

Fibroblasts in three dimensional matrices: cell migration and matrix remodeling

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Abbreviations: ECM, extracellular matrix; LPA, lysophosphatidic acid; S1P, sphingosine-1-phosphate

Abstract

Fibroblast-collagen matrix culture has facilitated the analysis of cell physiology under conditions that more closely resemble an *in vivo*-like environment compared to conventional 2-dimensional (2D) cell culture. Furthermore, it has led to significant progress in understanding reciprocal and adaptive interactions between fibroblasts and the collagen matrix, which occur in tissue. Recent studies on fibroblasts in 3-dimensional (3D) collagen matrices have revealed the importance of biomechanical conditions in addition to biochemical cues for cell signaling and migration. Depending on the surrounding mechanical conditions, cells utilize specific cytoskeletal proteins to adapt to their environment. More specifically, cells utilize microtubule dependent dendritic extensions to provide mechanical structure for matrix contraction under a low cell-matrix tension state, whereas cells in a high cell-matrix tension state utilize conventional acto-myosin activity for matrix remodeling. Results of collagen matrix contraction and cell migration in a 3D collagen matrix revealed that the use of appropriate growth factors led to promigratory and procontractile activity for cultured fibroblasts. Finally, the relationship between cell migration and tractional force for matrix remodeling was discussed.

Keywords: cell culture techniques; cell movement; cytoskeletal proteins; extracellular matrix; signal transduction

Introduction

The extracellular matrix (ECM) is an important component in establishing the cellular environment and provides mechanical support and a framework for cells and tissues in the body (Holmbeck and Szabova, 2006; Discher *et al.*, 2009; Wang *et al.*, 2009). Cells may be completely surrounded by ECM, as is the case for chondrocytes and fibroblasts, or may attach to the ECM only at one surface, as is the case for epithelial and endothelial cells. A diverse range of binding sites for cell-matrix interaction as well as plasticity and molecular remodeling of the matrix are critical for the organization of diverse tissues, such as skin, brain and muscle, that have specific structural and mechanical properties (Rauch, 2004; Bershadsky *et al.*, 2006; Halayko *et al.*, 2008).

The 3 dimensional (3D) matrix-cell culture system is a useful model to analyze functional and biomechanical features of cell-matrix interactions and pathogenesis, such as wound healing (Kim, 2005; Rhee and Grinnell, 2007; Carlson *et al.*, 2008; Lee *et al.*, 2008). Human fibroblasts cultured in 3D collagen matrices encounter completely new environments relative to those experienced on the more commonly used 2D surfaces. Especially, the fibril structure of the 3D collagen matrix confers a mechanically compliant environment to the cells, which provides a reciprocal effect on cell and matrix mechanics. There are a limited number of adhesion domains accessible to the cells. In addition, the cells not only adhere through integrin-dependent 3D cell-matrix interactions, which differs from the adhesion mechanism of conventional 2D matrix-cell interactions (i.e. focal adhesion) (Cukierman *et al.*, 2002; Petroll and Ma, 2003), but also there is a change in mechanical stability due to cell-matrix entanglement (Jiang and Grinnell, 2005). In addition, by characterizing global and local matrix remodeling induced by cell activity in the 3D collagen matrix, it would be possible to better understand the different mechanisms of cell migration in a 3D environment.

In this review, we summarize recent findings regarding fibroblast signaling in response to collagen matrix contraction and the key issues involved in understanding how the fibroblast-collagen matrix model system has been used to examine fibroblast plasticity and cell migration.

Fibroblast-mediated matrix remodeling in 3D collagen matrices

Fibroblasts are the primary cell type responsible for collagen synthesis and the buildup of connective tissue, where the cell itself is responsible for normal tissue homeostatic processes such as tissue repair in response to injury (Hinz and Gabbiani, 2003; Desmouliere *et al.*, 2005; Broughton *et al.*, 2006), tissue pathology associated with fibrosis (Kisseleva and Brenner, 2008a; 2008b), and cancer progression due to stromagenesis (Beacham and Cukierman, 2005; Pavlakis *et al.*, 2008; Castello-Cros and Cukierman, 2009). In addition, fibroblast-ECM remodeling is a pivotal design element in tissue engineering (Lutolf and Hubbell, 2005; Geutjes *et al.*, 2006).

3D culture systems are used to mimic the natural ECM features found *in vivo* and, thus, they are believed to provide a tissue-like environment to cells cultured under these conditions (Kim, 2005; Pedersen and Swartz, 2005; Lee *et al.*, 2007, 2008; Rhee and Grinnell, 2007; Yamada and Cukierman, 2007; Carlson *et al.*, 2008). Fibroblasts in a 3D collagen matrix have unique morphologies ranging from dendritic to bipolar depending on matrix stiffness and tension. Moreover, fibroblasts associate with collagen fibrils, which develop reciprocal mechanical interactions with the collagen matrix. The interactions between cells and the collagen matrix result in the formation of a mechanical entanglement that transmit fibroblast force to their surrounding matrix and induces local and global matrix remodeling (contraction, compaction) (Grinnell, 1994; Jiang and Grinnell, 2005) to achieve tensional homeostasis.

Matrix remodeling induced by fibroblasts depends on various factors including growth factors and the mechanical environments. To better understand the relationship between these factors and matrix remodeling, several experimental model systems were developed to examine how the cell's internal factors, such as exerted force, and extracellular factors, such as growth factors, affect matrix remodeling (Lee *et al.*, 2003; Rhee and Grinnell, 2006; Jiang *et al.*, 2008).

As illustrated in Figure 1, three experimental models, the floating matrix, attached matrix and restrained matrix, were developed to study the process of matrix remodeling. These models were shown to be excellent systems in regards to study the mechanism of how cells exert force to induce matrix remodeling before or after developing isometric tension (Grinnell, 2000; Lee *et al.*, 2003). In the floating matrix model, the matrix was released after polymerization at which point the matrix started to become remodeled. Current evidence

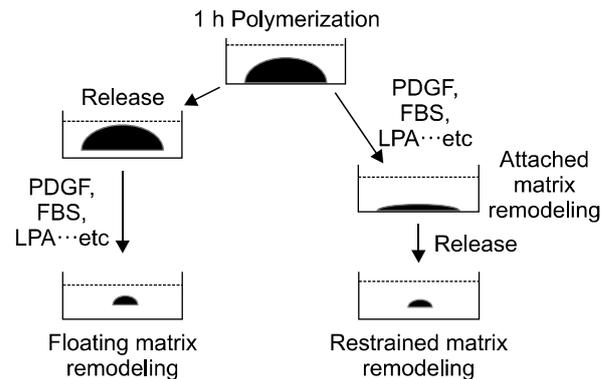


Figure 1. Comparison of *in vitro* collagen matrix remodeling. After one hour of collagen polymerization with fibroblasts, the matrices were either immediately released from the culture dish (floating matrix) or cultured under attached conditions (attached matrix). In the Restrained (stressed) matrix, isometric tension was developed overnight, after which the matrix was released to initiate remodeling.

suggests that floating matrix remodeling occurs as a result of the motile activity of cells, which are attempting to migrate throughout matrix (Grinnell *et al.*, 1999; Rhee and Grinnell, 2006; Jiang *et al.*, 2008). In contrast, cells in the attached matrix become bipolar along the line of tension and develop massive stress fibers resembling myofibroblasts (Tomasek *et al.*, 2002; Hinz and Gabbiani, 2003). As matrix remodeling progresses in the attached matrix, the stiffness of the matrix increases and the overall mechanical properties of the matrix changes, which affects the cells' tractional activity as determined by stress fiber and focal adhesion formation (Grinnell, 2000; Shreiber *et al.*, 2001; Tamariz and Grinnell, 2002).

In addition, a study on matrix remodeling in the restrained matrix model provided insight into understanding how an increase in the matrix mechanical loading changes the cellular mechanics. In the restrained matrix, which is under isometric tension, the mechanics controlling the cell became dependent on Rho kinase activity when LPA was used to induce remodeling (Lee *et al.*, 2003).

Signaling for matrix remodeling in fibroblast-3D collagen matrices

Floating matrix contraction was stimulated by PDGF, LPA and serum, whereas FBS and serum were found to be better than PDGF in inducing attached matrix and restrained matrix remodeling, indicating that cells utilize different motors to stimulate matrix remodeling based on whether they are under isometric tension or not (Abe *et al.*, 2003; Lee *et al.*, 2003). Interestingly, PDGF-induced floating matrix contraction was required for

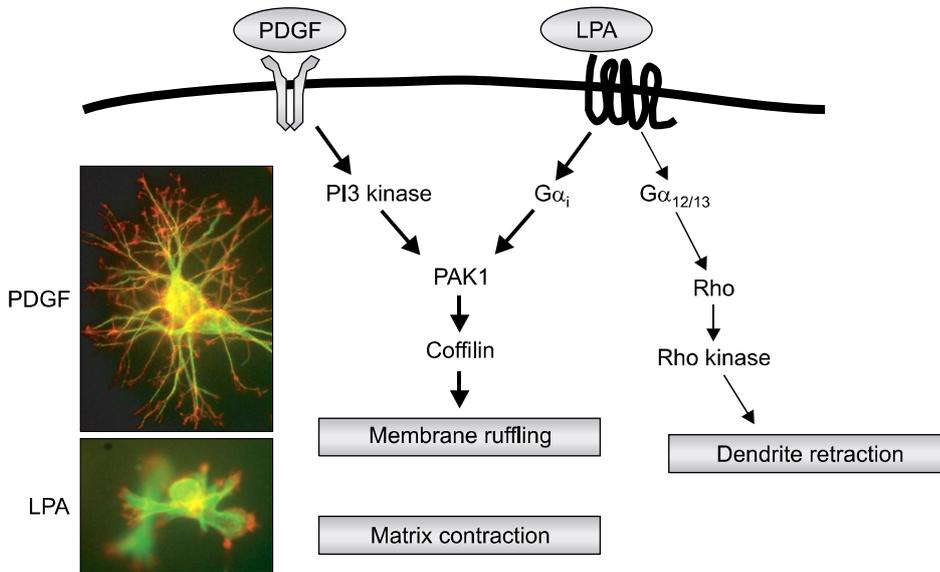


Figure 2. Membrane ruffling of fibroblast in 3D collagen matrix. The dendritic extensions of fibroblasts in 3D collagen matrices expanded in response to PDGF and retracted in response to LPA stimulation (Upper panel). Membrane ruffling was induced at the margin of the membrane in both PDGF- and LPA-stimulated fibroblasts (Low panel), which provides mechanical activity for the floating matrix. Rho kinase and mDia interacted with PAK1 for PDGF and LPA-mediated matrix contraction, respectively. See Figure 3 and 10 in (Rhee and Grinnell, 2006) for more detail.

phosphatidylinositol 3 kinase (PI3K) and Rho kinase activities but LPA-stimulated remodeling was independent of Rho kinase (Abe *et al.*, 2003). By using siRNA and pharmacological inhibitors, it was demonstrated that signaling during PDGF and LPA induced floating matrix contraction was dependant on the p21-activated kinase 1 (PAK1)-cofilin signaling pathway. The different Rho effectors were coordinated with PAK1 to regulate this remodeling process, where the Rho kinase was involved with the antagonist PDGF and mDia1 was involved with the antagonist LPA. Moreover, the 3D collagen matrix predominately induced a cellular ruffling activity in response to both growth factors instead of lamella formation, which may play a major role in the mechanical activity of the cell during matrix remodeling (Rhee and Grinnell, 2006) (Figure 2).

However, in the case of restrained matrix remodeling, which is under isometric tension, Rho kinase-dependent myosin light chain phosphorylation was required for LPA-mediated matrix remodeling (Lee *et al.*, 2003). Because no comparable switch occurs for PDGF-induced restrain matrix remodeling, although it has been reported that different integrins respond to specific signaling (Cooke *et al.*, 2000), the mechanism behind PDGF-induced restrain matrix remodeling still needs to be more clearly elucidated.

Fibroblast plasticity: effect of tension

It has been extensively demonstrated that the overall morphology of fibroblasts grown in a collagen 3D matrix culture was determined by the

physical environment of the cell (Carlson *et al.*, 2008; Miron-Mendoza *et al.*, 2008; Doyle *et al.*, 2009). Indeed, it has been shown that fibroblast morphology depends on the matrix organization. In low tension states, which can be created using a floating collagen matrix, actin stress fibers were not visible in the cells, few focal adhesion phenotypes were observed and matrix biosynthesis was not observed. Interestingly, these cellular morphological traits have also been observed in resting tissue *in vivo*, such as during quiescence/apoptosis (Fringer and Grinnell, 2001; Graf *et al.*, 2002; Tamariz and Grinnell, 2002; Tian *et al.*, 2002). However, in stiff, restrained matrices, which provide a high tension state, the cell undergoes massive cytoskeletal reorganization to induce the formation of stress fibers and focal adhesions (Petroll and Ma, 2003). This process then turns on matrix biosynthesis, which is analogous to cells found in active tissues such as granulation tissue and wound contraction (Kessler *et al.*, 2001; Tomasek *et al.*, 2002).

Changes in collagen biosynthesis and matrix stiffness by fibroblasts have also been implicated in skin aging (Papazafiri and Kletsas, 2003; Fu *et al.*, 2005; Fisher *et al.*, 2009). A major feature of aged skin is fragmentation of the dermal collagen matrix, which alters the mechanical properties of skin. Changes in the mechanical properties of dermal collagen can be caused by alterations in the biochemical reactions of dermal fibroblasts. Collagen synthesis in fibroblasts from aged skin was reduced and the activity of collagen-degradation enzymes was increased, which causes a tensional unbalancing of the dermal collagen ma-

trix (Fisher *et al.*, 2008).

Microtubule function in fibroblast-3D collagen interactions

Although it is believed that the mechanical and physical features of the extracellular environment significantly affects cell morphology (Cukierman *et al.*, 2002; Engler *et al.*, 2006; Vogel, 2006), it is not clear what regulatory mechanisms are involved in this process. Since fibroblasts in relaxed 3D collagen matrices have a unique morphology that contains microtubule-mediated dendritic formation, which are an indispensable factor for neurite protrusion (Lankford *et al.*, 1990; Johnson and Stoothoff, 2004; Schaefer *et al.*, 2008), it is reasonable to assume that microtubule-mediated signaling may be critical for determining fibroblast shape in response to different physical environments.

Figure 3 shows the differential effect of microtubules on cell spreading under 2D and 3D environments. Normally, cells cultured under 2D conditions, such as on a glass coverslip, can spread out with elongated morphologies. Interfering microtubule formation by nocodazole treatment impaired cell elongation but the extent of cell spreading without microtubules was similar to normal cells. In contrast, nocodazole treatment completely inhibi-

ted cell spreading in 3D collagen matrix environments (Rhee *et al.*, 2007). These findings indicate that the effect of microtubules on fibroblast spreading in a 3D collagen matrix depends on the mechanical conditions of the matrix. Although there are several differences between 2D and 3D conditions, these observations suggest that if the matrix is stiff (e.g., glass coverslip), microtubules determine cell polarity but if the matrix is soft (e.g., collagen matrix), the microtubule participates in cell spreading (Polte *et al.*, 2004; Rhee and Grinnell, 2007; Gauthier *et al.*, 2009).

Role of the promigratory and procontractile environment in fibroblast mechanics

Cells in a 3D matrix have distinct patterns of morphology and migration compared with those on a 2D planar surface (Friedl and Brocker, 2000; Friedl, 2004; Zaman *et al.*, 2006). Especially, when fibroblasts are on a 2D planar surface, the tractional force against a stiff substrate results in cell migration. However, when cells are in a 3D collagen matrix, the tractional force can be utilized as a mechanical inducer for matrix remodeling both locally and globally. Therefore, understanding the molecular mechanism of fibroblast migration in a 3D matrix can be important for understanding various pathological diseases and fibrotic diseases including fibrosis and cancer metastasis (Desmouliere *et al.*, 2004; Abraham *et al.*, 2007; Kenny *et al.*, 2007).

It was originally shown that fibroblasts can migrate out of tissue explants embedded in collagen matrices (Sawhney and Howard, 2002) and also migrate from contracted cells containing collagen matrices into fibronectin-fibrin matrices (Greiling and Clark, 1997; Clark *et al.*, 2003; Lin *et al.*, 2005). Researchers have recently taken advantage of these observations to develop the nested collagen matrix system (Grinnell *et al.*, 2006). In this system, dermal equivalents, which were prepared from precontracted floating collagen matrices, were embedded in cell-free collagen matrices. Unlike the process of durotaxis, where cell migration occurs under a gradient of matrix rigidity (Lo *et al.*, 2000), fibroblast migration can occur from a hard matrix to a soft matrix in a nest collagen matrix along the chemoattractants gradient, which is a distinct migration pattern in the context of the 3D matrix (Grinnell *et al.*, 2006; Miron-Mendoza *et al.*, 2008).

Several interesting findings in relation to fibroblast migration were obtained using the nested matrix. Initially, there was an 8-16 h lag phase before migration began, which has never been

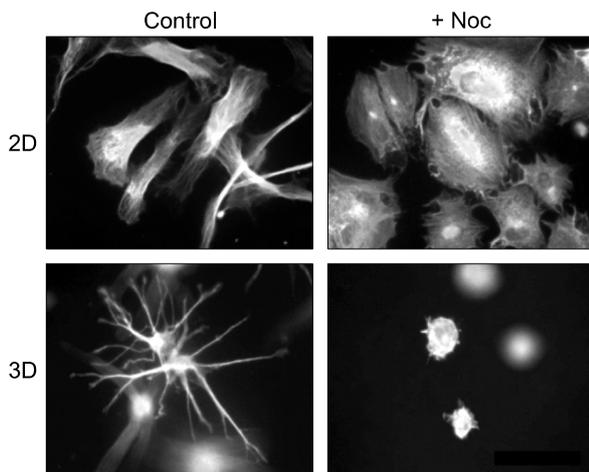


Figure 3. Different roles of microtubules in fibroblast spreading on collagen-coated coverslips compared to collagen matrices. The actin cytoskeleton was visualized and imaged by fluorescence microscopy. Fibroblasts cultured for 4 h on collagen-coated coverslips (2D) spread into an elongated, flattened morphology. Disrupting microtubules with nocodazole (+Noc) inhibited cell polarization but not spreading. Fibroblasts cultured inside of collagen matrices for 4 h (3D) spread by protrusion of dendritic extension. Dendritic extensions in 3D collagen matrices were not observed in cells that were cultured with Noc. See figure 1 in (Rhee *et al.*, 2007) for more detail.

observed on a 2D planar surface. However, it has not yet been clearly determined why the cell needs such a long lag phase before migration starts. One plausible explanation is that a certain period of time is required for the cells to overcome the difference in matrix stiffness between the inner and outer matrix. Another possible reason is that the cells need to develop biosynthetic features to simulate a resting dermis, since almost all signaling would already have been turned off in a dermal equivalent (Grinnell *et al.*, 2003). In addition, by examining fibroblast migration in the nested matrix, collagen fibril flow was shown to occur from the outer matrix toward the inner matrix prior to fibroblast migration (Miron-Mendoza *et al.*, 2008). Furthermore, this method can be used as a useful tool for screening of oncogenic transformation (Menezes *et al.*, 2008).

Experiments on matrix contraction and nested matrix cell migration have provided a better understanding of the molecular mechanisms responsible for fibroblast activity in the presence of different growth factors (Jiang *et al.*, 2008). In the process of wound healing, especially, fibroblasts might encounter various growth factors that regulate cell migration and matrix contraction. During wound repair, fibroblasts migrate into the wound area and consequently develop mechanical stress and matrix contraction (Fu *et al.*, 2005; Davidson, 2007). Therefore, it is important to understand the molecular mechanisms by which growth factors participate in distinct cellular processes in wound environments. Jiang *et al.* tested several purified agonists and serum for matrix contraction and cell migration in a nested collagen matrix. In this study, they demonstrated that PDGF was the only growth factor capable of inducing cell migration in a nested matrix. However, PDGF displayed weak activity under stress matrix contraction. In contrast, lipid variant agonists including serum, LPA and S1P were found to promote stress-released matrix contraction but not cell migration. Moreover, S1P worked as a master regulator for balancing the promigratory and procontractile conditions (Jiang *et al.*, 2008).

Conclusion

The development of 3D model systems in cell biology has recently emerged as an important tool to study cellular behavior since cell physiology is quite different in a 2D versus 3D environment. In addition, 3D experiments allow one to capture the complex 3D tissue physiology under *in vitro* conditions (Grinnell, 2003; Griffith and Swartz,

2006; Krause *et al.*, 2008; Kuo and Tuan, 2008). Furthermore, 3D model systems provide useful tools for understanding the mechanobiology of how individual cells sense and generate mechanical force in response to their surrounding environment (Pedersen and Swartz, 2005).

Thus far, most studies on cell mechanics have been carried out in high tension and 2D environments, which is presented as a one-way signaling event; however, the fibroblast-3D matrix system permits cells to have reciprocal, two-way interactions between the matrix and cells. Cells interacting under low-tensional matrix conditions can convert the matrix mechanics from soft to stiff in response to growth factor stimulation, which can eventually promote high-tensional interactions between cells. During these events, all the dynamic changes in the cell-matrix interactions under different mechanical environments greatly influence cell signaling and behavior.

In addition to the mechanical environment, the presence of different growth factors can also influence cell behavior in a 3D matrix. In this regard, differences in cell signaling, cytoskeleton modulation and integrin-focal dynamics during promigratory processes, such as in the presence of PDGF and procontractile processes, such as in the presence of serum and FBS, are critical for cell mechanics and cell-matrix interaction. Therefore, the combination of growth factor signaling and changes in the mechanical environment by cell-matrix interactions provides a new concept for understanding the relationship between cellular behavior and matrix mechanics (so called four quadrants) (Rhee and Grinnell, 2007).

Finally, cellular mechanics in different biomechanical and growth factor environments constitutes a new research field that still needs to be developed. For example, pathological diseases such as cancer progression and wound repair as well as aging require growth factor stimulation, which can alter the mechanical environment and change the cell-matrix interaction. It has been accepted that growth factor signaling changes the cell behaviors and dynamics; but, the effect of growth factors on mechanical changes in a matrix environment cannot be credibly established when experiments are carried out in 2D culture systems. However, these limitations can be overcome using a 3D culture system. 3D culture systems allow researchers to decouple multiple molecular and dynamic interactions between cells and a surrounding matrix, since the system acts as an *in vivo* mimic of biological tissue. Further studies exploring in greater detail the process of *in vitro* remodeling using fibroblast-3D matrix culture systems will be highly valuable in

resolving some of the challenges currently facing the field of 3D biology.

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