Biomaterials 32 (2011) 9584–9593

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Synergistic regulation of cell function by matrix rigidity and adhesive pattern

Shinuo Weng[a], Jianping Fu[a,b,*]

[a] Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI 48109, USA
[b] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA

ARTICLE INFO

Article history:
Received 18 August 2011
Accepted 1 September 2011
Available online 28 September 2011

Keywords:
Extracellular matrix (ECM)
Matrix rigidity
Adhesive pattern
Polydimethylsiloxane (PDMS)
Mechanotransduction

ABSTRACT

Cell–extracellular matrix (ECM) interactions play a critical role in regulating cellular behaviors. Recent studies of cell–ECM interactions have mainly focused on the actomyosin based and adhesion mediated mechanosensing pathways to understand how individual mechanical signals in the cell microenvironment, such as matrix rigidity and adhesive ECM pattern, are sensed by the cell and further trigger downstream intracellular signaling cascades and cellular responses. However, synergistic and collective regulation of cellular behaviors by matrix rigidity and adhesive ECM pattern are still elusive and largely uncharacterized. Here, we generated a library of microfabricated polydimethylsiloxane (PDMS) micropost arrays to study the synergistic and independent effects of matrix rigidity and adhesive ECM pattern on mechanoresponsive behaviors of both NIH/3T3 fibroblasts and human umbilical vein endothelial cells (HUVECs). We showed that both cell types were mechanosensitive and their cell spreading, FA formation, cytoskeletal contractility, and proliferation were all strongly dependent on both substrate rigidity and adhesive ECM pattern. We further showed that under the same substrate rigidity condition, smaller and closer adhesive ECM islands would cause both cells to spread out more, form more adhesion structures, and have a higher proliferation rate. The influence of adhesive ECM pattern on rigidity-mediated cytoskeletal contractility was cell type specific and was only significant for NIH/3T3. Morphometric analysis of cell populations revealed a strong correlation between focal adhesion and cell spreading, regardless of substrate rigidity and adhesive ECM pattern. We also observed a strong correlation between cellular traction force and cell spreading, with a substantially smaller independent effect of substrate rigidity on traction force. Our study here had determined key aspects of the biomechanical responses of adherent cells to independent and collective changes of substrate rigidity and adhesive ECM pattern.

1. Introduction

Environmental sensing to external signals is widespread in almost every cell type, from prokaryotes to multicellular organisms. Cells sense, analyze and integrate these external signals, both chemical and physical, and subsequently change its morphology, dynamic behaviors, and eventually, fate. For adherent cells to grow and function, it is crucial for them to maintain their tight association with the diverse connective tissue components that form the extracellular matrix (ECM). In recent years, it has become increasingly apparent that the cellular responses to microenvironmental signals go far beyond the ability of the cells to chemically sense specific ECM ligands, and it encompasses a wide range of physical cues that are generated at, or acted on the adhesive interface between the cells and the surrounding matrix [1–5]. Numerous studies have demonstrated the capacity of cells to respond to changes in multiple biointerfacial parameters, including adhesive ligand density and pattern [6–8], surface compliance (or ECM rigidity) [9–15], and ECM dimensionality and anisotropy [16–18], among other characteristics. It is generally believed that the cellular sensory machinery is capable of integrating this complex information into a coherent environmental signal, by globally altering cell shape and actin cytoskeletal organization [3,19–21], and by locally modulating adhesion formation and signaling [1,2,4].

Conventional methods to study cell–ECM interactions have largely relied on natural and synthetic hydrogels [5,22,23]. Although these hydrogel systems have proven important to deepen our understanding of cellular sensing of environmental signals, they still suffer from certain limitations that prevent their applications in some detailed investigations of biointerfacial cellular phenomena [3,22,23]. For example, conventional methods using hydrogels derived from natural ECM proteins (such as collagen-I, fibrin, and Matrigel) are known to have difficulty controlling their

* Corresponding author. Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI 48109, USA. Tel.: +1 734 615 7363; fax: +1 734 647 7303.
E-mail address: jpfu@umich.edu (J. Fu).

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.09.006

bulk mechanical properties. Further, in these natural hydrogels, changes in gel stiffness cannot be decoupled from other parameters such as ligand density or gel fiber thickness. The synthetic hydrogel systems such as polyacrylamide (PA) or polyethylene glycol (PEG) gels present a significant advancement for studies of cell–ECM interactions [14,24,25], as they have well-defined bulk mechanical properties and are chemically inert and can be functionalized with adhesive peptides and proteins using linker chemistry. Yet, these synthetic hydrogel systems are still not immune to molecular-scale changes in porosity, wettability, hydration, polymer chain mobility and binding properties of immobilized adhesive ligands that accompany changes in their bulk stiffness [3,23]. It has been shown that these molecular-scale changes can have their profound independent effects on cellular functions [26–28]. For example, a very recent study using synthetic hydrogels for human pluripotent stem cells indicates that these molecular-scale changes in wettability and surface topology can have significant effects on stem cell survival and cloning efficiency [26]. There are other reports that clearly suggest that the nanoscale local alterations in receptor–ligand binding characteristics could strongly influence cellular sensing of ECM surface properties and thus downstream cellular behaviors [27,28].

The importance of development of new tools for investigating biointerfacial cellular phenomena has been well recognized and documented [23,29,30]. Although the natural and synthetic hydrogel systems discussed above will continue to be important in characterizing and controlling cell–material interactions, there is a great current need for new classes of synthetic materials in which surface chemistry, adhesive pattern, topography, and mechanics can be independently controlled to facilitate the quest for design principles and material selection rules to control cellular responses. Recently, our group and some others have proposed an idea to use microfabricated elastomeric polydimethylsiloxane (PDMS) micropost arrays to regulate substrate rigidity independently of effects on adhesive and other material surface properties [31,32]. Our approach involves a library of replica-molded arrays of hexagonally spaced PDMS microposts from microfabricated silicon masters, which present the same surface geometry but different post heights to control substrate rigidity. The spring constant K of the PDMS micropost is solely determined by its geometry and by the *Young*'s modulus E of PDMS, and K can be approximately calculated using the *Euler–Bernoulli* beam theory as $K = 3\pi ED^4/(64L^3)$ [32–34], where D and L are the PDMS post diameter and height, respectively. The substrate rigidity of the PDMS micropost array can be further characterized using an effective *Young*'s modulus E_{eff} of a continuous elastic substrate, and E_{eff} is calculated using the expression of $E_{\mathrm{eff}} = 9K/(2\pi D)$ [32]. Thus, the rigidity of the PDMS micropost array can be modulated simply by varying the post height L and diameter D while keeping all other aspects of the substrate such as surface chemistry, ligand density, and bulk and nanoscale mechanics of the PDMS unchanged. In our previous study, we have shown that indeed, the rigidity of the PDMS micropost array can significantly impact cell morphology, focal adhesion (FA) formation, cytoskeletal contractility, and stem cell differentiation [31,35].

Most of the existing biomechanics studies characterizing biointerfacial cellular phenomena, including our own previous report examining the PDMS micropost array, have focused on determining how individual biophysical parameters in the cell microenvironment, such as substrate rigidity and adhesive ECM pattern, are sensed by cells and further trigger downstream intracellular signaling cascades and cellular responses. Thus, synergistic and collective regulations of cellular behaviors by a combination of different biointerfacial parameters are still elusive and largely uncharacterized, which will clearly hamper future efforts to design efficient synthetic materials and biointerfaces containing multiple biophysical signals to collectively direct cellular behaviors. The current incomplete understanding of cell–ECM interactions is largely due to the limited number of experimental techniques and approaches that can precisely and independently regulate multiple biointerfacial parameters simultaneously in the same experimental system. In this work, to specifically address this technical limitation and thus advance our current understanding of the combined influences of different biointerfacial parameters on cellular behaviors, we took advantage of the unique characteristic of the PDMS micropost array where the mechanical rigidity of the PDMS micropost array could be easily and completely decoupled from its surface properties including ECM ligand density and presentation and adhesive ECM pattern. In this work, we specifically studied the synergistic and independent effects of matrix rigidity and adhesive ECM pattern on cell spreading, FA formation, cytoskeletal contractility, and proliferation for different types of adherent mammalian cells.

2. Materials and methods

2.1. Fabrication of PDMS micropost array

Elastomeric PDMS micropost arrays were fabricated using conventional semiconductor microfabrication techniques and replica molding, as previously described [31,35]. Briefly, silicon micropost array masters were fabricated using high-resolution projection photolithography and deep reactive ion-etching (DRIE) techniques. By controlling the mask design of the micropost array structure and the DRIE etch time, we could determine precisely different geometrical factors of the silicon micropost array master, including post diameter, post center-to-center (c.t.c) distance, and post height. These silicon masters were silanized with (tridecafluoro-1,1,2,2,-tetrahydrooctyl)-1-trichlorosilane (United Chemical Technologies, Bristol, PA) for 4 h under vacuum to aid subsequent release of the negative PDMS (Sylgard 184, Dow-Corning, Midland, MI) mold from the silicon master. The PDMS micropost array was then generated by replica molding. In brief, 1:10 (w/w, curing agent:base) ratio PDMS prepolymer was poured over the silicon micropost master, cured at 110 °C for 20 min, peeled off, oxidized with air plasma for 1 min (200 mTorr; Plasma Prep II, West Chester, PA), and silanized with (tridecafluoro-1,1,2,2,-tetrahydrooctyl)-1-trichlorosilane vapor for 40 h to obtain the PDMS negative mold. To generate the final PDMS micropost array, 1:10 ratio PDMS prepolymer was poured over the negative PDMS mold, degassed under vacuum, cured at 110 °C for 40 h, and peeled off the negative mold. When peeling induced collapse of the PDMS microposts, we regenerated the free-standing PDMS microposts by sonication in 100% ethanol for 30 s followed by dry-release with liquid CO_2 using a critical point dryer (Samdri®-PVT-3D, Tousimis, Rockville, MD). Geometries of the silicon post master and PDMS micropost array were examined under optical microscopy and were further characterized using a surface profilometer (Prometrix P-10, KLA-Tenco Co., CA) and scanning electron microscopy (JEOL6320FV, JEOL USA, Inc., Peabody, MA).

2.2. Mechanical characterization of PDMS micropost array

The commercial finite element analysis (FEA) suite ABAQUS (SIMULIA, Dassault Systèmes) was used to analyze the nominal spring constant K of the PDMS micropost, as described previously [31]. The PDMS micropost was modeled as a neo-hookian hyperelastic cylinder with a *Young*'s modulus E of 2.5 MPa, and was discretized into hexahedral mesh elements. The bottom surface of the PDMS micropost was fixed in all degrees of freedom. A horizontal load F was then applied uniformly at all of the nodes on the top of the micropost. FEA analysis was performed to determine displacement δ of the micropost top due to F. From the force (F)–displacement (δ) curve, the nominal spring constant K of the PDMS micropost was computed by linearly extrapolating F to zero post deflection δ as $K = \mathrm{d}F/\mathrm{d}\delta$ ($\delta \rightarrow 0$). For our comparative studies for the two sets of PDMS micropost arrays with different post diameters (D(0.8) *vs.* D(1.83)), we further converted the nominal spring constant K of the PDMS micropost into an effective *Young*'s modulus E_{eff} of a continuous elastic substrate, using the expression of $E_{\mathrm{eff}} = 9K/(2\pi D)$ [32]. The two sets of PDMS micropost arrays reported in this work (D(0.8) and D(1.83)) produced a more than 10,000-fold range of rigidity from 0.10 kPa (D(0.8)L(0.42)) to 1200 kPa (D(0.8)L(13.45)) (Fig. 1e).

2.3. Surface functionalization of PDMS micropost array and flat PDMS surface

As described previously [31,35], we used microcontact printing to functionalize the top of the PDMS micropost with ECM proteins to promote cell attachment. Briefly, a flat 1:30 PDMS stamp was prepared and inked with fibronectin (BD Biosciences, San Jose, CA) at a saturating concentration of 50 μg mL^{-1} in distilled