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Translating Textiles to Tissue Engineering: Creation and Evaluation of Microporous, Biocompatible, Degradable Scaffolds Using Industry Relevant Manufacturing Approaches and Human Adipose Derived Stem Cells

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Abstract

Polymeric scaffolds have emerged as a means of generating three-dimensional tissues, such as for the treatment of bone injuries and non-unions. In this study, a fibrous scaffold was designed using the biocompatible, degradable polymer poly-lactic acid in combination with a water dispersible sacrificial polymer, EastONE. Fibers were generated via industry relevant, facile scale-up melt-spinning techniques with an islands-in-the-sea geometry. Following removal of EastONE, a highly porous fiber remained possessing 12 longitudinal channels and pores throughout all internal and external fiber walls. Weight loss and surface area characterization confirmed the generation of highly porous fibers as observed via focused ion beam/scanning electron microscopy. Porous fibers were then knit into a three-dimensional scaffold and seeded with human adipose-derived stem cells (hASC). Confocal microscopy images confirmed hASC attachment to the fiber walls and proliferation throughout the knit structure. Quantification of cell-mediated calcium accretion following culture in osteogenic differentiation medium confirmed hASC differentiation throughout the porous constructs. These results suggest incorporation of a sacrificial polymer within islands-in-the-sea fibers generates a highly porous scaffold capable of supporting stem cell viability and differentiation with the potential to generate large three-dimensional constructs for bone regeneration and/or other tissue engineering applications.

Keywords

Porous scaffolds; bone tissue engineering; Islands-In-The-Sea; human adipose-derived stem cells

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1. Introduction

Of the 6.2 million fractures that occur annually in the United States, approximately 5-10% of them experience impaired or delayed healing, or result in a non-union¹. Critical sized defects, described as the “smallest size intraosseous wound that will not heal spontaneously during the lifetime of the animal,” require medical intervention to achieve proper healing of the non-union². One potential intervention method may include pre-seeding a biodegradable scaffold with adult stem cells, inducing osteogenic differentiation, and then implanting this construct in the defect site³.

Previous research has indicated that cells cannot migrate more than 500µm from the surface of a scaffold or implant, a depth governed by the lack of nutrients and oxygen supply⁴. This lack of nutrients and means of waste removal prevents cells from migrating into the center of the scaffold, resulting in continued cell proliferation along the periphery. Proliferating cells on the periphery then serve as a barrier to nutrient and oxygen diffusion to cells in the scaffold center, resulting in cell viability only at the scaffold surface⁴. Over time, this can lead to the development of a necrotic core, as cells which had migrated either from surrounding tissues or initial seeding on the scaffold surface experience a premature death. To combat this problem, the human body uses blood vessels to supply nutrients and oxygen to cells throughout the entire tissue. This vasculature also serves a critical role in recruiting progenitor cells and delivering angiogenic growth factors to the site of injury⁵. The incorporation of hollow channels within fibers, therefore, are of interest as a means of facilitating and increasing mass transport of nutrients throughout a scaffold. A degradable scaffold possessing such a porous network could enable cell migration from the surrounding tissue, as well as deliver nutrients, excreted hormones, and cytokines to cells within the construct.

Porous biodegradable scaffolds are currently being examined for use as bone graft substitutes by a variety of researchers⁶⁻¹⁵. Biodegradable tissue engineered scaffolds are particularly attractive as no removal operation is required once the tissue has healed, unlike procedures where a metal plate or screw is implanted during the initial alignment and healing phase and later removed¹⁶. Consequently, biodegradable fracture fixation devices and scaffolding constructs are often more appealing than more permanent structures, such as those comprised of titanium. Biodegradable materials have successfully been used in many medical applications, such as cartilage, meniscal, or bone repair, as well as fracture fixation and drug delivery^{17,18}. However, in the context of bone (and some other tissues), it is important that the scaffold degradation rate match the rate of bone regeneration as closely as possible, both to provide mechanical stability and an appropriate environment for new tissue growth¹⁹. Poly (lactic) acid (PLA) is a commonly used degradable polymer employed in tissue engineering applications, as its degradation rate can be altered through modifications in the degree of crystallinity, hydrophobicity, molar mass, scaffold size and geometry, as well as the temperature and pH of the environment depending on the particular end application^{13,15,20-23}. Therefore, the degradation kinetics of a PLA scaffold can be modified based upon factors such as implant size and location to achieve an optimal tissue ingrowth/implant degradation relationship.

PLA undergoes hydrolytic degradation through de-esterification, producing lactic acid as a by-product^{4,20}. Lactic acid is also produced under physiologic conditions in the human body and thus can be removed by naturally occurring metabolic pathways, such as the tricarboxylic acid cycle⁴. However, the release of lactic acid during PLA degradation reduces the pH of the surrounding environment, which can result in autocatalysis of the polymer, further accelerating the degradation rate. Without proper diffusion of the by-products, autocatalysis can lead to increased degradation at the scaffold core, jeopardizing the mechanical integrity of the construct well before fracture union or tissue healing has occurred¹⁷. Healing can be further compromised by the adverse effects of the highly acidic environment on cellular function²⁴.

An approach to combat the accumulation of degradation products and reduce the effects of autocatalysis is to incorporate a network of pores within the scaffold. This network would aid the transport of acidic by-products away from the scaffold center and allow cells in surrounding tissue to metabolize the lactic acid molecules. Such an approach is of particular interest for bone tissue engineering applications, as porous scaffolds that possess increased diffusional properties would not only facilitate removal of lactic acid, but also aid the delivery of nutrients to cells throughout the structure. Porous scaffolds have been generated using a variety of techniques including gas foaming, solvent casting, particulate leaching and thermally induced phase separation^{9,10,25-27}. Other methods to produce porous structures include electrospinning, rapid prototyping, emulsion freeze-drying and three-dimensional (3D) printing techniques^{11,13-15,28}. Though these methods yield porous scaffolds, they are limited in their ability to achieve a specified, directed porous geometry that can be obtained through a commercially relevant, industry standard islands-in-the-sea (INS) extrusion process and the simple addition of warm water to remove a sacrificial polymer. In addition, the solvents and extreme temperature changes utilized by other methodologies can affect not only the polymer structure, but also cellular viability.

Therefore, the purpose of this study was to use an industry standard approach to generate a biocompatible, degradable, porous scaffold for use in bone tissue engineering applications, though the technology developed and evaluated here would benefit any tissue engineering applications that require enhanced mass transport capabilities. We incorporated a sacrificial polymer into a PLA matrix in combination with INS melt-spinning manufacturing techniques, an industry standard approach with the benefit of facile commercial scale-up²⁵. INS technology commonly utilizes a polymer matrix surrounding individual fibrils, or islands²⁹. The spinpack geometry dictates the number of islands observed within each fiber, which has included as many as 1200 islands (personal communication, Hills Inc., West Melbourne, FL). Although a common application of this fiber geometry is to fibrillate the polymer sea, releasing the sub-micron island fibers, we incorporated the sacrificial polymer into the islands as well as a percentage of the sea matrix³⁰. The fiber geometry was comprised of 12 islands that allowed for creation of hollow, longitudinal channels within the fiber after removal of the sacrificial polymer.

Nutrient delivery and waste removal are critical considerations in scaffold design, and can be improved through enhanced fluid flow properties of the matrix. We hypothesized that the use of INS technology would allow us to fabricate a scaffold possessing multiple hollow

channels along the length of the fiber and interconnected micropores throughout all walls of the fiber, connecting the hollow channels both to each other and to the fiber surface. Such a fiber would be generated after washing the extruded fiber in water, removing the sacrificial polymer islands, yielding 12 channels within each fiber. These INS fibers were then knitted into 3D fabrics, characterized through a variety of techniques, and seeded with human adipose-derived stem cells (hASC) to examine cell viability and osteogenesis within the tissue engineered constructs.

2. Materials and Methods

2.1 Fiber Extrusion and Scaffold Fabrication

Four fiber combinations were generated with an INS geometry (Figure 1). The compositions of the four fiber types fabricated are described in Table 1 and will subsequently be referred to using the abbreviations in that table. The PLA (Ingeo™ Biopolymer 6201D, NatureWorks LLC, Minnetonka, MN) is reported to have a glass transition temperature of 55-60 °C and crystalline melt temperature of 155-170 °C. The EastONE Water Dispersible Polymer is an amorphous polymer produced by the Eastman Chemical Company (Kingsport, TN).

Amorphous polymers do not exhibit a melt temperature, but will soften over a relatively wide temperature range. The temperature of the melt for all polymer combinations was set to 235 °C. The pressure for both the islands and sea extruders was set to 750 psi. The draw ratio used during fiber uptake was 2:1 and the denier of extruded fibers was 870. Each INS fiber combination was knitted into individual fabrics, using a 1×1 knit, 7 cut structure on a Brevets Dubied knitting machine (Dubied Machinery Company Inc., NY).

2.2 Fabric Washing For EastONE Polymer Removal

Knitted fabrics were submerged in deionized water and placed on a benchtop shaker table (SteadyShake 757 GYROMAX Benchtop Incubator Shaker, Amerex Instruments Inc., Lafayette, CA) for 2 hr, at 70 °C and 100 rpm. The fabrics were then transferred to an ultrasonicator (Cole-Parmer Instrument Company, Chicago, IL) and sonicated for 2 hr at 69 °C.

2.3 Weight Loss Analysis

Knitted fabrics were cut into 1 × 1.25 in rectangles. Fabrics were dried in a dessicator for 3 days, then weighed on a balance (AB 104-S, Mettler Toledo, Columbus, OH). Fabrics were then individually washed in 40 mL deionized water in 50 mL conical tubes (Corning, Tewksbury, MA) according to the EastONE polymer (Easton Polymer, Easton, PA) removal protocol, described above. Washed fabrics were dried overnight in normal atmosphere before being placed in a dessicator for 1wk. Fabrics were then transferred to an oven (Precision, Winchester, VA) set at 70 °C for 2 days to complete the drying process. Dried fabrics were weighed again, and the percent weight loss after EastONE removal calculated.

2.4 BET Surface Area Analysis

The Brunauer, Emmett and Teller (BET) method was used to compare the available surface area of each sample³¹. Washed fabric samples were cut into small sections, approximately 1mm in length, and 15-25 mg of each sample (n=1) was placed in the U-tube of the

Micromeritics FlowSorb II 2300 (Micromeritics, Norcross, GA) using 30.0% N₂ (balance He) gas (National Welders Supply Co., Charlotte, NC). Each sample was dried by repeated N₂ adsorption/desorption to remove excess water prior to analysis. The surface area of each sample was measured 3-4 times and reported as surface area/mass (m²/g) for each sample.

2.5 Focused Ion Beam Imaging

A FEI Quanta 200 3D Dual Beam FIB system (FEI Company, USA) was used for focused ion beam (FIB) cross-sectioning and imaging. All FIB nanomachining and imaging were performed using 30kV Ga⁺ as described previously in Wong et al.³². Briefly, all fibers were coated with gold-palladium using a sputter coater and a 3 µm thick platinum strip was deposited over the surface of regions to be sectioned, serving as a protective cap. A 20nA Ga⁺ ion beam was used to rapidly remove most of the polymer material and expose the fiber cross-section, then employed in a stepped line-by-line fashion to provide increasingly fine polishing of the exposed cross-section. After the mass removal and polishing processes, an ion beam was used to image the cross-sections at a working distance of approximately 30 mm and at an incident angle of ~52°. FIB induced secondary electron micrographs were acquired with dwell times of 55 µs per pixel until contrast was optimized.

2.6 Sterilization and Cell Seeding

Washed fabrics were dried in a dessicator for 2 days and cut into circular scaffolds 1.25 cm in diameter (McMaster-Carr, Aurora, OH). Scaffolds were then soaked in 70 % ethanol for 15 min, the ethanol aspirated, and the remainder evaporated over 16 hr. Scaffolds were then soaked in complete growth medium, consisting of alpha-MEM, 1% penicillin/streptomycin, 10% fetal bovine serum, and 2 mM L-glutamine, for 3 hours prior to cell seeding, and placed in 24 well non-tissue culture treated plates (Sarstedt, Inc., Newton, NC).

Human ASC were isolated from three age, race, and gender matched donors as previously described^{11,33} and seeded on the circular scaffolds, at a final density of 60,000 cells/scaffold, in three phases. The use of centrifugal seeding has previously been shown by others to increase the seeding efficiency of porous, degradable scaffolds³⁴. Therefore, a combination of centrifugal and static cell seeding was chosen to improve cell dispersion. In the first phase, 20,000 cells in 20 µL medium were added to each scaffold. Scaffolds were centrifuged at 500 g for 2 min, then incubated for 15 min. Next, another 20,000 cells were added to each scaffold then placed in the incubator for 15 min. All scaffolds were then turned over, seeded with an additional 20,000 cells, placed in the incubator for 15 min, then flooded with 1 mL complete growth medium.

2.7 Human ASC Viability

Effects of the scaffolds on cell viability was assessed via the Live/Dead assay (Live/Dead® Viability/ Cytotoxicity Kit, Molecular Probes, Eugene, OR) at days 1, 7, 14, and 21. At each time point, one scaffold of each type was rinsed twice with phosphate buffered saline solution. Scaffolds were then submerged in 1 mL calcein AM and ethidium homodimer-1 stain and kept in the dark. Scaffolds were individually imaged using a Zeiss LSM 710 confocal microscope (Carl Zeiss Microimaging, Inc., Thornwood, New York) with a 10X objective.

2.8 Osteogenic Differentiation of hASC

Human ASC from three donors were cultured in complete growth medium for 1 week on three scaffolds of each fiber type (n=9 analyses per time point). Following one week of culture in complete growth medium, cells were then cultured in osteogenic medium (growth medium supplemented with 0.1 μ M dexamethasone, 50 μ M ascorbic acid, and 10 mM β -glycerolphosphate) for up to 3 weeks. At 14, 21, and 28 days following initial cell seeding, scaffolds were frozen at -20°C until all samples were collected and simultaneously assayed using the Calcium Liquicolor Kit (Stanbio, Boerne, TX) per the manufacturer's protocol using techniques we have previously reported^{11,35,36}.

2.9 Statistical Analyses. Weight Loss Analysis

A one-way analysis of variance (ANOVA) model was specified in Proc GLIMMIX (SAS version 9.2, SAS Institute, Cary, NC) with scaffold type being the only variable. Tests to identify statistically significant pair-wise differences were conducted with a confidence level of 0.05 and a Tukey correction. *Cell-Mediated Calcium Accretion*. A two-way ANOVA was performed to detect differences in calcium accretion between scaffold types and the control scaffold comprised of solid PLA fibers. A Bonferroni correction was applied to maintain an overall Type I error rate of 0.05.

3. Results

3.1 Weight Loss Analysis

The percent weight loss after washing for each of the four fabric types was quantified (Figure 2, n=5 per condition). Significance is represented by differing letters. The results reflect the mean percent weight loss of each fabric type and statistical analysis indicated a significant decrease in scaffold weight as the concentration of EastONE polymer increased in each fiber type. Very little weight loss was noted for the PLA/PLA fabrics (<2%), however there was approximately 53% weight loss for PLA/EastONE fibers, resulting in the formation of 12 hollow channels. This weight loss increased significantly for the fibers containing EastONE in the sea matrix, to approximately 68% and 69% for the 90/10 and 80/20 PLA/EastONE sea combinations, respectively. The difference between these samples comprised of different sea concentrations was statistically significant, but may not be considered practically relevant as the difference in percent weight loss was relatively small.

3.2 BET Surface Area Analysis

The surface area of each washed fabric type was analyzed using the BET method (Figure 3). Statistical significance was not appropriate to perform as the analyses are of multiple measurements from single samples, but a trend of increasing surface area is observed as the concentration of EastONE polymer increased.

3.3 Imaging of Fiber Cross-Sections and Three-Dimensional Scaffold

FIB imaging was used to section and image both washed and unwashed INS fibers (Figure 4). Figure 4A shows the longitudinal and radial view of an unwashed 80/20 fiber. Smooth areas represent EastONE islands, and the surrounding polymer corresponds to the sea

matrix. Figure 4B is a representative cross-section of a fiber of the same composition at a higher magnification, in which small pores are visible in the sea matrix though these fibers were not yet washed. Figure 4C shows the removed EastONE islands in both the radial and longitudinal direction. The protective cap, added to prevent fiber degradation during the mass removal stage, is indicated by the arrow. Figure 4D also shows a washed 80/20 fiber at a higher magnification. This image reflects the results of incorporating EastONE polymer followed by removal with water, revealing hollow longitudinal channels and smaller pores within the remaining matrix. Scanning electron microscopy (SEM) images of the final knitted structure following removal of the sacrificial EastONE polymer confirm multiple fibers with hollow, longitudinal channels (Figure 5). Figure 5A is a representative image of the surface of the knitted scaffold. A side view of the scaffold is also shown in Figure 5B, as well as a side view at higher magnification (Figure 5C), confirming the presence of the longitudinal channels and pores throughout the 3D knit. The INS fibers consisted of channels approximately 6-10 μm in diameter separated from each other by approximately 1 μm of sea matrix, for a total fiber diameter of approximately 50 μm .

3.4 hASC Viability

Human ASC viability was assessed after 1, 7, 14, and 21 days using a Live/ Dead stain. Viable cells appear green, and dead cells appear red. In general, cell attachment, spreading and proliferation increased over time for hASC seeded on both PLA/PLA (Figure 6) and PLA/EastONE scaffolds (Figure 7).

3.5 Osteogenic Differentiation of hASC

Human ASC calcium accretion increased with increased culture time in osteogenic differentiation medium (Figure 8). Significant increases in calcium were observed after 28 days of culture in both groups which contained EastONE polymer in the sea matrix (90/10 and 80/20) compared to solid PLA fibers ($P < 0.05$).

4. Discussion

Large, 3D scaffolds have consistently failed to perform as needed with respect to mass transport requirements both *in vitro* and *in vivo*. In particular, significant challenges remain unmet for provision of adequate nutrients to cells throughout a thick scaffold, particularly to cells in the center, while simultaneously removing metabolic wastes. A variety of porous scaffolds have been developed to address the concerns of nutrient delivery, however interconnectivity of the pores has remained a challenge. To address this issue, we developed and present here for the first time, a novel 3D porous scaffold containing 12 longitudinal hollow channels. Both micropores and channels were incorporated into the fiber structure to support cell viability and osteogenic differentiation. These fibers were created using commercially relevant manufacturing approaches (melt-spun, INS) and then knit into a much larger scaffold capable of addressing large defects *in vivo*. We confirmed individual fibers within the knit structure maintained the hollow channel and interconnected micropore morphology and supported hASC viability and osteogenic differentiation throughout the entirety of the construct for up to 4 weeks. Cell viability over long-term culture *in vitro*

suggests these highly porous scaffolds may yield an implantable bone-graft substitute capable of supporting cell viability *in vivo* until adequate capillary formation is achieved.

Other researchers have attempted to prevent the development of a necrotic core within a scaffold by designing fibers that possess channels for enhanced fluid flow. Ellis and Chadhurri designed a porous hollow fiber membrane to allow mass transfer of molecules through the media stream in the lumen and cells on the outer surface³⁷. Poly (lactide-co-glycolide) fibers were generated with a 700 µm outer diameter, 250 µm inner diameter and pores ranging from 0.2-1 µm on the surface and were spun with 1-methyl-2-pyrrolidinone and 1,4-dioxane as solvents. While they reported that attachment and proliferation of immortalized osteogenic cells were observed on the fiber surface, the INS fibers described in this study possess thinner fiber walls and do not require the use of solvents during the spinning process. In another study, the incorporation of a hollow fiber core and porous structure facilitated cell growth by providing adequate diffusion of cell and polymer degradation byproducts³⁸. Drawing from these and other works, we designed a structure which would contain a greater number of channels and thinner fiber walls to reduce the mass of polymer to be degraded as well as the distance through which the pores must extend and connect. Further, we did this using industry standard, commercially relevant manufacturing approaches to arrive at a methodology that should facilitate commercial scale-up of these scaffolds.

An INS geometry was used to generate fibers with increased fluid flow properties. A water-dispersible polymer was incorporated to produce voids in the PLA matrix after washing with water. Removal of the EastONE polymer was confirmed by weighing fabric samples before and after washing. The quantitative determination of the percent weight loss after washing suggested successful removal of EastONE polymer from the fibers. These weight loss results confirm the successful removal of the EastONE polymer from the INS fibers after washing, as a significant increase in weight loss was observed as the concentration of the EastONE sacrificial polymer in the unwashed fiber increased. A slightly greater percent weight loss than might be expected was observed in the 90/10 and 80/20 combinations (10.3 and 6.7%, respectively). A possible cause for the discrepancy between expected and observed weight loss could be the presence of some voids in the PLA/EastONE fibers observed during FIB sectioning and believed to be a result of the extrusion process. This will be further investigated in future studies. Nonetheless, the trend observed is similar to the one observed for percent weight loss, further confirming the removal of EastONE polymer after washing, forming hollow channels within each fiber which contributed to an increase in surface area.

Focused ion beam provided an excellent analysis technique to fully evaluate the fibers. Nanomachined images obtained after removal of the sacrificial polymer revealed the presence of longitudinal channels and micropores in the INS fibers, further confirming successful polymer removal. Micrographs of washed EastONE/PLA fibers indicated that even though the islands were removed, the fibers retained their structural integrity during the FIB nanomachining process. It is interesting to note that the porosity of the cross-section of the sea in these fibers is significantly greater than would have been predicted from their 80/20 PLA/EastONE composition. As mentioned above, this unexpected void content is

believed to be a result of the extrusion process combined with the removal of the EastONE polymer. Some porosity was also evident in the unwashed fiber which is believed to be induced by the extrusion process, as similar porosity was also observed in 100% PLA fibers extruded under the same conditions. Evaluation of biaxial cross-sections additionally suggest that the overall porosity must be relatively uniform and contiguous, as water induced dissolution of the EastONE would not have occurred otherwise. Successful removal of the EastONE polymer throughout the entirety of the fibers further suggested pore interconnectivity, allowing water to reach the pockets of the sacrificial polymer and generate the observed pores. The cell culture medium can then travel through the pores created by removal of the sacrificial polymer, thus improving nutrient diffusion to the cells. These FIB images provided visual confirmation that both channels and micropores could be fabricated within melt-spun fibers. This analysis was largely qualitative, but was reflective of the more quantitative weight loss and surface area analyses. Increased surface area observed with increasing EastONE content further confirmed the internal porosity of the fibers after removal of the sacrificial polymer. In addition to images of the nanomachined single fibers, SEM images of the final knitted scaffold were also obtained. These images further indicated that fibers retained the structural properties of the longitudinal channels and micropores seen in the individual fibers, even in the scaffold center, as desired.

Human ASC were seeded on the PLA/PLA and PLA/EastONE scaffolds to examine cell viability over 4 weeks. Confocal images after one week of culture revealed viable cells, many of which had already attached to and spread along the fiber surfaces. With continued culture duration, hASC increased in cell number and appeared to cover the surface of the fabrics. Some autofluorescence of the fibers occurred, resulting in the red appearance seen most clearly in Figure 5A. In general, our results indicate that cell viability can be maintained on both solid and porous fiber scaffolds. More importantly, these results suggest that residual EastONE polymer does not affect cell viability over long-term culture. Though there appears to be a slightly greater number of cells present on the solid PLA/PLA fibers at later time points, this may be due to greater initial hASC attachment observed on day 1. After day 7, the culture medium was changed to osteogenic differentiation medium, which other studies have suggested results in a decrease in cell proliferation³⁹. Higher initial hASC seeding densities or longer culture times in growth medium should be investigated in future studies to address this difference.

Osteogenic differentiation was determined through quantification of cell mediated calcium accretion. No significant difference in calcium accretion was observed between the various scaffold types on days 14 and 21. However, by day 28 a significant increase in cell-mediated calcium accretion was observed on the 80/20 and 90/10 scaffolds compared to the control PLA/PLA scaffolds. This significant increase in calcium suggests there may be more viable cells seeded on the surfaces of the fibers throughout the depth of the 3D scaffold than could be observed via the superficial imaging of the Live/Dead micrographs. Such an increase in cell number could be due to increased fluid-flow properties of these scaffolds due to the channels and pores observed via imaging, weight loss and surface area analyses, increasing nutrient delivery to the scaffold center and thus facilitating continued cellular proliferation. As the cells are found on the exterior fiber surface for all fiber types, there is similar available area for growth on all combinations. However, we believe that as cells become

confluent at approximately day 21, the channels begin to play a larger role in nutrient delivery, resulting in the significant increase in calcium accretion observed at day 28. Although confocal microscopy allows for imaging in several focal planes, it was not possible to fully determine viability in the scaffold center as some surface fibers block the light path, limiting imaging to approximately 150 μm . Additionally, differences in surface porosity may have affected calcium concentrations, though scaffolds without cells were cultured under the same medium conditions and no differences were detected between control PLA scaffolds and the three experimental groups (PLA/EastONE, 90/10, and 80/20 PLA/EastONE) at day 28.

Some limitations of this study exist and will be addressed in future work. First, the effect of the porous, channeled design on the fluid flow properties of the fibers needs to be further elucidated. Additional modifications to the channel geometry may ultimately affect the final fiber design, though the work presented here confirms our ability to generate highly porous fibers that support cell growth and differentiation in an *in vitro* culture environment. Further, additional methods for downstream fabrication of the 3D structure, including adjustment of the weaving and knitting parameters, or using staple or spunbond processes, should be tested to optimize flow through the large spaces between the fibers. These large pores between fibers may also be enhanced through the incorporation of laser ablation, which we have previously used in other PLA scaffolds¹³. Finally, we would like to confirm enhanced healing properties at the tissue level in an animal model.

5. Conclusions

The design and characterization of a biocompatible, degradable scaffold with interconnected micropores and hollow channels has been described. Characterization of the knitted scaffolds indicates the use of a water-dispersible additive may produce fibers possessing a controlled porous geometry. The use of the water dispersible additive in the surrounding sea matrix further increases the porosity of the fabric after washing. Viability analyses indicate that human adipose-derived stem cells remain viable on these 3D constructs throughout a four week period. Further, assessment of cell-mediated calcium accretion over three weeks of culture in osteogenic medium indicate hASC are capable of osteogenic differentiation on and within this porous scaffold. This is the first study to describe an INS fiber designed specifically using industry standard, facile scale-up manufacturing approaches for tissue engineering purposes through the incorporation of sacrificial islands in addition to a porous sea matrix. Future studies will examine cell viability and differentiation in a critical sized defect model to determine its potential as a bone tissue engineering scaffold *in vivo*.

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REFERENCES

1. Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. *Injury*. 2005; 36(12):1392–404. [PubMed: 16102764]
2. Schmitz JP, Hollinger JO. The Critical Size Defect as an Experimental-Model for Craniomandibulofacial Nonunions. *Clinical Orthopaedics and Related Research*. 1986; 205:299–308. [PubMed: 3084153]
3. Tanaka T, Hirose M, Kotobuki N, Tadokoro M, Ohgushi H, Fukuchi T, Sato J, Seto K. Bone augmentation by bone marrow mesenchymal stem cells cultured in three-dimensional biodegradable polymer scaffolds. *J Biomed Mater Res A*. 2009; 91(2):428–35. [PubMed: 18985782]
4. Sachlos E, Czersuska JT. Making tissue engineering scaffolds work. Review: the application of solid freeform fabrication technology to the production of tissue engineering scaffolds. *Eur Cell Mater*. 2003; 5:29–39. discussion 39–40. [PubMed: 14562270]
5. Krishnan L, Willett NJ, Guldberg RE. Vascularization strategies for bone regeneration. *Ann Biomed Eng*. 2014; 42(2):432–44. [PubMed: 24468975]
6. Kang Y, Kim S, Fahrenholtz M, Khademhosseini A, Yang Y. Osteogenic and angiogenic potentials of monocultured and co-cultured human-bone-marrow-derived mesenchymal stem cells and human-umbilical-vein endothelial cells on three-dimensional porous beta-tricalcium phosphate scaffold. *Acta Biomater*. 2013; 9(1):4906–15. [PubMed: 22902820]
7. Hernandez A, Reyes R, Sanchez E, Rodriguez-Evora M, Delgado A, Evora C. In vivo osteogenic response to different ratios of BMP-2 and VEGF released from a biodegradable porous system. *J Biomed Mater Res A*. 2012; 100(9):2382–91. [PubMed: 22528545]
8. Lee HY, Jin GZ, Shin US, Kim JH, Kim HW. Novel porous scaffolds of poly(lactic acid) produced by phase-separation using room temperature ionic liquid and the assessments of biocompatibility. *J Mater Sci Mater Med*. 2012; 23(5):1271–9. [PubMed: 22382734]
9. Tayton E, Purcell M, Aarvold A, Smith JO, Kalra S, Briscoe A, Shakesheff K, Howdle SM, Dunlop DG, Oreffo RO. Supercritical CO₂ fluid-foaming of polymers to increase porosity: a method to improve the mechanical and biocompatibility characteristics for use as a potential alternative to allografts in impaction bone grafting? *Acta Biomater*. 2012; 8(5):1918–27. [PubMed: 22307029]
10. Son JS, Appleford M, Ong JL, Wenke JC, Kim JM, Choi SH, Oh DS. Porous hydroxyapatite scaffold with three-dimensional localized drug delivery system using biodegradable microspheres. *J Control Release*. 2011; 153(2):133–40. [PubMed: 21420453]
11. Haslauer CM, Moghe AK, Osborne JA, Gupta BS, Lobo EG. Collagen-PCL sheath-core bicomponent electrospun scaffolds increase osteogenic differentiation and calcium accretion of human adipose-derived stem cells. *J Biomater Sci Polym Ed*. 2011; 22(13):1695–712. [PubMed: 20836922]
12. Sumanasinghe RD, Haslauer CM, Pourdeyhi B, Lobo EG. Melt spun microporous fibers using poly(lactic acid) and sulfopolyester blends for tissue engineering applications. *J Applied Polym Sci*. 2010; 117:3350–3361.
13. McCullen SD, Gittard SD, Miller PR, Pourdeyhi B, Narayan RJ, Lobo EG. Laser ablation imparts controlled micro-scale pores in electrospun scaffolds for tissue engineering applications. *Ann Biomed Eng*. 2011; 39(12):3021–30. [PubMed: 21847685]
14. McCullen SD, Miller PR, Gittard SD, Gorga RE, Pourdeyhi B, Narayan RJ, Lobo EG. In Situ Collagen Polymerization of Layered Cell-Seeded Electrospun Scaffolds for Bone Tissue Engineering Applications. *Tissue Eng Part C Methods*. 2010; 16(5):1095–105. [PubMed: 20192901]
15. Asli MM, Pourdeyhi B, Lobo EG. Release profiles of tricalcium phosphate nanoparticles from poly(L-lactic acid) electrospun scaffolds with single component, core-sheath, or porous fiber morphologies: effects on hASC viability and osteogenic differentiation. *Macromol Biosci*. 2012; 12(7):893–900. [PubMed: 22648935]
16. Li Y, Jiang X, Guo Q, Zhu L, Ye T, Chen A. Treatment of distal tibial shaft fractures by three different surgical methods: a randomized, prospective study. *Int Orthop*. 2014

17. An YH, Woolf SK, Friedman RJ. Pre-clinical in vivo evaluation of orthopaedic bioabsorbable devices. *Biomaterials*. 2000; 21(24):2635–2652. [PubMed: 11071614]
18. Agrawal CM, Niederauer GG, Athanasiou KA. Fabrication and Characterization of PLA-PGA Orthopedic Implants. *Tissue Engineering*. 1995; 1(3):241–252. [PubMed: 19877903]
19. Liu X, Ma PX. Polymeric scaffolds for bone tissue engineering. *Ann Biomed Eng*. 2004; 32(3): 477–86. [PubMed: 15095822]
20. Rezwani K, Chen QZ, Blaker JJ, Boccaccini AR. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. *Biomaterials*. 2006; 27(18):3413–3431. [PubMed: 16504284]
21. Pitt CG, Gratzl MM, Kimmel GL, Surles J, Schindler A. Aliphatic polyesters II. The degradation of poly (DL-lactide), poly (epsilon-caprolactone), and their copolymers in vivo. *Biomaterials*. 1981; 2(4):215–20. [PubMed: 7326315]
22. Reed A, MaG DK. Biodegradable polymers for use in surgery—polyglycolic/poly(lactic acid) homo- and copolymers: 2. In vitro degradation. *Polymer*. 1981; 22(4):494–498.
23. Li SM, GHaV M. Structure-property relationships in the case of the degradation of massive aliphatic poly-(alpha-hydroxy acids) in aqueous media. *J of Mater Sci*. 1990; 1:123–130.
24. Kohn DH, Sarmadi M, Helman JJ, Krebsbach PH. Effects of pH on human bone marrow stromal cells in vitro: Implications for tissue engineering of bone. *Journal of Biomedical Materials Research*. 2002; 60(2):292–299. [PubMed: 11857436]
25. Tuin SA, Pourdeyhi B, Lobo EG. Interconnected, Microporous Hollow Fibers for Tissue Engineering: Commercially Relevant, Industry Standard Scale-up Manufacturing. *J Biomed Mater Res A*. 2013
26. De Nardo L, Bertoldi S, Cigada A, Tanzi MC, Haugen HJ, Fare S. Preparation and characterization of shape memory polymer scaffolds via solvent casting/particulate leaching. *J Appl Biomater Funct Mater*. 2012; 10(2):119–26. [PubMed: 23015372]
27. Ghanbar H, Luo CJ, Bakhshi P, Day R, Edirisinghe M. Preparation of porous microsphere-scaffolds by electrohydrodynamic forming and thermally induced phase separation. *Mater Sci Eng C Mater Biol Appl*. 2013; 33(5):2488–98. [PubMed: 23623059]
28. Montjovent MO, Mathieu L, Hinz B, Applegate LL, Bourban PE, Zambelli PY, Manson JA, Pioletti DP. Biocompatibility of bioresorbable poly(L-lactic acid) composite scaffolds obtained by supercritical gas foaming with human fetal bone cells. *Tissue Eng*. 2005; 11(11-12):1640–9. [PubMed: 16411809]
29. Yeom B, Poudeyhi B. Aerosol filtration properties of PA6/PE islands-in-the-sea bicomponent spunbond web fibrillated by high-pressure water jets. *Journal of Materials Science*. 2011; 46(17): 5671–5767.
30. Fedorova N, Pourdeyhi B. High strength nylon micro- and nanofiber based nonwovens via spunbonding. *Journal of Applied Polymer Science*. 2007; 104(5):3434–3442.
31. Brunauer S, Emmett PH, Teller E. Adsorption of gases in multimolecular layers. *Journal of the American Chemical Society*. 1938; 60:309–319.
32. Wong KC, Haslauer CM, Anantharamaiah N, Pourdeyhi B, Batchelor AD, Griffis DP. Focused ion beam characterization of bicomponent polymer fibers. *Microsc Microanal*. 2010; 16(3):282–90. [PubMed: 20233498]
33. Haslauer CM, Springer JC, Harrysson OL, Lobo EG, Monteiro-Riviere NA, Marcellin-Little DJ. In vitro biocompatibility of titanium alloy discs made using direct metal fabrication. *Med Eng Phys*. 2010; 32(6):645–52. [PubMed: 20447856]
34. Roh JD, Nelson GN, Udelsman BV, Brennan MP, Lockhart B, Fong PM, Lopez-Soler RI, Saltzman WM, Breuer CK. Centrifugal seeding increases seeding efficiency and cellular distribution of bone marrow stromal cells in porous biodegradable scaffolds. *Tissue Engineering*. 2007; 13(11):2743–2749. [PubMed: 17880269]
35. McCullen SD, Zhu Y, Bernacki SH, Narayan RJ, Pourdeyhi B, Gorga RE, Lobo EG. Electrospun composite poly(L-lactic acid)/tricalcium phosphate scaffolds induce proliferation and osteogenic differentiation of human adipose-derived stem cells. *Biomed Mater*. 2009; 4(3): 035002. [PubMed: 19390143]

36. McCullen SD, Zhan J, Onorato ML, Bernacki SH, Lobo EG. Effect of Varied Ionic Calcium on Human Adipose-Derived Stem Cell Mineralization. *Tissue Eng Part A*.
37. Ellis MJ, Chaudhuri JB. Poly(lactic-co-glycolic acid) hollow fibre membranes for use as a tissue engineering scaffold. *Biotechnol Bioeng*. 2007; 96(1):177–87. [PubMed: 16894632]
38. Morgan SM, Tilley S, Perera S, Ellis MJ, Kanczler J, Chaudhuri JB, Oreffo RO. Expansion of human bone marrow stromal cells on poly-(DL-lactide-co-glycolide) (PDL LGA) hollow fibres designed for use in skeletal tissue engineering. *Biomaterials*. 2007; 28(35):5332–43. [PubMed: 17822756]
39. Sikavitsas VI, van den Dolder J, Bancroft GN, Jansen JA, Mikos AG. Influence of the in vitro culture period on the in vivo performance of cell/titanium bone tissue-engineered constructs using a rat cranial critical size defect model. *J Biomed Mater Res A*. 2003; 67(3):944–51. [PubMed: 14613243]
40. McCullen SD, Haslauer CM, Lobo EG. Fiber-reinforced scaffolds for tissue engineering and regenerative medicine: use of traditional textile substrates to nanofibrous arrays. *J Mater Chem*. 2010; 20:8776–8788.

Figure 1.

A) Representative image of fiber with Islands-In-The-Sea (INS) geometry. Fiber contains 12 “islands” surrounded by a polymer “sea.” Scale bar represents 10 μm . **B)** Diagram illustrating cells seeded on the surface of the fibers and **C)** medium is allowed to flow through (arrow) the channels and pores created by removal of the sacrificial polymer.

Figure 2.

Weight loss of the four scaffold types (n=5 per condition) after washing procedure to remove the EastONE polymer. Bars reflect standard deviation of the measured mean for each sample. Different letters denote significance at $P < 0.05$. PLA/PLA = PLA islands within PLA sea, PLA/EastONE = EastOne islands within PLA sea, 90/10 = EastONE islands within a 90% PLA and 10% EastONE sea, and 80/20 = EastONE islands within a 80% PLA and 20% EastONE sea. Increased EastONE concentrations present prior to washing resulted in increased weight loss following polymer removal.

Figure 3.

Mean fiber surface area (m²/g) for each of the four samples measured via BET method after washing procedure. Error bars reflect standard deviation. PLA/PLA = PLA islands within PLA sea, PLA/EastONE = EastOne islands within PLA sea, 90/10 = EastONE islands within a 90% PLA and 10% EastONE sea, and 80/20 = EastONE islands within a 80% PLA and 20% EastONE sea. Mean fiber surface area increased with higher concentrations of EastONE polymer following the washing procedure.

Figure 4. Focused ion beam (FIB) images of 80/20 PLA/EastOne fibers before washing (A and B) and after EastOne removal (C and D). FIB polymer removal reveals longitudinal channels (A and C) as well as pores in the PLA sea matrix (B and D). Arrow indicates protective cap (C).

Figure 5.

Representative scanning electron microscopy images of knitted INS fibers after EastOne removal. (A) top-view of washed scaffold, (B) cross-section of scaffold, (C) zoomed cross-section revealing channels. Scale bars represent 500 μm (A and B) or 50 μm (C). Adapted from McCullen et al.⁴⁰.

Figure 6. Confocal microscope images of hASC seeded on (washed) PLA/PLA control scaffolds after A) 1day, B) 1 week, C) 2 weeks, D) 3 weeks. Calcein AM was used to stain viable cells green and ethidium homodimer-1 was used to stain dead cells (arrows) red. Scale bar represents 100 μm .

Figure 7.

Confocal microscope images of hASC seeded on washed 80/20 (EastONE islands within a 80% PLA and 20% EastONE sea) scaffolds after A) 1 day, B) 1 week, C) 2 weeks, D) 3 weeks. Calcein AM was used to stain viable cells green and ethidium homodimer-1 was used to stain dead cells (arrows) red. Scale bar represents 100 μm .

Figure 8.

Cell-mediated calcium accretion by hASC cultured on PLA/PLA (PLA islands within PLA sea), PLA/EastONE (EastOne islands within PLA sea), 90/10 (EastONE islands within a 90% PLA and 10% EastONE sea), and 80/20 (EastONE islands within a 80% PLA and 20% EastONE sea) scaffolds following washing and culture for one week in complete growth medium prior to the addition of osteogenic supplements. Calcium accretion increased over the culture period and was significantly increased in scaffolds with hollow channels in combination with a porous matrix (90/10 and 80/20 structures). Asterisks indicate significant differences from PLA/PLA samples at $P < 0.05$, $n = 9$ analyses per time point.

Table 1

Polymer compositions for island and sea matrices for the four fiber types.

Sea	Islands	Abbreviation
PLA	PLA	P/P
PLA	EastONE	P/E
80% PLA / 20% EastONE	EastONE	80/20
90% PLA / 10% EastONE	EastONE	90/10