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Mechanical function near defects in an aligned nanofiber composite is preserved by inclusion of disorganized layers: Insight into meniscus structure and function ☆

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ABSTRACT

The meniscus is comprised of circumferentially aligned fibers that resist the tensile forces within the meniscus (i.e., hoop stress) that develop during loading of the knee. Although these circumferential fibers are severed by radial meniscal tears, tibial contact stresses do not increase until the tear reaches ~90% of the meniscus width, suggesting that the severed circumferential fibers still bear load and maintain the mechanical functionality of the meniscus. Recent data demonstrates that the interfibrillar matrix can transfer strain energy to disconnected fibrils in tendon fascicles. In the meniscus, interdigitating radial tie fibers, which function to stabilize and bind the circumferential fibers together, are hypothesized to function in a similar manner by transmitting load to severed circumferential fibers near a radial tear. To test this hypothesis, we developed an engineered fibrous analog of the knee meniscus using poly(ε-caprolactone) to create aligned scaffolds with variable amounts of non-aligned elements embedded within the scaffold. We show that the tensile properties of these scaffolds are a function of the ratio of aligned to non-aligned elements, and change in a predictable fashion following a simple mixture model. When measuring the loss of mechanical function in scaffolds with a radial tear, compared to intact scaffolds, the decrease in apparent linear modulus was reduced in scaffolds containing non-aligned layers compared to purely aligned scaffolds. Increased strains in areas adjacent to the defect were also noted in composite scaffolds. These findings indicate that non-aligned (disorganized) elements interspersed within an aligned network can improve overall mechanical function by promoting strain transfer to nearby disconnected fibers. This finding supports the notion that radial tie fibers may similarly promote tear tolerance in the knee meniscus, and will direct changes in clinical practice and provide guidance for tissue engineering strategies.

Statement of Significance

The meniscus is a complex fibrous tissue, whose architecture includes radial tie fibers that run perpendicular to and interdigitate with the predominant circumferential fibers. We hypothesized that these radial elements function to preserve mechanical function in the context of interruption of circumferential bundles, as would be the case in a meniscal tear. To test this hypothesis, we developed a biomaterial analog containing disorganized layers enmeshed regularly throughout an otherwise aligned network. Using this material formulation, we showed that strain transmission is improved in the vicinity of defects when disorganized fiber layers were present. This supports the idea that radial elements within the meniscus improve function near a tear, and will guide future clinical interventions and the development of engineered replacements.

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1. Introduction

Menisci are semi-lunar shaped fibrocartilaginous wedges located between the femur and the tibia and support the mechanical function of the knee joint [1–3]. Each meniscus is comprised of circumferentially aligned collagen I bundles, which function to convert the axial compressive forces of the knee joint into tensile hoop stresses [3,4], thereby distributing axial loads across the tibial plateau. In addition to circumferential fibers, the meniscus also contains a population of radial tie fibers (RTFs). These RTFs originate at the meniscus periphery, and interdigitate amongst the circumferential fibers [1–3,5]. Comprised of types I and II collagen [1], RTFs vary spatially in both size and in their arborization [5]. Morphological characterization of RTFs, first detailed by Skaggs et al., indicate that they are thin and numerous in the anterior region of the meniscus, and posteriorly become thicker and longer, reaching into the inner one third of the tissue [3]. Serial sections of menisci further show that RTFs generate a unique three-dimensional network within the tissue, with RTFs extending into continuous sheets that weave between the circumferential collagen bundles [6,7]. Meniscal specimens that have a greater density of RTFs have higher tensile properties in the radial direction, suggesting that RTFs contribute to the overall mechanical function of the tissue [3]. Indeed, RTFs are thought to bind circumferential bundles together and may further function to prevent longitudinal splitting of the circumferentially-aligned collagen bundles.

Because this unique structural organization begets its mechanical function, the meniscus can be compromised when its structure is damaged. Meniscal injury results in approximately 850,000 surgical procedures annually in the United States and is the most common pathology of the knee joint, with an annual incidence of 66 per 100,000 people [8,9]. The surgical procedure most commonly performed to treat meniscus pathology is partial meniscectomy (excision of the damaged tissue). This method has limitations, however, with meta-analyses suggesting that the amount of meniscal tissue removed is predictive of future osteoarthritic changes in the joint space [10]. Partial meniscectomy increases the likelihood of OA development as it decreases area over which load is transferred from the femur to the tibia, increasing stress on the articular cartilage [11]. While partial excision of the meniscus increases contact stresses, a recent study showed that radial tears, which sever the circumferential fibers, do not increase contact stresses until the tear reaches ~90% of the meniscus width [11]. While the current dogma (directing clinical practice) suggests that a tear through circumferential fibers should result in a loss of tensile hoop stresses and functionality of the meniscus, this experimental observation suggests that the remaining meniscus tissue surrounding a radial defect provides some degree of mechanical function.

One possibility as to why radial tears do not impair meniscal function is that RTFs may transmit strain between circumferential fibers, thereby loading the severed fibers near a radial tear. To test this hypothesis, we developed an engineered fibrous analog of the knee meniscus. While many meniscal replacement have been developed [12–16], few consider the structural features of the native tissue that underlie its function. We previously developed anisotropic nanofibrous electrospun scaffolds with a pronounced fiber direction to mimic the circumferential fibers of the knee meniscus [17–19]. Likewise, we generated layers of cell-seeded aligned fiber constructs to produce hierarchical complexity, with different fiber alignment in each layer. Analysis of these more complicated structures showed that individual layers interact with one another mechanically, generating complex 3D interactions [19–21]. To further this line of inquiry, in this study, we developed acellular composite scaffolds consisting primarily of aligned fibers (to mimic the circumferential fibers of native meniscus) that were modified to contain thin layers of disorganized fibers (to mimic the RTFs of native tissue). Mechanical evaluation of these composite scaffolds indicated that the inclusion of disorganized layers within the otherwise aligned fiber network preserved the mechanical properties of scaffolds with a 'radial' defect and increased strain transmission to the region adjacent to the defect. This finding will both inform clinical practice (by supporting limited excision of native tissue near defects) as well as motivate new strategies for engineering meniscal replacement constructs that more faithfully replicate the structural complexity of the native tissue that enables its function.

2. Materials and methods

2.1. Nanofibrous scaffold fabrication

Poly(ε-caprolactone) (PCL) nanofibrous scaffolds were created via electrospinning as described previously [22]. A 14.3% w/v solution of PCL (80 kDa, Shenzhen Bright China Industrial Co., Hong Kong, China) was dissolved in a 1:1 solution of N,N-dimethylformamide and tetrahydrofuran (Fisher Chemical, Fairlawn, NJ) and heated (37 C) with constant stirring over 48 h. Once dissolved, 10 mL of the polymer solution was extruded via a syringe pump (KDS100, KD Scientific, Holliston, MA) at a rate of 2.5 mL/h through an 18G blunt needle, located 13–15 cm from an aluminum mandrel, which served as a collecting surface. A power supply (Gamma High Voltage Research, Inc., Ormond Beach, FL) applied a 13 kV potential to the needle, resulting in a potential difference between the needle and grounded mandrel (Fig. 1A). An additional power source was used to apply a 7 kV potential to aluminum shields that focused fiber deposition onto the mandrel. To create nonaligned scaffolds (NA) and elements, the collecting surface was rotated at ~3 m/s (Fig. 1B). To produce Aligned (AL) scaffolds, the speed of the mandrel was increased to a surface velocity of ~10 m/s (Fig. 1C) [17].

2.2. Composite scaffold fabrication

To fabricate composite scaffolds, i.e. scaffolds with variable orientation between layers, in which seven discrete but connected layers were included in one scaffold, the speed of the mandrel was decreased and increased to create subsequent NA and AL layers, respectively. Each composite scaffold had four AL layers (including the outermost layers) and three internal NA layers. Scaffolds with differing percentages of NA content were produced, where the percentage (i.e. 33%) was split between the three NA layers (11% each) and the remainder (67%) split between the four AL layers (16.8%) (Fig. 2). A custom-built stepper motor (Fig. 1A) was used to modulate the speed of the mandrel, allowing for precise acceleration and deceleration of the mandrel to each rotation speed. Single layer AL and NA, as well as composite scaffolds were collected for four hours and had an average thickness of 0.555 ± 0.101 mm. To confirm the presence of differential alignment in layers, PCL solution was doped with Nile Red (Sigma, St. Louis, MO) at 0.01% w/v and electrospun onto glass coverslips affixed to the rotating mandrel (Fig. 3). Fibers were collected for 12 min (6 min aligned, 6 min non-aligned) and imaged to a depth of 10 μm at 60× using a confocal microscope and the NIS Elements software (A1R, Nikon Instruments, Melville, NY). The top and bottom 3 μm were then maximally projected and fiber alignment was quantified using FibrilTool, a FIJI plug in [23]. This image processing tool utilizes pixel intensity levels in a given region of interest (ROI) to define a vector characterizing directionality for each identified object in that ROI. The average of each vector is used