent beads are also introduced and tethered in the fibrin mesh. Displacement data are obtained through tracking of beads from the stressed configuration to the relaxed, stress-free configuration. The latter is imposed by treatment with Cytochalasin D to inhibit cell contractility. A 3D displacement map is then extracted around the cells contained in each region of interest. These displacement maps evidence higher bulk displacement values after relaxing contractility as the cross-link density decreases. The mean of the bead displacements around single cells in the case of the lowest cross-link density fibrin was indeed about 30% higher than in the case of the highest cross-link density. However, in terms of vascular network formation, preliminary results indicate that the lowest cross-link density is not optimal, as vascular networks forms with fewer branches and the structure is more planar. This might suggest that gels with very low cross-linking do not provide enough support for cell protrusions and connections in early vasculogenesis. However, a deeper investigation on the strain that each cell feels is required to confirm that. We also plan Brownian dynamics simulation of 3D fiber networks (Mak et al., 2014) to interpret these results, particularly taking into account fiber nanomechanics and cross-linking, thus mimicking the fibrin gel microarchitecture. We explore the multiscale mechanical factors in the extracellular matrix that govern the propagation of force signals and regulate vascular tissue formation and maintenance.

Acknowledgements:
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References:

Whisler, J. A., Chen, M. B., and Kamm, R. D. (2014). Tissue Eng. Part C. Methods 20, 543–52.
Mak, M., Kamm, R. D., and Zaman, M. H. (2014). PLoS Comput. Biol. 10, e1003959.

12:20pm Mechanical Reinforcement of Proteins with Polymer Conjugation

Technical Presentation. NEMB2016-5995

***Elizabeth P. DeBenedictis, Elham Hamed, Sinan Keten**, Northwestern University, Evanston, IL, United States*

Many proteins are subject to mechanical stress in vivo, have load-bearing and load-sensing functions, and structures sensitive to external forces. Mechanical reinforcement of proteins is highly relevant to tissue engineering, immunology, biosensing, biomaterials, and drug delivery. Conjugating poly ethylene glycol (PEG) to peptides, also known as PEGylation, is proven to increase the thermodynamical stability of peptides, and has been successfully applied to prolong the lifetime of peptide-based vaccines and therapeutic agents. While it is known that protein structure and function can be altered by mechanical stress, whether PEGylation can reinforce proteins against mechanical unfolding remains to be ascertained. In this work, all-atomistic Molecular Dynamics simulations of alpha-helical peptides undergoing constant tensile forces to induce unfolding are employed to assess protein stability. With mechanical stresses ranging over an order of magnitude, our results show that PEGylated alpha-helices consistently require a longer time to unfold compared with their native counterparts, indicating that polymer conjugation provides a mechanical reinforcement effect. Three regimes can be distinguished for force-induced unfolding response of the alpha-helix: small, intermediate, and large force regimes, with the PEG stabilization effect being most pronounced for small forces. Our results illustrate that unfolding progresses with backbone H-bonds breaking, which is followed by the exposed polar groups forming H-bonds with the surrounding water molecules. In PEGylated peptides, the likelihood of hydrogen bond exchange between the backbone and surrounding water molecules is reduced, which leads to greater unfolding times. PEGylation is found to increase the unfolding time through two mechanisms. We see that first, the unfolding rate of a he-

lical segment is decreased through prolonged plateau regimes where the peptide helical content remains constant, and second, the proportion of re-folding to unfolding is increased. These mechanisms are achieved through a shielding effect imparted by the PEG chain surrounding the helix surface. This serves to prevent competing water molecules from replacing forcibly exposed backbone hydrogen bonds, evidenced by an increase in time required for irreversible breakage of backbone hydrogen bonds and formation of backbone-water hydrogen bonds. Furthermore, conformation and proximity of PEG to the helix surface can be tied to specific unfolding events, the proportion of which dictates overall unfolding time, indicating that methods to control PEG conformation such as controlling sequence distribution, conjugation site, or molecular weight are promising avenues for improving protein stability. Our findings open a new field of research by demonstrating the feasibility of improving peptide mechanical stability with conjugation. This provides a basis for future studies on optimizing conjugation location and chemistry to build novel biomolecules with tunable mechanical properties and tailored functionalities.

12:40pm Viscoelastic Properties of Dental Pulp Tissue for Biomaterial Development

Technical Presentation. NEMB2016-5925

***Burak Ozcan**, TOBB University of Economics and Technology, Ankara, Turkey, **Dilhan M. Kalyon**, Stevens Institute of Technology, Hoboken, NJ, United States, **Jian Zhou, Sahn G. Kim, Jeremy J. Mao**, Columbia University, New York, NY, United States, **Cevat Erisken**, TOBB University of Economics and Technology, Ankara, Turkey*

A critical step in biomaterial selection effort is the determination of material as well as biological properties of the target tissue. Previously, selection of biomaterials and carriers for dental pulp regeneration has based on empirical experience. However, effective tailoring of biomaterials for tissue regeneration strictly requires availability of properties of native tissues. The objective of this study is to, for the first time, characterize the linear viscoelastic material functions and compressive properties of dental pulp tissue obtained from miniature pig using small-amplitude oscillatory shear and uniaxial compression. These properties were also compared with the properties of select hydrogels (agarose, alginate and collagen), that are widely used as biometarials in tissue regeneration.

Pulp tissue is the only soft tissue in tooth, and serves primarily to maintain its own physiological functions as well as those of dentin through blood supply and nerves. It is a reservoir of multiple cell types including fibroblasts populated in a matrix of blood vessels and nerve endings. Extracellular matrix of dental pulp is also rich in terms of collagenous (mostly type 1 and 3) and non-collagenous (sulfates and proteoglycans) proteins [1]. The cells and the organic components of the dental pulp all together determine the structural and functional nature of pulp tissue, with collagen type I likely contributing to its biomechanical properties [2], and proteoglycans mostly contributing to its viscoelasticity [1]. Despite reported clinical success, endodontically treated teeth become de-vitalized and brittle, as well as susceptible to re-infections due to coronal leakage or microleakage [3]. If dental pulp can be regenerated, these complications may be avoided, and many teeth can be saved to function as native teeth.

The comparisons of the linear viscoelastic material functions of the native pulp tissue with those of the three hydrogels revealed the gel-like behavior of the pulp tissue over a relatively large range of time-scales, i.e., over the frequency range of 0.1-100 rps. At the constant gelation agent concentration of 2%, the dynamic properties, i.e., storage and loss moduli, and the tan delta, of the collagen-based gel approached those of the native tissue. Under uniaxial compression, the peak normal stresses and compressive moduli of the agarose gel were similar to those of the native tissue, while alginate and collagen exhibited significantly lower compressive properties.

Findings suggest that these properties of dental pulp tissue should provide valuable inputs for the selection of an appropriate biomaterial for dental pulp regeneration. In this regard, current study is expected to set a benchmark for the upcoming similar research studies as well as in the development of clinically feasible biomaterials.

[1] Goldberg M + Crit Rev Oral Biol Med 2004; [2] Gelse K + Adv Drug Deliv Rev 2003; [3] Yuan Z + Tissue Eng Part B Rev 2011

4-2
NEURAL PROCESSES

Navarro 9:30am - 11:00am

Session Organizer: **Ying Li**, *University of Connecticut, Storrs, CT, United States*

9:30am Memory under tension

Keynote. NEMB2016-6147

***Taher Saif**, University of Illinois At Urbana-Champaign, Urbana, IL, United States*

The most profound question in biology is how a cluster of neurons gives rise to memory, learning, and ultimately our consciousness and individuality. Over the last century, the working principles of neurons and their circuits have been looked at from a biochemical and electrical paradigm. Mechanical force played no role. In contrast to muscles and bones, neurons are not part of any load bearing or generation system. Over the last three decades, it has been observed that neurons in vitro generate force, and the direction of the growth cone advance depends on the force on the axons. But the link between neuronal force in vitro and neuronal functionality in vivo was not clear. During embryogenesis, growth cones of developing neurons form junctions with muscle or other neurons creating synapses. They cluster neurotransmitters, packed is small (50 nm) vesicles, at the presynaptic terminal of the synapse. When an action potential arrives at the synapse, neurotransmitters are released through exocytosis of some of the vesicles. These neurotransmitters excite the post-synaptic terminal. It is well understood that memory and learning in animals is mediated by neurotransmission at the synaptic junctions. The more a synapse is used, higher is the neurotransmission efficiency (plasticity), i.e., the junction "remembers" its use in the near past, and modifies accordingly. This usage dependent plasticity offers the basic mechanism of memory and learning. A central dogma in neuroscience is that, clustering of neurotransmitter vesicles is the result of a complex biochemical signaling process. We show, using embryonic Drosophila (fruit fly) nervous system, that mechanical tension in axons is essential for vesicle clustering. Axons of the motor neurons develop tension after forming the synapse by shortening. They maintain the rest tension actively, i.e., if the axon is slackened, it develops tension by shortening. On the other hand, axons relax by growing if tension is increased by mechanical stretching. In search of the origin of the tension, we employed a suite of cytoskeleton inhibitory drugs. We found that axons employ actin-myosin II machinery to generate tension. If tension is relaxed by severing the axon, vesicle clustering disappears, but reappears if tension is re-supplied to the severed axon. Vesicle clustering increases with increase in tension in axons applied mechanically. We propose a hypothesis that links axonal tension with vesicle clustering. Initial evidence supporting the hypothesis is presented. The study offers a new paradigm in understanding neurological diseases.

10:00am Axonal SK channels Revealed by Force Nanoscopy

Technical Presentation. NEMB2016-6019

***Krithika Abiraman, Anastasios Tzingounis, George Lykotrafitis**, University of Connecticut, Storrs, CT, United States*

Small-conductance calcium-activated potassium (SK) channels are a family of potassium channels that are voltage independent and are activated solely by intracellular calcium (Ca2+) through constitutively bound calmodulin. SK channels exist within microdomains of Ca2+ sources and mediate afterhyperpolarization and regulate neuronal excitability. The location and density of ion channels in neurons are important determinants of their functional and physiological impact. Although the three subtypes of SK channels, SK1, SK2 and SK3, have partially overlapping yet distinct distributions in the brain, SK2 subtype is the most abundant. SK2 channels reside primarily in dendrites with lower expression in the soma. However, it remains unclear if SK channels are present on neuronal axonal membranes.

In this study, we employed single molecule atomic force microscopy (AFM) combined with a natural toxin, to test for the presence of SK channels in axons. We exploited the selective block of SK channels by bee venom toxin, apamin, to detect SK channels on the axonal membrane. Single molecule AFM records force-distance curves of an approach/retract cycle of the apamin-functionalized AFM cantilever to/from the axonal surface. The unbinding forces obtained from the force-distance curves correspond to the presence of a SK channel. When 1μm2 scan areas along the axon was probed using an apamin-functionalized AFM cantilever, we observed mean unbinding forces of 20±8.0pN in about 5.6% of the sampled sites (n=6). In order to test whether the unbinding forces observed were indeed between apamin and SK channel unbinding, we repeated the experiment on cells pretreated with apamin. This would cause SK channels to be occupied by apamin, thereby becoming unavailable for detection by the AFM probe and presumably leading to lower frequency of unbinding forces. Indeed, we observed a significant decrease in the frequency in the unbinding forces (0.58%, n=3) when the cells were pretreated with apamin. Additionally, we transfected HEK cells with SK2-S (short SK2 channel splice isoform) channels and found mean binding forces of 28±5pN in 4.5% of the sampled sites. However, when the HEK cells were pretreated with apamin, the frequency of unbinding forces decreased to 0.3 %. These experiments demonstrate the specificity of AFM to detect SK channels.

Next, we tested the effect of cyclic adenosine monophosphate (cAMP) activated protein kinase A (PKA) on axonal SK channel surface expression as PKA controls SK channel expression in other neuronal compartments (soma and dendrites). When cells were pretreated with Rp-CAMPS (100μM for 30 min), a cAMP analog that prevents the activation of PKA by cAMP, we found a higher mean frequency of unbinding events (μ=20±9pN, surface density = 10.1%, n= 5). Similar increase in surface density was obtained when the cells were pretreated with PKA inhibitor KT5720 (1μM for 30 min). This indicates that cAMP-PKA regulates the expression of axonal SK channels.
Therefore, we show that apamin-sensitive SK channels reside on the axon and are under the control of cAMP-PKA.

10:20am Movement of a voltage sensor within a bilayer

Technical Presentation. NEMB2016-6078

Mehdi Torbati, Vikash Chaurasia, *University of Houston, Houston, TX, United States,* ***Kranthi Mandadapu***, *University of California, Berkeley, Berkeley, CA, United States,* ***Ashutosh Agrawal***, *University of Houston, Houston, TX, United States*

Neurons communicate via a traveling wave of electrical excitation that propagates down an axon at speeds in the range of 10-100 m/s. This electrical wave is triggered and regulated by opening and closing of voltage-gated ion channels. These channels have been traditionally believed to be sensitive to the local electrochemical environment. A growing number of experiments now conclusively show that lipid-associated mechanical stimuli can control the channel response. We develop a quantitative model to elucidate the interaction of a voltage sensor domain with it's neighboring lipids. We predict the impact of key lipid/protein properties on the transmembrane movement of the sensor.

10:40am 3D Bioprinting Conductive Nano Scaffold with Multi-walled Carbon Nanotube for Improved Nerve Regeneration

Technical Presentation. NEMB2016-5977

Se Jun Lee, *George Washington University, Washington, DC, United States,* ***Lijie Zhang***, *The George Washington University, Washington, DC, United States*

Neural defect resulted from various traumas and diseases, represents a critical clinical problem all over the world. Currently fully neural functional recovery is very difficult to achieve. Traditional surgical procedures such as nerve tissue graft may encourage and guide axonal regeneration between the ends of the severed axons and are effective for small nerve bundles with short gap distance. However, the repair of more complex defects with larger nerve gap remains problematic. In order to overcome these limitations, synthetic nerve scaffolds are being developed to mimic natural neural extracellular matrix that would encourage neuronal differentiation, growth and axon elongation across the gap. Nerve scaffolds can be fabricated by various methods. Amongst them, 3D printing techniques have drawn great interest because they can prepare scaffolds with highly controlled spatial architecture and complexity to meet the customized requirements. The main objective of this study is to create an innovative nerve scaffold with a biomimetic nano to micro architecture by integrating advanced 3D printing technique and conductive multi walled carbon nanotubes (MWCNTs). MWCNTs are widely investigated in neural interfacing applications due to its unique physical, chemical and electrical properties. Unlike other conductive polymer materials, their electrical conductivity remains high over a long period of time under harsh condition. It is expected that MWCNT scaffolds may allow the electrical stimulation and promote excitability of neurons. In addition, amine-functionalized MWCNTs will be used as the key component of our 3D printing inks and will add further controlled chemical signaling cues and mechanical strength to promote directed neural cell growth and differentiation for our printed constructs.

A series of mixture solutions of 0.01%, 0.025%, and 0.05% amine-functionalized MWCNTs and biocompatible poly (ethylene glycol) diacrylate (PEG-DA) hydrogel were successfully printed by our custom made stereolithograpy (SL) 3D bioprinter. SL printed scaffolds were designed as square pattern with small, medium, and large pores geometry (corresponding to 31%, 52%, 66% porosity) using computer aided design software. Our results shows that the MWCNTs were homogenously distributed inside the 3D printed scaffolds. Neural stem cells (NSCs, ATCC) were further seeded onto prewetted scaffold and evaluated for adhesion and proliferation study. 4 hour cell adhesion study showed the scaffolds with 52% porosity can significantly improve cell attachment compared to scaffolds with smaller pores. Then three more groups of scaffolds (52% porosity) with different concentration of MWCNTs were evaluated for proliferation study. Compared to any other groups, NSCs proliferate significantly on scaffolds with 0.01% MWCNTs after 5 day of culture. Through this study, amine-functionalized MWCNTs were effectively 3D bioprinted into a novel neural scaffold and greatly improved neural stem cell adhesion and proliferation, thus promising for future neural regeneration applications.

4-4
SELF-ASSEMBLY

Harris 4:00pm - 5:30pm

Session Organizer: **Sinan Keten**, *Northwestern University, Evanston, IL, United States*

4:00pm Understanding and Manipulating Nano-scale Self-assembling in Living Systems for Regenerative Medicine

Keynote. NEMB2016-5940
Mingjun Zhang, *Ohio State University, Columbus, OH, United States*

This talk will report results of our recent studies on understanding the underlying self-assembling processes in living systems used to generate unique nano-scale mechanical and photonic phenomena for realizing biological functions, including high-strength adhesive and fluorescent properties. Examples will be presented to illustrate how these processes can be manipulated to fabricate fluorescent peptide nanoparticles and tunable adhesive hydrogels for regenerative medicine.

4:30pm Designing Biological Interactions of DNA-Assembled Nanoparticle Superstructures

Technical Presentation. NEMB2016-5959

***Leo Chou**, Dana-Farber Cancer Institute, Boston, MA, United States, **Kyryl Zagorovsky, Vahid Raeesi, Warren Chan**, University of Toronto, Toronto, ON, Canada*

Nanoparticle drug delivery systems must overcome a series of physiological barriers to reach their sites-of-action. Although the in vivo behaviour of engineered nanomaterials can be tuned as a function of their physical parameters, design principles developed for one behavior often diverge from another. In other words, it is likely that the optimal size, shape, and surface chemistry of nanoparticles for traversing physiological barriers must evolve with their location and time within the body. To address this challenge, we explored the construction of dynamic nanoparticle systems, using DNA assembly as tool for prototyping. We postulated that DNA programmability provides stringent control over the structure and dynamics of nanomaterials for controlling their interactions with biological systems. Here, we describe how DNA-assembled nanoparticle superstructures interact with serum proteins, cells, and tissues as a function of their design, specifically in the context of drug delivery and discuss emerging strategies for therapeutic design.

Our study used spherical and rod-shaped metal nanoparticles of varying sizes (e.g. 3, 5, and 15 nm) as models. We functionalized these nanoparticles with oligonucleotides and poly(ethylene glycol) as ligands, and combined them in different combinations using complementary oligonucleotides linkers to form superstructures with distinct architectures. We characterized these materials using dynamic light scattering and electron microscopy, followed by a systematic assessment of their interactions with serum proteins, cells, and tissues. Superstructure interactions with serum were analyzed by polyacrylamide gel electrophoresis (PAGE); cellular uptake and biodistribution was measured by inductively-coupled atomic emission spectroscopy, and visualized by electron microscopy and whole-animal fluorescence imaging.

Our results show that DNA assembly can synthesize a large number of nanoparticle superstructures with controlled size, surface chemistry, and architectures. Architecture was especially important in determining the accessibility of ligands to nuclease degradation and recognition by macrophages. In essence, it determines what the biological system “sees” when encountering a superstructure nanomaterial. By controlling architecture and surface chemistry of the superstructures, we were able to engineer DNA-linked superstructures that are serum stable for up to 8 hours and resist macrophage uptake and sequestration. In vivo, these design optimizations improved superstructure accumulation within a tumor xenograft model, and permitted tumour-specific imaging contrast at 24 hours post-injection. We also investigated the use of DNA assembly to integrate photothermal gold nanorods and the chemotherapeutic doxorubicin into superstructures. This design strategy placed DNA melting and consequently superstructure integrity under optical control, thereby allowing light-triggered drug release and intracellular uptake. Overall, we showed that DNA assembly of dynamic nanoparticle systems permits emerging therapeutic design strategies as well as new ways to design their interactions with biological systems.

4:50pm Computational study on self-assembly of Au-Polyethylene glycol-lipid nanoparticles

Technical Presentation. NEMB2016-5988

***Ying Li, zhiqiang Shen**, University of Connecticut, Storrs, CT, United States*

Through nanomedicine, game-changing methods are emerging to deliver drug molecules directly to diseased areas. One of the most promising of these is the targeted delivery of drugs and imaging agents via drug carrier-based platforms. Such drug delivery systems can now be synthesized from a wide range of different materials, made in a number of different shapes, and coated with an array of different organic molecules, including ligands. If optimized, these systems can enhance the efficacy and specificity of delivery compared those of non-targeted systems. Up to date, there are several classes of nanoparticles (NPs) demonstrating the promising properties as therapeutic carriers, such as solid lipid NPs, liposomes, quantum dots, dendrimers and polymer micelles. By loading the drug molecules into these NPs, their bio-distribution, pharmacokinetics and toxicity can be dramatically improved, in comparison to their freely administrated counterparts. In this work, we have computationally designed a novel drug carrier, Au-Polyethylene glycol-lipid NP. This NP is formed by the self-assembly of the Au- Polyethylene glycol NPs, with random lipid molecules. Large scale coarse-grained molecular simulations have been performed to understand how the grafting density and molecular weight of tethered chains will affect the formed Au-Polyethylene glycol-lipid NPs. It has been found that the Au-Polyethylene glycol-lipid NPs could be more easily self-assembled when the grafting density and molecular weight of polyethylene glycol are enlarged and reduced, respectively. The phase diagram, in terms of the grafting density and molecular weight of tethered polyethylene glycol chains, is also computationally constructed, which could be used to guide experimental synthesis of these Au-Polyethylene glycol-lipid NPs. The endocytosis of Au-Polyethylene glycol-lipid NPs is further explored through molecular simulations and compared with liposomes.

5:10pm Exploring the Impact of Local Ligand Flexibility on B Lymphocyte Signaling via a Platform DNA Origami Nanostructure

Technical Presentation. NEMB2016-6008

***Christopher Lucas, Emily N. Briggs, Randy A. Patton, Molly Y. Mollica, John C. Byrd, Virginia M. Sanders**, The Ohio State University, Columbus, OH, United States, **Carlos Castro**, Ohio State university, Columbus, OH, United States*

Scaffolded DNA origami molecular self-assembly allows for the generation of two- and three-dimensional DNA nanostructures that may be functionalized in a highly specific manner with biological molecules to induce molecular and cellular responses that may be studied at the single cell scale. Previous work demonstrated that the level of intracellular signaling is dependent upon ligand proximity, an effect that may be controlled through the employment of DNA nanostructures. However, whether local flexibility of a specific ligand affects the level of intracellular signal generated within single B cells remains unexplored. Here we describe the generation of a Ligand Presentation Platform (LPP) DNA nanostructure that allows for precise control of the number, spatial arrangement, and local flexibility via the length of linker attaching the ligand to the platform. The ligand linker is a single-stranded biotinylated DNA oligo that extends from the nanostructure allowing for streptavidin and biotinylated anti-CD40 antibody attachment and precise control over ligand linker length. Well-formed LPP DNA nanostructures were confirmed via agarose gel electrophoresis, transmission electron microscopy, and atomic force microscopy. Effective streptavidin and biotin-labeled anti-CD40 antibody incorporation to LPP DNA nanostructures was assessed by fluorescent agarose gel electrophoresis and transmission electron microscopy. In addition, fluorescent microscopy experiments revealed that anti-CD40 functionalized LPP DNA nanostructures attached to the CH12.LX B cell surface in a specific manner relative to a species-/isotype-matched control antibody functionalized LPP nanostructure. Our initial functional experiments revealed that immobilization of an anti-CD40 antibody on a platform DNA nanostructure induced significant increases in NF-kappaB activation in single murine CH12.LX B cells relative to soluble antibodies at equal concentrations. Furthermore, the strength of signal induction of CD40-dependent

NF-kappaB activation in individual murine CH12.LX B cells was observed to vary inversely with respect to the ligand linker length suggesting that signal strength may depend on local flexibility of the receptor-ligand complex, which likely mediates the force transmitted between the two molecules. Together, these findings suggest that strength of intracellular signal induction depends on the local mechanical environment, which may provide a means to tune signal strength by employing nanostructures assembled by DNA origami. Our device could inspire novel designs used to study multiple intracellular signaling pathways in a variety of biological systems as well as enhance efficacy of antibody-mediated therapeutics. Funded in part by start-up funds provided to Dr. Castro by the Department of Mechanical and Aerospace Engineering at The Ohio State University and in part by a Specialized Center of Research from the Leukemia and Lymphoma Society, P50-CA140158.

TUESDAY, FEBRUARY, 23

4-5
NANO-DESIGN CONCEPTS

Hidalgo **9:30am - 11:00am**

Session Organizer: **Ashutosh Agrawal**, *University of Houston, Houston, TX, United States*

9:30am Meeting the Challenges of Adoptive Cell Immunotherapy with Protein Nanorings

Keynote. NEMB2016-6153

***Carston Wagner**, University of Minnesota, Minneapolis, MN, United States*

The recent development of chimeric antigen receptors (CARs) T-cells is one of the most exciting recent developments in anti-cancer therapy research. Typically, T-cells from a cancer patient are genetically engineered to express a single chain antibody (scFv)-CD3 fusion protein that can target a cancer cell surface biomarker. After re-introduction into the patient, CAR-expressing T-cells selectively eliminate the target cancer cells. While successful, the genetic engineering of cell surfaces is time consuming and irreversible. Furthermore, because there are no general non-invasive methods for monitoring the movement and location of modified T-cells in vivo, it is difficult to correlate the therapeutic effect of CAR T-cells with their biodistribution.

Our group has shown that two dihydrofolate reductase molecules (DHFR2) fused to single chain antibodies (scFv) or peptides can be engineered to spontaneously self-assemble upon the addition of the chemical dimerizer, bis-methotrexate (BisMTX), into either highly stable octavalent chemically self-assembled nanorings (CSANs). CSANs have been prepared with BisMTX containing a third arm, thus enabling it to be conjugated to oligonucleotides, fluorophores, radiolabels and drugs. Recently, we have prepared bispecific anti-CD3 CSANs that are capable of simultaneously targeting a cancer antigen and the pan T-cell antigen, CD3. The bispecific CSANs rapidly (min) and stably (days) bind to CD3 on T-cell membranes, thus forming chemically self-assembled prosthetic antigen receptor (PAR) T-cells. Upon incubation of the PAR T-cells with antigen positive cancer cells, rapid and selective cell killing was observed. A unique feature of our approach is the ability to remove the CSANs from the T-cells by incubation with the FDA-approved non-toxic antibiotic trimethoprim at clinically relevant concentrations, thus allowing us to deactivate the cells pharmacologically. In addition, we have demonstrated that the CSANs can be site specifically radiolabeled and used to prepare radiolabeled PAR T-cells enabling our ability to track the cells in vivo by PET/CT. We have also demonstrated that targeted radiolabeled CSANs can be used to image tumors. This lecture will discuss these and recent results of our application of nanotechnology to provide a non-genetic and reversible method for the monitoring and preparation of targeted T-cells that can be applied to the clinical development of anti-cancer immunotherapy.

10:00am Assessment of Exploiting Protein Corona for Active Targeting of Nanoparticles to Desired Cells

Technical Presentation. NEMB2016-6115

***Vahid Mirshafiee, Raehyun Kim**, University of Illinois at Urbana-Champaign, Urbana, IL, United States, **Soyun Park**, University of California, Berkeley, Berkeley, CA, United States, **Morteza Mahmoudi**, Stanford University School of Medicine, Stanford, CA, United States, **Mary Kraft**, University of Illinois at Urbana-Champaign, Urbana, IL, United States*

Upon exposure of nanoparticles (NPs) to the physiological environments, proteins and other biomolecules adsorb onto the nanoparticles' surfaces, and form a biomolecule layer, known as the "protein corona." Previous studies have shown that this biomolecule corona could significantly reduce the NPs' targeting efficiency. Here, we investigated whether the NP's surface could be engineered to promote the binding of proteins that have natural targeting capabilities, thereby forming a functional protein corona that could be utilized for actively targeting NPs to the desired cells. We selected the well-established opsonin-mediated phagocytosis of NPs as a simple model system to test the feasibility of this strategy. In order to promote binding of opsonins to the NPs upon exposure to the plasma proteins, NPs were first pre-coated with gamma-globulins, followed by incubation with human plasma for protein corona formation. Analysis of protein corona composition indicated that the protein corona of gamma-globulin pre-coated NPs was enriched with opsonins. Nonetheless, cellular uptake studies showed no significant difference in the uptake of pre-coated and uncoated NPs that had been exposed to human plasma by macrophages. Furthermore, our studies indicated the opsonins in the protein corona of pre-coated NPs were unable to interact with their binding partners, most likely because these opsonins were blocked by other plasma proteins in the corona. Thus, our study shows that the protein corona not only needs to be enriched with proteins that have natural targeting abilities, but their spatial orientations and availabilities must also be controlled in order to utilize them for active NP targeting.

10:20am Delineating the cellular uptake properties and intracellular Trafficking Pathway of Targeted Nanorods of Defined Aspect Ratios

Technical Presentation. NEMB2016-6152

***C.H. Jonathan Choi**, The Chinese University of Hong Kong, Shatin,Hong Kong*

The past decade has witnessed more investigations into the application of anisotropic nanoparticles as intracellular delivery carriers of therapeutic and imaging agents, yet their "bio-nano" interactions with the cell remain poorly elucidated.

In this work, we will systematically explore the effect of nanoparticle geometry on the mechanism for cellular uptake as well as the pathway of intracellular trafficking of anisotropic nanoparticles. For our model nanoparticle system, we have chosen gold nanorods due to their easily tunable aspect ratios. Coating gold nanorods with poly(ethylene glycol) (PEG) chains on their surface renders them biocompatible with living cells, supporting our subsequent analysis of their "bio-nano" interactions solely based on aspect ratio. To ascertain whether these geometry-based conclusions will still hold in the context of cellular targeting, we have also prepared gold nanorods that are surface-modified with DNA oligonucleotides, noting that, by literature precedent, three-dimensional DNA nanostructures can naturally target Class A scavenger receptors (SR-A) on the cell surface. By tracking how PEG-coated or DNA-coated gold nanorods interact with C166 cells (mouse endothelial cells with an appreciable expression level of SR-A), we will address two questions. (1) How do aspect ratio and receptor-mediated targeting collectively dictate the uptake of nanorods by the cell? (2) How do both parameters influence the movement of nanorods inside the cell?

Based on a purely geometric perspective, the degree of cellular uptake monotically decreases with the aspect ratio of PEG-coated, non-targeting nanorods. However, when active receptor-mediated targeting is enabled via the attachment of DNA oligonucleotides, shorter nanorods can enter the cell more readily than nanospheres and longer nanorods of the similar nanoparticle volume. After cellular entry, most nanorods, independent of aspect ratio, traffic from the early endosome to the late endosome. Moreover, nanorods of all aspect ratios will eventually reside in the late endosome but not the lysosome. Intriguingly, sorting of nanoparticles to the late endosome appears to be faster for longer nanorods than their shorter counterparts.

Our preliminary data will shed light on the effect of nanoparticle geometry on cellular uptake and intracellular trafficking. They may also point to important materials design rules for drug delivery carriers based on anisotropic nanoparticles.

10:40am Can rigid proteins make a membrane softer? — the curious case of HIV induced membrane softening

Technical Presentation. NEMB2016-6011

***Himani Agrawal**, University of Houston, Houston, TX, United States, **Liping Liu**, Rutgers University, New Brunswick, NJ, United States, **Pradeep Sharma**, University of Houston, Houston, TX, United States*

A key step in the HIV infection process is the fusion of the virion membrane with the target cell membrane and the concomitant transfer of the viral RNA. Experimental evidence appears to suggest that the fusion is preceded by considerable elastic softening and thinning of the cell membranes and the formation of well-defined pores. What are the precise mechanisms underpinning the elastic softening of the membrane upon peptide insertion? A clear understanding of this could potentially pave the way for intelligent drug design to combat the epidemic caused by this deadly virus. State-of-the-art experiments to understand the HIV peptide insertion with T-cell membranes have been conducted recently. Using diffuse X-ray scattering, they deduced the bending modulus of the membranes upon HIV fusion peptide addition. Depending on the type of membrane, they found that the bending modulus (i.e. the property which dictates how resistant a membrane is to mechanical bending) can reduce between 3-13 times! This enormous mechanical softening greatly facilitates the subsequent fusion and infection process. While the experimental findings are quite interesting, very little atomistic insights were gleaned. In short, modeling or simulations are necessary to interpret the aforementioned experiments and then provide guidelines for computationally driven rationale drug design. Predicated on the hypothesis that understanding, at the atomistic level, the membrane softening due to HIV peptide insertion will enable counter-measures, we have conducted large-scale molecular dynamics simulations on the interaction between HIV fusion peptide and cell membrane. Such simulations require modeling millions of atoms that interact with each through a complicated set of forces. The dynamics of such an ensemble was then studied and interpreted. For example, although the experiments were able to measure the overall reduction in bending modulus of the membrane upon interaction with the HIV peptide—-the key physics lies in what is happening locally at the peptide-membrane insertion interface. What exactly happens there that causes an overall softening of the membrane? In principle, insertion of rigid proteins or peptide in membranes ought to stiffen the membrane not soften it thus rendering the experimental observations even more perplexing. To this end, we have devised a numerical “experiment” which involves (computationally) sticking a needle into the membrane region of interest. Through derived theoretical formulae, and observation of the response of the atoms in the simulation when subject to the needle probe, we estimated the elastic behavior of a small and local patch of the membrane as opposed to the entire membrane itself. This, and the direct observation of the atomic behavior, allowed us to understand precisely what occurs at the peptide-membrane interface.

4-6
CELLULAR INTERACTIONS

Bexar/Travis 11:30am - 1:10pm

Session Organizer: **Ashutosh Agrawal**, *University of Houston, Houston, TX, United States*

11:30am Mechanical Phase Transition of the Active Cytoskeleton

Technical Presentation. NEMB2016-6080

***Michael Mak**, Massachusetts Institute of Technology, Cambridge, MA, United States, **Taeyoon Kim**, Purdue University, West Lafayette, IN, United States, **Muhammad Zaman**, Boston University, Boston, MA, United States, **Roger Kamm**, Massachusetts Institute of Technology, Cambridge, MA, United States*

The motor-driven actin cytoskeleton enables cells to perform mechanical functions, such as migration, shape change, and force generation. These functions are important especially during development, when transient, pulsatile contractile events lead to the reorganization of tissues, e.g. Drosophila gastrulation, and in cancer, in which metastatic cells can generate enhanced contractile forces potentially leading to mechanical remodeling of the extracellular matrix and promoting invasion. While the key molecules constituting the cytoskeleton are known, it is less clear how large numbers of these components interact and integrate in an active network to enable diverse global morphologies and dynamic control of internal tension. Specifically, the cytoskeleton is capable of maintaining homogeneous morphologies while generating a steady-state prestress, but can also undergo phase separation and foci formation. It is not well known how these different global mechanical states emerge spontaneously from a solution of randomly mixed cytoskeletal proteins.

Through Brownian dynamics simulations, we demonstrate how vital nanoscopic features – the polymerization/depolymerization of actin filaments, walking and local contractility of myosin II motors, and force-sensitive unbinding of actin crosslinking proteins – regulate global mechanical states and drive nonequilibrium phase transition. In particular, our results show that actin turnover, which enables the cytoskeletal network to self-regenerate, is a master regulator of cell mechanics, from the precise tuning of tension to driving the morphological phase transition from homogeneous to clustered actomyosin networks. Myosin activity and crosslinker density shift the critical actin turnover rate required for phase transition. We present multidimensional phase maps revealing how the local biochemical reaction kinetics and concentrations of these basic cytoskeletal components enable the emergence of diverse structure and functionality in the cytoskeleton. Furthermore, we demonstrate that pulsations across phase transition enable much larger global contractile forces, which would otherwise disconnect the percolated cytoskeleton, to be generated over prolonged periods. Finally, we complement our computational results with live-cell imaging studies of the cytoskeleton and demonstrate the dynamics of reversible actin foci formation due to the disruption of actin polymerization dynamics.

11:30am Therapeutic Properties and Cellular Impact of pH-Responsive Hybrid Nanoparticles

Technical Presentation. NEMB2016-6060

***Alessandro Parodi, Claudia Corbo**, Houston Methodist Research Institute, Houston, TX, United States, **Micheal Evangelopoulos**, Houston Methodist Research Institute, Hosuton, TX, United States, **Ennio Tasciotti**, The Methodist Hospital Research Institute, Houston, TX, United States*

In order to be effective, a new generation of therapeutics (siRNA, miRNA, mmRNA) has to be delivered directly into the cell cytoplasm of the diseased target site. This goal can only be achieved only by a delivery platform that simultaneously accomplishes two tasks: its accumulation at the cancer lesion and the endolysosomal escape of its payload. In this study, we describe the fabrication protocols of a new drug delivery systems able to enhance the effects of siRNA based treatments. We developed a new one-pot synthesis protocol to fabricate hybrid nanoparticles formed by a nanostructured, inorganic silica core and an organic pH-responsive hydrogel shell. This easy-to-perform oil-in-water emulsion process synthesizes fluorescently-doped silica nanoparticles wrapped within a tunable coating of cationic poly(2-diethylaminoethyl methacrylate) hydrogel. Electron microscopy and dynamic light scattering analysis demonstrated that the nanoparticles were uniformly coated with the hydrogel and dispersed in the aqueous phase. Thermogravimetric analysis showed that the formation of covalent chemical bonds between the silica and the polymer increased the stability of the organic phase around the inorganic core. The cationic nature of the hydrogel was responsible for the pH buffering properties of the nanostructured system and this was assessed by titration experiments. Zeta-potential analysis showed that the charge of the system reversed when it passed from acidic to basic pH and vice versa. Consequently, small interfering RNA (siRNA) could be loaded and released in an acidic pH environment, enabling the hybrid particles and their payload to avoid endosomal sequestration and enzymatic degradation. These nanoparticles loaded with specific siRNA molecules directed towards the transcript of the membrane receptor CXCR4 significantly decreased the expression of this protein in the human breast cancer cell line MDA-MB-231. In addition, when siRNA-loaded nanoparticles were administered in vivo by intravenous injection in orthotopic mouse models of human breast cancer, they preferentially accumulated in the tumor where they efficiently silenced CXCR4. A full characterization of the response of endothelial cells to the endolysosomal escape showed that pH-responsive nanoparticles transiently affected key physiological cellular functions, such as the formation of capillary-like structures on matrigel. A full biomolecular analysis implemented with confocal microscopy was performed to understand the nature of this effect and discovered that the nanoparticles dispersed in the cytoplasm interfered with the physiological rearrangement of the actin structure. In response to the damages caused by the escape from the endolysosomal compartment, the cells produced multilaminar bodies surrounding and engulfing back the nanoparticles. The vesicles were able to first isolate the escaped nanoparticles from the other cellular compartments and then to clean the cytoplasm through exocytosis of the vesicles. In conclusion, the cytoplasmic dispersion of nanoparticulate can reveal some effects on cellular physiological function, even though our data demonstrate that, at least in vitro, they are transient due to the natural ability of the cells to recover from this kind of stress.

12:10pm Synthesis, fabrication and characterization of biocompatible conductive hydrogel composites

Technical Presentation. NEMB2016-6137

***Pranav Soman, Ping Dong, Yibo Wu**, Syracuse University, Syracuse, NY, United States*

Significance: Electrically conductive hydrogels have recently generated much attention, as they have significant potential to serve as bioactive scaffolds with the ability to electrically stimulate cells and modulate their functions. These smart materials not only possess the unique properties of hydrogels such as tissue-like mechanical properties, high water content and good biocompatibility, but also possess the properties of electrically conductive materials. However, one challenge still faced by nearly all electrically conductive hydrogels is their low processability, which limit their applications in tissue engineering and other biomedical engineering fields. In this work, we present the synthesis and fabrication of new conductive hydrogels by combining intrinsically conductive polyaniline (Pani) within two hydrogel matrices, namely poly (ethylene glycol) diacrylate (Pegda) and gelatin methacrylate (Gelma) hydrogel.

Methods: In this work, we synthesize photosensitive Pegda and Gelma hydrogels using established protocols. Pegda or Gelma precursor solution mixed with 0.25% Irgacure 2959 photoinitiator was injected into a Teflon rectangular mold and subsequently shone with UV light to prepare a solid crosslinked hydrogel samples. Hydrogels samples were subsequently immersed in 1M HCL solution containing different amounts of ammonium persulfate (APS) and hexane solution with aniline monomers, to obtain hydrogel-Pani composites. Fourier transform infrared spectroscopy (FTIR) was used to confirm the presence of aniline within the hydrogel matrix. Swelling ratio, compressive modulus and contact angle measurements were carried out. The biocompatibility and ability to adhere to composite samples, were tested by seeding C3H/10T1/2 murine mesenchymal progenitor cells (10T1/2s). Viability and morphology of adhered cells were quantified. Digital projection stereolithography, which consists of a UV light source, a digital light processing (DLP) chip and computer controlled stages (Newport 426/433 series) was used to print hexagonal microgeometries. Electrochemical Impedance Spectroscopy (EIS) was used to test the electrical properties of Pegda/Gelma-Pani composites using a three electrode system at a frequency range of 0.02-20k Hz and an AC perturbation of 10 mV, as applied by Solartron Analytical 1280Z working station. A custom-made direct current resistance measurement system composed of a Data Acquisition (DAQ) system and a planar gold measurement chip, was also used to quantify electrical properties of samples.

Results: Interfacial polymerization approach of fabricating conductive hydrogels provides marked improvement over the conventional approach of forming conductive hydrogels by mixing conductive particles within hydrogel matrix. Based on the swelling, compression and biocompatibility testing, the Pegda/Gelma-Pani composites are similar in properties to the pure biomimetic hydrogels, and therefore closer to the properties of in vivo extracellular matrix. This means that although more electro-conductive as compared to other biomaterials, hydrogel-Pani composites would elicit cellular responses similar to pure Gelma and other biomaterials of similar properties, and would serve as a suitable conductive scaffold. The presence of Pani within Pegda/Gelma matrix was verified using FTIR. Electrical properties of Gelma and Gelma-Pani samples were evaluated using Electrochemical Impedance Spectroscopy (EIS) and a custom-made resistance-test-chip. EIS results were plotted in the form of Nyquist and Bode Plots and fitted with a standard Randles cell (RC) equivalent circuit model. Compared to pure Pegda/Gelma, Pani composite samples exhibited lower impedance especially at physiologically-relevant low frequencies. Custom-made resistance testing chip also demonstrated a significant increase in conductivity for the Pegda/Gelma-Pani samples as compare to pure hydrogel controls. We also demonstrate that Pegda/Gelma-Pani composites with user-defined patterns can be fabricated. We used digital stereolithography to demonstrate that Gelma-Pani composite can be fabricated in complex user-defined geometries. This work can be potentially extended to fabricating variety of complex geometries using several photosensitive biopolymers for developing next-generation electrical biointerfaces.

12:30pm Dissecting the Cell-Nanoneedle Interface to Elucidate Intracellular Delivery of Payloads

Technical Presentation. NEMB2016-6056

***Jonathan Martinez**, Houston Methodist Research Institute, Houston, TX, United States, **Ciro Chiappini**, Imperial College London, London, United Kingdom, **Enrica De Rosa, Xuewu Liu**, Houston Methodist Research Institute, Houston, TX, United States, **Molly M. Stevens**, Imperial College London, London, United Kingdom, **Ennio Tasciotti**, The Methodist Hospital Research Institute, Houston, TX, United States*

Nanowire and nanoneedle-based approaches provide a facilitated access to the cytosol of the cell, and are able to interact with the intracellular environment by sensing their surrounding, capturing molecules and metabolites, transferring a payload or recording the electrical activity. However, the mechanism by which these materials achieve or induce cytosolic delivery (i.e., nanoinjection) remains unclear. Current evidence suggests that nanoneedles do not completely cross the plasma membrane but may only be tightly associated with its outer face. Hence, how cells and tissue respond

after nanoneedle insertion and the safety associated with this approach must be elucidated to further advance these platforms. Biological barriers serve to impede the delivery of biologics, for example: for nucleic acids to be effective, they must be shuttled in their active form to the cytosol or nucleus bypassing the cell membrane and endolysosomal compartment. Currently, the in vivo delivery of nucleic acids remains impaired by issues of safety, limited site accessibility, inadequate scalability, and inefficient transfection. The direct nanoinjection of nucleic acids into the cytosol of cells successfully addressed these issues. Nanoneedles did not induce any significant acute inflammation or alteration in the normal tissue architecture or cellular ultrastructure. Nanoinjection permits the delivery of a payload to a confined area, effectively impacting only a specific set of cells while avoiding areas of the tissue that are not of interest. This localized transfer of payloads enables to temporarily alter tissue structure and function by initiating biologically driven cellular processes (i.e., angiogenesis) in live tissues. Nanoporous silicon nanoneedles were produced using metal-assisted chemical etching to obtain needles with tip diameter of <50 nm, 5µm length, and 600nm base diameter. We investigated their interface with cells using electron and confocal microscopy and used quantum dot to illustrate the ability of nanoneedles to achieve cytosolic delivery. In addition, a payload of siRNA and DNA was used to demonstrate the efficient delivery of functional nucleic acids in vitro and in vivo. Microscopy techniques revealed how nanoneedles interacted with the cells and permitted a kinetic study to provide insight on how nanoneedles gained access to the cytosol of cells and induced the remodeling of the nuclear envelope. Furthermore, we compared the seeding of cells over a nanoneedles substrate with the forceful admini¬¬stration of nanoneedles to highlight the similarities and differences between the two interfacing strategies. In both cases the nanoneedles gained access to the cytosol but completed this process over different timescales, with forceful nanoinjection gaining access quicker. Using quantum dots as a model payload enabled the monitoring of the direct cytosolic delivery of nanoparticles within the cell. In addition, these nanoneedles allowed for the sensing of intracellular pH without inducing apoptosis demonstrating their successful negotiation of the endolysosomal system.
The delivery of nucleic acids was assessed using GAPDH siRNA, that was successfully delivered intracellularly by the nanoneedles, achieving a >>90% knockdown efficiency. Co-loading of Cy3-siRNA and a GFP DNA plasmid demonstrated that nanoneedles co-transfected cells nearly at 100% efficiency. In vivo studies demonstrated that nanoinjection yielded localized delivery of the payload to the superficial layer of various tissues. The nanoinjection of VEGF-165 triggered neovascularization and induced a localized 6-fold increase in blood perfusion exhibiting functional new vessel formation. In summary, nanoneedles represents a promising delivery strategy able to interface with cells and tissues with minimal toxicity to deliver sensitive bioactive payloads.

12:50pm Multidomain Particle Dynamics Toolkit HPC

Technical Presentation. NEMB2016-6024

Vi Q., Ha, George Lykotrafitis, *University of Connecticut, Storrs, CT, United States*

A large-scale, fast paced, lightweight, and easily customizable C++ particle dynamics engine designed to run on distributed-memory parallel computing clusters is introduced. It uses message passing interface (MPI) distributions for parallel computing operations and at the same time is intuitive and user friendly. It also employs a pointer system that can easily access all suitable components of the molecular dynamics (MD) engine for customization.
The program currently has three main sets of interaction domains. The first set is a spatial domain distributed accordingly to available processors for short-range MD simulations. The spatial decomposition utilizes the linked list method to build a very efficient cell list algorithm. The spatial decomposition algorithm allows extreme fast paced and efficient computations of large-scale MD simulations with interactive potentials within a cut off radius. However, the cell list method cannot be employed in interactions between particles separated at distances larger than the cut-off distance without losing computational efficiency. To address this problem we developed a second interaction domain, the global network domain. The global network represent particles as nodes and connects them via edges. This allows efficient interaction between particles regardless of their spatial position or the processors they reside in within the computing cluster. The third interaction domain that we introduce is the continuum space domain which allows communication between particles or between particles and their environment via diffusion or wave propagation. Currently the appropriate equations are solved by utilizing the explicit finite difference algorithm. The program allows particles from the network domain and spatial domain to interact via the continuum domain.

Users can develop their own customized interacting domains based on the three C++ domain classes. Programming employing these three domains is made user friendly as the computational environment provides classes to easily and efficiently access the interacting particles. These classes are designed to allow users to generate subclasses for specific problems. In addition, particles have properties that can adapt and be altered throughout the course of the simulation. The code is designed to accommodate complex particle behaviors while the particles can store their individual properties such as mass, size, and electrical charge.

The code can be used in numerous cases. The spatial domain allows users to simulate common short-range MD simulations. Users can also easily simulate more complex scenarios such as diffusion of cell membrane proteins. The network domain allows user to simulation long distance protein-protein interactions while the continuum space allows simulation of chemotaxis.

4-7
MOLECULAR INTERACTIONS AND SIMULATIONS

Hidalgo **4:00pm - 5:40pm**

Session Organizer: **Sinan Keten**, *Northwestern University, Evanston, IL, United States*

4:00pm Multiscale Modeling of Ebola Virus Glycoprotein

Technical Presentation. NEMB2016-6028

Ashley Guy, Alan Bowling, *University of Texas at Arlington, Arlington, TX, United States*

This work investigates the application of a multiscale method to the modeling of proteins in dynamical simulations. High-resolution protein simulations are defined at the nanoscale and integrated at the femtoscale. These nanoscale simulations infamously require significant computational resources to generate even modest time evolutions due to bottlenecks in the forward dynamics: point-wise potential calculations and evaluation of the equations of motion. The goal of the work is to reduce the computation time associated with solving the equations of motion.

An existing method to reduce computation time is to use the over-damped Langevin equation, which assumes a large damping ratio and eliminates the mass terms from the equations of motion. However, recent work has empirically observed underdamped behavior in nanoscale systems using optic tweezers, showing that the mass term may not be eliminated. Our method retains the mass terms while providing greater savings than the massless approximation.

Coarse graining methods reduce model resolution by eliminating small discrete bodies, like hydrogen ions, or grouping clusters of rigidly attached bodies into a single approximate body. These approaches simplify the model and allow for larger integration time steps. Our method builds upon a coarse graining approach to further reduce computation time.

The multiscale method used in this work scales the terms in the equations of motion and brings those terms into the same order of magnitude. The theoretical justification comes from a perturbation method showing that many of the active forces cancel. If two forces cancel without producing a net

moment, then those forces do not contribute to the dynamics and therefore may be eliminated from the model. The use of this method greatly reduces the forward dynamics computational burden without significant deviation in results. This method has previously been applied to dynamic simulations of motor proteins and estrogen molecules.

The model used in this work is the Ebola virus glycoprotein. This virally encoded glycoprotein is bound in the virion envelope and has been shown to play a pivotal role in the mechanism of infection. Simulations of the interaction of this protein with host cell receptors could greatly aid on-going research for effective treatments.

The glycoprotein model has full atomic resolution and is assembled piecewise from a user-input sequence of amino acids. Given the large number of degrees of freedom, the Featherstone algorithm is used to numerically solve the forward dynamics. This algorithm is ideal for biopolymer simulations as it precludes a symbolic model and is easily parallelizable. Active forces include electrostatic and Lennard-Jones interaction potentials, viscous fluidic damping, and stochastic Brownian motion arising from non-modeled collisions with the medium. This work is the first application of the multiscale method to a high-resolution protein model and a simulation using Featherstone algorithms.

4:20pm Adhesion Mechanisms of Curli Subunit CsgA

Technical Presentation. NEMB2016-5997

***Elizabeth P. DeBenedictis, Jenny Liu, Sinan Keten**, Northwestern University, Evanston, IL, United States*

Naturally occurring and making up a large portion of the world's biomass, biofilms are present in a number of industrial and medical applications. They comprise bacterial communities housed in a scaffold of proteins and polysaccharides, which along with water make a slime-like substance that is difficult to remove. Because of their strong adhesion and persistence, biofilms are often associated with pathogenicity and nuisance, yet possess enviable adhesive and survival properties. Curli is a specific fiber that grows on the surface of E. Coli and plays a structural role in its biofilms, where it has been shown to be critical for adhesion. Additionally, it has been shown that genetically engineered biofilms can be created to exploit the curli self-assembly process and to synthesize materials with molecularly precise features. Yet, so far there is no clear link between the sequence, chemistry, and topology of curli and resulting mechanical properties of their biofilms. Here, we investigate the adhesion mechanisms of CsgA, the beta-helical subunit that self-assembles into curli fibers. All-atomistic Molecular Dynamics simulations of the protein subunit adsorbing on hydrophobic and charged surfaces in explicit water solvent are employed to assess adhesion. Using these, we explore the connection between cooperative motion among residues, change in secondary structure, and surface contact. Groups of residues in the structure are seen to experience correlated changes in secondary structure and flexibility, which can guide mutation location choices to induce changes in subunit mechanical properties. While loss of secondary structure can result in reduced stiffness, it often occurs in protein regions in contact with the surface. We find adhesion to be mediated by different mechanisms: aromatic residues facilitate adhesion onto graphene, while negatively charged residues play a larger role in adhesion to silica. Additionally, rows of mutations are incorporated within the subunit, with various compositions and positions. These are investigated in regard to changes in structural dependencies between residues, adhesion energy, and alignment between aromatic mutations. These results can be applied to determine optimal mutation location and type for (1) enhancing adhesion and (2) facilitating pi-stacking for electron conduction and can be used as a guide for choices in genetically modifications. These findings lend insight into the mechanisms governing the impressive adhesive properties of curli, which can be applied to development of strong adhesives. Results concerning mutations set the stage for development of methods to enhance adhesion and use curli's self-assembling power to create ordered nanostructures and microbial nanowires.

4:40pm Computational characterization of materials response based on results of nanoindentation tests

Technical Presentation. NEMB2016-6134

***Taha Goudarzi, Nahil A. Sobh**, UIUC, Urbana, IL, United States*

The analytical tools at our disposal for characterizing the mechanical behavior of indented materials in a nanoindentation test with a spherical probe (usually suitable for soft/bio materials) are limited to the Hertz's contact solution and its extensions. To overcome this deficiency many researchers have used finite element simulations to model the nanoindentation tests.
To characterize the behavior of indented materials in an inverse manner using the finite element simulation, one needs to run the nanoindentation simulation with a guess for the material properties which are the unknown of the problem and then compare the simulation results with the experimental data to see how good of a guess has been made. The computational cost of such an approach has been an obstacle in development of inverse computational methods and tools for material characterization based on results of nanoindentation tests. We have developed an online software (https://nanohub.org/tools/nanoindentation) that can characterize the mechanical behavior of indented materials in an iterative approach using finite element simulations. In order to maintain the computational cost under a reasonable level very effective optimization methods have been utilized.
In this online tool that is the first to a series of tools focusing on more complicated problems (e.g. inelastic constitutive behavior and boundary conditions) the concentration is on the nanoindentation of isotropic elastic materials with spherical probes under arbitrary boundary conditions.

5:00pm Atomistic-to-continuum rod modeling of DNA mechanics

Technical Presentation. NEMB2016-5986

***Wonmuk Hwang, Xiaojing Teng**, Texas A&M University, College Station, TX, United States*

Mechanical properties of DNA is crucial for genome organization and interaction with proteins. A challenge in modeling DNA mechanics arises due to the co-existence of atomistic behaviors and mesoscale behavior of DNA as a chain molecule. For the latter, order parameters must be identified that properly describe elastic deformation of DNA. We apply theory of elastic rod to the atomistic molecular dynamics simulation of B-DNA oligos, to identify local principal axes of bending. To achieve this, we perform a total of 0.9 microsecond all-atom molecular dynamics simulation of DNA oligos possessing repeating sequences. For each base pair, we assign a local coordinate basis (triad) and follow its trajectory relative to the triad of the neighboring dinucleotide step. In this way, major and minor bending axes are identified, where the distributions of bending angles are well-approximated by Gaussian. For a given dinucleotide step (there are 10 possible dinucleotide steps formed by G, C, A, T), we calculate the four elastic stiffness of the major/minor bending, twist, and extension. DNA is the most compliant in major bending, and we find that traditional description based on helicoidal parameters do not adequately capture conformational fluctuation around the equilibrium conformation. This is because the 16 helicoidal parameters, although they describe atomic structure of DNA well, they are not based on principal axes of DNA. For any given oligo sequence, we can calculate the corresponding persistence length that is consistent with experimental measurement. Based on our calculated parameters, we build coarse-grained model of DNA that effectively captures its sequence-dependent elastic properties. The coarse-grained simulation of DNA oligos further reveals a finite size effect where the persistence length measured by following the end to end distance fluctuation and applying the wormlike chain model, is shorter than one for an ideal, infinitely long DNA that is calculated based on their bending stiffness and sequence composition. This is due to the nonzero intrinsic curvature. We also decompose elastic energy in 1381 x-ray structures of protein-DNA complexes. In most cases, DNAs are only mildly deformed with the total elastic energy less than two times the thermal energy. In this case, twist is the dominant mode of deformation. For structures with total elastic energy greater than about four times thermal energy, major bending becomes dominant. The present results elucidate the atomistic origin for the elastic behavior of DNA and how it is used for interaction with proteins. Our approach is applicable to DNAs in different conformational states as well as to other filamentous proteins.

5:20pm Instability-driven vesicle growth in high membrane tension environment

Technical Presentation. NEMB2016-6077

***Nikhil Walani, Jennifer Torres, Ashutosh Agrawal**, University of Houston, Houston, TX, United States*

Clathrin-mediated endocytosis (CME) is a key metabolic pathway that plays a central role in the delivery of nutrients and drug carriers into cells. In this work, we model the interactions of lipid membranes with different types of protein scaffolds and active forces to provide mechanistic insights into CME. To this end, we develop and employ an extended theoretical framework of lipid membranes that entertains spatial heterogeneity and local anisotropy that could arise from membrane-protein interactions. We show that a departure from homogeneity and isotropy can lead to a variable surface tension field, conventionally assumed to be a constant parameter. We model the impact of resting tension in a cell and discuss its consequences on the minimal protein machinery needed to complete vesicle formation. Based on our quantitative model and findings, we highlight the physical principles that unify CME in apparently distinct yeast and mammalian cells.

WEDNESDAY, FEBRUARY, 24

4-8
CELLULAR ENGINEERING

Harris 9:30am - 11:00am

Session Organizer: **Himani Agrawal**, *University of Houston, Houston, TX, United States*

9:30am Engineering programmable, dynamic materials using bio-inspired communication

Keynote. NEMB2016-5941

***Cheemeng Tan**, UC Davis, Davis, CA, United States*

Dynamic, bio-mimetic materials operate autonomously by sensing and adapting to their surrounding environment. Engineered to respond to a multitude of extracellular signals (e.g., proteases, pH, light), these materials generally react by releasing small molecules into their surroundings. While these state-of-the-art engineered materials can sense their environment, two-way communications, like those prevalent in natural systems, remain difficult due to the myriad of interactions inherent in cell-like environments. Here, we exploit synthetic biology approaches to develop the first modular dynamic material that can perform two-way communications with natural cells. The dynamic material, also called artificial cell, mimics several key properties of natural cells, including synthetic membranes, molecular transport, gene expression, and cell-cell communication. They are assembled from the bottom up using lipids, DNA, protein synthesis machineries, NTP, amino acids, and various accessory proteins and chemicals. We demonstrate cell-cell communication under three scenarios: artificial cells signaling bacteria, bacteria signaling artificial cells, and artificial cells signaling each other. To guide the control of the systems, mathematical models are developed to describe the genetic circuitry of each system as well as the spatial distribution of the transmitters, receivers, and the diffusing signal molecules. We illustrate potential applications of the artificial cells by implementing artificial cells that induce the synthesis of an antimicrobial peptide in bacteria. The antimicrobial peptide inhibits bacterial growth, providing a possible alternative strategy for the treatment of antibiotic-resistant bacteria. The development of communication between artificial cells and living cells provides further insight into cell communication in general, opens the door for new therapeutic uses of artificial cells, and expands the capacity of artificial systems to mimic living ones.

10:00am Tuning inflammation towards regeneration: a new strategy in tissue engineering.

Technical Presentation. NEMB2016-6046

***Francesca Taraballi**, Department of NanoMedicine, Houston Methodist Research Institute, Houston, TX, USA, Houston, TX, United States, **Bruna Corradetti**, Department of Life and Environmental Sciences, Universita' Politecnica delle Marche, Ancona, Italy, **Claudia Corbo, Laura Pandolfi**, houston Methodist Research Institute, Houston, TX, United States, **Silvia Minardi**, Department of NanoMedicine, Houston Methodist Research Institute, Houston, TX, USA, Houston, TX, United States, **Fernando Cabrera, Xin Wang**, Houston Methodist Institute Research, houston, TX, United States, **Jeff Van Eps**, Houston Methodist Research Inst., Houston, TX, United States, **Sebastian Powell**, Houston Methodist Institute Research, houston, TX, United States, **Bradley Weiner**, Houston Methodist Hospital, Houston, TX, United States, **Ennio Tasciotti**, The Methodist Hospital Research Institute, Houston, TX, United States*

The foreign body response can be described as a non-specific immune response to any implanted foreign materials. It is usually characterized by the massive infiltration of inflammatory cells (including macrophages and T cells) at the surface of the implant. Physiologically this process aims at the clearance of the foreign material, and at the regeneration of the damaged tissue. This inflammatory phase is also elicited by the implantation of any tissue engineered scaffold, and it could dramatically result in the failure of the implant. Although the foreign body reaction could appear detrimental, it could be cunningly exploited to trigger and boost the regeneration process towards functional recovery.

Conventional tissue engineering approaches have been developed to stimulate the stem cell niche towards healing. On the contrary, in this study, we focused on the ability to design and develop an immune-instructive scaffold for tissue engineering, able to tune the fate of inflammatory cells, and to achieve faster tissue remodeling. Using a biomimetic approach, we functionalized a porous collagen scaffold with chondroitin sulfate (CSCL), a glycosaminoglycan vastly present in the extracellular matrix of different tissues and known to have anti-inflammatory properties. The efficacy of such functionalization was assessed firstly in vitro, to elucidate the molecular mechanism in an in vitro model of inflammation, and then in a rat subcutaneous implant model by evaluating the effect of CSCL in tuning the inflammation. We characterized at a molecular, cellular and tissue level (qPCR, flow cytometry and immunofluroescence) the effects of this scaffold at early (1, 3 and 7 days) and late (21 days) time points in comparison with an unmodified collagen scaffold (CL). After 1 day, we found both scaffolds completely infiltrated by cells. We characterized the populations of the infiltrated cells by flow cytometry, finding that 95% of the total cells recruited by CSCL were macrophages, while in CL represented only the 40%. Moreover, the 90% of these cells was positive for anti-inflammatory associated markers, such as CD206 and IL-10. The same results were achieved with CL only 7 days after the implant. We further investigated by PCR arrays, which molecular pathways was activated by such cells, and we confirmed that cells recruited by CSCL expressed higher levels of chemokines and cytokines involved in the positive regulation of the immune system processes and chemotaxis. This massive activation was progressively turned off during the analyzed time points and at a faster rate for CSCL, rather than CL. The final outcome of these early events culminated in the differences found between treatment groups after 21 days. We demonstrated a marked reduction in the expression of markers associated to chronic inflammation (IL-6) and fibrosis (F13a1, Fga, Plat, and Plaur), together with an overexpression of the markers of vascularization and tissue remodeling in CSCL. Finally, through histological evaluation at 21 days, the scaffolds appeared fully integrated within the surrounding tissue, showing higher and homogenous level of vascularization, as anticipated by the molecular analysis at the earlier time points. Altogether, these data suggest that targeting the inflammatory phase induced by implanted biomaterials is a promising strategy to accelerate the tissue integration of the scaffold and achieve faster healing of the defect.

10:20am High Performance Diagnostic Platforms For Uncovering Micro/Nanoenvironmental Heterogeneity Within Aggressive Cancer Cells

Technical Presentation. NEMB2016-6121

***Mandana Veiseh**, Palo Alto Research Center (PARC, a Xerox Co), Palo Alto, CA, United States, **Abhishek Ramkumar, Felicia Linn**, Palo Alto Reserach Center (PARC, a Xerox Co), Palo Alto, CA, United States, **Jeng Ping Lu**, Palo Alto Research Center (PARC, a Xerox Company), Palo Alto, CA, United States*

Background: Metastatic cancer progression follows complex and heterogeneous molecular and structural changes in tissue architecture and function at multiple scales. While there is extensive literature describing how the transformation from health to malignancy alters the architecture of cells and their microenvironment, little is known about the role of heterogeneous cellular nanoenvironments in tumor aggression. This is partly due to infancy of the nano-scale architectural profiling strategies within proper three dimensional (3D) contexts and the increased detectable heterogeneity by high spatiotemporal resolution approaches. We are exploring spatiotemporal characteristics and physicochemical identities of solid breast cancer cells and their micro/nanoenvironments in relation to tumor aggression. Methods: High-performance live cell sensing, bio-microelectromechanical systems (Bio-MEMS) with tunable physicochemical properties for high resolution imaging, and multi-scale imaging (fluorescent, scanning electron and optical microscopy), and tumor microenvironmental probes are being used to monitor human cancer cells of different subtypes (e.g. basal vs. luminal breast cancers) under two and three dimensional (2D, 3D) culture conditions. Cancer cells grown in optically-compatible 2D or 3D MEMS (plain or patterned) are assessed for morphology, 3D structure, physicochemical signatures, nano-topography of cell surface and adjacent environments, tumor microenvironmental probe uptake, and growth. Results and conclusions: Breast cancer cells grown on optically thin gold substrates revealed distinct 3D phenotypic characteristics (including different forms and sizes of nano-scale surface domains and tendrils), and growth according to aggression state. Same cells (grown under 2D or 3D culture conditions) responded to distinct structural and chemical environments induced by opaque silicon-based MEMS. Cells formed stable adhesions and structures on both plain and patterned MEMS within 4 days, and exhibited distinct surface topologies, 3D structures, and growth over time. The spatiotemporal characteristics and functions differed in different tumor subtypes and within cell subpopulations of the same subtype. This study may enable development of new diagnostic platforms for identification of aggressive subpopulations within cancer cells. Such platforms may uncover a previously undetected composition or a new mode of action for metastatic cells and their nanoenvironments. With the long-term goal of deciphering and targeting nano-scale metastatic events within malignant breast and brain cancers, our work may impact both early cancer detection and prevention.

Keywords: Cell-based sensing, Heterogeneity, Bio-MEMS, Imaging, Tumor microenvironment, 3D cultures, Cancer, Metastasis.

10:40am Accurate and High-Coverage Immune Repertoire Sequencing Reveals Characteristics of Antibody Repertoire Diversification in Young Children with Malaria

Technical Presentation. NEMB2016-5989

***Ning Jiang**, The University of TX At Austin, Austin, TX, United States, **Di Wu, Mingjuan Qu, Ben Wendel, Chengfeng He**, University of Texas at Austin, Austin, TX, United States*

Accurately measuring the immune repertoire sequence composition, diversity, and abundance is important in studying repertoire response in infections, vaccinations, and cancer immunology. Using molecular identifiers (MIDs) to tag mRNA molecules is an effective method in improving the accuracy of immune repertoire sequencing (IR-seq). However, the lack of a general framework for designing, implementing, and performing quality control of IR-seq experiments using MIDs has prevented the wide use of this technology in small amount of clinical samples to achieve a high coverage of the repertoire diversities. This is especially challenging in studying infections and vaccinations where B cell subpopulations with fewer cells, such as memory B cells or plasmablasts, are often of great interest to study somatic mutation patterns and diversity changes. Here, we describe a generalized approach of IR-seq based on the use of MIDs in combination with a clustering method that can reveal more than 80% of the antibody diversity in a sample and can be applied to as few as 1,000 B cells. We applied this to study the antibody repertoires of young children before and during an acute malaria infection. We discovered unexpectedly high levels of somatic hypermutation (SHM). There appears a separation between infants who are younger than 12 months and toddlers who are older than 12 months based on SHM load, suggesting that the secondary diversification of the antibody repertoire may have a developmental threshold that is around 12 months. However, SHMs in both age groups are similarly selected and extensive clonal lineage structures are also similarly present in both age groups. These results highlights the vast potential of antibody repertoire diversification in young children that has not been realized previously, which would have a profound impact on immunization in children.

TRACK 5 NANOMATERIALS DESIGN AND MANUFACTURING

MONDAY, FEBRUARY, 22

5-1
MICRO- AND NANO-MATERIALS DESIGN FOR CONTROLLED RELEASE

Harris 9:30am - 11:00am

9:30am Light and magnetic field activated release of anticancer drugs

Keynote. NEMB2016-6162

***Fuyu Tamanoi**, UCLA, Los Angeles, CA, United States*

Controlled release of anticancer drugs in response to external stimuli such as light and magnetic field is one of the major goals of nanoparticle based drug delivery. When combined with tumor targeting of nanoparticles, this type of external control could provide precise spatial and temporal control over anticancer drug delivery. This could significantly reduce side effects associated with current chemotherapy and change the way cancer therapy is carried out. We have exploited relative stability of mesoporous silica nanoparticles (MSNs) to confer mechanisms for controlled release in response to external stimul. MSNs are prepared by sol-gel method and the use of surfactants results in the generation of a material with thousands of pores. We have shown that anticancer drugs can be stored in the pores and can be released. Safety and biocompatibility of the material have been evaluated in a number of animal experiments.

To achieve light activated drug release, we took advantage of azobenzene that changes conformation upon light exposure. Azobenzene is incorporated into MSNs by lining up the wall of the pores where anticancer drugs are loaded. In addition to azobenzene, we add fluorophore that can capture energy from two photon light source. The captured energy is then transferred to azobenzene causing release of anticancer drugs. Operation of this type of system was demonstrated in aqueous solution as well as in human breast cancer cells. A slightly different type of light activated release system uses azobenzene incorporated into nanovalve caps that are placed at the opening of the pores. Use of rotaxane or pseudorotaxane as nanovalves provides an open and close function for anticancer drugs.
For a system that responds to magnetic field, we have used MSNs that have iron oxide core. Because of superparamagnetic property of iron oxide, exposure to oscillating magnetic field results in increase of temperature of nanoparticles. We use the heat to open nanovalves that enable to release of anticancer drugs. Operation of this system was demonstrated in aqueous solution as well as in human cancer cells. In the cell experiment, cells with Doxorubicin containing MSNs taken up are placed in a magnetic coil that generates oscillating magnetic field. Release of doxorubicin inside the cell was confirmed by red fluorescence of this drug. In addition, cell killing due to doxorubicin was observed.

The above study was carried out as a long standing collaboration with Dr. Jeffery Zink (Dept. of Chemistry and Biochemistry, UCLA). The study may have implications for the design of medical equipment that can carry out therapy in response to light and magnetic field. It may be possible to modify endoscopy to detect tumor and expose to light. In addition, medical equipment to generate magnetic field could be envisioned in the future. Our development of novel nanoparticles that respond to external stimuli and release anticancer drug release may have impact on future clinical settings. These will be discussed.

10:00am Getting the most from droplet microfluidic platforms in nano/bio-materials synthesis

Technical Presentation. NEMB2016-5948

***Amy Shen**, Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan*

Microfluidics has emerged in recent years as a versatile method of manipulating fluids at small length-scales, and in particular, for generating and manipulating micron size droplets with controllable size and functionality. For example, many research groups developed microfluidics devices for cell encapsulation, and synthesizing functionalized polymer microspheres and inorganic nanoparticles with precise control over their shapes and sizes. In this talk, I will showcase droplet microfluidic platforms to highlight their versatility and potential applications in nano-materials synthesis.

(a) A droplet microfluidics method to fabricate alginate microspheres while simultaneously immobilizing anti-Mycobacterium tuberculosis complex IgY and anti-Escherichia coli IgG antibodies primarily on the porous alginate carriers for specific binding and binding affinity tests. The binding affinity of antibodies is directly measured by fluorescence intensity of stained target bacteria on the microspheres. We demonstrate that the functionalized alginate microspheres yield specificity comparable with an enzyme-linked immunosorbent assay. We can easily modify the size and shape of alginate microspheres, and increase the concentration of functionalized alginate microspheres to further enhance binding kinetics and enable multiplexing.

(b) Cell microencapsulation technology involves immobilization of the cells within a polymeric semi-permeable membrane that permits the diffusion of the molecules, such as the influx of oxygen, growth factors and so on for cells living, and the outward diffusion of waste products and useful proteins. Recently, microfluidic techniques have proved useful for cell encapsulation by using alginate droplets as the microcapsules. This approach preserves cell viability as well as monodispersity of the microcapsules. A novel droplet microfluidics method to image oxygen in single islets (pancreatic cells) for glucose sensing. Individual islets and a fluorescent oxygen-sensitive dye were encased within a thin alginate polymer microcapsule for insulin secretion monitoring. The sensing system operated similarly from 2-48 hours following encapsulation, and viability and function of the islets were not significantly affected by the encapsulation process. This approach should be applicable to other cell types and dyes sensitive to other biologically important molecules.

(c) Based on part (b), proof of concept studies of a cryopreserved microcapsule-based quality control assays are presented for single islets. Individual rat pancreatic islets and fluorescent oxygen-sensitive dye (FOSD) are encapsulated in alginate hydrogel microcapsules via a microfluidic device. To test the susceptibility of the microcapsules and the FOSD to cryopreservation, the islet microcapsules containing FOSD are cryopreserved and the islet functionalities (adenosine triphosphate, static insulin release measurement, and oxygen consumption rate) are assessed after freezing and thawing steps. The cryopreserved islet-capsules with FOSD remain functional after encapsulation and freezing/thawing procedures, validating a simple yet reliable individual-islet-based quality control method for the entire islet processing procedure prior to transplantation. This work also demonstrates that the functionality of cryopreserved islets can be improved by introducing trehalose into the routinely-used cryoprotectant dimethyl sulfoxide. The functionalized alginate hydrogel microcapsules with embedded FOSD and optimized cryopreservation protocol presented in this work serve as a versatile islet quality assay and offer tremendous promise for tackling existing challenges in islet transplantation procedures.

10:20am Tailor-made Polyurethane-based Multi-block Nanomaterials as Drug Delivery Systems

Technical Presentation. NEMB2016-5992

***Gianluca Ciardelli, Clara Mattu, Monica Boffito, Susanna Sartori**, Politecnico di Torino, Torino, Select State/Province, Italy*

In virtue of their high chemical versatility, Polyurethane (PUR)-based biomaterials represent a very promising alternative to traditional polymers as both, drug delivery nanovectors and tissue engineering supports. PUR synthesis is quite straightforward: it requires a first reaction between a diol and a diisocyanate to form a prepolymer, which is then reacted with a chain extender to obtain the polymer. A wide variety of starting reagents can be used to obtain PURs with the desired physical, mechanical and biological properties [1]. In my group a two-step synthesis procedure has been optimized and a wide library of PURs based on polyether (polyethyleneglycol, PEG or Pluronics) and polyester (poly(ε-caprolactone), PCL) blocks or a combination thereof (at varying PCL/PEG ratio) has been obtained. These polymers have been used to prepare nanoparticles, nano-micells, and injectable hydrogels-based drug delivery vectors for several active principles [2].

For instance, Pluronic-based polyurethane hydrogels with sol-gel thermal transition at desired temperature and concentration have been obtained. These polymers are readily soluble in water at low temperature and rapidly undergo a phase transition from sol to gel at body temperature through micellar aggregation. Moreover, their ability to sustain the release of both, hydrophilic and hydrophobic, drugs has been demonstrated.

The hydrophobic core of the micelles has been exploited to host hard-to-administer hydrophobic drugs, obtaining a drug reservoir for the treatment of localized pathologies. For instance, Curcumin, Resveratrol and Dexamethasone, 3 model hydrophobic drugs, as well as Ampicillin and Bovine Serum Albumin (BSA) as model hydrophilic drugs have been entrapped inside the hydrogel and their release kinetics have been studied. In all cases, the polymer formed a stable hydrogel in less than 5 min at 37 °C (body temperature) and was able to sustain the release of the hydrophobic drugs for up to 25 days, whereas the release of hydrophilic molecules was faster and completed in about 2 weeks. Moreover, BSA integrity was demonstrate by SDS-page analysis of the released protein, indicating no adverse interaction between the protein and the hydrogel. In addition, the PUR hydrogel could be extruded into continuous filaments able to host cells, opening the possibility of using PURs in bioprinting applications [3,4].

PURs with modulated hydrophilic/hydrophobic balance have also been proposed for the preparation nanoparticles for the controlled release of the anticancer drug Paclitaxel. The effect of the polyurethane composition on cellular internalization, drug release and nanoparticles properties has been studied.

For active cancer recognition, surface coating and surface functionalization strategies with the monoclonal antibody Trastuzumab have been implemented. In the first case, hydrophilic/hydrophobic interactions have been exploited to obtain antibody-coated PUR nanoparticles, whereas for surface coupling we took advantage of the presence of BOC-protected amino functionalities, previously inserted in the polyurethane backbone, to covalently couple the antibody to PUR nanoparticles. In both cases, high drug loading, sustained release and good selectivity towards the selected cell type has been achieved [5,6].

Our group has demonstrated that PURs hold a number of advantages over traditional polymers and that they are optimal candidates to build tailor-made nano-scale drug delivery systems.

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10:40am Design and Characterization of Biomimetic Vesicles Deriving from Leukocyte Plasma Membrane

Technical Presentation. NEMB2016-6055

***Roberto Molinaro, Claudia Corbo**, **Francesca Taraballi**, Houston Methodist Research Inst., Houston, TX, United States, **Silvia Minardi**, Department of NanoMedicine, Houston Methodist Research Institute, Houston, TX, USA, Houston, TX, United States, **Michael Evangelopoulos**, Houston Methodist Research Institute, Houston, TX, United States, **Michael Sherman**, Univ. of Texas Medical Branch, Galveston, TX, United States, **Kelly A Hartman, Alessandro Parodi**, Houston Methodist Research Institute, Houston, TX, United States, **Ennio Tasciotti**, The Methodist Hospital Research Institute, Houston, TX, United States*

To date, a multitude of micro- and nanocarriers have been developed to improve the systemic delivery of pharmaceuticals. All these carriers are subjected to a number of biological barriers that limit their optimal biodistribution, providing one of the main obstacles to an effective drug delivery. Bio-inspired approaches have been utilized as alternative treatments to evade the mononuclear phagocytic system and facilitate the transport across the endothelial vessel wall. In the past decades, bottom-up and top-down approaches have been developed to formulate these biomimetic vesicles. However, both approaches are not without their own set of limitations. In fact, although bottom-up approaches have the great advantage of providing superior physicochemical control over the final formulation, current chemical conjugation methods remain inadequate to synthetically reproduce the complexity of the cellular membrane on the surface of nanocarriers. On the other hand, top-down strategies lacked the control of the physical parameters (i.e. size, homogeneity), and a poor encapsulation and retention of payload molecules (i.e. hydrophilic, amphiphilic, and lipophilic small drugs).

Here, we describe the formulation of a biological material (the plasma membrane) into nano-sized vesicles. Our method combines both bottom-up and top-down approaches, to produce a biomimetic nanovesicle with original drug delivery properties. At the same time, we transferred some functional properties of the plasma membrane onto the synthetic nanoparticles. This new class of biomimetic nanoparticles were extensively characterized for both their physical (dynamic light scattering, cryoTEM, atomic force microscopy, Fourier-transform infrared spectroscopy, and differential scanning calorimetry), as well biological (protein composition and protein orientation and activity) properties. We leveraged on the high versatility of liposomal assembly methods, to formulate a highly standardized and stable product. Theoretical calculations were used to quantify the number of copies of selected proteins per particles and per surface area unit (μm^2). From the physical standpoint, the biomimetic formulation of the membrane proteins produced particles with homogeneous size (around 120 nm, with >>90% unilamellarity), as demonstrated by low magnification cryo-TEM and the polydispersity index (PDI) from DLS, and negative surface charge (-13.8 mV). High magnification cryo-TEM revealed that the incorporation of the membrane proteins produced a 1.3 fold-increase in bilayer thickness compared to bare liposomes. Additionally, the proteomic analysis showed that this method permitted the transfer of over 180 membrane-associated proteins to the particle's surface, conserving their orientation, post-transcriptional modifications, functions, and their cooperation with other proteins. To the best of our knowledge, this approach represents the first time such a complex material as the plasma membrane is formulated into a lipid vesicle, using a reproducible method, to exploit the incorporation of membrane proteins into a lipid bilayer.

TUESDAY, FEBRUARY, 23

5-2
NANOMATERIAL PROPERTIES AND SENSING APPLICATIONS

Harris 11:30am - 1:10pm

11:30am Polydispersity Of Gold Nanoparticles: A New Metric For Quantitative Photothermal Conversion In Biomedical Applications

Technical Presentation. NEMB2016-6109

***Zhenpeng Qin**, University of Texas at Dallas, Richardson, TX, United States, **Yiru Wang**, University of Minnesota, Minneapolis, MN, United States, **Jaona RANDRIANALISOA**, GRESPI University of Reims Champagne-Ardenne, Reims,France, **Vahid Raeesi, Warren Chan**, University of Toronto, Toronto, ON, Canada, **Wojciech Lipinski**, The Australian National University, Canberra,Australia, **John Bischof**, Univ Of Minnesota, Minneapolis, MN, United States*

Gold nanoparticles (GNPs, including sphere, rod, and shell etc.) are increasingly used for biomedical applications due to unique optical properties, established synthesis methods, and biological compatibility. Despite recent developments in using GNP plasmonic heating for thermal therapy, imaging, and diagnostic applications, the lack of quantification of the heat generation from plasmonic nanostructures has led to difficulties in predicting the therapeutic and diagnostic outcome and comparing the heat generating capability for new plasmonic nanostructures. In this study, we quantitatively measured the heat generation for GNPs, and examined the validity of theoretical predictions. Surprisingly, we showed that the agreement between experiment and theory is dependent on the type of GNP studied. Specifically, we found that the heat generation of gold nanospheres matches well with theoretical prediction, while the measured heat generation for gold nanorod significantly deviates from theory, with only one third of the value predicted from theory at peak absorption. This then led to a surprising finding that the polydispersity, i.e. the deviation of nanoparticle size and shape from nominal value, significantly influences the optical properties of gold nanorod including heat generation (>>70% reduction), but has very limited influence (<10%) on gold nanospheres. We further demonstrate the importance of accounting for the polydispersity by an example comparing the photothermal absorption for gold nanosphere and nanorod with similar volume, leading to more realistic predictions. This work suggests the significance of polydispersity in determining the plasmonic nanoparticle heat generation and provides a method to quantitatively compare the heating capability for new plasmonic nanostructures.

11:50am Synthesis and Evaluation of Polymer Dynamic Organic Spheres (PolyDOTS)

Technical Presentation. NEMB2016-5952

***Nicole Levi-Polyachenko**, Wake Forest School of Medicine, Winston-Salem, NC, United States, **Elizabeth Graham**, Wake Forest University health Sciences, Winston-Salem, NC, United States, **Sneha Kelkar**, Wake Forest School of Medicine, Winston-Salem, NC, United States, **Christopher MacNeill**, Wake Forest University health Sciences, Winston-Salem, NC, United States, **Eleanor E. McCabe**, Wake Forest School of Medicine, Winston-Salem, NC, United States*

Theranostic agents combine both a therapeutic agent and an imaging platform within the same material to simultaneously diagnose and treat diseases, such as cancer. This report describes the development of donor-acceptor polymers into aqueously stable spherical nanoparticles that have the capacity to act as unique theranostic agents. We term these particles, PolyDOTS, for polymer Dynamic Organic Theranostic Spheres. In this application, PolyDOTS were created using the donor-acceptor conjugated polymer, polycyclopentadithiophene benzoselenadiazole (PCPDTBSe), which has tunable optical properties correlating to the molecular weight of the polymer. Various molecular weight fractions were combined at specific ratios to develop a hybrid nanoparticle capable of both fluorescence imaging and heat generation upon exposure to light. PolyDOTS have an approximate hydrodynamic diameter of 90nm and stable fluorescence following multiple heating/ cooling cycles, as well as minimal changes in the optical properties after a 30day exposure to ambient light. Upon exposure to 800nm light, PolyDOTS generated a 42 degrees Celsius temperature increase above ambient temperature. PolyDOTS were evaluated as effective photothermal agents against breast epithelial cells. Minimal cytotoxic response was observed in the absence of infrared light. In the presence of 800nm light, PolyDOTS incubated with MDA MB 231 breast cancer and MCF10A non-tumorigenic cells generate significant heating, resulting in a 90% decrease in cell viability at concentrations of 50ug/ml and higher. Fluorescence microscopy revealed that PolyDOTS localized throughout the cell but were excluded from the nucleus, and no photobleaching occurred during prolonged imaging. These results demonstrate that PolyDOTS represent a new class of theranostic nanoparticles built from the platform of conjugated polymers by selectively combining fluorescent and heat generating polymers into one stable nanoparticle that shows no fluorescence quenching and excellent photothermal capacity.

12:10pm Dual-functional moiré metasurfaces for nanospectroscopy and phototherapy

Technical Presentation. NEMB2016-6027

***Zilong Wu, Yuebing Zheng**, The University of Texas at Austin, Austin, TX, United States*

Metasurfaces with moiré patterns, featured by a large number of component sets and high rotational symmetry, are promising for multi-functional applications. With the hierarchical arrangements of plasmonic nanostructures of variable shapes and sizes, moiré metasurfaces can support multiple plasmonic modes. We have developed high-throughput, cost-effective method known as moiré nanosphere lithography (MNSL) for the moiré metasurfaces with multiple and tunable plasmonic modes. Furthermore, we have designed a metal-insulator-metal (MIM) patch structure with a thin Au moiré metasurface layer and an optically thick Au layer separated by a dielectric spacer layer. Benefiting from the combination of moiré patterns and field enhancement from the MIM configuration, the moiré metasurface patch exhibits the strong absorption (>>95%) of incident light across a broad range of electromagnetic spectrum, i.e, near-infrared (NIR) range from 900 nm to 1700 nm and mid-infrared (MIR) range (~5 um). Due to the broadband absorption in the NIR and MIR range, the moiré metasurface patch is proposed as a dual-functional substrate for spectroscopy and phototherapy.

12:30pm Investigation Of The Use Of Ordered Nano-patterned Polycarbonate Films For Detecting Alzheimer`s Protein

Technical Presentation. NEMB2016-5985

***Sevde Altuntas, Fatih Buyukserin**, TOBB University of Economics and Technology, ANKARA,Turkey*

Today, Alzheimer's disease is significantly more pronounced and it is responsible for irreversible neural damage of cerebral cortex and hippocampus. It seems possible to develop early diagnostic tools to decrease or annihilate harmful effects of the disease which seems to originate from the Amyloid-β protein that accumulates in brain in the form plaques as dictated by several literature reports. The basic problem of such a diagnostic tool for clinical or R & D study is the low amount of protein that cannot be detected properly in body liquids such as blood, saliva or urine. To solve this problem, tests like ELISA or PCR are proposed which are expensive, require specialized personnel and can contain complex protocols.

Surface-enhanced Raman Spectroscopy (SERS) can potentially allow even

single molecule detection in solutions or solid surfaces. In addition, SERS signal from a target molecule can be further increased by using nano patterned surfaces when compared to smooth counterparts. Moreover, Raman signals are specific to molecules, so one can comment about content of a sample. In this context, our study proposes to fabricate diagnostic test models that utilize Au-coated nano patterned polycarbonate (PC) surfaces modified with Thioflavin - T to detect low concentrations of Amyloid-β protein in water and artificial saliva medium by the enhancement of protein SERS signal.

The nano patterned PC surface that was used to enhance SERS signal was fabricated by using Anodic Alumina Membranes (AAM) as template. It is possible to produce AAMs with different column structures and varying thicknesses depending on voltage and anodization time. After fabrication process, the pore diameter of AAMs can be arranged with dilute acid solution treatment. In this study, two different columns structures were prepared. After a surface modification to decrease their surface energy, AAMs were treated with PC solution. Following the solvent evaporation, nano patterned PC films with tunable pillared structures were peeled off from the membrane surface. The PC film was then modified with Au and Thioflavin-T for the detection of Amyloid-β protein. The protein detection studies were conducted first in water via this biosensor platform. Same measurements are currently being conducted in artificial saliva to detect the presence of Amyloid-β protein. SEM, SERS, FTIR, fluorescence microscopy and contact angle measurements were carried out for the characterization of different surfaces and further demonstration of the protein attachment.

This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) Grant No: 214Z167.

12:50pm A Quartz Crystal Microbalance (QCM) sensor modified with Parylene C coating for cellular monitoring

Technical Presentation. NEMB2016-6014

***Jon Furniss**, New York Institute of Technology, Old Westbury, NY, United States, **Ioana voiculescu**, City College of New York, New York, NY, United States, **Fang Li**, New York Institute of Technology, New York, NY, United States*

Cell-based biosensors (CBBs), combined with living cells, have emerged as promising biotechnical tools in various fields, including drug development, medical diagnostics, cell-based therapy, and detection of chemical and biological agents. Among various CBB techniques, acoustic wave sensors have attracted great attention due to their simple structure, compact size, excellent time resolutions, high reliability, and sensitivity to the mechanical properties of materials on the sensor's surface. Especially, QCM sensor is the most commonly used acoustic wave device for cellular sensing due to its simple structure and mature measurement technologies. Poly(chloro-para-xylylene) or Parylene C is a material with a long history of use in the field of medical industry as a biocompatible encapsulant of implantable biomedical devices. Recently, it has been gained particular interest as a material for in vitro cell culture studies. It has been demonstrated that the surface properties of parylene C films, including the nanoroughness and wettability, could be tuned through oxygen plasma treatments, which alters the cell adhesion to it. Studies have shown that the surface-treated parylene-C substrates exhibit adhesion levels comparable to commercially available tissue culture-treated polystyrene. In this paper, we investigate the parylene C modified QCM sensor for dynamic and noninvasive cellular monitoring. The parylene-C layer coated on the sensor surface serves as a sensitivity-enhancing layer through two aspects: (1) controlled surface nanoroughness increases the attachment sites for cells, and the hydrophilic surface promotes cell adhesion; and (2) the parylene C coating layer serves as an acoustic matching layer. Due to the big difference in acoustic impedance between quartz substrate and cells, the acoustic waves transmitted from quartz substrate are reflected off. The parylene C layer inserted between the quartz substrate and cell layer can promote acoustic wave propagation into cell layer, therefore enhancing sensor's sensitivity. In this study, theoretically, transfer matrix method is used to study the effect of parylene C layer thickness on the sensor's sensitivity. Experimentally, the parylene C films with three different thicknesses are coated onto sensor surfaces and treated with oxgen-plasma with different conditions. H9c2 cells with the same cell seeding density are added onto sensor surface and admittance spectrum of the sensors is recorded during the cell attachment. The sensor's sensitivity is evaluated by using spectral signatures (i.e., peak frequency shift, peak admittance value shift) divided by cell numbers at 6 hours after cell seeded onto the sensor surface. The effect of surface roughness and parylene C layer thickness on sensor's sensitivity is investigated.

5-3
SELF-POWERED OR LOW ENERGY POWERED BIODEVICES

Hidalgo **11:30am - 1:00pm**

11:30am Highly efficient storage in Li3V2(PO4)3/C cathode Li-ion batteries for self-powered biomedical devices

Keynote. NEMB2016-5970

***Guozhong Cao**, University of Washington, Seattle, WA, United States, **Xihui Nan**, Beijing Institute of Nanoenergy and Nanosystems, Beijing, Beijing, China*

Lithium ion batteries are playing indispensible role in our modern life full of portable electronics and in constant move. However, the advancement of lithium ion batteries has lagged way behind electronics, in spite of extensive research efforts across research venues and industries. Search for better electrode materials with both high energy and power densities as well as excellent cyclic stability must consider (1) proper chemical reaction at the interface between electrode and electrolyte: alloying, conversion reaction and intercalation, (2) crystal structure with available space to accommodate guest species such as spinel, olivine and layer structured crystals, (3) reversible phase transition, and (4) manageable volume change. In addition, the charge/discharge voltage and transport kinetics play determining roles in the energy density and power density as well as the energy storage efficiency of the resulting batteries.

In this presentation, I will take a high performance Li3V2(PO4)3/C material synthesized by low-cost hydrothermal method followed with subsequent annealing treatment as an example to demonstrate the efficient storage of the high frequency pulse power generated by a triboelectric nanogenerator for biomedical applications. Not only does the Li3V2(PO4)3/C exhibit a discharge capacity of 128 mAh g-1 at 1 C with excellent cyclic stability (capacity retention is 90% after 1000 cycles at a rate of 5 C) in Li-ion battery, but also shows outstanding energy conversion efficiency (83.4%) compared with the most popular cathodic materials: LiFePO4 (74.4%), LiCoO2 (66.1%), and LiMn2O4 (73.6%) when it was charged by high frequency and large current electricity.

12:00pm Bubble-Pen Technique for Low-power Optical Manipulation and Patterning of Cells and Biomolecules

Technical Presentation. NEMB2016-6041

***Xiaolei Peng, Yuebing Zheng, Linhan Lin**, The University of Texas at Austin, Austin, TX, United States*

Versatile, non-invasive manipulation and patterning of cells and biomolecules play a critical role in early disease diagnosis, medicine, tissue engineering, and fundamental studies in life sciences. Despite their high versatility, optical tweezers have remained challenging in the non-invasive operation. Herein, we develop a new method - bubble-pen technique - to non-invasively pattern cells and biomolecules on substrates using microbubbles generated and controlled by low-power light beams. Briefly, a single laser beam generates a microbubble at the interface of the solution and a plasmonic substrate via plasmon-enhanced photothermal effects. The mi-

crobubble captures and immobilizes the biological particles on the substrate through coordinated actions of Marangoni convection, surface tension, gas pressure, and substrate adhesion. Through directing the laser beam to move the microbubble, we create arbitrary patterns of particles and cells with different architectures. With the low-power operation, versatility, and biocompatibility, the bubble-pen lithography will find a wide range of applications in biology and medicine.

12:20pm Investigation Of Anodic Alumina-Based Biomaterials For Improved Nerve-Material Interaction

Technical Presentation. NEMB2016-5983

***Sevde Altuntas**, TOBB University of Economics and Technology, ANKARA,Turkey, **Buket Altinok, Belma Aslim**, Gazi University, ANKARA,Turkey, **Necmi Biyikli**, UNAM National Nanotechnology Research Center, Bilkent University, ANKARA,Turkey, **Fatih Buyuksegrin**, TOBB University of Economics and Technology, ANKARA,Turkey*

Biomaterials that allow the utilization of electrical, chemical and topographic cues for improved neuron-material interaction and neural regeneration hold great promise for nerve tissue engineering, neural implant as well as nerve recording applications. The nature of anodic aluminum oxide (AAO) membranes intrinsically provides delicate control over topographic and chemical cues for enhanced cell interaction, and hence they are widely studied in bone tissue engineering applications. The use of AAO in nerve tissue engineering is still very limited, however, and the related studies mainly focus on the role of topography on neural behavior. In this project, in addition to topographic factors, chemical and electrical cues are used for the first time to control neural behavior on AAO membranes. In this context, AAO films with uniform 100 and 250 nm diameters were first synthesized in different electrolytes, and a parafilm protecting layer was used to selectively dissolve metallic Al. This process yields free-standing AAO membranes with ~ 50 cm2 areas. These substrates were then coated with a thin layer of C to obtain conducting carbon nanotube membranes (CNM). SEM, AFM, EDX, XPS and I-V measurement were then carried out for the detailed morphological, electrical and chemical characterizations of CNMs which was followed by the cell studies.

The cell studies were conducted by using PC12 cell line. The viability and adhesion data dictates that 100 and 250 nm pore-sized AAO samples are more suitable for the cells compared to same pore-sized CNMs. In addition to this surface chemistry factor, weaker cell adhesion were observed for flat TCPS and alumina controls that shows the influence of topographical cues on cell behavior. When the average neurite length and number were compared between different substrates, electrically stimulated (E+) 100 and 250 nm-CNMs demonstrated the best results, and hence, illustrate the effectiveness of electrical factors in this context. Two substrates was then chosen for NGF doping, namely, 100 nm-AAO that provides the best cell adhesion and electrically stimulated 100 nm-CNM that provides the best neurite extension. From these NGF-doped 100 nm-AAO, NGF-doped 100 nm-CNM-E+ and standard 100 nm-CNM-E+ substrates, the former one provided the best cell adhesion as well as comparable neurite extension to that of standard 100 nm-CNM-E+ which has provided the best neurite extension under standard experimental conditions. Overall, our studies demonstrate that the natural topographic and chemical form of nanoporous 100 nm-AAO substrate provide an optimum surface for cell viability and adhesion. Upon NGF doping, this nanomaterial further provides opportunities for neurite formation and hence can have potential applications in neural implant and nerve-recording electrodes.

This project was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) Grant No: 111M686.

12:40pm Electric Fields Guided Manipulation of Catalytic Nanomotors for Cargo Delivery and Assembly of Chemically Powered NEMS

Technical Presentation. NEMB2016-6017

***Jianhe Guo**, University of Texas at Austin, Austin, TX, United States, **Donglei Fan**, The University of Texas At Austin, Austin, TX, United States*

Catalytic nanomotors, which are autonomous self-propelled nanoscale devices powered by the conversion of chemical energy into mechanical motion, have attracted keen interest due to their potential in the near future to revolutionize emerging topics in multidisciplinary nanotechnology, medicine, sensing, and environmental science. Recent efforts aimed at enhancing the performance of catalytic nanomotors resulted in an increased speed and power along with a larger cargo-towing force. However, the movement directions of these catalytic nanomotors are usually random and constantly changing with time. It is highly desirable to control the transport directions of the catalytic nanomotors for practical usage. The most widely used technique reported previously is the magnetic tweezers for guiding the moving directions of catalytic nanomotors. The catalytic nanomotors with magnetic components have a deterministic motion guided in an external magnetic field, but also easily aggregate due to magnetic attraction.

In this study, for the first time, we demonstrated guided manipulation of catalytic nanomotors by electric tweezers for applications in cargo delivery to designated microdocks and assembling of catalytic nanomotors for chemically powering rotary nanoelectromechanical system (NEMS) devices. With the electric tweezers based on the combined AC and DC electric fields, the motions of nanowire catalytic motors can be readily aligned along the direction of AC electric fields and their speed can be readily modulated by the DC electric fields. A large array of catalytic nanomotors can be transported along arbitrary trajectories with tunable speeds depending on the applied DC E-field. Assisted with the electric fields applied in the vertical (Z-) direction in a three orthogonal microelectrodes setup, the transport of catalytic nanomotors can be instantly initiated and stopped in the 2-D X-Y plane and moved in the vertical (Z-) direction at suitable electric voltage. With strategically designed microelectrodes, we further realized swamp behaviors of catalytic nanomotors, where a large group of nanomotors can be simultaneously assembled and released on demand. Finally, the powerfulness of the manipulation of chemical motors by the electric tweezers is demonstrated in two applications: firstly, without any chemical/magnetic assistance, indispensable in previous work, we facilely employed catalytic nanomotors to attach, transport, and release cargos to assemble on pre-patterned microdocks with induced electric fields. Secondly, we precisely assembled a catalytic nanomotor to a designed rotary NEMS device and successfully powered its rotation. The innovations demonstrated in this work open a new, facile, and rational route in realizing many promising applications of chemical nanomotors in biomedical and NEMS/MEMS devices.

5-4
NOVEL PRINTING AND SYNTHESIS TECHNIQUES FOR BIOMATERIAL SCAFFOLDS

Navarro 11:30am - 1:00pm

11:30am Nano and Microscale Rapid 3D Printing for Regenerative Medicine

Keynote. NEMB2016-5943

***Shaochen Chen**, UC San Diego, La Jolla, CA, United States*

The goal of our laboratory is to develop micro- and nano-scale bioprinting and 3D printing techniques to create 3D designer scaffolds for tissue engineering and regenerative medicine. In this talk, I will present my laboratory's recent research efforts in femtosecond laser nano-printing and projection 3D bioprinting to create 3D scaffolds using a variety of biomaterials. These 3D biomaterials are functionalized with precise control of micro-architecture, mechanical (e.g. stiffness and Poisson's ratio), chemical, and biological properties. Design, fabrication, and experimental results will be discussed. Such functional biomaterials allow us to investigate cell-microenvironment inter-

actions at nano- and micro-scales in response to integrated physical and chemical stimuli. From these fundamental studies we can create both in vitro and in vivo tissue models for precision tissue engineering and regenerative medicine.

12:00pm Flow-induced one-step process to synthesize functionalized nanoporous scaffolds

Technical Presentation. NEMB2016-5949

***Amy Shen**, Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan*

Recently, we introduced a microfluidic assisted irreversible gelation procedure to create stable nanoporous scaffolds with proper hydrodynamic conditions for a given surfactant solution, under ambient conditions. The irreversible gelation results from the large shear and extension rates and total strain generated by the flow through the microdevice containing micropost arrays. This microfluidic approach provides a simple platform for biomolecule encapsulation in a porous scaffold. For proof of concept studies, we used the micropost design to encapsulate enzyme horseradish peroxidase (HRP) in an ionic surfactant-based nanogel for hydrogen peroxide sensing. To extend this work, novel and versatile processing method based on microfluidics is developed to synthesize functionalized nanoporous scaffolds for a variety of sensing applications.

(a) Glucose is an important carbohydrate that acts as a metabolic intermediate and an energy source for cells. Glucose biosensors have found applications in biological and clinical studies (e.g, blood glucose sensing), food processing, and textile industry. Electrochemical glucose sensors are widely used due to their low-cost, fast time-response, and simple operation procedures. In an electrochemical glucose sensor, molecular recognition enzymes such as glucose oxidase (GOx) need to be immobilized on electrodes. In the presence of molecular oxygen, immobilized GOx then catalyzes the oxidation of glucose to gluconolactone, and generates hydrogen peroxide as a byproduct, where two protons and two electrons are released during the reaction. The transfer of the released electrons (current) is the physical parameter sensed during the redox reaction between GOx and glucose. Here, a simple microfluidic platform is utilized to immobilize glucose oxidase (GOx) in a nonionic micellar scaffold. The immobilized GOx are verified by using a combination of cryogenic electron microscopy (cryo-EM), scanning electron microscopy (SEM), and ultra-violet spectroscopy (UV). Chronoamperometric measurements are conducted on nanogel-GOx scaffolds under different glucose concentrations, exhibiting linear amperometric responses. Without impacting the lifetime and denaturation of GOx, the nonionic nanogel provides a favorable microenvironment to GOx as in biological media. This flow-induced immobilization method in the nonionic nanogel host matrix opens up new pathways for designing simple, fast, biocompatible, and cost-effective process to immobilize biomolecules that are averse to ionic environments.

(b) A simple and rapid flow-induced microfluidic process is employed to disperse and encapsulate low volumes of single-walled carbon nanotubes (SWCNTs) in wormlike micellar networks, thereby forming an electro-conductive porous scaffold. SWCNTs with anionic surfactant sodium dodecyl sulfate (SDS) are first mixed with an aqueous wormlike micellar solution consisting of cationic surfactant cetyltrimethylammonium bromide (CTAB) and organic salt sodium salycilate (NaSal). The precursor mixture is then pumped through a microfluidic device containing hexagonal microposts at room temperature and ambient pressure, developing a soft scaffold with entangled bundle-like structures, containing interconnected SWCNTs and wormlike micelles. One-step microfluidic process presented in this work opens a new pathway to disperse and encapsulate SWCNTs in a micellar matrix without involving chemical reactions under ambient conditions, with promising potentials for sensing, encapsulation, and catalysis applications.

12:20pm 4D printed biomimetic nano smart bone scaffold

Technical Presentation. NEMB2016-5957

***Shida Miao, Wei Zhu, Nathan Castro, Lijie Zhang**, The George Washington University, Washington, DC, United States*

The gold standard method for repair of bone defect is utilizing an autologous bone graft, but the availability of autologous bone graft is limited and severe complications may generate in the graft harvesting. To replace autologous bone, great efforts have been made on grafting materials and fabricating techniques. In this study, we aim to 4D print nano smart biomimetic gradient bone scaffolds by a 3D printing guided approach to fill bone defects through shape memory effect. The term “4D print” refers to added shape transformation of printed construct on specific stimulation according to requirements to achieve minimally invasive surgeries and perfect end-to-end joint connection. The term “smart” originates from the shape memory behavior of the synthesized biocompatible polymers. To reach these goals, novel shape memory polymers with excellent biocompatibility and tunable shape changing effects are synthesized and cured with biomimetic nanocrystalline hydroxyapatite (nHA) in the presence of 3D printed sacrificing structures which are subsequently dissolved to create controllable and gradient porosity in the scaffolds. Morphology, thermal, mechanical and biocompatible properties as well as shape memory effect of the resultant porous scaffolds are characterized using varied techniques. Fourier transform infrared spectroscopy (FTIR) and gel content analysis confirm the formation of chemical cross-linking in obtained smart polymers which are synthesized by reacting polycaprolactone triol and plant oil with multi-isocyanates. Differential scanning calorimetry reveals an adjustable glass transition temperature in a range from 0 to 35 oC. Transmission electron microscopy analysis shows that the nHA is 50-100 nm in length and 20-30 nm in width of grain sizes similar to natural human bone nHA. Compression testing indicates that the obtained polymers have close compression modulus to polycaprolactone which is widely utilized as bone scaffold material, and fully interpenetrating polymeric networks are formed in fully cross-linked polymer networks. Scanning electron microscope analysis shows that the nHA is distributed evenly in the scaffold and micro pores are present in gradient from top to bottom of the scaffold, which mimics the non-uniformly distributed porosity in natural bone. Shape memory test results show that the 4D printed nanocomposite scaffolds display finely tunable recovery speed and exhibit greater than 90% shape fixing at -18 or 0 oC and full shape recovery at human body temperature. With polycaprolactone serving as a control, human bone marrow-derived mesenchymal stem cell shows significant higher proliferation and osteogenic differentiation. The current work will advance the future design and development of novel and functional bone healing nanocomposite scaffolds with advanced 4D printing technology and highly biocompatible smart biomaterials.

12:40pm Electrospun Patches for the Controlled Release of Growth Factors to Enhance Vascularization in Regenerative Medicine Applications

Technical Presentation. NEMB2016-6091

***Laura Pandolfi**, Houston Methodist Research Institute, Houston, TX, United States, **Francesca Taraballi**, Houston Methodist Research Inst., Houston, TX, United States, **Silvia Minardi**, Department of NanoMedicine, Houston Methodist Research Institute, Houston, TX, USA, Houston, TX, United States, **Xuewu Liu, Mauro Ferrari**, Houston Methodist Research Institute, Houston, TX, United States, **Ennio Tasciotti**, The Methodist Hospital Research Institute, Houston, TX, United States*

Tissue restoration is a complex process orchestrated by the presence of specific bioactive molecules and by their spatio-temporal distributions. A combination of growth factors (GFs) with different release kinetics has been shown to be beneficial in boosting tissue regeneration. In particular, the synergistic effect of platelet-derived growth factor (PDGF-BB) and vascular endothelial growth factor (VEGF) has been shown to enhance proliferation, migration and growth of endothelial cells, enhancing in situ neovascularization.

The ability to control the spatio-temporal release kinetics of GFs is crucial

to ensure the desired therapeutic effect and avoid the emergence of side effects due to the uncontrolled, systemic release of the GFs. We developed biocompatible nanoporous silicon multistage vectors (MSV) to allow the efficient loading of a variety of bioactive molecules. To prevent the burst release from the open nanopores and provide enhanced protection to the payload we engineered a tunable poly(DL-lactide-co-glycolide) acid (PLGA) coating of the MSV. By controlling the physical and chemical properties of the PLGA layer we provided a further control on the long-term release of therapeutic molecules. To test the ability of this platform to achieve sustained temporal release and localized spatial distribution, we integrated it in an electrospun gelatin based patch. The patch was inspired by the biological properties and composition of the extracellular matrix (ECM) to avoid the immune rejection of the graft. To increase the early vascularization of the implant we loaded the PLGA-MSV with VEGF and PDGF-BB. The controlled release of the two GFs can improve the local formation of neo-vasculature at the site of the implant by locally enhancing and supporting angiogenesis. The development of this biomimetic electrospun patch, functionalized with a tunable delivery platform consisting of MSV and PLGA (PLGA-MSV), allows for the programmable delivery of GFs in space and time. By orchestrating the release of signaling molecules, this patch could be used in support of any tissue restoration process requiring early vascularization. We characterized the patch using scanning electron microscopy, Fourier transform infrared spectroscopy, and confocal microscopy. The release of VEGF and PDGF-BB from PLGA-MSV was performed in physiological like conditions (PBS, 37°C, under mild agitation). Samples were collected up to 4 weeks and GF's release kinetics was determined by ELISA. The patch allowed to prevent the burst release of the loaded GFs and to prolong their delivery up to 4 weeks. Furthermore the stability and bioactivity of the loaded molecule was preserved during the duration of the release process. We tested the biocompatibility of the electrospun gelatin patches using human mesenchymal stem cells (hMSC). Early passage hMSC were seeded onto the patch to assess cell growth and differentiation up to 1 month. The results of the in vitro tests indicated uncompromised cell viability and full cell adhesion to the patch.

The presented strategy provides the controlled release of VEGF and PDGF-BB in a controlled fashion to enhance patch's vascularization. Further investigations are ongoing to verify the efficacy of this platform to induce in vivo vascularization, the localized delivery of GF and we expect to inevitably boost the regenerative microenvironment by promoting early angiogenesis at the site of the implant.

5-5
ASSEMBLED MATERIALS

Harris 4:00pm - 5:40pm

4:00pm NanoCluster Beacons Enable Enzyme-Free N6-Methyladenine Detection

Technical Presentation. NEMB2016-6000

Judy Obliosca, Yu-An Chen, Yen-Liang Liu, Cong Liu, Mary Gwozdz, Tim Yeh*, University of Texas at Austin, Austin, TX, United States*

NanoCluster Beacons (NCBs) are a new type of activatable molecular probes that are low cost, easy to prepare and have high fluorescence enhancement ratios. NCBs employ DNA-templated, few-atom silver nanoclusters (DNA/Ag NCs, with about 2~20 silver atoms per cluster) as reporters which can significantly "light up" through interactions with a nearby DNA sequence (called an enhancer). Taking advantage of this fluorescence tunability by altering the surrounding ligands, a property that is not commonly seen among existing reporters, NCB soon evolved to a multicolor probe, termed chameleon NanoCluster Beacon (cNCB), for single-nucleotide polymorphism (SNP) detection. Here we bring the NCB detection to the next level by designing a new NCB specifically for N6-methyladenine (hereafter denoted as m6A) detection. m6A is a methylation modification abundant in prokaryotic genomes, and also found in lower eukaryotes and higher plants. So far detection of m6A relies on methods such as TLC, HPLC, MS, and enzymatic reactions, which are often laborious, expensive, with low specificity and varying reactivity. Unlike 5-methylcytosine (m5C), there is no chemical treatment that can facilitate the m6A detection. Whereas high-resolution melting (HRM) analysis is able to detect a single m6A modification within a target DNA via the destabilizing effect of m6A, HRM cannot pinpoint the location of m6A in the sequence. A simple and cost-effective way to identify single m6A at any specific sites is therefore highly desired. Here, we developed a robust, simple, enzyme-free and hybridization-based method for m6A detection with "pinpoint specificity", using a new type of silver cluster-based DNA probe which we term methyladenine-specific NanoCluster Beacon (maNCB). To date, there is no hybridization technique that has the potential to reach these remarkable results. To date, there is no hybridization technique that has the potential to reach this remarkable result.

References:
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4:20pm Entropically controlled nanomechanical sensing with DNA origami

Technical Presentation. NEMB2016-6063

Michael Hudoba, Yi Luo, Michael Poirier*, The Ohio State University, Columbus, OH, United States,* ***Carlos Castro****, Ohio State university, Columbus, OH, United States*

DNA nanotechnology has emerged as a promising technology for applications such as single molecule sensing, super-resolution imaging, and manipulating molecular components. Major advances in the last decade have enabled the precise design and fabrication of DNA nanostructures with unprecedented geometric complexity; however, relative to natural biomolecular machines, the functional scope of DNA nanotechnology is limited by an inability to design dynamic mechanical behavior such as complex motion, conformational dynamics, or force generation. Taking inspiration from methods used in macroscopic machine design, we have recently developed DNA nanostructures with well-defined 1D, 2D, and 3D motion, and we have demonstrated the ability to actuate that motion via binding or competitive release of DNA strands on the timescale of ~1 minute. We have recently developed dynamic nanostructures that exhibit multiple stable states separated by tunable energy barriers designed to allow thermally driven conformational changes at room temperature. In addition, we have demonstrated the ability to tune the kinetics of transition between the stable states through structure design parameters that regulate the conformational entropy by constraining thermal fluctuations in specific states. In particular, we have designed a dynamic DNA Origami Two-state nanodevice (DOT) consisting of two stiff double-stranded DNA bundle components connected by 6 flexible single-stranded DNA linkers arranged symmetrically around the circumference of the bundle. One of the linkers can close into a loop via binding of a single DNA base-pairing interaction yielding a dynamic equilibrium between a closed state where the two bundle components are pinned together and an open state where they can exhibit large relative motion. This closing interaction was designed to allow for transient binding into the closed state with short lifetimes on the timescale of ~1 second. Equilibrium behavior of the DOT measured both by transmission electron microscopy and single molecule fluorescence resonance energy transfer experiments confirm a distribution of closed and open states. We further demonstrated the ability to tune the kinetics of conformational transitions between states through structural design parameters that regulate conformational entropy. A major goal of our laboratory's work is to develop devices where dynamic behavior can be exploited to probe nanoscale physical properties or interactions (e.g. molecular forces). Here we demonstrated that both the kinetics and equilibrium behavior of DOTs serve as a readout of depletion forces that result from molecular crowding using polyethylene glycol as a crowding reagent. Given the length scale of ~100nm, this device could ultimately be implemented to measure depletion forces inside cells where molecular

crowding is known to affect molecular motor transport, enzyme function, and other molecular interactions.

4:40pm Regulation-at-a-Distance of Biomolecular Interactions Using a DNA Origami Nanoactuator

Technical Presentation. NEMB2016-5954

***Yonggang Ke**, Emory School of Medicine, Atlanta, GA, United States, **Travis Meyer**, Emory University/Georgia Institute of Technology, Atlanta, GA, United States*

The creation of nanometer-sized structures that exhibit controllable motions and functions is a critical step towards building nanomachines. Recent developments in the field of DNA nanotechnology have begun to address these goals, demonstrating complex static or dynamic nanostructures made of DNA. Here we have designed and constructed a rhombus-shaped DNA origami "nanoactuator" that uses mechanical linkages to copy distance changes induced on one half ("the driver") to be propagated to the other half ("the mirror"). We demonstrated the ability to control this dynamic DNA origami with long-range allosteric activation properties, wherein the binding of an effector molecule controls its global shape. By combining this nanoactuator with split Enhanced Green Fluorescent Protein (eGFP), we have constructed a DNA-protein hybrid nanostructure that demonstrates tunable fluorescent behaviors via long-range allosteric regulation. In addition, the nanoactuator can be used as a sensor that responds to specific stimuli, including changes in buffer composition and the presence of restriction enzymes or specific nucleic acids.

We expect the same mechanism can potentially be applied to other proteins, small molecules, and nanoparticles for constructing DNA-controlled artificial nanodevices with tunable enzymatic, photonic, or plasmonic functions. In addition, the nanodevice can serve as a general platform for studying weak molecular interactions at the single-molecule level (for instance, weak protein-protein interactions). Single-molecule experiments are generally limited to strongly interacting pairs because the short binding time between weak pairs often make it impossible to monitor. In addition, single-molecule methods typically require low concentrations of molecules, which further reduce the frequency of weak molecular interactions. Our device provides a potential solution for observing these weak interactions: by increasing the local concentration of molecules and by providing rationally designed spatial confinement, we may enhance the weak interactions to enable monitoring such events at the single-molecule level.

This novel mechanism could also potentially enable construction a group of sensors that respond to a wide range of stimuli besides what have been demonstrated in this work. In these sensors, the sequences of the detecting strands were designed independently without regard to the underlying DNA origami structures that carried the fluorescence reporters. Therefore, these sequences can be modified to enable the DNA origami nanodevice to sense other types of stimuli ranging from small molecules to large protein complexes. For instance, implementing aptamer sequences can potentially allow the device to detect specific proteins.

Finally, the nanodevice could be adapted to serve as a quantitative platform for a low-cost electrophoretic mobility shift assay. The allosteric mechanism and the large size of the origami devices are two of the advantages for stimuli detection using this technique. Alternatively, the drastic conformational change of the device in presence of specific targets can be visually detected at the single-molecule level through atomic force microscopy or electron microscopy. For fluorescence-based detection, the three-dimensional shape and the chemical addressability of the origami device allows for the incorporation of several dye/quencher pairs along the arms of the device, thereby making read-out of the nanoswitches more sensitive.

5:00pm Controlled Assembly Of Biocompatible Metallic Nanoaggregates Using A Small Molecule Crosslinker

Technical Presentation. NEMB2016-6087

***Jacob Berlin**, City of Hope, Beckman Research Inst., Duarte, CA, United States*

The controlled assembly of biocompatible nanoparticle aggregates using small molecule crosslinker has been a long standing challenge, likely owing to difficulties in controlling rates of initiation, propagation and termination. Here we demonstrate that adjusting the concentration of the starting nanoparticles or the crosslinker allows for the preparation of relatively homogenous aggregates from metallic nanoparticles of varied composition and size, presumably by controlling the rates of initiation and propogation. Capping reactive thiols on the formed aggregates with PEG-maleimide provides a termination step and renders the aggregates stable and biocompatible. The size of the aggregates can be systematically adjusted. The aggregates are biocompatible and show no toxicity when incubated with cells. Finally, the aggregates are highly stable and appear unchanged after uptake by cells. It is expected that this straightforward and inexpensive assembly of highly stable nanoparticle aggregates will expand the biological applications of this class of materials. Furthermore, this method for preparing aggregates is highly modular as the crosslinker, the building block nanoparticles and the exterior coating can all be independently varied and the use of alternative crosslinkers and capping agents will enable applications in diverse material applications.

5:20pm Heat-Shrunken Hierarchical Silica Nanomembrane for Solid Phase DNA Extraction

Technical Presentation. NEMB2016-6013

***Ye Zhang**, Johns Hopkins University, Baltimore, MD, United States, **Yi Zhang**, Institute of Bioengineering and Nanotechnology, Singapore,Singapore, **Kelvin Liu**, Circulomics Inc., Baltimore, MD, United States, **Tza-huei Wang**, Johns Hopkins University, Baltimore, MD, United States*

Introduction: The ability to obtain large quantities of high molecular weight and high purity DNA is of rising importance as genetic analysis tools become increasingly sophisticated. Conventionally, liquid phase DNA extraction techniques, such as Phenol/Chloroform precipitation, are used for high quality sample preparation; however, this approach is laborious, time-consuming and highly operator-dependent. Solid phase DNA extraction via silica adsorption, such as spin columns and magnetic particles, are gaining popularity these days due to their speed, ease of use, and generally high performance. However, they tend to induce shear forces that fragment DNA and reduce DNA quality. In this paper, we present a simple strategy to create a high surface area silica nanomembrane that contains a high density of hierarchical micro- and nanoscale features. This material is created by depositing silica onto a thermoplastic polyolefin (PO) film and heat-shrinking in an oven. The resultant membrane exhibits overlaying hierarchical structures from nano to micro scale, which can be fine-tuned through silica deposition thickness. These nanostructures significantly increase the total surface area of silica on the membrane with exceptional adsorption capacity for DNA as a novel substrate for solid phase extraction. DNA isolated with the proposed nanomembrane shows higher recovery yield and better integrity comparing with commercial columns and particles, as well as comparable performance with gold standard Phenol/Chloroform precipitation.

Materials and Methods: The nanomembrane was fabricated by depositing silica onto both sides of the PO film using electron beam physical vapor deposition. Then the silica-coated film was baked at 300°F for 3 min to induce surface wrinkling. The resulted shrunk film is smaller than 10% of its original size and exhibits hierarchical nanostructures under scanning electron microscope. Nucleic acid extraction can then be performed using a standard bind, wash, and elute protocol in as little as 45 minutes.
Results and Discussion: The nanomembrane surface topography can be fine-tuned by varying the thickness of silica deposition. With 2nm silica, the membrane displays only micro-scale ridges. With 20nm and 50nm silica, nanoscale wrinkles and flakes emerge and interweave with micro-ridges, forming overlaying hierarchical nanostructures. Once the silica layer exceeds 100nm, a high density of nano-scale flakes interweave to form secondary

microstructures replacing those ridges completely. These hierarchical structures significantly enlarge the specific surface area of silica, enhancing DNA absorption capability on the nanomembrane. We compared the performances of our nanomembrane with commercial particles for DNA re-isolation in three aspects: recovery yield, purity and integrity. We were able to recover about 3.2 microgram (80%) using our nanomembrane from 4 microgram genomic DNA, while magnetic particles only recovered about 20% DNA under the same condition. DNA shearing induced by commercial particles was also observed by running resulting DNA in gel electrophoresis. DNA isolated using small particles (100nm in diameter) were sheared much more significantly compared with using large particles (5um in diameter). In contrast, DNA isolated using the silica nanomembrane retained their integrity. We also performed DNA extraction from cultured cells utilizing our nanomembrane, and gel electrophoresis result demonstrated that the yield DNA had significantly high molecular weight DNA, over 50kb, comparable with those extracted through phenol/chloroform method in both yield and quality. Moreover, with the same amount of cultured cells as input (about 3 million), DNA yield using column and magnetic particles are only about 42% and 56% of that using our nanomenbrane.

Conclusions: In summary, we have demonstrated a novel method to fabricate silica nanostructures based on self-wrinkling induced by thermal shrinkage. The formation of overlaying hierarchical nanostructures on the membrane is closely related to the thicknesses of silica deposition. These hierarchical nanostructures have vastly enlarged the specific area of silica, thus enabling us to implement the nanomembrane in solid phase DNA extraction. We applied the silica nanomembrane for DNA extraction and demonstrated its better performance than commercial columns and particles, in terms of DNA yield and quality. Therefore, we expect this hierarchical nanomembrane to be widely adapted in various DNA analyses.

WEDNESDAY, FEBRUARY, 24

5-6
NANOPARTICLE SYNTHESIS

Hidalgo **9:30am - 11:00am**

9:30am Engineering magnetic nanoparticles for biomedical applications in time-varying magnetic fields (Invited)

Keynote. NEMB2016-6038

***Carlos Rinaldi**, University of Florida, Gainesville, FL, United States*

Magnetic nanoparticles are unique among nanomaterials due to our ability to control their translation and rotation, and actuate thermal release, through the application of magnetic field gradients and time-varying magnetic fields. Furthermore, because of their biocompability and the fact that magnetic fields penetrate through the body, magnetic nanoparticles possess tremendous potential for biomedical applications. In this talk I will discuss our recent work aimed at engineering magnetic nanoparticles to tailor their response to time-varying magnetic fields and for their application in thermal cancer therapy, magnetic particle imaging, and probing the mechanical properties of biological environments.

10:00am Size Dependency for Magnetic Resonance Contrast and Magnetic Fluid Hyperthermia

Technical Presentation. NEMB2016-6023

***Christopher Quinto**, Georgia Institute of Technology, Atlanta, GA, United States, **Sheng Tong, Gang Bao**, Rice University, Houston, TX, United States*

Magnetic iron oxide nanoparticles (MNPs) possess the unique ability to be used in both a diagnostic and therapeutic capacity for cancer treatment by utilizing their inherent magnetic resonance contrast and potential to generate local heat when exposed to an alternating magnetic field (AMF). Recent advances in the synthesis of MNPs through thermal decomposition have enabled fine size tuning within a wide range for distinct and monodisperse populations. This level of control prompts an in-depth investigation of the nano size effects in magnetic fluid hyperthermia (MFH) and magnetic resonance imaging (MRI). Here we synthesized an extensive array of MNPs and examined the influence of nanoparticle size for MRI and MFH at the solution, cellular, and tissue levels.

In this study, 8 different magnetite nanocrystals from 6 to 40 nm were synthesized by thermodecomposition of iron acetylacetonate. The room temperature M-H curves for all nanocrystals exhibit minimal hysteresis and high saturation magnetization approaching that of bulk magnetite. The nanocrystals below 10 nm in diameter have a reduced saturation magnetization in contrast to larger nanocrystals, suggesting the influence of a disordered surface layer on magnetization increases with the surface to volume ratio. To confer aqueous solubility, the nanocrystals were coated with phospholipid-PEG using a dual solvent exchange method. Dynamic light scattering measurements showed that the coated MNPs were monodisperse in water. The minimal hysteresis loop in the M-H curves of these MNPs suggests that the primary mechanism of AMF heat generation can be attributed to Néelian and Brownian relaxation. We found that when aqueous solutions of MNPs were exposed to AMF, there was very low heating below 10 nm and a linear relationship between the specific absorption rate (SAR) and the nanoparticle size above 10 nm. The maximum SAR was achieved with the 40 nm nanocrystals at 2844 W/g with an AMF setting of 23.8 kA/m and 325 kHz. When the MNPs were dispersed in glycerol, there was no significant change in SAR, indicating that Néelian relaxation is dominating in heat generation. In addition, our numerical analysis showed that the size dependence of SAR could be explained by accounting for the contribution of the surface layer to the anisotropic energy of MNPs. In the meantime, the MRI T2 contrast of MNPs was assessed with a 0.47T Minispec Analyzer. Interestingly, there is also a linear increase in the relaxivity with respect to the size of MNPs, with the highest relaxivity occurring in the 40 nm MNPs at 850 mM-1s-1.
The tumor tissue represents a heterogeneous microenvironment for MRI and complicated thermodynamics for MFH due to homeostatic temperature regulation. To this end, MNPs of 6 nm, 19 nm or 40 nm were examined in a mouse xenograft tumor model. After intratumoral injection of 50 μg of MNPs, MR images clearly delineated the injection volume of MNPs of all sizes. However, AMF treatment of the tumors injected with the 6 nm MNPs did not induce an increase in temperature, while the 19 nm and 40 nm MNPs caused an increase of 2.5 and 10.1 degree respectively. In histological examination, only the 40 nm MNP-treated tumors showed strong TUNEL staining, which is consistent with the temperature measurements.

In summary, our study shows a linear correlation of SAR and T2 relaxivity with respect to the size of magnetite nanocrystals as a result of the size-dependency of their magnetic properties. Developing MNPs for MRI and MFH applications requires careful consideration of the physicochemical properties of the particles in order to achieve peak efficiency.

10:20am Large-Scale Nanoparticle Synthesis through High Precision Feedback Control of Parallelized Microfluidic Reactors

Technical Presentation. NEMB2016-6112

***Michael Toth, YongTae Kim**, Georgia Institute of Technology, Atlanta, GA, United States*

A wide range of nanoparticles (NPs) has been developed with the potential to revolutionize therapeutics and diagnostics through targeted delivery of drugs/genes and selective combination of imaging agents to specific cells/tissues. Recent approaches using microfluidics produced NPs with higher reproducibility by facilitating the micro- and nanoscale interactions of precursors in synthesizing several types of NPs than that of conventional bulk synthesis. However, these microfluidics-based NP syntheses have been largely limited to small-scale production of specific single component NPs. There

is no reliable and practical approach available for scalable manufacturing of multicomponent and multifunctional NPs to industrially relevant level (~kg/h or even higher as needed). This is not only because the underlying mechanism of flow-induced NP assembly in current microfluidic methodology remains elusive, but also current early-stage microfluidic approaches have relied on conventional syringe pumps, which are not robust methodology for scalable manufacturing of NPs due to several problems including the limited syringe size and the open-loop control-based operation unable to compensate for unexpected disturbances in the manufacturing process resulting in non-robust production operations. Here, we present a new large-scale integration (LSI) of parallelized microfluidic reactors with high-precision microfluidic pressure control system, which allows for mass production of multicomponent therapeutic NPs. In this study, we use our LSI microfluidics technology to demonstrate a representative example of scale-up synthesis of lipid-PLGA NP (LPNP) in a parallelized microfluidic array (PMA). Each single microfluidic reactor in the PMA operates at a Reynolds number of 250 to create optimal microvortex flow patterns to strongly mix an organic solution of poly(D,L-lactide-co-glycolide)(lactide:glycolide (50:50)) (PLGA) in acetonitrile with an aqueous solution of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-Distearoylsn- glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2000-DSPE) in a 3:7 molar ratio in 4wt% ethanol. We evaluate the size uniformity of produced NPs using dynamic light scattering (DLS) and transmission electron microscopy (TEM). Computational fluid dynamics (CFD) simulations allowed optimization of a single microfluidic reactor dimensions (a cylinder of diameter of 2mm and height of 5mm) and the operating flow rate condition (Reynolds number of 250). The PMA model has 25 single microfluidic reactors parallelized into a 5x5 array system. This array system was designed and optimized using an electrical circuit model and CFD simulations to operate at the same condition for each reactor while minimizing pressure difference among 25 single microfluidic reactor inlets. We utilized one simplified electrical circuit model to optimize the ratio of the impedances between the flow path of the array network to have as same flow rates as possible at each reactor inlets in the PMA system. The developed PMA model that allows for a production rate of 315g/h, which is approximately 1000 times higher than that of our previous approaches. Our custom high-precision control system was developed by modeling a fluidic circuit using two variable resistances controlled by linear actuator displacements, with control over the input of a high pressure source and a low pressure outlet. The system was tuned using a proportional-integrative-derivative (PID) controller to achieve fast response less than 0.3 s settling time, and long term stability, error less than 0.5%. The pressure range of the control system is sufficient for the PMA system to acquire optimal flow conditions at the inlet and has been designed for long term experimentation, over multiple hours or days. Our LSI microfluidic technology will generate a significant contribution to improving the design, predictability, efficiency, and control of many NP manufacturing techniques, thereby addressing a critical need in nanoengineering and nanomanufacturing. When combined with the latest advances in the application of low-cost and high-performance computing for production operations, this integrated system-based approach will enable flexible and reconfigurable operations for rapid product generation and scaling of multicomponent NPs.

10:40am Bioinspired Rotary Micromachines for Microfluidic Applications

Technical Presentation. NEMB2016-6070

Kwanoh Kim, Minliang Liu, Donglei Fan*, The University of Texas at Austin, Austin, TX, United States*

Micromachines are miniaturized mechanical devices that can convert different energy sources into controlled motion. Recently, with vigorous progress in nanotechnology, remarkable advances have been made in the development and application of miniaturized machines. Various working mechanisms have been reported, and sensors and actuators based on micromotors have been demonstrated using nanoentities with different shapes, sizes, and material properties. However, while those functional nanoentities are essential to further expand the applications of micromachines, it is arduous to obtain three-dimensional (3D) micro/nanostructures with complex geometries and desired properties. It usually requires costly and time consuming processes with sophisticated skills and instrument. In nature, on the contrary, biological systems have created delicate and refined 3D nanostrucrtures for a long time. Due to their unique optical/mechanical properties and geometry, not to mention their abundance and excellent biocompatibility, biological nanomaterials have inspired researchers to directly utilize or replicate them for micro/nanoelectromechanical systems (MEMS/NEMS).

In this work, we report an innovative type of rotary micromotors assembled from biological 3D micro/nanostructures for microfluidic applications. The micromotors are comprised of diatom frustules, patterned magnetic microdisks, and quadruple microelectrodes working as rotors, bearings, and stators, respectively. Diatoms are unicellular photosynthetic algae living in marine ecosystems. They create silica cell walls called frustules with ordered micro/nanoscale pores on the surface in different geometries and dimensions resembling photonic crystals. The frustule rotors were prepared through a simple cleaning and filtration process of diatomaceous earth powders followed by deposition of a thin film of Cr/Ni/Au. These metal-coated frustules can be readily manipulated by the electric tweezers based on combined DC and AC electric fields with a maximum speed of ~140 μm/s and assembled onto prepatterned micromagnets into working rotary micromotors. The micromotors can rotate both clockwise and counterclockwise to a maximum speed of ~2800 rpm, controlled by the frequency and intensity of the electric fields. Multiple frustule motors can be assembled and actuated in ordered arrays with fully controlled rotation speed and orientation. With understanding of the frequency-dependent rotation behaviors, for the first time, we realized individually controllable rotary micro/nanomotors, which are highly essential for many applications including nanorobotics and nanomachineries. Finally, the micromotors were successfully assembled and actuated in microfluidic channels as micromixers and micropumps. The innovations reported in this work could provide a cost-effective and facile approach for the fabrication of sophisticated micromachines with functional three-dimensional nanostructures, and they are relevant to microfluidics, MEMS, biosensing, and lab-on-a-chip architectures.

TRACK 6 POSTERS

SUNDAY, FEBRUARY, 21

6-1
NANOIMAGING

Grand Ballroom 5:00pm - 8:00pm

Activatable and Cell-Penetrable Multiplex FRET Nanosensor for Profiling MT1-MMP Activity in Single Cancer Cells

Poster Presentation. NEMB2016-6092

***Eddie Chung, Peter Yingxiao Wang, Shaoying Lu**, UCSD, La Jolla, CA, United States*

We developed a quantum-dot-based fluorescence resonance energy transfer (QD-FRET) nanosensor to visualize the activity of matrix metalloproteinase (MT1-MMP) at cell membrane. A bended peptide with multiple motifs was engineered to position the FRET pair at a close proximity to allow energy transfer, which can be cleaved by active MT1-MMP to result in FRET changes and the exposure of cell penetrating sequence. Via FRET and penetrated QD signals, the nanosensor can profile cancer cells.

Generalized Syntheses of Tumor Targeted Yolk/Shell Structured Multifunctional Nanosystems

Poster Presentation. NEMB2016-5965

***Sixiang Shi**, University of Wisconsin-Madison, Madison, WI, United States, **Feng Chen, Stephen A. Graves**, University of Wisconsin-Madison, Madison, WI, United States, **Shreya Goel, Todd E. Barnhart**, University of Wisconsin-Madison, Madison, WI, United States, **Weibo Cai**, University of Wisconsin-Madison, Madison, WI, United States*

Objectives: Hollow mesoporous silica nanoparticles (HMSNs), with a large cavity inside each original mesoporous silica nanoparticle (MSN), have recently gained increasing interest due to their tremendous potential for future cancer imaging and therapy. Dual-modality imaging can be achieved by integrating various types of inorganic functional nanocrystals into HMSN nanostructures, which provides synergistic advantages over each modality. Our goal is to develop a generally applicable protocol for yolk/shell structured multifunctional nanosystems, to combine positron emission tomography (PET) with other imaging modalities for image-guided drug delivery.

Methods: Upconversion nanoparticle (UCNP, with NIR-in-NIR-out upconversion luminescence) was used as the initial example. UCNP was first coated with a dense silica (dSiO2) shell, forming UCNP@dSiO2, followed by regrowth of a shell-thickness controllable MSN to form UCNP@dSiO2@MSN. A Na2CO3 etching protocol was then used to selectively etch away dSiO2, leaving behind yolk/shell structured nanoparticles denoted as UCNP@HMSN. A step-by-step surface engineering process was then adopted to conjugate NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid), polyethylene glycol (PEG) linkers, TRC105 (an antibody that binds to CD105, a receptor overexpressed on tumor vasculature), and 64Cu radiolabel to form 64Cu-UCNP@HMSN-PEG-TRC105. Both hydrophobic (i.e. Sunitinib) and hydrophilic (i.e. Doxorubicin) drugs could be loaded inside UCNP@HMSN. Systematic in vivo PET imaging and biodistribution studies were performed in 4T1 tumor-bearing mice to evaluate and confirm tumor targeting capability, validated by in vitro/ex vivo studies. Similar protocol was also applied to prepare quantum dot (QD)@HMSN and superparamagnetic iron oxide nanoparticle (SPION)@HMSN core/shell structured nanosystems.

Results: TEM confirmed successful synthesis of UCNP@HMSN with high uniformity. By changing the "yolk" to QD or SPION, we confirmed the general applicability of this protocol. The stability of as-designed nanosystem in PBS was significantly improved due to the incorporation of PEG. In vitro CD105 targeting in HUVEC (CD105+) and MCF-7 (CD105-) cells showed strong/specific binding of FITC-conjugated UCNP@HMSN-PEG-TRC105 to CD105+ cells with negligible non-specific binding. In vivo tumor targeting and PET imaging demonstrated CD105-specific targeting of 64Cu-UCNP@HMSN-PEG-TRC105 in 4T1 tumor-bearing mice, with peak tumor uptake of ~6.5 %ID/g at 6 h post-injection. CD105 specificity was confirmed by blocking and ex vivo histology studies. With large interior cavity inside the core/shell structure, enhanced drug loading was achieved with UCNP@HMSN compared to UCNP@dSiO2@MSN (same nanoparticle without a hollow cavity inside).

Conclusion: We report the generalized syntheses of yolk/shell structured nanosystems for tumor targeted PET imaging and drug delivery. With UCNP, QD, SPION or other nanocrystals inside each yolk/shell structure, this nanoplatform is highly versatile for future multimodality image-guided drug delivery. By introducing tumor vasculature targeting, the as-designed nanosystems will benefit from reduced side-effects and enhanced therapeutic efficacy due to enhanced targeting specificity.

Synthesis and Characterization of Dextran-Polycaprolactone Diblock Copolymers for the Preparation of Highly Stable Micelles with Facile Surface Functionalization

Poster Presentation. NEMB2016-6006

***Elizabeth Higbee-Dempsey, Lesan Yan, Andrew Tsourkas**, University of Pennsylvania, Philadelphia, PA, United States*

Nanoparticle-based imaging probes are often coated with so-called "stealth" polymers: hydrophilic neutrally-charged polymers that help prolong blood circulation by minimizing particle aggregation and minimizing interactions with serum and tissue proteins. Currently, the most popular choice of polymer for this purpose is polyethylene glycol ("PEG"). Despite its near-ubiquity, PEG has several important limitations. For example, PEG can only be modified at its two ends, which limits its ability to be crosslinked. Crosslinking may be desirable if improved stability (e.g. lower critical micelle concentration) is required or if drug leakage is high. Also, while PEG's stealth properties help particles avoid nonspecific recognition, PEG brushes can also unintentionally conceal targeting ligands or other surface modifications. Due to the flexibility of linear PEG chains, terminal moieties may become buried within a PEG corona, particularly if these moieties are not highly hydrophilic.

To overcome these particular challenges, we have developed an alternative polymer micelle system based on dextran, a glucose polymer. We have synthesized a biodegradable diblock copolymer combining dextran ("Dex") and the hydrophobic polymer polycaprolactone (PCL). Click chemistry was employed in order to obtain the copolymer Dex-PCL in high yield and purity, and the product was thoroughly characterized using NMR and IR spectroscopy. These polymers were then self-assembled into nanoparticles using a two-phase emulsion method, yielding spherical micelles with narrow polydispersity. The resulting nanoparticles can be aminated and chemically crosslinked in order to minimize cargo leakage and improve stability at low micelle concentration. Furthermore, while aminated dextran-coated nanoparticles are known to display favorable cellular uptake profiles, for cell tracking studies, these Dex-PCL particles can also be further chemically modified in order to introduce targeting ligands on the micelle surface.

To explore the potential of these materials to be used as imaging probes, a variety of substances were encapsulated within the hydrophobic micelle cores. Micelles were loaded with superparamagnetic iron oxide (SPIO) in order to form nanoparticles with magnetic resonance (MR) contrast capabilities. Compared to previously reported dextran-coated iron oxide nanoparticles, these Dex-PCL micelles contain a significantly higher SPIO payload, which should result in greater contrast sensitivity per particle concentration. Dex-PCL micelles were also loaded with indocyanine green (ICG), a near-infrared dye that can be used for both fluorescence, absorbance, and

photoacoustic imaging. Many previously-reported ICG-containing nanoformulations are prone to leakage, due to ICG's moderate hydrophilicity; however, Dex-PCL micelles are expected to have much greater stability, due to crosslinking of the outer dextran layer. Altogether, these materials represent a promising alternative to PEG-based options in several important nanoimaging applications.

A Porphyrin-PEG Polymer With Rapid Renal Clearance

Poster Presentation. NEMB2016-5960

***Haoyuan Huang**, University of New York at Buffalo, Buffalo, NY, United States, **Weibo Cai, Reinier Hernandez**, University of Wisconsin-Madison, Madison, WI, United States, **Jumin Geng**, **Haotian Sun, Wentao Song**, University of New York at Buffalo, Buffalo, NY, United States, **Feng Chen, Stephen A. Graves**, **Robert J. Nickles**, University of WisconsinMadison, Madison, WI, United States, **Chong Cheng**, **Jonathan Lovell**, University at Buffalo, Buffalo, NY, United States*

In the past decades, there was a volatile expansion of developing nanosized probes for in vivo imaging. More and more diseases can be diagnosed fast and easily by a variety of imaging techniques. Porphyrins and tetra-pyrrolic derivatives have long been used as theranostic agents. The inherent optical properties make porphyrin and its derivatives hold great potential as probes used in these imaging techniques. Besides the novel optical properties, the high activities of the central two hydrogens of porphyrins, aromatic rings make them can be easily chelated with a lot of metals in smooth conditions. The chelation of metals improves the optical skills of porphyrins and the derivatives, and makes metallo-porphyrins become optimal probes for different imaging techniques. Although the hydrophobicity of some porphyrins limits their water solubility, it could be increased dramatically just simply by conjugating with hydrophilic polymer chains. According to these knowledge, in this presentation, we will introduce our newest finding of porphyrin-PEG conjugation polymer working as probes for detecting renal system functions and diseases. Acute renal failure was chosen in this study, due to it causes around 1-2 per thousand people suffered from this disease around the world. Current detection method is evaluating serum levels of endogenous biomolecules such as creatine, urea and cystatin C. These testing methods usually require arduous blood sample and take long time for detection. So, with these drawbacks of current ways, an efficient and fast detection method is required. In our work, tetracarboxylic porphyrins and polyethylene glycol (PEG) diamines were crosslinked in conditions that gave rise to a water-soluble porphyrin polyamide. Using PEG linkers 2 kDa or larger prevented fluorescence self-quenching. This networked porphyrin mesh was retained during dialysis with membranes with a 100 kDa pore size, yet passed through the membrane when centrifugal filtration was applied. Following intravenous administration, the porphyrin mesh, but not the free porphyrin, was rapidly cleared via renal excretion. The process could be monitored by fluorescence analysis of collected urine, with minimal background due to the large Stokes shift of the porphyrin (230 nm separating excitation and emission peaks). In a rhabdomyolysis mouse model of renal failure, porphyrin mesh urinary clearance was significantly impaired. This led to slower accumulation in the bladder, which could be visualized non-invasively via fluorescence imaging. Without further modification, the porphyrin mesh was chelated with ^{64}Cu for dynamic whole body positron emission tomography imaging of renal clearance. Together, these data show that small porphyrin-PEG polymers can serve as effective multimodal markers of renal function.

Multifunctional nanoparticles for improved stem cell function and photoacoustic tracking

Poster Presentation. NEMB2016-6066

***Isaac Adjei, Huabei Jiang, Carlos Rinaldi, Jon Dobson, Blanka Sharma**, University of Florida, Gainesville, FL, United States*

Introduction: The success of stem cell-based therapies is significantly hindered by a rapid decline in survival/function of implanted cells and limitations in clinically relevant technologies for monitoring cell fate in vivo. The overall goal of this research is to integrate a therapeutic delivery system with a novel cell imaging system to address critical barriers in translating stem cell therapies. We have developed a multifunctional nanoparticle system capable of 1) delivering nucleic acids to MSCs to direct their behavior, 2) enriching the population of engineered stem cells for in vivo implantation, and 3) providing multi-modal imaging of cells during and post-implantation, using conventional MRI and emerging photoacoustic tomography (PAT), in order to better understand their role in the regenerative process. PAT has great potential for cell imaging, as it has greater temporal and spatial resolution compared to MRI, with the cost and convenience of a hand-held scanner. Therefore, unlike MRI, PAT could be used clinically at point-of-care to aid in the implantation of the stem cells as well as quantitative monitoring/tracking the cells over time. The objective of this study was to engineer the multifunctional NP system and demonstrate proof-of-principle for gene delivery and PA imaging of MSCs.

Materials and Methods: Multifunctional NPs were formulated to take advantage of the controlled release properties of poly(lactic-co-glycolic acid) (PLGA) and the magnetic and imaging contrast properties (by MRI and PAT) of iron oxide. PLGA NPs encapsulating iron oxide NPs (IONP) and plasmid DNA (magPLGA-NPs) were formulated by a modified double emulsion method. NPs were characterized for size and morphology, as well as DNA loading and integrity. Cellular uptake of NPs and transfection with reporter gene GFP were evaluated by fluorescence microscopy and flow cytometry, while cytotoxicity was determined by MTS assay. Magnetic selection of cells that have taken up NPs was performed with magnetic-activated cell sorting (MACS) separation column coupled to a custom external magnet array. PA characterization of NPs and NP-loaded MSCs were performed with input wavelengths (λ) of 532 or 720 nm and 1 MHz motorized immersion acoustic transducer.

Results and Discussion: The magPLGA NPs were 350 nm in size, with IONPs uniformly distributed within the PLGA polymer matrix, and were responsive to magnetic fields. The formulation technique resulted in 84% DNA loading efficiency into the NPs, which was retained in supercoiled conformation, a characteristic necessary for gene expression. Encapsulation of IONPs into PLGA did not alter their absorption coefficients, resulting in linear correlation between PA signal and NP concentration for both 532 nm and 720 nm input λ, with 532 nm producing stronger PA signal. MagPLGA-NPs were taken up by MSCs without any cytotoxicity, and provided sustained expression of the encapsulated plasmid DNA. Significantly, the NPs were taken up by MSCs at levels sufficient for magnetic selection of the cells at 86% efficiency, which will enable the enrichment and use of only the engineered MSCs for downstream applications and/or allow active targeting of MSCs to tissues of interest using external magnetic field. Loading NPs into MSCs enabled their imaging by PAT with 200,000 readily imaged with 532 nm and 720nm input λs. Based on cellular uptake studies and PA signal from magPLGA-NPs, we estimate a detection limit of 10000 cells and 3000 cells for 720 nm and 532 nm input λs respectively.

Conclusions: This multifunctional NP platform has the potential to advance translation of stem cell-based therapies, by improving stem cell function and consistency via sustained drug/gene delivery, and providing a promising new tool to monitor cells in a clinically relevant manner.

Imaging and quantitative analysis of the nanoscaled brain interstitial space

Poster Presentation. NEMB2016-6166

***Hongbin Han**, Peking University, Beijing, Select State/Province, China*

Brain interstitial space provides the accomodation of brain cells. It occupies 20% of the total brain volume and is in width of 20-60nm. The transport process in the cortex has been widely explored and the communication between ISF and CSF in the superficial cortex was recognized. Functional lym-

phatic vessels lining the dural sinuses were identified as an important step in the drainage of the interstitial fluid into cerebrospinal fluid and the periphery. The brain ISF flow in the deep nuclei of the brain is also recently studied by using a tracer-based MRI, and a non-uniform flow was demonstrated with different distribution and flow speeds in different regions. More interestingly, the transportation of the brain ISF in the thalamus could not reach the ISS in the Caudate nucleus (Cn), and vice versa, although these two regions located so close to each other. The result indicates that the brain ISS is not a highly connected system as speculated before, and there seems to exist transportation barrier to the brain ISF flow in the ISS. The separated regions were characterized with different diffusion parameters, different distribution and clearance process of the traced brain ISF. We also demonstrated that the flow of ISF slowed significantly following neuronal activity in the thalamus. This reduction in ISF flow continued for hours and was not accompanied by slow diffusion into the ISS. The tracer-based MR imaging is a novel method developed by our group, which is the only measurement technique providing a three-dimensional visualization of the dynamic drainage flow of the brain ISF on a whole-brain scale. Gadolinium-diethylene triamine pentacetic acid (Gd-DTPA) is used to label the water in the brain ISS and trace the flow of the brain ISF [1]. The water-soluble chelate Gd-DTPA is a stable extracellular MR imaging contrast agent. After being introduced into the brain ISS, Gd-DTPA can shorten the spin-lattice relaxation time of hydrogen nuclei in water molecules within a distance range of 2.41-2.5 angstroms. These affected water molecules show a high signal on a T1-weighted MR image (T1WI), and the flow process of the traced brain ISF can be dynamically imaged using a series of MR scans [2]. To calculate the biophysical parameters of the brain ISS, the sequential MR images at various time points are co-registered and the images before injection subtracted. The net signal enhancement can be converted to the tracer's concentration using a pre-calibrated fitting curve. According to the classical diffusion equation, the diffusion coefficient D and clearance coefficient k of the brain ISS can be calculated from the concentration-time profile. Moreover, the flow properties of the traced brain ISF can be quantitatively measured and depicted as Vdmax, time toVdmax, and half-life ($t_{1/2}$,).

1. Shi C, Lei Y, Han H et al. Transportation in the Interstitial Space of the Brain Can Be Regulated by Neuronal Excitation. Sci Rep. 5:17673 (2015). doi:10.1038/srep17673.

2. Han H, Shi C, Fu Y etal. A novel MRI tracer-based method for measuring water diffusion in the extracellular space of the rat brain. IEEE J Biomed Health Inform. 18(3):978-83 (2014). doi: 10.1109/JBHI.2014.2308279.

6-2
NANOPARTICLE-BASED DELIVERY

Grand Ballroom 5:00pm - 8:00pm

Theranostic Nanoparticles for Photothermal Ablation Therapy and Fluorescence Imaging of Murine Breast Cancer

Poster Presentation. NEMB2016-5953

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Theranostic materials that can simultaneously detect and treat diseases hold great promise for treatment of debilitating diseases like cancer. The small size of nanoparticles (NP) allows for preferential accumulation in tumors due to enhanced permeability and retention effect. Our group has developed polymer dynamic organic theranostic spheres (PolyDOTS) which are near-infrared (NIR) fluorescent and generate hyperthermia upon 800 nm laser irradiation. The formulation comprises of a NIR heat generating, semiconducting polymer poly[4,4-bis(2-ethylhexyl)-cyclopenta[2,1-b;3,4-b']dithiophene-2,6-diyl-alt-2,1,3-benzoselenadiazole-4,7-diyl] (PCPDTBSe) and a NIR fluorescent polymer poly[(9,9-dihexylfluorene)-co-2,1,3-benzothiadiazole-co-4,7-di(thiophen-2-yl)-2,1,3-benzothiadiazole] (PFBTDBT10). The therapeutic formulation was optimized to achieve the highest thermal and fluorescence quantum efficiency. The PolyDOTS are spherical with hydrodynamic diameter of 120 nm and zeta potential of -16.8 mV. The NP showed excellent colloidal stability in acidic (pH 4), basic (pH 10), high salt buffer (10X PBS) as well as serum (10% FBS) conditions for over a month. The in vitro efficacy of photothermal ablation (PTA) therapy with the PolyDOTS was evaluated in non-tumorigenic mouse epithelial cells (Balb/C.CL7) as well as two types of mouse breast cancer cell lines, 4T1 and E0771 using various laser parameters and NP concentrations. An in vivo model utilized luciferase transfected murine breast cancer cells (4T1) injected into the mammary fat pad of Balb/C mice. A 100 µL NP solution at 0.1mg/mL concentration in PBS was injected into the tail-vein. The fluorescence imaging was conducted at 0, 6, and 24 h to determine optimum time for NP accumulation in a tumor. The tumors were then treated with NIR laser (K-laser 800 nm, 2W/2min) 24 h after NP injection and a response to PTA was monitored by measuring tumor size twice a week over forty days. In addition to NP + laser treatment group, NP + no laser, PBS + laser and PBS + no laser served as control groups. Our results indicated significant decrease in tumor size as well as significant increase in overall survival in NP + laser group compared to control groups. Due to preferential tumor accumulation, PolyDOTS can be of great use to highlight solid tumors, tumor margins and micro-metastasis during surgery.

Towards the generation of functionalized iron nanowires to target leukemic cells

Poster Presentation. NEMB2016-5980

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In recent years, magnetic nanowires (NWs) have been widely used for their therapeutic potential in biomedical applications. The use of iron NWs combines two important properties, biocompatibility and an anisotropy that allows remote manipulation by magnetic fields. In addition the NWs can be coated and functionalized to target cells of interest and, upon exposure to an alternating magnetic field, have been shown to induce cell death on several types of adherent cells, including several cancer cell types. For suspension cells, however, using these NWs has been much less effective primarily due to the free-floating nature of the cells minimizing the interaction between them and the NWs.

Leukemic cells express higher levels of the cell surface marker CD44, compared to normal blood cells. The goal of this study was therefore to functionalize iron NWs with a specific monoclonal antibody towards CD44 in order to target leukemic cells (HL-60 cells). This approach is expected to increase the probability of a specific binding to occur between HL-60 cells and iron NWs.

Iron NWs were fabricated with an average diameter of 50 nm and a length around 3-4 µm. Then, they were coated with bovine serum albumin (BSA) in order to conjugate them with an anti-CD44 antibody (i.e. anti-CD44-iron NWs). This antibody covalently reacts with the amine group in the BSA via the 1-Ethyl-3-3-dimethylaminopropyl-carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) coupling. The NWs functionalization was confirmed using a number of approaches including: infrared spectroscopy, Nanodrop to measure the concentration of CD44 antibody, as well as fluorescent-labeled secondary antibody staining to detect the primary CD44 antibody. To determine that the antigenicity of the CD44 antibody was maintained following its functionalization to the NWs, a series of CD44 immunoprecipitation and western blot studies were performed. To confirm that the anti-CD44-iron NWs and bare iron NWs, in the absence of a magnetic field, were not toxic to HL-60 cells, cytotoxicity assays using XTT (2,3-Bis-2-Methoxy-4-Nitro-5-Sulfophenyl-2H-Tetrazolium-5-Carboxanilide) were performed and resulted in a high level of biocompatibility.

Further studies will focus on analysis of the specificity of these newly generated iron NWs and their internalization into cells. We aim to use this method as an anti-cancer therapy to specifically target and kill leukemic cells and allow healthy differentiating blood cells to thrive.

Circulating Peptidome to Indicate the Tumor-resident Proteolysis

Poster Presentation. NEMB2016-6084

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Tumor-resident proteases (TRPs) are regarded as informative biomarkers for staging cancer progression and evaluating therapeutic efficacy. Currently in the clinic, measurement of TRP is dependent on invasive biopsies, limiting their usefulness as monitoring tools. Here we identified circulating peptides naturally produced by TRPs, and evaluated their potential to early diagnose a cancer or monitor the efficacy of anti-tumor treatments.
In a biomarker study of early tumor diagnosis, we used a breast cancer mouse model. The nature and extent of peptide cleavage by CPN was investigated by fragment profiling using nanopore fractionation and mass spectrometry. The fragment profiles in interstitial fluid correlated with concentrations of CPN-catalyzed peptides in blood samples taken from the tumor-bearing mice, healthy women, and breast cancer patients. CPN expression in the same set of samples was further examined by immunohistochemistry and immunoblotting. We showed that generation of C3f_R1310-L1319 specifically correlated with the CPN expression level. In both the mouse and clinical patient samples, CPN was clearly increased in tumor tissues compared with normal breast tissue, whereas corresponding CPN abundance in blood remained constant. Concentrations of 6 CPN-catalyzed peptides predominantly increased in sera taken from the mice (n = 8) at 2 weeks after orthotopic implantation. Six homologous peptides displayed significantly higher expression in the patients' plasma as early as the first pathologic stage of breast cancer.

In another biomarker study of evaluation of anti-tumor efficacy, we established a mouse model for ovarian cancer development and treatment by orthotopic implantation of the human drug-resistant ovarian cancer cell line HeyA8-MDR, followed by porous silicon particle- or multistage vector (MSV) - enabled EphA2 siRNA therapy. Immunohistochemistry staining of tumor tissue revealed decreased expression of matrix metallopeptidase 9 (MMP-9) in mice exhibiting positive responses to MSV-EphA2 siRNA treatment. We demonstrated, via an ex vivo proteolysis assay, that C3f peptides can act as substrates of MMP-9, which cleaves C3f at L1311-L1312 into two peptides (SSATTFRL and LWENGNLLR). Importantly, we showed that these two C3f-derived fragments detected in serum were primarily generated by tumor-resident, but not blood-circulating, MMP-9.
Our results suggested that the presence of the circulating fragments specially derived from the localized cleavage in tumor microenvironment can be used for early diagnosis of cancer or evaluation of therapeutic efficacy of anti-cancer treatment, assessed through a relatively noninvasive and user-friendly proteomics approach.

Multi-edged lightning silica nanoparticles preparation and its application on bio-imaging

Poster Presentation. NEMB2016-5933

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Amongst inorganic nanoparticles, silica nanoparticles named as "GRAS" (Generally Recognised As Safe) due to its biocompatibility being endogenous to most living organisms[1]. Recent activities on development of biomedical imaging and therapy, more efforts towards the design and synthesis of functionalised silica nanoparticles with various prominent properties. In this work we have evaluated a microemulsion technique for incorporation of fluorophore into the silica matrices, optimised it for enhanced encapsulation and free from monomers. Highly fluorescent silica nanoparticles with different edges, intended for fluorescent cell imaging, were prepared via simple modification. Transmission Electron Microscopy, Fourier Transform Infrared Spectroscopy, Dynamic Light Scattering, and Zeta Potential measurements were used to characterize the novel silica nanoparticles by particle size, functionality, and morphology, as well as by ζ-potential and physical stability. Interaction of the protein for each of the different nanoparticle terminals was unique, indicating a strong dependence on surface chemistry[2]. Our studies indicate that the silica matrix inhibits the aggregation and decomposition of cores with outside environment leads to efficient bio-imaging property. Furthermore the particle toxicity, indicated by membrane integrity and mitochondrial activity was measured by lactase dehydrogenase (LDH) release, and tetrazolium reduction (MTT), in both normal and cancer cells. As a result compared to bare dye, multi edged encapsulated dyes showed less toxicity to normal cells which is high toxic to cancer cells.

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Preparation of a Nano Structure Generation 4.5 Poly (amidoamine) Dendrimer and Monoclonal Anti-IL-6 Antibody Conjugate as Bioimaging Probe and Drug delivery

Poster Presentation. NEMB2016-5917

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In this study, interleukin-6 (IL-6)-conjugated anionic generation 4.5 (G4.5) poly (amidoamine) (PAMAM) was synthesized through EDC/NHS coupling chemistry followed by encapsulation of anticancer drug. Chemical interactions were confirmed using ζ potential, UV-visible, emission fluorescence and NMR spectroscopy. After IL-6 conjugation, nanoparticles size increased to approximately 70 nm by forming core-shell nanostructure and zeta potential increased from -56.5 ± 0.2 to -19.1 ± 2.4 mV due to neutralization of negatively charged G4.5. Wide-angle X-ray scattering (WAXS) suggested that a layered nanoparticles structure was formed by the G4.5/IL-6 conjugate. Furthermore, the cellular uptake of the conjugates by HeLa cells was significantly enhanced in comparison to free G4.5; these results indicated that the described system may be a potential bioimaging probe in vitro. The drug encapsulation efficiency of the dendrimer-antibody conjugates was found 51.3 %. The cellular uptake and the binding efficiency of drug in the dendrimer-antibody conjugate were also evaluated by confocal microscopy and flow cytometry in vitro against cervical carcinoma (HeLa) cell lines.

Chitosan Coated PLGA Nanoparticle as Drug Delivery System

Poster Presentation. NEMB2016-6129

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Poly (lactide-co-glycolide) (PLGA) based microparticulate formulations have increasing attention in delivery applications due to theirs capability for sustained and controlled drug release characteristics as well their biocompatible and biodegradable properties. However due to the shortage of active functional groups on surface of PLGA, the wide applications of these formulations are always limited in targetable drug delivery. In this research, PLGA nanoparticles were modified with chitosan (CS), a cationic polysaccharide bears repetitive amine groups in backbone through physical adsorption. We

used a model drug, Diclofenac (DS) a nonsteroidal anti-inflammatory drug (NSAID), which is commonly utilized in the treatment of arthritic disorders, to study the drug release characteristics by using this nanoparticle. We first developed DS encapsulated CS coated PLGA nanoparticle and use in vitro set up to study drug release properties. PLGA nanoparticles were synthesized by double- emulsion solvent evaporation technique. The resulted nanoparticles were evaluated based on their particle size, zeta potential, entrapment efficacy, FTIR, and effect of pH in drug release study. About 400 nm of average diameters and uniform morphology of the particles were confirmed by scanning electron microscope (SEM) imaging and dynamic light scattering (DLS) measurement. Chitosan coating over PLGA surface was confirmed by FTIR study and positive zeta potential value for coated sample. Drug entrapment efficacy was up to 60%. Chitosan coated sample showed a pH responsive drug release in in-vitro. The release was about 60% more at pH5.5 than at pH7.4. The results of our study indicated the development of chitosan coating over PLGA nanoparticle for pH dependent controlled release DS drug for therapeutic applications.

Object Shape and Elasticity Impact Interactions in the Tumor Microenvironment

Poster Presentation. NEMB2016-6146

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Cancer chemotherapy necessitates the contrivance of a therapeutic system to successfully negotiate biobarriers and efficiently deliver therapeutics to the diseased cells without affecting healthy sites. In Nature, blood-borne objects like erythrocytes, leukocytes and platelets possess non-spherical shape and elasticity due to which they exhibit various circulatory patterns, biological interactions as well as the ability to extravasate through fenestrations in the vascular endothelium, in response to stimuli in the tumor microenvironment. We hypothesize that systemically administered particles with cell-like physical attributes such as geometry and elasticity can likewise, marginate through vasculature, extravasate through endothelial fenestrations that are much smaller than their dimensions, and interact with the cells in the tumor microenvironment. For this purpose, we engineered biocompatible, Elastic Microparticles (EM), which are “soft” hollow polymeric microcapsules (shells) with spherical and cubical (edge-possessing) shapes by layer-by-layer (LbL) assembly of hydrogen-bonded tannic acid/ poly(N-vinylpyrrolidone)/ (TA/PVPON) polymers on sacrificial templates. We compared the 2 micron-sized spherical and cubical shells with their rigid counterparts displaying the same surface properties - core shells or Rigid Microparticles (RM), in their interaction with cells present in the tumor microenvironment namely, macrophage (J774A.1), endothelial (human microvascular endothelial, HMVEC) and breast cancer cells (4T1, MDA-MB-231 and SUM159). The hollow elastic shells are unrecognized by macrophage cells in contrast to the rigid core-shells that are internalized within 15 minutes of contact, whereas there is no significant difference between the uptake of spherical and cubical core-shells or shells. Cubical shells are internalized 5-fold more efficiently by human microvascular endothelial cells in static conditions and 2.5 and 6 fold more efficiently by breast cancer cells (SUM159 and MDA-MB-231, respectively). At physiological flow conditions similar to the tumor vasculature, shear rate of 100 s-1, the cubical objects interact substantially more than spherical objects, but at a normal vascular shear rate of 10 s-1, shape does not seem to play a significant role. We also explored the extravasation potential of the polymeric systems by passing them through a nylon membrane with 0.8 micron-sized pores, resembling the fenestrations in the tumor endothelium and found that the EM are capable of squeezing thorough pores one-third their dimension unlike RM that were retained on the membrane.

Our study demonstrates that cubical shape favours the interaction with breast cancer cells and elasticity prevents recognition by immune cells. Our study signifies how the various mechanisms occurring in nature can be applied in overcoming the multiple barriers encountered in drug delivery and the employment of cell-like microparticles as tools to investigate the various phenomena in biological interactions.

Polyvinylpyrrolidone Coating of Ironoxide nanoparticles as magnetic drug delivery agents

Poster Presentation. NEMB2016-6150

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Iron-oxide particles recently are researched for the potential applications of targeted drug delivery due to the magnetic properties. The surfaces of the iron-oxide particles must be modified to reduce the toxicity and to load the drug to the particles. Biopolymers are good surface modifier of the colloidal particles such as iron-oxide particles. The degree of surface coverage of the colloidal iron-oxide particles affects the stability, toxicity, magnetic properties and drug loading efficiency. In this study, the interactions of iron-oxide (Fe_3O_4) particles and PVP were determined with the colloidal properties. The proper concentration of PVP for the whole coverage of the iron-oxide particles was found for the possible magnetic drug delivery applications by controlling the colloidal properties of the dispersions. Magnetic properties and toxicity of the fully covered bioiron-oxide was also determined for the possible applications.

Colloidal Properties and in-Vitro Evaluation of Hydroxyl Ethylene Cellulose Coated Iron Oxide Nanoparticles for Targeted Drug Delivery

Poster Presentation. NEMB2016-6151

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In this study, superparamagnetic iron oxide (Fe_3O_4) nanoparticles were prepared for the targeted drug delivery applications by controlling the colloidal properties with cellulosic polymer, Hydroxyethyl cellulose (HEC). Fe_3O_4 particles were treated with HEC in a variable range of polymer concentration. Rheological, electrokinetical, magnetorheological and morphological properties of the dispersions were investigated to have stable and fully covered surfaces of Fe_3O_4 particles by coating with HEC and to obtain non-toxic biocompatible multifunctional magnetic particles. The fully HEC covered iron-oxide particles were thermally, magnetically characterized and tested for toxicity. The cancer drugs, DOX, were adsorbed on the particles. The effects of these particles on the cancer cells were examined to report a nanodrug system, which is potentially open up new possibilities in the design of therapeutic agents.

Engineering Gold Nanoconstructs for Targeted Delivery and Controlled Release of Antibiotics

Poster Presentation. NEMB2016-6068

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Drug delivery systems with targeted capability and on-demand controlled release mechanism are particularly appealing for designing optimal medications in many disease treatments. Controlled release systems for drug delivery using nanocarriers have been developed and studied for more than three decades. Gold nanostructures have been used as drug delivery vehicles in chemotherapy because of their biocompatibility, facile surface

modification, and robust optical properties. While drug molecules can be covalently immobilized on the nanoparticles' surface, non-covalent interactions are particularly appealing because they minimize modification of the drug molecules, whose efficacy is then largely retained upon release. In this work, we have developed a controlled-release system for delivery of antibiotics based on gold nanoconstructs and demonstrated the synergistic effect of photothermal and antibiotic therapies. The gold nanoconstructs are capable of converting light into heat and triggering the release of the encapsulated antibiotics. Upon light irradiation, the photothermal effect of gold nanoconstructs can initiate an instantaneous release, and thus control of the release kinetics, demonstrating on-demand drug release. The surface of these gold nanoconstructs can be readily functionalized with specific moieties for targeting biomarkers at the pathological sites. We have demonstrated that an appropriate antibiotic (daptomycin) can be incorporated into gold nanoconstructs and that daptomycin-loaded gold nanoconstructs can be conjugated to antibodies targeting a species-specific surface protein (staphylococcal protein A) as a means of achieving selective delivery of the nanoconstructs directly to the bacterial cell surface. We demonstrated that laser irradiation at levels within the current safety standard for use in humans can be used to achieve both a lethal photothermal effect and controlled release of the antibiotic, thus resulting in a degree of therapeutic synergy capable of eradicating viable bacteria. The use of this nanoconstruct to synergize these therapeutic modalities was successfully demonstrated for the methicillin-sensitive S. aureus strain UAMS-1 in planktonic culture and more importantly for the methicillin-resistant S. aureus strain LAC in both planktonic culture and a clinically-relevant biofilm model. The system was initially validated using planktonic bacterial cultures and was subsequently shown to be effective in the context of an established biofilm, thus indicating that this approach could be used to resolve intrinsically-resistant biofilm infections. Further utilizing the optical properties of gold nanoconstructs, these systems can achieve theranostics through diagnosis via gold nanoconstructs as contrast-enhanced molecular imaging and multi-modal treatment via photothermal and antibiotic therapies.

Combinatorial Approach for Targeting Anti-Tuberculosis Treatment via Geometry-Based Nanovectors and Thioaptamers

Poster Presentation. NEMB2016-6148

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Tuberculosis (TB) remains the most frequent and important infectious disease causing morbidity and death worldwide. One-third of world's population is infected with Mycobacterium tuberculosis (MTB), the etiologic agent of TB, which usually resides in the alveolar macrophages. The major problem in eradicating TB is caused by the ability of Mycobacterium to survive and replicate inside macrophage phagosomes, and current tuberculosis treatment regimen requires at least 6 months of chemotherapy. The long course of treatment causes low compliance and often leads to the selection of multidrug resistant strains. The scientific community generally agrees that the key to improving TB therapy relies on shortening its duration and making it more effective. In this study we propose for the first time, that hemodynamics in the lung and the pathological changes in the infected cells and inflamed vascular structures result in the alteration of the macrophage/vascular endothelium phenotype (e.g., surface receptor expression), causing the selective targeting of the nanovectors to the infected tissue.

We used thioaptamers (TA) with E-selectin (ESTA) and a CD44 targeting moiety, selectively based on the combinatorial approach and conjugated to discoidal silicon mesoporous particles (SMP) nanovectors. ESTA-SMP and CD44TA-SMP were tested in vitro for targeting human macrophages infected with M.smegmatis, M.avium and M.tuberculosis were analyzed for the particle uptake, resulting macrophage mortality, and the cytokine release. Further, the formulations were tested on balb/c mice pre-infected with M. tuberculosis for 14 days, and mice were sacrificed 2 hour and 8 hours after treatment. Silicon particle disposition was analyzed by inductively coupled plasma atomic emission spectroscopy (AES-ICP) as well as by fluorescence microscopy of tissue sections. Colony forming units (CFU) of M. tuberculosis were assessed by growing tissue homogenized on Middlebrook 7H11 agar plates and counting after 2 weeks of growth.

In vitro, the uptake and killing efficacy increased along with increasing pathogenicity of Mycobacterium strains. 40 and 25 pSi-ESTA and pSi-CD-44TA were taken up, respectively, by M. tuberculosis-infected macrophages. In control macrophages and M. smegmatis-infected macrophages, the numbers were significantly lower, ranging below 10, while M. avium-infected macrophages ingested around 10 and 15 particles. Higher number of ingested particles led to higher cell death, where less than 50% of M. tuberculosis- and M. avium-infected macrophages survived the treatment with both formulations. Furthermore, in vivo results showed a 4-fold and almost 2-fold accumulation of pSi-ESTA and pSi-CD44TA in the lung of infected mice, but no increased accumulation in uninfected mice. Further analysis of the lung tissue also revealed an increased T-cell accumulation with the treatment, where 2-times and 4-times amount of T-cells were found in pSi-ESTA- and pSi-CD44TA-treated lung of infected mice after 8 hours, respectively, when compared to the control infected mice. Additionally, the treatment, even without any drug content, was proven to be able to reduce the number of live M. tuberculosis found in the lung of the infected mice by 70% within 8h. The nanovectors developed in this study were proven to enhance accumulation to alveolar macrophages based on targeting moieties and preferential localization in the lungs based on their geometry. The mechanism of the designed system may include a combination of increased uptake of particles leading to the infected macrophage death, as well as activation of immune response to TA, causing an increased T-cell accumulation in the treated lungs. Ongoing studies with TA-SMP with anti-TB agents can open new avenues in the TB management. This can potentially reduce the length of treatment and increasing the drug concentration, thus avoiding multi-drug-resistance due to long-term exposure to antibiotics.

Mathematical Modeling for Dual Ligand Conjugated Micro/Nano Particle Adhesion for Targeted Drug Delivery

Poster Presentation. NEMB2016-6135

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Drug delivery system based on micro/nano particles is one of the promising techniques for targeting cardiovascular diseases or cancers (Ferrari, 2005). To avoid side effects and toxicities of the drug, it is important for the particles to be attached to the diseased cells specifically. To enhance the efficiency and specificity of the drug delivery, many studies on the surface modified particle have been performed by several research groups through attaching adhesion-related ligand or antibody (Farokhzad et al., 2004, 2005, Paulis et al., 2012, Kolhar et al., 2013). The ligands conjugated onto the surface of the particle specifically bind to their receptors expressed on the inflammatory cells. Moreover, multiple molecules are expressed on the diseased cell and it is possible to choose several adhesion-related molecules for targeting. For example, for leukocyte binding to the inflammatory endothelium, the adhesion procedure is related to two types of molecule, i.e. ICAM and selectin family. Thus, dual ligand-receptor interaction model for the specific targeting of the particle to the cell needs to be considered for particulate system design. The stochastic approach of the adhesion of two different ligands conjugated particle to the cell under the hydrodynamic flow is derived mathematically, assuming that each ligand-receptor interacts specifically and independently. It is verified that there are several parameters affecting on the adhesion of the particle to the cell. First, a separation distance determined

by the force balance between the non-specific attractive force and the specific repulsive force modeled as the elastic spring that supports the particle is considered. With the stiff ligand-receptor pair, the equilibrium distance is lengthened so that the hydrodynamic force on the particle increases. Secondly, the number of the receptors on the cell could be an important factor on the probability of adhesion. When the number of the receptors expressed on the cell increases, the receptors could easily interact with the ligands conjugated on the particle so that the probability of adhesion is expected to increase. Third, the kinetic binding affinity between the ligands and the receptors is one of the important parameters on the probability of adhesion. The higher kinetic affinity, the higher probability of adhesion is expected. Lastly, the different length of each ligand-receptor pair is an important factor. In this presentation, considering the biophysical conditions of dual ligand-receptor pairs that influence on the particle-cell adhesion probability, the several design parameters for multiple ligand conjugated micro/nano particle based delivery system are suggested.

Gold Nanovaccine Platform for Delivery of Tumor-Associated Antigens

Poster Presentation. NEMB2016-6020

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The goal of cancer vaccines is to stimulate the immune system such that tumor cells expressing a particular tumor-associated antigen are recognized and destroyed. However, vaccinations with peptide antigens have failed to elicit sufficiently strong anti-tumor immunity due to poor delivery to antigen presenting cells. Gold nanoparticles (AuNPs) are ideal carriers to address this delivery hurdle because AuNPs can be easily functionalized with peptide antigens and naturally distribute to the spleen and relevant immune cell populations therein.

Previously, our group used gold nanoparticles to improve delivery of the exogenous peptide antigen ovalbumin (OVA) in vivo. The AuNP-mediated delivery of the OVA peptide resulted in significantly stronger antigen-specific immunity and anti-tumor activity compared to OVA alone. AuNP-OVA treatment prevented tumor formation and extended survival in prophylactic and therapeutic B16-OVA tumor models [1]. We have since expanded upon this initial success of our gold nanovaccine platform by demonstrating improved antigen-specific immunity when delivering the melanoma-associated antigen, Trp2.

Trp2 peptides were conjugated to 30 nm gold nanoparticles via an intermediate polyethylene glycol (PEG) layer and EDC/Sulfo-NHS chemistry. To evaluate the immune response in vivo, C56BL/7 mice were primed on day 0, boosted on day 7, and their spleens were harvested on day 17. An Enzyme Linked ImmunoSpot (ELISpot) assay illustrated the amount of IFN-γ producing splenocytes following incubation with Trp2 peptides, which corresponds to the antigen-specific immune response. AuNP-Trp2 demonstrated significantly improved antigen-specific immunity compared to administration of Trp2 alone ($p<0.01$).

Our results indicate that the improvement of antigen-specific immunity imparted by gold nanoparticle delivery is sustained when incorporating an endogenous tumor-associated antigen, Trp2. This result is important in two ways. First, the hydrophobic Trp2 peptides are more difficult to incorporate on AuNPs because the hydrophobic surface interactions can lead to particle instability under certain synthesis conditions. Thus, our successful synthesis and administration indicate that we can incorporate a range of peptides with our established synthesis approach. Second, it is more difficult to elicit antigen-specific immunity of endogenous tumor-associated antigens due to immune tolerance mechanisms. Thus, the ability of AuNP-Trp2 to induce strong immunity toward a melanoma-associated antigen demonstrates the potential for clinical translation of gold nanovaccines.

To further substantiate the effectiveness of gold nanovaccines, studies evaluating the anti-tumor properties of these AuNP-Trp2 particles are underway. In addition, because these gold nanovaccines improve antigen delivery, their mechanism of action is complementary to those of checkpoint inhibitors such as PD-1, making them good candidates for combination therapies as their effects are predicted to be synergistic. Our aim is to continue to demonstrate the ability of the gold nanovaccine platform to result in specific and clinically relevant immune responses for use in cancer vaccine therapies.

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Co-delivery of siRNA and anti-cancer drug using polymeric nanoparticles

Poster Presentation. NEMB2016-6120

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The development of acquired chemoresistance is a persistent clinical problem limiting the successful treatment of malignancies and considerable work has been done to identify the molecular mechanisms involved. Many possible mechanisms have been suggested for anti-cancer drug resistance emergence, such as drug efflux, apoptosis inhibition among others. Due to the lack of effective inhibitors and the undruggable feature of some targets, it is not easy to screen and validate the target combinations, especially in vivo. Since synthetic siRNA has emerged as a class of attractive therapeutics for treatment of various cancers by silencing genes that cause diseases. Given the ability to target and silence nearly any gene of interest, specific siRNA can be constructed to target genes encoding proteins involved in DNA repair and the acquisition of multidrug resistance. Although a powerful means to silence the expression of target genes, the safe and effective systemic delivery of siRNA therapeutics remains an important challenge. Herein we have developed an innovative chemo-RNAi nanotherapeutic strategy that can simultaneously deliver platinum drugs and multiple siRNAs. We hypothesize that the NP-mediated delivery of multiple siRNAs against different platinum resistance mechanisms will allow the validation of key targets. We aim to improve the efficacy of chemotherapy through additive or synergistic effects coupled by platinum drug and siRNAs targeting different drug resistance mechanisms. As proof-of-concept, we have used poly(lactide-co-glycolide)-b-poly(ethylene glycol)-lipid hybrid nanocarriers as a delivery platform, translesion DNA polymerases Rev1/3L as model target proteins responsible for platinum resistance, and prostate cancer as a model disease. We have successfully created polymer-lipid NPs that exhibited simultaneous entrapment of siRNA (up to 99%) and cisplatin (around 10%). The size of the NPs ranges from 180 to 220 nm and he drug release profile shows a controlled release of cisplatin and siRNA over 10 days. We demonstrated the potency of the siRNA-containing NPs to efficiently knockdown target genes both in vitro and in vivo.

Predicting Therapy Response of Pancreatic Cancer Patients by Evaluating Tumor Hypoxic Status with Circulating Hydroxylated Bradykinin in the Blood

Poster Presentation. NEMB2016-6083

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Background: Using hypoxic status to predict outcomes in patients undergoing chemotherapy and radiotherapy meets impediments in clinic due to the lack of a reliable, accurate, prompt, and noninvasive detection methodology. In the blood, the circulating peptide bradykinin (BK) and its hydroxylated derivative hyp-BK can directly reflect the activity of prolyl 4-hydroxylase subunit alpha-1(P4HA1), which is tightly regulated by hypoxia. In the current study we

propose to use the ratio of hydroxylated bradykinin to bradykinin (hyp-BK/BK) in the blood as a reliable hypoxic marker for predicting the prognosis of anticancer treatment for pancreatic cancer.

Materials and Method: We first tested the regulation of hypoxia on P4HA1 in multiple pancreatic cancer cell lines by Western blot, quantitative real-time PCR, and chromatin immunoprecipitation (ChIP). An in vitro assay for evaluating the activity of P4HA1, combined with nano-trap and MALDI-MS, was established to correlate the hyp-BK/BK ratio and hypoxia. Finally, clinical serum samples from pancreatic cancer patients before any treatment were evaluated for the levels of hyp-BK/BK, which was subject to correlation analysis to these patients' prognosis after anticancer therapies.

Results: Hypoxic pancreatic cancer cells showed elevated levels P4HA1, as shown by increased mRNA and protein levels of P4HA1 and HIF-1 (hypoxia-inducible factor-1). ChIP and immunofluorescence microscopy confirmed that P4HA1 was subject to direct regulation by HIF-1. Importantly, immunoprecipitated P4HA1 from COLO-357 cells cultured under hypoxic conditions produced significantly higher levels of hyp-BK/BK. Furthermore, when we included a HIF-1-specific inhibitor, digoxin, under the hypoxic conditions, the ratio was significantly reduced, suggesting that hypoxia directly affected the readout of hyp-BK/BK. We could demonstrate that the hyp-BK/BK ratio provided much more consistent results compared with the use of either hyp-BK or BK alone, as multiple cycles of freezing/thawing and prolonged storage of samples would not affect the readout of the hyp-BK/BK ratio. Measurement of hyp-BK/BK in 22 clinical pancreatic cancer samples has shown that there was a significant correlation between high levels of hyp-BK/BK and poor treatment response. The marker is specific to the pancreatic cancer because significantly elevated level of hyp-BK/BK was only observed in clinical blood samples from the cancer patients, but not in normal or pancreatitis samples.

Conclusions: Hypoxia positively regulates HIF-1 and its downstream effector P4HA1 in pancreatic cancer cells. Consequently, the major P4HA1 catalytic product hyp-BK may be released into the blood. The ratio of hyp-BK to the unmodified BK (hyp-BK/BK) in the blood may serve a hypoxia-specific marker and could perform consistently in clinical sample measurement by MALDI-TOF MS. Results from clinical measurement support the notion that the pre-therapy levels of hyp-BK/BK are significantly elevated in pancreatic cancer patients who later showed poor response to anticancer therapies.

Leukocyte Membrane Cloaking Enables Synthetic Carriers to Target and Overcome Tumor Vasculature

Poster Presentation. NEMB2016-6054

Michael Evangelopoulos, Roberto Palomba, Alessandro Parodi, Claudia Corbo, Mauro Ferrari*, Houston Methodist Research Institute, Houston, TX, United States,* ***Francesco Salvatore****, CEINGE Biotecnologie avanzate, Naples, Italy,* ***Ennio Tasciotti****, The Methodist Hospital Research Institute, Houston, TX, United States*

The lack of an efficient targeted transport of chemotherapeutics remains one of the primary reasons for poor cancer patient survival. Over the past decades, nanomedicine has provided several delivery platforms able to enhance the delivery of chemotherapeutics. Nanoparticles provide an efficient means to modulate the biodistribution of injectable drugs and to favor their accumulation at tumor sites. Nanoparticles accumulation mainly relies on exploiting the superior permeability of tumor vasculature, a phenomenon commonly referred to as the enhanced permeability and retention (EPR) effect [1]. However, the EPR effect is not characteristic of all tumor vasculature, prompting a deeper investigation into alternative vector-associated modifications and tumor characteristics. In particular, cancer vasculature provides several opportunities to develop targeted therapies by leveraging the adhesive proteins over-expressed on the inflamed vasculature, such as intercellular adhesion molecule-1 (ICAM-1). In tumor-associated vasculature, ICAM-1 overexpression fosters the recruitment of immune cells to the inflamed tissue in an attempt to restore homeostasis. The activation of the ICAM-1 pathway through surface interactions between leukocytes and inflamed vasculature induces the loss of endothelial intercellular junctions, thereby favoring immune cell infiltration. Immune cells have been demonstrated to employ adhesive membrane proteins to target inflamed vasculature, locally increase vascular permeability, and extravasate across inflamed endothelium during leukocyte diapedesis. Inspired by the physiology of circulating leukocytes, we recently developed a procedure to transfer leukocyte membranes onto biocompatible Multistage Nanovectors (MSV) [2], yielding Leukolike Vectors (LLV) [3]. The LLV coating contains bioactive adhesive molecules, such as lymphocyte function-associated antigen-1 (LFA-1) and macrophage-1 antigens (Mac-1), which are critical in interacting with inflamed endothelium and have been shown to increase blood vessel permeability by activating the ICAM-1 pathway. Herein, we provide a comprehensive analysis demonstrating that the transfer of leukocyte membrane proteins onto LLV maintains their biological functions. We demonstrated that the LLV effectively interacted with receptors on the endothelial surface, facilitating enhanced transport into the subendothelial space. We demonstrated that using the same mechanisms as leukocytes, LLV preferentially accumulated at the cell border under physiological flow conditions in vitro, and was able to increase tumor blood vessel permeability in vivo. We demonstrated that the purified leukocyte membrane coating maintained an active role in the interaction with tumor associated blood vessels, leading to an increase in vascular diffusion into the subendothelial space. Furthermore, these steps are critical to further define and exploit the leukocyte features that are fundamental for drug delivery. Among these, the targeting and bioactivity towards inflamed vasculature provides promising clinical significance, as this is a characteristic of a multitude of diseases, including cancer.

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6-3
NANO AND MICROFLUIDICS

Grand Ballroom 5:00pm - 8:00pm

Local and Sustained Delivery of Tamoxifen for the Prevention of ER+ Breast Cancer Using a Nanochannel Delivery Platform

Poster Presentation. NEMB2016-5961

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A high incidence (~75%) of primary breast cancers are estrogen receptor positive (ER+), and a large fraction of these patients can pursue chemopreventive therapies. However due to adverse side effects, only 5% to 20% of the tens of thousands of women at high risk who could benefit from chemotherapeutics enroll in preventive treatment. There is a clear need for alternative preventive strategies that minimize side effects and improve enrollment and compliance. Selective estrogen receptor modulators, such as tamoxifen (TMX), have been shown to reduce ER+ breast cancer incidence by up to 50% among high-risk women. Importantly, along with raloxifene, it is one of only two FDA-approved drugs for breast cancer prevention. TMX has already been in use for over 40 years and has a proven record in pre- and post-menopausal women. However, the drug is marred by side effects, the most common being symptoms of menopause. Further, women treated systemically and chronically with TMX were found to have an increased inci-

dence of endometrial carcinoma. Although considered rare, this side effect, along with other serious adverse effects (such as blood clots, strokes, and cataracts), has resulted in a debate concerning the benefits versus risks of TMX use in cancer prevention.

As the key to success for breast cancer chemoprevention relies upon long-term delivery of drugs while circumventing side effects, we have developed a novel local delivery strategy for the constant and sustained administration of TMX. We maintain a long-term, local release of TMX in mammary tissues by utilizing a novel implantable nanochannel Delivery System (nDS). The nDS consists of a bioinert, implantable, and mechanically robust silicon membrane which houses an exact number of densely packed slit-nanochannels as small as 2.5 nm with tight tolerances on size, geometry, and surface properties. Providing steady levels of TMX at the mammary gland target tissue through nDS delivery maximizes the therapeutic index while limiting the unwanted secondary effects, which will ultimately improve patient compliance.

In this work we chemically induced tumorigenesis in Sprague-Dawley rats by N-methyl-N-nitrosourea (NMU) injection to promote the development of estrogen-dependent tumors. We then performed ovariectomies seven days after NMU injection to mimic post-menopausal biology. nDS implants loaded with either TMX or PEG400 (negative control) were inserted under the left abdominal mammary gland to determine the effects of nDS-TMX on tumor growth and tumor biomarkers. Utilizing LC/MS we were able to quantitatively determine the amount of TMX released from the nDS into the plasma. Rats were also examined for palpable tumors to assess breast tumor incidence, latency to onset, and multiplicity. Our results show that the nDS implant enables the effective delivery of TMX in this breast tumor model. Further, this technology has the potential to rapidly provide long-term breast cancer protection with significant improvement in the quality of life of patients at high risk, thereby saving thousands of lives every year.

Nanotrap-based assay for absolute quantification of iron regulator-hepcidin from human bodily fluids

Poster Presentation. NEMB2016-6082

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Endogenous serum peptides that carry important information of disease are considered to be great potential biomarkers for clinical diagnosis. However, due to the extremely high dynamic range of protein concentration in serum and the interference of highly abundant and large proteins, the detection of the serum peptide biomarkers remains a challenge. Herein, we developed a silica nanopore-based (Nanotrap) assay to selectively enrich and quantify a low-abundance peptide, hepcidin, from human body fluids (HBF).
Hepcidin consists of 25 amino acids (Hep-25) that could down-regulating cellular iron. Pathological excess or deficiency of hepcidin could lead to a variety of iron disorders and be used as a diagnostic tool in clinic. Thus, we believe the measurement of hepcidin levels in HBF is urgently needed to facilitate the personalized medicine. Several methods have been developed for quantifying hepcidin. However, antibody-based method are lack of the selectivity to differentiate Hep-25 from the other two N-terminal truncated hepcidin isoforms, Hep-20 and Hep-22, which are not expected to play significant roles in iron metabolism. In regards to the MS-based methods still require large volume sample and time-consuming pre-treatment. In this study, we developed a high-throughput approach for peptides extraction using Nanotraps with different nanotextures (pore size, surface, and structure) specifically and precisely tailored for hepcidin enrichment. We further investigated the mechanisms of hepcidin enrichment in nanopores, including size-exclusion, surface charge, and pore morphology effects, and provide a basis for understanding the interaction of the target peptide with NPS thin films, which is highly useful for adapting this material for a variety of biomedical and clinical applications by using chemical functionalization of nanotextured surfaces. The silicone masks were placed on top of the NPS films to normalize the area of sample exposure. Serum and urine samples were first spotted into each well and then incubated at room temperature. Only small proteins and peptides can diffuse into the nanopores, while large proteins are excluded and subsequently removed by washing. The small peptide fractions were extracted by elution buffer. Using this procedure, Hep-25 can be enriched in the optimized nanopores, and then analyzed by MALDI-TOF MS. To achieve absolute quantification of our target peptide biomarker, we introduced heavy Hep-25 (Glu1-Thr17-Hepcidin) into human serum samples as internal standards. Our method requires only microliter sample volume and eliminates time-consuming sample pretreatment, while still maintaining a high degree of precision, accuracy, and sensitivity. In a clinical validation of our technique, Hep-25 levels were quantified in both serum and urine from 119 healthy volunteers and 19 patients suffering from inflammation. The levels of hepcidin were found to be gender, menopausal, and inflammation status dependent. The application of and proof-of-concept for this new approach could help not only to improve our understanding of iron disorder diseases, but also consequently enable hepcidin to become a viable diagnostic biomarker for clinical uses.

ILISA for the Diagnosis of Infectious Diarrhea

Poster Presentation. NEMB2016-6016

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Infectious diarrhea or gastroenteritis is one of the leading causes of death worldwide, particularly in children. Viral, bacterial, or parasitic infection can all lead to serious and long lasting syndrome. Early and accurate diagnosis and targeted therapy will undoubtedly improve the survival rate. A growing list of biomarkers, including proteins, genes, and carbohydrates from both the patients and the pathogens, has been indicated to have great potential in early detection of infectious diarrhea. However, a more sensitive, specific, and reliable method for clinical diagnosis is still lacking. In this study, we developed an antibody-based iron oxide-linked immunosorbent assay (ILISA) for the detection of virus- and bacteria-induced diarrhea. By combining nanotechnological tools with serum biomarkers for diagnosis and prognosis, our method not only has the aforementioned sensitivity, specificity, and reliability, but also shows significant advantages over diagnostic methods clinically used.

Rotavirus has been the most common cause of acute gastroenteritis requiring hospitalization among children. However, immunity to rotavirus is still very poorly understood. Tests including rotavirus-specific IgG, stool IgA, and neutralizing antibodies have been used in the past, but data in humans are conflicting. Serum IgA is the most widely used correlate. Here we developed a novel assay for IgA detection, which takes advantage of the dense atom packing in iron oxide nanocrystals. By combining acid-dissolution of nanocrystals and a ferrous ion-induced stoichiometric chromogenic reaction, our method can easily achieve high signal amplification. To be brief, we synthesized iron oxide nanoparticles of different sizes and conjugated to antibodies against human IgA. By optimizing various experimental conditions, including the species of detection and capture antibodies (mouse, rabbit, or goat), the concentration of the reducing agent (2-Mercaptoethylamine HCl) for antibody reduction, the selection of antibody fragments (half IgG or Fab') for conjugation, the concentration of nanoparticles used for detection, and the incubation time, we can accurately reach the concentration of IgA as low as 1.5 pM with high reliability and repeatability. With nanoparticles of larger diameter, we further increased our detection sensitivity to 0.2 pM.
Several bacterial pathogens can also cause serious diarrhea and lead to death due to severe dehydration. Clostridium difficile (C. difficile) infection is the most commonly reported case in hospitalized patients. Traditional stool test measures stool leukocytes and lactoferrin levels, which provides low accuracy. In our study, we provide a novel and reliable diagnostic method by assessing the level of C. difficile toxins in serum. Different from rotavirus infection, we used biomarkers derived from the pathogens as the analyte,

which is more specific. Besides, our nanoparticle-based detection assay is fast and easy to perform and could become the new gold standard for clinical diagnosis of infectious diarrhea.

Micro-Sample Exosome Detection Assay For Pancreatic Cancer

Poster Presentation. NEMB2016-6086

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Exosome have gained increasing interest as a novel target for cancer diagnostics. However, detection of tumor-derived exosomes is technically challenging and often requires extensive sample purification from human serum. Here we developed an ultrasensitive plasmonic scattering assay that could directly level tumor-derived exosome from down to 1 µl serum. Our strategy integrated specific three-probe recognition system and plasmonic coupling effect colorimetric scatter, caused by the proximity of diverse gold nanoparticles, to enhance local signal intensity of scattering light in dark field microscope. By incorporating tumor-identified maker EphA2, we could distinguish pancreatic cancer patients from pancreatitis and normal controls with high specificity. Quantification of circulating tumor-derived exosome was informative in staging tumor progression and patients' early response to neoadjuvant therapy.

A binding cooperativity study of cholera toxin-mixed gangliosides using a high-throughput nanocube-based cell membrane array

Poster Presentation. NEMB2016-6093

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Lectins often consist of multiple binding subunits that exhibit specific or semi-specific glycan recognition. The cooperative action between multiple bound receptors often strongly enhances the binding avidity and specificity. Although the binding subunits of lectins are often identical and many lectins preferentially bind to the same glycan structure, they still exhibit unique binding patterns to various cell surfaces. We hypothesize that the unique lectin binding patterns on heterogeneous cell surfaces are achieved via cooperative interaction between bound glycan moieties.

To better understand the essential nature of binding cooperativity in multivalent binding mechanism, quantitative analysis of multivalent membrane recruitment onto the cellular surface is critical. We have developed a nanocube sensor coupled with complex reaction analysis to quantitatively explore the multivalent binding mechanism. The nanocube sensor is surrounded by a lipid bilayer that possesses the same physical and chemical properties as cell membranes. This novel sensor is an ideal tool for studying binding cooperativities because receptors can freely diffuse and rotate on 2D fluidic cellular membranes allowing receptor self-organization to enable multivalent interactions. This label-free sensing platform can be conducted in standard 384-well microplate; therefore, its high-throughput utility enables the complex analysis of multivalent lectin binding. In addition, the simple protocol ("mix-and-then-detect") allows any end users to perform the analysis in their own laboratories.

Recently, we studied the classic pentameric lectin, cholera toxin subunit B (CTB), binding to GM1-like gangliosides (GM1, fucosyl-GM1, and GM2). We performed experimental measurements and theoretical analysis of a stepwise binding model to investigate the influence of cooperativity on CTB binding. Two important phenomena were found. First in contrast to GM1, the cooperative interaction between bound fucosyl-GM1 molecules is negative. Surprisingly, such negative binding cooperativity increases the binding capacity of CTB on a cell membrane surface. We confirmed and explained this result with a computational model of a stepwise binding mechanism. Second, we found the strong binding ligand (fucosyl-GM1) could activate the very weak binding ligand (GM2). A fucosyl-GM1/GM2 mixture increased the maximum binding of CTB on membrane surfaces. As such, the attenuation or enhancement of CTB binding is not simply controlled by the concentration of strong ligands; the cooperative actions among gangliosides in a complex can influence the overall binding. These unexpected discoveries not only demonstrate the essence of cooperativity in the multivalent lectin binding, but also significantly impact lectin-based glycomic analysis. In summary, our nanocube-based cell membrane array provides an excellent tool to dissect complex multivalent interactions; its easy-to-use and high-throughput features will make this tool immediately available to biological communities.

A fully integrated impedance biosensing platform for point-of-care diagnostics

Poster Presentation. NEMB2016-6012

***Tae-Hoon Kim, Jungkyu Kim**, Texas Tech University, Lubbock, TX, United States*

An impedance biosensor is a well-known label-free system simplifying overall bioassay procedures. However, the readout sensitivity afforded by label-free affinity impedance biosensors is inferior to other label-free techniques such as conventional ELISAs. Micro/nanoparticles are a common signal amplification technique to improve the sensitivity of an impedance based biosensing platform. However, utilizing the micro/nanoparticle increases complexity of overall assay procedures. In this work, we developed a fully integrated impedance biosensing platform which enables high sensitivity improving SNR (signal to noise ratio) with a microparticle. Also, we incorporated a particle labeling method to further improve sensitivity in impedimetric bioassay, and evaluated the minimum detectable number of polymeric microparticles on an interdigitated electrode (IDE) surface over a wide range of frequencies using the developed impedance analyzing platform.

A custom-made impedance measurement system consists of four major parts: 1) a gold (Au) IDE array chip, 2) an impedance analyzing circuit, 3) a data acquisition (DAQ) board and 4) a LabVIEW software. The Au IDE array chip having a finger width and spacing of 10 um was fabricated on a glass wafer, and 4 sets of IDEs were aligned on the single chip. The surface of each IDE was functionalized with anti-TNF alpha capture antibody using a standard silane functionalization and bio-conjugation methods. For a sample handling processor, Lifting Gate microfluidic valves were developed for delivering all required samples and reagents, and washing non-specifically bound targets and microparticles during this immunoassay. As a labeling, a streptavidin-coated polystyrene (PS) particle was used for impedance measurement. Also, the impedance signal from solely distilled (DI) water was utilized as the baseline. After adding the microparticles with different concentrations through the pumping system, the magnitude of impedance signals was measured in the specified range of frequency (11 kHz to 91 kHz).

We found a significant decrease of impedance magnitude as the number of microparticle increases on the working electrode when compared with label-free approach. Considering the surface coverage rate, the absolute magnitude of impedance showed a significant difference with microparticle numbers. This result shows a promising possibility of the developed impedance system in differentiating the amount of surface charge for unknown microparticles. This fact can be directly related to selectivity and sensitivity of the proposed instrumentation in bioassay. The developed system also successfully detected a single-particle having larger diameter than the finger spacing. In addition, the pneumatic pump system was successfully applied from delivering the native particles to washing the unbounded particles, which can enable fast and automated analysis in various bioassays. With these results, we will further improve limit of detection (LOD) by optimizing several parameters including the area of the probe surface, a flow rate during mass transport, hydrodynamic washing step, and the physical properties of micro/nanoparticles.

Expanded Control of Ionic Diffusion Through Enhanced Nanoscale Confinement

Poster Presentation. NEMB2016-6025

R. Lyle Hood, Giacomo Bruno, Priya Jain, Alessandro Grattoni, *Houston Methodist Research Institute, Houston, TX, United States*

Physiological hormone regulation in complex organisms is precisely controlled down to picomolar specificity. Emulation of these systems can be accomplished through nanofluidic controlled delivery, which also offers potential solutions in many other applications, including drug delivery, desalination, and filtration, among others.

Previously, this group has demonstrated controlled release with a broad set of drugs and biomolecules both in vitro and in vivo, leveraging transport phenomena unique to the nanoscale regime into clinically useful drug release. In this study, we present a set of experiments providing an unprecedented characterization of nanofluidic diffusion with a model cation (histamine) and anion (cefazolin) through nanochannels. Achieving sub-nanometer resolution and reliability in silicon membranes, we were able to observe several diffusion-driven regimes dependent on the ratio between the nanochannels' height and ions' hydrodynamic radii.

The diffusive release of histamine (111 Da, +2e at pH 3.54) and cefazolin (455 Da, -1e at pH 7.61) were examined through a scaled series of nanochannel membranes with distinct sizes: 2.5, 3.6, 5.7, 13, 20, 40, and 200 nm in height. Release experiments were quantified through UV-Vis spectroscopy and conducted with the ions solubilized in aqueous 10 mM NaCl solutions and at the pH stated above over 24-48 hours. Size scaling, exponential bulk diffusion was observed as the major transport phenomenon for both ions within the largest nanochannels (200 nm). In the intermediate range (5.7-40 nm), histamine demonstrated near-surface diffusion behavior speculated to arise from positively charged ions diffusing along the negative silicon surfaces (2D dominated transport). This was evidenced by overall transport rates remaining nearly identical regardless of channel size and following an exponential profile. Cefazolin demonstrated substantially different transport in the 5.7-40 nm channels, as release rates correlated linearly with channel size and maintained a zero-order profile. This is attributed to repulsion from the negative channel surfaces restricting ionic diffusion to the center of the channel (gated diffusion, 3D dominated transport).

However, transport within the finest nanochannels (2.5 and 3.6 nm) exhibited previously unobserved charge independent behaviors. For both ions, the release rate dropped by 1-2 orders of magnitude, a particularly marked decline for histamine following the identical rates for the 5.7-40 nm channels. In this domain, sustained zero-order release profiles scaling with nanochannel height were observed for both ions. A noteworthy finding was the linearization of small cation transport (histamine), which, to our knowledge, is an original demonstration. This study highlights the relevance of nanofluidic systems as tools for investigating novel transport phenomena and demonstrates the potential for controlled, zero-order delivery of clinically relevant ions regardless of their expressed charge, overcoming a primary limitation of the approach.

Tunable Control of Therapeutics Release through Electric Field Modulated Transport in Nanochannels

Poster Presentation. NEMB2016-6029

Giacomo Bruno, Thomas Geninatti, R. Lyle Hood, *houston methodist research institute, houston, TX, United States,* ***Giovanni Scorrano***, *Rice university, Houston, TX, United States,* ***Alessandro Grattoni***, *Houston Methodist Research Institute, Houston, TX, United States,* ***Danilo Demarchi***, *Politecnico di Torino, Turin, Italy*

Nanofluidic devices have been investigated for over a decade as promising platforms for the controlled release of therapeutics, but their potential can be extended to several different systems for filtration, sorting, or desalination, among others. The nanochannel drug delivery system (nDS) is an implantable platform employing a silicon, nanochannel membrane and applied electric fields for the active and remote modulation of therapeutic transport from the inner reservoir to the outer environment. The nanochannels were manufactured using industrial grade processes and etched in parallel to achieve a highly dense array (over 100,000 nanochannels per mm2) while maintaining sub-nanometer precision. This group has previously demonstrated the advantages of nanochannels in drug delivery by releasing different types of therapeutics over long periods (6 months) both in vitro and in vivo. In nanofluidics, where molecules are confined into nano-spaces, the diffusion redistribution results as the major transport phenomenon. This leads to a zero-order flux of drugs through the membrane and into the patient, reducing potential side effects and enhancing the efficacy and efficiency of the healing process. The next generation nDS leverages the use of a strong electric field (greater than 1kV/m) to modulate the release of molecules from the implantable device. A small potential of 1.5 V was applied between two platinum electrodes sputtered on the faces of the silicon membrane and controlled via Bluetooth by a microprocessor incorporated within the nDS. Depending on the size of the nanochannels, different phenomena occurred when a electrical potential was present. For large nanochannels (greater than 20 nm) the electro-kinetic transport regulated the therapeutic flux depending on the polarity of the potential applied. Au contraire, smaller nanochannels exhibit a phenomenon termed Ionic Concentration Polarization (ICP), which resulted in a complete stop of drug passage. To investigate this further, a thorough evaluation of ICP though a wide range of nanochannels was conducted. Experiments employed channels 2.5, 3.6, 5.7, 13, 20, 40, 200 nm in height and different ionic concentrations ranging from 100 µMol to 1 Mol NaCl. The results highlighted three separate regions: ICP did not occur, ICP limited the transport velocity, or ICP blocked the ionic flux, which allowed decoupling of the phenomenon involved. The novel properties of the next generation nDS allows active and external control of drug transport, provides tuning of the therapeutic administration over time, and motivates future medicine possibilities such as personalized and telemedicine treatment protocols. The capability of this nanotechnology platform to temporally control the diffusive release of molecules offers potential solutions in management of several chronic diseases such as cancer, heart disease, circadian dysfunction, hypertension, pain, and stress, as well as directly enabling modern treatment regimens such as chronotherapy.

Nanochannel Membranes for Sustained HIV Prophylaxis

Poster Presentation. NEMB2016-6032

Priya Jain, R. Lyle Hood, Eugenia Nicolov, *Houston Methodist Research Institute, Houston, TX, United States,* ***Roberto Arduino***, *The University of Texas Health Science Center at Houston, Houston, TX, United States,* ***Alessandro Grattoni***, *Houston Methodist Research Institute, Houston, TX, United States*

Introduction:
The Human Immunodeficiency Virus (HIV) affects over 35 million people globally with 2 million new infections arising in 2014. Truvada, a combination of tenofovir and emtricitabine, has been demonstrated to be an effective drug for HIV prevention; however, adherence to this once-a-day regimen has been shown to be of primary clinical concern. Our BioMEMS nanofluidic nanochannel Delivery System (nDS) introduces a method to overcome this adherence issue by developing an implantable device that provides constant, long-term delivery. This system employs a silicon membrane containing more than 100,000/cm2 nanochannels that can be varied in size from 2.5-250 nm to adapt for the drug of interest and desired dosage rate. Developing a novel approach to refill the device transcutaneously provides a minimally-invasive method to release drugs over a period of years instead of months.

Materials and Methods:
Emtricitabine (FTC) and tenofovir alafenamide (TAF) were kindly provided by Gilead Sciences to test with our implantable nDS. In vitro experiments were performed using nanochannel membranes mounted within PEEK capsules

with 250 µl reservoirs. FTC and TAF were mixed separately in aqueous, isotonic solutions near their solubility limits (110 and 20 mg/ml, respectively) and syringe loaded individually into implant reservoirs. The implants incorporated membranes with nanochannels either 20 or 250 nm in height. The drug concentration was measured using an HPLC protocol developed by our group. The transcutaneous refill method was tested by loading a 600 µL capsule with a known FTC concentration and placing it under the skin of a chicken thigh. Venting and loading needles were inserted into the ports of the device through the skin, and 1.5 ml of air was circulated through the device to remove all liquid before pushing either 1, 1.5, 2, and 2.5 ml volumes of fresh FTC solution through the reservoir. Drug concentration was then measured using UV-Vis spectroscopy.

Results and Discussion:
Constant, sustained delivery was measured for up to 2 weeks in vitro for the 20 nm membranes. In addition, FTC's higher loading concentration (about 6X) and smaller size resulted in a higher average daily release of 650 µg than 120 µg for TAF. The 250 nm nanochannels demonstrated a burst release on Day 1 with a release of 4 mg and 3 mg followed by an average daily release of 420 µg and 160 µg for FTC and TAF, respectively. This initial burst release is associated with insufficient nanoconfinement, leading to an exponential decay of drug from the implant reservoir, which is in contrast to the more clinically desirable linear decay seen in the 20 nm membranes. The transcutaneously refilled drug solution was removed from the reservoir and measured to be 87%, 93%, 96%, and 97% of the loading solution concentration for the 1, 1.5, 2, and 2.5 ml refill volumes, respectively. This indicates that at least 2 ml of the FTC drug solution must be used to achieve at least a 95% nominal drug concentration. This volume may be reduced by increasing the FTC drug concentration to approximately 105% of the desired loading concentration.

Conclusion:
We have been able to demonstrate controlled, long-term release of FTC and TAF for HIV pre-exposure prophylaxis using our nDS technology. An in vivo pharmacokinetic study on the release of these drugs from the nDS within macaque models is currently ongoing. These results demonstrate that the nDS approach has the potential to revolutionize the standard of care for HIV prophylaxis.

Boron Doped NanoCrystalline Diamond Sensor Integrated Lab On A Chip Device For Blood Gas Sensing Using Electrochemical Approach.

Poster Presentation. NEMB2016-6033

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Blood gas measurement are used to evaluate a person's lung function and acid /base balance. The proposed sensor integrated lab on the chip device was manufactured to severe the diagnostic purposes of such applications. Hydrogen sulfide (H2S) has emerged as an important signaling molecule that plays significant roles in health and disease. Work from many labs has revealed that H2S bioavailablilty and exogenous hydrogen sulfide therapy regulate numerous disease states including inflammation, cancer, cardiovascular, neurological and gastrointestinal diseases. This proposed work investigates and demonstrates new H2S detection techniques well suited to disease detection and biomedical application through a Lab on a Chip (LOC) approach.

As the significance of H2S grows, the ability to foster clinical detection and promote treatment becomes increasingly critical. Current technology relies on elaborate methodologies that impede real world use in a clinical setting. Present day small scale systems are incapable of micro-molar (µM) H2S detection. This is a critical limitation because disease detection requires this degree of precision. This constraint further hampers the ability to expand detection and treatment to medical fields in third world applications or other environments where cost is a significant issue.

Currently, the novel chip was designed to detect the level of hydrogen sulfide present in the blood. A unique LOC device has a various parts which serves as specific purpose. The micro-fabricated layer was coated with the sulfide liberation buffer. The liberated gas was then passed via a highly selective polymer membrane and then collected at the final chamber for its quantification. The electrochemical detection was made possible at this chamber using boron doped nanocrystalline diamond electrode (BDUNCD) electrode of various sizes. Both direct and indirect detection have been investigated and compared. Detection of sulfide via the catalytic electrochemical oxidation of ferrocyanide was much promising and low power consumption. This research highlights the optimization of sensor integrated lab on the chip device to detect sulfide in biological range in a water based sample. The working of the device proof detection of hydrogen sulfide or other blood gas in similar fashion. The optimized sensor have a linear detection which makes the sensor possible for calibration. The limit of detection was 0.1 µM. The detection of the gas was less labor intensive, fast and low cost compared to the any other techniques due to its possibility of the miniaturization and batch fabrication. The first commercialized product will server for peripheral artery disease (PAD) patients.

A Robust Oil-Free DC Digital Microfluidic Device By Induced Lateral Electric Field (LEF)

Poster Presentation. NEMB2016-6034

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Digital microfluidics (DMF) is used in numerous lab-on-a-chip (LOC) applications including proteomic sample preparation, enzyme assays, polymerase chain reaction, immunoassays, clinical sample processing and applications involving cells. A combination of electrowetting and electromechanical force applied by DC or AC electric field enables manipulation of droplet surrounded by oil or air, respectively. While DC based DMF can be a cheap and portable LOC device, AC based DMF contradicts portability since AC amplifiers are more expensive and larger in size compare to DC. However, breakdown of the dielectric layers at micro-nano scale is unpredictable for DC voltage in air medium and DC-based DMF devices working in air medium suffers from electrolysis and charge accumulation. Silicone oil is commonly used as surrounding fluid to solve this problem by reducing droplet surface tension, which limits the applicability of the DMF device in biological processes. Once silicone oil is applied to the device surface, it's also nontrivial to clean device surface. In this study, a robust oil-free DC digital microfluidics device is developed, which can be long term operational without electrolysis by increasing electrostatic force by electric field modulation.

DMF chip is fabricated by subsequent coating of 2.7 µm parylene and 60 nm Cytop on 100 nm chromium coated glass slide (75 mm x 25 mm) as bottom electrode and an ITO coated glass slide (50 mm x 25 mm) is used as top ground electrode. Bottom electrodes were fabricated using standard photolithography technique and 1.5 mm x 1.5 mm electrodes were created. The device top to bottom plate spacing was kept at 90 µm. Droplets of DI water were dispensed and manipulated by applying + 120dc to electrode adjacent to droplet and negative ground to droplet containing electrode along with top ground electrode to induce lateral electric field (LEF), thus increasing electromechanical force in the direction of droplet translation. By appying LEF threshold voltage was reduced down to 80 Vdc while it was >>300 Vdc without LEF for 2.7 µm parylene coated electrode. Voltage was amplified using EMCO Q03-24 and controlled by a custom built switching board, which can simultaneously apply positive high voltage and negative ground to the electrodes. Repetitive droplet dispensing, transport, split and merging were performed without any electrolysis in air medium. Dispensing was performed for more than 100 times without any failure with at least three droplets generated from a reservoir volume of 3 µL. Average volume of droplets was found to be 230 nL with only 3.9 % deviation for reservoir to control electrode surface area ratio of 10. In continuation of our current observation, we will present the effect of electric field, effect of charge density and polarity of droplet on the droplet motion and effect on the biomolecules.

Fabrication And Characterization Of A Sensor Integrated on Lab-On-A-Chip To Detect H2S In Biological Samples.

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Blood gas measurement are used to evaluate a person's lung function and acid /base balance. The proposed sensor integrated lab on the chip device was manufactured to severe the diagnostic purposes of such applications. Currently, the novel chip was designed to detect the level of hydrogen sulfide present in the blood. Hydrogen sulfide (H2S) has emerged as an important signaling molecule that plays significant roles in health and disease. Work from many labs has revealed that H2S bioavailablilty and exogenous hydrogen sulfide therapy regulate numerous disease states including inflammation, cancer, cardiovascular, neurological and gastrointestinal diseases. As the significance of H2S grows, the ability to foster clinical detection and promote treatment becomes increasingly critical. Current technology relies on elaborate methodologies that impede real world use in a clinical setting. Present day small scale systems are incapable of micro-molar (µM) H2S detection. This is a critical limitation because disease detection requires this degree of precision. This constraint further hampers the ability to expand detection and treatment to medical fields in third world applications or other environments where cost is a significant issue.

This proposed work investigates and demonstrates new H2S detection techniques well suited to disease detection and biomedical application through a Lab on a Chip (LOC) approach. A unique LOC device has a various parts which serves as specific purpose. The micro-fabricated layer was coated with the sulfide liberation buffer. The liberated gas was then passed via a highly selective polymer membrane and then collected at the final chamber for its quantification.

A sensor integrated LOC device to detect all forms of H2S in biological samples has been fabricated and characterized. The carbon nanotube modified boron doped-UNCD electrode was designed to detect the H2S in micromolar to submicromolar range in biological sample. Both direct and indirect detection have been investigated and compared. Detection of sulfide via the catalytic electrochemical oxidation of ferrocyanide was much promising and low power consumption. This research highlights the modification of sensor integrated lab on the chip device to detect sulfide in biological range in a water based sample. The optimized sensor have a linear detection which makes the sensor possible for calibration. The limit of detection was 0.1 µM. The detection of the gas was less labor intensive, fast and low cost compared to the any other techniques due to its possibility of the miniaturization and batch fabrication.

Nano Droplets of Ferrofluids

Poster Presentation. NEMB2016-6100

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Ferrofluids are fluids with properties tailored on the nanometer scale. They consist of single domain magnetic nano-particles dispersed in a liquid carrier. As their properties are remotely tunable by a magnetic field, ferrofluids are exploited in a broad range of disciplines including magnetic drug targeting, cancer treatment by magnetic hyperthermia, contrast agent in magnetic resonance imaging (MRI), magnetic-capillary self-assembly, optics, sensors, and mechanical sealing and acoustic. In these implementations, dispensing a small volume of fluid is in demand. Current systems for dispensing ferrofluid droplets at small volumes are mostly based on microfluidics flow-focusing approaches, induced acoustic actuation, or conventional injection systems, requiring complicated dispensing systems. Here, we present a facile approach to continuously/intermittently dispense nano/pico liter ferrofluid droplets from a ferrofluid reservoir. Once a jet of ferrofluid is subjected to a constrained flux through a membrane and an inhomogeneous magnetic field, the jet experiences a curvature-driven instability and transforms to a droplet. In this approach, a ferrofluid reservoir is covered with a membrane. An imposed inhomogeneous localized magnetic field to the reservoir generates a continuous jet of ferrofluid. Once the jet flows through the membrane, the jet experiences a constrained flux. The combination of constrained flux and inhomogeneous magnetic force results in the thinning of the ferrofluid jet. As the ferrofluid jet becomes thin enough, the developed Laplace pressure in the ferrofluid jet leads to capillary instability and back-flow of the ferrofluid to the reservoir. This back-flow leads to breaking of the fluid jet into a droplet. Volumes of the dispensed droplets are tuned in the range of 0.1-1000 nanoliter by the induced magnetic field and hydraulic resistance of the membrane. Furthermore, the frequency of droplet dispensing is adjusted with the magnetic field and type of the membrane. A mathematical model is developed to elucidate the dynamics of the ferrofluid jet. This mathematical model includes two parts. In the first part, the dynamics of ferrofluid jet-front between the reservoir and the magnet is modeled. In the second part, the dynamics of thinning of the ferrofluid jet is studied. In this mathematical model, we solve Darcy equation along with mass and momentum conservations to predict the dispended volume of the ferrofluid. Once the ferrofluid properties, characteristics of the membrane, and the spatial form of magnetic field are given, the model predicts the dispensed volume of the ferrofluid. There is an excellent agreement between the model's predictions and the measurements. The developed approach promises a simple route to dispense miniature volume of ferrofluid in biotechnologies, optics, energy systems, and even aerospace technologies with no need of complex droplet generation devices.

Turning the Sensitivity of Point-of-Care Test by Gold Nanoparticle Aggregation: Effects of Nanoparticle Concentration and Size

Poster Presentation. NEMB2016-6103

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Point-of-Care (POC) diagnosis of infectious diseases continues to be in urgent need and can lead to early diagnosis and treatment. POC diagnosis requires a fast assay time, minimal infrastructure, and sufficient sensitivity and specificity. Among the various infectious disease biomarkers, the amplification and detection of pathogen's nucleic acid sequence has been shown to be highly sensitive and specific. To detect the target nucleic acid sequence, a gold nanoparticle (GNP)-based aggregation assay has shown great promise to meet the POC requirements. For this assay, GNPs were conjugated with two different DNA sequences that are complementary to target nucleic acid sequence to create "probes". The probes hybridize with the target nucleic acid to form GNP aggregates which induce a color change due to the altered plasmon resonance. Previous work has reported the optimized reaction conditions and detection methods. The goal of this work is to investigate the effects of GNP probe concentration and size on the assay performance. Malaria parasite genus Plasmodium was chosen as a clinically relevant target. A poly A-tail was included in the probes to increase flexibility of the sequence. The HPLC-purified probes were synthesized and purchased from Bio Basic Canada Inc. GNPs were prepared by reducing gold chloride by sodium citrate. To conjugate DNA probes to GNPs, we followed a pH-assisted and surfactant-free method reported earlier. The complementary target was then hybridized with GNP probes for 30 min at room temperature in the presence of a hybridization buffer which consisted of 20 % Formamide, 16% dextran sulfate and 0.6 M NaCl. The optical spectrum was measured using a Beckman Coulter UV-Vis spectrophotometer (model DU800). The probe nucleic acid conjugation on GNP leads to plasmon resonance peak shift from 518 to 524 nm and hydrodynamic diameter increase by 9 nm for 15nm GNP. Within the concentration range studied here, increasing the GNP probe concentration shifts the dynamic range of the assay to higher target sequence concentrations and thus compromises the limit of detection. For instance, a low probe concentration (0.4nM) can detect down to 0.1nM while a higher probe concentration (10nM) can only detect down to ~10nM.

We hypothesize that the large excess of non-aggregated GNPs (thus background) for higher probe concentration explains the less sensitive detection. Increasing the GNP size (from 15nm to 50nm) improves the sensitivity as well as peak shift (thus signal) by up to 3-fold. Current sensitivity is similar to lateral flow assay and future work is needed to improve sensitivity. Our work presents guidelines to rationally design this nanoparticle assay for better sensitivity and signal strength.

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Development of a Microfluidic Device with Dual Channels for Growth Factor Gradient on Breast Cancer Chemotaxis in Three Dimensions

Poster Presentation. NEMB2016-6107

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An important factor in tumor development and metastatic potential is directed migration of cancerous cells. Spatial and temporal gradients intrinsic to the tumor microenvironment, significantly mediates migration. Current research suggests that the altered tumor microenvironment may drive cancer aggression and metastatic potential [1]. Steep growth factor gradients in tumor microenvironment, result from the combined effects of high interstitial fluid pressure, dense extracellular matrix (ECM), and poor perfusion [2, 3]. It has been hypothesized that tumor motility and aggressive migration may be caused in part by chemotaxis in response to these gradients [1]. In particular, breast tumor cells are susceptible to gradients in epidermal growth factor (EGF) due to overexpression of EGF receptors and related proteins and receptors [4,5].

With recent advances in tissue engineering, microfluidic techniques and fabrication, using soft lithography-based two-dimensional polydimethylsiloxane (PDMS) perfusion chambers, of in vitro tissue microenvironments. Some studies have been performed investigating the effect of EGF concentration on chemotaxis [6, 7]. Additionally, other studies investigated how the shape of EGF gradients (linear or nonlinear polynomial) and the magnitude regulate chemotaxis of MDA-MB-231 metastatic human breast cancer cells [8]. Although, these studies are insightful, the complex mechanical, chemical, and perfusion components of the tumor microenvironment have not yet been successfully recapitulated. Previous studies have focused on understanding specific biological cues rather than improving the fidelity of the microenvironment platform. Experiments performed on 2D substrates, even those treated with extracellular matrix proteins such as collagen or fibronectin, are unable to provide the three-dimensional microenvironment which has been repeatedly shown to have a momentous effect on cell morphology, signaling, and migration through mechanical and structural interactions [9,10]. Studies in three-dimensional matrices generally lack direct perfusion of the microenvironment [11, 12] and therefore cells are subjected to gradients in all growth factors and nutrients. Furthermore, although the importance of the role of matrix mechanics in tumor aggression has been noted, previous chemotaxis studies have not considered parameters such as ECM stiffness [8]. Interactions between microenvironment mechanics and self-expressed chemical cues have been studied, but still at low cell concentrations relative to those found in vivo [13].

In this study, we developed a microfluidic tumor platform incorporating a physiological concentration of MDA-MB-231 cells in a collagen I hydrogel scaffold with a fully three-dimensional geometry. The device is perfused at physiological flow rates using two parallel channels (dual channels) with diameters comparable to microvasculature. We have quantified chemotaxis in response to EGF gradients of varying magnitude formed by diffusion of EGF from one channel into the surrounding hydrogel.

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Utilization of a 3D In Vitro Tumor Platform to Study Nanoparticle Transport

Poster Presentation. NEMB2016-6111

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Cancer is a major health problem worldwide, accounting for one in 4 deaths in the United States alone. Significant progress in nanomedicine has been made in order to develop therapies for cancer, particularly related to enhanced photothermal and photochemical treatments. These nanotechnology-based cancer treatments have the potential to provide localized, targeted therapies to enhance efficacy and reduce side effects, thereby improving patients' quality of life. Understanding the distribution and transport of nanoparticles (NPs) in the tissue as a function of physiological conditions (e.g. matrix properties and hemodynamics) and nanoparticle dimensionality is critical to optimizing particle design for maximizing delivery and efficacy. Most commonly, 2D in vitro cell cultures have been used to study NPs and their interaction with tumor cells, but do not capture the essential features of the tumor microenvironment including matrix mechanics, hemodynamics, and the elevated interstitial pressure. In vivo models have been used and do capture the tumor microenvironment, but they can be highly variable and a large number of animals are needed making them cost prohibitive for NP optimization. 3D in vitro platforms however are capable of characterizing the transport and efficacy of NPs as well as facilitating cell attachment, proliferation, and infiltration, reducing the expense associated with in vivo models and inaccuracy of 2D in vitro models.

In this study, a 3D microchannel system was employed previously developed by the Rylander lab. This 3D in vitro model contains a vascularized collagen type I tumor microchannel polymerized in FEP tubing. Within the microchannel we created an endothelialized vessel by fitting in a stainless steel needle capped with PDMS sleeves. After polymerization and removal of the stainless steel needle, the vessel was created and seeded with endothelial cells. Previous studies by the Rylander lab have employed the same 3D in vitro microchannel system to study tumor-endothelial signaling and vascular organization as a function of flow through the channel. This channel was able to withstand a range of normal, high and low flow shear stresses that are relevant to the tumor microvasculature.

To replicate the tumor vasculature and investigate the vessel properties on NP transport, we introduced inflammatory agonists such as thrombin (10 U/mL) or histamine (100 M), altering endothelium permeability. To measure vessel permeability, 70 kDa Oregon green-conjugated dextran was perfused into the channel and imaged to measure fluorescence intensity over time. The FEP tubing and the water bath were used to allow refractive matching for undistorted imaging. Flow was introduced into the microfluidic channel with a syringe pump producing wall shear stresses of 1-15 dynes/cm2. Nanoparticles of different sizes ranging from 50-100 nm were introduced into the flow of the microchannel and images were acquired using a Zeiss epifluorescent microscope in the span of 72 hours recorded at 10 minute intervals. By imaging the channels, we were able to determine the transport of nanoparticles as a function of time, permeability, and NP dimensionality. Use of our 3D in vitro system enabled investigation of the dynamic nanoparticle transport within a physiologically relevant system providing fundamental un-

derstanding for use in enhancing the effectiveness of nanoparticle therapies for cancer.

Red blood cells improve margination of micro-particles for drug delivery in microcirculation. The effect of particles size and shape

Poster Presentation. NEMB2016-6117

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In the last years, nano-carriers have been recognized as a promising strategy in the drug development process, thanks to the many advantages in comparison with current therapies. Exploiting host physiological mechanisms, nano-delivery systems can been engineered ad hoc. In particular, the use of nano-scaled systems for the treatment of cancer has been focused on the well-known enhanced permeability and retention effect (EPR) [1]. EPR results in an increase of vessels permeability and impaired lymphatic drainage within the pathological tissues, allowing preferential passage and retention of circulating drug carriers. A plethora of different carriers have been functionalized to exploit the EPR, but, most of them showed different limitations due to the biological barriers [2]. The interaction with red blood cells (RBCs) in the microcirculatory network, and the carrier's margination - the mechanism according to which particles migrate along vessel radius to the vessel wall - could represent the two main limitations in the blood stream for the delivery carrier [3]. Blood cannot be considered as a homogeneous fluid but as a concentrated suspension of RBCs. RBCs are deformable objects [4, 5], which are distanced from vessel wall creating a RBC-rich core in the center of the vessel and a cell-free layer (CFL) in proximity of the vessel's wall [6]. The phenomenon of margination happens once a carrier is injected in the blood stream and it is normally displaced in the proximity of the CFL [7] in a size and shape dependent manner [8]. Independently on the targeting mechanism of choice in the design of a drug delivery system, the margination propensity of a specific particle is an essential parameter to maximize the interactions with the vessel wall and potentially augment the targeting.

Although margination has been modeled by numerical simulations and investigated in model systems in vitro, experimental studies including RBCs are lacking. Here, we evaluated the effect of RBCs on different drug delivery systems margination through microfluidic studies in vitro and by intravital microscopy in vivo. We showed that margination, which is almost absent when particles are suspended in a cell-free medium, have been drastically enhanced by RBCs. This effect is size- and shape-dependent, larger spherical/discoid particles being more effectively marginated both in vitro and in vivo [8]. We demonstrated that our in vitro system could be a good model to study the margination of micro and nano-carriers. These results are relevant for the design of drug delivery strategies based on systemically administered carriers.

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A Microfluidic Assay Device for Study of Cell Migration on ECM Mimicking Suspended Nanofibers in Presence of Biochemical Cues

Poster Presentation. NEMB2016-6128

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Many in vivo biological processes including wound healing, cancer metastasis, and embryogenesis are driven by chemotaxis and guided by physical stimuli. Many research works have investigated chemotaxis and durotaxis separately; however, the combined effect, critical to fully recapitulating in vivo processes, is not well studied. The fibrous extracellular matrix (ECM) provides cells with simultaneous material (N.m-2) and structural stiffness (N.m-1) gradients. Durotaxis has traditionally been investigated by studying cell migration on gels of varying elastic modulus, while the role of structural stiffness gradients in cell motility remains largely unexplored. In this study, we present a microfluidic assay device that can be utilized to investigate the role of concurrent biochemical and biophysical cues of suspended fibers on single cell NIH/3T3 mouse fibroblast migration.

The non-electrospinning Spinneret-based Engineered Tunable Parameters (STEP) fiber manufacturing technique was used to deposit ECM mimicking, suspended, 500 nm diameter, polystyrene (PS) nanofibers on a 2 mm × 5 mm × 0.125 mm flat PS substrate with a 1.7 mm × 1.7 mm square cutout. The nanofiber scaffold was integrated in a gradient-generating microfluidic device with a quasi-steady, linear biochemical gradient. The microfluidic device was fabricated from polydimethylsiloxane (PDMS) using standard soft lithography techniques. The device is composed of diffusive mixing channels and an observation channel (2.2 × 16 mm2). A linear gradient of platelet-derived growth factor (PDGF) was established in the observation channel (i.e. biochemical cues) and scaffolds with suspended, aligned nanofibers were placed in the microfluidic device observation channel. The nanofibers are oriented in the direction of the biochemical gradient and provide a structural stiffness gradient (i.e. biophysical cues). In such a configuration, the structural stiffness is highest at the fixed ends and lowest in the middle of the fiber span length, whereas the biochemical concentration is the lowest at one fixed end and highest at the other end of the fiber span length. Cells were seeded onto the nanofibers in the microfluidic device and time-lapse images of the fibroblast cells were taken every 10 minutes for 12 hours. Control experiments without the biochemical gradient or without the structural stiffness gradient (flat) were also conducted to decouple the effects of these two stimuli on cell migration. The cell positions were used to evaluate velocity (displacement/time), persistence (displacement/total distance travelled), and chemotactic index (distance travelled in the direction of the biochemical signal/total distance travelled) as a function of the global biochemical gradient and local structural stiffness. Our preliminary results suggest durotactic gradients presented by the structural stiffness (N.m-1) of suspended fibers can influence cell response to the biochemical gradient. Cells migrating toward the region of increasing structural stiffness and increasing biochemical concentration tend to have higher persistence than cells moving toward the region of decreasing structural stiffness and increasing biochemical concentration. This platform enables us to quantitatively investigate cell migration with both cooperating and opposing biochemical and structural stiffness

gradients. This microfluidic platform is novel in combining biochemical and biophysical cues and can be used towards development of a new class of biological assays for investigating developmental biology and disease pathogenesis at the single cell resolution.

Plasmonic Biosensors Based on Gratings

Poster Presentation. NEMB2016-6144

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Surface Plasmon Resonance (SPR) biosensors are in demand for the detection of chemical and biological species for many applications including environmental monitoring, food safety monitoring, and for the pharmaceutical and medical industries. Such applications require devices that are highly sensitive and provide real-time sensing. SPR biosensors incorporate surface electromagnetic waves (surface plasmon polaritons) which propagate on the boundary of a metal-dielectric interface. Changes to the boundary, such as binding of biomolecules on the metal surface, produce changes in the optical signals to be measured. Thus, SPR devices can detect small concentrations of an analyte, in real-time, making them favorable for these applications. SPR sensors use several methods of optical excitation and this work investigated grating-coupled surface plasmon resonance (GSPR) for bio-sensing applications.

This paper presents the design of a GSPR device in order to realize a highly sensitive sensor. The fabrication process flow for this device is discussed and includes using interference lithography (IL) to create the periodic grating features. IL was used instead of electron beam lithography because it can expose bigger areas and does not need expensive photoresists. A metallic bi-layer of chrome and gold was deposited on the device using thermal deposition techniques. The final step of fabrication included functionalization of the device for biosensing capabilities. During processing, the materials were characterized using various techniques including atomic force microscopy (AFM) and a scanning electronic microscope (SEM). These results will be presented as well.

A Microengineered Model of the Human Placental Barrier

Poster Presentation. NEMB2016-6065

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During pregnancy, the maternal-fetal interface of the human placenta regulates the exchange of nutrients, oxygen, metabolic waste, and xenobiotics between the mother and fetus. During late gestation, this critical barrier in the placental chorionic villi consists of maternal villous trophoblasts and fetal capillary endothelial cells, which are located in close apposition to facilitate efficient exchange between the maternal intervillous space and fetal circulation. Our biomimetic model of the human placental barrier leverages microengineering technology to develop a miniaturized cell culture platform that reconstitutes the three-dimensional microarchitecture and dynamic microenvironment of the human placental maternal-fetal interface. This “placenta-on-a-chip” device enables compartmentalized co-culture of human villous trophoblasts and fetal endothelial cells on a thin, semipermeable polymeric membrane. The microdevice is composed of two poly(dimethylsiloxane) (PDMS) layers, each containing a hollow microchannel, fabricated using standard soft lithography techniques. A thin PDMS mortar layer is used to bond the two layers to the intervening porous membrane. The dual channel design facilitates simulation of physiological flow conditions while also enabling precise spatiotemporal control of the cell microenvironment in each compartment. Preliminary work has successfully demonstrated co-culture of transformed choriocarcinoma cells (BeWo) and primary human placental villous endothelial cells in this microengineered model. We have demonstrated cell-cell fusion in the BeWo cell population on-chip through activation of the protein kinase A pathway to mimic trophoblast syncytialization, a key process in placental development. Cell fusion was assessed through observation of changes in cell morphology and E-cadherin expression, as well as quantification of human chorionic gonadotropin (hCG) production, which is increased in syncytialized trophoblasts. Additionally, we have worked to characterize the molecular permeability of the trophoblast-endothelial cell interface within the placenta-on-a-chip device. Glucose is the chief energy substrate for the placenta and fetus and is an essential nutrient for fetal development. Thus, we have investigated rates of glucose transfer between the maternal and fetal compartments on-chip. This work represents the first steps towards development of a placenta-on-a-chip microsystem that will provide new capabilities as a research tool for placental biology research.

Point-of-Care Diagnosis of Hemoglobin Disorders with a Mobile Electrophoresis Chip

Poster Presentation. NEMB2016-6035

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In developing countries, diagnostic tests for homozygous (HbSS) or compound heterozygous (HbSC or HbS-Beta thalassemia) sickle cell disease (SCD) are not readily available at the point-of-care (POC). Very few infants are screened in Africa for SCD because of the high cost, time for sample transfer to a central laboratory (2-6 weeks), and level of skill needed to run traditional tests. The World Health Organization recognizes a crucial need for early detection of SCD in newborns, since it is estimated that 70% SCD-related deaths in Africa are preventable with early cost-effective interventions. The diagnostic barrier can be broken with affordable, POC tools that facilitate early detection immediately after birth or at the time of immunization. To address this unmet clinical need, we have developed a mobile electrophoresis platform (HemeChip) for reliable, affordable, and rapid diagnosis of SCD.

The HemeChip uses a microfabricated platform, with a material cost less than $0.87 per device, housing cellulose acetate electrophoresis to rapidly separate hemoglobin (Hb) types. Less than 5 microliters of blood, which can be obtained through a finger stick or heel stick, is processed on a piece of cellulose acetate paper via an applied electric field in alkaline buffer within 10 minutes. We clinically tested and benchmarked HemeChip against standard clinical methods using 51 blood samples from 14 patients.

The HemeChip reliably identifies and discriminates amongst Hb C/A2, S, F and A0. The HemeChip hemoglobin concentration results were correlated (Pearson Correlation Coefficient (PCC) of ~0.96 for all Hb types tested) with standard clinical hemoglobin screening methods, including high performance liquid chromatography (HPLC). The agreement between the HemeChip and HPLC results were assessed using the Bland-Altman plot, which showed a strong agreement between estimated (HemeChip) and actual (HPLC) hemoglobin percentages. The majority (95.5%) of the differences between actual and estimated hemoglobin percentages were within the limits of agreement. Furthermore, the receiver Operating-Characteristic (ROC) curves showed more than 0.89 sensitivity and 0.86 specificity for identification of hemoglobin types using the HemeChip, based on the band travelling distance from the sample application point.

We developed a web-based image processing application for automated and objective quantification of HemeChip results at the POC using cloud computing resources. This intensity-based mobile phone image quantitation method showed high correlation with HPLC results for tested patient blood samples (PCC=0.95). Moreover, the Bland-Altman analysis showed strong agreement between the HemeChip results analyzed with the mobile user interface and HPLC. The majority (91%) of the differences between actual (HPLC) and estimated (mobile user interface) were within limits of agreement.

HemeChip technology offers a low-cost, easy to use, rapid approach and an innovative solution to POC diagnosis of SCD and other hemoglobin disorders. HemeChip can distinguish between different patient phenotypes, including HbSS (HbS only), transfused HbSS (HbS and HbA), and Hemoglobin SC disease (HbS and HbC). In summary, the HemeChip identification and quantification of hemoglobin phenotypes, as a POC technique, were comparable to standard clinical methods. This platform has clinical potential in under-served populations worldwide, in which SCD is endemic.

One-touch-activated Blood Multidiagnostic System Using a Hollow Microneedle Integrated with a Paper-based Sensor

Poster Presentation. NEMB2016-6098

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Point-of-care testing (POCT) represents a system whereby simple, rapid, and low-cost medical diagnostic tools are used at the patient care sites, and has progressed dramatically in the last few years with advances in biomedical microelectromechanical systems, lab-on-a-chip devices, and micro total analysis systems. Blood, a rich source of human biological information, is used as the target diagnostic sample in various POCT biosensors such as glucose meters, lateral flow assay strips, and cholesterol meters. However, the separation of blood collection and sample injection in commercially available POCT devices has been shown to negatively influence the performance of biosensors. In addition to this, the use of hypodermic needles or finger pricking for blood collection and use of pipettes or disposable droppers for sample injection limit the miniaturization of the POCT system, resulting in patient inconvenience. Although the creation of an all-in-one POCT system by combining blood collection with biosensor components has been a longstanding goal for complete, real-time blood diagnosis, such a system has not yet been developed.

To become a useful tool in an all-in-one POCT blood diagnostic system, an improved miniature system must reduce the actuator complexity while simultaneously simplifying the operation process. Currently, paper-based biosensor technology has been recognized as a future alternative for POCT biosensors for detection and quantification of a broad variety of analytes due to the high specificity and sensitivity of these biosensors as well as their simple and cost-effective fabrication process. Various paper-based POCT biosensors such as rapid kits, dip sticks, and glucose meters are already commercialized and allow portable, on-site detection based on colorimetric methods, however, almost all of them are separated from the crucial sample collection process.

Here, we demonstrate a novel one-touch-activated blood multidiagnostic system (OBMS) that consists of a biocompatible minimally invasive hollow microneedle and paper-based multiplex biosensor. We took advantage of microneedle technology for pain-free blood sampling with minimal tissue damage and inflammation, and paper-based sensors that have a simple and cost-effective fabrication process. The OBMS integrated and automated all functions of blood collection, separation, and detection, sequentially, in a single device that only required one-touch activation by finger-power without additional operations. In an in vivo experiment, using a rabbit model we demonstrated that the OBMS could perform blood sampling and multidetection of glucose and cholesterol levels in a fully automated manner. This new integrated microneedle and paper-sensor based blood diagnostic platform shows great promise for future POCT and disposable biomedical applications.

Flow Controllable Lateral Flow Device

Poster Presentation. NEMB2016-6108

***Yuxin Liu, Veronica Betancur, Nianqiang Wu**, West Virginia University, Morgantown, WV, United States*

Lateral flow devices have been used and are promising in many applications, including point of care diagnosis in medical, clinical, food and environmental control and monitoring. In particular, disposable paper-based lateral flow strips utilize low-cost materials and do not require expensive fabrication instruments. However, there are constraints on tuning the flow rate and immunoassays functionalization in paper as well as technical challenge in integration of sensors and concentration units for low abundant molecular detection. In the present work, we demonstrated an integrated lateral flow device that applied the capillary forces with functionalized polymer-based microfluidics as a strategy to realize a portable, simplified, and self-powered lateral flow device for potentially biomarker detection with minimizing the need for off-chip equipment. The device combined the ability to separate plasma from human whole blood and create a controlled and steady flow. Lateral flow relies on surface tension and hydrophilic natural of the flow channel, and controlled flow is also a key variable for immunoassay based applications for providing enough time for protein binding to antibodies. To render polydimethylsiloxane (PDMS) surface of hydrophilicity as well as to control the flow rate, PDMS was functionalized with different concentrations of Pluronic F127. The results show that, in an integrated LFD, the flow rate was regulated by the combination of multiple factors, including Pluronic F127 functionalized surface properties of microchannels, resistance of the integrated flow resistor, the dimensions of the microstructures in the capillary pump, dynamic contact angles, and viscosity of the fluids. The predominant factors are the resistance of the integrated flow resistor and the surface hydrophilicity of Pluronic functionalized PDMS, which can be easily modified by the dimensions of the flow resistor and the Pluronic concentrations as well as post-functionalization treatment, respectively. The flow rates ranging between 0.5 and 18.6 nL/s were achieved. In addition, the highly asymmetric nature of a plasma separation membrane allowed the cellular components of the blood to be captured in the larger pores without lysis, while the plasma flowing down into the smaller pores on the downstream side of the membrane was driven into the lateral flow device by the capillary pump. The integrated lateral flow device required no external macroscopic actuators and bulky fluidic connections for initiating and controlling fluid flow, demonstrated the capability in using of whole blood, showed the potential for future diagnostic applications in home and point of care situations with low cost, small sample volume and portability.

Determining the Influence of Dynamic Paracrine Signaling on Tumor Progression in an Evolving Microenvironment

Poster Presentation. NEMB2016-6101

***Manasa Gadde**, University of Texas at Austin, Austin, TX, United States, **M Nichole Rylander**, Univeristy of Texas at Austin, Austin, TX, United States*

Cancer research aimed at developing anti-angiogenic therapies is limited by a lack of physiologically representative platforms that can be used to study the dynamic intercellular interplay and tumor stromal interactions within an evolving microenvironment. Greater understanding of the role of key players in the process of angiogenesis will lead to the development of improved treatments that will halt cancer progression. The objective of this study was to increase the complexity of an existing 3D in-vitro microfluidic tumor model comprised of a co-culture of microvascular endothelial cells and breast cancer cells with the incorporation of fibroblasts, a key component of the tumor stroma. This model was used to investigate the influence of fluid shear stress and paracrine signaling between these cells on the expression of pivotal markers of angiogenesis including Matrix Metalloproteinase 9 (MMP-9), Angiopoietin-1 (ANG-1), Angiopoietin-2 (ANG-2), and Vascular endothelial growth factor (VEGF). In-vitro tumor models were constructed using

collagen I hydrogels with monocultures, co-cultures, or tri-cultures of MDA-MB-231 breast cancer cells, normal human dermal fibroblasts (NHDF), and telomerase-immortalized microvascular endothelial (TIME) cells of varying cell seeding densities of 1 million cells/ml, 0.5 million cells/ml, and 50,000 cells/cm2 respectively. Both NHDF and MDA-MB-231 cells were seeded in 8 mg/ml solubilized collagen and polymerized at 37°C in a cylindrical fluorinated ethylene propylene (FEP) tube, while TIME cells were seeded in the center of the channel forming an endothelialized lumen through which flow is introduced. Overall, seven groups of cell cultures with different cells combinations were studied: MDA, NHDF, TIME, MDA+NHDF, MDA+TIME, NHDF+TIME, and MDA+NHDF+TIME. The influence of intercellular signaling on angiogenic response was analyzed by performing ELISA and quantitative PCR for the expression of ANG-1, ANG-2, MMP-9, bFGF, PDGF and VEGF and immunofluorescence staining for F-actin. Three different shear stresses, 4 dyn/cm2 (normal microvascular wall shear stress), 1 dyn/cm2 (low microvascular wall shear stress), and 10 dyn/cm2 (high microvascular wall shear stress) were used to test the angiogenic response. To determine the effect of flow on cellular behavior and angiogenic response of cells, the microfluidic tumor platform was compared to a static tumor model without flow. In the static model in the MDA+TIME and MDA+NHDF+TIME groups, TIME cells that were in direct contact with MDA-MB-231 cells underwent anoikis, a process when cells detach from the ECM and undergo apoptosis whereas this behavior was not present in the microfluidic platform with the flow. In the flow system, TIME cells proliferated, elongated and formed a confluent layer. Also, the secretion levels for VEGF has a similar trend in both systems but in the static system, the amount of VEGF expressed was much higher. This study improved the complexity of an existing in vitro tumor platform and revealed signaling between MDA-MB-231, TIME and NHDF cells impact morphology, viability, expression of angiogenic factors. Overall, we concluded that this system provides a mean to study and better understand the dynamic interplay between stromal cells and microenvironmental factors such as flow which play an important role in tumor progression and therefore must be better understood to create effective anti-cancer therapies.

6-4
NANO-PHENOMENA IN LIVING SYSTEMS

Grand Ballroom 5:00pm - 8:00pm

A force toolkit for cell and molecular research

Poster Presentation. NEMB2016-5981

Torsten Mueller, *JPK Instruments AG, Berlin,Germany,* ***Randy Beaubien***, *JPK INSTRUMENTS USA INC, Carpinteria, CA, United States,* ***Heiko Haschke, Torsten Jähnke***, *JPK Instruments AG, Berlin, Germany*

Topography, roughness, (bio)chemical cues, and mechanical properties of biomaterials are crucial parameters affecting cell adhesion, morphology, and differentiation. Atomic Force Microscopy (AFM) is a multipurpose technology suitable for imaging a wide range of different samples with nanometer scale resolution under controlled environmental conditions, but also for mapping mechanical and adhesive properties of sample systems and tissues.

We have developed a new distinct imaging mode called “Quantitative Imaging” which is based on fast force-distance curves with intelligent control, to simultaneously obtaining topographic, nanomechanical, and adhesive sample properties. Additionally, even more complex data like contact point images, Young´s moduli images, or recognition events can be achieved. To demonstrate the capability and flexibility of AFM mode, a variety of soft and hard samples have been investigated. A comparison between “Quantitative Imaging”, conventional force spectroscopy and traditional AFM imaging modes clearly reveals that supplementary information can be gained. Using Single Cell Force Spectroscopy (SCFS), early cell adhesion phenomena can be quantified. A specialized AFM platform has been improved to perform SCFS, and nano-indentation experiments on single cells, and 3D scaffolds with a need for up to 100 microns pulling length. A side view holder enables a side view on the cantilever tip-sample interaction while AFM experiment. This setup gives optical access to the contact interface during force spectroscopy, and provides complementary information without expensive optical z-stacking function.

A new range of experiments is opened up when light is used as a handle for the manipulation of cells or molecules rather than a cantilever. We improved our optical tweezers system for more flexible 3D experiment geometry, and a force sensitivity down to 0.1 pN. Using AOD steering, and multiplexing we have investigated the frequency dependent rheology on red bloods, and simultaneous up to 8 multiplexed traps.

Nano-contact Printing of Netrin-1 Digital Nano-dot Gradients on Ultra-soft Substrates: The Impact of Substrate Stiffness on Haptotaxis

Poster Presentation. NEMB2016-6110

Donald MacNearney, Bernard Mak, David Juncker, *McGill University, Montreal, QC, Canada*

Introduction:
Cellular navigation, migration, and motility are vital requirements for life, whether it be during the developmental stages of an organism, or throughout the life cycle - for example during tissue maintenance and wound healing. This process is regulated by continuous integration of multiple extracellular cues. Surface bound chemical cues regulate cell navigation in neutrophils, myoblasts, and developing neurons through a process known as haptotaxis, while mechanical cues, in the form of substrate stiffness, can also regulate cell motility, through a process commonly denoted durotaxis. To properly investigate cellular navigation in a meaningful context, it is necessary to understand the interplay of these different influences. Here, we present a technique to investigate cellular migration and navigation via surface bound gradients patterned on ultra-soft substrates (E = 1 kPa), and we use this method to compare the characteristics of haptotaxis on both hard and soft substrates.

Methods:
Continuous gradients of surface bound proteins were created by patterning digital nano-dot gradients on glass using lift-off nano-contact printing. The digital nano-dot gradients consist of 200 nm squares with varying pitch, from 15 um to 300 nm, resulting in a continuous surface density gradient of the printed protein. A modification of nano-contact printing was developed, which uses dissolvable thin films of polyvinyl alcohol as an intermediate substrate, and the same protein gradients were patterned on Sylgard 527, an ultra-soft formulation of PDMS. A reference surface solution was applied, consisting of 75% poly-ethylene glycol grafted with poly-lysine and 25% poly-D-lysine, to obtain the same background surface chemistry on both substrates. C2C12 myoblasts were cultured on the gradients and live-imaged overnight to observe cell phenotypes and cell migration with respect to the patterned cues.

Results:
The novel printing technique is shown to generate the desired gradients of surface bound cues quickly, accurately and reliably. The patterns were compared with the same patterns on glass, as well as with the computer generated design files, and excellent agreement was found in both cases. C2C12 myoblasts were observed to respond to patterned nano-dot gradients of Netrin-1, migrating towards higher surface densities of Netrin-1 over the course of 15-hour live imaging. Preliminary data suggests a stronger migratory response on the ultra-soft substrate. In addition, myoblast phenotype and migration speeds were compared between glass and the ultra-soft substrate, with significantly rounded phenotypes and increased migration velocities observed on the softer substrate.

Conclusion:
We developed a novel printing technique which allowed us to pattern con-

tinuous digital gradients of surface bound cues on ultra-soft substrates. We used this technique to compare myoblast growth and haptotaxis on soft and hard substrates. Our results show significant differences between the two substrates when looking at cellular phenotype, migration speed, and response to patterned gradients. These results provide insight into the potential interplay between the wide range of mechanical and chemical cues that cells are presented with in vivo, and our techniques may be used to probe these relationships more accurately in the future.

Cellular Plasma Membrane-Bound DNA Nanostructures

Poster Presentation. NEMB2016-6113

***Molly Y. Mollica, Ehsan Akbari, Christopher Lucas, Jonathan Song**, The Ohio State University, Columbus, OH, United States, **Carlos Castro**, Ohio State university, Columbus, OH, United States*

Structural DNA Nanotechnology techniques have enabled the design and synthesis of complex 3D nanostructures with dynamically controllable features that exploit molecular self-assembly principles. In order to enhance our understanding of interactions and phenomena that govern membrane function and behavior, multiple attempts have been made to anchor DNA nanostructures to artificially synthesized vesicles. While attaching DNA nanostructures to vesicles has increased our understanding of their dynamics on a 2D lipid bilayer along with studying plasma membrane fluidity, creating synthetic membrane channels, and programming vesicle transportation, attaching DNA nanostructures to living cells is imperative in order to dynamically study the transmembrane cellular interactions. In addition, the ability to attach DNA nanostructures with strategically designed features to a live cell enables novel techniques to study cell-cell and cell-substrate interactions. Due to obstacles such as membrane-bound protein interference, cellular endocytosis, and DNA nanostructure instability in cell culture conditions, however, living cell membrane-bound DNA nanostructures have not yet been reported. The objective of this study is to establish robust methods to integrate DNA origami nanostructures onto cell membranes. We hypothesize that with appropriate structure design and attachment scheme, we can overcome the aforementioned cell-specific barriers to nanostructure binding. To achieve this, a honeycomb lattice DNA origami platform was designed in caDNAno with strategically located overhang oligonucleotides that bind to cholesterol-modified oligonucleotides. Agarose gel electrophoresis and transmission electron microscopy were used to establish optimal folding conditions, confirm well-folded structures, and verify nanostructure stability in cell-culture conditions. Due to the hydrophobic nature of cholesterol, cholesterol-modified oligonucleotides embed into the cell's plasma membrane. This was confirmed by fluorescently labeled oligonucleotides with the reverse complement sequence of the cholesterol-oligonucleotides binding to the cell membrane in the presence of cholesterol-oligonucleotides and not binding in their absence. Attachment of the DNA nanostructure to live B lymphocytes was confirmed by fluorescently labeling nanostructures and imaging membrane binding via live cell fluorescence microscopy. These results are the first report of DNA nanostructure attachment to live cells and display the importance of nanostructure overhang design and stability considerations. Future studies include developing methods to attach the DNA origami nanostructures to other cell types. A particular future focus of this work is the utilization of membrane-bound DNA origami nanostructures to develop a FRET-output force sensor for inter-endothelial junctions in order to enhance our understanding of how the inter-endothelial interactions are regulated under the effect of both biomechanical and biochemical stimulations. Moreover, the attachment of cell membrane components to the DNA nanostructure can be utilized to examine the influence of spatial arrangement on juxtacrine-induced cell activation and mechanotransduction. An understanding of these inter-endothelial interactions and biophysical parameters will allow us to determine the influence of mechanics on cell growth and signaling, ultimately allowing us to better diagnose and treat diseases such as cancer.

An Experimentally Validated Biophysical Model for Candida albicans Interactions with Nanofiber-textured Surfaces

Poster Presentation. NEMB2016-6130

***Zhou Ye, AhRam Kim, Amrinder Nain, Bahareh Behkam**, Virginia Tech, Blacksburg, VA, United States*

Candida albicans is a common human pathogen that can cause infection of the skin, oral cavity, esophagus, vagina and vascular system. C. albicans biofilms formed on prosthetic devices and implants are resistant to most of the traditional antifungal treatments. Alternative strategies, such as applying micro/nano-scale textures on such biomedical surfaces are envisioned to provide an effective route to reduce the C. albicans adhesion and biofilm formation. Previous work by us and others has demonstrated the efficacy of nanopatterns in reducing microbial adhesion. However, a quantitative study of the effect of the geometry and size of nanopatterns on microbial adhesion and biofilm formation is lacking. Herein, we report a biophysical model that describes the total free energy of C. albicans cells adhered to nanofiber-textured surfaces as a function of the nanofiber diameter and spacing, and the relative position of the cell with respect to the nanofibers. This total free energy is comprised of the adhesion energy and the elastic deformation energy. Upon adhesion to a surface, cell undergoes deformation to gain energy by maximizing its contact area with the substrate. The adhesion process will also involve energy loss due to stretching of the cell membrane. The minimization of total free energy will then determine the final shape of the cell interacting with the fiber-textured surface. When the spacing of nanofibers is larger than the cell diameter, a cell can only interact with a single fiber. The cell shape and total free energy are both functions of the cell position relative to the fiber. Our experimental results show that, when interacting with a single fiber, a cell continuously moves and adjusts its position until it reaches the position with lowest free energy. Similar behavior is observed when the spacing of fibers is smaller than the diameter of the cell, and one cell interacts with multiple fibers. By extension, our model also predicts the extent of biofilm formation on nanopatterned surfaces wherein the surface design that yields the highest cell free energy is expected to yield the least cell attachment density. The modeling results were experimentally validated by quantifying C. albicans attachment density on polystyrene nanofiber-textured surfaces as a function of fiber diameter (diameter: 0.5 - 2 μm) for fixed fiber spacing of 2 μm. Consistent with the experimental findings, our model predicts a biphasic relation between cell attachment density and fiber diameter wherein the minimum cell attachment density is observed at 1 μm fiber diameter. This biophysical model can be extended to other nanostructures and microorganisms for ab initio biomaterial design.

Magnetite nano needles for single cell analysis

Poster Presentation. NEMB2016-5947

***Mincho Kavaldzhiev, Jose Perez**, King Abdullah University of Science and Technology, Thuwal,Saudi Arabia, **Jurgen kosel**, King Abdullah University of Science & Technology, Thuwal, Select State/Province,Saudi Arabia*

Single cell analysis and manipulation is an active research domain that studies the heterogeneity of cell populations, investigates with high precision the inner components of living cells and tries to improve the understanding of cell mechanisms. Developing tools to analyze, for example, drug efficacy, protein levels, RNA transcripts or pH is key to develop novel treatments and to better understand living organisms. This work focuses on the fabrication and characterization of magnetic nano needles as an in-vitro tool for cell analysis and manipulation. By using a magnetic material, the needles can be remotely actuated, for instance, to exert forces or to generate heat.

Two different sizes of iron needles were fabricated: 400 nm and 4 μm in diameter, with aspect ratios from 1:5 to 1:10. The fabrication of the needles was done using electron beam lithography coupled with reactive ion etching and iron electroplating.

The needles are inductively heated by an alternating magnetic field and their tips are deflected by a constant magnetic field. Cell viability studies of HCT 116 (human colorectal carcinoma) cells grown on substrates with the iron nano needles was analyzed via Calcein AM / Ethidium homodimer-1 fluorescent staining, revealing a high degree of biocompatibility for up to 24 hours of incubation. Fluorescence and confocal microscopy analysis showed the growth of cells on top of the needles, with the restructuring of the cell membrane around the needles. These properties make arrays of such magnetic nano needles on cell culture substrates promising tools for cell analysis.

The effect of surface corona on the interaction of silver nanoparticles with human serum and on toxicity to cancer cells

Poster Presentation. NEMB2016-5978

***Amanda N Abraham, Vipul Bansal, Ravi Shukla**, RMIT University, Melbourne, VIC,Australia*

Silver nanoparticles (AgNPs) incur the highest incorporation in consumer and medical products to date, due to their broad spectrum antibacterial and antifungal properties. They are widely used in catheters, wound dressings and various household products such as electronics, cosmetics, toothpastes, shampoos, air sanitiser sprays, bedding, clothing etc. Typically, silver nanoparticles are synthesized using a reducing agent and a stabilising agent, such as hydrazine and sodium borohydride. However, both these and many other reducing and stabilising agents used in nanoparticle synthesis are highly toxic to living organisms and the environment.

One alternative method of AgNP synthesis involves the use of plant extracts such as Aloe vera, cinnamon, edible mushrooms and neem. Evidence suggests that the polyphenol compounds present in these plant extracts are responsible for the reduction of the metal ions to nanoparticles while simultaneously coating the surface of the nanoparticles. In order to extend the usage of these AgNPs for biological applications, the mechanism of action needs to be fully understood. Currently, it is not known whether the effect on cells is elicited due to the polyphenolic coating (surface corona) or due to the core of the nanoparticle. Another aspect to keep in mind when considering the use of NPs for in vivo purposes is that NPs interact with biological fluids such as a serum before reaching the target organ. However, it is unknown what role the surface corona plays in interaction with proteins found in the blood or in cell culture media in in vivo and in vitro scenarios.

In order to shed some light on these aspects, this study used silver nanoparticles (AgNPs) synthesised with bi-and polyphenolic compounds viz. curcumin from turmeric and Epigallocatechin gallate (EGCG) from green tea, respectively. L-Tyrosine, a monophenolic amino acid was used as a control as it is an amino acid found in all living organisms and is not toxic to the body. The interaction of these AgNPs with human serum albumin (HSA) was elucidated using fluorescence spectroscopy and toxicity to prostate cancer (PC-3) cells was analysed using trypan blue dye exclusion assay and confocal microscopy. The results suggest that while the surface corona does dictate the interaction with serum and toxicity, the effect on toxicity appears to be a synergistic effect of both the surface corona and the silver core of the AgNPs.

Modelling Cancellous Bone: An Elastic Mixture Theory Approach

Poster Presentation. NEMB2016-5903

***Burhanettin Altan**, Giresun UNiversity, Giresun,Turkey*

Bones are common in all mammals as well as in birds and other animals which are typical examples of open cell porous media that can be found in the nature as light-weight structural elements. Man-made open cell porous media are also used as materials for light-weight structural elements with high specific strengths as well as thermal insulators. It is an ongoing active research field to develop methods to predict the mechanical, thermal, acoustical behavior of open cell porous media.

Open cell porous media can be envisioned as a collection of randomly interconnected struts. The properties, both the geometrical (cross- sectional area and length) and the mechanical (Young's modulus, Poisson ratio, fracture) ones of the struts may exhibit significant variations from cell to cell, even within a cell. The complexity of the structure of the open cell porous media poses difficulty for modelling their mechanical behavior. Finite Element Analysis (FEA) has been proven to be useful to model the mechanical behavior of open cell porous media. Li et al. [1] studied the mechanical response of an open cell porous medium with various cell size and strut cross sectional area. Another approach for modelling the mechanical behavior of porous medium is to replace it with an equivalent continuum for which Gibson and Ashby [2] can be referred to.

A homogenization method for modelling mechanical behavior of cancellous bone employing the theory of elastic mixtures is introduced. A short survey on cancellous bones and importance of its mechanical behavior is provided. The models available in the literature for the mechanical behavior of cancellous bones are summarized. The scheme for modelling the solid skeleton of cancellous bone is introduced in full detail. Wave propagation in cancellous bone is studied and it is shown that the constitutive equation obtained in the study is capable to represent positive and negative dispersion as well as attenuation. The effect of bone marrow on wave propagation is discussed by incorporating a generalized Biot's theory. This study is concluded by indicating that the quantitative ultrasound can be extended to obtain some estimation about the micro-architectural parameters of cancellous bone.

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Dense iron nanowire array for the culture and regulation of fate of mesenchymal stem cells

Poster Presentation. NEMB2016-5945

***Jose Perez**, King Abdullah University of Science and Technology, Thuwal, Makkah,Saudi Arabia, **Timothy Ravasi, Jurgen kosel**, King Abdullah University of Science & Technology, Thuwal, Select State/ Province,Saudi Arabia*

Mesenchymal stem cells are highly sensitive to mechanical stimuli, responding with the regulation of fate through the reorganization of the cytoskeleton and activation of specific signaling pathways. In this work, an array of iron nanowires was studied for its use as a substrate for the culture and mechanical stimulation of mesenchymal stem cells. For this purpose, a nanoporous alumina template was fabricated by a two-step anodization process, followed by a pulsed electrodeposition step of iron in the array of pores. Then, a partial release of the template was performed via wet chemical etching in order to remove a portion of the nanoporous alumina and reveal an array of dense, vertically aligned nanowires. In order to study cell growth, attachment and spreading behavior on the nanowire array, human mesenchymal stem cells were cultured for up to six days and then fluorescently stained for actin filaments and cell nuclei. Fluorescence staining of cells with Calcein AM and Ethidium homodimer-1 allowed the determination of cell viability at different incubation times. Additionally, the interface between the cells and the nanowire array was observed by scanning electron microscopy after critical point drying. Results showed a reorganization of actin filaments coupled to an altered, spherical cell shape. Actin filaments accumulated in bundles at the adhesion points of the cells on the nanowires, driving the change of cell shape. The cell viability of attached cells remained higher than 90% up to six days of incubation, though the overall number of attached cells was lower compared to the control. The interface between the cells and the nanowire array confirmed the reorganization of actin and showed the focal adhesion points on the iron nanowire array. The cells possess sufficient force to pull the nanowires towards themselves as they alter their shape. Further, multiple

filopodia were observed surrounding each cell, suggesting the probing of the array to find new adhesion points. Overall, the iron nanowire array is a potential platform for studying stem cell differentiation, as well as to influence it through magnetic field mechanical stimuli.

Protein-lipid synergy drives mitochondria fission

Poster Presentation. NEMB2016-6075

***Ehsan Irajizad**, University of Houston, Houston, TX, United States, **Rajesh Ramachandran**, Case Western Reserve University School of Medicine, Cleveland, OH, United States, **Ashutosh Agrawal**, University of Houston, Houston, TX, United States*

Mitochondria are one of the most important organelles in our cells and are responsible for breathing of the cell and supplying energy for our body. How they maintain their shape and undergo fission, however, continues to remain a mystery. Experimental studies have implicated various proteins and lipids such as Cardiolipin, Drp1, PE, Fis1 in fission but the energetics and pathway for mitochondria fission still remain elusive. In this study, we simulate protein-lipid interactions and provide novel insights into mitochondrial fission. Our study reveals that the mitochondrial fission is driven by an instability triggered by DRP1 proteins and PE lipids. The computed shapes from our simulation match closely with the shapes observed experimentally.

Numerical and Experimental Investigation of Gold Nanoparticle Heating-induced Protein Inactivation for Nano-Bio Interface Applications

Poster Presentation. NEMB2016-5976

***Peiyuan Kang, Oumar Fall**, The University of Texas at Dallas, Richardson, TX, United States, **Jaona RANDRIANALISOA**, GRESPI University of Reims Champagne-Ardenne, Reims,France, **Zhenpeng Qin**, University of Texas at Dallas, Richardson, TX, United States*

Gold nanoparticles (GNP) have received broad interest in biomedical applications due to their unique optical and thermal properties. Specifically, GNP can be designed to strongly absorb or scatter optical energy at different wavelengths of interest. The laser energy absorption can lead to significant temperature changes on the nanoparticle as well as the surrounding biological environment. Among the molecular components in the biological system, proteins are extremely thermally sensitive and play a critical rule in performing biological functions. Although the thermal effect on proteins are well understood in low temperatures (below 100°C) and longer time scale (seconds to minutes), the fate of protein under short time scales (enabled by nanosecond pulsed lasers) are less understood.

In this research, we investigated on the effect of GNP heating on proteins in the nanosecond and nanometer scale. Firstly, we performed numerical simulations to obtain the GNP optical absorption, thermal responses and protein inactivation, by using the Mie theory, heat equation and Arrhenius model, respectively. The protein inactivation was found to be strongly dependent on the laser pulse energy, GNP size, distance between the protein and GNP, and pulse number. However, the protein inactivation is independent of the laser pulse shape (Gaussian versus Rectangular with similar pulse width) and temperature dependent thermal properties of water. Thermal interface conductance (TIC) significantly increases the GNP temperature but exerts a relatively smaller effect on surrounding water due to the large specific heat of water. TIC was found to significantly reduce the heat flux from GNP to water and thus affect protein inactivation when the value is smaller than 100 MW/(m2 K).

Secondly, protein inactivation was studied experimentally by conjugating an enzyme (alpha-chymotrypsin) to 15nm GNP with a polyethylene glycol (PEG) spacer. GNP was heated by an Nd:YAG nanosecond pulsed laser at 532nm (Quantel USA) and the protein inactivation was measured by a colorimetric enzyme assay. The experiment shows that the protein inactivation is dependent on laser pulse energy and complete inactivation (up to 90%) is possible. When the enzyme is not attached to the protein, no inactivation was observed, suggesting a highly selective and localized effect.

In summary, this study proves the selective protein inactivation adjacent to gold nanoparticles during nanosecond pulsed laser heating and establishes a numerical and experimental approach to investigate and understand this important phenomenon. The protein inactivation can be controlled in the nanometer scale by laser energy, nanoparticle size, distance between protein and nanoparticle, and thermal interface resistance. Furthermore, our study has a broader impact in understanding the nano-bio interface involving laser nanoparticle heating, and designing novel nano-surgical tools to selective inactive proteins of interest in living systems.

6-5
NANOMATERIALS DESIGN AND MANUFACTURING

Grand Ballroom 5:00pm - 8:00pm

Plasmonic tweezers for biology and medicine: towards low-power and versatile manipulations of cells and biomolecules

Poster Presentation. NEMB2016-6067

***Linhan Lin, Xiaolei Peng, Yuebing Zheng**, The University of Texas at Austin, Austin, TX, United States*

Versatile, non-invasive manipulation and patterning of cells and biomolecules play a critical role in early disease diagnosis, medicine, tissue engineering, and fundamental studies in life sciences. Despite its capability of offering remote, real-time and versatile manipulations of particles, conventional optical tweezers require high laser power and have remained challenging in the non-invasive operation. Employing the near-field effect of the surface plasmons and its capability to concentrate light at the subwavelength scale, we develop a new type of plasmonic tweezers to non-invasively manipulate and pattern cells and biomolecules on plasmonic substrates by low-power laser beams. The plasmonic substrate is optimized to match the plasmonic resonance wavelength with the laser wavelength and have high-density “hot spots”, which improve the optical force and dramatically reduce the laser power. Through directing the laser beam, dynamic trapping and versatile manipulation of single/multiple biological particles is achieved. Furthermore, employing the plasmon-enhanced photothermal effect, a microbubble is generated to capture and immobilize the biological particles on the substrate through coordinated actions of Marangoni convection, surface tension, gas pressure, and substrate adhesion. Arbitrary patterns of particles and cells with different architectures are demonstrated. With the low-power operation, versatility, and biocompatibility, this new plasmonic tweezer will find a wide range of applications in biology and medicine.

Moire metasurfaces: from nanoengineering to applications in biology and medicine

Poster Presentation. NEMB2016-6058

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Moiré metasurfaces consist of arrays of plasmonic nanostructures in moiré patterns. Due to the high rotational symmetry and a wide arrange of nanostructure sizes and shapes, moiré metasurfaces exhibit unique optical property that can be harnessed for various applications. Herein, we report our recent progress in fabrication, characterization and applications of moiré

metasurfaces in biology and medicine. Firstly, we have developed moiré nanosphere lithography (MNSL) for the high-throughput and cost-effective fabrication moiré metasurfaces. We create moiré metasurface patch by stacking a thin Au moiré metasurface layer on an optically thick Au layer separated by a dielectric spacer layer. Secondly, we have employed both experiments and simulations to show that the moiré metasurfaces and their patches exhibit multiple and tunable plasmonic modes. For example, the moiré metasurfaces can have the strong absorption (>>95%) of incident light across a broad range of electromagnetic spectrum, i.e., near-infrared (NIR) range from 900 nm to 1700 nm and mid-infrared (MIR) range (~5 um). The multiband responses and strong field enhancements of the moiré metasurfaces patches are exploited for dual-functional applications in nanospectroscopy and phototherapy. Lastly, we have demonstrated that the moiré metasurfaces exhibit chiral optical responses in a wide range of wavelengths due to the rotational symmetry of the metasurfaces. The optical chirality underpins a new type of optical sensors that can detect the chirality of molecules for applications in nanomedicine and life sciences.

Fabrication of hybrid fibrous structure using drum collector-based electrospinning

Poster Presentation. NEMB2016-6123

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Nanofibers are very useful in biomedical engineering and filtration fields because they have various advantages such as high surface area to volume ratio, porosity, and good permeability. As a result, diverse kinds of nanofibers have been introduced to various applications of tissue engineering and cell biology. Electrospinning is known as one of the most typical methods to fabricate nanofibers, however, electrospun nanofibers have strong limitation in cell migration and porosity control. As a result, the seeded cells mainly covers a fibrous mat instead of going into inside of the mat. Thus, it is very difficult to find the cell migration over full thickness of fibrous mat.

In this study, we present a hybrid fibrous structure enhancing controllability of porosity as well as cell migration. The hybrid fibrous structure is fabricated using a drum collector electrospinning. The polymer for electrospinning was polycaprolactone with number average weight of 70,000 - 90,000. Its solutions with various concentration were prepared after dissolving in pure chloroform for 12 h. Also, a drum collector based electrospinning equipment was prepared to fabricate various types of electrospun fibers by varying process parameters including rotation speed of drum. It was equipped with a syringe pump, high voltage power supply, and a servo motor for rotating drum with higher velocity controllability as well as high speed. In the experiments, PCL fibers with variety of diameters, thickness and morphologies were fabricated under various electrospinning and drum speed conditions.

The morphologies of the electrospun fibers were observed using a field-emission scanning electron microscopy. From the observation results, it could be seen that the diameter of fibers changed with good consistency between 700 nm and 3 um according to the solution concentration and tip to collector distance. Also, it was shown that the level of fiber alignment could be controlled using drum speed. Also, the thickness of fiber mat was controlled using electrospinning time. As a result, three types of mat with different combinations of fiber diameter and thickness were fabricated to investigate the cell migration into the mats.

To indicate cell viability and mobility of the mat, in-vitro cell tests using fibroblast cell line (NIH-3T3) were performed. Cells were cultured in medium supplemented with 10% fetal bovine serum and 1% penicillin streptomycin under the typical culture conditions (humidified atmosphere of 5% CO2 in air at 37 °C). Cells were seeded at a concentration of 10,000 cells/sample, and then the media in each samples was replaced with fresh media at every two days for 7 days. Cell proliferation was assessed using DNA content in each mat. The cell morphology was assessed using SEM images and F-actin/DAPI staining. From the results, it was demonstrated that the fabricated mat with hybrid structure could provide significantly improvement in cell migration as well as cell viability.

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Development of a Low-Cost Electron Paramagnetic Resonance (EPR) Spectrometer to Measure Properties and Heating of Iron-Oxide Nanoparticles

Poster Presentation. NEMB2016-6022

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Abstract:
Iron oxide nanoparticles (IONPs) are in development in laboratories throughout the world for their capabilities in diverse biomedical applications.1 IONPs are designed for multifunctional uses, including providing contrast agent in imaging and for delivering therapies. Despite their potential for such applications, much remains to be learned about their physical properties, biocompatibility, and effectiveness for treating cancers using magnetic fluid hyperthermia (MFH). In this project, we are designing and building a new tool for studying IONP properties and MFH heating. Specifically, we are building a low-cost electron paramagnetic resonance (EPR) spectrometer using only a radiofrequency field.2 In our studies of IONPs, this system will be used for two purposes: First, we will use it to measure the EPR spectrum of different IONP formulations. Second, we will use our device to investigate the possibility to increase MFH heating using circularly-polarized radiofrequency irradiation to modulate the direction of the magnetization (as opposed to the conventional linearly-polarized RF field).

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Antibacterial Activity of Cationic-cellulose Nanofibers treated with Zataria multiflora Boiss. Extracts

Poster Presentation. NEMB2016-5901

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Cellulose-based textile have been widely used by consumers due to its outstanding properties such as no static electricity, biodegradability, good absorbency, comfort soft hand , breathability and hygroscopicity. For these reasons by discovering nanofibers in textile industry cellulose Nanostructures fiber also grab worldwide considerable attention besides, amongst all nanofibers, cellulose-based nanofibers are very fascinating due to easy fabrication via the electrospinning process, it is well known that cationizing cellulose by the cationic agent shows significant advantages in terms of improved dyeing properties because the repulsive charges on cellulose surface can eliminate with surface modification of cellulose by using cationic agents.

On the other hand, Antimicrobial textiles are becoming very important and demandable due to different health problems and global environmental awareness issues.

The present study was carried out to determine the antimicrobial properties of cationic-cellulose nanofibers which produces by electrospinig process then dyed with Zataria multiflora Boiss extracts(asa a bioactive antibacterial

agent), with cold pad batch method as an ecofriendly dyeing method, with different dye concentration.

Zataria multiflora Boiss. is a thyme-like plant belonging to the Lamiaceae family with a remarkable antibacterial and antioxidant activity. This plant with vernacular name of Avishan Shirazi (in Iran) has traditional uses such as antiseptic, anesthetic and antispasmodic and a popular spice in food or drinks. Nanofiber webs dyed with Zataria multiflora Boiss. extracts by padding method and the different dye concentrations for padding were selected (40 and 60 gram per litter). The color strength (K/S value) of all dyed swatches was evaluated by using UV/Vic spectrophotometer.
The antimicrobial activity of samples was considered against gram negative (Pseudomonas aeruginosa) and gram positive (Staphylococcus aureus) bacteria.

Antibacterial properties of cationic-cellulose nanofibers were estimated by standard AATCC Test Method 100-2004, all fabric swatches were sterilized before antimicrobial testes by U.V autoclave, the dilution medium was nutrient broth to evaluate the antimicrobial activities, the reduction in colony number between the treated and untreated nanofiber webs after incubation was specified.

All Dyed species illustrated very effective antimicrobial properties, more than 95% microbial reduction in all bacterial population. In higher concentration of Zataria multiflora Boiss. the antibacterial activity of samples increased remarkably. The obtained results show that the antimicrobial activity of nanofibers is due to the presence of Zataria multiflora Boiss therefore, Zataria multiflora can be used in eco-friendly dyeing and antimicrobial finishing of Cationic-cellulose Nanofibers, this colored textile with breathable and hygienic properties can potentially be considered as future apparel for surgical gowns or for other hospital usages, institutional articles, casual and fashion.

Secure IPN's Nano Fibers for Insulin Delivery

Poster Presentation. NEMB2016-6105

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SUMMARY
Novel triblock copolymers of poly(L-lactide)-poly(ethylene glycol)-sebacate-poly(ethylene glycol)-poly(L-lactide) were synthesized by Ring-Opening Polymerization (ROP) using Diazabicyclo-[5.4.0]- undec-7-ene (DBU) as catalyst and different ratio of L-lactide with pre-prepared poly(ethylene glycol)-sebacate-poly(ethylene glycol) polymers, namely poly(ethylene glycol) molecular weight, 2000, 4000, and 10000 g/mole respectively. These polymers were loaded with 50 IU insulin, and then they were crosslinked with hydroxyl ethyl cellulose to produce the nanofiber IPN's samples. The results simply supported that loading of insulin with prepared nanofiber IPN's can be used for controlled insulin release.

POLYVILLIC NANOSTRUCTURES FOR BIOMEDICAL APPLICATIONS: A SNOWFLAKE INSPIRED DESIGN

Poster Presentation. NEMB2016-5913

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Gold has continued to be of scientific interest throughout the majority of mankind's existence. Initially for its luster, used as currency and in recent decades gold has been found to have many other applications in electronics and medicine. At the nanoscale, gold has been applied toward drug delivery, diagnostic imaging contrast enhancement, and photothermal ablative therapies. We describe our preliminary findings on a novel synthetic methodology initiating morphological differentiation of highly controlled branched gold nanostructures. The morphological differentiation was fundamentally driven by incubation temperature, time, and environmental pH. Differentiated morphologies varied from dendritic, snowball, to polyvillic. This differentiation approach initiates a myriad of shapes using relatively low temperatures in aqueous solution wherein the branching can be finely tuned. The extent of branching, size of nanostructure and morphology found to be facilitating cellular internalization with significant loading which could be detected by bright field imaging, and Raman spectroscopy. Origin of near infrared absorbance in these nano-differentaiations enunciate their probable use in broad spectrum of biomedical uses ranging from in vitro diagnostic to tissue penetrating detection agents.

Fabrication of an Electrospun Chitosan-blend-Poly(Ethylene Oxide)-co-Fibrinogen Nanofibrous Scaffold with Biphasic Drug Delivery Potential

Poster Presentation. NEMB2016-5915

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Introduction:Electrospinning is a process that allows for a great deal of versatility in producing novel biomaterials and can be used to generate nanofibrous scaffolds. Electrospun nanofibrous scaffolds can be prepared with a high degree of structural control, thereby generating properties needed for biological success. The objective of this study was to fabricate chitosan (CS) and fibrinogen (FI)-based bioactive nanofibrous scaffolds, and to evaluate the drug loading potential, degradation, and biological and physical properties. The ability to electrospin nonwoven CS and FI-based nanofibers designed for controlled drug delivery is an important step in the development of a stable natural polymer-based wound dressing.

Methods: CS-based scaffolds were fabricated as a blend of CS with poly(ethylene oxide) (CS/PEO) at a 2:1 weight ratio, while FI-based scaffolds were fabricated as a homogenous solution. Both scaffolds were electrospun using a stationary plate electrospinning apparatus. CS/PEO-FI scaffolds were fabricated by co-electrospinning the two systems via a dual spinneret/mandrel apparatus. The morphology of the scaffolds was characterized by scanning electron microscopy (SEM). Scaffold degradation was evaluated for up to 48 hours in cell culture media at 37°C by dry weight and Fourier Transform Infrared (FTIR) spectroscopy. Platelet-derived growth factor (PDGF) was loaded into both electrospinning solutions and the release kinetics for CS/PEO and FI nanofibrous scaffolds assessed by ELISA. Finally, fibroblast viability and proliferation were evaluated using water-soluble tetrazolium (WST-1) assay to test biocompatibility.

Results: Evaluation of SEM micrographs showed randomly aligned CS/PEO (2:1), FI, and CS/PEO-FI nanofibers with a mean fiber diameter of 244 ± 70 nm, 488 ± 86 nm, and 353 ± 133 nm, respectively (n = 100). Scaffold degradation indicated a biphasic degradation of CS/PEO, in which PEO was degraded in a burst release. In contrast, FI degradation indicated a steady and sustained release of FI over 48 hours, and CS/PEO-FI scaffolds showed characteristics of both individual scaffolds. Chitosan/PEO released PDGF as predicted by degradation results, in a burst release within the first few hours. Fibrinogen PDGF release was sustained over 48 hours. None of the three scaffolds significantly impacted fibroblast viability or proliferation over five days.

Conclusion: CS/PEO- and FI-based scaffolds are biocompatible, have favorable degradation kinetics, and are capable of being co-electrospun with growth factors such as PDGF. Furthermore, these studies demonstrate the feasibility of fabricating co-electrospun CS/PEO-FI nonwoven nanofibrous scaffolds. Together, these studies demonstrate that CS/PEO and FI scaffolds are excellent candidates for future drug-release applications, and suggest

that a CS/PEO-FI nanofiber scaffold could be used as a tunable biphasic drug-delivery platform for wound dressing and other clinical applications.

Cell-On-Chip Stretchable Platform For Mammalian Cells Studies

Poster Presentation. NEMB2016-5944

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CELL-ON-CHIP STRETCHABLE PLATFORM FOR MAMMALIAN CELLS STUDIES

Research in stretchable electrodes has long history and broad range of materials and electrode designs were reported in literature. The stretchable electrodes could be fabricated from gold, platinum or single walls carbon nanotubes. The electrodes fabricated on stretchable substrate reported in the literature are passive electrodes used to in vivo record the electric field of the heart. The ECIS electrodes fabricated in this research are active electrodes that are exposed to ac voltage and are part of an electrochemical sensor. Endothelial function involved in endothelial damage, arterial degeneration, plaque formation. Mechanical stress caused by blood flow and pressure can influence the function of endothelia cells. Therefore, study the endothelial response to mechanical stimuli in vitro assists understanding the endothelial function and mechanisms. ECIS sensor has been shown to be able to evaluate cell function, morphology and behavior. This paper presents the fabrication and testing of electric cell-substrate impedance spectroscopy (ECIS) electrodes on a stretchable membrane, which can evaluate the influence of mechanical stimuli on endothelial functions underwent cyclic stretch. It's the first time the ECIS sensors were fabricated on a stretchable substrate and ECIS measurements on mammalian cells under 15% cyclic strain were successfully demonstrated. A chemical was used to form strong chemical bond between gold electrodes of ECIS sensor and polymer membrane, which enable the electrodes keep good conductive ability during cyclic stretch. The stretchable membrane integrated with the ECIS sensor can simulate and replicate the dynamic environment of organism and enable the analysis of the cells activity involved in cells attachment and proliferation in vitro. Bovine aortic endothelial cells (BAEC) were used to evaluate the endothelial function influenced by mechanical stimuli in this research because they undergo in vivo cyclic physiologic elongation produced by the blood circulation in the arteries. The ECIS sensor fabrication has two main steps. Firstly, a gold film was deposited on a glass slide using a shadow mask and gold sputtering technique. Secondly, a thin PDMS membrane that was pre-stretch 20%. Finally, the gold electrodes on glass slide were stamped onto stretched PDMS membrane. A probe station was used to evaluate the Au electrodes conductive ability, since the wrinkles and cracks will be generated on the Au electrodes during stretch. The inherent resistance values fluctuated between 120 Ω and 200 Ω during stretch, which was acceptable since the impedance of cell monolayer is more than thousands ohms. The BAEC were cultured on the stretchable ECIS sensors mounted on a linear motor. The impedance data was acquired at the end of each stretch/release cycle. Firstly, the BAECs were passively cultured for 50 hours. Then the cyclic stretch of 15% was applied for 35 hours. When the cyclic stretch was applied the impedance values of cell monolayer were 660 Ω. The impedance decreased during the initial 5 hours of cyclic strain due to the degradation of intercellular junction. In time, the cell attachment and proliferation was improved by the cyclic and the corresponding final impedance was 862.16 Ω. As reported in the literature endothelial cells exhibited an increase in proliferation in response to stretch. This study is the first time ECIS measurements were performed on a stretchable substrate, which enables the BAEC subject to cyclic strains for long period of time.

Improved Clinical Diagnosis of Tuberculosis by Antibody-Free Detection of Circulating Mycobacterium tuberculosis Antigen

Poster Presentation. NEMB2016-6085

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Tuberculosis disease (TB) by Mycobacterium tuberculosis (Mtb) infection poses a significant public health challenge to both the patients and the wider international community. The current "gold standard" for TB diagnosis and treatment monitoring in adults as well as pediatric patients relies heavily on time-consuming bacterial culturing methods. For this reason, rapid screening and accurate diagnosis of active TB is still challenging and critically needed for global TB control efforts. In this study, we present a rapid and streamlined technology, using nanoporous silica thin films, which are optimized for pore size, geometry, capillary force, and film thickness, to isolate Mtb antigens in laboratory and clinical samples for rapid TB screening. This technology, referred to here as NanoTrap, is integrated with the detection capability of the high-throughput matrix-assisted laser desorption/ionization time-of flight mass spectrometry (MALDI-TOF MS) to screen and identify fragments of the Mtb antigen, CFP-10, from complex human bodily fluid samples, without use of immunoaffinity agents. With the use of this comprehensive approach, we clearly distinguished a clinical isolate of active TB patients from nonTB species infection patients, latent TB infection patients, as well as healthy controls by detecting CFP-10 from the blood circulation. This assay can reach a detection limit of 1nM with a 90% sensitivity and a 99% specificity for the antigen CFP-10. Our strategy has significant potential to fill the conceptual and technical gaps in rapid diagnosis of active TB disease.

Minimally Invasive Curved Microneedle for Innocuous Treatment of Retinal Detachment

Poster Presentation. NEMB2016-6165

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Retinal detachment has been a serious vision threatening disease. Consensus treatment of retinal detachment is the re-attachment of retina onto choroid. Although there are various surgical methods for treatment of retinal detachment, no satisfactory visual outcome has been obtained without side effects such as unintended retinal tear, surrounding tissue damage and severe hemorrhage. In order to minimize surgical side effects, we developed novel curved microneedle, which has specific features such as 30° curved hollow metallic microneedle with 15° beveled tip, 80 µm inner diameter and 100 µm outer diameter for suction of high viscous sub-retinal fluid with minimally invasive and safe application. Stable drainage of sub-retinal fluid by UDM was demonstrated with in vitro and ex vivo experiments. Invisible retinal hole on the re-attached retina was made by UDM whereas 200 µm diameter of retinal large hole and incurring choroidal damage was induced by traditional hypodermic needle. In conclusion, UDM is a potential alternative to traditional surgical needle for innocuous treatment of retinal detachment.

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Built upon a solid foundation of research and development, K-Laser has earned worldwide recognition for its therapy and treatment solutions. The K-Laser development team is dedicated to offering the most advanced technologies to our clients, with a never-ending dedication to innovation. The K-Laser Class IV Laser Therapy accelerates wound healing, decreases inflammation, and increases joint flexibility with no known side-effects.

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Izon Science designs, manufactures and sells precision instrumentation for nano- and micro-scale particle analysis. Using its technology, Tunable Resistive Pulse Sensing (TRPS), thousands of particles can be accurately analyzed for their size, concentration and charge. The technology is highly flexible and directly measures a wide range of biological and synthetic particles with individual particle resolution. Izon's instruments are in use in research institutes, universities, pharmaceutical and biotechnology companies around the world.

Visual Sonics FujiFilm (www.visualsonics.com/)

FUJIFILM VisualSonics, Inc is the undisputed world leader in real-time, in vivo, high-resolution, micro-imaging systems, providing modalities specifically designed for preclinical research.

These cutting edge technologies allow researchers at the world's most prestigious pharmaceutical and biotechnology companies, hospitals and universities to conduct research in cardiovascular, cancer, neurobiology and developmental biology areas. These technologies support applications that include genetic research, phenotypic studies and drug development. VisualSonics platforms combine high-resolution, real-time in vivo imaging at reasonable cost with ease-of-use and quantifiable results.

MANCEF (www.mancef.org/)

MANCEF connects a global community focused on commercializing micro, nano, and emerging technologies through conferences and educational efforts. MANCEF organizes international conferences, trade shows, educational training sessions, seminars and internet-based forums focused on micro, nano, and emerging technologies enabled by the effects of miniature scale, including commercial and educational opportunities utilizing such technology. We provide a forum for a network of professionals to exchange information in a timely manner to help accelerate the acceptance and commercialization of emerging technologies.

Nanosurf

Nanosurf develops and manufactures unique scanning probe microscopes to help researchers easily and reliably acquire and analyze bio-mechanical structural information with picoNewton resolution. Our FluidFM system combines nanofluidics with the force sensitivity and positional accuracy of an atomic force microscope providing new possibilities in single-cell biology. Nanosurf has established a world-wide reputation of offering its customers a range of products and services that are reliable, cost-effective, and easy-to-use.

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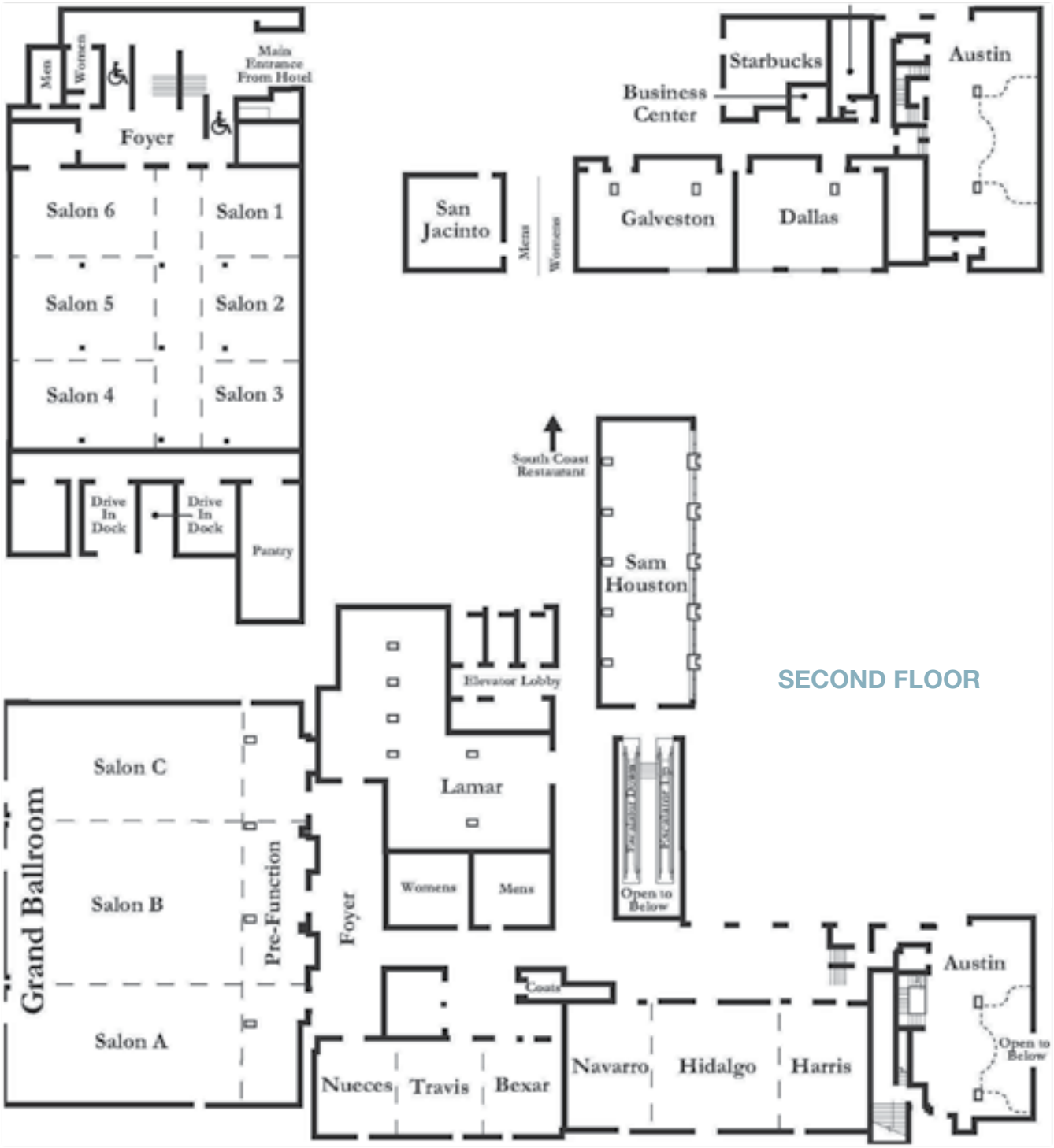
LIBERTY HALL



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ASME
SETTING THE STANDARD

NEMB

NanoEngineering for Medicine and Biology Conference