Microfluidics based single islet encapsulation with real time 3D oxygen sensing capability

Shen A., Chen W., Lisowski M., Khalil G., Sweet I.
University of Washington, Seattle, USA (amyshen@uw.edu)

INTRODUCTION

Oxygen consumption reflects multiple processes in pancreatic islets including mechanisms contributing to insulin secretion, oxidative stress and viability, providing an important readout in studies of islet function, islet viability and drug testing. Due to the scarcity, heterogeneity, and intrinsic kinetic properties of individual islets, it would be of great benefit to detect oxygen consumption by single islets. Recently, microfluidic techniques have advanced cell encapsulation by using hydrogels or other biopolymers as the microcapsules. This approach preserves cell viability and generates a uniform set of monodisperse microencapsulated cells necessary to obtain reliable individual cell behavior from a population of cells (Kim 2011).

In this paper, we used a micorfluidics setup to encapsulate single isolated pancreatic islets and a water-soluble, oxygen-sensitive, fluorescent dye inside a 180-μm sized alginate microcapsule (Chen 2012). The dye situates itself during the encapsulation process in the extracellular space between the islet cells and the alginate layer, and acts as a 3-dimensional real time oxygen sensor. The alginate microcapsule-based sensor was stable, sensitive to small changes in oxygen tension, and responded to various effectors of mitochondrial metabolism in real time.

MATERIALS AND METHODS

Krebs-Ringer bicarbonate solution (KRB), used for all perifusion analysis, contained 98.5 mM NaCl, 4.9 mM KCl, 1.2 mM potassium phosphate, 1.2 mM magnesium sulfate, 25.9 mM sodium bicarbonate, 2.6 mM $CaCl_2$, varying amounts of glucose , and 20 mM HEPES. The agents used in perifusion experiments were potassium cyanide (KCN), FCCP, and nimodipine (Sigma-Aldrich). Agents used in the encapsulation procedure were soybean oil, Sterile sodium alginate (endotoxins<100 EU/g) (Novamatrix, Oslo, Norway), and Span 80 (Sigma-Aldrich). The oxygen-sensitive dye, Pt(II) meso-Tetra(N-Methyl-4-Pyridyl)Porphine Tetrachloride (Frontier Scientific).

Islets were harvested from Sprague-Dawley rats (≈ 250 g, Charles River) anesthetized by intraperitoneal injection of sodium pentobarbital (35 mg/230 g rat). Islets were prepared and purified, and then cultured at 37°C in RPMI Media 1640 supplemented with 10% heat-inactivated fetal bovine serum for 18 hours prior to encapsulation.

As shown in Fig. 1, islets were encapsulated in alginate using a microfluidic system with three inflow channels (100 μm wide) and a single outflow channel (180 μm wide), fabricated using soft lithography. The cross section of each microchannel was rectangular with a depth of 180 μm. A mixed solution of vegetable oil and Span 80 (1% volume ratio), acting as the carrier phase, was pumped through the center microchannel at 150 μL/hr. Culture media containing 45 mM $CaCl_2$ was pumped through the lower microchannel at 60 μL/hr and another aqueous mixture of culture media containing islets, alginate (1% wt ratio), and oxygen-sensitive dye (20 μM) was pumped through the upper microchannel at 70 μL/hr.

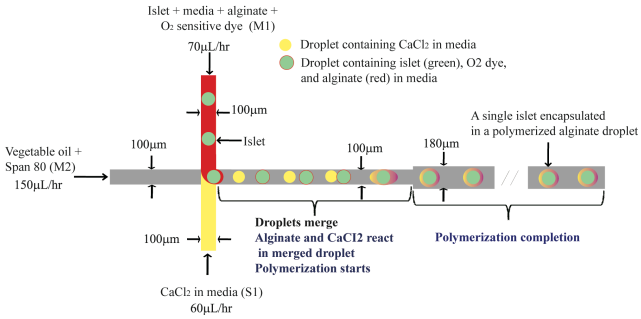

Figure 1: Schematic of microfluidics system used to encapsulate islets.

At the intersection of the microchannels, the aqueous phases enter the faster flowing oil phase in alternating fashion as discrete droplets in the face of competition of viscous and capillary forces. Once in the main channel, the less viscous and faster calcium droplet collides and merges with the more viscous alginate droplet containing the oxygen-sensitive dye and islet. Polymerization occurs in the outflow channel when the alginate comes into contact with Ca^{2+} thereby trapping the dye along with the islet. Encapsulated and dyed islets were collected from the outflow into a vial of culture media, and centrifuged at 1500 g for 3 minutes to separate the oil from the media and islets. Encapsulated islets were transferred to a petri dish with fresh culture media, and incubated in a CO_2/37 degree incubator for 2-48 hours prior to experiments.

RESULTS AND DISCUSSION

The microcapsule size of the encapsulated islet is determined by the width of the outflow microchannel (180 μm). The alginate layer is nearly transparent, but was visible in images obtained with brightfield

illumination (Fig. 2A and B); no layer was observed in the unencapsulated islet (Fig. 2C). Typically, the thickness of the alginate layer was uniform. For a 145-µm islet the alginate layer was about 15 µm thick (Fig. 2A) and for a 90-µm islet the layer was about 45 µm thick (Fig. 2B). When the oxygen-sensitive dye was included in the encapsulation solution, the resulting encapsulated islet fluoresced in all regions of the islet, most brightly in areas around cells in the islet (Fig. 2D).

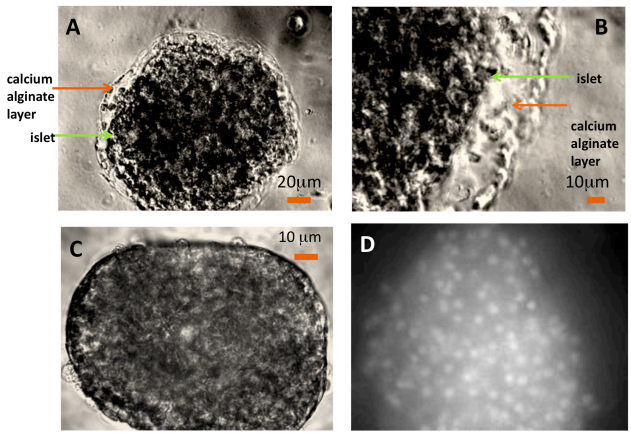

Figure 2: Images of encapsulated islets illustrating the polymerized alginate layer and dye fluorescence. Images of single islets (A. diameter = 140 µm; B. diameter = 95 µm) coated with transparent calcium alginate. C. Image of unencapsulated islet. D. Fluorescence image of an encapsulated/dyed islet.

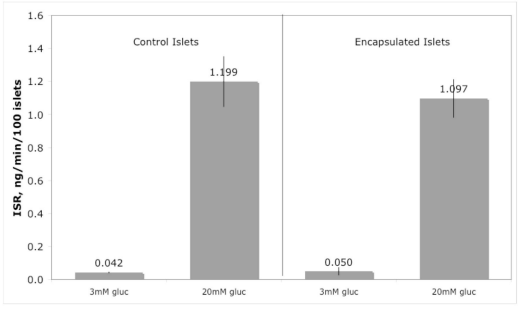

Figure 3: Insulin secretion by encapsulated and unencapsulated islets. After pre-incubation in KRB containing 3 mM glucose, islets were incubated in 96-well plates in the presence of 3 or 20 mM glucose for 60 min.

To gauge the functionality of the encapsulated islets, their ability to secrete insulin was compared to that of unencapsulated islets (Fig. 3). Insulin secretion by the encapsulated islets was normal. Fluorescent signal from the encased dye, detected using a standard inverted fluorescence microscope and digital camera, was stable and proportional to the amount of oxygen in the media (Fig. 4). When integrated into a perifusion system, the sensing system detected changes in response to metabolic substrates, mitochondrial poisons, and induced-oscillations. Glucose responses averaged 30.1 ± 7.1% of the response to a metabolic inhibitor (cyanide), increases were observed in all cases (n=6), and the system was able to resolve changes in oxygen consumption that had a period greater than 0.5 minutes [1]. The sensing system operated similarly from 2-48 hours following encapsulation, and viability and function of the islets were not significantly affected by the encapsulation process.

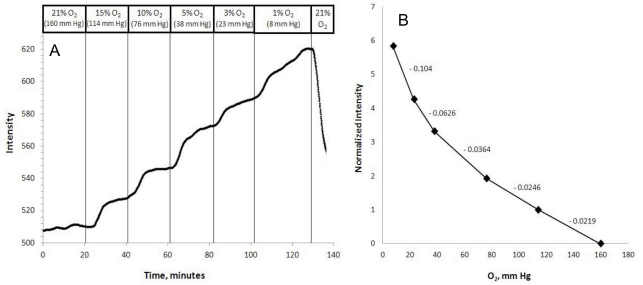

Figure 4: A. Effect of oxygen tension in the perifusate on dye in islet. Rat islets were encapsulated with the oxygen-sensitive dye and then loaded into the perifusion system. Fluorescence from the dye was measured after islet respiration was suppressed by 3 mM KCN. B. Steady-state intensity values for each oxygen level. Intensity was averaged over the final 5 minutes of each period, normalized to the change in intensity observed in response to decreasing oxygen tension from 142 to 101.5 mm Hg.

CONCLUSION

An oxygen-dependent dye situated around and within a pancreatic islet encapsulated by a thin layer of alginate was sensitive to changes in oxygen consumption, and was not harmful to the function or viability of islets over the course of two days [3]. The microcapsule-based sensing method is particularly suited to assessing the effects of compounds (dose responses and time courses) and chronic changes occurring over the course of days. The approach should be applicable to other cell types and dyes sensitive to other biologically important molecules.

REFERENCES

- Chen W. et al. (2012) *Microencapsulated 3-dimensional sensor for the measurement of oxygen in single isolated pancreatic islets*. PLoS ONE, 7 (3) e33070.
- Kim C. et al. (2011) *Generation of core-shell microcapsules with three-dimensional focusing device for efficient formation of cell spheroid*. Lab Chip 11 (2) 246-252.