P-041	**Increasing islet viability with protein microfibers in alginate microparticles**	

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INTRODUCTION AND OBJECTIVES

The effective treatment of type I diabetes mellitus requires the introduction of a controlled hormone replacement therapy to continually regulate blood glucose. The transplantation of pancreatic islets has proven to normalize blood glucose in both human and animal models, however, it necessitates immunoisolation to avoid the negative effects of immunosuppression therapy. Traditionally, this has been achieved by encapsulating islets within alginate microparticles, due to the cytocompatibility of ionic gelation and favourable immune response to highly purified alginates.

Despite advances in the field, a primary issue continues to be the maintenance of islet viability within the alginate microparticles over an extended period of time. One approach to improving viability is to restore the integrin-mediated bonds between the cells and the extracellular matrix (ECM) through the introduction of proteins. The result has been an up-regulation of insulin secretion and reduction in apoptosis (Weber 2008). A second approach has been to recreate a better model of the physical environment by culturing islets on fibrous matrices, showing increased viability and insulin secretion (Kawazoe 2009).

Previous attempts at combining the benefits of three-dimensional matrices, ECM proteins, and alginate microparticle immunoisolation have encountered an issue with scale. Preformed scaffolds must fit within a 300 µm microparticle for optimal diffusion, but the pores must be large enough to allow for the seeding of 150 µm diameter islets. Research on seeding dissociated islets onto porous gelatin microcarriers has proven that, while the use of a protein matrix displays benefits over the alginate control, the disruption of the islet aggregate was too detrimental to glucose sensitivity to be an effective solution (Del Guerra 2001).

The geometrical limitation has been overcome through the development of a novel one-step process which forms a gelatin microfiber matrix around an islet within a barium alginate microparticle under cytocompatable conditions.

The production of discrete gelatin microfibers by a calcium alginate mediated drawing technique was developed by Yang et al (2009) and further optimized in this study. Crosslinking of the fibers with genipin is necessary to ensure mechanical integrity and prevent protein leaching. Genipin, a naturally derived compound, reacts with primary amine groups produces a stable bond with non-cytotoxic byproducts (Liang 2002).

The focus of this study was 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

Pharmaceutical-grade Protanal LF 10/60 sodium alginate was purchased from FMC Biopolymers (Drammen, Norway) and further purified by a modified Klöck method (Langlois 2009). Genipin was purchased from Challenge Bioproducts Co., Ltd. (Taiwan). All encapsulation work was performed under sterile, endotoxin-free conditions.

Extruded fibers were produced from a heated solution of 1.5% sodium alginate and 1.5% gelatin in distilled water. The solution was filtered and extruded through a blunt tipped syringe immersed in a heated vortex of 1.5% gelatin, and 2% $CaCl_2$ to crosslink the alginate phase.

Fibers were placed within 0.25% genipin in TRIS-buffered $CaCl_2$ to crosslink the gelatin phase for 48h. After crosslinking, the alginate support matrix was removed by chelation. The gelatin fibers were then dried and sterilized in a sequential ethanol bath followed by vacuum drying. When required for encapsulation, the fibers were rehydrated in sterile phosphate buffered saline, and dissociated with ultrasonic homogenization.

Islets were isolated from male Sprague Dawley rats (Charles River Institute, St.-Constant, Canada) according to established techniques (Langlois 2009). All cultures were performed in 5.5 mM glucose RPMI supplemented with 10% fetal bovine serum, 1% pen-strep, and 0.25% AB at 37˚C, 5% CO_2. Animal work was performed under the approval of the Maisonneuve-Rosemont Hospital Animal Ethics Committee.

Fiber and islet co-encapsulation proceeded by electro-static mediated dropwise particle formation into buffered 10 mM $BaCl_2$. The extruded solution contained 40 vol% hydrated fiber, 60 vol% 1.6% purified sodium alginate dissolved in saline, and 2000 islets/mL.

Islet-laden particles were incubated for 1, 7, 14 and 21 days. Viability was assessed with propidium iodide-

acridine orange staining. Insulin secretion was determined by rat-insulin ELISA (Mercodia) performed on aliquots of media following 1h incubations of 50 particles in triplicate at either 3.3, 5.5, 11, or 16.5 mM glucose in RPMI. Apoptosis was visualized with Hoechst nuclear staining observed on a fluorescent microscope. All data was normalized to total DNA content quantified by PicoGreen assay (Invitrogen) after particle and cell dissociation by freeze-thaw cycling in 10 mM EDTA.

Fiber integrity and crosslinking efficiency were assessed by ninhydrin primary amine assay and BCA assay.

RESULTS AND DISCUSSION

With the optimization of the wet-drawing process, fiber diameter was reduced from a literature value of 181 ± 2 μm (Yang 2009) to 22.3 ± 0.4 μm (Fig 1A), with an average length of 223 ± 13 μm. Average particle size was 294 ± 4 μm, as can be seen in Figure 1B.

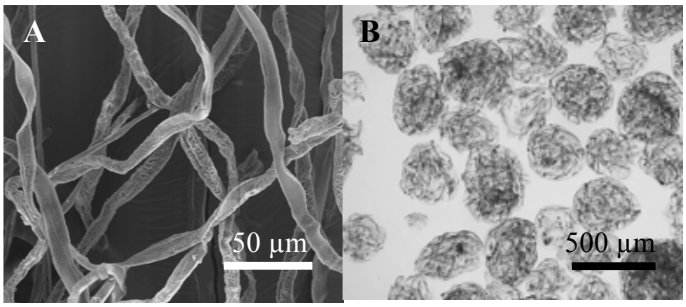

Figure 1. A) SEM image of dehydrated gelatin microfibers. B) Fiber and islet-laden microparticles.

The integrity of the fibers is important to ensure efficacy as a long-term support. Fiber samples were incubated in phosphate buffered saline at 37˚C for 10 weeks, releasing < 0.01% of the total protein mass. Crosslinking efficiency was determined to be 65 ± 8% of the available primary amines. The encapsulated fibers displayed no signs of degradation over 21 days in culture.

Adhesion of the islets to the protein fibers was observed within the particles (Fig 2), and within the encapsulation solution following only one hour of co-incubation.

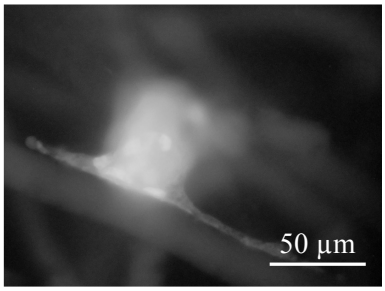

Figure 2. Islet adhesion to encapsulated gelatin fiber.

Viability, defined as > 50% of the cell mass staining viable with acridine orange is summarized in Table 1. An independent method confirmed significantly higher viability in capsules with fibers ($p < 0.01$) at days 7, 14 and 21. The increase in viability corresponded with a preservation of intact islet morphology.

Table 1. Islet viability as assessed by AO/PI staining

Day	Fiber Particles	Control Particles
1	79%	61%
7	87%	50%
14	82%	56%
21	71%	25%

Glucose-stimulated insulin secretion, was maintained in the fiber-laden system with an average 16.5 mM / 3.3 mM glucose stimulation index of 1.9 ± 0.7 compared to 1.2 ± 0.6 for the controls at days 7, 14, and 21. Relative rates of apoptosis, determined from nuclear morphology, are currently under analysis.

Future studies will build on the foundational work presented here, by investigating the modification of the microfibers with other ECM proteins and further improving particle consistency and sphericity.

CONCLUSIONS

A novel method was developed to produce discrete, genipin-crosslinked gelatin microfibers with a significantly reduced diameter of 22.3 ± 0.4 μm. These fibers were incorporated into ionically crosslinked 294 ± 4 μm barium alginate microparticles in the form of a interwoven scaffold, showing for the first time an original one-step process in which large cellular aggregates can be seeded within a microparticle-scale matrix without the limitations of pore-size. A preliminary in vitro study showed a significant increase in viability at 7, 14, and 21 days coinciding with an increased preservation of physiological morphology, while maintaining glucose sensitivity. Future work will focus on modifying the surface of the gelatin fibers with additional ECM proteins which have been shown to further enhance the viability and efficiency of transplanted islets.

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