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COMMENTARY

Making a Case: Nanofabrication Techniques in Encapsulated Cell Therapy for People with Diabetes

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Abstract

A nanoporous immunoisolative case/capsule that encases/encapsulates insulin-secreting cells vastly expands the source of therapeutic cells available for grafting in people with diabetes, including cells from animal sources, stem cells, and genetically engineered cells. These encapsulated cellular grafts potentially provide an endogenous, renewable, and long-term source of insulin without the need for pharmacological immunosuppression.

Micro- and nanofabrication techniques used principally in the semiconductor industry can play a positive role in encapsulated cell therapy. Many of these techniques do not have direct applicability in cell encapsulation, but can be leveraged to develop processes suitable for this application. This commentary highlights the salient features of an effective cell encapsulation system, enumerates limitations of existing encapsulation schemes, and touches upon progress in key areas of encapsulation technology; one example of how micro- and nanofabrication technology may be used to develop a more effective platform for cell encapsulation is presented. This commentary urges further exploration and expansion of techniques used traditionally in electronics and optics for cell-based therapy in people with diabetes.

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Introduction

The endocrine pancreas comprises functional units called *islets of Langerhans*, comprising α, β, and δ cells. β cells sense an increase in blood glucose levels and secrete insulin in response to it, which helps maintain stable blood glucose levels by facilitating glucose transport from blood to tissues.

Type 1 diabetes is characterized by the autoimmune destruction of β cells, which results in absolute insulin deficiency and loss of this glycemic control. When confronting a loss of cells or cell function, a logical and physiological approach is to replace the dead or dysfunctional cells with normally functioning cells. Therefore, type 1 diabetes may be reversed by transplanting functioning insulin-secreting cells from a donor source.

A Case for Cell Therapy

Numerous approaches can be used to overcome insulin deficiency. These approaches include insulin injection, oral or nasal insulin delivery, insulin pumps, and pancreas transplantation. For insulin injection and orally/nasally delivered insulin, the patient must have continual access to insulin and adhere to a strict regimen. Even the strictest adherence to regimen and carefully

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calculated doses do not mimic the complex and nuanced insulin response of normally functioning β cells. This inexact glycemic control leads to long-term damage and failure of organs and organ systems. Furthermore, this exogenous insulin can only be administered in discrete doses. Therefore, the patient may suffer from hyper- and/or hypoglycemic episodes. Insulin pumps also require the patient to refill and recharge the pump continually, which results in lifestyle restrictions such as curbs on travel. Although insulin pumps have more dynamic control over insulin delivery, a functional error in the insulin pump could result in a catastrophe. Transplantation of a pancreas is a more physiological and long-term therapeutic approach. However, whole-organ transplantation involves a complicated procedure often accompanied by postoperative complications, and the donor pool of pancreata is severely limited.

The challenges associated with the treatment options stated earlier make the transplantation of insulin-secreting cells an attractive strategy to treat people with diabetes. This may involve transplanting clusters of β cells, other insulin-secreting cells, or intact islets, which are syncytial aggregates of α, β, and δ cells, all of which play a role in glycemic control. These cells are a potential source for *de novo*, on-demand, and long-term insulin production whose transplantation involves minimal surgery.

A Nanoporous Case for Cell Therapy

For people with diabetes, the transplantation of functional β cells from a donor can result in the continuous sensing of blood glucose levels by these cells, followed by insulin secretion in a timely, dosed, and phasic manner to ensure strict glycemic control. However, the introduction of these donor cells triggers a response from the host's immune system that destroys the cellular graft. Graft rejection can be overcome by using pharmacological immunosuppression, but these immunosuppressants are harmful to both graft and host.[1,2]

Encasing/encapsulating the cellular graft in an immunoisolative barrier can eliminate the need for pharmacological immunosuppression. Such an immunoisolative barrier must be semipermeable, allowing the bidirectional transport of small molecules necessary for graft function, including ions, oxygen, glucose, and insulin. However, this barrier must impede the transport of large molecules such as complement molecules and antibodies that result in graft destruction by the immune system. This selective permeability is achieved by controlling the pore size of the immunoisolative barrier.

The salient characteristics of an effective encapsulation system are (1) precise dimensional control over every pore of the immunoisolative barrier, (2) long-term *in vivo* mechanical and chemical stability of the immunoisolative barrier, (3) adequate graft oxygenation, (4) ability to implant at desirable locations such as well-vascularized or immunoprivileged sites, (5) high-throughput, reproducible and cost-effective manufacturing, and (6) biocompatibility.

Currently, the most commonly employed technique to achieve this immunoisolation is to entrap the therapeutic cells in polymeric coatings, mainly made of alginates.[3,4] The porosity of the polymer coating regulates molecular transport between the graft and the host. Such encapsulation has been effective in short-term graft function. However, the use of these polymers presents challenges for long-term graft efficacy. First, these polymers have a range of pore sizes; therefore, while most pores prevent the passage of the large molecules of the immune system, some larger pores do not. Second, many of these polymers are mechanically and chemically unstable. For instance, in calcium cross-linked alginate, calcium ions are displaced by sodium ions over time. Third, the cellular grafts encounter mechanical stresses and episodes of insufficient graft oxygenation during the encapsulation process. Fourth, these cell entrapment approaches can result in the cellular graft occupying as little as 1% of the total graft volume. This large total graft volume limits the potential sites for grafting.[5]

Numerous advances in polymer science continue to improve the mechanical and chemical stability of these polymeric coatings, albeit with some lingering toxicity concerns[6,7]; several techniques used to coat pancreatic islets with a thin and/or conformal polymer coating aim to address issues related to graft oxygenation and graft volume.[5,8,9] Nonetheless, to exclude harmful molecules from accessing the graft, these systems rely on polymer matrices that exhibit a range of pore sizes. Because the theory of immunoisolative encapsulation is predicated on the size-based exclusion of molecules, there is a vital need to devise a biocompatible, mechanically and chemically stable, immunoisolative barrier with extremely precise and reproducible control over its porosity and to do so in a high-throughput manner for clinical utility.

Techniques employed in the semiconductor industry have impressive dimensional control over small features.

However, no such existing technique has immediate applicability to satisfy all the criteria that are desirable in a cell encapsulation system as detailed earlier. Therefore, new techniques must be developed to further advances in the micro- and nanofabrication fields for cell encapsulation application.

An Example

Nanofabrication technologies such as electron-beam lithography and nanoimprinting present an opportunity to precisely control the size of every nanopore of an immunoisolative membrane. This commentary seeks to highlight how these processes, developed for semiconductor manufacturing, can be adapted for cell encapsulation application. One such example is detailed here.

To achieve precise control over the membrane nanoporosity for a potential immunoisolative barrier, a method has been devised to create a large number of nanoslots in a membrane made of an epoxy—the width of the nanoslots can be controlled precisely for selective molecular transport (**Figure 1**).[10] First, a nanoimprint mold was created whose features were initially defined by conventional electron beam lithography. Because this process does not yield the desired feature dimensions, the feature dimensions of the mold were reduced drastically by controlled and cyclic oxidation and etching. The number of oxidation and etch steps can be modified to achieve any desired feature dimension, which in this case should result in a molecular cutoff for key humoral components.[11] Next, this mold was used to superficially imprint nanoslots in the epoxy membrane. Nanoimprinting was followed by the deposition of metal at an oblique angle to the membrane to protect the dimensional integrity of the superficial imprint features during the subsequent etch process without clogging the imprinted nanoslots. Next, nanoslots were etched chemically and anisotropically through the entire cross section of the membrane to yield the desired semipermeable membrane. This approach achieved several goals: (i) it rendered precise nanometer control over membrane porosity; (ii) it afforded extremely high-throughput fabrication, which has immediate clinical applicability, as the membrane encapsulating each islet requires thousands of nanopores and a single transplant recipient may require hundreds of thousands of encapsulated islets to become insulin independent; and (iii) use of a nanoimprint mold ensured that once the requisite mold was fabricated, all the membranes thus formed had reproducible nanoporosity. Molecular transport through these membranes remains to be thoroughly investigated and optimized for transplantation. Such an investigation includes optimizing the number of pores to support graft survival and function *in vivo*. Furthermore, because proteins can undergo conformational changes, the molecular cutoff of these membranes will depend on membrane thickness and pore architecture, which should also be fully investigated to ensure immunoisolation.

The nanoslotted membranes described earlier were integrated into the surfaces of a lithographically defined cuboid microcapsule made from the same epoxy whose biocompatibility has been studied previously.[12,13] The cuboid microcapsule provided structural support to the thin nanoslotted membrane, which was devised for rapid molecular transport between graft and host. The microcapsule was designed to house a single pancreatic islet, or a cluster of insulin-secreting cells of equivalent volume, with a 200 × 200 × 200-μm encapsulation space to ensure graft oxygenation.[14,15] The encapsulation space was in the base structure of the microcapsule, which was then closed with a nanoslotted lid. Several methods to assemble the components (base and lid) of these microcapsules that include geometric and magnetic assembly have been reported.[15,16] The lithographically defined microcapsules containing nanoslotted membranes impart the following characteristics to cell encapsulation: (1) low-cost manufacturing, (2) mechanical and chemical stability of the encapsulation system, (3) reduced mechanical and hypoxic stress on cells during encapsulation, (4) short molecular transit time between graft and host for improved oxygen flux and insulin secretion kinetics, (5) small graft volume that enables the study of transplant sites that were previously precluded due to large graft volume,[17] and (6) thin barrier between graft and host providing improved molecular and solvent diffusivity and therefore potentially more safe and effective cryopreservation of encapsulated cells prior to grafting. While preliminary data on molecular transport and graft oxygenation have been acquired,[15,16] a thorough understanding of these parameters requires the capsule to be sealed completely, with the only allowable molecular transport occurring through the membrane nanopores.

Not a Closed Case

The geometrically assembled microcapsules just described rely on a male–female locking mechanism between two components of the microcapsule to seal it shut.[15] However, because these components are defined lithographically, they do not have the dimensional integrity that precludes gaps in the interface that are on the order of a few nanometers. The same is true for the magnetically assembled microcapsules.[16] Over time,

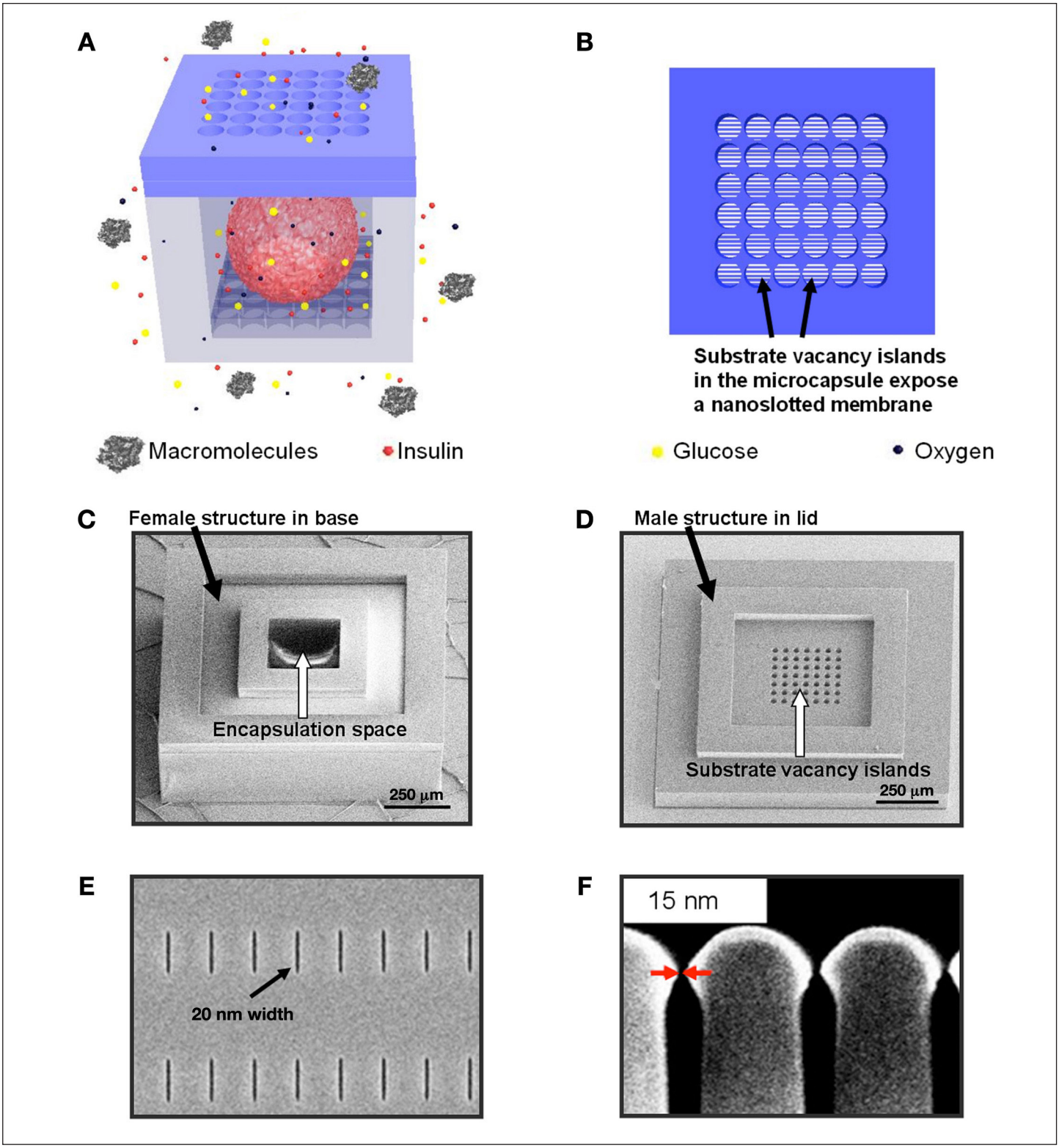

Figure 1. A schematic of an islet-encapsulating microcapsule with two opposing nanoporous surfaces **(A)**. Substrate vacancy islands in these microcapsule surfaces expose a thin nanoporous membrane for selective molecular transport between graft and host **(B)**. Scanning electron micrographs of the microcapsule base **(C)** and the lid **(D)**. (Note that these microcapsules have a large footprint for easy handling during *in vitro* testing, whereas the capsules for transplantation are smaller cuboid structures slightly larger than the typical islet.) Substrate vacancy islands of the lid expose a thin nanoslotted membrane **(E)**, seen here in top view. Nanoslots defined by nanoimprinting **(F)**, seen here in a cross-sectional view, have highly reproducible nanopores that can be fabricated in extremely large numbers with a single imprinting step. **(C–E)** Reprinted with kind permission of *Springer Science and Business Media*.[15] **(F)** Reprinted with permission from *American Vacuum Society*.[10]

some large molecules (antibodies, complements) may traverse to the interior of the microcapsule. Therefore, it is essential to develop methods to create a strong interfacial bond between the microcapsule components in biofriendly conditions between the components of the microcapsule to finally close the case, which is necessary for a thorough assessment of biocompatibility and graft function. Upon such assessment, it may be required to modify the microcapsule surface chemically to enhance biocompatibility.

Other Barriers

The aforementioned technological advances should help realize effective cell encapsulation within membranes that entirely disallow direct contact between the graft and the large molecules of the host's immune system. While the presence of an effective physical barrier between graft and host comprises an important and necessary component in encapsulated cell therapy, it does not ensure long-term graft function. The overall success of grafting encapsulated insulin-secreting cells in people with diabetes warrants other considerations. Graft rejection cannot be entirely attributed to immune rejection. Other factors, such as biocompatibility of the encapsulation system or lipotoxicity when islets are transplanted in the liver, must be fully explored. The role of shed antigens must be addressed along with strategies to reduce their deleterious effects.[18,19] It may be helpful to coencapsulate insulin-secreting cells with other cells that play a positive role in glycemic control, β-cell survival and function, or local immunosuppression; encapsulated cell therapy may require adjuvants for success, including glucagon or C-peptide administration. It may be necessary to engineer cells to secrete human insulin to avoid host production of anti-insulin antibodies. The lack of intraislet vascularization and innervation of encapsulated cells warrants further investigation. Geometries other than the one presented here, such as scaffolds for easy and periodic retrieval, may be explored. A detailed discussion of these issues is beyond the scope of this commentary. This commentary urges further investigation and leveraging of micro- and nano-fabrication techniques used in electronics and optics to create suitable processes for the effective encapsulation of insulin-secreting cells as an immediate solution for people with diabetes.

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