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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 with 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 ($P < 0.05$), with the tensile modulus near the edge of the scaffolds decreasing with increasing scaffold length. No such 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 orientations and mechanics, will enable the formation of functional anatomic meniscus constructs.

1. Introduction

The menisci are crescent-shaped fibro-cartilaginous 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-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 orthopedic surgery in the USA [5,6], despite the fact that meniscectomy increases stress on the underlying cartilage and increases the risk of osteoarthritis [6–8]. In cases of severe degeneration, meniscal allografts are used to replace the entire meniscus. However, these biological 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 mechano-biology 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 of 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

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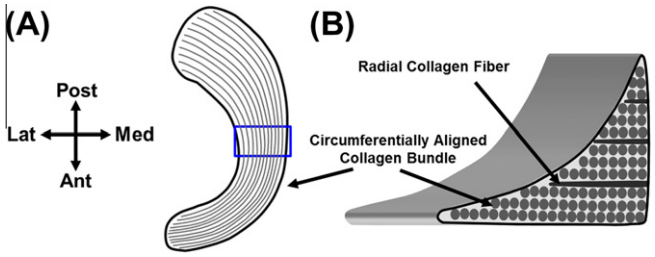

Fig. 1. Illustration of a meniscus showing (A) the generalized anatomic macrostructure and (B) a wedge-like cross-section displaying a simplified collagen fiber organization, with the majority of fiber bundles in the circumferential direction with occasional radial "tie" fibers.

the fibers within these scaffolds is unidirectional over a macroscopic length 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 the replacement of large portions of 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 with 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) (80 kDa) 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 h^{-1}. The spinneret was charged to +12 kV 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 towards 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^{-1} (Fig. 2A), as previously described [21,23–25]. To produce CircAl mats, the collection apparatus was modified such that the spinneret 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^{-1} was achieved at a radius of 30 mm.

2.2. Visualization and quantification of fiber alignment

To examine 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 Fig. 3A and 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 keV.

Additionally, thin films were collected for ~5 min on glass slides (n = 6 per scaffold type) and viewed by light microcopy (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 4× magnification over a 20 × 10 mm area using a motorized stage and an image stitching program (NIS Elements software, version 3.22). Individual 4× 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 (Mathworks, Inc., Natick, MA). The analysis is based on the principle that a fiber will produce a sharp intensity peak in the frequency domain that is perpendicular to the fiber direction. Thus for each angle over a 180° 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° span, and shifted by 90° 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 × 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 medium (Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and 1% penicillin/streptomycin/fungizone). Cells were trypsinized, counted, and pipetted directly and evenly onto each scaffold in 100 μL of DMEM (80,000 cells per scaffold) in non-adherent culture dishes. To promote cell adhesion, the constructs were incubated at 37 °C for 1 h with no other medium. Then 5 mL of basal medium was added per scaffold, and scaffolds were cultured for 5 days at 37 °C and 20% O_2/5%CO_2. 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 per 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 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 0.5 N, preconditioned from 0–3% strain for 15 cycles, and then extended to failure at a rate of 0.5% s^{-1}. 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