Tissue-to-cellular deformation coupling in cell-microintegrated elastomeric scaffolds

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Abstract Long term efficacy of tissue replacements or regenerative therapies rely on the critical processes of cell proliferation and differentiation, the production of organized matrix, and concurrent tissue remodeling or growth. Recent studies have shown that mechanical and chemical factors modulate cell function which has profound implications on tissue growth and remodeling. As such, creating engineered tissue replacement options requires a detailed command of the complex, dynamic, and reciprocal interactions which occur at the cell-ECM interface. To gain a better understanding of the coupled tissue-cellular deformation response, we propose the use of cell-microintegrated elastomeric scaffolds which provide a unique platform to investigate cellular deformations within a three dimensional fibrous scaffold. Scaffold specimens micro-integrated with vascular smooth muscle cells (VSMC) were subjected to controlled biaxial stretch with 3D cellular deformations and local fiber micro-architecture simultaneously quantified. Interestingly, local cellular deformations exhibited a non-linear deformation response with scaffold strain which was attributed to unique microarchitectural morphologies. Local scaffold microstructural changes induced by macro-level applied strain dominated cellular deformations, so that monotonic increases in scaffold strain do not necessitate similar levels of cellular deformation. This result has fundamental implications when attempting to elucidate the events of de-novo tissue development and remodeling in engineered tissues.

Introduction

The development of efficacious therapies for tissue repair, replacement, or regeneration rests in large part on our ability to managing the events of cellular mechanobiology. Controlling cellular processes in turn necessitates a strong fundamental knowledge of native biological or artificial material structure and function across multiple scales. Cells perceive and react to their mechanical environment through adhesion to local substrates as well as cellular deformations induced by the surrounding mechanical environment. The intricate interactions between cells and their environment dictate mechanotransduction of proteins critical for cell function and maintaining a mechanically sound, organized matrix. Being able to manage cellular processes through controlled exogenous cues has immense implications in the development of engineered tissue therapies. Though much is known about the arrangement and connectivity of load bear elements which transmit mechanical cues from the matrix, through the cell cytoskeleton, and on to the nucleus, contemporary knowledge is lacking a mechanistic understanding of how mechanical stimuli translates to the mechanotranstuction of proteins. This is a result of technical difficulties encountered when performing studies in native tissues, which are confounded by complex hierarchical structures and multiple cell and tissue types. The use of artificial scaffolds with controllable microstructure provides a unique model system to elucidate the fundamental mechanisms by which cells perceive and respond to their local environment. The following presents a review of recent studies to characterize electrospun scaffold structure-function relationships across multiple scales and to understand how cells embedded within these scaffolds respond to gross construct deformation [1, 2].

Methods

Specimen fabrication

For a detailed account of the process to produce cell microintegrated poly (ester urethane) urea (PEUU) constructs, the interested reader is referred to the work of Stankus et al. [3-5]. Electrospinning involves the deposition of a solubilized polymer delivered through a capillary tube across a large voltage gradient onto a collection surface. By altering the rotational velocity of the cylindrical collection mandrel, PEUU fiber alignment could be controlled. The resulting scaffold is a continuous non-woven mat that can be made to exhibit a wide range of mechanical properties while providing a suitable environment for cell proliferation and growth [6, 7].

Image acquisition and construct characterization

Briefly, an inverted Laser Scanning Confocal Microscope (LSCM, Olympus Fluoview 1000) was chosen to observe living cell nuclei stained with DRAQ5 and

polymer scaffold in situ. A custom stretching device was constructed for use with the LSCM capable of imposing controlled biaxial modes of deformation on the cell integrated constructs. A similar stretching device was constructed so that non-integrated scaffolds could be placed into the scanning electron microscope (SEM) enabling simultaneous specimen deformation and imaging of the specimen surface.

Nuclear aspect ratio (NAR) was used as a metric for overall cellular deformation while scaffold fiber morphologies such as fiber orientation, tortuosity, and diameter was measured with custom image analysis algorithms implemented in MATLAB (The MathWorks, Inc., Natick, MA, USA). Fiber orientation was quantified in the unstrained and deformed configurations. Methods for this custom image analysis software have been previously presented in detail [8-10]. Essentially, the software directly produces statistical distributions of fiber orientation probability, $R(\theta)$, from all measured fibers over the range of all possible orientations, $-90^{o} \leq \theta \leq 90^{o}$. Fiber tortuosity, as defined as the ratio of the total fiber length (or perimeter) to the fiber end-to-end length, was quantified for approximately 150 fibers across a mosaic image created from a 3 x 3 array of SEM micrographs. This process was accomplished via custom software which tracked fibers along their length in a semi-automated manner to quantify fiber tortuosity. Lastly, fiber diameter, defined as the distance between fiber edges perpendicular to the fiber axial direction, was manually determined from 50 randomly chosen fibers throughout the image.

Results

Scaffold micromechanics

The electrospun scaffolds investigated in this study exhibited complex, hierarchical architectures spanning multiple length scales (Fig. 1). These multi-scale structures combine to form a complex 3D scaffold with tunable tissue-level mechanical

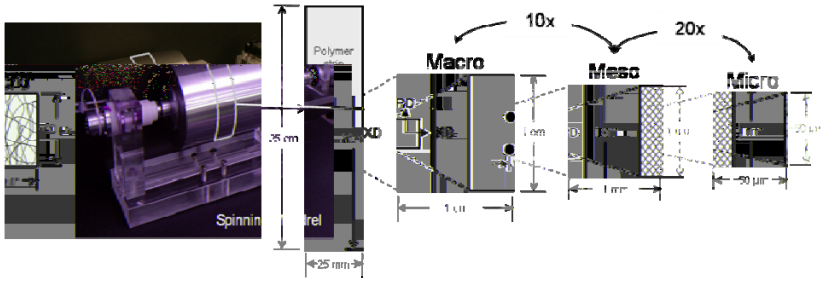

Fig 1. Electrospun PEUU scaffolds exhibit complex multi-scale hierarchical structures. Scaffold deformation was measured at three scales defined as micro, meso, and macro. At the micro and meso scales, the structural characteristics of fiber orientation, tortuosity, and diameter were quantified [2].

behavior that can be remarkably similar to the biaxial mechanical response of the native pulmonary valve (PV) leaflet [8]. However, understanding the mechanisms by which these materials deform and behave under various loading conditions is not an elementary task

In the reference configuration, the fiber angle and tortuosity measurements indicate that both fiber tortuosity and fiber orientation are dependent upon mandrel velocity (Fig. 2). When these results are combined to create a probability distribution relating fiber orientation and tortuosity, subtle changes are observed with increasing mandrel velocity as manifest by the evolution of secondary fiber populations exhibiting increased tortuosity in the preferred (PD, $\theta = 0^{o}$) and cross-preferred (XD, $\theta = 90^{o}$) fiber directions (Fig. 2c-d). Increased fiber tortuosity may function in conjunction with rotational fiber kinematics to produce the increasingly non-linear behavior observed with mandrel velocity and exemplifies the need to elucidate structural characteristics and their functional role at the macro scale.

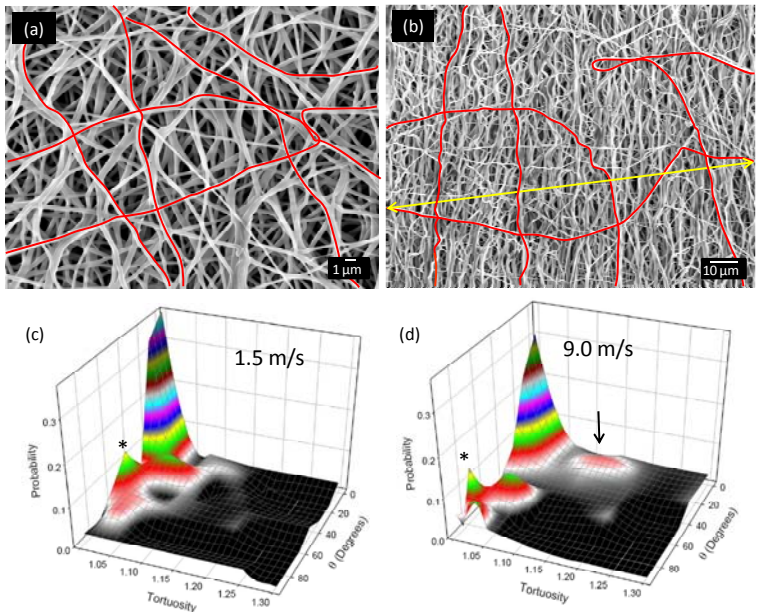

Fig 2. From the electrospinning process, a continuous mat is produced by successive layers of non-woven polymer. At the meso scale (a), PEUU fibers are seen to exhibit significant tortuosity, as highlighted in red, with this structural characteristic becoming much more prominent with increasing scale (b). (c-d) The complex relation between fiber tortuosity and its angle of orientation was observed to be a function of increased mandrel velocity. All scaffolds exhibited a primary probability peak about the origin (PD). Interestingly, a secondary probability peak was seen to develop in the PD with low to moderate tortuosity levels as mandrel velocity increased (denoted by the arrow) while the XP always exhibited a subpopulation of fibers with relatively low tortuosity values (indicated by "*").

At the micro scale a very heterogeneous deformation response is observed throughout the image plane with large changes in deformation (Fig. 3 left panel). In addition, a trend was observed wherein the PEUU fiber diameter decreased with increasing mandrel velocity during production. Furthermore, with increased specimen deformation, a monotonic decrease in PEUU fiber diameter was measured for all specimens. At the meso scale, substantially less variation is observed in the deformation response but the interpolated ranges in deformation remain relatively large. Lastly, at the macro scale, the electrospun polymer scaffold is capable of recapitulating the long, independently acting fiber response exhibited by the dense collagen network of the native PV (Fig. 3 right panel).

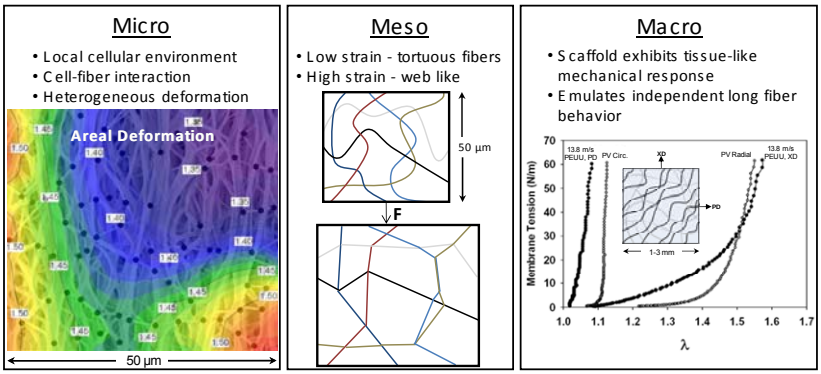

Fig 3. Despite exhibiting gross tissue-like mechanical response at the macro scale, the scaffold exhibits unique micro and meso mechanical behaviors. For instance, at the micro-scale a heterogeneous deformation response is observed. In addition, fibers in the unstrained configuration exhibit an undulated or tortuous morphology which transitions to a highly interconnected web-like architecture at finite strains [2].

It is not until the macro scale, on the order of ~1 mm, that a reasonably homogeneous deformation behavior is observed. As equi-biaxial deformation increased, fiber tortuosity was reduced in both the preferred and cross-preferred directions but not extinguished completely. The PEUU fiber intersections appear to be quite secure and while allowing relatively free rotation about the point of intersection, fiber slippage or translation was inhibited, limiting fiber straightening and resulting in a residual level of tortuosity.

Coupled cell-scaffold deformation

The cell micro-integrated electrospun scaffolds exhibited micro-fiber morphologies and kinematics that were shown to directly influence local cellular deformations. In the unstrained configuration the electrospun fibers exhibited a tortuous architecture which transitioned to a web-like network of straight, interconnected fibers at high levels of strain. The deformations of the micro-integrated cells were found to be primarily mediated by local fiber straightening. Furthermore, the mi-

crointegrated cells exhibit a very planar orientation in the non-deformed configuration and were observed to readily rotate into the direction of principle strain. Both fiber and local scaffold deformations were observed to be fully recoverable after large deformation. Initially, the integrated cells exhibited a rapid increase in NAR as fibers straightened and tortuosity was reduced. Once the PEUU fibers became straightened and the architecture transitioned to an interconnected web like structure, changes in NAR were observed to plateau (Fig. 4).

Discussion

The ability to create engineered tissue replacement options requires an understanding of how mechanical cues from the tissue or organ level are transmitted to the cell or cell components and elucidation of the signaling pathways which guide mechanotransduction events. Furthermore, the use of new technologies in the production of engineered scaffolds necessitates a detailed understanding of the structure-function relationships unique to these materials. Currently, the exact microstructural characteristics of engineered scaffolds often remain ill defined and presumably will have a profound influence on cellular function. Clearly, the effects of physical factors on cellular activity must be determined in engineered tissues. Knowledge of these signals may shorten the iterations required to replace a tissue successfully and direct cellular activity and phenotype toward a desired end goal.

In contrast to the compression mediated deformations observed in aortic valve interstitial cells (Fig. 4) [11], microintegrated cell deformation and kinematics were mediated by the local reduction of tortuosity or straightening of the electrospun fibers (Fig 5).

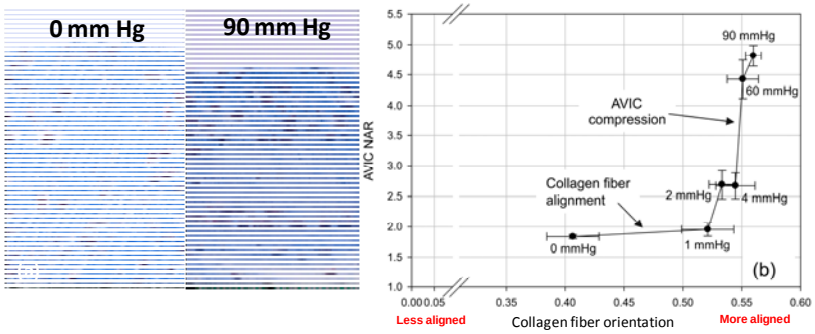

Fig 4. Native aortic valve interstitial cell (AVIC) nuclear deformation with tissue strain. (a) Here aortic valve tissue cross-sections are shown in the unloaded (0 mmHg) and full physiological loaded (90 mmHg) states. (b) In native tissues such as the aortic valve, cell nuclei undergo very large changes in aspect ratio induced by tissue stretch and compaction. Figure adapted from Huang et al [12].

Though an affine transformation is valid for gross fiber kinematics, it is likely that electrospun fiber translation is limited by the presence of secure interconnections between fibers [2]. Johnson et al. presented a similar hypothesis for reduced fiber mobility in ES polymers exhibiting "point bonding" [13]. Combining this behavior with the known scaffold biaxial response [8] suggests the possibility of successfully emulating gross native tissue behavior without exactly replicating their highly complex micro-architectures.

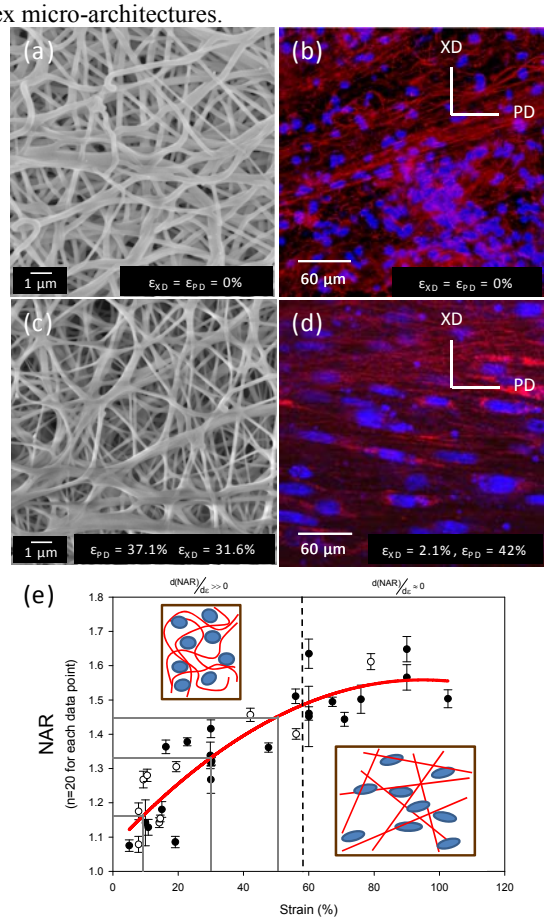

Fig 5. Strain induced changes in micro-architecture and resulting nuclei deformation. When exposed to biaxial modes of deformation, electrospun fibers were observed by (a,c) SEM and (b,d) LSCM to transition from a tortuous configuration in the unstrained state to an interconnected web-like architecture at high strains. (e) A composite of all NAR measurements (mean ± s.e.m) demonstrated a rapid increase to ~60% strain, after which a plateau was observed with further strain increases, indicating that nuclei deformations are dominated by local fiber straightening. Figure adapted from [1].

The scaffold and microintegrated cell characterization studies presented above will lay the basis for future modeling efforts and guide mechanical training studies aimed at the development of a tissue engineered pulmonary valve leaflet. Modeling efforts will focus on the multi-scale effects of the unique micro-architecture exhibited by these electrospun scaffolds, such as the presence of tortuous fibers, and how they dictate overall scaffold behavior.

We showed that monotonic increases in scaffold deformation do not necessitate comparable cellular deformations and thus may not correlate to incremental changes in mechanotransduction events (Fig. 5). Electrospun PEUU scaffolds provide a unique yet logical "next-step" from current fibrous scaffold technologies for tissue engineering such as needled non-woven biodegradable polymers [14-16] since they are able to fully recover after large deformation and exhibit structural and behavioral characteristics similar to native tissues.

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