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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

Sonia Bansal^{a,b,c}, Sai Mandalapu^a, Céline Aeppli^a, Feini Qu^{a,b,c}, Spencer E. Szczesny^{a,c}, Robert L. Mauck^{a,b,c}, and Miltiadis H. Zgonis^{a,c}

^aMcKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, U.S.A

^bDepartment of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, U.S.A

^cTranslational Musculoskeletal Research Center, Philadelphia Veterans Administration Medical Center, Philadelphia, PA 19104, U.S.A

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

*Address for Correspondence: Miltiadis Zgonis, M.D., Assistant Professor of Orthopaedic Surgery, McKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, Perelman School of Medicine, University of Pennsylvania, 424 Stemmler Hall, 36th Street and Hamilton Walk, Philadelphia, PA 19104, Phone: (215) 898-8653, Fax: (215) 573-2133, Miltiadis.Zgonis@uphs.upenn.edu.

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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.

Graphical Abstract

Keywords

electrospinning; meniscus; scaffold; mechanical properties

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 hours. 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/hr 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 (Figure 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 (Figure

1B). To produce Aligned (AL) scaffolds, the speed of the mandrel was increased to a surface velocity of ~10 meters/sec (Figure 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%) (Figure 2). A custom-built stepper motor (Figure 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 (Figure 3). Fibers were collected for 12 minutes (6 minutes aligned, 6 minutes non-aligned) and imaged to a depth of 10 μ m at 60x using a confocal microscope and the NIS Elements software (A1R, Nikon Instruments, Melville, NY). The top and bottom 3 μ m 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 to determine the orientation in the ROI and the circular variance is used to determine the anisotropy score, where 0 refers to a perfectly isotropic material and 1 refers to a perfectly anisotropic material. Overall anisotropy index was quantified using the entire image as an ROI, whereas the average and standard deviation of the fiber direction in each image was computed via 8 random polygonal regions of interest.

2.3. Mechanical Testing

Prior to mechanical testing, scaffold mats were cut into strips of 40×5 mm, with the long axis corresponding to the direction of mandrel rotation (i.e., direction of fiber alignment). Neighboring scaffolds cut from the same mat were paired, with one scaffold left intact, and the other cut with a scalpel to create a defect 2.5 mm long, perpendicular to the long axis of the strip at the vertical midpoint of the strip. A custom 3D printed mold was used to ensure that each scaffold received the same size defect at the same location. Scaffolds were then speckle coated with Rust-Oleum multi-purpose enamel black spray paint to allow for subsequent digital image correlation (DIC) analysis during mechanical testing. Prior to mechanical testing, sample cross-sectional area was measured via a laser-based measurement system [24]. Subsequently, uniaxial tensile testing was performed with an Instron 5848 equipped with a 50N load cell and vise grips supplemented with high-grit sandpaper (Instron, Canton, MA). Samples were placed in the grips with a gauge length of 20 mm. Samples, both intact and cut, were extended to 25% strain at 0.5 %/sec. Images of the scaffold during tensile testing were acquired via an externally mounted camera capturing images at 2 fps (A1021, Basler, Ahrensburg, Germany). Bulk construct stress and strain

were calculated from the load-displacement curve and sample geometry. The yield point was calculated by iterative determination of the slope (step size of 0.5% strain over intervals of 2.5% strain) of the inputted data until the segment was no longer linear [25]. The transition point and linear modulus were calculated via a continuous exponential-linear function to the stress-strain curve [26] [27,28]. Additional batches of scaffolds were subjected to a dynamic testing protocol in which samples were placed in grips (with a gauge length of 20 mm) and cycled 10 times between 2% and 8% of the gauge length at 0.1 Hz. The dynamic modulus was calculated by fitting a sine wave to the experimental data and dividing the maximum stress by the maximum strain [29]. The phase angle was determined from the offset of the load and displace curves as previously described in [30,31]. Video recordings from the test were used to determine the local Lagrangian strain along the direction of loading (E_{YY}) within the top half of defected scaffolds using the digital image correlation software (Vic2D, Correlated Solutions). A custom MATLAB script was used to determine the area in which local strain was equal to or less than one half the global applied strain at any given strain level.

2.4. Statistical Analysis

A simple mixture model was used to determine the expected composite mechanical properties. This was done by multiplying the ratios of non aligned and aligned percentages by the values for pure nonaligned and aligned scaffolds, respectively and summing. One and two-way analysis of variance (ANOVA) was carried out using GraphPad Prism (San Diego, CA). Mats of varying NA content spun within 48 hours were considered as one “batch” and three batches were tested using a 2-way ANOVA to verify that batch to batch variability was within a reasonable range [32]. Data are presented as mean $\pm$ standard error. 1-way ANOVA was used to compare the linear and toe moduli, as well as transition and yield stresses and strains between groups (n=8/condition-batch). A Fisher’s least significant difference posthoc test was performed to make comparisons between groups ($p < 0.05$).

3. Results

3.1. Production of Composite Fibrous Scaffolds

Composite scaffolds containing both Non-aligned (NA) and Aligned (AL) fibers were produced using an electrospinning device. The predominant direction of fibers can be readily visualized in SEM images (Figure 1B–C). Fluorescent-labeling of fibers and confocal imaging (depth dependent shading such that AL: blue, NA: yellow) further demonstrated that fibers could be successfully produced in discrete layers via sequential modulation of mandrel surface velocity during the electrospinning process (Figure 3A). Average fiber direction of AL fibers indicated a near perfect alignment ($89 \pm 1^\circ$) (Figure 3B) compared to NA scaffolds ($-16 \pm 26^\circ$) (Figure 3C). There was a larger spread in NA fiber direction, consistent with their lower anisotropy index compared to AL fibers (NA: 0.07, AL: 0.51 anisotropy index), where a score of 0 represents a perfectly isotropic material and a score of 1 represents a perfectly anisotropic material.

3.2. Quasi-Static Mechanical Properties of Single-Layer and Composite Scaffolds

To elucidate the effect of the inclusion of NA layers on the mechanical properties of otherwise aligned scaffolds, tensile testing was performed on single layer and composite (25%, 33%, 50%, 75%) scaffolds. Testing was carried out in the predominant fiber direction, with no visible delamination occurring in test specimens. Example stress-strain plots for each scaffold formulation showed distinct stress strain relationships for each group (Figure 4A). AL scaffolds had a higher linear modulus than NA scaffolds (AL: 29.50 ± 1.05 MPa; NA: 9.21 ± 0.29 MPa, $p < 0.001$). As the amount of NA content in composite scaffolds increased, the linear modulus decreased. Quantification of these results showed that all composite scaffolds had linear region moduli that were lower than AL scaffolds and/or greater than purely NA scaffolds ($p < 0.001$). Toe moduli showed a similar trend with changing NA content (Figure 4B). Additionally, both transition and yield strains were significantly higher in AL scaffolds than in NA scaffolds (AL, transition: 0.045 ± 0.001 ; NA, transition: 0.025 ± 0.001 , $p < 0.001$; AL, yield: 0.093 ± 0.003 ; NA, yield: 0.059 ± 0.003 , $p < 0.001$). Much like the linear modulus, both the transition and yield strain of composite scaffolds trended lower with increasing NA content (Figure 4C). Employing a simple weighted mixture model (taking into account the volume fraction of AL and NA components), we observed that the toe moduli of composite scaffolds over-performed expectations (red) while the linear moduli of composite scaffolds tended to under-perform expectations (Figure 4B).

3.3. Dynamic Mechanical Properties of Single-Layer and Composite Scaffolds

To elucidate the effect of NA inclusions on the dynamic mechanical properties of otherwise aligned scaffolds, dynamic tensile testing was performed on single layer and composite (25%, 33%, 50%, 75%) scaffolds. As above, testing was carried out in the predominant fiber direction. Example hysteresis loops showed distinct loading profiles for each group (Figure 5A). AL scaffolds had a higher dynamic modulus than NA scaffolds (AL: 31.66 ± 1.60 MPa; NA: 17.18 ± 0.50 MPa, $p < 0.001$). Further, as the amount of NA content increased, the dynamic modulus of composite scaffolds decreased. Quantification of these results showed that all composite scaffolds had dynamic moduli lower than AL scaffolds and/or greater than NA scaffolds ($p < 0.001$) (Figure 5B). The phase angle showed only minor changes with increasing NA content (Figure 5C).

3.4. Quasi-Static and Dynamic Properties of Scaffolds in the Context of a Radial Defect

While the above suggested that NA content influenced overall scaffold mechanics, we were also interested in how such inclusions impacted strain transfer in the context of defects that interrupted the primary fiber population. As above, testing was carried out in the predominant fiber direction and paired samples (taken from scaffold regions immediately adjacent to intact samples) were tested after a 50% radial defect, spanning half of the width of the scaffold at its vertical midpoint, was introduced (Figure 6A). The percent decrease in modulus was reported relative to intact scaffolds. Interestingly, defected AL scaffolds lost significantly ($p < 0.01$) more of their apparent modulus (30.0 ± 1.1 MPa to 18.3 ± 0.9 MPa) compared to fully NA scaffolds (9.2 ± 0.3 MPa to 6.5 ± 0.21 MPa). Compared to AL scaffolds, composite scaffolds with 33% and 75% NA content showed decreased attenuation

of this apparent modulus ($p < 0.05$ and $p < 0.01$ respectively) (Figure 6B). No significant differences were shown for the toe modulus, with all groups decreasing by ~20% when defected ($p = 0.5323$) (Figure 6C). No obvious trends for the dynamic modulus were observed across groups ($p = 0.870$) (Figure 6D).

3.5. Strain Reconstitution in the Vicinity of a Radial Defect in Composite Scaffolds

Bulk measures of scaffold mechanics suggested that inclusion of disorganized layers preserved some of the scaffold mechanical function in the presence of a defect. To understand the mechanical origin of this observation, we measured local strains in the direction of stretch (E_{yy}) near the radial defects (Figure 7). These analyses showed a significantly decreased ($p < 0.01$) area near the defects where the measured strain was less than 50% of the global applied strain (Figure 7D). That is, for constructs with disorganized layers, the region of attenuated strain near the defect was smaller compared to the purely aligned scaffolds. Further, when we determined the distance from the defect at which this 50% value was reached, this distance was significantly lower ($p < 0.05$) for composite scaffolds compared to fully aligned scaffolds (Figure S1). Taken together, this suggests that strain is reconstituted back towards the global strain over a smaller region and closer to the defect in composite scaffolds.

4. Discussion

Native dense connective tissues, such as the knee meniscus, the annulus fibrosus, and tendon/ligament show marked complexity in structural organization and mechanical function across multiple length scales [33–36]. In this study, we developed a biomaterial model of the knee meniscus, inclusive of radial tie fibers, by generating nanofibrous scaffolds that contained non-aligned (disorganized) layers enmeshed regularly throughout an otherwise aligned network. This was intended to mimic the radial tie fiber and circumferential fibers of the major fiber fractions of the native tissue. We hypothesized that the inclusion of disorganized RTF-like elements would maintain mechanical properties in materials with defects that severed a portion of the aligned fiber network, by redirecting load around the defect through the RTF-like network. Such a mechanism is similar to that described in tendon fascicles, wherein the interfibrillar matrix transfers loads around local interruptions in aligned collagen bundles [37–39]. If this mechanism existed in the meniscus, it might explain why the native tissue can tolerate tears of up to 90% of the tissue width before its functionality at the joint length scale is lost [11].

To test this concept, we electrospun fibrous scaffolds whose internal organization and alignment changed as a function of depth. Sequentially layering polymers has been explored by several investigators to produce multi-polymeric scaffolds with specific degradation or mechanical properties [40–42]. Here, by modulating the mandrel speed, we generated discrete layers of organization within the same scaffold, with continuous transitions occurring from one layer to the next (Figure 2). This process yielded a single-polymer scaffold with increased mechanical complexity, including an internal architecture that mimics the RTFs found in native meniscus tissue. By combining aligned and non-aligned layers in variable amounts, we were able to establish that the mechanical properties of

individual layers summed to produce composite scaffolds whose bulk mechanics reflected the relative contributions of the individual layers. That is, composite scaffolds with increasing non-aligned content had a decreased linear region modulus and dynamic modulus, as well as decreased transition and yield strains (Figure 3) compared to fully aligned scaffolds.

Having established that these scaffolds could be fabricated and that their mechanical performance met expectations, we next queried how local interruptions in the fiber architecture might influence the mechanical response, and whether it depended on internal scaffold organization. For this, we introduced defects that interrupted the aligned fibers across 50% of the scaffold width. In an aligned scaffold, where all fibers are directed in a primary direction, one would expect that a 50% width defect would result in a 50% reduction in the apparent modulus. Instead, if instead lateral interactions occurred between fibers, as would be the case with the non-aligned layers, then the apparent modulus might decrease by a lesser amount, depending on how quickly the ‘disconnected’ fibers adjacent to the defect were re-engaged by these lateral interactions. Interestingly, we found that, for fully aligned constructs, there was a 40% decrease in the apparent modulus with a 50% width defect (Figure 6B). This suggests that even in aligned scaffolds, some amount of fiber dispersion and/or lateral connectivity must exist, which can transfer energy into the disconnected regions. Supporting this idea, testing fully aligned scaffolds of shorter and shorter gauge length resulted in a measured apparent modulus that approached a 50% decrease with a 50% width defect (10 mm gauge length showed -48 ± 2 % decrease, while 20 mm gauge length indicates -41 ± 3 % decrease and 60 mm gauge length shows -23 ± 7 % decrease) (Figure S2). This implies that a shorter length scale likely limited the number of occasionally disordered fibers contributing to lateral energy transfer.

When on the other hand the disorganized (non-aligned) layers were purposefully introduced into composite scaffolds, this phenomenon was even more apparent. That is, with inclusion of NA layers, the apparent modulus decreased less than the fully aligned scaffolds in the context of a radial defect. This finding supports that notion that non-aligned layers are able to re-route energy around the defect, and so can serve to reengage disconnected aligned fibers in adjacent layers. This concept was supported by strain mapping in regions local to the defect (Figure 7C–D). These assays showed enhanced reconstitution of local deformation in ‘disconnected’ regions occurring closer to the defects for scaffolds that contained non-aligned elements. This finding in a biomaterial analog supports a similar potential role for the radial tie fiber network in the native meniscus. RTFs may serve to bind together and ensure strain transfer to the aligned, circumferential fibers in native meniscus tissue, even in the context of a local radial tear. Furthermore, given the variation in density of RTFs in different regions of the meniscus, certain regions may be more susceptible to loss of function with radial tears than others.

While our data support the notion of RTFs mediating resumption of strain transfer in the context of radial tears that interrupt aligned fibers, this study is not without limitations. First, the non-aligned elements are certainly not equivalent to radial tie fibers in native tissue. For instance, while the NA layers do interweave with the AL layers, they lack the 3D branched organization of native RTFs. Also, it is not clear how closely the polymer fiber mechanics

used here match that of the collagen within the RTFs in the native meniscus. Beyond knowing something of their composition, little is known regarding RTF internal collagen organization or mechanical properties of individual fibers. That said, our biomaterial model is sufficiently versatile such that additional features could be queried as new information is available on the native tissue. For example, one could easily compare smaller, more numerous layers to thicker, less numerous layers to determine which was more efficacious in restoring strain transfer near a tear. Likewise, the mechanical properties of the RTF-like elements could be increased or decreased (based on polymer selection) to determine how this influences strain reconstitution. Future studies will address these issues as well as native tissue samples with different RTF density and distribution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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.

Figure 1.

A) Schematic of stepper motor device used to modulate mandrel speed during scaffold production. The stepper motor clamps onto the speed controller and automatically changes the speed based on an Arduino input script controlling duration of deposition for each layer. B) SEM depicting non-aligned (NA, produced at ~3 m/s surface velocity) electrospun scaffold. C) SEM depicting aligned (AL, produced at ~10 m/s surface velocity) electrospun scaffold. Scale Bar = 10 μm .

Figure 2.

Schematic depicting single layer scaffolds, with non-aligned (NA) scaffolds in blue and aligned (AL) scaffolds in red, as well as composite scaffolds with seven discrete layers, alternating in alignment. Blocks indicate four composite groups, showing increasing non-aligned content from 25% to 75%.

Figure 3.

A) Confocal image stack (10 μm thick) depicting continuous transition between NA (yellow) and AL (blue) layer within a composite scaffold. Scale bar = 25 μm. B) and C) are maximum projections of 3 thickness segment from AL and NA region, respectively, with inset showing quantification of fiber directionality. Scale bar = 10 μm.

Figure 4.

A) Average stress-strain plots during quasi-static tensile testing of AL (Blue), 33% composite (Orange) and NA (Yellow) scaffolds, with 95% confidence interval depicted by bars. B) Toe and linear moduli of AL (0), composite (33 to 75% NA inclusion), and NA (100) scaffolds. Data are presented as the mean $\pm$ SEM; $n=24$ samples per group drawn from 3 separate production runs. Red exes denote predicted modulus from a simple mixture formulation based on fractional composition. $P<0.01$, unless n.s. indicated. C) Transition and yield strain of AL (0), composite (33 to 75% NA inclusion), and NA (100) scaffolds. Data are presented as the mean $\pm$ SEM; $n=24$ samples per group drawn from 3 separate production runs.

Figure 5.

A) Hysteresis loops depicting the final cycle of dynamic tensile test of AL (Blue), 33% composite (Orange) and NA (Yellow) scaffolds. B) Dynamic moduli of AL (0), composite (33 to 75% NA inclusion), and NA (100) scaffolds. Red exes denote predicted dynamic modulus from a simple mixture formulation based on fractional composition. C) Phase angle of AL (0), composite (33 to 75% NA inclusion), and NA (100) scaffolds. Data are presented as the mean $\pm$ SEM; n=24 samples per group drawn from 3 separate production runs.

*indicates $p < 0.01$, unless n.s. indicated.

A) Schematic illustrating defect testing protocol, where a radial cut spanning 50% of the width of the scaffold was made prior to testing. B) Change in apparent linear region modulus between intact and defect scaffolds. Significance indicated vs. AL scaffolds, where *indicates $p < 0.05$ and **indicates $p < 0.01$. C) Change in apparent toe modulus between intact and defect scaffolds. No significant differences were noted. D) Change in apparent dynamic modulus between intact and defect scaffolds. No significant differences were noted. Data are presented as the mean $\pm$ SEM; $n=24$ samples per group drawn from 3 separate production runs.

Figure 7.

A – Schematic depicting “reconstituted” and ‘non-reconstituted’ areas (light and dark blue, respectively), wherein local E_{YY} is between 50 and 100% or less than 50% of the applied strain. Y is the direction of stretch. B) Normalization distance indicates distance at which the global strain is fully reconstituted on the cut edge. C) Visual depictions of local strain as a function of group. D) Quantification of non-reconstituted area normalized to total area, showing a larger section of AL scaffolds with $E_{YY} < 50\%$ of the applied strain. Data are presented as the mean $\pm$ SEM; $n=3-6$ samples per group. ** indicates $p < 0.01$ and *** indicates $p < 0.001$.