# A BIO-ARTIFICIAL PANCREAS CREATED USING CELL ENCAPSULATION IN SELF-ASSEMBLED MICROCONTAINERS ON ALGINATE SHEET

J. Park<sup>a\*</sup>, C. L. Randall<sup>b</sup>, Y. V. Kalinin<sup>a</sup>, S. Pandey<sup>a</sup>, and D. H. Gracias<sup>a\*\*</sup>

<sup>a</sup>Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD 21218, USA <sup>b</sup>Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21205, USA

# ABSTRACT

This paper presents a bio-artificial pancreas fabricated with self-assembled, precisely structured 3D microcontainers on a rolled-up alginate sheet. We utilize a novel 3D membrane approach developed in our laboratory to package pancreatic  $\beta$ -cells so that they can be transplanted and function like an autonomous insulin-producing organ. Our approach provides stable and adequate diffusion of insulin from the microcontainers, as a prior step to the ultimate artificial pancreas which will eventually achieve precise immunoisolation of encapsulated cells using nanotechnology.

KEYWORDS: Self-Assembly, Bio-MEMS, Microfabrication, Artificial Pancreas

#### **INTRODUCTION**

Type 1 diabetes is a multifactorial autoimmune disease wherein pancreatic  $\beta$ -cells are attacked by the immune system, leading to their destruction. As an alternative for pancreas transplantation which requires extensive surgery and immunosuppression therapy, pancreatic islets can be transplanted. While the islet transplantation involves a much less risky surgery, patients still must have lifelong immunosuppressive drug treatment. Since the 1970s, researchers have been attempting to devise a way to avoid the need for lifelong immunosuppression, by encapsulating transplanted cells in a synthetic biocompatible membrane. These membrane-based devices, when used with encapsulated pancreatic islets, are commonly referred to as a bio-artificial pancreas, because their intended use is to mimic the natural insulin secretion of a healthy pancreas. This approach has not yet been successful, despite experimentation with numerous device architectures [1-4].

In this paper, we explore another method to enclose the cells inside array of microcontainers with micropores attached on an alginate sheet. Our approach overcomes two of the main obstacles limiting applicability of earlier efforts, namely achieving precise immunoisolation of transplanted cells by precisely patterning of nano-sized pores and providing adequate diffusion of every transplanted cell. Self-assembled 3D containers were fabricated by folding nets with heatactivated hinges as shown in Figure 1(a). To enhance practical throughput and quality, more steps and modifications were involved to the process that has been previously reported [5]. Pore density of side walls (15 %) and dimension of each container (0.3 mm × 0.3 mm × 5 mm) are decided based on our simulation to prevent hypoxia of cells encapsulated in the container. Figure 1(b)-(c) shows conceptual illustration of microcontainers on rolled-up sheet. After loading pancreatic  $\beta$ -cells into gold-electroplated containers, an alginate sheet was used for assembling the containers into a roll shape.



Figure 1: Illustration of microcontainer and rolled-up sheet. (a) Geometry of microcontainer before its self-assembly process. Faces consist of 8  $\mu$ m × 8  $\mu$ m windowed pores on 8  $\mu$ m thick Nickel. After the self-assembly, the container is coated with 2  $\mu$ m thick gold by electroplating for bio-compatibility. (b) Containers attached on alginate sheet using suture glue, spaced by 1 mm. (c) The alginate sheet is rolled-up after encapsulation of  $\beta$ -cells. (d)  $\beta$ -cell encapsulation and assembly process of containers on rolled-up alginate sheet.

978-0-9798064-4-5/µTAS 2011/\$20©11CBMS-0001

85

#### DEVICE FABRICATION AND EXPERIMENTAL SETUP

The containers are fabricated on 3" Silicon substrate. After spinning and curing 5.5 µm thick PMMA (950 PMMA A11, MicroChem) sacrificial layer, Chromium and Copper (30 nm / 200 nm) are deposited using thermal evaporation. Faces of container with 8  $\mu$ m × 8  $\mu$ m windowed pores are patterned with 10  $\mu$ m thick photoresist (SPR 220-7.0, MicroChem) and then 8  $\mu$ m thick Nickel is electroplated. The pore size will be reduced to 6  $\mu$ m × 6  $\mu$ m that makes 15 % pore density after gold-electroplating as the final step (Figure 2 (b)). Another 30 nm Chromium on the porous area is patterned using 5.5 µm thick photoresist (SC-1827, MicroChem) and thermally evaporated. This Chromium layer plays an important role on blocking the solder flowing into the pores during the self-assembly. 9 µm thick solder hinges are patterned using 10 µm thick SPR 220-7.0 and electroplated. Prior to removing the Chromium and Copper existing outside of the patterned structures, the Chromium on the porous area is protected from the etching process by patterning 3 µm thick SC-1827. The patterned structures are released by dissolving PMMA in NMP (1-Methyl-2-Pyrrolidinone, Sigma-Aldrich) at 100 °C. The released structures are heated to 250 °C in 10 % RMA (Flux #5 RMA, Indium Corp.) in NMP for self-assembly. 2 µm thick gold was electroplated as the final step for bio-compatibility. To make 20 µm thick alginate sheet, 8 mL of the 3 % alginate (Alginic acid sodium salt, Scientific Polymer Products) in DI water was poured onto 100 mm culture dish and dehydrated overnight, and then cured by adding 5 % calcium chloride in DI water. As illustrated in Figure 1(d), the containers were carefully aligned with the open face upward onto an 200 µm height SU-8 mold providing 1 mm spacing between the containers. High density pancreatic β-cell suspension was loaded and incubated at 37 °C overnight. The containers were attached on the alginate sheet using a non-toxic suture glue (Gluture, WPI), and then the sheet was rolled into a cylinder shape (Figure 1(c)).



Figure 2: (a) Magnified image of single container (open-face down). (b) SEM image of  $6 \mu m \times 6 \mu m$  pores after gold electroplating. (c)-(f)  $\beta$ -cell encapsulation in single container and rolled-up sheet and their fluorescent images of live cells with calcein indicator. The  $\beta$ -cells were grown for 1 day and the containers were washed with PBS before staining.

#### **RESULTS AND DISCUSSION**

To demonstrate the encapsulated  $\beta$ -cells can survive in the container and the rolled-up sheet, we applied a fluorescent indicator during the incubation (Figure (d) and (f)). Prior to insulin measurement, the devices were incubated under low-glucose condition for 12 hours. After replacing to high-glucose medium to watch glucose stimulation, 20  $\mu$ L of medium samples were taken at various time points. An insulin enzyme-linked immunosorbent assay (ELISA) was used for quantifying insulin. We observed that the concentration of produced insulin saturates at certain level in both cases as shown in Figure 3. In case of the rolled-up sheet consisting of 5 containers, the saturation level of its released insulin is lower than the 5 times of the insulin from single container. The reason for the difference comes from the fact that one face of the single container is open, whereas the open faces of the containers on rolled-up sheet are coved with alginate sheet.



Figure 3: Released insulin from single container (left) and rolled-up sheet with 5 containers (right).

In case of human, volume of  $\beta$ -cells producing insulin ranges from 0.65 to 1.2 mL [6]. Assuming that the single container is fully filled with  $\beta$ -cells, volume capacity of the single container is 0.45 µL, which means approximately 2000 containers are needed for encapsulating  $\beta$ -cells for human body. The number of containers can be reduced by making longer containers or by shortening the space between containers on alginate sheet within the range of avoiding hypoxia. The typical blood level of insulin is 8–11 µU/ml [7] (0.36 – 0.5 µg/mL), which is 24 – 33 times higher than the insulin level from the rolled-up sheet (15 ng/mL). Regarding the insulin-production-rate (IPR), typically the basal IPR of healthy man is 17 mU/min [8] which corresponds to 0.765 µg/min. We measured that the rolled-up sheet with 5 containers released 0.625 ng for 1 hour, which can be scaled up to 13.8 ng/min assuming the rolled-up sheet has 2000 containers for human pancreas. The IPR from rolled-up sheet could be increased by packing the cells with higher density and increasing the pore density.

### CONCLUSION

As a preliminary research for complete bio-artificial pancreas, we demonstrated that the cell encapsulation technology in microcontainers on rolled-up sheet have potential to investigate an artificial organ. Based on the simulation providing a guideline for hypoxia, dimensions of container and rolled-up sheet were determined. Fabrication process was modified to enhance quality and throughput during the microfabrication. An alginate sheet providing bio-compatibility was employed for assembly of multiple containers. Insulin release from the rolled-up sheet was quantified and its potential to be improved for artificial organ was evaluated. Our current research involves fabrication of nano-sized pores on the microcontainer with high precision, which will play an important role on releasing insulin and blocking immune responses. Additionally, since our fabrication process is compatible with microelectronic and sensor fabrication, we expect that we can easily add modules to the device to enable real time imaging of transplanted cells and wireless monitoring of the device performance even after implantation.

## REFERENCES

- [1] F. Lim and A. M. Sun, "Microencapsulation of Islets as Bioartificial Endocrine Pancreas", Science, 210, 908 (1980).
- [2] T. A. Desai, W. H. Chu, J. K. Tu, G. M. Beattie, A. Hayek, and M. Ferrari, "Microfabricated Immunoisolating Biocapsules", *Biotechnol Bioeng*, 57, 118 (1998).
- [3] J. T. Santini, M. J. Cima, and R. Langer, "A Controlled Release Microchip", Nature, 97, 335 (1999).
- [4] G. Lesinski, S. Sharma, K. Varker, P. Sinha, M. Ferrari, and W. Carson, "Release of biologically functional interferon-alpha from a nanochannel delivery system", *Biomed Microdevices*, 7, 71 (2005).
- [5] B. Gimi, T. Leong, Z. Gu, M.Yang, D. Artemov, Z. M. Bhujwalla and D. H. Gracias, "Self-assembled Three Dimensional Radio Frequency (RF) Shielded Containers for Cell Encapsulation", *Biomed Microdevices*, 7, 4, 341-345 (2005).
- [6] A. A. Elayat, M. M. El-Naggar, M. Tahir, "An Immunocytochemical and Morphometric Study of the Rat Pancreatic Islets", J Anat, 186, 3, 629–37, (1995).
- [7] H. Iwase, M. Kobayashi, M. Nakajima, T. Takatori, "The Ratio of Insulin to C-Peptide Can Be Used to Make a Forensic Diagnosis of Exogenous Insulin Overdosage", *Forensic Sci Int*, 115 (1–2): 123–127 (2001).
- [8] W. K. Waldhäusl, P. R. Bratusch-Marrain, M. Francesconi, P. Nowotny and A. Kiss, "Insulin Production Rate in Normal Man as an Estimate for Calibration of Continuous Intravenous Insulin Infusion in Insulin-Dependent Diabetic Patients", *Diabetes Care*, 5, 1, 18-24 (1982).

#### CONTACT

\* J. Park, tel: +1-410-982-8564; jaehyun0118@gmail.com \*\* D. H. Gracias, tel: +1-410-516-5284; dgracias@jhu.edu