O3-2	**Encapsulation of crosslinked gelatin nanofibers to better mimic ECM conditions within alginate microparticles**	

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INTRODUCTION AND OBJECTIVES

The immunoisolation of islet allografts and xenografts within alginate microspheres has succeeded in normalizing blood-glucose levels in different animal models without the use of immunosuppression; however, these systems require supraphysiological quantities of islets and exhibit reduced graft viability with time. The benefits of microspheres include a high surface area to volume ratio, high resistance to mechanical stress, relatively short diffusion paths leading to improved response rates, and access to various implantation sites (Hallé 2009).

The enzymatic treatment and washing protocols required to isolate islets from the pancreas have been shown to strip them of ECM components and growth factors required to up-regulate insulin production (Robitaille 2003). Incorporation of individual ECM proteins into hydrogels containing immortalized β-cells have shown significant increases in β-cell survival and corresponding decreases in apoptosis over controls (Weber 2008). Culture of whole islets on fibrous matrices has also led to increased viability and insulin secretion (Kawazoe 2009).

The benefits of ECM protein incorporation, three-dimensional fiber matrices, and alginate immunoisolation may be combined by incorporating a crosslinked gelatin fiber network into the traditional islet-laden alginate microspheres. However, due to the solubility of gelatin, the fibers must first be crosslinked. Traditional crosslinking techniques are known to have residual cytotoxic effects. Microbial transglutaminase (mTG), can be used to form covalent linkages between glutamine and lysine residues at physiological conditions without affecting cellular viability (Chau 2005). Genipin is another natural crosslinking agent which reacts with primary amine groups, producing a stable bond (Liang 2002).

The focus of this study will be to combine the best aspects of immunoisolation, ECM incorporation, and islet culture in fiber networks to increase the viability and insulin secretion rates of islets for bioartificial pancreas engineering.

MATERIALS AND METHODS

Activa-TI™ (Ajinomoto), a 1% mTG culinary product, was kindly donated by Thomas, Large & Singer Inc. (Montreal, Canada). Genipin was purchased from Challenge Bioproducts Co., Ltd. (Taiwan). Unless specified, all other chemicals were purchased from Sigma Aldrich (Oakville, Canada).

Nanofibers were electrospun from a 10% gelatin (type A, 300 bloom) solution dissolved in 50% acetic acid, 30% ethyl acetate, and 20% distilled water. The solution was extruded at 1 mL/hr through a 22G blunt needle with a +12 kV potential, over 10 cm to a grounded mandrel.

Extruded fibers were produced from a solution of 1.5% sodium alginate and 1.5% gelatin in distilled water, maintained at 45°C. The solution was filtered and extruded through a microcapillary (ID 0.1 mm) at a rate of 0.30 mL/min directly into a wet-draw 2% $CaCl_2$ bath mixed at 500 rpm to crosslink the alginate phase.

Fibers were placed within one of two solutions to crosslink the gelatin phase at 25°C for 48 hours: either a 5% solution of Activa-TI or 0.25% genipin and 50 mM $CaCl_2$. After crosslinking, the alginate support matrix is removed from the extruded fibers in a 37°C solution of 0.10 M sodium carbonate and 0.04 M citric acid with mixing. The gelatin fibers are then dried and sterilized in a sequential ethanol bath (50%, 75%, 95%) followed by vacuum drying. When required for encapsulation, the fibers are rehydrated in phosphate buffered saline, and dissociated with an ultrasonic homogenizer for 5 minutes (Cole-Parmer 4710-CV17, 60% duty, output 4).

Fiber encapsulation proceeded by a modified internal gelation emulsion (Poncelet 1992). The aqueous phase contained 2% sodium alginate, 3.5% gelatin fibers, and 0.3% ultrafine calcium carbonate (Specialty Minerals Inc, USA). The alginate mixture was dispersed 1:2 in sunflower oil at 330 rpm for 10 minutes, then acidified with glacial acetic acid in sunflower oil to solubilize the calcium carbonate and crosslink the alginate phase. After an additional 5 minutes, 50 mM $CaCl_2$ was added and the particles were allowed to settle. The oil phase was removed by aspiration and the particles were stored in 50 mM $CaCl_2$.

Light microscopy images were obtained on a Wild Heerbrugg M3 stereomicroscope. Scanning electron microscopy (SEM) was performed on vacuum-dried gold-sputtered samples with an accelerating voltage of 10kV. Fiber sizing was performed by hand using characteristic images and calibrated calipers.

RESULTS AND DISCUSSION

Electrospinning proved to be a very simple method for producing pure gelatin nanofibers, as shown in Figure 1. The average fiber diameter was 250 ± 140 nm.

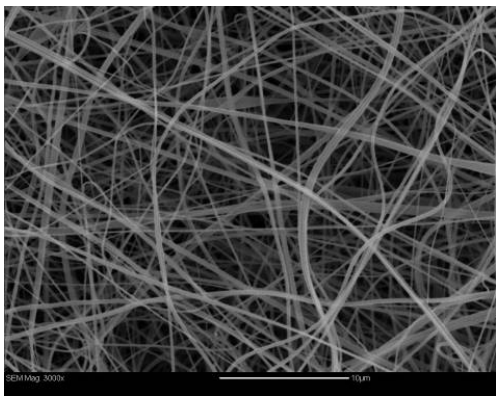

Figure 1. SEM image of electrospun gelatin fibers. Scale bar represents 50 µm.

However, the electrospun fibers were highly soluble in the aqueous crosslinking solutions, and alternative crosslinking agents, such as glutaraldehyde are deemed detrimentally toxic for this application. Additional research is being performed with low-temperature crosslinking to reduce gelatin solubility.

The process of extruding alginate-gelatin fibers with this wet-drawing process is a simple method, with a high degree of control, as the capillary diameter and wet-draw speed have a direct influence on the fiber diameter. The fibers produced have consistent diameters (Figure 2), with a wet-draw speed of 500 rpm corresponding to an average hydrated fiber diameter of 61 ± 7 µm.

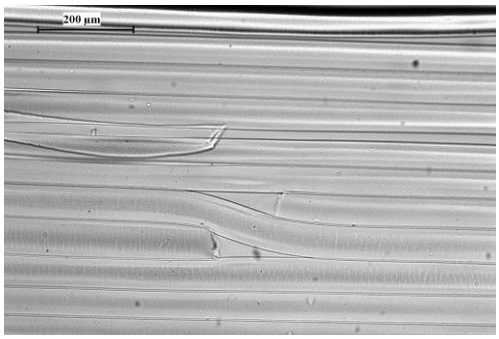

Figure 2. Extruded alginate-gelatin fibers. Scale bar represents 200 µm.

Crosslinking with transglutaminase, while effective on larger scales, produced insufficient and inconsistent crosslinking, resulting in few fibers remaining after the removal of the alginate phase. In comparison, genipin produced strong gelatin fibers over a period of 48 hours, and also produced a characteristic blue colouration which could be used to monitor the degree of crosslinking.

Genipin-crosslinked fibers encapsulated within an internally-gelled calcium alginate microparticle can be seen in Figure 3. The fibers are arranged such that they are contained within the particle, surrounding the core of the particle.

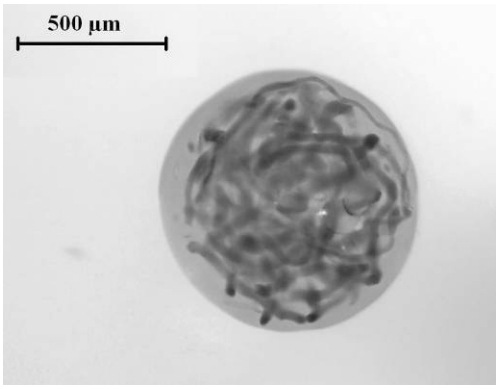

Figure 3. Genipin-crosslinked gelatin fibers encapsulated in an internally gelled calcium alginate microparticle.

The particles produced had a wide size range, as is characteristic of the emulsion method. Future studies will aim to narrow the range, and optimize particle size.

CONCLUSIONS

While a work in progress, the results to date are a proof of concept for the incorporation of crosslinked gelatin fiber networks within the traditional alginate micro-particle matrix using cytocompatible processes. In future studies, islets will be incorporated into the process and nestled within the fibrous gelatin network, providing a surface for adherent growth conditions. The use of polyionic membranes will also be investigated to allow for a liquid-core to facilitate cell-network adhesion.

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