



Building Nanoglands

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In the search for personalized medicine, nanochannel implants mimic the body's natural regulators of health.

When it comes to maintaining our health, there is nothing more effective than our own bodies. The body senses signals that can be chemical (e.g., variation in the plasma glucose concentration), biochemical (e.g., presence of virus), mechanical (e.g., accelerations such as impacts or pressure drops), or thermal, and thereby triggers a proportional response.

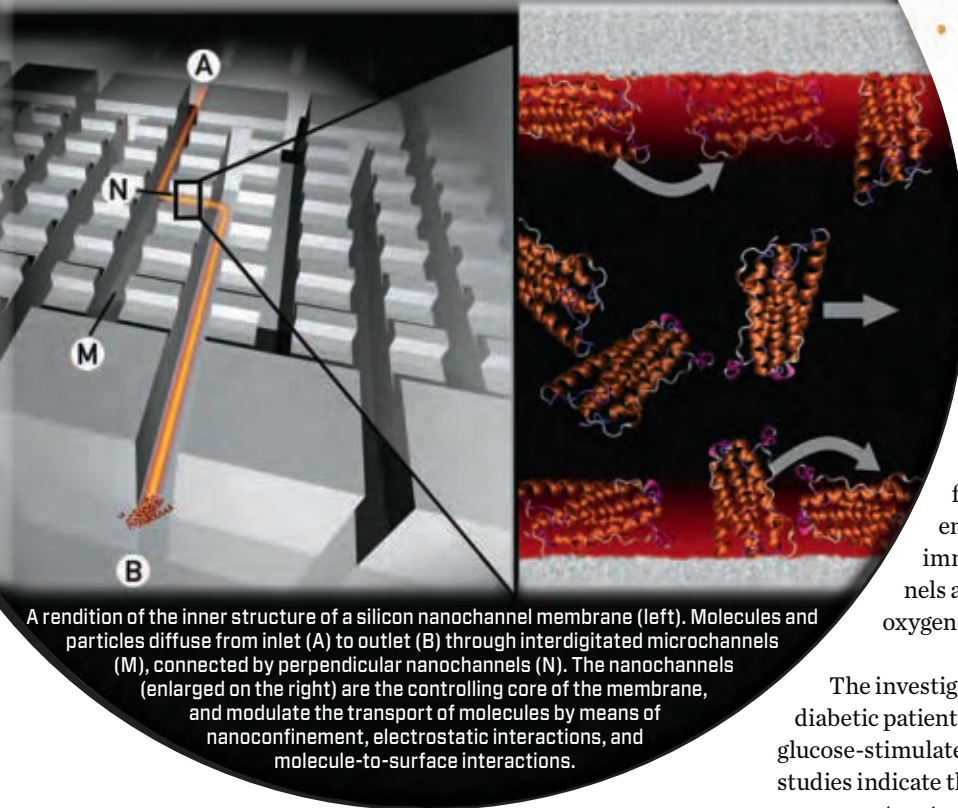
In this context, the glands of the body (pituitary, thyroid, parathyroid, adrenal, pancreas, ovary, and testis) work by providing molecules (typically hormones) when required and only in amounts that are determined by the need. This is true when there is a healing process involved (such as releasing antibodies to fight an infection) as well as when it is a matter of maintaining health: the release of insulin and glucagon from the pancreas, for instance, maintain the glucose level in the plasma in the normal, or euglycemic, range.

The combined action of insulin and glucagon introduces three important points about life. First, it reminds us that health is all a matter of balance (homeostasis). Insulin and glucagon have indeed opposing effects on glucose uptake, and their yin-yang is necessary to keep the system functional. Too much or too little of one or the other makes life impossible. Second, the “right” range changes

with time. Circumstances such as eating or exercising change the body's need of glucose. And third, the “right” release is often a combination of constant passive release (the so-called baseline insulin) and a time-variable component (active release).

Novel approaches which can balance the release of therapeutic molecules in accordance with need are an as-yet-unachieved goal of modern medicine.

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nanochannels with unprecedented control over dimensional tolerances.

It is impractical to transplant unprotected foreign cells into the body for long-term therapy because they will be rapidly destroyed by the immune system. By encapsulating transplanted cells within silicon implants, however, the research team proved that it is possible to maintain the viability and functionality of pancreatic cells for extended periods of time. The nanochannel enclosure physically protected the cells from immunorejection. The implants contained channels as small as 18 nm that allowed the exchange of oxygen and nutrients through the encapsulation.

The investigation, aimed at restoring normoglycemia in diabetic patients, showed that the cells could maintain both a glucose-stimulated and basal insulin secretion. This and other studies indicate that nanofluidic systems can be developed for permanent restoration of body functions that have been compromised by underlying medical conditions.

• • • Steady-Release Implants

Nanofluidic membranes reveal counterintuitive mass transport phenomena in nanochannels caused by fluid confinement. As opposed to the macroscale, where water molecules next to a pipe wall have zero velocity, in nanochannels fluid molecules slip at the channel surface, experiencing an enhanced convective transport. Ferrari and his colleagues showed that by shrinking the size of the channel to a few times the size of diffusing molecules, it was possible to achieve a saturated, concentration-independent transport through membranes: promising for controlled transport of molecules and therapeutics. ("Fantastic Voyages" by Mauro Ferrari appeared in this magazine in October 2006 and discussed the concept of encapsulating cells for long-term therapy.)

Since then, Ferrari's group has focused on the development of nanopores and nanochanneled membranes and on nanoscale fluid mechanics.

Medical practitioners have long realized the need for personalized treatment of significant pathologies and medical conditions, capable of synergizing with the innate response of the body to diseases. Despite this realization, the most common approaches to drug administration in current medicine are oral or intravenous delivery of bolus (a large amount of drug delivered all at once at regular intervals) or intravenous slow infusions.

The bolus administration is commonly associated with adverse side effects deriving from a temporary regional overdose, to which patients are subject following each administration. Infusions are required in case of highly toxic drugs (typical of numerous chemotherapeutics), which force patients to be in hospital beds for periods ranging from hours to days, to tolerate the severe side effects of treatments. This conventional practice represents a rudimentary and impersonal approach to drug delivery, where therapeutics are often coarsely and inefficiently delivered at maximum tolerated doses.

How can medical technology possibly mimic the body's natural control over the release of molecules with accurate dosing and precise timing? The answer can be found in nanotechnology and nanoscale fluid mechanics.

Over the past three decades, micro- and nanotechnologies have been actively pursued for biomedical applications. It was in the early 1990s that Mauro Ferrari led a group at the University of California at Berkeley that first demonstrated a breakthrough by the use of a silicon-based nanochannel membrane, de facto establishing the field of nanofluidics. They used the membrane as a molecular sieve capable of immune-isolation, thus enabling rejection-free transplantation of cell clusters. They used conventional photolithographic techniques and developed new protocols for the fabrication of membranes hosting arrays of

A network of biosensing nodes (BSN) communicates with the nanogland with feedback loops. The nanogland, remotely powered through radio frequency, responds to the input and modulates the release of therapeutics while communicating health status to a monitoring center (MC).



As a part of Ferrari's group, now located at the Methodist Hospital Research Institute in Houston, we have developed methods for fabricating mechanically robust devices with hundreds of thousands of densely packed nanochannels with precisely controlled size and surface properties. Advancing the technology, we have used sacrificial-layer techniques to reproducibly fabricate nanochannels as small as 3 nm.

We have also analyzed the nanoscale fluid mechanics, and developed and demonstrated new predictive mechanistic laws for osmotic pressure and constrained diffusion in nanochannels. At the nanoscale, molecular interactions with the channel wall dominate the transport of fluids to such an extent that the classical mechanical laws of diffusion (Fick's laws) break down.

With these highly structured membranes, we have attained our goal of constant release of therapeutics, over a broad range of molecular sizes, at release rates relevant for medical applications. The constant release was achieved with small molecules such as leuprolide, a common treatment for prostatic cancer, as well as with large molecules such as bevacizumab, widely used in the treatment of metastatic colon cancer.

Such an approach could be applied to achieve the goal of metronomic delivery of chemotherapeutics, a constant low-dose administration of drugs over a long period of time. Metronomic regimens have shown promise in the treatment of cancer with developed resistance to periodic administration of maximum tolerated doses. Targeted metronomic nanodevices, surgically or percutaneously implanted, could regionally deliver "seeds" of chemotherapeutics and be applied to prostatic, breast, or intracranial solid tumors, for example.

By exploiting nanochannels in passive systems we were able to achieve a controlled and constant delivery for extended periods of time, mimicking the basal and continuous release of molecules from natural glands. This functionality cannot be attained at the macro- or microscale without the use of complex pumping devices and other moving components, because the diffusion of molecules is Fickian, meaning that the release rate is dictated by the gradient of molecular concentration.

● ● ● Active Release

A time-variable rate of release can be also achieved through electrokinetic phenomena occurring when an electrical potential is applied between the inlet and outlet of channels. Electrokinetic phenomena such as electrophoresis and electroosmosis have been widely studied in microchannels. Albert van der Berg and his collaborators at the University of Twente in the Netherlands, Harold Craighead at Cornell University, and others have studied these phenomena and applied them to fluid sampling and processing for biomedical applications.

Promise of the Future

Implantable, mechanically engineered nanoglands, which closely mimic the body's natural response in healing processes, are promising for the final achievement of personalized treatment of many diseases. Not only would nanoglands provide enhanced therapeutic efficacy by administering a drug only when needed, they could lead to an overall reduction of drug doses and adverse side effects. They promise to deliver a local concentration of drugs in loci as opposed to systemic flooding of the body. Nanoglands may one day provide real-time and remote therapy outside the clinic, not only to improve the quality of life of patients, but also possibly to serve populations in underserved geographical areas.

As reliable nanoscale sensors evolve, it may be possible to invoke more complex, closed-loop systems for molecular delivery and health monitoring in extreme environments, for military medicine, athletics, and long-duration interplanetary space exploration, for example. In soldiers and athletes, nanoglands monitoring hydration, nutrition, and metabolic workload could trigger electrolyte or nutrient release, communicate health status to a remote monitoring center, or even trigger alerts to the individual via wireless means.

During protracted microgravity space flight missions, maintenance of bone density is a serious challenge, typically requiring up to two hours of resistive exercise each day. Nanoglands with hormonal or drug delivery systems adjusting to personal need might minimize some of the operational burden of lengthy daily exercise routines. Moreover, radioprotective pharmacologic agents might be administered based on need, both for astronauts and other radiation workers.

Furthermore, the achievement of bio-stable sensing units could lead to the goal of artificial nanomechanical devices for the replacement of body glands: the thyroid, involved in the regulation of the metabolism; the parathyroid, whose disorders may alter its fundamental role in controlling the amount of calcium in blood and bones; the hypothalamus, which controls body temperature, fatigue, sleep, hunger, thirst, and the circadian cycle; and adrenal glands, which are devoted to the release of hormones which, among other functions, regulate blood pressures and the body's reaction to stress. This technology could set the basis for the development of even more complex systems, such as artificial kidneys.

In most cases, however, the electroosmotic transport has been studied at high applied electrical potentials. In an electroosmotic flow, charges near the channel wall move along the surface following the gradient of electrical potential, and mechanically drag the bulk of fluid by viscous forces. In a nanochannel, viscous dragging becomes more effective and more energy-efficient electroosmotic transport. This may be highly suitable for implantable technologies, and functionality can be achieved using much lower applied voltages.

Our group has developed implantable nanochannel membranes with electrodes near the membrane inlet and outlet, and has reproducibly demonstrated the delivery of molecules

proportional to the applied electrical field up to ten times higher than the passive delivery. Additionally, by reversing the polarity of the applied electrical field the release could be completely stopped.

As opposed to the rudimentary conventional practice wherein drugs are administered, nanochannel technologies promise to allow for precise adjustment of timing, duration, and frequency of administration as needed. This approach, mimicking the time-variable release of molecules in the body, can enable new delivery regimens including chronotherapy—synchronization of drug delivery with optimal times during the circadian cycle of the body.

Chronotherapy was demonstrated in preclinical trials to improve the efficacy of therapies requiring smaller doses of therapeutics, with reduced adverse side effects. In this context, a sustained cyclic delivery can be attained with preprogrammed systems where the implantable capsule incorporates a battery and electronic control units for the modulation of the applied electrical potential.

Such a system could easily include communication hardware for remote external control of the activation, cessation, or modulation of the drug delivery. Ultimately, electrokinetic nanochannel architectures are suitable for incorporation in artificial glands hosting sensors, capable of transducing environmental, physical, and biological changes, with a logic unit regulating the administration of therapeutics.

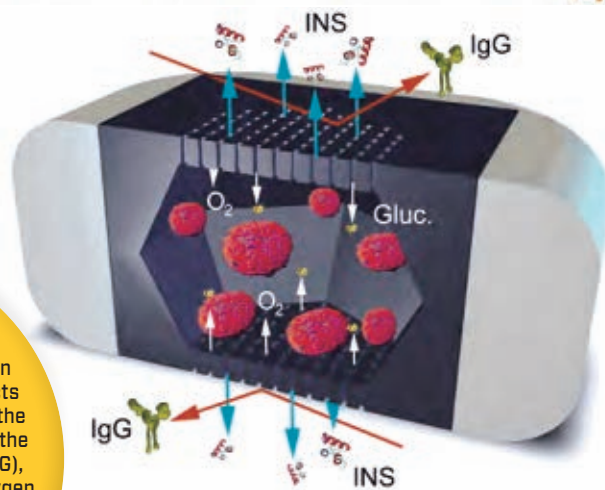
The ability to monitor the physiological state of patients and adjust the therapeutic regimen in real time would not only render current therapeutic regimes more efficient, but might also enable complete replacement of defective parts of the endocrine system. An artificial gland, for example, could restore the important regulatory function of the thyroid in controlling the body metabolism in cases of chronic conditions such as hypothyroidism.

A large variety of implantable sensors have been developed, including pressure transducers and ion selective probes, which would render artificial glands suitable for applications ranging from cardiovascular diseases to pain management.

• • • The Challenges

On one hand, development and preclinical translation of such sophisticated systems require a deep understanding of their behavior in vivo, where a harsh biological environment may change long-term functioning. This is particularly true for implantable sensors, whose viability is the critical factor limiting their use in extended in vivo applications.

A nanogland for cell transplantation embedded in a soft silicone rubber shell (light gray). The silicon nanochannel encapsulation protects pancreatic islets (red globes) from the body's immune system, preventing the permeation of immunoglobulins (IgG), while promoting the exchange of oxygen and nutrients, necessary for the islets' viability. In addition to a basal insulin (INS) secretion, the protected islets are able to respond to glucose stimuli from the body.



On the other hand, the design of the active components of membranes requires rigorous understanding of the mechanics of fluids at the nanoscale, which ultimately determines the transport of analytes through nanoconfined spaces. Although significant efforts have been spent in the theoretical investigation of nanoscale fluid mechanics and new theories have elucidated several aspects of confined fluid flow, the mechanism determining saturated diffusive transport is still far from being understood.

At the nanoscale, decoupling the interacting phenomena that affect the constrained transport of analytes is very complex: molecular interaction with channel walls, including adsorption and desorption mechanisms, molecule-to-molecule interactions, charge distribution, physical confinement, and fluctuations in fluid properties. Decoupling such mechanisms is theoretically challenging while experimentally impossible.

To untangle this complex problem, we have developed a microscale model of our nanofluidic system. In the absence of the gravitational field, microparticles in microchannels constitute a reliable substitute for molecules in nanochannels. Moreover, a microscale model can be more easily and well characterized thanks to the large number of techniques available, though limited when applied to the nanoscale.

Our experimental model was developed to be fully automatic and will reveal the relative importance of charge interaction and physical confinement over the saturated transport of particles. Supported by a recent Heinlein Trust Microgravity Award, the experiment will be performed aboard a SpaceX Dragon orbital spacecraft, which will fly in early 2011 for approximately five days.

Results of the experiment will be translated to the nanoscale for the development of a more comprehensive predictive model of the diffusive transport in nanoconfinement. The ultimate goal of this analysis will be the development of engineering tools for the rational design of implantable drug delivery systems, ultimately for rapid translation toward actual patient care. ■