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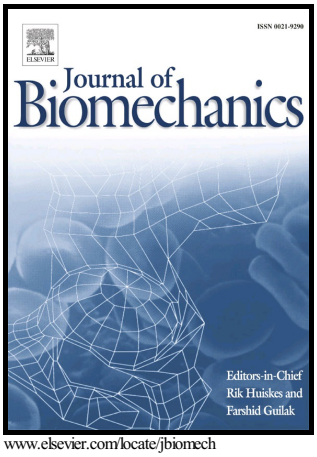

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The many ways adherent cells respond to applied stretch

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Abstract

Cells in various tissues are subjected to mechanical stress and strain that have profound effects on cell architecture and function. The specific response of the cell to applied strain depends on multiple factors, including cell contractility, spatial and temporal strain pattern, and substrate dimensionality and rigidity. Recent work has demonstrated that the cell response to applied strain depends on a complex combination of these factors, but the way these factors interact to elicit a specific response is not intuitive. We submit that an understanding of the integrated response of a cell to these factors will provide new insight into mechanobiology and contribute to the effective design of deformable engineered scaffolds meant to provide appropriate mechanical cues to the resident cells.

Keywords: Cell mechanics; Cytoskeleton; Mechanobiology; Tissue engineering

Word Count: 4071

Introduction

Nearly all cells in the human body are continually subjected to mechanical forces due to tensile stress and strain imposed at the tissue level. These mechanical forces have a crucial role in tissue development and homeostasis. A disruption in the ability of cells to properly respond to mechanical cues contribute to the etiology of many important diseases such as osteoporosis, deafness, atherosclerosis, cancer, osteoarthritis, muscular dystrophies, and developmental disorders (Ingber 2003, Jaalouk and Lammerding 2009, DuFort et al. 2011). A variety of methods have been developed to investigate the influence of tensile strain on cells cultured on elastomeric sheets with a common result that cells tend to align themselves perpendicular to the direction of stretch (Wang et al. 1995, Wang, Goldschmidt-Clermont et al. 2001, Neidlinger-Wilke et al. 2002, Moretti et al. 2004). This reorientation response has been found in many different cell types such as endothelial cells (Dartsch and Betz 1989), smooth muscle cells (Kanda et al. 1992), fibroblasts (Jungbauer et al. 2008), tumor cells and mesenchymal stem cells (Tondon and Kaunas 2014).

How cells respond to applied strain is highly dependent on the contractile state of the cell, the spatial and temporal pattern of applied strain, and the dimensionality and mechanical properties of the substrate (Figure 1). The responses range from morphological changes, signal transduction and functions such as cell proliferation, apoptosis, migration and differentiation. Mechanotransduction and mechanosensing of forces transmitted to cells from the extracellular matrix has been the focus of excellent recent reviews (Roca-Cusachs et al. 2012, Haase et al. 2014, Humphrey et al. 2014). This review seeks to instead highlight and compare results from stretching cells on and in synthetic and natural scaffold materials, focusing on the influence of the parameters that modulate morphological changes in response to mechanical stretch.

Cell Cytoskeleton and Contractility

The cell cytoskeleton, consisting of actin microfilaments, microtubules and intermediate filaments, forms the dynamic architecture of the cell. Each of these cytoskeletal protein networks contribute to cell polarity and migration (Etienne-Manneville 2013, Akhshi et al. 2014, Leduc and Etienne-Manneville 2015). Locally applied forces promote directed microtubule growth in the direction of force application (Kaverina et al. 2002), while a step increase in equibiaxial strain increases total polymerized microtubule mass (Putnam et al. 2001). Goldyn et al. (2009, 2010) demonstrated that a functional microtubule network is not required for cyclic stretch-induced cell alignment perpendicular to the direction of stretch, though the rate of realignment is dependent on microtubule stability. While it is well established that intermediate filaments contribute to the mechanical integrity and organization of cells (Kim and Coulombe 2007), their role in stretch-induced morphological changes is relatively minor. For example, myoblasts expressing mutated desmin showed altered dynamics of perpendicular alignment in response to cyclic stretching, yet still aligned to a comparable extent as myoblasts expressing wild-type desmin (Leccia et al. 2013). In contrast, disruption of the actin cytoskeleton completely blocks stretch-induced cell alignment (Kaunas et al. 2006, Goldyn et al. 2009).

Actin filaments are organized via actin-associated proteins into higher order structures, including lamellipodia and filopodia. Actin microfilaments can also form contractile bundles of parallel microfilaments, termed *stress fibers*, through interactions with nonmuscle myosin II. Stress fibers are the major tension-bearing structures in adherent, non-muscle cells (Burridge 1981) and are also critical to cell functions involving cell alignment (Goldyn et al. 2009, Hsu et al. 2009), contractility (Chrzanowska-Wodnicka and Burridge 1996), and cell adhesion (Tojkander et al. 2012). Stress fibers are anchored to focal adhesions, which consist of integrins and many other so-called focal adhesion proteins that serve to physically connect the extracellular matrix to the actin cytoskeleton and sense mechanical

forces (Cramer et al. 1997, Naumanen et al. 2008). Stretch-induced cell alignment is generally preceded by alignment of stress fibers (Goldyn et al. 2010, Faust et al. 2011).

Live cell imaging of fluorescently-labeled actin has revealed that stretch-induced stress fiber reorganization can occur through at least two mechanisms (Goldyn et al. 2009, Lee et al. 2010, Chen et al. 2012). The Kemkemer lab demonstrated focal adhesion sliding (Goldyn et al. 2009) and associated stress fiber rotation (Chen et al. 2012) perpendicular to the direction of cyclic uniaxial strain. Data from our group indicate that the process of stress fiber reorganization is a little more complex. In addition to some stress fiber rotation, there is also substantial stress fiber turnover and fusion (Lee et al. 2010, Hotulainen and Lappalainen 2006).

Modulation of myosin II activity substantially affects stretch-induced actin and cell reorganization. The extent of Myosin Light Chain (MLC) phosphorylation determines ATPase activity of the Myosin Heavy Chain, hence regulates the level of contractile force that can be generated. In a separate pathway, Rho-kinase and myosin light chain kinase (MLCK) regulate MLC activity both directly through MLC phosphorylation and indirectly through phosphorylation and deactivation of MLC phosphatase. Further, these MLC kinases regulate different populations of stress fibers (Totsukawa et al. 2000, Katoh et al. 2007). Specifically, the MLCK inhibitor ML7 primarily inhibits assembly of peripheral stress fibers, while the Rho-kinase inhibitor Y27632 inhibits assembly of central stress fibers (Totsukawa et al. 2000, Katoh et al. 2001). Subjecting cells treated with ML7 or Y27632 to cyclic uniaxial stretch results in the *de novo* formation of stress fibers parallel to the direction of stretch in the central and peripheral subregions of the cell, respectively (Lee et al. 2010). Rho-kinase activity is regulated by Rho GTPase and inhibition of Rho GTPase with a dominant-negative mutant RhoN17 has a similar effect as Y27632 treatment on stretch-induced stress fiber assembly parallel to the direction of stretch (Kaunas et al. 2005). In contrast,

a constitutively-active mutant of Rho GTPase increases the extent of stress fiber alignment perpendicular to the direction of stretch compared to cells with normal Rho activity (Kaunas et al. 2005).

Spatial Strain Pattern

A variety of methods have been developed to investigate the influence of mechanical strain on cells, with the most widely used methods involving flat elastomeric sheets (Table 1). The elastomer of choice is polydimethylsiloxane (PDMS), though polyurethane film has also been used (Barbee et al. 1994). By coating the sheet with extracellular matrix molecules, e.g. fibronectin and collagen, strain applied to the sheet is transmitted to the cells via matrix-integrin bonds. These coatings generally result in good cell adhesion with cells stretching in registry with the sheet, though thin cell processes and bipolar cells have been reported to experience strains different than that of the substrate (Barbee et al. 1994).

Early studies applying cyclic uniaxial strain by direct axial pulling on opposing ends of a sheet resulted in either perpendicular or oblique alignment of the cells and stress fibers relative to the strain axis (Buck 1980, Dartsch et al. 1986, Dartsch and Betz 1989). The extent of alignment is proportional to strain magnitude above a threshold level of 0.03 (Kaunas et al. 2005, Takemasa et al. 1997). Careful analyses of the substrate strains indicated that lateral contraction during axial pulling (i.e., Poisson effect) was responsible for oblique alignment and that the cells and stress fibers orient in the direction of lowest normal strain magnitude (Takemasa et al. 1998, Wang 2000, Wang et al. 2001b). In contrast, cyclic equibiaxial stretch results in no cell or stress fiber alignment (Wang et al. 2001b, Kaunas et al. 2006). A recent study investigating the effects of the ratio $r = -\varepsilon_l/\varepsilon_a$, where ε_a and ε_l are the substrate strains in the axial and lateral directions, demonstrated that the angle of alignment is not actually the direction of lowest normal strain (Livne et al. 2014). Instead, the cells oriented in a direction θ that minimizes elastic strain energy density and depends on the value of r and the elastic anisotropy of the cell, i.e. the ratio of

the elasticities along the cell major and minor axes. These authors went further to show that the rate of alignment τ could be described in terms of a characteristic time and that both θ and τ do not depend on the applied strain magnitude, frequency or substrate rigidity (0.02 – 1 MPa), though the strain frequency range tested (1.2-12Hz) was above the saturation frequency determined in previous reports (Jungbauer et al. 2008, Hsu et al. 2009).

Mechanotransduction is also dependent on the spatial pattern of strain. Hornberger et al. (2005) demonstrated that uniaxial and multiaxial stretch induced an increase in extracellular signal regulated kinase (ERK) and Protein Kinase B phosphorylation, but only multiaxial stretch induced ribosomal S6 kinase phosphorylation. Lee et al. (1999) reported that a transient uniaxial stretch induced a larger increase in fibronectin mRNA expression than that induced by equibiaxial stretch. Cyclic equibiaxial strain induces sustained activation of JNK, while JNK activation induced by cyclic uniaxial strain subsides as stress fibers align away from the stretch direction (Kaunas et al. 2006). These results demonstrate that stress fiber realignment provides a mechanism for modulating mechanotransduction events.

Local cell ruffling in lamellipodia is also affected by the spatial strain pattern. Katsumi et al. (2002) reported that equibiaxial step stretch uniformly and transiently decreases lamellipodia formation due to inhibition of Rac1 GTPase. In contrast, uniaxial step stretch increased lamellipodia formation and Rac1 activity at the axial ends of the cell, while decreasing ruffling along the lateral sides. This is consistent with the previous finding that tension applied to the cell suppresses lateral protrusive activity (Kolega 1986). It has been hypothesized that directional ruffling is a prerequisite for stress fiber reorientation to cyclic stretch. Huang et al. (2010) demonstrated that edge ruffling was concentrated perpendicular to the direction of cyclic uniaxial stretch axis, consistent with the eventual stress fiber alignment direction as reported previously (Goldyn et al. 2009). Inhibition of actin-related protein-2/3 (Arp 2/3), which

suppressed lamellipodia to result in a non-elongated morphology did, not block stress fiber reorientation perpendicular to the stretch direction (Huang et al. 2010), suggesting that directional edge ruffling is not a primary mechanism guiding cytoskeletal alignment in response to stretch. Decoupling of cell shape and cytoskeletal alignment in response to stretch has also been observed, where inhibition of stretch-activated cation channels or focal adhesion kinase (FAK) suppresses cell, but not stress fiber, alignment(Hayakawa et al. 2001, Hsu et al. 2010).

Temporal Pattern of Strain

The extent of cell and stress fiber alignment depends not only on strain magnitude, but also strain frequency (Jungbauer et al. 2008, Hsu et al. 2009). Specifically, alignment increases with increasing strain frequency up to a saturation value of ~1Hz, above which there is no increase in alignment (Jungbauer et al. 2008). Various theoretical models have been proposed to explain the dependence of stretch-induced alignment responses on strain frequency. Models based on actomyosin motor dynamics predict that no alignment occurs at low strain-rates when myosin motors are able to relax perturbations in stress fiber tension, while stress fibers are forced to align in the direction of lowest normal strain through stress fiber turnover and/or focal adhesion turnover and stress fiber rotation when strain-rates exceed the speed that myosin motors can respond (Safran and De 2009, Kaunas et al. 2011, Zhong et al. 2011, Chen et al. 2012). Models based on actin polymerization dynamics predicts that stress fibers disassemble in the direction of rapid fiber shortening, resulting in frequency-dependent alignment (Wei et al. 2008, Obbink-Huizer et al. 2013).

Modification of the typical temporal strain patterns typically used in cyclic stretch experiments, e.g. sinusoidal or sawtooth patterns, reveals that stretch-induced cell morphological changes are much more sensitive to strain rate than strain frequency (Tondon et al. 2012). A square-wave pattern applied at sub-

saturation frequencies (0.01 and 0.1Hz) induces significantly greater stress fiber alignment than a sawtooth pattern. Subjecting the cells to asymmetric sawtooth waveforms, i.e. rapid lengthening/slow shortening or vice-versa, reveals that the cells are much more responsive to the rate of lengthening than the rate of shortening. A theoretical model was developed that incorporated force-dependent bond disassembly (Bell 1978) in stress fibers to describe these observations. The model predicts stress fibers are more sensitive to positive perturbations in tension (increased tension) than negative perturbation (decreased tension).

Rapidly applied static strain causes transient responses, including inhibition of membrane ruffling (Katsumi et al. 2002), increased cell-matrix traction forces (Gavara et al. 2008, Mann et al. 2012), cytoskeletal fluidization and resolidification (Trepat et al. 2007) and intracellular signaling without substantial changes in stress fiber organization (Hsu et al. 2010) or filamentous actin content (Sato et al. 2005). Our group has demonstrated that a rapidly applied static strain transiently activates JNK, ERK and p38, but no activation is observed when the rate of strain is decreased by 100-fold (Hsu et al. 2010). Similarly, the activation of these kinases in response to cyclic stretching at 1 Hz is reduced at lower frequencies and absent upon stretching at 0.01Hz (Hosokawa et al. 2002, Hsu et al. 2010).

Research in the Fredberg lab has revealed that subjecting cells to a 4-second equibiaxial stretch-unstretch maneuver results in a sudden drop and gradual recovery in cell traction forces, which was attributed to fluidization and subsequent resolidification of the cytoskeletal lattice (Krishnan et al. 2009). The fluidization-resolidification response involves disassembly and reassembly of stress fibers and fluidization and this is not observed when a negative stretch-unstretch maneuver is applied, i.e. compression and release (Chen et. al 2010). Performing the stretch-unstretch maneuver with a uniaxial strain pattern also resulted in fluidization, but the traction forces increase above baseline (i.e.

reinforcement). Periodic application of uniaxial stretch-unstretch cycles results in a prompt fluidization response followed by slow resolidification typified by recovery of the traction forces, but the traction forces gradually align perpendicular to the stretch direction (Krishnan et al. 2012). Theoretical modeling predicts that myosin unloading during the unstretching immediately after stretching triggers stress fiber disassembly due to sudden loss of tension, while compression and release maneuver maintains positive tension at all times and thus does not lead to disassembly (Wu and Feng 2015). This suggests that the reorientation of the traction forces in response to periodic stretch-unstretching is an adaptative response that stabilizes the stress fibers oriented in a direction that avoids stretching.

Dimensionality

There are striking disparities between cell behavior in 2-D and physiologically relevant 3-D environments (Cukierman et al. 2001). Unlike cells cultured on flat substrates, cells in 3-D have no apical-basal polarity, interact with network of fibrils, and have integrin-mediated adhesions all around the cell surface (Baker and Chen 2012). Studies from 20-30 years ago demonstrated that cells embedded within collagen matrices spontaneously align as they contract the matrix in a manner that depends on the boundary constraints (Harris et al. 1984, Klebe et al. 1989, Kolodney and Elson 1993, L'Heureux et al. 1993). Barocas and Tranquillo (Barocas and Tranquillo 1997) developed an anisotropic biphasic theory that describes the simultaneous co-alignment of cells and fibrillar matrix network (i.e. contact guidance) as a result of cell traction and matrix reorganization. For cells in collagen matrix anchored at opposing ends, this theory correctly describes the co-alignment of cells and fibrils along the confined axis and predicts alignment is the result of cells pulling on and translocating along fibrils to result in fibril alignment in the direction of greatest resistance to gel compaction. Collagen synthesis and proteolysis via matrix metalloproteinases (MMPs) are also critical to tissue remodeling. Cyclic strain induces matrix expression

in both 2-D (Leung et al. 1976) and 3-D cultures (Trachslin et al. 1999). Cardiomyocyte alignment in collagen matrices is blocked in cultures treated with a general MMP inhibitor (Nichol et al. 2008).

Cells subjected to step or cyclic uniaxial strain in 3D collagen and fibrin gels constrained at opposing ends co-align with collagen fibrils parallel to the direction of strain to a greater extent than cells subjected to a static stretch (Nieponice et al. 2007). Moreover, strain-induced increases and decreases in tension are observed to relax perturbations in tension, suggesting that cells tend to maintain a homeostatic level of tension, i.e. tensional homeostasis (Brown et al. 1998). A step increase in strain can also cause a sudden loss of tension associated with dramatic actin cytoskeletal turnover as tension is restored (Nekouzadeh et al. 2008). Pang et al. (2011) subjected cells to a static uniaxial strain and observed initial cellular alignment parallel to the strain axis beginning within 2h, reaching completion after 6h when collagen fibril alignment localized to the front of cell protrusions became apparent. These authors proposed that collagen alignment occurs as a consequence of observed cell migration parallel to the direction of stretch. Interestingly, intact microvessels extracted from adipose tissue and suspended in collagen gels are also observed to align along the confined axis in non-stretched gels to a comparable extent as when subjected to static or cyclic uniaxial strain (Krishnan et al. 2008). Microvessel alignment was associated with collagen fibril co-alignment, suggesting that ensemble contractile forces generated by cells within individual microvessels behaved similarly to that of individual cells in the aforementioned studies with dispersed cell populations.

Cells are strongly oriented on engineered substrates containing contact guidance cues such as aligned micro- and nano-grooves or micro-patterned cell-adhesive islands (Clark et al. 1990, den Braber et al. 1996, Flemming et al. 1999, Teixeira et al. 2003, Ahmed et al. 2010). When cells cultured in micro-grooved surfaces are subjected to cyclic uniaxial strain parallel to the groove alignment, the cells remain

aligned along the grooves rather than aligning perpendicular to the strain direction (Wang and Grood 2000, Wang et al. 2000). Cyclic strain applied perpendicular to the grooves enhances cell alignment relative to that when the strain is applied parallel to the grooves (Loesberg et al. 2005). Ahmed et al. (2010) constrained cell alignment using matrix micropatterning to provided evidence that actin orientation is predominantly dictated by cyclic strain, while nuclei elongation is predominantly dictated by the micropattern. Importantly, Kurpinski et al. (2006) demonstrated that cyclic uniaxial stretching of mesenchymal stem cells in parallel with microgooves increased smooth muscle marker gene expression and proliferation, which is not observed when cyclic stretch is applied perpendicular to the microgrooves.

Substrate and Matrix Rigidity

It has become evident that cells respond to the local extracellular matrix stiffness to regulate cellular processes ranging from cell-cell and cell-substrate adhesions (Wang et al. 2002, Reinhart-King 2008), motility (Palecek et al. 1997, Pelham and Wang 1997, Lo et al. 2000, Wang et al. 2001a, Engler et al. 2004), cell spreading (Giannone et al. 2004) and differentiation (Engler et al. 2006). By pulling on the surrounding matrix through cell-matrix adhesions and sensing the mechanical resistance of the matrix, cells respond through modulation of cytoskeletal dynamics and organization (Pelham and Wang 1997, Engler et al. 2004, Saez et al. 2005). The extent of cell spreading and stress fiber formation are proportional to matrix rigidity (Yeung et al. 2005, Fu et al. 2010, Mih et al. 2012). Cells are only sensitive to differences in rigidity within a narrow range comparable to the rigidity of the cell (Zemel et al. 2010).

Given the central role of cell contractility in responding to matrix rigidity and applied strain, it is perhaps not surprising that the response of a cell to applied strain depends on substrate rigidity (and vice-versa). Cells cultured on thick collagen gels are observed to align their cell body and stress fibers parallel to the

direction of both step and cyclic uniaxial strain within a few hours (Tondon and Kaunas 2014). In contrast, applying uniaxial strain to these cells on collagen-coated silicone rubber membranes resulted in no alignment for step strain and perpendicular alignment for 1Hz cyclic strain. Cell alignment on the collagen gels occurred in the absence of collagen fibril co-alignment, likely due to boundary constraints and short timeframe of the experiments. Collagen matrix stiffness increases in response to strain (Gavara et al. 2008). Consequently, prestretching the matrix before cell attachment resulted in alignment, but to a lesser extent than applying strain to cells already attached to the matrix (Tondon and Kaunas 2014). Together these results suggest that cell and stress fiber alignment in these experiments are due to both anisotropic strain-stiffening of the matrix and mechanosensing by the cells, but not contact guidance.

The integrated cell response to strain and substrate rigidity is dependent on the substrate composition. Faust et al. (2011) reported that fibroblasts subjected to cyclic uniaxial strain at mHz frequency oriented toward the direction of zero strain on stiff (50kPa) fibronectin-coated PDMS substrates, but did not align at all on soft (1kPa) PDMS. Throm Quinlan et al. (2011) reported a similar dependence on substrate stiffness when cyclically stretching cells at 1Hz on soft (0.3kPa) vs. stiff (50kPa) polyacrylamide gels. Interestingly, this group also demonstrated that cells cultured on static soft gels were small and round, but spread and developed pronounced stress fibers upon application of cyclic equibiaxial stretch. Similarly, Cui et al. (2015) demonstrated that cyclic equibiaxial strain applied to cells on soft PDMS micropillar arrays resulted in cell spreading and stress fiber formation similar to that observed on a flat, rigid PDMS sheet. Of note, the stretch-induced rescue of stress fibers is similar to that observed in cells treated with inhibitors of Rho or Rho-kinase upon cyclic stretching on flat, rigid PDMS sheets (Kaunas et al. 2005).

Engineered Tissues

While we have learned much from studies performed on 2-D elastomeric sheets and extracellular matrix gels, engineered tissue scaffolds pose a new challenge to the study of stretch-induced cell remodeling and mechanotransduction. Synthetic hydrogels provide several advantages over naturally-derived extracellular matrix proteins, including precise control over material and chemical properties (Lutolf and Hubbell 2005). The mechanical resilience of matrices reconstituted from solubilized collagen or fibrin are suboptimal, however. Researchers have therefore used a combination of synthetic and natural polymers. Tomei et al. (2009) developed a composite hydrogel that consisted of fibroblasts suspended in collagen supported within a porous polyurethane sponge. The composite matrix did not undergo compaction in response to cell contractile forces. The dynamic modulus of the scaffold increased with increasing strain frequency, which was predicted to be due to interstitial flow generated by cyclic expansion and compression of the porous scaffold. Cells within the pores underwent myoblastogenesis and aligned parallel to the direction of strain, though interstitial flow may also have contributed (Ng and Swartz 2003, Ng et al. 2005).

Rubbens et al. (2009) developed a hybrid scaffold in which cells initially suspended in fibrin supported within a nonwoven polyglycolic acid scaffold. Cells subjected to two weeks of intermittent cyclic uniaxial strain regimen (3h on/3h off) deposited collagen that co-aligned with the cells, which was not observed in non-stretched controls. Further, the orientation of cells and collagen fibers shifted from a near-perpendicular orientation relative to the strain axis at the scaffold surfaces to a parallel orientation deeper within the scaffold. Subsequent experiments performed by Foolen et al. (2012) on collagen-only scaffolds showed that perpendicular alignment could be observed at the surface of the scaffold, but not the core (Foolen et al. 2012). This study also showed that perpendicular alignment would result if stretching was performed prior to complete collagen gelation. These authors thus argued that the

parallel alignment of cells with the direction of stretch often seen in collagen gels is due to contact guidance, and that the removal of this contact guidance allows the cells to instead align perpendicular to the stretch direction.

Polyethylene glycol diacrylate (PEGDA) hydrogels are elastic and can support encapsulated cells (Tibbitt and Anseth 2009). Like other synthetic polymers, PEG hydrogels are inert to matrix proteases, lack a fibrillar structure, and also have sub-micron pores. Consequently, encapsulated cells are confined by the surrounding polymer, which does not allow cell alignment or elongation in response to strain (Richardson et al. 2013). We encapsulated U2OS osteosarcoma cells within collagen microspheres suspended within PEGDA hydrogel and investigated the morphological changes to the cells when the composite scaffold was subjected to cyclic uniaxial strain (Figure 2). Strain measurements indicated the collagen spheres underwent comparable strain to the PEGDA scaffold. Cyclic uniaxial strain resulted in the cell alignment parallel to the strain axis, except in regions near the PEGDA-collagen interface (Fig. 2B). The extent of cell and collagen fibril alignment was quantified as a function of stretch frequency using the order parameter $\langle \cos 2\Theta \rangle$ (Tondon et al. 2012). Uniform or random distributions result in $\langle \cos 2\Theta \rangle = 0$, while alignment perpendicular or parallel to stretch results in value of -1 or 1, respectively. Cells near the surface of the collagen microspheres did not align in response to strain (Fig. 1C). Instead, these cells elongated along the PEGDA-collagen interface.

The unique behavior of the cells at the PEGDA-collagen interface may be due to their ability to respond to the stiffness of PEGDA at a distance. Cells perceive very thin gels as having a stiffness approaching that of the rigid material supporting the gel (Sen et al. 2009, Buxboim et al. 2010). For example, the extent of spreading of mesenchymal stem cells measured on very soft hydrogels (≈1 kPa) shows that cells spread little on thick gels, but below a threshold thickness of 20 um the cells spread increasingly more as the gel thickness decreases (Buxboim et al. 2010). Subsequent studies also suggested cells

spread more on soft gels (<10 kPa) with micron scale thickness as compared with thick ~100 μm gels (Engler et al. 2006, Maloney et al. 2008). Rudnicki and colleagues (2013) reported that cells cultured on fibrin or collagen matrices have much larger characteristic sensing distances (>65μm) than on linearly elastic synthetic materials due to the fibrous nature of these materials. Thus, while uniaxial strain provides the symmetry breaking cue necessary to drive alignment of the cells in the central region of the spheres, the cells located near the sphere interface appear to respond to symmetry breaking caused by the relatively rigid PEGDA interface.

Concluding comments

Clearly, mechanical stretching of a cell is complex process dependent on many interacting variables. Underlying themes emerge, however. Cell contractile forces are balanced by matrix reaction forces and these are modulated by applied strain and substrate rigidity. Dissipative processes due to myosin motor activity, cytoskeletal remodeling and turnover of cell-matrix adhesions relax perturbations in the force equilibrium. In the quest to engineer composite tissues that support appropriate stretch-induced cell remodeling and mechanotransduction, experiments must be designed that delineate the roles of these parameters. It is a daunting task to examine the overall effects of the many possible combinations of these parameters. The experiments described in this survey have inspired the development of numerous mathematical models capable of predicting the effects of varying multiple parameters (Hsu et al. 2009 and 2010, Kaunas et al. 2011, Livne et al. 2014, Safran and De 2009, Wei et al. 2008), paving the way for the development of more comprehensive models to interpret observations from many different experiments and thus identify the complex parameter interactions.

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Figure legends

Figure 1. Parameters that influence the cellular response to applied strains.

Figure 2. Cells encapsulated in collagen spheres suspended in PEGDA (a) were subjected to 6h of 7.5% uniaxial cyclic stretch at frequencies of 0, 0.01 and 1 Hz. The order parameters of cell and collagen fibril alignment are summarized as a function of stretch frequency (* indicates $p<0.01$, ANOVA followed by Student-Neumann-Keuls multicomparison testing) (B). Images of the cells (green) and collagen fibrils (red) are shown for cells in the center of the spheres and cells located near the PEGDA/collagen interface along with a diagram illustrating symmetry breaking due to strain and due to the nearby rigid PEGDA surface (C).

Conflict of interest statement

None declared.

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Table 1. Methods for Applying Tensile Strain to Cells on Elastomeric Sheets

Method of Deformation	Spatial Strain Pattern	Resulting Cell Morphology	References
Vacuum suction	Heterogeneous with high radial strain at periphery, low equibiaxial strain in the center	Circumferential alignment at the periphery and no alignment in the center	Mills et al. 1997, Gilbert et al. 1994
Vacuum suction with circular post	Equibiaxial above post	No alignment	Gavara et al. 2008 Trepat et al. 2007
Indenting with circular post	Equibiaxial above post	No alignment	Lee et al. 1996, Sotoudeh et al. 1998
Indenting square membrane with square / anisotropic posts	Equibiaxial / pure uniaxial in subregion above post	No alignment / Perpendicular alignment of cells to direction of stretch	Kaunas et al. 2006
Axial pulling without constraining lateral strain	Uniaxial in central region of membrane	Oblique alignment of cells to direction of stretch	Dartsch et al. 1986, 1989
Axial pulling with constrained lateral strain	Pure uniaxial in central region of membrane	Perpendicular alignment of cells to direction of stretch	Naruse et al. 1998, Wang et al. 2001b

Figure legends

Figure 1. Parameters that influence the cellular response to applied strains.

Figure 2. Cells encapsulated in collagen spheres suspended in PEGDA (a) were subjected to 6h of 7.5% uniaxial cyclic stretch at frequencies of 0, 0.01 and 1 Hz. The order parameters of cell and collagen fibril alignment are summarized as a function of stretch frequency (* indicates $p<0.01$, ANOVA followed by Student-Neumann-Keuls multicomparison testing) (B). Images of the cells (green) and collagen fibrils (red) are shown for cells in the center of the spheres and cells located near the PEGDA/collagen interface along with a diagram illustrating symmetry breaking due to strain and due to the nearby rigid PEGDA surface (C).