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Encapsulation of protein microfiber networks supporting pancreatic islets

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Abstract: Networks of discrete, genipin-crosslinked gelatin microfibers enveloping pancreatic islets were incorporated within barium alginate microcapsules. This novel technique enabled encapsulation of cellular aggregates in a spherical fibrous matrix <300 μm in diameter. Microfibers were produced by vortex-drawn extrusion within an alginate support matrix. Optimization culminated in a hydrated fiber diameter of 22.3 ± 0.4 μm, a significant reduction relative to that available through current gelatin microfiber spinning techniques, while making the process more reliable and less labor intensive. Microfibers were encapsulated at 40 vol % within 294 ± 4 μm 1.6% barium alginate microparticles by electrostatic-mediated dropwise extrusion. Pancreatic islets extracted from Sprague Dawley rats were encapsulated within the microparticles and analyzed over 21 days. Acridine orange and propidium iodide fluorescent viability staining and light microscopy indicated a significant increase in viability for islets within the fiber-embedded particles relative to fiber-free controls at days 7, 14, and 21. The fiber-embedded system also promoted cellular aggregate cohesion, reducing the incidence of dispersed islet morphologies within the capsules from 31 to 8% at day 21. Further enquiry into benefits of islet encapsulation within a protein fiber network will be the subject of future investigation. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 100A: 3384–3391, 2012.

Key Words: pancreatic islets, microfibers, encapsulation, scaffold, tissue engineering

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INTRODUCTION

Type 1 diabetes is caused by an autoimmune destruction of insulin-secreting beta cells.[1] Current exogenous insulin therapy, while effective, simply slows the progression of the disease as it fails to mimic the highly regulated physiological blood glucose control.[2] Transplantation of pancreatic islets has been investigated as islets can be isolated, encapsulated, and implanted, while retaining the complex glucose-modulating multicellular interactions of the endocrine pancreas functional subunit.[3] Encapsulation within alginate microparticles has been shown to protect islets from the bulk of the host immune response.[4–6] However, while major improvements in purity and subsequent islet viability have been made,[7] alginate, being a polysaccharide without adhesion domains, does not sufficiently mimic the physical or chemical microenvironment required for optimal islet function.[8]

The process of islet isolation by enzymatic digestion can be detrimental to the function of the islets, as the homeostasis of the extracellular matrix (ECM) microenvironment is disrupted, resulting in effects such as the down-regulation of insulin production.[9] The reintroduction of extracellular matrix components has been shown to increase islet viability and insulin secretion.[10–12] Previous work on the modification of alginate microparticles to reintroduce ECM components has focused on the incorporation of soluble growth factors,[9] adhesion peptides,[13] and ECM molecules.[14] The restoration of a structural fibrous matrix with cultures of whole islets on modified and unmodified poly(lactide-*co*-glycolide) fibers has increased viability, insulin secretion, and gene expression.[12,15–19]

Daoud et al.[16] showed that both the introduction of ECM-proteins and the provision of a fibrous support matrix within a collagen gel each resulted in increased gene expression and insulin production. Therefore, both the chemical and physical environmental alterations provided significant independent benefits. The geometrical limitations of porous scaffold seeding requires pore sizes 5–10 times larger than the cells to be seeded.[20] Therefore, to entrap a

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150-μm pancreatic islet within a 300-μm diameter fiber network, a new approach was required.

The system outlined within this investigation combines discrete crosslinked protein microfibers with islets and alginate at the time of particle production, generating a network of fibers around the islet as the particle is formed, all in a single step.

To form a fiber network within the microcapsules, discrete protein microfibers were required on a scale finer than those available from existing techniques. Gelatin was selected as it is readily crosslinked and easily modified due to the higher availability of unhindered primary amines, relative to triple-helical collagen.[21] A gelatin matrix also restores sufficient cell–ECM interactions to induce glucose-dependent insulin production in dissociated beta cells.[22]

Yang et al.[23] produced discrete porous gelatin microfibers 180 μm in diameter using an alginate support phase. This technique, while producing fibers too large for microencapsulation, displayed the potential for optimization. The production of fine gelatin nanofibers by electrospinning has also been investigated.[24,25] However, electrospun gelatin fibers are consistently fused into a mat when crosslinked.

Many crosslinking techniques suffer from both short- and long-term cytocompatibility issues.[26,27] Genipin is a natural crosslinking agent, food dye, and anti-inflammatory which reacts with primary amine groups producing a stable bond with extremely low toxicity.[27–29] Genipin-crosslinked protein biomaterials have demonstrated a greatly reduced inflammatory reaction and slower degree of degradation relative to glutaraldehyde crosslinked controls.[28,30]

There are numerous methods for the production of ionically gelled alginate microparticles. Electrostatic-mediated droplet production is effective and cytocompatible.[3] In this technique, islets are suspended in an alginate solution that is dispensed through a charged needle into a grounded divalent cation collection bath. Alternatively, emulsion encapsulation[31,32] uses pH-triggered internal gelation to form discrete ionically gelled alginate microparticles from a suspension of ultrafine calcium carbonate in alginate emulsified in oil. Hoesli et al.[33] optimized the internal gelation system to reduce the duration of the emulsion and acidification phases, increasing the cytocompatibility of the emulsion process.

The present investigation aimed to bring together developments in islet culture systems in a manner that had been previously limited by the size of cellular aggregates. These developments are alginate microparticle immunoisolation, the introduction of an ECM chemical microenvironment, and a fibrous support scaffold.

The key was to form a matrix of protein fibers around an islet during the encapsulation process, eliminating the need for islet seeding geometries, allowing for greatly enhanced contact, and increasing the surface area for islet–ECM integrin-mediated connections.

The investigation can be divided into three main studies: protein fiber production, fiber-embedded particle production, and a demonstration of islet viability.

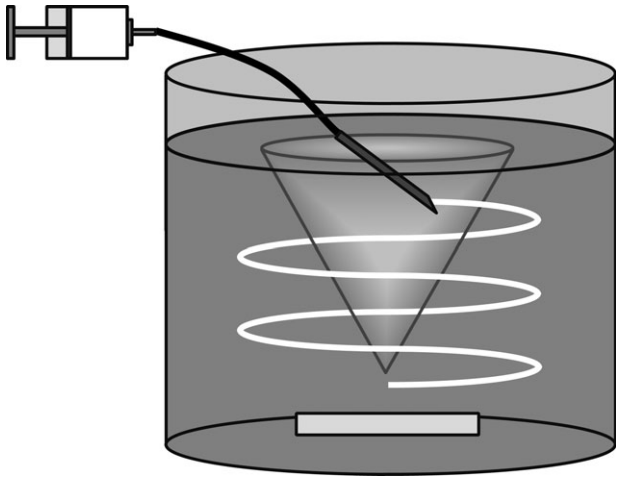

FIGURE 1. Extrusion of gelatin/alginate solution into a vortex of gelatin and $CaCl_2$.

MATERIALS AND METHODS

Pharmaceutical-grade Protanal LF 10/60 sodium alginate was purchased from FMC Biopolymers (Drammen, Norway) and purified by a modified Klöck method.[7] Genipin was purchased from Challenge Bioproducts (Taiwan). Polysucrose 400 (Ficoll), glucose (EuroCollins) solution, and electrolyte additive solution were purchased from Mediatech (Herndon). Unless otherwise specified, alginate purification reagents were purchased from Fisher Scientific. All other materials were of analytical grade and purchased from Sigma-Aldrich Canada (Oakville, Canada). All investigations using islets were performed under endotoxin-free conditions. When possible, disposable endotoxin-free materials were used. All solutions were prepared with endotoxin-free labware using sterile water and additional sterile filtration.

Drawn gelatin microfibers

Through a series of optimizations, a wet-drawn vortex system was developed by radical modification of the alginate-mediated extrusion method originated by Yang et al.[23] A 45°C solution of 1.5% sodium alginate (Sigma Aldrich) and 1.5% gelatin (Type A, 300 Bloom) was extruded through a 35-G blunt-tipped needle at a rate of 2.4 mL/h. The tip of the needle was submerged at a 45° angle into a 45°C, 700 rpm vortex of 1.5% gelatin and 2% $CaCl_2$ dissolved in distilled water as illustrated in Figure 1. The syringe and vortex were maintained at 45°C with a temperature-controlled waterbath.

Gelatin–alginate fibers were transferred to a protein-crosslinking solution of 50 m*M* $CaCl_2$, 100 m*M* Tris (pH 8.0), and 0.25 wt % genipin. Fibers were crosslinked for 48 h at 25°C. The alginate support matrix and uncrosslinked gelatin were removed from the fibers by chelating in a 37°C solution of 55 m*M* sodium citrate, 30 m*M* ethylenediaminetetraacetic acid (EDTA), and 0.15 *M* NaCl with agitation, changing the chelation solution three times. The gelatin fibers were then dried and sterilized in a sequential ethanol bath (50%, 75%, 95%) followed by vacuum drying.

When required for encapsulation, the fibers were rehydrated in sterile pH 7.4 phosphate buffered saline (PBS) and dissociated into discrete 200 μm fibers with an ultrasonic homogenizer for 5 min (Cole-Parmer 4710-CV17, 60% duty, output 4). The sonicated suspension of fibers in PBS was allowed to settle for 10 min before the unsettled fraction was collected and pelleted at 2000×*g* for 10 min.

Fiber integrity

A ninhydrin assay was performed to measure the concentration of free primary amines in genipin-crosslinked fibers relative to uncrosslinked fibers. Dehydrated samples were placed in 2 mL of 0.1 *M* pH 5.0 phthalate buffer. All vials were heated in a water bath at 85°C for 5 min and then cooled to room temperature. Once cooled, 1 mL of 2% ninhydrin in dimethyl sulfoxide (DMSO) was added to each vial, and the vials were placed in a boiling water bath for 10 min. The samples were cooled for 10 min, and 5 mL of methanol was added. The absorbance at 570 nm was measured by spectrophotometer. The extent of crosslinking was determined by Eq. (1).

$$\%\,\text{Crosslinked} = 1 - \frac{\text{Abs}_{\text{Crosslinked}} - \text{Abs}_{\text{AV Blank}}}{\text{Abs}_{\text{Uncrosslinked}} - \text{Abs}_{\text{AV Blank}}} \quad (1)$$

Fiber integrity was analyzed by a 70-day 37°C incubation of crosslinked samples in sterile PBS followed by soluble protein quantification by micro bicinchoninic assay (BCA, Thermo Scientific) of the supernatant.

Emulsion encapsulation

Early acellular studies used a modified internal gelation emulsion encapsulation.[23,24] The aqueous phase contained 4% sodium alginate, 0.3% ultrafine calcium carbonate (Specialty Minerals), and 50 vol % hydrated gelatin fibers (concentrated at 2000×*g*, 10 min). The alginate mixture was dispersed 1:2 in 60 mL sunflower oil with a 42-mm marine impeller at 330 rpm. At 5 min, the system was acidified with 120 μL of glacial acetic acid in 10 mL sunflower oil. After 1 min of acidification, 50 m*M* $CaCl_2$ was added and the particles were allowed to settle. The oil phase was removed by aspiration and the particles were stored in 50 m*M* $CaCl_2$.

Islet isolation

Islets were isolated from 10-week old male Sprague Dawley rats (Charles River Institute, St.-Constant, Canada) according to established techniques.[7,34] The protocols for the animal manipulations performed in this study were approved by the Maisonneuve-Rosemont Hospital Animal Ethics Committee and all procedures were performed accordingly (Approval # 2006-28). Briefly, the bile duct was cannulated and infused with chilled 1 mg/mL collagenase V (Sigma Aldrich C9263; lot 128H8632) in Hank's balanced salt solution (HBSS), and then incubated at 37°C for 30 min. The digest was washed with 4°C HBSS and Eurocollins solution, filtered on an 800-μm sieve, and purified on a discontinuous Euroficoll gradient (1.108, 1.096, 1.069, 1.034 mg/mL). Islets were then handpicked and cultured overnight. The absence of islets in the remaining acinar and exocrine cell tissue pellet was confirmed by dithizone staining. All cultures were performed in 5.5 m*M* glucose RPMI-1640 supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin, 0.25% amphotericin B, 15 m*M* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 23 m*M* sodium bicarbonate at 37°C and 5% CO_2.

Electrostatic-mediated dropwise encapsulation

Islet studies were performed exclusively with the electrostatic-mediated dropwise encapsulation technique. The extruded solution contained two parts hydrated fiber (concentrated at 2000×*g* for 10 min), three parts 1.6% purified sodium alginate dissolved in 0.9% saline, and 2000 islets/mL. Hydrated fibers and islets were homogenously distributed within the alginate by repeated pipetting.

The fiber-embedded solution was extruded from a 22-G needle at 2 mL/h, whereas the fiber-free control solution was extruded at 5 mL/h. The alginate solution was charged with 11 kV of potential pulsed for 2.5 ms every 25 ms and extruded into grounded 10 m*M* $BaCl_2$ buffered at pH 7.4 with 12.5 m*M* HEPES. Particles were washed with complete media before culturing in nonadhesive polystyrene dishes. Islet-laden particles and controls were incubated in nonadhesive polystyrene dishes for 1, 7, 14, and 21 days at 5% CO_2 and 37°C.

Islet viability assessment

Viability was assessed by incubation of 100 particles in 50 μg/mL propidium iodide (PI) and 0.25 μg/mL acridine orange (AO) in sterile PBS for 10 min at days 1, 7, 14, and 21. Under fluorescent microscopy with 490 nm excitation, viable cells fluoresce green and dead cells fluoresce red. The islet viability gradient was divided into five classifications: 1. completely red, 2. more than 50% red, 3. more than 50% green, 4. less than 10 red cells, 5. completely green. The rankings were averaged for each time point and condition.

Electron microscopy

Samples were vacuum-dried, fixed to aluminum stubs with carbon tape, gold sputtered, and imaged on a JEOL 840 scanning electron microscope (SEM) with an accelerating voltage of 10 kV.

Statistical analysis

All values are average ± standard error for replicate data sets unless otherwise stated. All statements of significance are reported for two-sided Student's *t*-tests with $p < 0.05$ unless otherwise stated.

RESULTS

Drawn gelatin microfiber development and optimization

The optimized vortex-drawn extrusion system, with sonication to render discrete fiber lengths, produced crosslinked gelatin fibers with an average hydrated diameter of 22.3 ± 0.4 μm and average length of 223 ± 13 μm with a distribution as shown in Figure 2. Maintaining spinning conditions allowed for reproducible fiber diameters, and filtering over

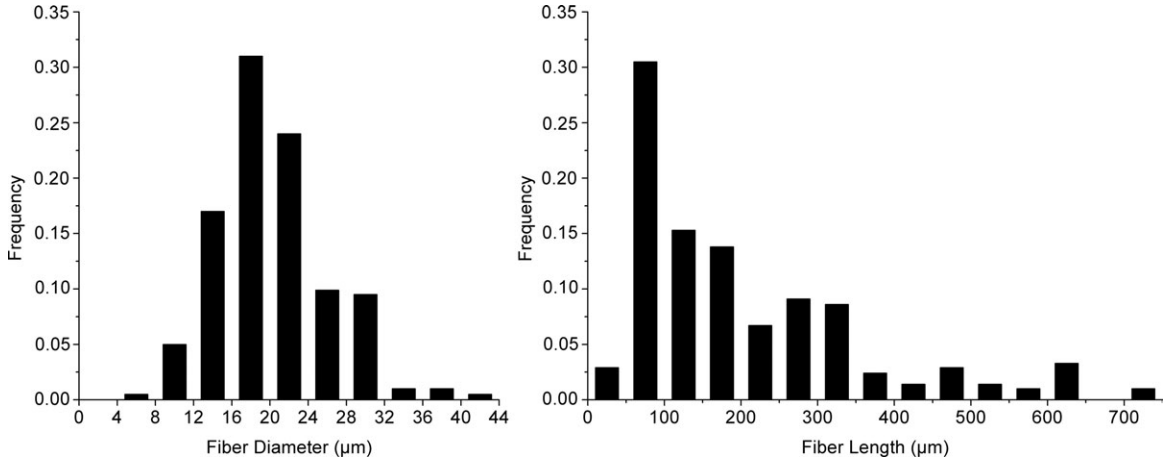

FIGURE 2. Dimensions of rehydrated genipin-crosslinked 1.5% gelatin microfibers. Left: hydrated diameter, av = 22.3 ± 0.4 μm (n = 296); right: length, av = 223 ± 13 μm (n = 214).

a coarse 710-μm filter between rounds of sonication allowed for a consistent fiber length distribution. Fibers were imaged by SEM in a dehydrated state to demonstrate that they were discrete and uniform as shown in Figure 3.

Genipin was found to be a very effective crosslinking agent, providing superior mechanical strength and crosslinking kinetics relative to microbial transglutaminase (data not shown).

Fiber integrity and crosslinking

Microfibers crosslinked in 0.25 wt % genipin solutions in pH 8.0 Tris buffer for 48 h were analyzed by ninhydrin assay for the presence of primary amines, the reactive site for genipin crosslinking. The crosslinked samples exhibited a 65 ± 8% crosslinking efficiency (n = 4), defined as the reduction in primary amines relative to uncrosslinked controls [Eq. (1)]. This is comparable to the observed crosslinking efficiency plateau of 75 ± 2% for the genipin–gelatin system.[35]

Encapsulation-ready microfiber samples were incubated at 37°C for 70 days in sterile PBS to measure uncrosslinked or hydrolyzed gelatin leaching from the fibers. The supernatant was analyzed by BCA assay, which indicated a negligible loss of <0.002% of the mass of the fiber sample over 70 days.

Emulsion encapsulation optimization

Varying the emulsion times and fiber concentrations allowed for a wide variety of fiber-laden microparticles to be produced, as illustrated in Figure 4.

A preliminary trial with the emulsion encapsulation system used 50–150 μm, 1.08 g/mL fluorescein isothiocyanate (FITC) labeled gelatin particles to simulate islets. The labeled gelatin particles were readily encapsulated and observed centralized within the fibrous emulsion.

The fiber-laden emulsion system produced a bimodal distribution of a few large dense particles and a large number of fine fiber-free particles as shown in Figure 5.

Electrostatic-mediated dropwise encapsulation

Electrostatic-mediated dropwise extrusion produced fiber-embedded particles with a narrower size distribution and allowed for the production of 40 vol % fiber concentrate in 1.6 wt % purified alginate particles within the <300 μm range of interest. To ensure a high fiber density on the addition of islets, this process was optimized to reproducibly produce 294 ± 4 μm (n = 260) particles.

Incorporation of islets into fiber-embedded microparticles

Islets were isolated from nine male Sprague Dawley rats and encapsulated in 294 ± 4 μm fiber-embedded microparticles formed by electrostatic-mediated dropwise extrusion as shown in Figure 6. Viability was assessed with PI–AO staining. A significant improvement in viability is exhibited by the fiber-embedded particles over the control particles at days 7, 14, and 21 (n = 100, $p < 0.01$). The average viability rankings for the fiber-embedded particles and fiber-free controls more than 21 days are displayed in Figure 7. At

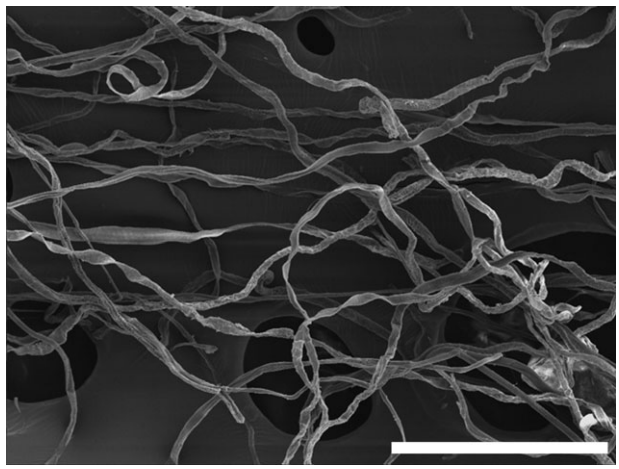

FIGURE 3. SEM images of dehydrated microfibers. Crosslinked fibers are discrete and uniform (scale bar represents 200 μm).

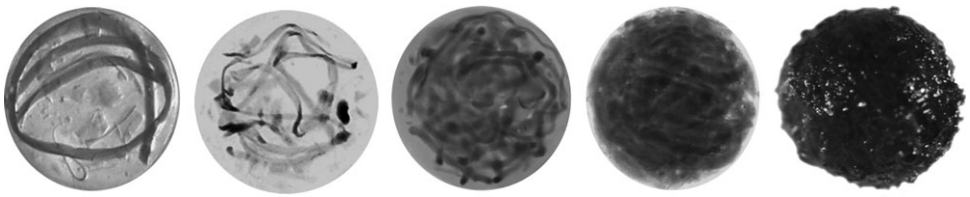

FIGURE 4. A distribution of fiber densities from different external gelation emulsion systems. Particles ranged from 600 μm to over 3 mm in diameter.

days 14 and 21, a trend toward a disrupted distribution was observed in both the fiber-embedded and control particles, however, this trend was more pronounced in the control particles which exhibited a 31% incidence of disruption at day 21 compared to only 8% for the fiber-embedded particles as shown in Figure 8.

Adhesion between the islets and the fibers was observed in the short time before encapsulation within particles, as observed in Figure 9, but further *in vitro* studies are required to determine the true nature of the islet–fiber interaction.

Throughout the course of the 3-week incubation, the fiber-laden barium alginate particles maintained equivalent structural integrity as the fiber-free control particles, and could be handled with minimal particle breakage.

DISCUSSION

Microfiber optimization

The initial extrusion system used a collection bath and mandrel to produce and draw gelatin/alginate microfibers. Fibers of varying gelatin concentrations (0–2 wt %) with a fixed 1.5 wt % alginate concentration were produced with the goal of minimizing fiber diameter and maximizing gelatin concentration. Increased gelatin concentration reduced the strength of the fibers produced, causing breakage at lower draw ratios with correspondingly larger fiber diameters. Fibers produced by the optimization of this method were uniform, significantly smaller than those produced in the literature, with higher gelatin content (50% dry mass), but still too large to produce a microencapsulated network.

Fiber breakage at higher gelatin concentrations and drawing velocities was overcome by replacing the $CaCl_2$ bath and mandrel with a vortex of $CaCl_2$ solution. The substitution allowed for uninterrupted spinning, as the shear force imparted on the extruded stream by the vortex acted as a mandrel, drawing out the fibers. As fiber-breakage would no longer interrupt the process, vortex speeds, and therefore, draw ratios could be increased. To further optimize the system, the extrusion needle was reduced to 35 G, and the extrusion system was maintained at 45°C to prevent thermosetting of the gelatin.

The final alteration to the extrusion system addressed the issue of fiber inconsistency. When the extruded stream did break, a small mass would form at the tip of the needle until the shear force on the buildup exceeded the surface tension at the needle tip. To overcome this difference in surface tensions while improving the laminar nature of the vortex at higher rotational speeds, 1.5% gelatin was added to the collection bath. The more viscous collection bath improved fiber consistency and reduced breakage by reducing the turbulence in the solution at higher rotational velocities.

The development of a method to produce discrete crosslinked gelatin microfibers on a scale amenable to microencapsulation has, to our knowledge, not been published.

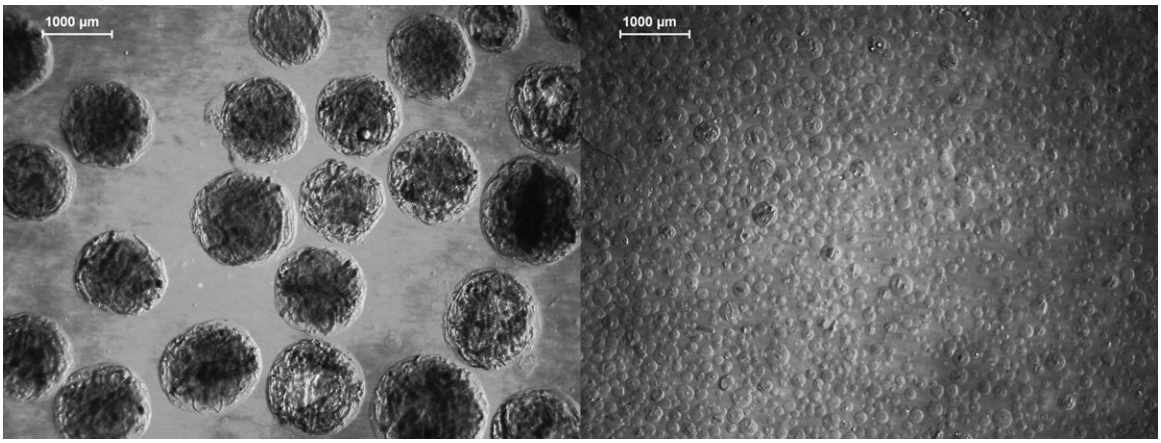

FIGURE 5. Bimodal distribution of large, dense particles (left), and small fiber-free particles (right) produced by an 8-min emulsion (scale bars represent 1000 μm).

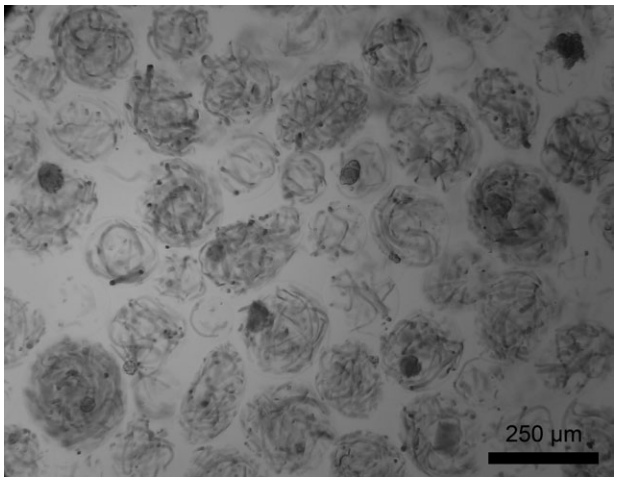

FIGURE 6. Fiber- and islet-laden particles used for the *in vitro* study produced by electrostatic-mediated dropwise extrusion.

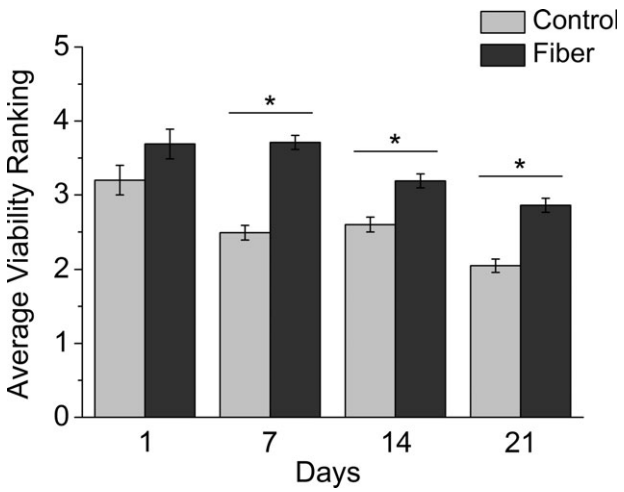

FIGURE 7. Average viability of rat islets encapsulated within fiber-laden and no-fiber control particles over a period of 21 days, (mean ± SE, day 1: $n > 50$, days 7–21: $n > 100$), *statistically different ($p < 0.01$).

Previous work on gelatin fiber formation has only produced >100 μm microfibers, and fused electrospun mats of fibers <1 μm.[23–25,36]

Encapsulation optimization

The initial approach to the production of a microfibrous matrix within a microparticle was the internal gelation emulsion system. The emulsion system appeared ideal as it would concentrate fibers within the center of the particles and weave the fibers into a tight matrix.

In a conventional emulsion, an equilibrium between particle coalescence and disruption is observed, producing a normal distribution of particle diameters. However, in a fiber-laden emulsion, a bimodal system was observed to develop. On coalescence of fiber-laden alginate droplets, the fiber networks interpenetrate and are centralized within the droplet. Shear force on the surface of the droplet draws off the fiber-free alginate polymer solution, further concentrating the fibers within the droplet. Fiber core coalescence continues until the system is ionically crosslinked by acidification, resulting in the observed bimodal distribution. Issues with multivariate optimization of the emulsion system coupled with the intrinsically broad particle size distribution from emulsion systems led to the investigation of electrostatic-mediated dropwise particle production as an alternative.

Electrostatic-mediated dropwise extrusion is a well-established technique that produced particles within the size range of interest, while sacrificing the fiber-wrapping effect of the emulsion system. Despite this drawback, it was readily reproducible and produced a homogenous batch of particles for analysis.

The emulsion system warrants further investigation to take full advantage of the unique network morphology produced.

In vitro study

The islet isolation method used well characterized and tested procedures for the preparation of a pure pancreatic

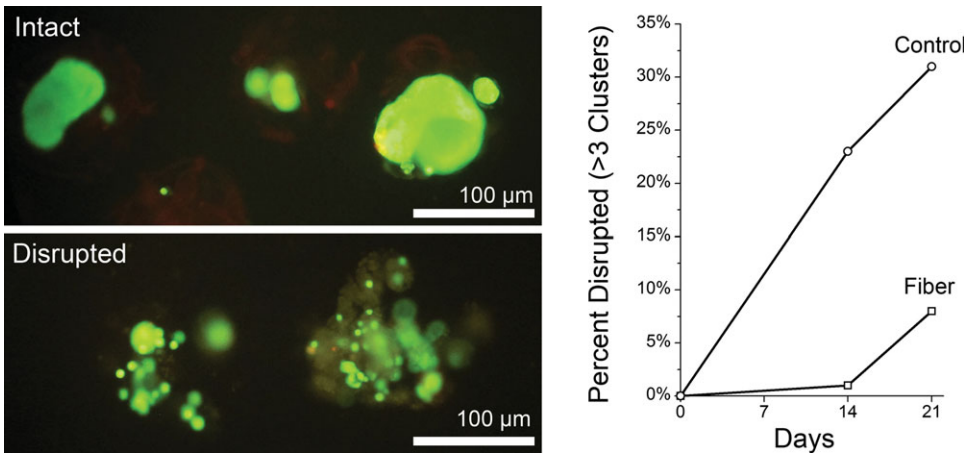

FIGURE 8. Upper left: intact islet morphology under AO/PI staining, lower left: disrupted morphology with (>3 clusters), right: percent of viable islets disrupted for the fiber-laden (□) and fiber-free (○) systems as a function of time ($n > 100$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

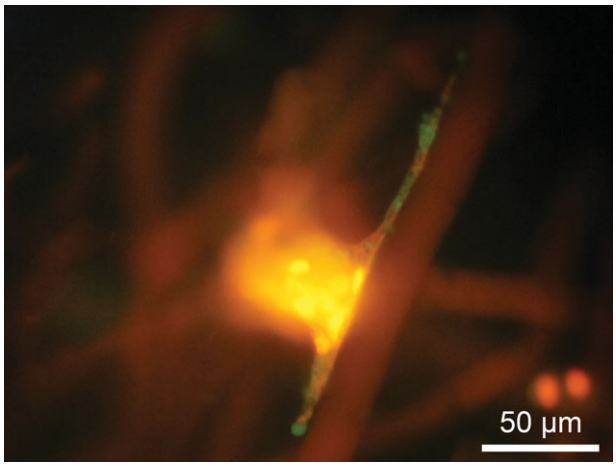

FIGURE 9. Islet–fiber adhesion observed at 24 h, visualized with PI/AO staining and fiber autofluorescence. As the fiber network was subjected to strain, the islet–fiber bonds were maintained, resulting in the distended morphology observed. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

islet population. A EuroCollins incubation was used to lyse residual acinar tissue and the EuroFicoll density gradient further concentrated the islets. This bulk purification was followed by a positive and negative hand-pick by qualified technicians. The cellular aggregates obtained stained positive with dithizone, indicating that they were β-cell rich islets. Therefore, it can be stated with considerable certainty that the adherent cells originated from the pancreatic islet. In future studies, the adherent cell type will be identified by immunohistochemistry.

The viability of encapsulated islets was assessed by PI/AO viability staining, scored on a scale of 1–5 and averaged for all time points. Scoring islets using a bimodal live/dead scale, while a valid approach,[9] was felt to over-state the scale of our observed improvement. Therefore, it was felt that an average provided a more accurate depiction of the trend along the continuum of islet viability. The PI/AO staining protocol provided additional information on the morphology of viable islets over the 21-day investigation. On isolation, islets are composed of a single dense cluster of viable cells, appearing as a single cohesive mass under viability staining protocols. The observed transition to a disrupted morphology was more pronounced in the fiber-free particles. As cell–cell contact is so important in the regulation of glucose secretion, a loss of intact morphology is very detrimental to islet function.[22]

Although the mechanical integrity of the particles was sufficient for *in vitro* manipulation, in future studies, alginate particles can be fortified to withstand *in vivo* loading conditions with higher alginate concentrations and/or the addition of an outer polyelectrolyte coating.

The increase in viability indicates the potential of this technique to restore both the chemical and physical microenvironments. It was hypothesized that the increase in viability was evidence of a reduction in apoptotic events as opposed to necrotic deaths, as the primary source of encapsulated islet necrosis is diffusional limitations, which were consistent throughout the samples and would not be expected in <300 μm capsules.[37] Previous studies have also shown increases in islet viability through the modification of the microenvironment, but none have achieved this in an immunoisolated microparticle geometry.[12,15–19] Although the bulk of the islet cells are not in direct contact with the introduced ECM, intra-islet cell–cell communication via paracrine and autocrine signaling as well as Cx36 gap junctions and cell–cell coupling may be responsible for the improvements observed throughout the islet regarding viability and cohesion.[38]

This investigation is a proof-of-concept study for a system with considerable potential to improve the culture conditions for cellular aggregates. The next stage in the research will be the surface modification of the fibers. Gelatin was selected for its physical and chemical properties, with some evidence that it would restore the islet–ECM connections lost during extraction.[22] However, the intent is to build on the extensive pool of literature and incorporate specific proteins that are known to produce additional improvements in islet viability and insulin secretion. This batch modification of the gelatin substrate is economical, as the bulk is composed of a commodity protein that can be readily modified via carboxylic acid functional groups as genipin binds only to primary amines. By maintaining an outer capsule of alginate within and around the fibers and islets, the surfaces of the particles can also be modified with any method developed for traditional alginate capsules, such as coatings.

The opportunities for modification and optimization of the system outlined for the first time within this study are numerous, as it allows the implementation of various surface modifications developed for macroscopic geometries, but within an immunoisolated microparticle.

CONCLUSIONS

The production of discrete crosslinked protein microfibers was optimized, reducing the hydrated fiber diameter to 22.3 $\pm$ 0.4 μm, while making the process more reliable and less labor intensive. The optimized microfibers were encapsulated within 294 $\pm$ 4 μm barium alginate microparticles by an electrostatic-mediated dropwise extrusion system. The encapsulated microfibers formed a novel system incorporating an immunoisolated, interwoven, fibrous gelatin network with the ability to envelop a cellular aggregate far larger than the network pore size. Islets were extracted from Sprague Dawley rats and encapsulated within the particles for a 21-day preliminary *in vitro* study. Fluorescent viability staining and light microscopy indicated a significant increase in viability, and a trend toward retained islet cohesion.

The primary contribution of this work to the field of tissue engineering is the development of a method to produce discrete gelatin fibers in the 20–100 μm size range. There are currently no published techniques for spinning gelatin fibers in this intermediate range, finer than mandrel-drawn fibers (>100 μm) and larger than electrospun fibers (<1 μm). Fiber-embedded emulsion encapsulation has the potential to be optimized into a very promising technique. However, the emulsion kinetics must be studied to produce reproducible, relatively monodisperse particles in <300 μm

range. This work will act as a foundation for a comprehensive *in vitro* investigation and the further development of the one-step immunoisolated protein–fiber matrix encapsulation method for cellular aggregates.

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