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Organized Nanofibrous Scaffolds That Mimic the Macroscopic and Microscopic Architecture of the Knee Meniscus

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Abstract

The menisci are crescent-shaped fibrocartilaginous tissues whose structural organization consists of dense collagen bundles that are locally aligned, but show a continuous change in macroscopic directionality. This circumferential patterning is necessary for load transmission across the knee joint and is a key design parameter for tissue engineered constructs. To address this issue, we developed a novel electrospinning method to produce scaffolds composed of circumferentially aligned (CircAl) nanofibers, quantified their structure and mechanics, and compared them to traditional linearly aligned (LinAl) scaffolds. Fibers were locally oriented in CircAl scaffolds, but their orientation varied considerably as a function of position ($p < 0.05$). LinAl fibers did not change in orientation over a similar length scale ($p > 0.05$). Cell seeding of CircAl scaffolds resulted in a similar cellular directionality. Mechanical analysis of CircAl scaffolds revealed significant interactions between scaffold length and region ($p < 0.05$), where the tensile modulus near the edge of the scaffolds decreased with increasing scaffold length. No differences were detected in LinAl specimens ($p > 0.05$). Simulation of the fiber deposition process produced “theoretical” fiber populations that matched the fiber organization and mechanical properties observed experimentally. These novel scaffolds, with spatially varying local orientation and mechanics, will enable the formation of functional anatomic meniscus constructs.

Keywords

Tissue Engineering; Meniscus; Nanofibrous Scaffold; Electrospinning; Mechanical Properties

1. Introduction

The menisci are crescent-shaped fibrocartilaginous tissues that function to transmit and distribute loads between the femur and tibia of the knee joint. As such, the meniscus experiences complex loads, including tension, compression, and shear. Due to its wedge-

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7. Disclosures

No disclosures or conflicts of interest.

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shaped cross-section, substantial tensile hoop stresses are generated within the meniscus when it is compressed. To efficaciously transmit such loads, bundles of highly aligned collagen circumnavigate the tissue between insertion sites on the tibial plateau (Fig. 1) [1]. These aligned collagen bundles endow the tissue with highly anisotropic mechanical properties in tension, which are highest in the primary collagen orientation [2, 3].

Unfortunately, injuries to the meniscus are common, and suture repair of the injured tissue is often ineffective or impossible [4, 5]. As such, partial removal of the meniscus (meniscectomy) is the most commonly performed orthopaedic surgery in the U.S. [5, 6], despite the fact that meniscectomy increases stress on the underlying cartilage and increases the risk of osteoarthritis [6–8]. Moreover, in cases of severe degeneration, meniscal allografts are used to replace the entire meniscus. However, these biologic implants suffer from limited availability as well as issues related to size matching, disease transmission, and long term viability [9].

Given the clinical need, the field of meniscus tissue engineering has gained great interest, and a number of strategies, centered on hydrogels, non-woven fabrics, and lyophilized foams, have been developed to produce engineered constructs with anatomic geometries [10–17]. Indeed, several commercial products to replace the meniscus, including both natural materials (such as reconstituted collagen) and synthetic polymers (such as poly(ϵ -caprolactone)) have reached the marketplace [18, 19]. Yet, these materials do not recreate the unique structure and organization of the collagenous matrix of the meniscus, important for both tensile functionality and cellular mechanobiology under physiological conditions.

To address this critical issue in engineering the knee meniscus, we have produced aligned nanofibrous scaffolds that can recapitulate the mechanical anisotropy of native tissue [20]. In the fiber direction, the tensile properties of these scaffolds are approximately 30 times larger than in the perpendicular direction, with a tensile modulus on the order of the native meniscus [2, 3]. Seeding these scaffolds with human meniscus cells increased this critical property with time in culture by directing organized tissue formation [21]. However promising, the preferential direction of the fibers within these scaffolds is unidirectional over a macroscopic scale, while the fibers within the native tissue have a pronounced c-shaped organization (change in direction of ~ 160 – 200° over ~ 7 – 10 cm in humans [22]).

To provide a tissue engineering solution for replacement of large portions or the entire meniscus, we developed a new electrospinning method to collect locally organized nanofibers while mimicking the macroscopic change in orientation of the meniscus. To do so, fibers were collected on the planar surface or a rapidly rotating circular plate. The objectives of this study were to 1) quantify the structure and mechanics of nanofibrous scaffolds created in this manner, and 2) compare these scaffolds to aligned scaffolds obtained from a traditional electrospinning approach using a rotating mandrel. We hypothesized that circumferentially aligned (CircAl) scaffolds would behave similarly to linearly aligned (LinAl) scaffolds over short length scales, but exhibit marked differences as the length scale increased.

2. Materials and Methods

2.1 Scaffold Fabrication

Nanofibrous scaffolds were created via electrospinning as previously described [21, 23]. Briefly, poly(ϵ -caprolactone) (PCL, 80kDa) was dissolved in equal parts dimethylformamide and tetrahydrofuran (Fisher Chemical, Fairlawn, NJ) with constant stirring at 40°C . The polymer solution was driven through a 10 ml syringe attached to an 18G needle (the spinneret) via a syringe pump (KDS100, KD Scientific, Holliston, MA) at 2

mL/hr. The spinneret was charged to +12kV using a power supply (Gamma High Voltage Research Inc., Ormond Beach, FL) and placed ~150 mm away from a grounded mandrel to create an electric field and guide the resultant polymer jet toward the mandrel.

To create scaffolds with preferentially aligned fibers, the mandrels were designed to produce a high surface velocity in different orientations, as fibers align along the direction of movement [24, 25]. For LinAl mats, the spinneret was directed perpendicularly to the long axis of a cylindrical mandrel rotating at a linear surface velocity of ~10 m/s (Fig. 2A), as previously described [21, 23–25]. To produce CircAl mats, the collection apparatus was modified such that the polymer was centered above and perpendicular to the plane of a spinning, thin aluminum collecting plate (Fig. 2B). In this configuration, the direction of movement is along the circumferential direction of the rotating circular surface. The plate was rotated such that a linear velocity of ~10 m/s was achieved at a radius of 30 mm.

2.2 Visualization and Quantification of Fiber Alignment

To examine the spatial changes in fiber alignment, CircAl and LinAl mats of ~0.75 mm thickness were fabricated and small pieces of scaffold were excised every 10 mm over a range of 60 mm, as depicted in Figure 3A&B. Samples were AuPd sputter coated and imaged with a Philips XL 20 scanning electron microscope (Penn School of Medicine Microscopy Core) operating at an accelerating voltage of 10 kV.

Additionally, thin films were collected for ~5 minutes on glass slides (n=6/scaffold type) and viewed by light microscopy (Eclipse 90i, Nikon Instruments, Melville, NY). For CircAl fibers, slides were centered at a radius of 25 mm on the mandrel. Images were collected at 4X magnification over a 20 mm by 10 mm area using a motorized stage and an image stitching program (NIS Elements software, version 3.22). Individual 4X images were used for quantification. For CircAl fibers, images at a radius of 30 mm were utilized.

To quantify fiber alignment, images were analyzed utilizing Fast Fourier Transform analysis, using methods similar to those previously described [26]. Black and white images were converted to the frequency domain in ImageJ (National Institutes of Health, Bethesda, MD) and then analyzed using a custom MATLAB script. The analysis is based on the principle that a fiber will produce a sharp intensity peak in the frequency domain, which is perpendicular to the fiber direction. Thus, for each angle over a 180 degree span, pixel intensity values were summed along the radial direction over a distance equal to the radius of the image. These values were then normalized by the sum of the pixel intensities over the entire 180 degree span, and shifted by 90 degrees to match the orientation of the original images. Using circular statistics, the mean angle of the distribution was calculated for each image.

2.3 Cellular interactions

Scaffolds (30 mm by 10 mm, n=3) of ~0.7–0.8 mm thickness were utilized to assess cellular morphology. Juvenile bovine mesenchymal stem cells (MSCs) were harvested as previously described [21, 23] and cultured up to two passages in basal media (Dulbecco's modified Eagle's medium with 10% fetal bovine serum and 1% penicillin/streptomycin/fungizone). Cells were trypsinized, counted, and pipetted directly and evenly onto each scaffold in 100 μ l of DMEM (80,000 cells/scaffold) within non-adherent culture dishes. To promote cell adhesion, the constructs were incubated at 37°C for one hour with no other media. Then, 5 mL of basal media was added per scaffold, and scaffolds were cultured for 5 days at 37°C and 20% O₂/5% CO₂. Constructs were then fixed with 4% paraformaldehyde, permeabilized, and stained with phalloidin-Alexa488 (Invitrogen, Grand Island, NY) for F-actin and 4',6-

diamidino-2-phenylindole (DAPI) for cell nuclei. Stained samples were coverslipped and imaged by fluorescence microscopy (Eclipse 90i, Nikon Instruments, Melville, NY).

2.4 Mechanical Testing

To evaluate mechanical properties, LinAl and CircAl mats of ~0.7–0.8 mm thickness were formed. Scaffolds were excised at two lengths (30 or 60 mm) with a width of 3 mm ($n=7$ /scaffold type). For CircAl scaffolds, specimens were taken tangential to the rotational axis and centered at a radial position of 30 mm (Fig. 6A). The cross-sectional area of the samples was measured using a custom laser-based device. Samples were speckle coated with Verhoeff's stain and placed in custom-made grips. Clamp-to-clamp length was set at 15 mm and 45 mm for the short and long strips, respectively. Mechanical evaluation was performed using a materials testing machine (model 5848, Instron, Canton, MA) in a saline bath at 37°C. Samples were preloaded to 0.5N, preconditioned from 0–3% strain for 15 cycles, and then extended to failure at a rate of 0.5%/sec. Images were acquired at a rate of 2 frames per second. Lagrangian strain in an area at the scaffold center (equal to the central 25% of the short specimens) and an area of equal size near the grips was computed using Vic2D (Correlated Solutions, Columbia, South Carolina) for both short and long scaffolds. The average strain in the loading direction (E_{yy}) and transverse to the loading direction (E_{xx}) as well as the average shear strain (E_{xy}) were computed within each region. Stress was calculated as the force divided by the original cross-sectional area of the specimen. Tensile modulus for each region was calculated as the slope of the linear region of the stress-strain curve between 1–3% strain. Poisson's ratio was calculated as $-E_{xx}/E_{yy}$.

2.5 Modeling Fiber Organization

To better understand the experimental findings, a model of fiber deposition onto a rotating mandrel was created using MATLAB (Mathworks, Inc., Natick, MA). The total area for deposition was defined as a square (115 mm × 115 mm) for LinAl scaffolds and a circle (65 mm radius) for CircAl scaffolds. This allowed an approximate surface area of ~16,900 mm² for both groups. For each simulation, 50,000 fibers were deposited. The fiber population was assumed to have a normal distribution centered at the axis of rotation and a standard deviation equal to 10°. For each fiber, a random initial position was selected within the pre-defined total area of the mandrel. Each fiber was randomly assigned an initial starting angle relative to the axis of rotation based on the distribution of the fiber population. Since the axis of rotation was dependent on position for the CircAl scaffolds, the simulation was run in an iterative manner to allow realignment of the fibers given their new positions and angle relative to the axis of rotation. In each iteration, the fiber length was increased in 1 mm intervals, and this process was repeated 150 times for each fiber. The number of fibers and the number of iterations for each fiber were based on preliminary studies in which these values were independently varied. With increasing repetitions (either higher numbers of fibers or iterations per fiber), the solutions converged, so values were selected which maintained an error of less than 4%, while substantially reducing computational time.

Following simulation of fiber deposition, images were extracted from the model with similar dimensions and orientation as those collected experimentally. Six separate simulations were run with each scaffold type to compare to the experimental data. To allow comparisons of the fiber distribution, each computer generated “scaffold” was analyzed in the same manner as the experimental data.

To compare the tensile response of the computer generated and experimental scaffolds, regions of equivalent size and location were used. Specifically, direct comparison of the edge and center regions were made to demonstrate the ability of the model to detect differences between these regions in long and short scaffolds. For each region of interest, it

was assumed that all fibers contributed to the modulus. The contribution of each fiber to the overall modulus of the region was defined as

$$E_{\text{tot}} = \sum_{i=1}^n E_i \cos \theta \frac{A_i}{A}$$

where n represents the number of fibers, E_i represents the fiber modulus, θ represents the angle between each fiber and the loading direction, A_i represents the area of each fiber, and A represents the total area of the region. Simulations were repeated seven times, and the ratio of the edge modulus to the center modulus was calculated for each.

2.6 Statistical Analyses

All statistical analysis was performed using SPSS (version 15.0.1, IBM Corp., Armonk, New York). For quantification of fiber alignment, Pearson correlation coefficients were calculated between the mean of the fiber distribution and spatial location, with statistical significance set at $p < 0.05$. For mechanical analysis, a two-way repeated measures ANOVA was performed for each scaffold type, followed by paired t-tests between individual groups if statistically significant interactions were found. To account for multiple comparisons, a Bonferroni correction was performed such that statistical significance for individual t-tests was set at $p < 0.01$. For comparisons between experimental and computational data, an unpaired t-test was performed ($p < 0.05$).

3. Results

Within individual scanning electron microscopy images, locally aligned fibers were observed in all scaffolds and at all positions (Fig. 3A&B). For LinAl scaffolds, the fiber distribution at each point in the scaffold over the 60 mm span was similar, and all distributions were centered at 0° (Fig. 3C). Conversely, intensity distributions for the CircAl scaffolds had a similar range, but varied in primary orientation from -90° to 90° over the same span (Fig. 3D). These orientations correlated roughly with the circumferential direction of the mandrel.

In terms of continuous changes in fiber alignment, the orientation of LinAl fibers did not vary with position over 20 mm ($R = -0.111$, $p > 0.05$) and were centered at 0° (Fig. 4). For CircAl fibers deposited at a radius of 30 mm, primary fiber orientation varied linearly across a similar distance, ranging from $\sim 13^\circ$ to $\sim -13^\circ$. Statistical analysis confirmed this relationship with a Pearson correlation coefficient of -0.963 ($p < 0.05$). In terms of cellular interactions (Fig. 5), the cytoskeletal and nuclear morphology was similar between MSCs seeded on CircAl and LinAl scaffolds, with elongated nuclei and spindle-shaped actin following the local directionality of the scaffold. However, alterations in cellular alignment were clearly observed over a macroscopic scale for the CircAl scaffolds (Fig. 5B).

Mechanical analysis of CircAl scaffolds revealed significant interactions between scaffold length and region within the scaffold for all parameters measured ($p < 0.05$). As such, comparisons between groups were done on an individual basis. For short specimens, no significant differences were found in the modulus between the center and edge regions of the CircAl specimens ($p > 0.01$, Fig. 6C). Conversely, for long CircAl specimens, the modulus was 36% lower near the edge of the scaffolds compared to the center ($p < 0.01$). In terms of varying scaffold length, the modulus of the central region of the CircAl specimens was similar between short and long specimens ($p > 0.01$); however, within the edge region, values for the short CircAl specimens were 72% higher than the respective long specimens

($p < 0.01$). No statistically significant differences could be detected between LinAl specimens as a result of varying specimen length or region within the scaffolds ($p > 0.05$).

These differences in modulus are further highlighted by a graphical depiction of the Lagrangian strain (E_{yy}) in the direction of loading (Fig. 6B). For the long CircAl scaffolds, the strain in the edge region was approximately two times that of the center region, while all other scaffolds featured a relatively uniform strain distribution. Quantitatively, the average E_{yy} in the edge region of long CircAl scaffolds was 63% higher than respective short specimens ($p < 0.01$, Fig. 6D). Similarly, within the long CircAl specimens, the average E_{yy} of the edge region was 55% higher than the center region ($p < 0.01$). No other statistically significant effects due to specimen length or region of interest were detected for CircAl ($p > 0.01$) or LinAl scaffolds ($p > 0.05$). Similar results were obtained for the strain transverse to loading (E_{xx}) (Table 1). As a result, the Poisson's ratio was similar between all CircAl groups ($p > 0.05$) and between all LinAl groups ($p > 0.05$). Finally, the average shear strain (E_{xy}) within the edge region of long CircAl specimens was four-fold greater than that of the central region (Fig. 6E) ($p < 0.01$). Again, no statistically significant differences were detected within short CircAl ($p > 0.01$) or LinAl scaffolds ($p > 0.05$).

The model developed to simulate fiber deposition on the mandrel during the electrospinning process was able to capture the gross organization of both the LinAl and CircAl scaffolds which aligned in the vertical and circumferential directions, respectively (Fig. 7A). In terms of quantification of fiber alignment, the model captured the behavior of both the LinAl and CircAl scaffolds observed under brightfield imaging (Fig. 7B), with a maximum difference of 4° between actual and predicted angles (Fig. 7C). In terms of predicted mechanical properties, the model generated scaffolds predicted a similar pattern of edge-to-center modulus as was observed in the experimental findings (Fig. 7D, $p > 0.05$). Most interestingly, for long CircAl scaffolds, the model showed a drop in the edge to center ratio (0.68), which was similar to the experimental group (0.72); all other values were ~ 1 .

4. Discussion

A successful tissue engineering approach to replace an injured meniscus will restore both the microscopic as well as macroscopic features of the meniscus that are necessary for mechanical function. In this work, we developed a novel approach to produce electrospun nanofibrous scaffolds with a spatially varying macroscopic fiber orientation, similar to the meniscus, and compared their structural and mechanical features to traditional linearly aligned scaffolds.

On the microscopic scale, this new methodology preserved important features of nanofibrous scaffolds. Locally, a similar degree of fiber alignment was found when compared to a traditional electrospinning approach, as observed and quantified via SEM and brightfield microscopy. Likewise, MSCs seeded on these scaffolds showed similar local morphology, with the cytoskeleton and nucleus aligned with the fibers. Over a larger size scale, clear differences could be readily observed. CircAl fibers followed a pattern roughly parallel to the circumferential direction of the disc on which they were collected, creating a gradient in fiber angle and resulting in MSCs with a similar change in morphology over the macroscopic scale, in support of our hypothesis. These changes are also consistent with the human meniscus anatomy, in which the fibers run in the circumferential direction [1–3]. Additionally, the human medial meniscus generally completes a full 180° arc with a radius of approximately 30 mm based on measurements obtained via magnetic resonance imaging [22]. Thus, the “curvature” of the native tissue is similar to that of the CircAl scaffolds proposed in this study.

In terms of mechanical properties, these scaffolds behaved similarly to linearly aligned scaffolds over a short spatial domain, but varied considerably as specimen length increased. This finding further supported our hypothesis that continuous, but spatially varying, fiber orientation could elicit functional outcomes in scaffold mechanics. Assuming circumferential alignment of the fibers on the mandrel, one would expect the fibers to be aligned along the direction of loading in the center region, while those near the edge region would be at an angle and sometimes exit the scaffold margin before reaching the grips, particularly for longer specimens. This would explain the larger longitudinal and shear strains in this region, as well as the decreased modulus relative to the central region. Interestingly, even in the central regions of the CircAl scaffolds, the modulus was approximately half of that obtained for respective LinAl scaffolds, despite having a similar mean alignment. The CircAl scaffolds also showed a trend of higher Poisson's ratio (1.6–1.9) compared to LinAl scaffolds (0.9–1.3). Together these results suggest that complex interactions between fibers or differences in their distribution may exist. For example, fiber-fiber welding or packing may be more likely in LinAl scaffolds compared to CircAl scaffolds [27]. Additionally, the ability of specific fibers to span the entire clamp-to-clamp distance for tensile testing may impact the results. It is likely that LinAl fibers would have a much greater probability of this occurrence, given the circumferential nature of the CircAl fibers. Nevertheless, additional work characterizing the distribution and contribution of the CircAl fibers to overall scaffold mechanics will need to be performed to fully explain these results. Specifically, the unique architecture of the fibers as well as the complex loading experienced *in vivo* warrants evaluation of the mechanical properties in multiple directions via biaxial testing [28]. It is also important to note that the values for tensile modulus obtained in this study had a similar order of magnitude, but were lower than the native tissue (50–200 MPa) [2, 3]. This limitation can be overcome either by varying the polymer composition [29] or culturing with cells, which will then produce aligned matrix and increase the mechanical properties of the construct [21, 30].

A model for the deposition of fibers onto the mandrel was developed to further explain our experimental findings. For the CircAl scaffolds, the model was prescribed to center the fiber distribution along the circumferential direction of the mandrel. Similarity between the model and experimental data provided confirmation that the fibers in our experimental studies generally followed the circumferential direction of the mandrel. An important feature of the model was that the fibers were allowed to contact the mandrel many times, and thus, realign over a short length scale. For the CircAl fibers, this allows the fibers to “curve” as the mandrel moves. In preliminary studies, models that prevented realignment were not able to match the experimental data, providing some additional insight into the fiber deposition process (i.e., the frictional interactions between the mandrel and the fibers as they are deposited). In addition, by prescribing the fiber orientation, the model was able to predict changes in tensile properties of CircAl scaffolds with varying specimen length and location of analysis. Of note, the model was only able to capture these trends if all fibers were included in the analysis, rather than only fibers spanning the entire vertical distance. These data along with those collected by others [31] suggest complex interactions exist between fibers in these scaffolds, and is supported by the significant shear strains we observed experimentally, particularly at the edge regions where the fibers are generally more oblique to the loading axis.

The complex geometry of the native meniscus and unique mechanics engendered by its hierarchical and macroscopically oriented collagen fiber population present challenges for tissue engineering. Several tissue engineered scaffolds to replace the entire meniscus have considered the geometric form. In such instances, hydrogels or foams are commonly implemented, as they can be easily cast in pre-formed molds that replicate the anatomy; however, these materials have tensile properties two or more orders of magnitude less than

the meniscus [10, 11, 17, 32, 33], resulting in mechanical failure *in vivo* [15]. In order to address this, sutures or non-woven fibers have been embedded within the bulk scaffolds [32, 34, 35]. One limitation of these approaches is the large size scale of the fiber elements (hundreds of microns in diameter), which do not emulate the nanometer size collagen fibril diameters with which cells interact and increase complexity in manufacturing. Another important consideration is the large mechanical mismatch between different materials, which could result in wear or fracture of the overall construct or extrusion of the fiber element through the gel or polymer under repetitive loading [15]. A major advantage of electrospinning is the ability to create nanofibrous meshes that are instructive and direct cells to produce a locally aligned matrix [21, 36, 37]. Furthermore, the circumferential arrangement of the fibers within CircAl mats occurs naturally and does not require additional handling or construction. Since this technology does not alter any of the fundamental variables regarding electrospinning, it can be broadly applied to a wide range of polymers. Previous methods to introduce increased porosity by simultaneously depositing a water-soluble fiber fraction or to further vary mechanical properties by simultaneously spinning multiple polymers can likewise be applied [23, 29, 38].

The major limitation of this work to date is the relatively thin (<1mm) nature of the scaffolds, which fails to recreate the height of the native meniscus (>10mm). Additionally, the meniscus features a slightly concave surface (Fig. 1B), which interacts with the convex femoral condyle. Ultimately, to solve both issues, individual CircAl layers of different shapes/sizes might be stacked to create an anatomic meniscus replacement. These layers will need to be physically joined together for easy handling and appropriate load transmission. This might be accomplished by culturing cell-seeded layers one atop another. We have previously shown that three-dimensional structures with complex hierarchy can be fabricated in this fashion [39, 40]. Indeed, ongoing studies look to compare the organization of ECM produced by cells on CircAl vs. LinAl scaffolds. Based on previous studies by our research group [21, 41–43], we expect the cells to produce a collagenous matrix with similar alignment as the scaffold fiber direction, resulting in sustained differences in the bulk mechanical properties of these constructs as they mature *in vitro*. Alternatively, we have recently developed methods to spin nanofibers containing gold nanorods [44]. A short exposure to focused infrared light results in rapid heating of these tiny inclusions (3nm × 8nm), and the nanorods can reach ~80°C, a temperature well-above the melting temperature of PCL (~60°C), resulting in local melting of the PCL and the formation of “point welds” between two apposed scaffold layers. This method could be easily adapted to join multiple layers in a sequential fashion, and so construct an anatomic meniscus from layers possessing macroscopic curvature. Finally, this study utilized juvenile bovine cells for these proof-of-concept studies, as they adopt a similar morphology as adult human cells on aligned scaffolds [21, 41]. For future studies aimed at *in vivo* translation, a more clinically translatable cell source will be utilized.

5. Conclusions

This study demonstrated a novel and simple methodology for producing nanofibrous scaffolds with both a microscopic and macroscopic fibrous organization similar to the native meniscus. On a microscopic scale, fibers were similar to those produced using traditional electrospinning approaches. However, on the macroscopic scale, these fibers were oriented along the circumferential direction of the disc-shaped collecting mandrel. While further work is required to scale up this technology to match the full anatomic dimensions of the meniscus, these results suggest that electrospinning onto a rotating plate may provide an easy and repeatable method to direct the formation of an anatomic meniscus construct with direction dependent mechanical properties for the repair and/or replacement of the knee meniscus.

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Figure 1.

Illustration of meniscus showing generalized anatomic macrostructure (A) and wedge-like cross-section displaying a simplified collagen fiber organization, with the majority of fiber bundles in the circumferential direction with occasional radial “tie” fibers (B).

Figure 2. Schematic illustration of electrospinning set-up, anticipated fiber direction, and map of linear velocity on mandrel surface designed to produce linearly aligned (A) and circumferentially aligned (B) scaffolds.

Figure 3.

Schematic displaying locations for samples for scanning electron microscopy for linearly aligned (A) and circumferentially aligned (B) scaffolds (scale bar = 5 μm). Intensity distributions derived from FFT analysis of SEM images of LinAl (C) and CircAl (D) scaffolds plotted as a function of position. Colors correspond to images in (A) and (B).

Figure 4.

Merged brightfield images (4X magnification) of LinAl (A) and CircAl (B) fibers over a 20 mm expanse. Arrows indicate mean fiber angle from individual images (one arrow per image). Quantification of mean fiber angle derived from FFT analysis as a function of position along the scaffold (C).

Figure 5. Fluorescent imaging of actin (green) and nuclei (blue) in mesenchymal stem cells seeded on LinAl (A) and CircAl (B) scaffolds (scale bar = 100 μm).

Figure 6.

Mechanical properties of LinAl and CircAl scaffolds. Schematic showing lengths of scaffolds tested (short, long) and regions of analysis (center, edge) (A). Color maps of Lagrangian strain in the testing direction (E_{yy}) within individual regions (B). Quantification of tangent modulus (C), strain along direction of loading (E_{yy} , D), and shear strain (E_{xy} , E) (*p < 0.01 between short and long scaffolds, +p < 0.01 between center and edge regions).

Figure 7.

Model of theoretical fiber deposition. Typical simulations of fiber populations for LinAI and CircAI fibers (A, scale bar = 10mm). Comparison of fiber appearance between model generated and brightfield images of experimental samples (B, scale bar = 500 μ m) and quantification of the fiber direction in model generated and experimental scaffolds (C). Comparison of edge-to-center modulus ratios between model generated and experimental scaffolds (D). Dashed line indicates equal modulus in edge and center regions.

Table 1

Mechanical properties of CircAl and LinAl scaffolds

		Modulus (MPa)	E _{yy} (%)	E _{xx} (%)	E _{xy} (%)	Poisson's Ratio
<u>LinAl</u>	Center	17.2 ± 6.6	3.0 ± 0.1	-3.4 ± 1.7	0.4 ± 0.4	0.9 ± 0.4
	Edge	17.2 ± 6.9	2.8 ± 0.2	-3.2 ± 1.2	0.2 ± 0.2	1.0 ± 0.4
	Center	15.5 ± 3.8	3.0 ± 0.2	-3.6 ± 1.6	0.4 ± 0.2	1.2 ± 0.5
	Edge	17.3 ± 3.4	2.8 ± 1.1	-3.6 ± 1.4	0.4 ± 0.2	1.3 ± 0.5
<u>Short</u>	Center	8.2 ± 1.4	3.0 ± 0.1	-5.0 ± 1.8	0.9 ± 1.0	1.7 ± 0.6
	Edge	7.2 ± 1.6	2.9 ± 0.6	-5.2 ± 2.1	0.7 ± 0.9	1.9 ± 1.2
	Center	6.5 ± 2.5	3.0 ± 0.1	-5.6 ± 2.8	0.6 ± 0.3	1.8 ± 0.9
	Edge	4.2 ± 1.0 ^{*,†}	4.7 ± 0.9 ^{*,†}	-7.3 ± 0.9	2.2 ± 0.5 ⁺	1.6 ± 0.4
<u>CircAl</u>	<u>Long</u>					

* p<0.01 vs. respective short scaffold,

† p<0.01 vs. respective center region