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Fibroblast mechanics in 3D collagen matrices ☆

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Abstract

Connective tissues provide mechanical support and frameworks for the other tissues of the body. Type 1 collagen is the major protein component of ordinary connective tissue, and fibroblasts are the cell type primarily responsible for its biosynthesis and remodeling. Research on fibroblasts interacting with collagen matrices explores all four quadrants of cell mechanics: pro-migratory vs. pro-contractile growth factor environments on one axis; high tension vs. low tension cell–matrix interactions on the other. The dendritic fibroblast — probably equivalent to the resting tissue fibroblast — can be observed only in the low tension quadrant and generally has not been appreciated from research on cells incubated with planar culture surfaces. Fibroblasts in the low tension quadrant require microtubules for formation of dendritic extensions, whereas fibroblasts in the high tension quadrant require microtubules for polarization but not for spreading. Ruffling of dendritic extensions rather than their overall protrusion or retraction provides the mechanism for remodeling of floating collagen matrices, and floating matrix remodeling likely reflects a model of tissue mechanical homeostasis.

Keywords: Adhesion; Migration; Contraction; Extracellular matrix; Wound repair; Tissue engineering; Tensegrity; Platelet-derived growth factor; Lysophosphatidic acid

Contents

1. Introduction

Connective tissues begin as embryonic mesenchyme and develop in the adult into a variety of tissues ranging from blood to bone. Rich in extracellular matrix (ECM), most connective tissues provide mechanical support and frameworks for the other tissues of the body. Our laboratory has been particularly interested in so-called *ordinary* connective tissue such as found in the dermis of skin. Type 1 collagen is the major protein component of ordinary connective tissue, and fibroblasts are the cell type primarily responsible for its biosynthesis and remodeling.

Plasticity and molecular remodeling are key mechanical features of ordinary connective tissue in which collagen and other ECM molecules can stretch, slip, and undergo stable reorganization relative to each other [1]. As a result, the tissue is in a sense *tunable* according to the mechanical needs of the body. Such tunability or matrix remodeling has been implicated

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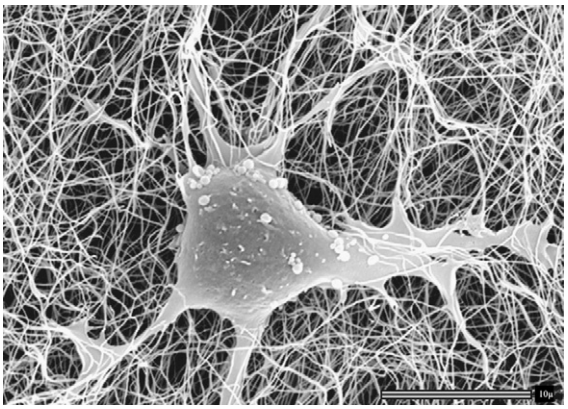

Fig. 1. Human fibroblasts interacting with 3D collagen matrices. Scanning electron microscopic image showing cells incubated on collagen matrices in PDGF-containing medium. Cell extensions penetrate into the matrix and become entangled with collagen fibrils. See Fig. 6 in [17] for additional details.

in diverse aspects of physiology including control of interstitial fluid pressure [2], aging [3], repair [4–6], fibrosis [7,8] and tumorigenesis [9]. Not surprisingly, matrix remodeling also is an important consideration for tissue engineering [10–16].

2. The four quadrants of cell mechanics

When fibroblasts interact with collagen matrices — unlike planar surfaces — the cells can penetrate into the substance of the matrix and become entangled with matrix fibrils (Fig. 1) [17]. Cells interacting with collagen matrices exhibit distinct patterns of signaling and migration [18–21] and remodel matrices both locally and globally [5,6,22–24] to achieve tensional homeostasis [25,26]. While cells on planar surfaces can modulate their cytoskeletal function in response to surface mechanics [27,28], they have little capacity to modulate the overall molecular organization and mechanical properties of the ECM-coated planar surface itself.

To emphasize the plasticity of fibroblasts in collagen matrices compared to planar surfaces, Fig. 2 illustrates the four quadrants of cell mechanics. One axis (arbitrarily selected as the *x*-axis in the figure) depends on the growth factor environment — pro-migratory in the case of platelet-derived growth factor (PDGF) and pro-contractile in the case of lysophosphatidic acid (LPA) or fetal bovine serum (FBS). The other axis depends on the tension state of cell–matrix interactions — high tension (formation of stress fibers and focal adhesions) with routine culture surfaces such as glass or plastic coverslips and low tension with relaxed collagen matrices. While pro-migratory and pro-contractile growth factor agonists can be distinguished empirically as will be described later, the idea of dual growth factor environments originates from studies that identified PDGF and LPA respectively as activators of the small G proteins Rac and Rho and discovered that LPA rather than PDGF was the serum growth factor responsible for stimulating cell contractile activity [29,30].

The extensive body of research on the mechanism and regulation of cell adhesion and migration has almost all been carried out within the pro-contractile, high tension state quadrant where fibroblasts exhibit lamellipodia, stress fibers and focal adhesions [31–39]. Even work using more flexible surfaces to examine cell behavior at a low tension state still typically incorporates pro-contractile (i.e., serum-containing) conditions [40–43].

Fig. 2 shows that in the high tension state quadrants, differences between the morphology of human fibroblasts under

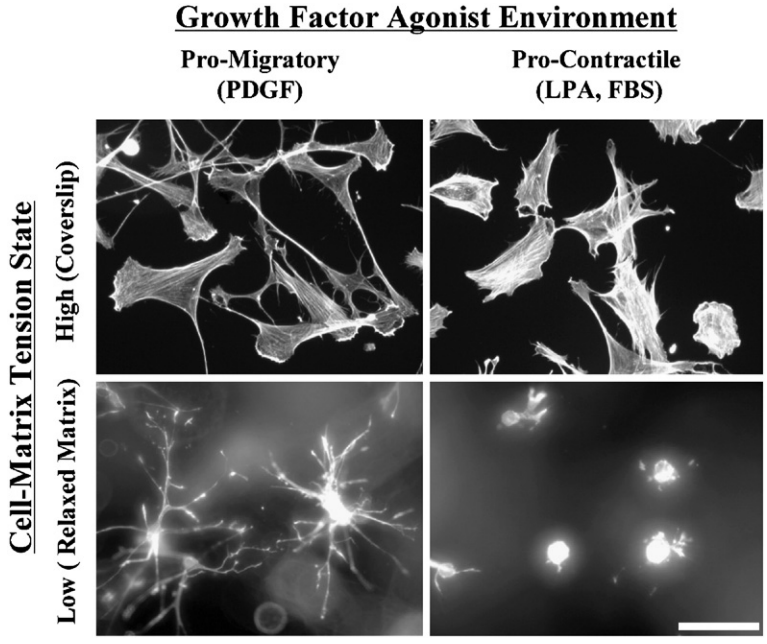

Fig. 2. Four quadrants of cell mechanics. Fluorescence images of cells stained to show the actin cytoskeleton. On coverslips, cells spread with lamellipodia and develop increased ruffles under pro-migratory conditions or stress fibers under pro-contractile conditions. In collagen matrices, cells spread with dendritic extensions under pro-migratory conditions or have retracted extensions under pro-contractile conditions. See Fig. 1 in [44] for additional details.

pro-migratory (more ruffling) vs. pro-contractile (more stress fibers) conditions are subtle compared to the more profound changes that occur in the low tension state quadrants. In the later, cells protrude dendritic extensions under pro-migratory conditions, whereas dendritic extensions undergo transient retraction under pro-contractile conditions [44]. Cells in a pro-contractile, low tension state environment eventually re-protrude their extensions but in a more bipolar morphology [45]. Fibroblasts exhibiting dendritic/bipolar morphologies resemble tissue fibroblasts under resting conditions [46–50], whereas cells with prominent stress fibers and focal adhesions cannot typically be observed in tissues except during activated conditions such as wound repair and fibrosis [5–8].

3. Microtubule function and tension state of cell–matrix interactions

Fibroblast dendritic extensions have microtubule cores and actin rich tips [44]. Recently, we found that interfering with cytoplasmic microtubules prevents fibroblasts in relaxed collagen matrices from forming dendritic extensions. Fibroblasts on collagen coverslips can form lamellipodia extensions and spread completely without microtubules although they cannot become polarized [51]. Time-lapse microscopic studies showed that cells interacting with collagen matrices or coverslips protrude dendritic extensions initially, but that on coverslips these protrusions rapidly merge and form lamellipodia.

These findings suggested that fibroblasts use microtubules differently when they interact with collagen-coated coverslips vs. relaxed collagen matrices. Collagen matrices differ from coverslips in many ways: stiffness, topographic organization of potential adhesion sites, and adhesion site density. We suspected, however, that the key difference was tension state. If we added the myosin II inhibitor blebbistatin [52] to block high tension interactions, then fibroblasts on collagen coverslips were unable to develop lamellipodia and instead protruded dendritic extensions whose formation was microtubule-dependent. Conversely, if we prepared precontracted collagen matrices on which cells could spread at a high tension state, then the fibroblasts became less dependent on microtubules for formation of dendritic extensions and more dependent on microtubules for polarization.

The tensegrity hypothesis of cell shape [53] predicts that microtubules will act as non-compressive structures to resist contractile tension of the actin cytoskeleton. For cells in relaxed collagen matrices, microtubules might have been necessary for formation of dendritic extensions to resist contractile tension, whereas cells on coverslips could have transferred contractile tension to the non-compressive rigid culture surface. This explanation did not appear to be the case, however. As described above, blocking contractile tension failed to make cells in matrices less dependent on microtubules for cell spreading but rather caused fibroblast extensions to become more microtubule-dependent.

Rather than tensegrity, our findings are consistent with the clutch hypothesis [54,55] shown in Fig. 3. That is, the key difference between microtubule function in low and high tension states appears to be how cells control the balance between actin polymerization and depolymerization [56,57]. At a high tension state, polymerization is promoted by an external clutch, e.g., adhesion-dependent mechanisms [37,58,59], in which case the

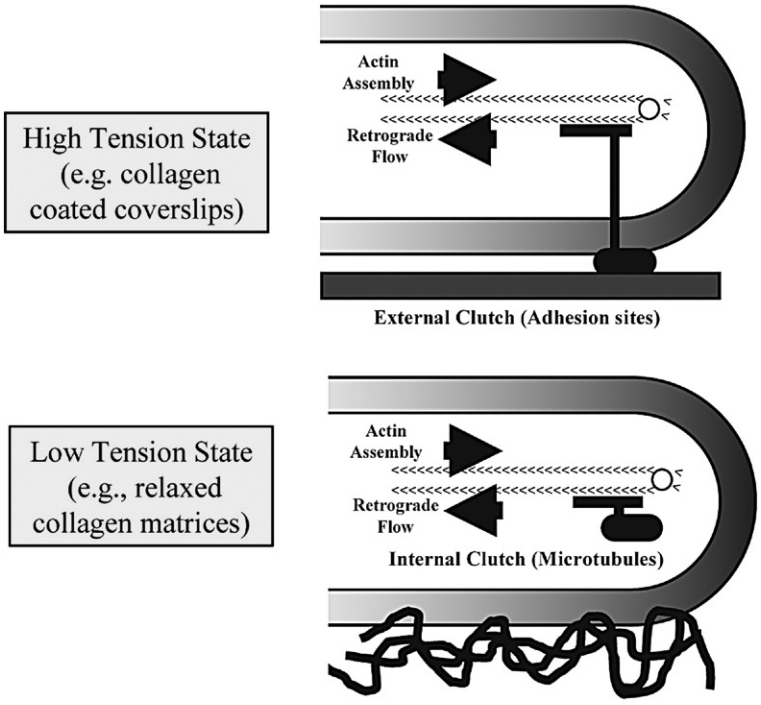

Fig. 3. Microtubule function in fibroblast spreading varies with tension state of cell–matrix interactions. At a high tension state, adhesion sites act as an external clutch for actin polymerization and microtubules function in cell polarity. At a low tension state, microtubules act as an internal clutch for actin polymerization. Adapted from [54]. See [51] for additional details.

function of microtubules will be primarily to determine cell polarity [60–62]. At a low tension state, polymerization is promoted by an internal clutch provided by microtubule polymerization, e.g., Rac1 activation [63,64].

4. Multiple mechanisms of collagen matrix remodeling

The observation that the cellular dendritic network expanded with PDGF and retracted with LPA was difficult to reconcile with the observation that both PDGF and LPA were able to stimulate remodeling of floating collagen matrices. How could opposite movements of dendritic extensions lead to a similar degree of matrix remodeling? These were not the first data suggesting a paradox in the ability of PDGF and LPA to stimulate floating collagen matrix remodeling.

Bell and coworkers introduced the floating collagen matrix model and called their discovery *collagen lattice contraction* and the tissue-like structures that formed as a result of matrix remodeling *dermal equivalents* [65]. The original work was carried out in medium containing FBS, and the presence of serum subsequently was shown to be required [66]. Later work implicated PDGF as the serum agonist involved [67]. Yet when cells were placed under tension and stimulated with diverse growth factors, it became evident that either FBS or LPA were much more effective than PDGF in stimulating cell contractile force generation [68], reminiscent of the findings for Rho activation [29].

Fig. 4 illustrates the floating collagen matrix model and compares it to restrained matrices. What distinguishes restrained

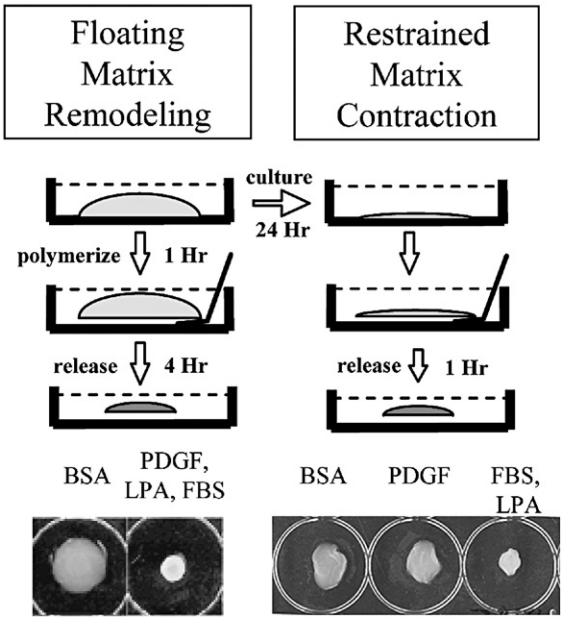

Fig. 4. Comparison of floating and restrained collagen matrix remodeling. Floating matrices are released and allowed to initiate remodeling immediately after polymerization whereas restrained matrices develop tension overnight before they are released. Floating matrix remodeling is stimulated above basal levels (BSA) by PDGF, LPA or FBS; whereas restrained matrix remodeling is stimulated above basal levels (BSA) by LPA or FBS but not by PDGF. See Figs. 1 and 2 in [69] for additional details.

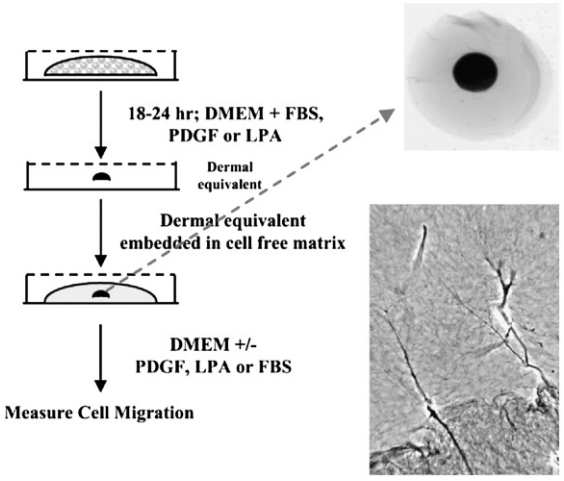

Fig. 5. Fibroblast migration in nested collagen matrices. Floating matrices remodeled overnight (dermal equivalents) were re-embedded in outer collagen matrices and then incubated for an additional 24 h. After a lag phase of several hour, fibroblasts with leading dendritic extensions (inset, H&E stained section), can be observed. See Figs. 1–3 in [72] for additional details.

from floating matrix remodeling is that cells have already developed tension in the matrices before they are released. When we directly compared floating and restrained collagen matrices, we found that floating matrix remodeling was stimulated similarly by FBS, LPA or PDGF, but that restrained matrix remodeling was stimulated by FBS or LPA much better than PDGF [69]. In short, whether or not cells were under tension changed the mechanism that they use to remodel collagen matrices, and restrained matrices appeared to be a better measure of cell contractile activity than floating matrices. Indeed, so-called *collagen lattice contraction* by fibroblasts in floating collagen matrices probably does not depend on cell contraction at all.

It also had been suggested that floating matrix remodeling might be a result of attempted cell migration rather than contraction [70]. Fibroblasts were shown to migrate out of dermal equivalents into fibrin matrices [71], so we developed a similar model to study growth factor specificity of fibroblast migration in collagen matrices [72]. Fig. 5 illustrates the nested collagen matrix model and shows the border between the inner dermal equivalent and surrounding outer matrix. Fibroblasts with leading dendritic extensions can be seen migrating into the outer matrices. Using this model, we found that cell migration was stimulated primarily by PDGF and not by FBS or LPA. Therefore, attempted cell migration could not explain floating collagen matrix remodeling because remodeling occurred under both pro-migratory and pro-contractile conditions.

Recently, we gained further insight into the floating matrix remodeling dilemma by focusing on the convergence of PDGF and LPA signaling pathways. Previous work had established that p21-activated kinase-1 (PAK1) is a downstream effector for both PDGF [73] and LPA-mediated signaling [74]. Our findings, which are summarized in Fig. 6 [75], showed that PDGF and LPA regulate floating collagen matrix contraction through signaling pathways that converge on PAK1 and its downstream

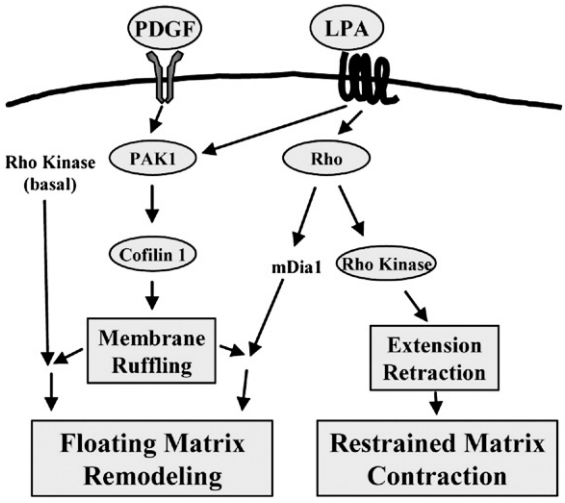

Fig. 6. Signaling pathways in floating collagen matrix contraction. Model showing convergence of PDGF and LPA signaling on PAK1 and cofilin1, cell ruffling, and collagen matrix contraction. Rho kinase cooperates with PAK1 for PDGF-stimulated contraction, whereas mDia1 cooperates with PAK1 for LPA-stimulated contraction. Rho kinase also is required for LPA-stimulated retraction of dendritic extensions. See Fig. 10 in [79] for additional details.

effector cofilin, and that contraction depends on cellular ruffling activity rather than the overall movement of cell extensions. Interestingly, different Rho effectors were observed to cooperate with PAK1 in regulating contraction, Rho kinase in the case of PDGF and mDia1 in the case of LPA. The Rho effectors appeared to act in parallel to and cooperatively with the PAK1 signaling pathway and details of this cooperative interaction remain to be worked out.

Nested collagen matrices and restrained collagen matrices can distinguish pro-migratory (PDGF) and pro-contractile (LPA, FBS) growth factor agonists. That either pro-migratory or pro-contractile agonists can stimulate floating collagen matrix remodeling leads us to speculate that this process may represent a more general feature of tissue homeostasis. Fibroblasts in floating collagen matrices develop a dendritic network interconnected by gap junctions [44] analogous to the interconnected dendritic network of osteocytes — another connective tissue cell. The osteocyte dendritic network functions as the regulatory sensor to detect changes in tissue mechanics in bone [76,77]. Perhaps the fibroblast dendritic network plays a similar mechanoregulatory function in ordinary connective tissue.

5. Conclusions and future directions

Most research on cell adhesion and migration has been carried out in the pro-contractile, high tension environment. Moreover, collagen matrices differ from planar culture surfaces in terms of penetration, entanglement and remodeling. Even when remodeled matrices permit cells to develop high tension state interactions, the properties of matrix stiffness, adhesion site density and topography will be distinct from conventional planar surfaces. Sorting out the biological consequences of these differences and exploring in greater detail the other three quadrants of cell mechanics provides a robust and challenging research agenda for the future.

Thinking about the four quadrants of cell mechanics emphasizes that there are dual modes of cell signaling, one arising from the growth factor environment and the other from cell–matrix interactions. To the extent that growth factors and focal adhesions converge on downstream effector pathways, then focal adhesions will act as amplifiers for growth factor signals. At low tension states, the amplification will be turned down.

Finally, the distinction between pro-migratory and pro-contractile environments raises important questions about physiological tissue environments. During wound repair, for instance, fibroblast migration is an early event, whereas fibroblast (myofibroblast) contraction is a late event. In general, the possibility that the growth factor environment might change to facilitate migration or contraction has not be considered. Although the wound environment is rich in growth factors released from platelets, it also contains locally secreted factors including proteolytic enzymes [78–80]. Perhaps the highly proteolytic chronic wound environment becomes pro-contractile and unable to support fibroblast migration because of the destruction of PDGF [81], which might help explain the ability of recombinant PDGF (Regranex) to stimulate repair of chronic human wounds.

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