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A Scalable Microfluidic Device for the Mass Production of Microencapsulated Islets

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Abstract

The objective of this research was to test the viability and function of islets microencapsulated using a scalable microfluidic device that is suitable for the mass production of encapsulated islets for transplantation. A 3-D microfluidic device consisting of eight outlets with an inner fluid inlet and an outer concentric inlet to the device has been designed and fabricated using the stereolithography rapid prototyping technique. Islets were isolated from normal Wistar-Furth rat pancreas using the procedure of collagenase digestion of pancreatic tissue. Following purification, islet suspensions in 1.5% sodium alginate were pumped into the fluid inlet of the microfluidic device, which distributed the flow equally to all the eight channels according to the design. The air plenum distributed compressed air uniformly through the eight concurrent outlets, and with one fluid pump and air source, the device produced eight microencapsulations simultaneously. After encapsulation, the islets were tested for functionality using the dynamic perfusion procedure with low- and high-glucose concentrations. The device is capable of producing eight channels of steady

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stream of monodisperse microencapsulations of a range of diameters depending on the design and process parameters. Using this prototype device, encapsulated islets were shown to be viable in the functional tests that we performed. Thus, the mean $\pm$ standard deviation rate of insulin secretion increased from a basal rate of 0.165 ± 0.059 ng/10 islets/min to a stimulated rate of 0.422 ± 0.095 ng/10 islets/min ($P < .05$, $n = 3$), when the glucose concentration was changed from 5.5 mmol/L to 27.5 mmol/L, and this glucose stimulation index was not different from that observed with unencapsulated islets under same conditions. In summary, the high-throughput prototype device that we have designed can produce encapsulated islets that are viable and suitable for transplantation studies.

THE USE OF ALGINATE MICROBEADS to encapsulate various materials has found applications in drug delivery, biosensors, microelectronics, bioanalyses, coded imaging aids, cell delivery, and cell transplantation primarily due to the biocompatibility and physical properties of alginate including its ease in forming hydrogels under mild conditions such as in the presence of divalent cations.¹⁻⁴ A widely researched application of the microencapsulation technology is the use of alginate encapsulated islet cells as a form of bioartificial pancreas to treat type 1 diabetes.⁵⁻¹⁰

One major drawback of the current technologies in use for microencapsulation, namely, the air syringe pump droplet generator¹¹ and the electrostatic generator,¹² is that they are incapable of producing sufficient numbers of microcapsules in a short period to permit mass production of encapsulated and viable islets for transplantation in large animals and human.^{13,14} Obviously, the prolonged process of encapsulation inherent in using currently available technologies adversely affects the viability of the cells. It has been estimated that one million islets are needed for transplantation in a diabetic human subject and that about 100 hours would be required to complete the encapsulation of this number of islets, assuming one islet/microcapsule. In practice, however, it has actually been estimated that the duration of the process would be closer to 200 hours because of the additional steps involved in the encapsulation procedure, such as perm-selective coatings and washings, following the generation of the initial islet containing alginate microspheres.¹³

Fabrication of microfluidic devices has been previously accomplished with the use of various manufacturing techniques like the rapid prototyping technology along with polydimethylsiloxane softlithography and PDMS, micromachining, and micromolding.^{2,15-17} In this article, we describe a novel microfluidic approach for producing large numbers of alginate microspheres to encapsulate islets. We present this new approach and reduce it to practice, by designing and building a prototype, and assessing the quality of the products made with the device. Specifically, we assessed the viability and function of islets encapsulated with our prototype device.

METHODS

Prototype Device Fabrication and Microbead Generation

The microfluidic device was designed using a commercial CAD package (SolidWorks 2008, Dassault Systèmes SolidWorks Corp, Concord, Mass, USA) and then stored as a STL file format. The STL file was then further analyzed for defects and features that may not form and then prepared for high-resolution build using the 3D Lightyear software. It was subsequently sent to a Viper si2 SLA System (3D Systems Corporation, Rock Hill, South Carolina, USA) in which the microfluidic chip was fabricated.

Figure 1 shows the different views of the microfluidic prototype device. It consists of a 3-D air supply, an alginate cell mixture inlet, and a multinozzle outlet as shown in Fig 1 a and b

for highly monodisperse microcapsule generation. There are two outlets provided near the top of the microfluidic device (Fig 1a) to allow entrapped air to escape while filling the device with alginate suspension and any air bubbles that might enter the system. The alginate-cell suspension enters the device through the alginate inlet shown in Fig 1a. This mixture is then split equally into eight parts, which flow through the inner concentric nozzle shown in Fig 1c. The compressed air enters the system along the $\times$ direction through the inlet shown in Fig 1a where it takes a 90-degree turn and flows along the Y direction, where it is split equally into eight parts before it takes another 90-degree turn to flow in the Z direction in the outer concentric nozzle. The compressed air flowing in the outer concentric nozzle shears off the alginate-cell mixture flowing in the inner nozzle at the interface to form droplets, which are collected in a calcium chloride bath where they cross-link to form highly monodisperse microbeads.

Islet Isolation, Microencapsulation, and Functional Test

Using an IACUC-approved protocol, normal wister-furth rats were euthanized and islets were isolated after collagenase digestion of the pancreatic tissue following intraductal infusion of the enzyme, distension, and subsequent incubation of the pancreatic tissue at 37°C.¹⁸ After washing and purification, the islets were cultured overnight in Memphis serum-free medium using standard conditions prior to encapsulation as recently described,¹⁹ and the islets were then tested for function using the dynamic perfusion test with low (5.5 mmol/L) and high (27.5 mmol/L) glucose concentrations in HBSS, samples were collected as previously described.²⁰ The effluent samples collected during perfusion tests were stored frozen at -20°C until radioimmunoassay for insulin content.

Statistical Evaluation of Data

The basal rate of insulin secretion at 5.5 mmol/L glucose was compared to the maximal rate of secretion at 27.5 mmol/L glucose using Student *t* test, and a value of $P < .05$ was accepted as significant.

RESULTS

Using the prototype device, islets were microencapsulated one islet/capsule, as illustrated in Fig 2, which is a low-resolution image of microencapsulated islets as seen with a dissecting microscope. Using a flow rate of 2 mL/min and air pressure of 18 psi, the microcapsules measured approximately 400 μ m in diameter. During the functional testing of the microencapsulated islets (Fig 3), insulin secretion increased from a mean $\pm$ standard deviation basal rate of 0.165 ± 0.059 ng/10 islets/min to a stimulated rate of 0.422 ± 0.095 ng/10 islets/min ($P < .05$, $n = 3$), which was not different from the response of unencapsulated islets to glucose stimulation under same conditions (data not shown).

DISCUSSION

We have designed a prototype microfluidic device capable of microencapsulating isolated islets that are functionally viable as shown in the dynamic perfusion test of insulin response to glucose stimulation. The prototype device herein described can increase the production rate of microspheres eightfold compared to currently available devices and can be scaled up easily to higher multiples. Based on the manufacturing capability of this microfluidic encapsulation device, we estimate that it could potentially reduce by more than two orders of magnitude the time to encapsulate ~1 million microencapsulated islets required for human transplantation, which would improve the viability of microencapsulated islets and hopefully the outcome of their transplantation. Invariably, one big advantage of this new microencapsulation device is that it would provide good product quality control because a

large batch of encapsulated islets produced within a few minutes will be subjected to the subsequent manual steps of perm-selective coating and washings at the same time, resulting in significantly improved viability of the encapsulated islets used for transplantation.

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Fig 1.

Various sections of the prototype eight-channel microfluidic device. It consists of a 3-D air supply, an alginate cell mixture inlet, and a multinozzle outlet **(a)** and **(b)** for highly monodisperse microcapsule generation. There are two outlets provided near the top of the microfluidic device **(a)** to allow entrapped air bubbles to escape, which might enter the system.

Fig 2.
Rat islets microencapsulated with the microfluidic device. Low-resolution image of microencapsulated islets as seen under a stereomicroscope.

Fig 3.

Glucose stimulation of insulin secretion by microencapsulated islets. The response of encapsulated islets to glucose stimulation was tested using dynamic perfusion of low glucose (5.5 mmol/L) and high glucose (27.5 mmol/L) solutions. Effluent samples collected during the perfusions were stored frozen until assayed for insulin content by radioimmunoassay.