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Mechanical Interactions and Crosstalk between Corneal Keratocytes and the Extracellular Matrix

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Abstract

The generation of cellular forces and the application of these physical forces to the ECM play a central role in mediating matrix patterning and remodeling during fundamental processes such as developmental morphogenesis and wound healing. In addition to growth factors and other biochemical factors that can modulate the keratocyte mechanical phenotype, another key player in the regulation of cell-induced ECM patterning is the mechanical state of the ECM itself. In this review we provide an overview of the biochemical and biophysical factors regulating the mechanical interactions between corneal keratocytes and the stromal ECM at the cellular level. We first provide an overview of how Rho GTPases regulate the sub-cellular pattern of force generation by corneal keratocytes, and the impact these forces have on the surrounding ECM. We next review how feedback from local matrix structural and mechanical properties can modulate keratocyte phenotype and mechanical activity. Throughout this review, we provide examples of how these biophysical interactions may contribute to clinical outcomes, with a focus on corneal wound healing.

Keywords

Extracellular Matrix; Corneal Stroma; Corneal keratocytes; Cell Mechanics; Mechanobiology

1. Introduction

The cornea is an optically clear tissue that forms the front surface of the eye, and accounts for nearly two-thirds of its refractive power. The corneal stroma, which makes up 90% of corneal thickness, is a highly ordered structure consisting of approximately 200 collagen lamellae (Pepose and Ubels, 1992). Corneal stromal cells (keratocytes) reside between the collagen lamellae, and are responsible for secreting extracellular matrix (ECM) components required to develop and maintain normal corneal structure and function (Chakravarti et al.,

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2000; Funderburgh et al., 2003; Hassell and Birk, 2010). In addition to their role in matrix synthesis, the mechanical activity of corneal keratocytes (i.e. the generation of cellular forces and the application of these physical forces to the ECM) plays a central role in mediating fundamental processes such as developmental morphogenesis and wound healing. In general, cellular forces organize ECM into tissue-specific patterns during embryonic development, and feedback between cell and matrix mechanics is a key factor regulating this process (Bard and Higginson, 1977; Engler et al., 2006; Krieg et al., 2008; Stopak and Harris, 1982; Stopak et al., 1985).

As in development, corneal wound healing following lacerating injury, penetrating keratoplasty or refractive surgery involves an ordered sequence of cell-matrix mechanical interactions (Jester et al., 1999b; Netto et al., 2005; Stepp et al., 2014; Wilson, 2012). In the corneal stroma, quiescent keratocytes normally have a dendritic morphology and a cortical distribution of f-actin (Jester et al., 1994). From a mechanical standpoint, resting keratocytes are considered quiescent; they do not express stress fibers or generate substantial contractile forces (Jester et al., 1994; Lakshman et al., 2010). Following injury or surgery, corneal keratocytes surrounding the area of injury generally become activated by cytokines present in the wound environment, and transform into a fibroblastic repair phenotype (Garana et al., 1992; Moller-Pedersen et al., 1998b; Stramer et al., 2003). Corneal fibroblasts proliferate, develop intracellular stress fibers, migrate into the wound and reorganize the ECM through the application of mechanical forces.

In certain wound types, the presence of transforming growth factor beta (TGF β) in the wound induces transformation of corneal fibroblasts to a myofibroblasts phenotype. Corneal myofibroblasts express α -smooth muscle actin, generate even stronger forces on the matrix and synthesize a disorganized fibrotic ECM (Blalock et al., 2003; Jester et al., 1999a). Together these processes can alter corneal shape both through the addition of tissue (Moller-Pedersen et al., 2000), as well as the redistribution of mechanical load bearing and tension within the stroma (Dupps and Wilson, 2006; Ruberti et al., 2011). Fibrotic wound healing can also reduce transparency due to increased light scattering by both cells and the newly synthesized ECM (Boote et al., 2012; Jester et al., 2012; Moller-Pedersen et al., 1998a). Overall, a better understanding of the underlying cellular and molecular mechanisms that regulate the biomechanical activities corneal fibroblasts could ultimately lead to more effective approaches to modulating the wound healing response *in vivo*.

The Rho-family of small GTPases such as Rho, Rac, and Cdc42 play a central role in mediating the changes in cell mechanical activity in response to growth factors and other cytokines in a variety of cell types (Amano et al., 2010; Hall, 2005; Jaffe and Hall, 2005; Wang et al., 2013). These GTP binding proteins function as molecular switches; alternating between the active GTP-bound state and the inactive GDP-bound state. In fibroblasts on rigid 2-D substrates, activated Rho stimulates the formation of stress fibers and the development of focal contacts (Anderson et al., 2004; Parizi et al., 2000; Rottner et al., 1999; Sander et al., 1999; Totsukawa et al., 2000), and these cytoskeletal changes are dependent on actomyosin contraction (Jester and Chang, 2003; Rottner et al., 1999). Activated Rho binds to and activates Rho kinase, which inhibits myosin light chain (MLC) phosphatase, resulting in elevated MLC phosphorylation and increased cell contractility

(Amano et al., 1998; Amano et al., 1996; Chrzanowska-Wodnicka and Burridge, 1994; Kimura et al., 1996; Kolodney and Elson, 1993; Parizi et al., 2000; Rayan et al., 1996; Sanderson et al., 1992; Tomasek et al., 1992). In contrast to Rho, Rac induces cell spreading, via the creation of smaller focal complexes and actin polymerization (Demali and Burridge, 2003; Rottner et al., 1999; Sander et al., 1999; Svitkina and Borisy, 1999; Totsukawa et al., 2000). Rac activation enhances both cell spreading and migration within 3-D collagen matrices (Andresen et al., 1997; Grinnell et al., 2006).

In addition to growth factors and other biochemical factors that can modulate the keratocyte mechanical phenotype, another key player in the regulation of cell-induced ECM patterning is the mechanical state of the ECM itself. In the cornea, large shifts in the global distribution of ECM tension can be induced by lacerating injury, penetrating keratoplasty, or refractive surgery (Dupps and Wilson, 2006; Ruberti et al., 2011). In addition, the provisional matrix produced during wound healing is generally less dense and more compliant than normal corneal tissue. Diseases such as keratoconus can also produce changes in stromal structural and mechanical properties. Keratoconus corneas generally have reduced mechanical stiffness (Ali et al., 2014; Andreassen et al., 1980; Edmund, 1989; Morishige et al., 2007), and thinning of the central cornea in keratoconus patients induces a redistribution of tension within the stromal ECM (Ambekar et al., 2011). In contrast, treatment of keratoconus with UV cross-linking increases corneal stromal rigidity (Beshtawi et al., 2013). Overall, changes in ECM structure, stress and elasticity have the potential modulate both the acute and long-term responses of corneal keratocytes to a range of clinical conditions (Winkler et al., 2011).

In this review we provide an overview of the biochemical and biophysical factors regulating mechanical interactions between corneal keratocytes and the stromal ECM at the cellular level. We first provide an overview of how Rho GTPases regulate the sub-cellular pattern of force generation by corneal keratocytes, and the impact these forces have on the surrounding ECM. We next review how feedback from local matrix structural and mechanical properties can modulate keratocyte mechanical activity. Throughout this review, we provide examples of how these biophysical interactions may contribute to clinical outcomes, with a focus on corneal wound healing.

2. Modulation of Corneal Keratocyte Mechanical Behavior by Rho and Rac

2.1 Regulation of Keratocyte Contractility and Matrix Reorganization by Rho and Rho kinase

The Rho GTPases are prime candidates for regulating the mechanical interactions between corneal keratocytes and the stromal ECM during various phases of wound healing. In corneal fibroblasts, time-lapse imaging of cells plated on top or within restrained 3-D collagen matrices have shown that activation of Rho using lysophosphatidic acid (LPA), induces retraction of cell processes and a corresponding pulling in of the surrounding ECM (Petroli et al., 2008b; Roy et al., 1999). In contrast, inhibiting Rho kinase with the inhibitor Y-27632 induces rapid cell body elongation, formation and extension of dendritic cell processes, and a corresponding relaxation of cell-induced tension on the matrix (Figure 1) (Vishwanath et al., 2003). Static imaging of collagen fibril organization surrounding isolated corneal fibroblasts in 3-D culture has directly demonstrated increased ECM compaction and

alignment when Rho is activated by serum (Hay, 1985; Kim et al., 2006; Tomasek et al., 1982). When Rho kinase is inhibited, this cell-induced matrix reorganization is significantly reduced (Kim et al., 2006). In order to quantify cell-mediated changes in matrix tension, Brown and coworkers developed a culture force monitor system in which cell-seeded collagen matrices are suspended between a fixed bar and a force transducer (Eastwood et al., 1994). A similar system was recently used to measure cellular force generation by corneal fibroblasts. Culture in serum induced a gradual rise in cell-induced matrix tension, which reached a plateau after 24 hours. Subsequent addition of Y-27632 produced a 64% decrease in the measured force produced by corneal fibroblasts (Zhou, 2014). Furthermore, pre-incubation with Y-27632 blocked the initial rise in matrix tension.

In addition to serum and LPA, Rho kinase has also been shown to mediate fibroblastic transformation of keratocytes in response to basic fibroblast growth factor (FGF2) treatment, as well as myofibroblast transformation in response to TGF β (Chen et al., 2009; Lakshman and Petroll, 2012; Yamamoto et al., 2012). Specifically, treatment with Y-27632 inhibits stress fiber formation, and blocks the induction of α -SM-actin expression and the increase in cell-induced matrix remodeling normally induced by TGF β . Inhibition of Rho/Rho kinase has also been shown to block the decrease in keratin sulfate proteoglycan synthesis normally associated with corneal myofibroblast transformation, suggesting a linkage between increased cell contractility and altered ECM synthesis (Chen et al., 2009).

The role of Rho kinase in regulating corneal fibroblast migration mechanics has also been investigated, using a nested 3-D collagen matrix model that facilitates dynamic imaging of cell-matrix interactions during cell translocation. In this model, corneal fibroblasts cultured in serum-containing media generate significant tractional forces on the matrix during migration, as indicated by inward displacement and reorganization of collagen in front of cells (Karamichos et al., 2009; Kim et al., 2010; Zhou and Petroll, 2010). When Rho kinase is inhibited, cells became more elongated and form dendritic cell processes, and the rate of cell migration is significantly reduced. Interestingly, these dendritic cells are still able to generate tractional forces at the leading edge. Overall, these data suggests that Rho kinase impacts corneal fibroblast migration by affecting morphology, polarization, and mechanical coordination between the leading and trailing edges of cells (Amano et al., 2010; Zhou and Petroll, 2010).

Taken together, previous studies demonstrate that Rho kinase plays a central role in regulating corneal fibroblast contractility, migration and matrix remodeling *in vitro* (Anderson et al., 2004; Kim et al., 2006; Petroll et al., 2008b; Roy et al., 1999; Vishwanath et al., 2003). Recently, Koizumi and coworkers investigated whether topical application of Y-27632 could modulate *in vivo* corneal wound healing following lamellar keratectomy in a rabbit model (Yamamoto et al., 2012). After 21 days of treatment with Y-27632, the expression of α -SM actin was suppressed in the center of the wound, but not the periphery. In addition, while both treated and control corneas showed similar amounts of type I collagen matrix deposition and keratan sulfate during healing, the amount of Type III collagen was reduced in the Y-27632 group. Epithelial resurfacing was also delayed in animals treated with Y-27632. Since TGF β 1 can be produced by the corneal epithelium (Wilson et al., 2001), this delay in resurfacing may explain, in part, why the reduction in

myofibroblast transformation was limited to the central region of the wound. Temporal assessment of the corneal keratocyte response to Y-27632 at earlier time points may shed additional light on its potential therapeutic role. It should be noted that Rho kinase inhibitors are also being evaluated as potential therapeutics for glaucoma, since they can inhibit trabecular meshwork contraction and thereby increase outflow facility (Inoue and Tanihara, 2013; Wang et al., 2013).

2.2 Regulation of Corneal Keratocyte Spreading, Migration and Tractional Force Generation by Rac

Rac is activated by platelet derived growth factor BB (PDGF BB) in both corneal and dermal fibroblasts (Grinnell, 2000; Sander et al., 1999). In time-lapse studies of corneal fibroblasts in 3-D matrices, PDGF-induced Rac activation stimulated rapid cell spreading, via elongation, ruffling and random branching of pseudopodial processes (Petroll and Ma, 2008; Petroll et al., 2008a). In general, relaxation (decompression) of the ECM was observed along the cell body, whereas tractional forces were generated by extending cell processes, as indicated by centripetal displacement of collagen fibrils at the ends of cells. Thus overall, there was a shift in the tractional forces on the matrix from the center to the periphery of corneal fibroblasts in response to Rac activation.

For quiescent corneal keratocytes maintained in serum-free conditions (in which the level of Rho activation is low), both global and local ECM reorganization induced by PDGF BB are comparable to that produced by basal serum-free media (Figure 2) (Lakshman and Petroll, 2012). Nonetheless, PDGF still stimulates more rapid cell migration through collagen matrices under serum free conditions (Kim et al., 2012a; Zhou and Petroll, 2014). During migration, PDGF induces repeated extension and retraction of dendritic cell processes which produce only small, transient matrix deformations. Local matrix reorganization produced by migrating cells in PDGF is significantly reduced as compared to serum or TGF β 1 (Figure 3). Following lacerating injury or incisional surgery, contractile force generation is needed to facilitate wound closure and prevent loss of the mechanical integrity of the cornea. However, following refractive surgical procedures such as PRK or LASIK, it is preferable to minimize cellular force generation and fibrosis during stromal repopulation, since these processes can alter corneal shape and transparency (Dupps and Wilson, 2006; Jester et al., 1999b; Moller-Pedersen et al., 2000; Moller-Pedersen et al., 1997; Netto et al., 2005; Petroll et al., 1992). PDGF stimulates keratocyte proliferation and has been shown to up-regulate synthesis of normal stromal ECM (Etheredge et al., 2009; Jester and Chang, 2003). Thus it is interesting to speculate that PDGF BB may contribute to stromal repopulation following injury or surgery through up-regulation of both proliferation and migration, without producing fibrotic tissue or generating large forces which can alter corneal shape and transparency.

3. The Impact of ECM Mechanical Properties on Corneal Keratocyte Behavior

3.1 Modulation of Cell Spreading, Migration and Matrix Reorganization by ECM Stiffness and Anisotropy

It is well established that mechanical signals from the ECM play a key role in regulating growth and function in a variety of cell types (Brown, 2000; He and Grinnell, 1994; Ingber and Folkman, 1989; Liu et al., 1999; Sadoshima and Izumo, 1997; Shyy and Chien, 2002; Tummina et al., 1998; van Bockxmeer et al., 1984). Increasing substrate stiffness can facilitate formation of actin stress fibers and focal adhesions in contractile cells (Miron-Mendoza et al., 2010; Pelham and Wang, 1997; Yeung et al., 2005), and both in vitro and in vivo studies have demonstrated that these structures tend to align along the tensile axis under anisotropic conditions (Kolodney and Wysolmerski, 1992; Takakuda and Miyairi, 1996; Wakatsuki and Elson, 2003). Alignment and global force generation parallel to the axis of greatest mechanical resistance by dermal fibroblasts, myoblasts and bone marrow stem cells has also been documented in 3-D culture models (Cheema et al., 2003; Eastwood et al., 1994; Eastwood et al., 1998; Huang et al., 1993; Mudera et al., 2000).

Studies using corneal fibroblasts have shown similar cross-talk between cell and matrix mechanics. In 3-D culture models, significant differences in cell alignment, morphology, and matrix reorganization are observed between constrained (anisotropic) and unconstrained (isotropic) rectangular matrices (Karamichos et al., 2007). Cells align nearly parallel to the long axis of the construct in matrices constrained at the ends, whereas cells in unconstrained matrices show no preferential orientation. Corneal fibroblasts also tend to align and compact collagen parallel to the axis of greatest effective stiffness under anisotropic conditions. Furthermore, in regions of high cell density within constrained matrices, bands of compacted and aligned collagen spanning between adjacent corneal fibroblasts are generally observed, suggesting mechanical coupling of cells through the ECM which leads to local amplification of matrix patterning (Karamichos et al., 2007; Petroll et al., 2004a).

Cell migration and spreading can also be influenced by the mechanical stiffness of the substrate. In 3-D matrices pre-fabricated with directional gradients in collagen density, fibroblasts migrate towards the stiffer region (Hadjipanayi et al., 2009), a phenomenon called "durotaxis" (Lo et al., 2000). To evaluate the effect of matrix mechanical properties on corneal keratocyte migration, migration has been compared in constrained and unconstrained nested matrix constructs (Kim et al., 2012a). Consistent with previous results using dermal fibroblasts (Grinnell et al., 2006), invasion of keratocytes into the outer matrix in 10% FBS was significantly reduced in unconstrained matrices, in which effective stiffness is reduced. Interestingly, the dependency of corneal fibroblast migration on matrix constraint was eliminated when Rho kinase was inhibited.

Migration of keratocytes cultured in PDGF BB, in which low levels of contractility are maintained, was also unaffected by changes in the effective stiffness of the ECM (Kim et al., 2012a). Thus while durotaxis appears to modulate the spreading and migration of corneal keratocytes that have transformed to a contractile fibroblastic phenotype, it does not appear

to impact the motility of keratocytes that maintain a quiescent, low contractility mechanical phenotype. This is consistent with the concept that durotaxis only regulates contractile cells (Grinnell and Petroll, 2010)

3.2 Modulation of Keratocyte Mechanical Activity and Matrix Remodeling by Temporal Changes in ECM Stress and Strain

Time-dependent changes in ECM stress have also been shown to impact cell mechanical behavior. Using a tensioning culture force monitor system, Brown and coworkers demonstrated that stretching of 3-D matrices seeded with dermal fibroblasts resulted in a rapid increase in the measured force (Brown et al., 1998), which was immediately followed by a gradual cell-dependent reduction in force toward the baseline level. In contrast, reducing strain on the gel caused an initial loss of tension, followed by a cell-dependent increase back to the baseline level. Thus in both cases cells responded to mechanical loading in a way that maintained “tensional homeostasis” (constant tension) in their surrounding matrix. Consistent with this model, fibroblast force generation in 3-D matrices reaches a constant value that is independent of matrix stiffness (Freyman et al., 2002). Tensional homeostasis may be fundamental to the regulation of tissue tension under normal conditions, during development and also in response to injury.

Using a modified culture force monitor system (Zhou, 2014), a similar response to changes in matrix strain has been observed using serum cultured corneal fibroblasts (personal observation). In addition, the effect of dynamic changes in ECM tension on corneal fibroblasts has been investigated at the sub-cellular level, by using micropipettes to displace the collagen fibrils near a cell (Petroll and Ma, 2008; Petroll et al., 2004b). In these studies, reducing effective ECM stiffness by pushing toward the front of a cell resulted in rapid cellular shortening with corresponding ECM compression along the cell body (Figure 4A–D). This initial contraction is likely due to the release of isometric cellular contractile forces, since it is blocked by treatment with Y-27632. Following contraction, pseudopodial extension (spreading) was then observed at both ends of the cell. Interestingly, the ECM was pulled inward during this secondary spreading, and rapid turnover of focal adhesions was observed along extending pseudopodia. FEM analysis demonstrates that following the initial reduction of tension induced by the needle push, fibroblasts partially re-establish baseline tension during secondary spreading, consistent with the tensional homeostasis model.

In contrast to ECM compression parallel to the long axis of cells, compressing the ECM perpendicular to the long axis of corneal fibroblasts had little effect on cell morphology or mechanical activity (Figure 4E–H). This is also consistent with the tensional homeostasis model, since the cytoskeleton, focal adhesions and contractile forces are all aligned parallel to the long axis of bipolar cells, and therefore reducing the effective stiffness of the ECM alongside of the cell should have little impact on cellular tension. Overall, while durotaxis has been shown to regulate cell alignment and migration within collagen matrices under static conditions (Hadjipanayi et al., 2009), tensional homeostasis may modulate cell behavior in response to more transient changes in ECM tension in a 3-D environment. During developmental morphogenesis and wound healing, such dynamic changes in local

matrix tension could be produced by the mechanical activity of nearby cells, and/or externally applied forces.

Recently, the effects of cyclic stretch on the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) by corneal fibroblasts was also investigated (Liu et al., 2014). The results suggest that stretching magnitude determines whether the cornea undergoes matrix degradation or synthesis, by changing the balance between MMPs and TIMPs secreted by corneal fibroblasts. Overall, 15% stretching increased expression of MMPs and decreased expression of TIMPs, whereas 5% stretching increased expression of TIMPs and reduced expression of MMPs. These data suggest that fluctuations in corneal strain, produced by changes in IOP or eye rubbing, may induce or modulate keratocyte remodeling of the stromal ECM. These fluctuations would be larger under conditions in which the overall corneal stiffness is reduced, such as in keratoconus or following injury or refractive surgery.

3.3 Modulation of Keratocyte Growth Factor Responses by ECM Stiffness

The response of corneal keratocytes to growth factors can also be modulated by changes in ECM stiffness. Several studies have shown that FGF2 induces fibroblastic transformation of keratocytes on rigid 2-D substrates, as indicated by changes in cell morphology and development of stress fibers and focal adhesions (Chen et al., 2009; Jester and Chang, 2003; Jester et al., 2002). However, within hydrated 3-D collagen matrices (which have high compliance), FGF2 stimulates ruffling of keratocyte processes without inducing major changes in cell morphology, formation of stress fibers, or collagen matrix organization (Lakshman and Petróll, 2012). When corneal keratocytes are seeded within compressed 3-D collagen matrices, fibroblastic transformation is again observed. Both glass and compressed collagen matrices are several orders of magnitude stiffer than a hydrated collagen matrix, thus substrate mechanical properties appear to modulate the phenotypic changes induced by FGF2.

Matrix stiffness also impacts the response of ocular cells to TGF β treatment. For example, increased matrix stiffness has also been shown to enhance TGF β -induced myofibroblast transformation of human tenon fibroblasts (Meyer-ter-Vehn et al., 2011). Consistent with these results, Murphy and coworkers recently demonstrated that corneal fibroblasts grown compliant polyacrylamide substrates had fewer stress fibers and expressed significantly reduced amounts of α -SMA as compared cells plated on rigid 2-D substrates (Dreier et al., 2013). In hydrated 3-D collagen matrices, treatment with TGF β 1 & 2 increases cell contractility, as indicated by the formation of stress fibers and stimulation of cell-induced ECM reorganization (Jester and Chang, 2003; Lakshman and Petróll, 2012). However, α -SM-actin labeling is negative for cells plated at low cell density within these compliant matrices. In contrast, approximately 20% of cells show positive labeling for α -SM-actin localized to the stress fibers at high cell density, where mechanical cross-talk between cells increases the tension within the matrix. A similar increase in stress fiber formation and myofibroblast transformation of corneal keratocytes is observed within compressed 3-D collagen matrices; further demonstrating that increased substrate stiffness enhances TGF β -induced myofibroblast transformation of corneal keratocytes.

Both connective tissue growth factor (CTGF) and PDGF have been shown to participate in TGF β -induced myofibroblast differentiation through an autocrine feedback loop, which would be amplified at higher cell density (Garrett et al., 2004; Jester et al., 2002). While stress fibers were observed in all cells within compressed ECM irrespective of cell density, the percentage of cells with α -SM-actin incorporated into stress fibers was greater at higher cell density (~60% versus ~20%), suggesting that both mechanical stiffness and autocrine signaling promote myofibroblast transformation *in vitro* (Lakshman and Petroli, 2012). Interestingly, myofibroblasts tend to develop towards the end of the corneal wound repair process when cell density is high and the wound environment is stiffer, thus similar processes may be involved during *in vivo* healing.

Unlike FGF and TGF β , keratocytes cultured in insulin growth factor (IGF) or PDGF BB maintain a quiescent mechanical phenotype over a range of ECM environments, including rigid 2-D substrates, compliant hydrated matrices and compressed collagen matrices (Lakshman and Petroli, 2012). Thus, the effects of these growth factors do not appear to be modulated by matrix stiffness. IGF increases keratocyte proliferation and stimulates synthesis of ECM components resembling normal corneal stroma, and also stimulates network formation (Berthaut et al., 2011; Etheredge et al., 2009; Jester and Chang, 2003). Thus it has been suggested that IGF may be involved in maintenance of normal corneal structure and could contribute to a regenerative wound healing phenotype (Etheredge et al., 2009; Jester and Chang, 2003).

3.4 Regulation of Corneal Keratocyte Differentiation by ECM Topography

In addition to matrix mechanical properties, nano-scale surface topography can also have a profound impact on cell behavior. Recent advances in micro and nano scale photolithographic techniques have led to the development of substrates with surface topographies (e.g. micropillars, grooves, ridges and pits) modeled after *in vivo* tissue matrices (Frey et al., 2006; Ghibaud et al., 2009; Kim et al., 2012b; Kriparamanan et al., 2006). Interestingly, small changes in topographical parameters (i.e. height, depth, width and spacing) can produce significant changes in cell morphology and migration mechanisms (Ghibaud et al., 2009; Teixeira et al., 2003; Teixeira et al., 2004). Aligned surface grooves have been reported to inhibit the transformation of corneal fibroblasts to myofibroblasts normally observed in response to TGF β (Myrna et al., 2012). They also increase the alignment of cells and matrix within each layer of self-assembled sheets derived from corneal fibroblasts (Guillemette et al., 2009). On Transwell filters which have parallel, aligned grooves on the surface, human corneal fibroblasts stimulated with ascorbate analogs secrete and organize a more cornea-like ECM as compared to fibroblasts plated on planar substrates or disorganized collagen ECM (Guo et al., 2007; Karamichos et al., 2014; Karamichos et al., 2010; Ren et al., 2008; Saeidi et al., 2012). Thus substrate topography can modulate both the organization and differentiation of corneal fibroblasts.

3.5 Biophysical Regulation of Keratocyte Differentiation of Stem Cells

Differentiation of both embryonic and adult stem cells is also regulated by the chemical and physical characteristics or their microenvironment (Engler et al., 2006; Kshitiz et al., 2012; Reilly and Engler, 2010). Adult corneal stromal stem cells (CSSC), which express genes and

proteins characteristic of quiescent corneal keratocytes (Du et al., 2005), are influenced by substrate structure, attachment and topography. In 2-D culture on planar substrates, CSSC cells do not secrete an abundant ECM, but as free-floating 3-D pellets they produce an ECM containing stromal-like molecular components and regions of aligned collagen (Du et al., 2007). Furthermore, when CSSC are cultured on an aligned nanofibrous substrata, they form a parallel lamellar ECM similar to that of adult corneal stroma (Karamichos et al., 2014; Wu et al., 2013; Wu et al., 2012). In contrast, on substratum of randomly oriented nanofibers, CSSC secretion and organization of a stroma-like ECM is significantly reduced (Wu et al., 2012). Thus overall, topographic cues from the substratum have a significant impact on the ability of CSSC to produce a cornea-like ECM. Differentiation of human embryonic stem cells into corneal keratocytes is also regulated by the physical environment, as indicated by the upregulation of numerous keratocyte markers when these cells were cultured as substratum-free 3-D pellets (Chan et al., 2013).

4.0 Conclusions

The mechanical interactions between corneal keratocytes and the stromal ECM are regulated not only by biochemical signals from growth factors and other cytokines, but also by mechanical signaling and feedback due changes in ECM structure, stress and elasticity. Changes in the activity of Rho GTPases appear play a central role in modulating corneal keratocyte mechanical behavior in response to both biochemical and biomechanical cues. Together these signals determine the pattern and amount of cellular force generation and ECM reorganization produced following injury or refractive surgery. Feedback between biochemical and biophysical signals can also modulate keratocyte differentiation, myofibroblast transformation, and matrix synthesis and degradation. Overall, mechanical interactions and crosstalk between corneal keratocytes and the ECM likely impact a range of fundamental processes in the cornea in both health and disease.

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Highlights

Keratocyte mechanical activity can alter corneal stromal shape and transparency

Rho kinase plays a central role in mediating increased cell contractility and ECM reorganization

ECM structural and mechanical properties also modulate keratocyte mechanical behavior

Figure 1.

FEM strain maps generated using ANSYS, showing regions of matrix tension and compression surrounding a human corneal fibroblast expressing GFP- α -actinin within a 3-D collagen matrix. **A.** Cell-induced ECM deformation is observed following culture in media containing 10% fetal bovine serum (S+). **B.** The magnitude of this deformation is reduced when the cell is switched to Y-27632 (10 μ M). **C.** Stain on the matrix is reestablished after switching back to S+. Strain is shown relative to the “relaxed” matrix configuration determined by treating cells with Cytochalasin D and TritonX-100. (From Vishwanath et al, 2003)

Figure 2.

Keratocyte mechanical behavior in constrained 3-D collagen matrices. **A.** Global matrix contraction assay. Cell-induced matrix contraction (decrease in matrix height) was significantly greater in media containing 10% fetal bovine serum (FBS) as compared to PDGF BB (50 ng/ml) or basal serum-free media, after both 24 hours and 4 days of culture. **B–D.** Day four overlays of phalloidin (green) and collagen (red) obtained using confocal fluorescence and reflection imaging, respectively. Corneal keratocytes developed a stellate morphology in 3-D matrices (B), with a cortical, membrane associated f-actin organization. In contrast, cells cultured in 10% FBS generally developed a more polarized morphology with thicker pseudopodial processes (C). Following culture in PDGF, cells developed long dendritic processes (D). In general, minimal compaction and realignment of collagen fibrils was observed surrounding cells cultured in basal media or PDGF. In contrast, collagen at the ends of cells in 10% FBS appeared to be compacted and aligned parallel to the ends of pseudopodial processes. (*- Significantly greater than all other conditions) (From Kim et al., 2012a)

Figure 3.

Maximum intensity projections of f-actin (green) and collagen fibrils (red) at the interface between the inner and outer matrices of nested constructs. **A)** Following culture in 10% FBS, migrating cells developed a bipolar morphology with occasional stress fibers along the cell body. Collagen fibrils were compacted and aligned parallel to the long axis of pseudopodia. **B)** Following culture in TGFβ1 (10 ng/ml), cells developed a broad morphology and intracellular stress fibers were observed. Collagen fibrils were compacted both around and between the cells. **C)** Migrating cells in PDGF BB (50 ng/ml) were more

elongated and had branching processes. Collagen fibrils remained more randomly aligned around the cells. (From Kim et al., 2010)

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Figure 4.

Fibroblast response to ECM compression using small microneedle. A–D. Human corneal fibroblast following 2 days of culture in media containing 10% FBS, inside a 3-D collagen matrix. Needle was inserted axially into the matrix above the cell (A) without inducing changes in cell behavior (B). Pushing on the ECM towards the leading edge of a cell induced rapid cellular contraction and ECM compression along the cell body (C, arrows). This initial contraction was followed by re-spreading and tractional force generation (D, red tracks; crosses mark position immediately after needle push). E–H. Fibroblast response to ECM compression adjacent to cell body. Human corneal fibroblast 1 day after plating inside

collagen matrix. Pushing small microneedles toward the side of the cell had no significant effect on cell morphology or tractional force generation. (From Petroll and Ma, 2008)

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