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Lateral boundary mechanosensing by adherent cells in a collagen gel system

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ABSTRACT

Cell adhesion responses to in-depth physical properties such as substrate roughness and topography are well described but little is known about the influence of lateral physical cues such as tissue boundaries on the function of adherent cells. Accordingly, we developed a model system to examine remote cell sensing of lateral boundaries. The model employs floating thin collagen gels supported by rigid grids of varying widths. The dynamics, lengths, and numbers of cell extensions were regulated by grid opening size, which in turn determined the distance of cells from rigid physical boundaries. In smaller grids (200 μm and 500 μm wide), cell-induced deformation fields extended to, and were resisted by, the grid boundaries. However, in larger grids (1700 μm wide), the deformation field did not extend to the grid boundaries, which strongly affected the mean length and number of cell extensions (~60% reduction). The generation of cell extensions in collagen gels required expression of the β1 integrin, focal adhesion kinase and actomyosin activity. We conclude that the presence of physical boundaries interrupts the process of cell-mediated collagen compaction and fiber alignment in the collagen matrix and enhances the formation of cell extensions. This new cell culture platform provides a geometry that more closely approximates the native basement membrane and will help to elucidate the roles of cell extensions and lateral mechanosensing on extracellular matrix remodeling by invasion and degradation.

1. Introduction

The composition and structure of extracellular matrices vary widely and depend on tissue type and the state of health or disease of the tissue [1,2]. In health and disease, the extracellular matrix in which cells reside provides microenvironments with diverse mechanical properties [3,4] that vary on a length scale of microns to mms. Biophysical approaches for modulating the mechanical properties of the matrix have shown that substrate elasticity, topography and roughness influence cellular processes such as spreading, migration, phagocytosis and differentiation [5–8]. The ability of cells to sense and respond to the mechanical properties of the extracellular matrix is dependent in part on application of actomyosin-dependent contractile forces [9]. The responses of cells to the mechanical properties of the matrix and force-induced matrix deformation can be measured by characterizing the deformation fields created in the extracellular matrix [10]. Unlike synthetic hydrogels (e.g. polyacrylamide gels) that exhibit nearly ideal linear elastic deformation, naturally-occurring matrix biopolymers demonstrate complex mechanical behavior after application of forces. For example, collagen gels display non-linear viscoelastic behavior when subjected to cell-generated forces and may undergo strain-stiffening [11,12], fiber alignment [13], or irreversible network compaction [14] depending on the magnitude and duration of deformation.

Elastic or inelastic matrix deformations enable adherent cells to detect inhomogeneous physical properties of the matrix, such as the presence of a rigid foundation [15,16] subjacent to the matrix or of adjacent cells that are located several hundred microns distant [17,18]. Notably, adherent cells can mechanosense relatively farther on fibrillar matrices than on elastic hydrogels [19]. This marked difference may be explained by the non-linear strain-stiffening behavior of fibrillar matrices [18] and/or by fiber alignment in collagen gel networks [20]. Notably, stiff fibrillar matrices such as cross-linked collagen exhibit lower rates of contraction and deformation than soft matrices [21].

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Cells explore the mechanical properties of their immediate microenvironment by generating filopodia, relatively short cell extensions that act as biomechanical sensors [22]. In addition to mechanosensing at cell adhesion sites to the matrix, cell extensions like filopodia play important regulatory roles in other cellular processes including cell migration, wound healing, matrix remodeling and degradation [5,23,24]. Cells adherent to fibrillar networks such as collagen gels form filopodia that enable remodeling and alignment of the collagen fibers in the network. The sensory, exploratory and matrix remodeling functions of filopodia are dependent on the generation of tensile forces and by the resistance of the matrix to deformation [25]. For example, tensile forces generated at the tips of filopodia are thought to mediate local network compaction and to enable further cell spreading [26].

Much of the work on cellular mechanosensing has examined the influence of the mechanical properties of an underlying substrate on adherent cells [27–29]. However, cells respond to physical cues that are lateral to, but are located well beyond, the cell–substrate interface. We differentiate these lateral cues from those in-depth cues that are positioned directly underneath the cell surface, which we denote as the mechanical properties of the substrate that are out-of-plane. Tissue boundaries are one example of laterally-oriented structures in extracellular environments that provide environmental cues [30]. During migration through extracellular matrices or in metastatic invasion of remote tissues, cells encounter microenvironments with wide variations of tissue boundary sizes [31], but which are not in direct contact with cells. Notably, Jacobson and Moury [32] investigated the role of tissue boundaries in neurulation and described how cell behavior is altered when cells approach a tissue boundary. They showed that disruption of the boundaries by incision, blocked neuronal cell extensions, neural plate elongation and tube formation. Neurons can also respond to the topography of their underlying substrate (i.e. in-depth mechanical properties); when cultures on pillar surfaces, neuronal cells demonstrated neurite outgrowth on narrow repeating topography [33]. These two examples demonstrate in neurons, the importance of in- and out-of-plane mechanical cues for regulating the formation of cell extensions.

To examine the impact of physical boundaries on cell behavior and cytoskeletal organization we developed a new model system in which nylon meshes with defined openings (200 μm wide and larger) provide defined physical boundaries in the plane of the supported matrix. Previously, several groups [34–36] have examined the effect of matrix geometry on cell function and, in particular, contractility. With the use of micropatterning techniques, cell spreading is controlled by the area and shape of a patterned, highly adhesive surface so that cells conform to a configured geometry to which they are confined. With this approach it appears that the magnitude and spatial distribution of cell-generated forces are not primarily dependent on cell spreading area [37]. Instead, a more important determinant of traction forces is the distance from the cell centroid to the perimeter of the adhesive area: when comparing cells of equal areas, more elongated cells generate stronger traction forces [38]. A similar model was used to examine pre-configured patterns of traction forces in three dimensional gels, which showed that the strongest inwardly directed traction forces originated from the tips of long, matrix-probing pseudopodia [39]. Further, with the use of matrices of defined geometry the direction and magnitude of local forces were correlated with the area and elongation of mature focal adhesions [34]. The results of such studies have suggested a linear relationship between net traction force and the size of focal adhesions in unconstrained spread cells. However, these approaches required a thin matrix protein coating (e.g. collagen) on a rigid foundation (i.e. out-of-plane physical boundaries) such as glass [35] or polydimethylsiloxane gel surfaces [34]. Notably, Fraley and colleagues found that when cells were adhered on their ventral surfaces to planar substrates and were then exposed on their dorsal surfaces to a matrix protein, focal adhesions still formed on the dorsal surface but these adhesions were greatly decreased in size and number [40]. However, for cells that were remote from the supporting glass surface (i.e. remote from rigid physical boundaries), no focal adhesions were detected. This study also reported that while focal adhesion proteins such as vinculin and focal adhesion kinase contribute to cell-induced matrix deformation in two-dimensional models, these adhesion molecules are not significantly involved in the generation of cellular forces in three-dimensional environments. Collectively, these studies highlight the importance of rigid physical boundaries in assessing cell function and contractility.

In our model system, the grid openings are coated with fibrillar collagen gels and then floated on growth medium so that in-plane boundaries are not altered by interference from an underlying rigid support. In this model the strain-stiffening nature of collagen fibrils and the alignment and compaction of fibrils by cells are important determinants of in-plane boundary sensing. As the material properties of the liquid foundation (i.e. growth medium) do not significantly affect the mechanics of the hydrogel, the effect of physical boundaries on collagen compaction and remodeling can be more readily examined. With this model system, acute control of matrix geometry and physical boundary conditions can be achieved, which provides new insights into the mechanical interplay between cells, substrate mechanics and static boundary conditions. In particular, matrix fiber alignment can be quantified based on the distance of adherent cells to rigid boundaries; with this approach the influence of spatial and mechanical effects on cell responses can be decoupled. In this report we determined whether the presence of physical boundary conditions impacts cellular protrusive behavior, a useful indicator of cellular mechanosensing [41].

2. Materials and methods

2.1. Preparation of nylon meshes

Nylon mesh sheets with square openings (200 μm wide) were obtained from Dynamic Aqua-Supply (Surrey, BC). Mesh was cut into 2 cm × 2 cm pieces that fit into 35 mm non-tissue culture plastic dishes. For some experiments, single nylon fibers were removed vertically and horizontally to create grid opening sizes with defined geometries (e.g. 200 μm × 500 μm; 500 μm × 500 μm; 1700 μm × 1700 μm). With this approach, the thickness of the collagen gel (~100 μm) and the nylon fiber diameters were kept constant while the grid opening size was varied.

2.2. Collagen gels

Collagen gels were prepared from pepsin-treated, bovine dermal type I collagen (6.0 mg/ml; ~97% type I collagen; Advanced BioMatrix, San Diego, CA). Prior to experiments, collagen solutions were neutralized with 0.1 M NaOH to pH = 7.4 and diluted to a final concentration of 1 mg/ml collagen. Glass dishes were covered with stretched parafilm to make a smooth, hydrophobic surface. Collagen solutions (~70 μl) were poured on the prepared hydrophobic surface. The nylon mesh was then placed on to the collagen, which filled the nylon grids with collagen solution. The amount of collagen solution poured on each gel was adjusted depending on the area of each nylon mesh that was created. Samples were incubated at 37 °C in 5% CO_2 until collagen polymerization was complete (>90 min). Polymerized, collagen-coated nylon meshes were gently detached from the hydrophobic surface by addition of 1× PBS and the floating collagen-coated meshes were inverted and immersed in cell culture medium.

2.3. Cells

3T3 fibroblasts, β1-integrin-deficient GD25 fibroblast-like cells, focal adhesion kinase knockout fibroblasts (ATCC; CRL-2644) or their corresponding wild-type controls, or 3T3 cells with filamin A knockdown [42] or their corresponding controls treated with irrelevant shRNA, were plated on collagen gels supported by nylon mesh grids. Cells were plated in DMEM medium supplemented with 10% calf serum and 10% antibiotics at low density to avoid the inclusion of more than one cell in a single grid space. Cells adhered rapidly to the collagen gels and spread within 3 h.