Acta Biomaterialia 57 (2017) 304–312

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Spatial distributions of pericellular stiffness in natural extracellular matrices are dependent on cell-mediated proteolysis and contractility

M. Keating [a,1], A. Kurup [a,1], M. Alvarez-Elizondo [b], A.J. Levine [c], E. Botvinick [a,*]

[a] University of California, Irvine, Department of Biomedical Engineering, Irvine 92697-2730, USA
[b] Technion, Israel Institute of Technology, Department of Biomedical Engineering, Technion City, 32000, Israel
[c] Departments of Physics & Astronomy, Chemistry & Biochemistry, and Biomathematics, University of California, Los Angeles, CA 90095, USA

ARTICLE INFO

Article history:
Received 17 January 2017
Received in revised form 31 March 2017
Accepted 4 May 2017
Available online 5 May 2017

Keywords:
Microrheology
Cellular remodeling
Pericellular stiffness
Tissue mechanics
Extracellular matrix
Hydrogel

ABSTRACT

Bulk tissue stiffness has been correlated with regulation of cellular processes and conversely cells have been shown to remodel their pericellular tissue according to a complex feedback mechanism critical to development, homeostasis, and disease. However, bulk rheological methods mask the dynamics within a heterogeneous fibrous extracellular matrix (ECM) in the region proximal to a cell (pericellular region). Here, we use optical tweezers active microrheology (AMR) to probe the distribution of the complex material response function ($\alpha = \alpha' + \alpha''$, in units of μm/nN) within a type I collagen ECM, a biomaterial commonly used in tissue engineering. We discovered cells both elastically and plastically deformed the pericellular material. α' is wildly heterogeneous, with $1/\alpha'$ values spanning three orders of magnitude around a single cell. This was observed in gels having a cell-free $1/\alpha'$ of approximately 0.5 nN/μm. We also found that inhibition of cell contractility instantaneously softens the pericellular space and reduces stiffness heterogeneity, suggesting the system was strain hardened and not only plastically remodeled. The remaining regions of high stiffness suggest cellular remodeling of the surrounding matrix. To test this hypothesis, cells were incubated within the type I collagen gel for 24-h in a media containing a broad-spectrum matrix metalloproteinase (MMP) inhibitor. While pericellular material maintained stiffness asymmetry, stiffness magnitudes were reduced. Dual inhibition demonstrates that the combination of MMP activity and contractility is necessary to establish the pericellular stiffness landscape. This heterogeneity in stiffness suggests the distribution of pericellular stiffness, and not bulk stiffness alone, must be considered in the study of cell-ECM interactions and design of complex biomaterial scaffolds.

Statement of Significance

Collagen is a fibrous extracellular matrix (ECM) protein widely used to study cell-ECM interactions. Stiffness of ECM has been shown to instruct cells, which can in turn modify their ECM, as has been shown in the study of cancer and regenerative medicine. Here we measure the stiffness of the collagen microenvironment surrounding cells and quantitatively measure the dependence of pericellular stiffness on MMP activity and cytoskeletal contractility. Competent cell-mediated stiffening results in a wildly heterogeneous micromechanical topography, with values spanning orders of magnitude around a single cell. We speculate studies must consider this notable heterogeneity generated by cells when testing theories regarding the role of ECM mechanics in health and disease.

1. Introduction

Interactions between cells and their extracellular matrix (ECM) are bi-directional. On one hand, the mechanical properties of the ECM have been shown to regulate key processes in cells; for example, increasing bulk ECM stiffness has been correlated to invasion of mammary epithelial cells [1], differentiation of mesenchymal stem cells [2,3], and maturation of cardiomyocytes [4]. On the other hand, cells actively alter their ECM through context-dependent degradation, remodeling, and deposition of new ECM [5]. Thus, quantifying the mechanical interactions between the cell and its ECM both spatially and temporally, at a scale relevant to the

* Corresponding author.
E-mail address: elliot.botvinick@uci.edu (E. Botvinick).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.actbio.2017.05.008
1742-7061/

interaction, is imperative to study how cells are regulated in physiological and pathological processes.

One interesting aspect of cell-ECM physical interactions is the distribution of traction forces that cells exert onto their local ECM. 3D traction force microscopy (TFM) has been developed for cells fully embedded within a linear, homogenous, nano-porous, synthetic PEG hydrogel containing tracer microbeads [6,7], which can be modified to contain sites for cell adhesion and cell mediated degradation [8]. The strain field can be calculated by tracking bead displacement. Then, an estimation of traction forces can be computed under the assumption of hydrogel linear elasticity, homogeneity, and without consideration of cell-mediated degradation and deposition of new ECM. While these methods are elegant and provide important insight, results may not be generalizable to physiologically relevant tissues because these gels do not share the native architecture, pore size, or nonlinear properties of natural matrices [9]. Additionally, cells may remodel the synthetic hydrogels differently than they would a fibrous gel of natural origin. Heterogeneities in local ECM architecture and stiffness have hindered efforts to extend TFM to natural matrices particularly in the pericellular space. For example, it has been reported that stresses within the ECM cannot be determined from bead displacements alone under the assumption of homogenous mechanical properties [7] and without accounting for local degradation [10]. Furthermore, stiffness of natural, type I collagen fibrous matrices increases non-linearly with deformation, and cannot be determined from collagen concentration alone [7,11]. Instantaneous stiffness should be determinable from strain if the nonlinear relationship between strain and stiffness is known a priori. However, such a calculation requires knowledge of the current stress free state of the material, which may not be available once cells plastically remodel the local matrix. Thus, the study of how pericellular stiffness changes over time requires the use of a technique that can directly measure stiffness locally.

Here we use optical tweezers active microrheology (AMR) to measure the complex material response function at multiple sites around cells grown in 3D type 1 collagen gels and observe the dependence of material property heterogeneity on both cell contractility and matrix metalloproteinase (MMP) activity. One potential way a cell can modulate its pericellular mechanical topography is through cytoskeletal contractile forces that locally deform the ECM and stiffen it through strain-hardening [12], a process that's also essential for cell mechanoresponsiveness [13]. Another way in which a cell may modulate its local mechanical topography is through degradation of its local ECM, mediated by cell synthesized MMPs [14]. MMP mediated matrix degradation has been shown to be critical in processes including angiogenesis [15,16], cancer metastasis [17], and skeletal formation [18]. Thus, both cytoskeletal contractility and MMP activity are logical targets to explore the role of a cell in establishing or maintaining its pericellular stiffness, which we have shown can be significantly stiffer than values reported by bulk rheology [19–21] and are consistent in order-of-magnitude to stiffness reported by other groups using AMR in type I collagen [22], Matrigel, hyaluronic acid, and zebrafish *in vivo* [23]. In earlier studies, we used AMR to discover that during capillary morphogenesis, the pericellular space surrounding the tip of a sprouting capillary had increased stiffness as compared to distal regions [24]. We also showed that mouse skeletal stem cells required MMP14 (MT1-MMP) activity to stiffen the pericellular space within 3D collagen gels, a result that was associated with osteogenic fate commitment *in vivo* [25]. Here we use AMR to measure the distribution of pericellular stiffness surrounding isolated dermal fibroblasts and smooth muscle cells embedded within collagen gels and observe important new insights into how cells modulate their mechanical microenvironment in a contractility and MMP-dependent manner.

2. Methods

2.1. Cell culture

Dermal fibroblasts (DFs) were acquired from Lonza (CC-2511) and were cultured in DMEM (Fisher) with 10% FBS (Gibco) and 1% penicillin streptomycin (Gibco). Human Aortic smooth muscle cells (HAoSMCs) were acquired from ATCC (PCS-100-012) and the media plus bullet kit (CC-3182) from Lonza. All cells in this study were used prior to passage 7.

2.2. Collagen hydrogel formation

Type 1 collagen was chosen for these studies given both its abundance as the one of the main structural proteins of ECM within the body [26] and its relative prevalence within the natural context of the investigated cell types [27,28]. Collagen hydrogels were made at a final concentration of 1.0 mg/mL or 2.0 mg/mL using acid extracted rat tail type 1 collagen from vendors Advanced Biomatrix or Corning, respectively. Collagen of this type has been previously reported to vary significantly from lot to lot [29]. Therefore, collagen lots and concentrations were kept consistent for each set of cells: 1 mg/mL for DF experiments and 2 mg/mL for HAoSMC experiments. Structure (as assessed by reflection confocal microscopy) and mechanical properties (as probed by AMR) were roughly matched between the two cell-free conditions. For DF experiments, 3 cells in 3 separate gels were measured per condition. For HAoSMC experiments, 3 cells were measured within a single gel per condition.

Collagen gels were prepared with 10× PBS (Life Technologies), 1 N NaOH (Fisher), sterile-filtered DI H_2O, 2 μm carboxylated silica microbeads (0.8 mg/ml, Bangs Laboratories), and cells (100 k/ml) in 35 mm glass bottom dishes (MatTek). The samples were placed in a standard tissue culture incubator at 37 °C for 40 min during the polymerization process after which media was added to each dish.

Cells in control conditions were fed with normal media at the time of gelation. In the BB94 conditions, cells were fed with normal media supplemented with 10 μM BB94 (Sigma) after gelation. All dishes were incubated for 24 h in a standard tissue culture incubator. Prior to AMR measurements, the culture media was supplemented with HEPES (20 mM) and the dish placed on the microscope stage. A custom-built incubation system plus an objective heater maintained temperature in the dish at 34 °C. Gels were allowed to equilibrate to temperature for at least 1-h to prevent focus drift [30]. Y27632 conditions were supplemented with 20 μM Y27632 (Sigma) during this 1-h, on-stage incubation period.

2.3. AMR system

The AMR apparatus is illustrated in Fig. S1a. The optical tweezers microbeam is generated by a continuous-wave fiber laser with emission at 1064 nm (IPG Photonics), hereafter referred to as the trapping beam. A pair of galvanometer mirrors (ThorLabs) placed conjugate to the back focal plane of the microscope objective lens steers the trapping beam focus in the transverse plane of the microscope objective. A glass coverslip reflects a small fraction of the beam power and directs it onto a quadrant photo diode (QPD, Newport) labeled as trapQPD in Fig. S1a. The trapQPD outputs analog signals proportional to the deflection of the trapping beam. A low power laser diode with emission at 785 nm (World Star Technologies), hereafter referred to as the detection beam, detects the probe microbead response. A long pass dichroic beam splitting mirror (D1, Semrock) combines the two laser beams and