

Fabrication of Reinforced Alginate Fibers for Cell Based Therapies

By

Sydney C. Carter

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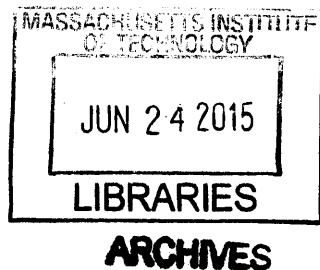
Signature of Author: _____
Department of Mechanical Engineering
May 21, 2015

Signature redacted

Certified by: _____
Robert Langer
Professor of Chemical Engineering
Thesis Advisor

Signature redacted

Accepted by: _____
Anette Hosoi
Undergraduate Officer



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ABSTRACT

Type I Diabetes is a condition in which the pancreas does not produce enough insulin, leading to dysregulation in glucose metabolism. Most Type I diabetics take insulin injections after each meal in order to remain euglycemic. Transplantations of islet cells in immunoprotective alginate capsules has been shown to be promising as a long-term cure for Type I Diabetes. In this study, reinforced alginate fibers were fabricated as an alternative to alginate capsules for islet transplantation. Surgical thread was covered in alginate-cell solution by the microfluidic nozzle system to give a stronger structure to be used for implanting the cells. The thickness of the cell-encapsulated alginate layer produced by microfluidic nozzle processing system was controlled by varying the flow rate of alginate being pumped through the nozzle as well as by varying the speed by which the thread was extracted from the nozzle. The study showed that higher flow rates of alginate and lower thread speeds result in thicker alginate layers for encapsulating more cells within the fiber. The cells remained viable over a month, suggesting plausibility of cell-encapsulated microfibers as a long term solution to diabetes treatment.

Thesis Supervisor: Robert Langer

Title: Professor of Chemical Engineering

Direct Supervisor: Suman Bose, Ph.D.

Table of Contents

ABSTRACT.....	3
INTRODUCTION	6
Diabetes	6
Current Treatments for Diabetes.....	6
METHODS	9
Nozzle Design.....	9
Fabrication of Alginate Fibers	10
Encapsulation of Cells	11
RESULTS	12
Processed Microfibers.....	12
Cell Growth.....	15
CONCLUSIONS.....	17
REFERENCES	19

INTRODUCTION

Diabetes

Diabetes is the seventh leading cause of death in the United States. Approximately 9.3% of Americans have diabetes, with 1.7 million cases being diagnosed annually (National Diabetes Statistics Report, 2014). Diabetes is a disorder in which the body experiences difficulty in regulating blood sugar. In a normal body, beta cells in the pancreas release insulin when blood sugar levels rise after consuming food. The secreted insulin attaches to cells and activates transport proteins that will allow the cells to absorb the glucose and convert it to energy (Rorsman & Renstrom, 2003). Type I Diabetes, often called Juvenile Diabetes, is a condition in which the immune system destroys the beta cells in the pancreas so it does not produce enough insulin to regulate blood sugar. There are several complications that diabetics may experience including vision loss with diabetic retinopathy, nerve damage with neuropathy, and kidney damage.

Current Treatments for Diabetes

Type I Diabetes yields a poor quality of life, as blood sugar must be monitored throughout each day and insulin must be injected after each meal. Glucose meters used to measure the concentration of glucose in the patient's blood usually require patients to obtain a drop of blood for the measurement through a finger-prick, one of the most unpleasant aspects of self-care. Insulin pumps, which automatically pump insulin into their blood stream, are available as an alternative to manually taking insulin injections following food consumption.

To minimize the energy spent managing Type I Diabetes with insulin injections, pancreatic and islet cell transplants are currently being researched as long term solutions. Type I patients with serious complications occasionally receive transplants of fragments or a whole pancreas from

cadaver organ donors. Pancreas transplants are rare, as the demand greatly exceeds the supply. After the pancreatic transplant, patients may experience better regulation of blood sugar without taking insulin, reduction or delay in symptoms of complication, and occasionally improvement in those with nerve damage (Troppman, et al., 1996). The Edmonton protocol is an islet transplantation procedure that involves extracting islet cells from a fully functional pancreas and inserting them into the body of a diabetic through minor surgery (Shapiro, et al., 2006). Although islet transplants are safer than the major surgery required to insert a pancreas, both pancreas implants and implanted islet cells provoke a foreign body response. The body often kills the islet cells, and they must be replanted periodically. To counteract the immune rejection, patients must take immunosuppressants that significantly increase their vulnerability to disease and infection (Shapiro, et al., 2006).

The safety and efficiency of the Edmonton Protocol is currently being investigated in several studies. Alginate fibers embedded with pancreatic islet cells have been initially tested with implants into diabetic mice. After successes in normalizing glucose in mice through islet transplantation, the procedure has recently been tested further in humans. A recent study in human implants has suggested that the benefits of the success in resulting insulin independence from the treatment outweigh the risks of an immunosuppressed body (Ryan, et al., 2015). However, one of the more consistent adverse effects of islet transplants in humans was increased creatinine levels, which signals kidney failure (Shemesh, Golbertz, Kriss, & Myers, 1985). The process of islet cell transplants must be developed further to reduce the foreign body response.

Hydrogels are one class of medium in which cells are embedded to be implanted into the body (Silva, et al., 2004). Hydrogels such as alginate – an FDA-approved biomaterial extracted from brown seaweed (Kang, et al., 2011), are often used for implanting tissues, directing cell

growth, and guiding cell differentiation in the body. Alginate offers protection to the implanted cells from the immune system of the patient to decrease the foreign body response. Alginate microfibers that are often used for constructing fabric meshes and three dimensional scaffolds can be processed by several methods, including electrospinning and microfluidic channels (Kang, et al., 2011). This work takes advantage of microfluidic processing to produce alginate fibers reinforced with surgical threads. The thickness of the cell-encapsulated alginate layer produced by our microfluidic nozzle processing system was controlled by varying the flow rate of alginate being pumped through the nozzle as well as by varying the speed by which the thread was extracted from the nozzle. The study showed that higher flow rates of alginate and lower thread speeds result in thicker alginate layers for encapsulating more cells within the fiber. The encapsulated cells remain viable over a month, demonstrating plausibility of cell-encapsulated microfibers as a long term diabetes treatment solution.

METHODS

Nozzle Design

The nozzles were designed by CAD and 3D printed in ABS plastic using an industrial printer. A 2-core, and later a 3-core nozzle was designed to fit two different sized needles: 16 gauge and 22 gauge. The inner diameter of the 22 gauge need was just large enough to encompass the size 4.0 non-porous surgical thread. The 3D printed model was finished by reinforcing the holes for the needles with a drill before dull needles were fitted and glued to the nozzle. The final design for the nozzle is shown below in Figures 1 and 2.

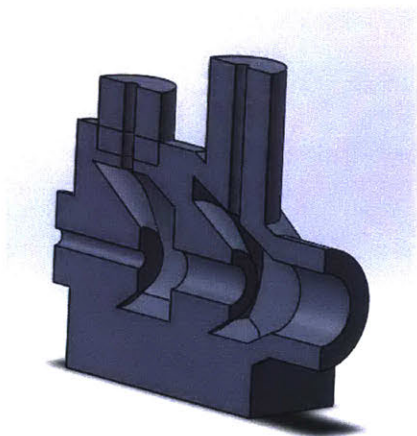


Fig. 1. A cross-section of the final 3-core nozzle design



Fig. 2. The 3D-printed nozzle assembled with needles.

As shown in the images above, needles were embedded into the 3 core nozzle. In the microfluidic biomaterial threading system, the surgical thread was inserted into the needed through the inlet at the back of the nozzle and through to the outlet pipe in the front, concentric with the 16 gauge needle. The alginate was pumped into the tallest injection port on top of the nozzle to flow into the outer most layer of the nozzle and the composite thread. The injection port with the shorter pipe on top of the nozzle was designed for encapsulated cell solution to flow into the inner region

of the nozzle. The increasing diameters of the pathways between each region from the inlet port through to the end of the nozzle allow for each layer to be distinct so that the thicknesses of the layers stay uniform about the center.

Fabrication of Alginate Fibers

After assembling the nozzle, a syringe of alginate solution was placed into a fluid pump for regulating the mass flow rate of the alginate being pumped into the nozzle. The alginate solution was pumped into the tallest injection port on top of the nozzle via a clear tube. The alginate was pumped at several different mass flow rates onto the thread during these experiments. A 7.4 V bipolar stepper motor was attached to a spool to regulate the speed at which the thread would be pulled from the nozzle. A potentiometer was installed for changing the rotational speed of the spool on the motor, with an LED screen to display the revolutions per minute. The nozzle was placed into a large circular dish and submerged in 20 mM BaCl 0.9% NaCl solution. The 1.2% alginate being used would be solidified into a gel when soaked in the barium chloride solution in about one minute.



Fig. 3. Assembly of alginate-thread depositing system. From left to right: syringe pump, nozzle at base of dish, motor with spool attached to side of dish, breadboard with Arduino chip and LED for spool speed display.

Once the alginate-cell layering system for the thread was assembled, the diameters of each composite thread were measured as alginate was pumped at volumetric flow rates of 100, 150, and

200 $\mu\text{l}/\text{min}$. The speed of the thread being pulled by the spools were also varied at 5 rpm and 10 rpm for linear velocities of 4.58 mm/s and 9.16 mm/s, respectively. After threads were deposited at each combination of speeds, they were allowed to soak in the BaCl solution to solidify before being imaged under a light microscope. Images of the threads were taken under the microscope for quantitative analysis of thread diameter.

Encapsulation of Cells

Hela cells were suspended in a 1.2% alginate solution at a concentration of 10,000 cells/ml. and allowed to incubate. Next, the cell-alginate solution was inserted into a syringe to be pumped through the nozzle. The solution of alginate and cells was then used following the previous procedure to synthesize a layer over the surgical threads. This time, the solution was pumped at 150 $\mu\text{l}/\text{min}$ with spool speed at 4.58 mm/s and 9.16 mm/s, and at 500 $\mu\text{l}/\text{min}$ with spooling at 9.16 mm/sec. The threads were successfully made at both flow rates and thread speeds. The threads were either imaged directly or cultured in 1ml of DMEM with 10% FBS in an incubator. For viability detection, the cell-layered threads were dyed with Cell Tracker Orange [®] (Invitrogen) to stain the live cells. Viability measurements were done at day 3 and day 20.

RESULTS

Processed Microfibers

The following images show the layered threads processed by the alginate nozzle system. The first set of images in Fig. 4-A show the threads that were processed with only a layer of alginate solution. The second set of images in Fig. 4-B show the threads that were processed with alginate solution incubated with cells. In all cases, the threads with the largest flow rate of alginate solution being pumped through the nozzle resulted in thicker layers of alginate on the threads. In addition, the slower thread speed 4.58 mm/s resulted in thicker fibers than those with spooling speed 9.16 mm/s at equivalent alginate pumping rates.

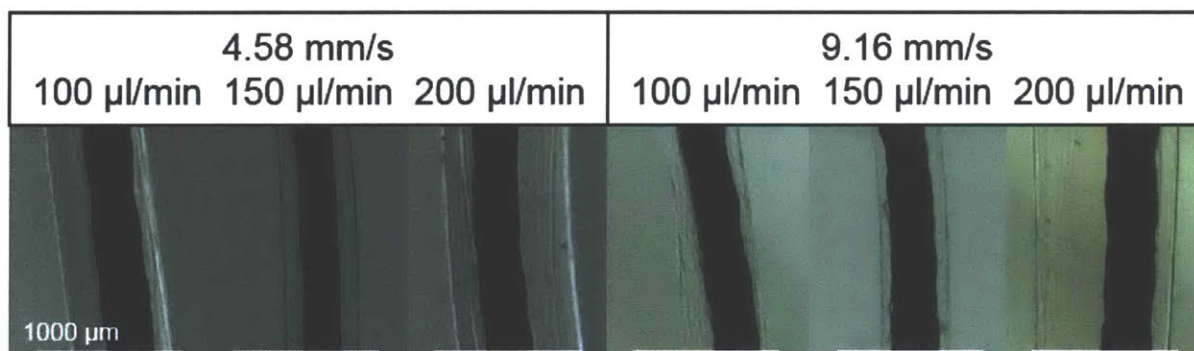


Fig. 4-A. Images of alginate fibers produced at different operating parameters.

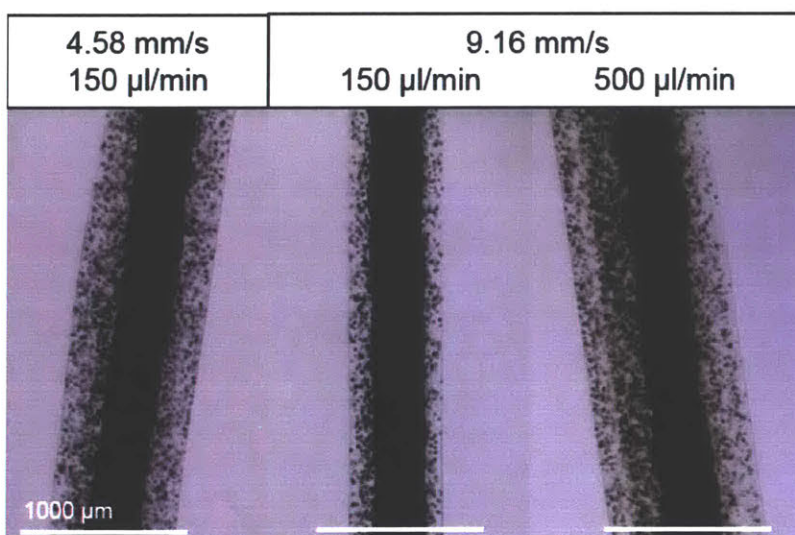


Fig. 4-B. These images show the threads produced when covered with alginate incubated with Hela cells.

A mass conservation approach was used to derive the relation between different operating parameters to compare the experimental measurements with theoretical calculations. The physical process of fabrication of the alginate fiber is shown in Fig. 5. The alginate solution in which cells would be encapsulated is depicted in the light green, with Q representing the rate at which the alginate would be pumped through the nozzle. The black line in the center of the diagram represents the thread to be covered with alginate, with d representing its diameter before being processed. U represents the linear velocity by which the spool would extract the thread from the nozzle. Lastly, D is the net fiber diameter after processing. The net fiber diameter is related to the other parameters as shown in Eqn. 1:

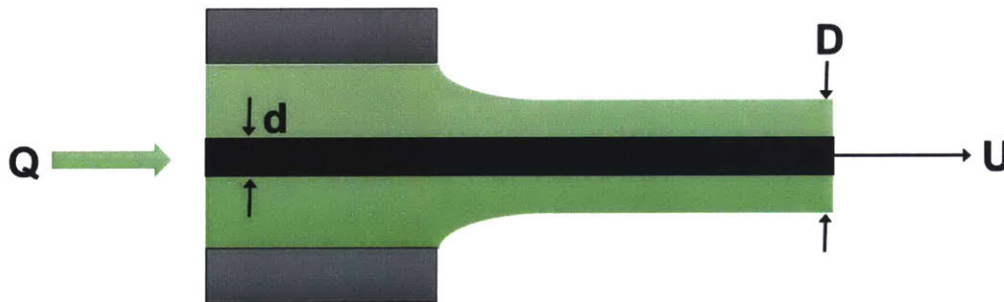


Fig. 5. Diagram of alginate layering system.

$$D = \sqrt{\frac{4Q}{\pi U} + d^2}$$

Eqn. 1. Total diameter D of fiber processed through microfluidic system, where Q represents volumetric flow rate of alginate, d is the diameter of the plain thread, and U is the linear velocity of the thread being pulled from the nozzle.

Fig. 6-A. Graph of thread diameters at linear velocity 4.58 mm/s.

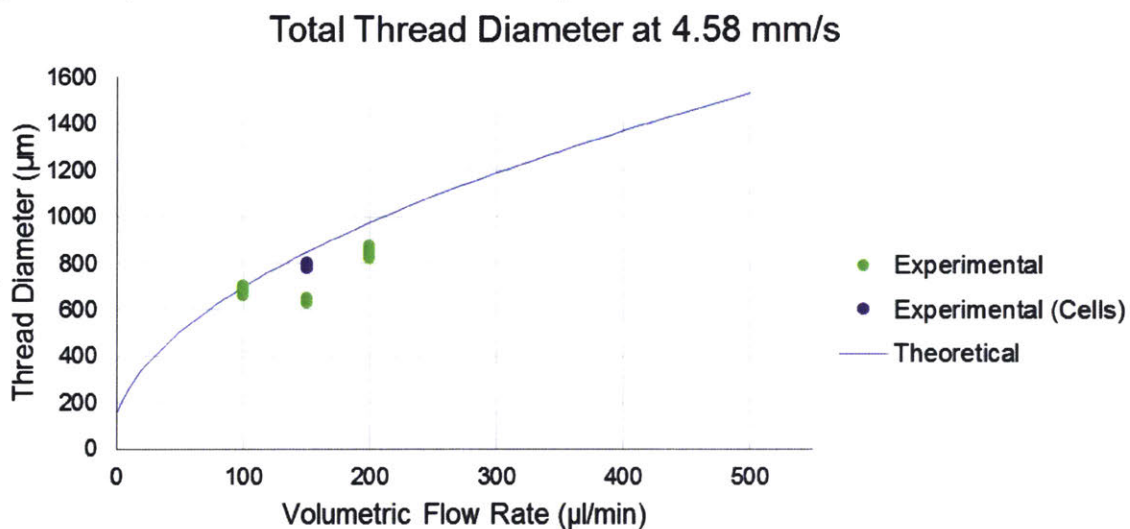


Fig. 6-B. Graph of thread diameters at linear velocity 9.16 mm/s.

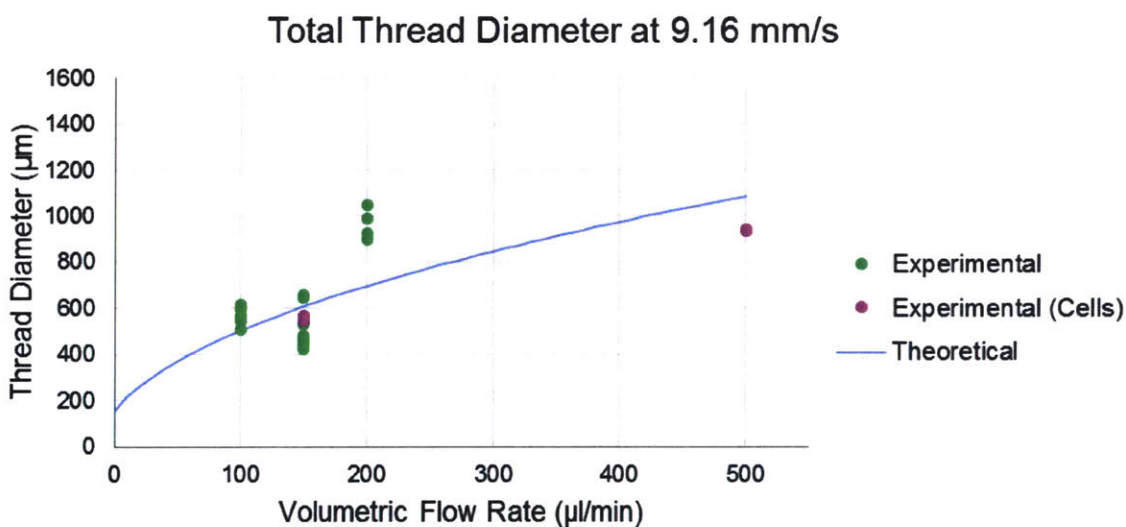


Fig. 7. Comparisons of average measured diameter against calculated diameter.

Cell Presence	Thread Speed (mm/s)	Flow Rate (µl/min)	Theoretical Diameter (µm)	Average Diameter (µm)	% Error
	4.58	100	696.91	680.88	2.30%
	4.58	150	846.92	790.69	6.64%
with cells	4.58	150	846.92	790.69	6.64%
	4.58	200	974.10	846.74	13.07%
	9.16	100	504.07	574.51	13.97%
	9.16	150	608.18	589.01	3.15%
with cells	9.16	150	608.18	557.38	8.35%
	9.16	200	696.91	965.56	38.55%
with cells	9.16	500	1086.49	939.38	13.54%

Equation 1 was used to compare the observed diameters with the theoretical values. It appears that a slower speed by which thread was pulled resulted in a thicker layer of alginate on the surgical thread. The thickness of the alginate layer also increased as the volumetric flow rate being pumped increased. The purple and magenta data points shown in the Fig. 6 graphs show the thread that includes the cell-alginate solution. One can conclude that the addition of the cell solution to the alginate does not significantly change the nature or viscosity of the solution and its thread quality from the large overlap between the theoretical thread diameters with the experimental diameters.

Cell Growth

The set of images in Fig. 8 shows the live cells on the thread three days after the thread was processed and cultured in an incubator. The images show lines of cells growing along the thread. These lines are cell colonies where the original cells planted on the thread have grown and replicated while embedded in the alginate.

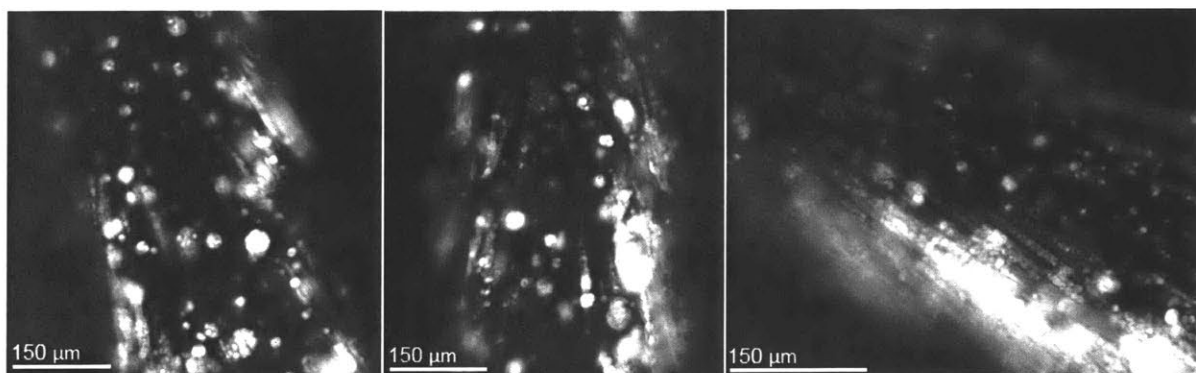


Fig. 8. Images of live cells on alginate processed thread after three days of incubation.

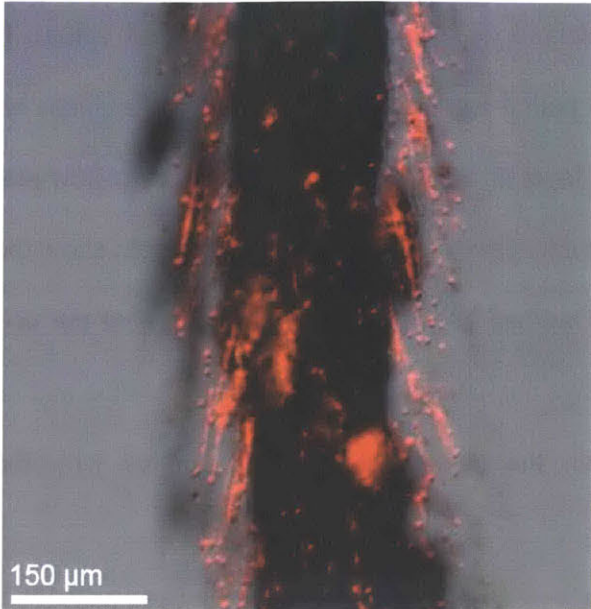


Fig. 9. Live cells after incubating for 21 days.

The lines of viable cells along the alginate layer on the thread demonstrate that the cells can not only survive, but also grow on the alginate threads. Extended cell life is imperative for reducing the frequency of islet transplant surgeries in diabetic patients. The proliferation of the cells suggests that an alginate covered surgical thread could be a plausible medium for long-term islet transplantation.

CONCLUSIONS

In this study, a microfluidic nozzle system was designed to coat surgical threads in cell-encapsulated alginate. This system contributes to the cultivation of microfiber fabrication necessary for islet transplantation in Type I Diabetes. The alginate offers protection to the embedded cells from foreign immune system responses while they release insulin to be diffused the hydrogel. The results demonstrated that the total diameter of the cell-embedded alginate layer increased with higher alginate flow rates pumped through the nozzle and decreased with the speed by which the thread was pulled from the multi-core nozzle. Although non-porous surgical thread was used for its strength as a structure for the cell-encapsulated alginate layer, other thread materials may also be used for processing the fibers. The thread in this case should enhance the ease of transport in the cases that implanted fibers must be removed from the body. In addition, it was found that the cells reproduced upon incubation in the processed alginate thread, which suggests a good survival rate is probable for cells if the thread is used for islet transplantation. The threads processed from this experiment demonstrate that layers of alginate not only provide a significant space for cells to be implanted under protection from a patient's immune system, but for islet cells to grow and colonize. Maximizing the concentration of cells to be occupied on a fiber as well as the length of time that cells can thrive is essential to reducing the frequency of surgeries and optimizing the efficiency of microfiber islet transplants.

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