Review article

Cell-matrix mechanical interaction in electrospun polymeric scaffolds for tissue engineering: Implications for scaffold design and performance

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ABSTRACT

Engineered scaffolds produced by electrospinning of biodegradable polymers offer a 3D, nanofibrous environment with controllable structural, chemical, and mechanical properties that mimic the extracellular matrix of native tissues and have shown promise for a number of tissue engineering applications. The microscale mechanical interactions between cells and electrospun matrices drive cell behaviors including migration and differentiation that are critical to promote tissue regeneration. Recent developments in understanding these mechanical interactions in electrospun environments are reviewed, with emphasis on how fiber geometry and polymer structure impact on the local mechanical properties of scaffolds, how altering the micromechanics cues cell behaviors, and how, in turn, cellular and extrinsic forces exerted on the matrix mechanically remodel an electrospun scaffold throughout tissue development. Techniques used to measure and visualize these mechanical interactions are described. We provide a critical outlook on technological gaps that must be overcome to advance the ability to design, assess, and manipulate the mechanical environment in electrospun scaffolds toward constructs that may be successfully applied in tissue engineering and regenerative medicine.

Statement of Significance

Tissue engineering requires design of scaffolds that interact with cells to promote tissue development. Electrospinning is a promising technique for fabricating fibrous, biomimetic scaffolds. Effects of electrospun matrix microstructure and biochemical properties on cell behavior have been extensively reviewed previously; here, we consider cell-matrix interaction from a mechanical perspective. Micromechanical properties as a driver of cell behavior has been well established in planar substrates, but more recently, many studies have provided new insights into mechanical interaction in fibrillar, electrospun environments. This review provides readers with an overview of how electrospun scaffold mechanics and cell behavior work in a dynamic feedback loop to drive tissue development, and discusses opportunities for improved design of mechanical environments that are conducive to tissue development.

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1. Introduction

Scaffolds for tissue engineering applications are designed to mimic the properties of the extracellular matrix (ECM) of the target tissue to guide tissue regeneration and repair [1]. When cells are placed within a scaffold, tissue development is driven, in part, by microscale mechanical interactions between cells and the matrix [2]. The local mechanical properties of the scaffold, such as elasticity, act in concert with physicochemical and biochemical properties to drive cell behaviors including migration, proliferation, and differentiation [3,4]. Cells, in turn, exert forces on the matrix and remodel its structure throughout tissue development. Thus, the ability to monitor and manipulate the micromechanical environment is crucial in designing scaffolds for tissue engineering, as well as in developing physiologically relevant platforms for studying tissue development and pathology in vitro.

Over the last decade, studies of cell-matrix mechanical interaction, or mechanobiology, in tissue engineering have emerged [5,6], as well as micro- and nano-fabrication and characterization techniques that have advanced [7,8], allowing exploration of biophysical problems on this length scale. These studies have primarily relied on smooth or micro-patterned hydrogel substrates, which have controllable structure and elasticity, but do not recapitulate the fibrillar ECM, inducing cell behaviors distinct from those in vivo [9]. On the other hand, studies of cell-matrix interaction in fibrous collagen matrices provide insight to the 3D native environment [10], but the material properties of these constructs are difficult to control. Electrospinning has emerged as a favored technique for developing biomimetic scaffolds with controllable, nano- to micro-scale structures and chemical properties [11,12]. This technology employs electrostatic forces to extract nanofibers from a polymer solution, which are deposited layer-by-layer to form a scaffold. The resulting constructs can be tailored to mimic a range of tissues, including musculoskeletal [13,14], skin [15] and vascular tissues [16], and templates may be used to further define the 3D macrostructure [17]. A range of natural and synthetic biodegradable polymers may be electrospun [18], and blending of natural and synthetic polymers, incorporation of ceramics, and development of core-shell fiber geometries all provide pathways for controlling the mechanical properties of electrospun scaffolds over multiple length scales [19].

Previous reviews have reported on the biological performance of electrospun polymeric scaffolds [20–22], considering the effects of parameters such as fiber microarchitecture and pore size on cell behavior. The impact of the physicochemical and biochemical properties of electrospun, biodegradable polymers and their blends on cell behavior has also been reviewed [18,23–25]. Many studies have emphasized the bulk, or macroscale, mechanical properties of electrospun scaffolds, as these are critical to the ability to provide support in load-bearing tissues and to degradation kinetics [19], but these are not representative of the local, microscale properties sensed by cells. Multiscale studies have used computational modeling to explore how microstructure impacts macroscale mechanical behavior of electrospun scaffolds [26,27], and, conversely, how extrinsic loads applied on a bulk level are transferred via individual, electrospun fibers to cells on a local level [28]. Although excellent models have been developed for explaining these multiscale biomechanics, they do not provide insight into the intrinsic micromechanical properties at the length scale at which cells interact with their environment.

In this review, we report on a number of recent studies that reveal how the micromechanical properties of electrospun scaffolds impact cell behavior, and how, in reciprocal fashion, cellular forces and extrinsic loading remodel the micromechanical properties of scaffolds during tissue development. Throughout, we consider the implications for in situ performance of these scaffolds. A deeper understanding of mechanical phenomena in these environments will contribute to development of improved biomimetic scaffolds that successfully promote tissue regeneration.

2. The engineered microenvironment as a mechanical system

2.1. Defining mechanical properties

Tissues and biomimetic scaffolds exhibit complex mechanical properties that may be described as nonlinear, anisotropic, viscoelastic, and poroelastic [29]. However, for practicality in quantifying mechanical properties, tissues are often assumed to be linear, isotropic, elastic solids; that is, their mechanical behavior is assumed to be independent of the amount and direction of applied deformation, and independent of time. In this simplified case, elasticity, or stiffness, is quantified by Young's modulus, \( E \), defined as the ratio of stress, \( \sigma \), to strain, \( e \): \[ e = \frac{\sigma}{E}. \] In the micromechanical context of electrospun scaffolds, stresses and strains on the matrix may be due to, for example, a cell pushing against a fiber to initiate migration, or physiological loads such as hydrostatic pressure. Electrospun scaffolds may be viewed as “strut and tie” systems, in which the fibers (struts) may undergo tension, compression, and bending, depending upon cell-induced tractions. In these cases, it is also helpful to quantify the bending modulus of fibers [30], which is dependent upon geometry, as well as material constituents.

At large deformations, tissues and scaffolds can exhibit mechanical nonlinearity, and effects such as strain stiffening (increase in elasticity with strain) should be considered when analyzing mechanical behavior [31]. At the macroscale, mechanical nonlinearity is thought to help prevent rupture of tissues, whilst at the microscale, nonlinearity has a role in cell-cell communication, as straining of the matrix by one cell changes the apparent stiffness sensed by a neighboring cell, leading to migratory...
patterns not exhibited on mechanically linear substrates [32]. The strain stiffening exhibited by biopolymers can be attributed to bending and stretching of the fibers, reorientation of the cross-links that mediate interfiber force transmission, and changes in the network microstructure [33]. Conversely, synthetic polymers tend to be made up of highly coiled chains that when straightened reduce the entropy of the system, resulting in a more linear mechanical response [34]. However, the nonlinear elasticity of electrospun synthetic polymers may be tailored to mimic that of tissues using strategies such as core-shell electrosprining [35] or blending of synthetic and natural polymers [36] to combine properties of two mechanically distinct materials. At the nanoscale of individual fibers, however, electrospun collagen actually exhibits strain softening (decrease in elasticity with strain) when loaded in a bending configuration, highlighting the importance of testing scaffolds across length scales and loading directions to fully characterize their mechanical behavior [30].

The mechanical properties of many fibrous tissues, including cartilage, skeletal muscle, and vasculature, are direction-dependent, or anisotropic. Typically, these tissues exhibit higher elasticity when loaded along the direction of fiber alignment, and lesser elasticity when loaded perpendicular to fiber alignment. Mechanical anisotropy lends function to load bearing tissues; for example, aligned fibers in the tendon allow support of tensile loads, whereas the ECM of vasculature is arranged to best support circumferential loads. At the microscale, cells in anisotropic tissues and in aligned electrospun scaffolds orient themselves along the direction of fiber alignment [37], and this anisotropy can direct distinct migration patterns and stem cell differentiation [12].

Tissues and many polymeric electrospun scaffolds also exhibit time-dependent, or viscoelastic properties [38], which describe the tendency to relax to an undeformed state following loading, or the extent to which a tissue creeps, that is, has a delayed strain response to a load. Such properties are critical to the mechanical function of certain tissues, such as vascular tissues, which undergo cyclic stresses [39]. Cells and subcellular components are also viscoelastic; for example, a cell may not respond instantaneously to an extrinsic load, but may continue to change shape in the minutes following loading [40]. When quantifying forces experienced in cell-matrix interaction, omitting the time-dependency of the mechanical response can lead to significant errors [41]. Closely related to the viscoelastic properties of a scaffold are its poroelastic properties, which take into account the biphasic nature of scaffolds in situ, in which fluids move throughout the porous network and affect the stresses and strains experienced locally at the microscale [42].

2.2. Cellular machinery and mechanotransduction

Whilst mechanical properties may be measured at any spatial scale, our goal is to consider these properties at the cellular scale, requiring an understanding of the mechanisms by which cells sense their mechanical environment. Mechanotransduction refers to the molecular pathways via which cells respond to forces and apply forces to a substrate [43,44]. At all stages of tissue development, the cells involved, from stem cells to mature cells, sense their mechanical environment via mechanoreceptors on the cell surface [4]. When a cell first comes into contact with the matrix, integrins, the adhesive molecules on the cell surface, bind with ligands on the matrix surface, forming large protein complexes called focal adhesions that tether the cell cytoskeleton to the matrix. These nanoscale events direct the cell's attachment points and morphology, and, in turn, their function [45].

Focal adhesions repetitively tug on the matrix to gauge its stiffness and act as local, nanoscale sensors [46]. Through physical attachment to actin filaments in the actomyosin network of the cell, focal adhesions also allow cells to pull or push themselves along on a matrix during migration. In order for cells to travel with persistent direction, the matrix must have sufficient stiffness to resist deformation by cell tractions, otherwise cells may simply contract the matrix without traveling. On 2D, planar substrates, cells tend to migrate toward stiff substrates in a process called durotaxis [47]. Molecular pathways underlying cell proliferation are also regulated via contractile forces imparted by the actomyosin network and sensed by focal adhesions, and have been shown to be dependent on matrix elasticity in 2D substrates [48]. However, mechanosensing is more complex in the 3D, fibrillar environments of native ECM and electrospun scaffolds. Here, cells can pull or push on fibers from any direction [49], and the elasticity depends on direction of the cell-induced load; for example, the effective elasticity sensed by the cell may be best quantified by the tensile modulus or the bending modulus, depending on directionality.

Mechanotransduction also plays a role in directing stem cell fate [50]. Incorporation of stem cells in scaffolds can “kickstart” tissue regeneration, and scaffolds for tissue engineering should provide an environment that directs stem cells to the target lineage. Stem cells seeded on 2D hydrogel substrates with elasticities matching those of native ECM of various tissues, from neural to bone tissues, express precursor genes for the cell lineage typically found in those respective tissues, in many cases without the presence of specific growth factors [50]. Again, whilst studies on such simple substrates have been key to elucidating mechanical interactions in stem cell differentiation, only recently have these questions been addressed in 3D, biomimetic, electrospun scaffolds. All of the above cell behaviors (migration, proliferation, and differentiation) are affected not only by direct interactions with the matrix, but also by extrinsic loads. For example bulk loads are transferred to cells via local deformations in the ECM, and fluid flow through pores in the matrix induces additional mechanical signaling [28,51]. Thus, cells respond to a combination of intrinsic and extrinsic mechanical forces that are inextricably linked to cell structure and signaling. The concept of tensional homeostasis or
2.3. Techniques for measuring and visualizing mechanical interaction

Bulk properties of electrospun scaffolds do not translate to the local properties at the cell-sensing scale [53], though many studies use bulk values as the sole indicators of stiffness. Here we describe techniques used to quantify mechanical properties of single fibers, as well as imaging techniques that visualize microscale mechanical heterogeneity in scaffolds.

2.3.1. Single fiber mechanics

Manipulating nanoscale, electrospun fibers and sensitively measuring their deformation is a challenging technical problem. With its nanoscale resolution and adaptable cantilever tips to perform testing in a variety of geometries, atomic force microscopy (AFM) has emerged as the key modality for testing individual nanofibers [54]. AFM techniques have been developed for tensile testing to quantify Young’s modulus, three-point bending testing to quantify bending modulus, and nanoindentation to quantify elasticity via contact models. These techniques have previously been reviewed in detail [54–56]. Fig. 2 illustrates the various geometries in which these tests are performed. Often, AFM or other nano-tensile testers are combined with optical or electron microscopy to observe failure modes and shape changes during testing [55,57–59], providing additional insight to behavior of fibers.

Tensile, bending, and indentation tests using AFM generally extract elastic properties of fibers, but tests may be adapted to probe more complex, dynamic properties. For example, one study employed a novel fiber anchoring technique using UV-sensitive adhesive to measure the viscoelastic properties of electrospun polycaprolactone (PCL) fibers [59], which exhibited stress relaxation patterns that were age-dependent, revealing how nanoscale mechanical properties may degrade over time in situ. Resonance measurements of anchored fibers may also extract dynamic information [55]; however, such high-frequency techniques do not necessarily recapitulate the cell-induced or physiological loading that fibers experience in scaffolds in situ.

Table 1 lists values reported in the literature for individual fiber modulus as measured using AFM, compared to values of the macroscale electrospun mat modulus. In most instances, the elasticity of individual fibers is greater than that of the bulk electrospun mat. However, fiber modulus can vary widely depending on loading technique; for instance, PCL measured using nanoindentation has modulus 2–3 orders of magnitude lower than that measured using bending. Whilst many studies have compared single-fiber mechanical properties to bulk properties of electrospun mats [58], highlighting the disparate values obtained depending on length scale, only recently have a few studies related single-fiber elasticity to subsequent cell behavior on these fibers [60], as detailed in Section 3.

2.3.2. Imaging techniques for visualizing local micromechanics

Many studies continue to rely on bulk measurements to explain mechanical effects on cell behavior, perhaps due to the convenience of bulk testing compared to the complexity of single-fiber techniques. The imaging techniques described in this section fulfill an intermediate spatial niche between the single-fiber and bulk

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**Table 1**

Mechanical properties of single electrospun fibers versus electrospun mats.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Polymer</th>
<th>AFM technique</th>
<th>Fiber diameter (nm)</th>
<th>Single-fiber modulus (MPa)</th>
<th>Electrospun mat modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[58]</td>
<td>PCL</td>
<td>3-Point bending</td>
<td>250–700</td>
<td>3700 ± 700</td>
<td>3.8 ± 0.8 [58]</td>
</tr>
<tr>
<td>[59]</td>
<td>PCL</td>
<td>3-Point bending (fiber anchoring)</td>
<td>440–1040</td>
<td>62.3 ± 25.6</td>
<td></td>
</tr>
<tr>
<td>[61]</td>
<td>PCL</td>
<td>Nano tensile (non-AFM)</td>
<td>350–2500</td>
<td>~250–350</td>
<td></td>
</tr>
<tr>
<td>[63]</td>
<td>PCL</td>
<td>Nanoindentation</td>
<td>~1500</td>
<td>~1.1</td>
<td></td>
</tr>
<tr>
<td>[64]</td>
<td>PLGA</td>
<td>Nanoindentation</td>
<td>760 ± 210</td>
<td>0.42 ± 26</td>
<td>400 [65]</td>
</tr>
<tr>
<td>[66]</td>
<td>PLLA</td>
<td>Nano tensile (non-AFM)</td>
<td>890 ± 190</td>
<td>1000 ± 1600</td>
<td>9.6 ± 0.1 [67]</td>
</tr>
<tr>
<td>[68]</td>
<td>PVA</td>
<td>3-Point bending</td>
<td>~100–600</td>
<td>~3000–13,000</td>
<td>~3 [69]</td>
</tr>
<tr>
<td>[70]</td>
<td>PVA</td>
<td>AFM-based tensile</td>
<td>131 ± 57</td>
<td>470 ± 270</td>
<td></td>
</tr>
<tr>
<td>[71]</td>
<td>PEO</td>
<td>AFM-based tensile</td>
<td>700</td>
<td>~45</td>
<td></td>
</tr>
<tr>
<td>[72]</td>
<td>Silk</td>
<td>3-Point bending</td>
<td>520</td>
<td>~5000</td>
<td>515 [73]</td>
</tr>
<tr>
<td>[74]</td>
<td>Silk</td>
<td>3-Point bending</td>
<td>300–600</td>
<td>~17,000</td>
<td></td>
</tr>
<tr>
<td>[75]</td>
<td>Silk/PEO 80:20</td>
<td>Nanoindentation</td>
<td>800</td>
<td>750 ± 60</td>
<td></td>
</tr>
<tr>
<td>[30]</td>
<td>Collagen type I</td>
<td>3-Point bending</td>
<td>160–783</td>
<td>2800 ± 400</td>
<td>52.3 ± 5.2 [76]</td>
</tr>
<tr>
<td>[60]</td>
<td>Hyaluronic acid</td>
<td>3-Point bending</td>
<td>744 ± 45</td>
<td>1060 ± 60</td>
<td>0.0025 (compression)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>601 ± 36</td>
<td>8600 ± 1180</td>
<td>0.0035 (compression)[60]</td>
</tr>
</tbody>
</table>

PCL: polycaprolactone; PLGA: poly(lactic acid – glycolic acid); PLLA: poly(L-lactic acid); PVA: poly(vinyl alcohol); PEO: poly(ethylene oxide).
levels, providing novel pathways for studying cell-matrix mechanical interaction within scaffolds. In contrast to single-fiber measurements, which provide a single value for a mechanical property, images of the micromechanical properties of scaffolds allow assessment of heterogeneity throughout the matrix, and a fuller appreciation of the 3D context.

In addition to its use for single-fiber measurements, AFM has been used to map the surface elasticity of electrospun scaffolds, giving a sense of the mechanical landscape at the length scale of cells and focal adhesions. Using cantilever tips of varying stiffness, AFM can quantify a large range of Young's modulus values, from ~100 Pa (e.g., brain tissue) to several MPa (e.g., mineralized bone tissues) [77]. Although AFM provides an accurate measure of local elasticity in simple sample geometries, the contact models typically used in AFM to estimate elasticity are not accurate in electrospun geometries due to complex boundary conditions [72]. Advanced AFM techniques using dynamic loading can overcome this limitation; Fig. 3a depicts an AFM image of an electrospun silk scaffold, color coded for local stiffness, formed using a dynamic technique [72]. The image highlights that stiffness is not necessarily constant for a given fiber; local stiffness depends on whether the fiber is supported by underlying fibers or is suspended between fibers. Despite its advantage of nanoscale resolution, AFM is restricted to in vitro studies and can only probe mechanical properties in 2D at the sample surface. Another disadvantage is that AFM is time-consuming, especially if a large field of view (greater than ~100 μm²) is desired.

Elastography is a novel technique that has emerged for probing the mechanical properties of scaffolds [78], using medical imaging to track 3D deformation of a sample under a load. Elasticity or strain is estimated from the measured deformation and plotted onto an image. Elastography was originally developed using ultrasound and MRI, and more recently, using the high-resolution technique optical coherence tomography (OCT). OCT-based elastography, or OCE, has a resolution of ~10 μm, can image 1–2 mm into turbid tissues, and is sensitive to tissue motion on the order of nanometers [79], making it a promising candidate for exploring the impact of micromechanical properties on cell behavior [80]. A key advantage of OCE over AFM is its capacity for in vivo imaging [81], meaning it may assess scaffolds throughout their life cycle, from fabrication to implantation. OCE also goes beyond the surface maps obtained using AFM to provide 3D maps, as in the example in Fig. 3b, in which strain distinguishes the microscale layers of mouse aortic tissue. Besides OCT, other high-resolution techniques including micro-computed tomography [82] and confocal microscopy [83] have been used to map local strains within fibrous scaffolds.

The above techniques map matrix properties, but do not map cell-induced forces on the matrix. To quantify these forces, traction force microscopy (TFM) uses confocal microscopy to track displacement of fluorescent beads embedded in a substrate of known stiffness [84]. When cells are seeded on the substrate, TFM allows measurement of how much cells “push” or “pull” on the substrate; that is, traction forces. With the use of an on-stage incubator, TFM may be performed on live cells, lending potential for imaging throughout the development of tissue in culture. However, a limitation of TFM is that cells are typically removed from the culture or scaffold and reseeded onto a substrate of known elasticity (typically polyacrylamide gel) that may not accurately represent native ECM mechanical properties. Using TFM to observe the behavior of cells on an electrospun fibrous matrix, one study incorporated fluorescent beads directly into a solution of hyaluronic acid, electrospun a thin layer of fibers onto a coverslip, and seeded cells onto the fibers [60]. The bending moduli of fibers were characterized.
This study also highlighted that cell-induced contraction of the unique morphology of a cell migrating along an electrospun fiber, such as formation of bleb-like structures at the cell’s leading edge, but over-contractility led to inefficient migration pathways, actin network and less persistent migration. Fig. 4a highlights the adhesions on planar surfaces led to increased tension in the cell’s tracks. The wider cell spreading and spatial distribution of focal adhesions on fibers compared to the diffuse distribution of focal adhesions on planar tracks. The wider cell spreading and spatial distribution of focal adhesions on planar surfaces led to increased tension in the cell’s actin network and less persistent migration. This, in turn, affects the magnitude and spatial profile of the loads that a cell imparts on its matrix, as well as intracellular tension, which directs cell phenotype [43]. In the above study on electrospun PCL, varying fiber diameter from 0.3 to 1.3 μm did not affect cell behavior [86]. However, varying fiber diameter over a much wider range (2–170 μm) significantly altered migratory patterns of epithelial cells [90]. On fibers with diameters 2–40 μm, individual “lead” cells were able to pull away from the cell monolayer, initiating collective migration. Thicker fibers, >40 μm, promoted cell morphology and migration more similar to those observed on planar surfaces, and collective migration was prohibited altogether on sub-micrometer fibers, consistent with studies showing a diameter threshold for adequate formation of focal adhesions [91]. Comparing these patterns to those on planar surfaces, it was deemed that the ability of the cells to wrap around fibers was key to their migratory behavior, again pointing to the localization of focal adhesions [90]. However, this study was carried out on drawn glass fibers; further investigation should determine if these patterns are conserved on electrospun materials that more closely mimic native ECM.

Varying the fiber diameter also changes the inherent fiber stiffness. For instance, smaller-diameter electrospun PCL fibers have increased crystallinity, resulting in higher elastic modulus, especially at diameters <700 nm [61,62]. Larger-diameter fibers may have a less aligned molecular structure as they are deposited, leading to more amorphous intra-fiber properties and lower modulus [30,92,93]. This trend was also observed in polyvinyl-alcohol fibers [68]. However, as seen in Table 1, reported values for single-fiber modulus vary widely between studies, likely due to variance in AFM methods, solution preparation (e.g., polymer molecular weight), and mechanical models used to estimate modulus [93]. For instance, nanoindentation, which estimates modulus via contact models, has been used to characterize electrospun fibers [93]; however, such models are not appropriate here, due to the complex geometry and boundary conditions [72]. Techniques that accurately and consistently characterize individual fiber stiffness are needed to facilitate inter-study comparison. Ideally, to correlate stiffness with cell behavior, fiber mechanics should be measured at cell-sensing spatial scales, deformation amplitudes, and loading rates. One study recapitulated cell-induced forces in AFM by using a focal-adhesion-sized cantilever tip and loading velocities mimicking those at which cells pull against their substrates (20–120 nm/s) [94]. Another study that performed AFM at the focal adhesion scale (~1 μm) showed that mechanical heterogeneity of a collagen matrix is much greater at this scale than on the whole-cell scale (~30 μm) at which many studies probe matrix properties [95]. Future studies investigating how fiber diameter relates to single-fiber stiffness, and, in turn, impacts a cell’s ability to push and pull against fibers, will aid design of fibers that promote particular cell migration, proliferation, and differentiation patterns.

### 3.1. Impact of fiber geometry

In fibrillar environments, such as electrospun scaffolds, the physical connections between cells and the matrix are distinct from those on planar surfaces due to the curvature of the fibers [9]. For example, the actomyosin mechanisms involved in cell migration on electrospun, suspended PCL fibers were distinct from those on microprinted tracks, a trend that held for 11 cell types, including fibroblasts, epithelial, and neuron-like cells [86]. On fibers, cells generated fin-like projections that propagated along a fiber and initiated migration. Fin formation was due in part to the highly localized distribution of focal adhesions on fibers compared to the diffuse distribution of focal adhesions on planar tracks. The wider cell spreading and spatial distribution of focal adhesions on planar surfaces led to increased tension in the cell’s actin network and less persistent migration.

**Fig. 4.** Electrospun scaffold geometry affects cell micromechanics. (a) On the curved surfaces of electrospun fibers, cells exhibit a cytoskeletal organization and localized focal adhesions that promote unique migration patterns compared to planar substrates. Dashed box highlights that the leading protrusion of the cell is not adhered to the fiber, allowing participation of actin filaments in formation of fin-like projections that guide migration. Adapted from [86], (b) Left: Cells spread along single suspensions when pores are much larger than the cell (~100 μm), resulting in large cell tractions (thick arrows) and bridge across pores to form several attachment points when pores are smaller than the cell (~20 μm), resulting in smaller forces (thin arrows) with a distinct spatial distribution. Adapted with permission from Elsevier [50]. Right: Fluorescence images of fibroblasts in electrospun PCL scaffolds with large (top) and small (bottom) pore sizes demonstrate the varying arrangement of cells in these constructs. Adapted with permission from Elsevier [101].

#### 3.1.1. Impact of fiber diameter

Previous studies have shown a correlation between fiber diameter and cell behavior in electrospun scaffolds [88,89]. From a mechanobiology perspective, altering fiber diameter alters cell morphology, including cytoskeletal and focal adhesion arrangement. This, in turn, affects the magnitude and spatial profile of the loads that a cell imparts on its matrix, as well as intracellular tension, which directs cell phenotype [43]. In the above study on electrospun PCL, varying fiber diameter from 0.3 to 1.3 μm did not affect cell behavior [86]. However, varying fiber diameter over a much wider range (~2–170 μm) significantly altered migratory patterns of epithelial cells [90]. On fibers with diameters 2–40 μm, individual “lead” cells were able to pull away from the cell monolayer, initiating collective migration. Thicker fibers, >40 μm, promoted cell morphology and migration more similar to those observed on planar surfaces, and collective migration was prohibited altogether on sub-micrometer fibers, consistent with studies showing a diameter threshold for adequate formation of focal adhesions [91]. Comparing these patterns to those on planar surfaces, it was deemed that the ability of the cells to wrap around fibers was key to their migratory behavior, again pointing to the localization of focal adhesions [90]. However, this study was carried out on drawn glass fibers; further investigation should determine if these patterns are conserved on electrospun materials that more closely mimic native ECM.

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#### 3.1.2. Impact of fiber alignment

Cell-matrix interaction is also influenced by the anisotropy, or degree of fiber alignment, in electrospun scaffolds. Alignment
impacts cell morphology (Fig. 1), including actin network structure and distribution of focal adhesions [96], as well as adhesion size [97]. The resulting directionality of mechanical interactions leads to migration, proliferation, and differentiation behaviors distinct from those in scaffolds with randomly oriented fibers. Electrospun fiber alignment patterns mimicking the ECM architecture of tissues such as tendons and ligaments [13], nervous tissues [98], and blood vessels [99] have been shown to direct cell phenotypes toward generation of the target tissue. Although fiber alignment does not directly impact the inherent modulus of individual fibers [91], the stiffness sensed by cells depends on the direction of cell tractions. Effective fiber stiffness is higher along the direction of alignment than orthogonally, which may further explain why cells tend to align and migrate along the fiber direction: they are better able to “push” and “pull” against the matrix in this direction, as opposed to causing fiber buckling in the orthogonal direction [49]. The effective stiffness also depends on the packing density of aligned fibers; in a study in collagen matrices, bundling of fibers resulted in increased effective stiffness (from \( \sim 1 \) kPa for a single collagen fiber to \( \sim 5 \) kPa for fiber bundles, measured using a 1-\( \mu \)m AFM tip), leading to more stable cell adhesions and greater rates of migration [95].

### 3.2. Impact of pore size

Pore size is a principal determinant of the performance of electrospun scaffolds for tissue engineering, as it impacts cell attachment and infiltration, nutrient flow, vascularization, and mechanical integrity. From the perspective of a cell, pore size impacts the geometry in which the cell attaches to the matrix, and, thus, the forces it can impart [100]. For example, pores larger than the cell can result in cells growing along single fibers, whereas cells may bridge across smaller pores to attach at multiple points in 3D [50,101]. These arrangements are demonstrated in images of fibroblasts in electrospun PCL scaffolds with large and small pores, Fig. 4b. The fibroblasts, \( \sim 20 \) \( \mu \)m in size, spread along matrix walls in scaffolds with pore sizes \( >20 \) \( \mu \)m, and better infiltrated the scaffold. For pore sizes \( 12.5 \) \( \mu \)m or smaller, cells bridged across the voids, seemingly enhancing inter-cell communication and boosting ECM production. This suggests that a mix of large and small pores may be needed to promote a combination of these tissue regenerative behaviors [101].

Besides directing attachment, pore size also affects progenitor cell differentiation, as this is partially dictated by the dimensions in which the cell may spread [102]. Pore size was also found to be a determinant of macrophage polarization in electrospun polydioxanone scaffolds; larger pores promoted cell infiltration and enabled cells to acquire a spread morphology, which was linked to anti-inflammatory signaling [103]. However, pore sizes in nanofibrous, electrospun constructs are typically of the order of 1 \( \mu \)m, prohibitively small for cell infiltration [21], and promoting infiltration remains a key challenge in these scaffolds [19,104].

Pore size scales with fiber diameter, especially at sub-micrometer diameters, where pore size decreases as the fiber-fiber contacts per unit length increase [105]. The packing density of fibers, particularly in aligned constructs, also affects the amount of void space through which cells may infiltrate the scaffold [13]. In cases where this void space is smaller than the cell, the cell may still migrate by amoeboid movement [106,107], deforming its cytoskeleton to “squeeze” through confined spaces. However, a recent study in reconstituted collagen matrices showed that the size and rigidity of the cell nucleus set a threshold pore size of \( \sim 10\% \) the nucleus size, below which the cell may no longer penetrate by deformation alone, but must rely on proteolytic processes, such as activation of matrix metalloproteinases to degrade the matrix [107]. This study found that the ability of a cell to deform and force through these small pores did not depend on matrix stiffness; however, only a small modulus range of 28–51 Pa was considered, measured on the cellular scale using a 10-\( \mu \)m AFM tip. Furthermore, nanofibers in electrospun scaffolds may be more conducive to cell migration through confined spaces, as fibers typically lie loosely on one another, compared to the bundled fibrils in native and reconstituted ECM matrices, which vary in density [108], impacting effective rigidity of the pore walls [95].

Further study of the mechanisms by which cells move through pores in electrospun scaffolds is needed to identify the optimal size range for infiltration. The solution may lie in a spatially varying distribution of micro- and nanofibers and pore sizes, promoting a combination of excellent cell attachment on nanofibrous surfaces with small pores, and infiltration through larger pores [21,109]. Perhaps a key obstacle in visualizing and quantifying cell movement and tractions within the pores of electrospun constructs is development of nondestructive imaging techniques that can penetrate these often opaque scaffolds, with sufficient resolution to capture cellular processes [110]. Micromechanical imaging techniques such as TFM are relatively straightforward to adapt to observe the effects of fiber properties within the first \( \sim 100 \) \( \mu \)m of a scaffold [60,84], but much more complex to extrapolate to the subsurface, turbid environment within 3D porous matrices.

The varying and sometimes contradictory findings of studies on scaffold geometry and cell behavior demonstrate that no single set of fiber diameter, alignment, and pore size values will promote particular behaviors for all cell types, and the target application must always be considered in interpreting results. For example, a range of pore sizes from 20 to 1500 \( \mu \)m have been reported to promote favorable cell behavior, depending on the application [111]. Designing scaffolds to direct cell morphology, and, therefore, mechanics, is dependent on the typical dimensionality of cells in the target tissue; for example, neurons have an elongated, 1D form, whereas chondrocytes tend toward spherical, 3D morphology [102].

### 3.3. Impact of material constituents

The micromechanical properties of electrospun scaffolds can be controlled by changing the fiber diameter and pore size; however, it is often desirable to maintain architectural parameters and vary mechanical properties independently. This can be achieved by manipulating the material constituents of the fiber, via altering the polymer chemistry or employing core-shell fiber technology.

#### 3.3.1. Altering polymer chemistry

Photoactivatable, crosslinking polymers allow tuning of mechanical properties by modifying polymers with varying levels of photoreactive groups or by varying photopolymerization time [112]. These polymers have been implemented into electrospun constructs to control micromechanics independently from geometry. A key study achieved direction of stem cell fate via mechanical cues on electrospun scaffolds of photoactivatable hyaluronic acid [60]. Single-fiber AFM determined the bending modulus of fibers, and the effects of soft (1.06 GPa bending modulus) versus stiff (8.6 GPa bending modulus) fibers on stem cell migration, proliferation, and gene expression were assessed, with the goal of directing stem cells toward chondrogenesis. Fiber mechanics affected gene expression, with softer fibers promoting chondrogenic markers, but did not significantly affect proliferation, migration, nor focal adhesion formations; they were found to depend more strongly on fiber adhesivity, varied by increasing ligand density. These findings are supported by studies in hydrogels that decoupled the effects of ligand distribution and substrate mechanics to show that both parameters direct stem cell fate [113]. To further understand the mechanical interactions at play, TFM experiments...
were performed by electrospinning a thin layer of fibers onto a glass coverslip and incorporating fluorescent beads for tracking fiber motion. Cells were able to displace soft fibers, whereas stiff fibers showed little to no displacement. This is one of the first studies to study cell tractions directly in an electrospun fiber network rather than on bulk hydrogels.

Another study changed fiber modulus by varying the photopolymerization time of electrospun polyethylene glycol dimethacrylate scaffolds [114]. As in the above study, fiber diameter and pore size were held constant, whilst modulus varied over a range of 2–15 kPa (as measured by compression testing). Upon seeding of stem cells onto the scaffolds, the softest scaffolds (~2 kPa) resulted in upregulation of endothelial cell markers, whilst the stiff scaffolds (>10 kPa) elicited smooth muscle cell (SMC) markers. In blood vessels in vivo, the endothelial cell layer is softer than the SMC layer, showing correspondence with the stem cell behavior in this study. The degree of cell spreading was greater on higher modulus scaffolds; this may have led to differences in the SMC line, as SMCs have greater striation and, therefore, the aptitude of cells to attach and mechanically interact with their environment [67]. Core-shell, or coaxial, fibers offer an attractive alternative in which mechanical properties may be controlled independently of surface chemistry and scaffold geometry [116, 117]. Stiffness of these constructs has been shown to scale with core diameter, and in one study led to improved adaptation, allowing optimization of cell contractility versus proliferation toward favorable phenotypes for vascular regeneration (Fig. 5a, right). Although this study did not characterize scaffold elasticity on the microscale, it reinforced the importance of both mechanical factors and surface factors (ligand density) in determining cell behavior on electrospun scaffolds.

Incorporation of natural components or minerals into electrospun scaffolds, intended to improve biocompatibility, can also impact micromechanics. One study showed improved chondrogenesis in electrospun scaffolds made from a blend of PCL and cartilage-derived matrix, but found that the elasticity of these scaffolds was only 1% of that of neat PCL scaffolds, rendering them unsuitable for implantation [63]. In bone tissue engineering, hydroxyapatite has been incorporated in electrospun scaffolds to improve osteoinductivity and bulk elasticity [23]. However, at the microscale, AFM measurements of fiber bending modulus showed that hydroxyapatite up to only ~20% by weight reinforced fiber mechanics; beyond this, modulus decreased (from ~25 GPa at 20% to ~18 GPa at 40%), which can adversely affect cell behavior [74]. These studies highlight potential tradeoffs in tuning the biological and micromechanical environments in scaffold design.

### 3.3.2. Core-shell fibers

A drawback of altering fiber mechanics via techniques such as photopolymerization and blending is that an interdependence of mechanics and surface chemistry is introduced, and their effects on cell behavior are difficult to separate. For instance, polymer blends can alter surface roughness and hydrophilicity of fibers, and, therefore, the aptitude of cells to attach and mechanically interact with their environment [67]. Core-shell, or coaxial, fibers offer an attractive alternative in which mechanical properties may be controlled independently of surface chemistry and scaffold geometry [116, 117]. Stiffness of these constructs has been shown to scale with core diameter, and in one study led to improved
mechanical performance of engineered skin [118]. Combining the mechanical properties of two polymers also offers the ability to mimic complex properties, such as mechanical nonlinearity of smooth muscle, which is essential for cardiac function [34,119]. Cardiomyocytes seeded onto nonlinear scaffolds formed from poly(glycerol sebacate)/poly(l-lactic acid) core-shell fibers exhibited beating phenotypes for several weeks in culture, with physiological beating rates [119]. Another study employing poly(ether sulfone)(PES)/PCL core-shell fibers showed that altering stiffness by core-shell design could direct stem cell fate, whilst holding geometry and surface chemistry constant [120]. Stiff, core-shell fibers (30.6 MPa) promoted high density of stress fibers in progenitor cells, leading to osteogenesis, whilst softer PCL fibers (7.1 MPa) tended to promote rounded cell morphologies and chondrogenesis. This trend was linked to the varying ability of cells to deflect individual fibers, as shown using a beam-bending model, Fig. 5b [120].

Although core-shell fibers show promise for controlling cell-matrix mechanical interaction independently of surface chemistry, none of the above studies have measured the micromechanics of core-shell fibers on the cell-sensing scale. Recently, a multiscale mechanical study developed models for predicting individual core-shell fiber modulus based on material constituents, and macroscale modulus based on scaffold porosity and mass density [121]. This work aimed to enable customizable micro- and macro-scale mechanical properties in electrospun scaffolds and, importantly, reinforced that it is possible to control individual fiber stiffness independently of both surface chemistry and bulk scaffold stiffness. However, further studies using AFM or other micromechanical imaging techniques are needed to validate how the core-shell geometry impacts individual fiber mechanics, and, in turn, how cells respond.

4. Mechanical remodeling of electrospun scaffolds during tissue development

Reciprocally to direction of cell behavior by matrix micromechanics, cells alter the mechanical properties of their environment throughout tissue development, via deposition of new ECM, cellular tractions, and tissue remodeling [122]. Additionally, an electrospun matrix undergoes dynamic mechanical changes throughout its lifetime, due to degradation and responses to extrinsic loading. In this section, we describe such longitudinal mechanical changes and consider their implications for tissue regeneration.

4.1. Impact of matrix turnover: ECM production and scaffold degradation

Production of new ECM by cells that infiltrate electrospun scaffolds, both in vitro and in vivo, affects the scaffold’s mechanical properties. Several studies toward engineering of load bearing tissues have shown that ECM deposition in electrospun PCL scaffolds over periods of several weeks rendered constructs with improved mechanical properties (Young’s modulus and yield strain) more similar to those of native tissues [13]. The viscoelastic and nonlinear properties of these constructs also tended toward those of native tissues with increasing ECM deposition [13]. Furthermore, the orientation of deposited collagen, and thus, the anisotropic mechanical properties, depended on electrospun fiber alignment [13]. A key study used the mechanical effect of ECM deposition to overcome one of the paradoxes in electrospun scaffolds: the need to increase porosity whilst maintaining mechanical integrity. Scaffolds were electrospun containing two fiber types: PCL fibers and up to 60% water-soluble, sacrificial poly(ethylene oxide) fibers, lending increased porosity and allowing complete cellular infiltration of the scaffolds. After 12 weeks in culture and 4 weeks implanted in mice knee menisci, the bulk tensile properties of the high porosity scaffolds actually exceeded those of the PCL-only scaffolds, due to increased production of collagen in 3D throughout the scaffold [123]. This opens the possibility to engineer tissues that quickly adapt to fulfill their mechanical function in vivo.

Measurements of mechanical properties at the microscale following ECM deposition, however, showed a different response: AFM measurements (using a 25-μm spherical tip) on stem-cell-seed PCL electrospun scaffolds after 28 days in culture showed lower Young’s modulus than acellular scaffolds [63]. This was attributed to the production of new tissues by the cells on the scaffold surface, which are softer than the PCL fibers, as supported by previous AFM studies of stem cell mechanical properties [124]. However, in contrast to the above studies on bulk mechanical properties of aligned electrospun scaffolds, these scaffolds had a random fiber orientation, and, thus, the collagen produced did not exhibit an aligned structure, which may have contributed to the lower modulus values. At an even smaller scale, as measured by AFM nanoindentation using a 20-nm tip, cell seeding and ECM deposition by fibroblasts after 10 days in a collagen/chitosan hydrogel increased the scaffold Young’s modulus. Whilst this was not in an electrospun environment, it again highlights differences in mechanical properties across length scales [125].

To reconcile these disparate findings on the effect of ECM production on mechanics across length scales and scaffold types, techniques to visualize deposition of ECM matrix proteins, namely collagen, may elucidate the structure-property relationships at play. One solution may be to use combined microscopy techniques, such as second harmonic generation to reveal detailed information about collagen structure and organization [126], and elastography to quantify local mechanical properties [78]. Importantly, both techniques may be translated in vivo for monitoring implanted constructs [78,127].

At the same time that cells deposit ECM, the original electrospun scaffold degrades via hydrolytic and cell-mediated proteolytic processes [23]. The effect of degradation on mechanical properties is material-dependent. For example, the bulk tensile properties of electrospun PCL (a slowly degrading polymer) remained relatively unchanged after 63 days in culture, but when PCL was blended with PLGA, significant mass loss and reduction of tensile properties of the electrospun scaffold was observed over this time period [65]. Whilst these and other synthetic polymers degrade mainly through hydrolysis, native ECM also degrades through cell-mediated enzymatic activity [128]. As shown in hyaluronic acid hydrogels, as stem cells degraded their local matrix, they were able to change morphology and apply tractions that helped direct them toward osteogenesis (as opposed to adipogenesis when enzymatic matrix degradation was inhibited) [129]. Similar studies of local degradation and its interplay with cellular tractions are needed in electrospun environments.

4.2. Impact of extrinsic loading

Electrospun scaffolds experience mechanical loads from two key sources: physiological loading (fluid flow, load bearing or muscular contraction), and cellular tractions. Physiological loading is dependent on the application; for example, the loading cycles of engineered skin differ from those of cardiac tissue or ligaments. Physiological loads play an important role in cell behavior and tissue development via mechanotransduction. The mechanisms by which extrinsic loads contribute to tissue development, and how these loads are recapitulated in bioreactors, are reviewed elsewhere [51,130]. Here, we focus on how loading directly impacts electrospun scaffold mechanical properties at the microscale.
In fibrous tissues such as cartilage, loads applied at the bulk scale are attenuated at the local cellular and nuclear levels by 35–70%. However, in aligned, electrospun PCL scaffolds, similar loads were conserved (15–25% attenuation), causing significant elongation of cells and their nuclei [131]. This disparity in microscale behavior between native and engineered cartilage was hypothesized to be due to the nonfibrous, proteoglycan-rich microdomains within native cartilage, in which cells are more rounded and experience less force, as bulk loading is transferred via fibers. Similar nonfibrous microdomains were incorporated into electrospun PCL scaffolds by seeding pellets of fibrochondrocytes and stem cells in layers in the scaffold. These heterogeneous constructs closely mimicked native ECM microstructure (Fig. 6a, top), micromechanics (according to increased heterogeneity in local strain in scaffolds under tension, Fig. 6a, bottom panels) and mechanobiology (reduced calcium signaling due to tensile loading) [132]. Thus, cells do not interact solely with fibrous tissues, but also with nonfibrous regions, which contribute to overall health of tissue, and should perhaps be incorporated in any truly biomechanic scaffold. Furthermore, bulk loads can be attenuated in tissues by microstructural effects such as sliding and reorientation of fibers and uncrimping of the wavy structure characteristic of collagen [133]. Similar fiber sliding and rotation has been observed in electrospun collagen scaffolds under tensile loading [134]. Also, self-crimping electrospun poly(D,L-lactide-co-D,L-lactide) fibers have been fabricated that mimic collagen structure and promote ECM production by fibroblasts [135]. Finally, it is worth considering whether, beyond scaffold heterogeneity and fiber microstructure, the material constituents of fibers may impact load attenuation. For instance, blending of natural and synthetic polymers in electrospun fibers can result in a nonlinear stress-strain response of the construct [36]; perhaps, then, the transfer of stress from fibers to cells may also increase nonlinearly with strain. Experiments that investigate the impact of fiber constituents on macro- to microscale load transfer would provide valuable insight to scaffold design.

In anisotropic tissues, it is imperative to consider the direction of the applied load relative to fiber alignment. Cells and their nuclei tend to orient their morphologies in the direction of fiber alignment in anisotropic tissues such as cartilage, a behavior mimicked in anisotropic electrospun scaffolds [40,136]. In one study, tensile loading of electrospun PCL scaffolds in the direction of fiber alignment further elongated seeded stem cells and their nuclei, whilst loading perpendicular to the fibers had a relatively minor effect on cell morphology. These loads were shown to be transmitted to the cell via its actin networks, rather than microtubules or vinculin networks [40]. This study also showed a time-dependent response to loading, as the cells and nuclei relaxed back toward their undeformed state when loading of the electrospun matrix was held constant. Further work is required to analyze cellular deformation in 3D to fully characterize this anisotropic behavior, perhaps using advanced confocal imaging. Imaging of cells that have infiltrated into the scaffold, rather than those at or near the surface, would also provide needed insight into mechanical behavior in truly 3D environments.

The magnitude of loading must also be considered in directing cell behavior. A recent study examined the effect of cyclic strain on macrophage phenotype when seeded on electrospun PCL scaffolds [137]. The effect of scaffold mechanics on macrophage phenotype is an important emerging area of study, as these immune cells play a key role in determining whether implanted biomaterials are integrated (via anti-inflammatory and pro-angiogenic signaling) or rejected (via pro-inflammatory and tissue-destructive signaling) [138]. The study found that 7% cyclic strain promoted...
macrophage polarization toward an anti-inflammatory phenotype, whereas larger strains resulted in pro-inflammatory signaling [137]. Further study is required to uncover the mechanisms underlying macrophage response to electrospun scaffold morphology and mechanics.

Electrospun fibers also experience shear stresses due to fluid motion through the scaffold. Whilst shear stresses experienced by cells can significantly alter phenotype via mechanotransduction [51], these stresses are typically not sufficient to permanently alter scaffold microstructure and intrinsic mechanical properties. However, the scaffold structure itself partially determines the magnitude of the stresses; for example, smaller pore size leads to increased shear stresses at the pore walls and at cell surfaces. Such an effect led to increased osteogenic activity (calcium deposition) in electrospun PCL scaffolds seeded with stem cells in a perfusion bioreactor; as cells produced ECM over a 16 day period, the pore size of the scaffolds decreased, and shear stresses increased [139]. In a vascular tissue engineering application, electrospun PCL/collagen scaffolds with aligned fibers, designed to withstand the cyclic shear stresses due to blood flow, induced endothelial cell phenotypes with improved attachment to the matrix under physiological flow conditions [140]. Favorable smooth muscle cell and endothelial cell phenotypes have also been induced in electrospun silk vascular grafts under dynamic flow conditions [141].

Cellular tractions, imparted to sense the local mechanical environment, also change the environment [142]. A common manifestation of cell-mediated forces is scaffold contraction; seeding cells onto electrospun scaffolds can cause shrinkage if the elasticity is insufficient to resist cellular loads [143]. However, some degree of local contraction is necessary for cell processes including migration and differentiation. In collagen matrices, fibers must be able to bend and move relative to each other; fixing fibers in place halts cell motility [144]. In addition, cell contraction of collagen matrices causes local increases in matrix stiffness due to its nonlinear mechanics, which, in turn, alters the mechanical environment of neighboring cells, providing a means of inter-cellular communication [32]. This points to a need for similar studies on cell tractions in electrospun matrices, including quantification of cell-induced fiber deformation, to better understand the interplay between cell contraction, motility, and downstream behaviors including differentiation.

Toward this end, a recent study observed cell-fiber interaction in electrospun dextran scaffolds [145]. The scaffolds induced similar cell morphology and matrix contraction as in native collagen matrices, but with the advantage of greater control over fiber stiffness (via photo-crosslinking), diameter (via polymer concentration) and alignment (via collector rotation). The authors showed that in hydrogel substrates, cell spreading and proliferation increases with substrate stiffness, whereas in 3D fibrillar constructs, increasing stiffness of individual fibers (measured using AFM) inhibits cell proliferation. Observation of cell-induced fiber deformation showed that, in softer dextran fibers (bending modulus 140 MPa) cellular forces were sufficient to recruit nearby fibers, effectively increasing local ligand concentration and promoting cell proliferation, as seen in Fig. 6b. Fiber deformation in stiff fibers (bending modulus 3.1 GPa), however, was negligible, and cell proliferation was commensurately reduced [145]. This study highlights the added complexity of cell behaviors in 3D, fibrillar environments compared to hydrogels, and calls into question the generality of mechanobiology concepts such as durotaxis.

5. Outlook and conclusion

Throughout these studies, an inextricable link emerges between electrospun matrix microstructure, inherent matrix stiffness, and cell response. The microstructure, including fiber diameter, pore size, and scaffold alignment, dictates the cell morphology, cytoskeletal and focal adhesion arrangements, and, therefore, the spatial profile with which a cell can load its substrate. The inherent matrix stiffness, then, altered via polymer concentration (fiber diameter, Section 3.1), polymer crosslinking (Section 3.3.1), and matrix material components (blend fibers, Section 3.3.1, and core-shell fibers, Section 3.3.2), determines the degree of matrix deformation (strain) induced by cell tractions. The degree of matrix deformation has implications for intracellular mechanosignaling, leading to distinct differentiation pathways in stem cells, and also determines the cell’s ability to alter its local environment, via fiber recruitment to effectively increase local ligand density and promote proliferation, or via nonlinear strain-hardening of the local matrix, critical to migration patterns and inter-cellular communication. Fig. 7 illustrates this reciprocity between matrix microstructure, stiffness, cell tractions, and cell behavior, and summarizes key parameters that alter the local matrix stiffness in electrospun environments.

These microscale mechanical phenomena, many of which are distinct from those observed previously on smooth, hydrogel substrates, have only come to light by studying mechanics and cell behavior in fibrillar, electrospun scaffolds. Techniques to accurately measure and image microscale mechanical properties and cell tractions, such as AFM and TFM, have been critical to elucidating how mechanics drive cell behavior, and, reciprocally, how mechanical properties are altered over time by cell-mediated matrix deformation and turnover. Noninvasive, 3D imaging is expected to play an increasing role in monitoring matrix mechanics, including second harmonic generation microscopy for visualizing matrix alignment [126], and rapid, motion-sensitive techniques such as optical elastography for mapping microscale elasticity [79]. Systematic comparison of such techniques to the values obtained using the more common AFM would indicate how mechanics differ across the nano- to micro-length scales. In addition, further extension of 3D TFM to electrospun environments will be key to quantifying the role of cellular forces in guiding behavior. Translation of these techniques in vivo is also critical for monitoring scaffold mechanics in animal and human studies. Noninvasive, high speed, in vivo optical elastography and nonlinear microscopy of skin and other epithelial tissues are feasible using handheld scanners [146,147] or endoscopic probes [148]. For accessing deep, solid...
tissue for high-resolution elastography, the optics may also be delivered via novel needle probes [149]. Although much progress has been made over the past decade in using electrospun scaffolds to guide cell behavior, further development is needed to more closely mimic in vivo cell-matrix interaction. For example, a number of studies have been carried out on relatively hydrophobic fibers, such as PCL, whereas hydrophilic substrates, in as native ECM, give rise to distinct cell attachment and morphology [67], impacting downstream mechanical processes. In addition, most electrospun mats are relatively thin, of the order of 0.1–1 mm, and development of truly 3D constructs is imperative, not only to produce anatomically appropriate constructs for implantation, but also to study how cells behave in realistic, “thick” tissues, as dimensionality has been shown to be as important to cell behavior as stiffness and fiber alignment [150]. Increasingly flexible control over scaffold properties is also needed to pinpoint how various environmental stimuli direct cells. Use of core-shell fibers to independently control fiber mechanics and surface chemistry is expected to provide excellent adaptability to trigger a spectrum of cell responses. Repeatability and refinement of the electrospinning process must also be considered, as significant intra- and inter-sample variation confounds comparison of results and restricts development of robust computational models to predict scaffold mechanical behavior [122,151].

In moving toward scaffolds that promote tissue regeneration in vivo, all of the above must be considered in the context of the target application. Whilst the emphasis in this review has been on fibrillar extracellular environments, it is worth noting that tissues such as the brain and the liver exhibit more amorphous microstructures, and electrospinning alone may not be sufficient for replicating these structures. In such cases, composite scaffolds combining electrospun fibers with hydrogels [152] may provide an adaptable solution.

Finally, the concepts explored here extend beyond the field of regenerative medicine to that of tumor mechanobiology and its role in cancer genesis, progression, and metastasis. The biomechanics and controllability offered by electrospinning may be used to engineer tumor environments, providing a platform for studying cell-matrix mechanical interaction in cancer and for developing therapeutics [153]. Furthermore, electrospun scaffolds may serve as platforms for investigating the mechanics of tissue morphogenesis, especially interaction of stem cells and progenitor cells with their environment in vitro [154,155].

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