Acta Biomaterialia 38 (2016) 106–115

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Guiding cell migration with microscale stiffness patterns and undulated surfaces

Jonathan T. Pham [a,*], Longjian Xue [a,1], Aránzazu del Campo [b,c], Marcelo Salierno [a,d,*]

[a] Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany
[b] INM-Leibniz Institute for New Materials, Campus D2 2, 66123 Saarbrücken, Germany
[c] Saarland University, Campus D2 2, 66123 Saarbrücken, Germany
[d] Department of Biological Chemistry, School of Sciences, University of Buenos Aires, IQUIBICEN-CONICET, CABA, Argentina

ARTICLE INFO

Article history:
Received 8 December 2015
Received in revised form 14 April 2016
Accepted 18 April 2016
Available online 19 April 2016

Keywords:
Cell migration
Migration efficiency
Stiffness patterns
Surface topography
Focal adhesions

ABSTRACT

By placing stiff structures under soft materials, prior studies have demonstrated that cells sense and prefer to position themselves over the stiff structures. However, an understanding of how cells migrate on such surfaces has not been established. Many studies have also shown that cells readily align to surface topography. Here we investigate the influence of these two aspects in directing cell migration on surfaces with 5 and 10 μm line stiffness patterns (a cellular to subcellular length scale). A simple approach to create flat, stiffness-patterned surfaces by suspending a thin, low modulus polydimethylsiloxane (PDMS) film over a high modulus PDMS structure is presented, as well as a route to add undulations. We confirm that cells are able to sense through the thin film by observation of focal adhesions being positioned on stiff regions. We examine migration by introducing migration efficiency, a quantitative parameter to determine how strongly cells migrate in a certain direction. We found that cells have a preference to align and migrate along stiffness patterns while the addition of undulations boosts this effect, significantly increasing migration efficiency in either case. Interestingly, we found speed to play little role in the migration efficiency and to be mainly influenced by the top layer modulus. Our results demonstrate that both stiffness patterns and surface undulations are important considerations when investigating the interactions of cells with biomaterial surfaces.

Statement of Significance

Two common physical considerations for cell-surface interactions include patterned stiffness and patterned topography. However, their relative influences on cell migration behavior have not been established, particularly on cellular to subcellular scale patterns. For stiffness patterning, it has been recently shown that cells tend to position themselves over a stiff structure that is placed under a thin soft layer. By quantifying the directional migration efficiency on such surfaces with and without undulations, we show that migration can be manipulated by flat stiffness patterns, although surface undulations also play a strong role. Our results offer insight on the effect of cellular scale stiffness and topographical patterns on cell migration, which is critical for the development of fundamental cell studies and engineered implants.

1. Introduction

Cells are the smallest unit with self-governing functionality in a living organism. They constantly sense and react to their surroundings, which ultimately determine their fate and function. In particular, cell alignment and migration are critical for animal morphogenesis and wound healing, as well as for undesired cancer metastasis [1,2]. Technologically, accruing evidence demonstrates that controlling alignment and motility is crucial for tissue regeneration and for establishing successful implants [2–4]. Inside of a tissue, cells reside in a rich microenvironment with a variety of heterogeneities and anisotropic properties, both biochemical and physical in nature [5–9]. While much effort has been placed on understanding the biochemical aspects of cell sensing and

* Corresponding authors at: Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany.
E-mail addresses: phamj@mpip-mainz.mpg.de (J.T. Pham), salierno@gmail.com (M. Salierno).
[1] Present address: School of Power and Mechanical Engineering, Wuhan University, South Donghu Road 8, Wuchang Wuhan, Hubei 430072, China.

http://dx.doi.org/10.1016/j.actbio.2016.04.031
1742-7061/

migration, there is continued interest in cell response to mechanical and geometric cues [10–12]. From a biomaterials development point of view, these physical variations are interesting because they provide a route towards manipulating cell migration without requiring special molecules.

The stiffness of surfaces significantly affects a number of factors in adherent cells, such as spread area, cell morphology, and differentiation [10,13–19]. When substrates have spatial variations in stiffness, cells migrate towards stiffer regions in a phenomenon known as mechanotaxis [20,21]. Utilizing various patterning methods to create stiffness gradients, studies have demonstrated the ability to direct cell migration and location by spatially tuning the mechanical properties [22,23]. Many of these experiments are conducted with hydrogels, such as polyacrylamide (PAAm) and polyethylene glycol (PEG), because it is easy to control their mechanical properties and because they are biologically inert. By simply changing the ratio of the polymer and crosslinker, the modulus can be tuned over a range of 3 orders of magnitude. Although this approach is successful in tailoring the modulus, the different stiffness materials possess different molecular network properties, which may also lead to differences in the binding density of adhesive molecules at the cell-material interface [14,24,25].

An alternative approach to spatially tuning stiffness is by placing high modulus materials underneath a thin layer of a soft gel. In contrast to tailoring the crosslinking density, this leads to control over the stiffness while keeping the polymer network constant, providing an equal opportunity for cells to adhere to any location across the substrate (i.e., planar surfaces with homogeneous adhesive molecules and crosslinking density). Using this approach, cells have demonstrated a preference to reside in stiffer regions [26–28]. However, these studies did not characterize how directional migration behavior is affected by the underlying stiffness patterns. Moreover, due to the inherent swellability of hydrogels, it can be difficult to eliminate undulations at the surface and control the local mesh size [27]. One can eliminate swelling-induced undulations by utilizing materials that do not swell in aqueous environments. For example, previous work has taken this approach using a polydimethylsiloxane (PDMS) elastomer to demonstrate the preference of cells towards stiffer regions [26]; however, these stiffness patterns were on size scales larger than a single cell. Although Degand el al. have utilized nanoscale colloids as the high modulus component, controlled placement of particles under the layer and elimination of topography was experimentally challenging [29].

Here we present a simple method to fabricate substrates with a stiff structure underlying a soft thin layer to pattern the stiffness of flat surfaces comprising a homogeneous, non-swelling elastomeric material. The length scale of the underlying patterns is comparable to the width of a single migrating cell in order to manipulate single cell migration [30,31]. We then introduce mild undulations to elucidate the effects of both stiffness patterns (i.e., flat surfaces) and surface undulations (i.e., topography) on cell migration directions (see Fig. 1). Physiologically, physical heterogeneities smaller than a single cell are found in normal tissues, making such size scales relevant for biological systems [5].

2. Materials and methods

2.1. Substrate preparation

The fabrication procedure is summarized in Fig. S1. A master mold is first prepared by traditional photolithography. Silicon wafers (p-type, 2 in. diameter) were obtained from Crystec Kristalltechnologie in the 100 orientation. SU-8 photoresist type 2010 and developer mr-Dev 600 from MicroChem was used as received and as direct by the manufacturer to create structures with depths of 10 μm on silicon wafers. Photolithography was conducted on a MJB 3 UV 400 mask aligner (Süss Microtec Lithography) equipped with a PL-360 LP filter (Omega Optical) to eliminate wavelengths under 350 nm. Photolithography masks were purchased from Compugraphics Jena. Prior to being used for molding PDMS structures, the SU-8 molds were fluorinated. The molds were first exposed to oxygen plasma (Plasma Technology) under vacuum for 20 s to activate the surface, and then placed in an evacuated desiccator for 1 h with ~30 μL of 1H,1H,2H,2H-perfluorodecyltricholosilane (Alfa Aesar) for vapor silanization. The substrate was then baked at 90 °C in an oven for 1 h to complete the silanization process.

PDMS elastomer kits (Sylgard 184) were obtained from Dow Corning. To create the underlying structure, the prepolymer was mixed with the crosslinker at a 10:1 ratio, degassed under vacuum, poured over the SU-8 master mold, fully cured for ~15 h at 60 °C, and removed. For the top layer, glass coverslips were obtained from VWR with a circle diameter of 25 mm and fluorinated in the same manner as the SU-8 molds. PDMS was mixed at a 60:1 ratio, degassed, and then spin-coated onto the fluorinated glass at 10 k RPM for 15 min. The films were then partially cured at 60 °C for 1 h (60:1 top layer) or 5 min (10:1 top layer). The stiff PDMS microstructure was then placed onto the films and allowed to cure for ~15 h. The structures were then peeled away from the glass to leave the desired substrates. Fibronectin was coupled to the surface by first activating the PDMS with oxygen plasma for 10 s and subsequently incubated in a diluted aqueous solution of fibronectin (20 μg/mL) for ~15 h in a closed high humidity chamber. To introduce minor surface undulations, 60:1 PDMS was mixed, degassed, and placed at the ends of the microchannels after a partial curing step. The samples were instantly placed into the oven at 60 °C and allowed to fully cure.

2.2. Characterization of surface morphology

Surfaces were examined by scanning electron microscopy by first sputtering a ~2 nm layer of platinum (Bal-Tec, MED 020). Images were obtained on a LEO 1530VP Gemini scanning electron microscope. To measure the depth of the subsurface structures, a NanoFocus μsurf confocal microscope was used. The thickness of the PDMS thin films on glass were measured by making a cut with a razor blade and observing with the NanoFocus microscope, which were ~2 μm. Optical images were taken on an upright microscope equipped with a 50× objective.

2.3. Mechanical testing

The prepolymer to crosslinker ratio was varied from 10:1 for the stiff underlying structure material to 60:1 for the soft top film. Thin films were created of 400 μm thickness in a polystyrene dish, controlled by the volume, and cured for ~15 h at 60 °C. Dog-bone shaped samples were stamped with a 4 mm width and 20 mm gauge length and measurements were conducted on a Zwick/Roell Z005 materials testing machine equipped with a 50 N load cell. Young's modulus was calculated by performing a linear fit to the data in the small strain regime. For AFM measurements, tipless cantilevers were obtained from MikroScience with a spring constant ~2–8 N/m. A silica sphere (Bangs Laboratories Inc.) with a diameter of 1.5 μm was attached to the cantilever tip with a thermal glue (Epikote 1004, Hexicon Specialty Chemicals), allowed to sit overnight, and then mounted onto the AFM for measurement (JPK Instruments). AFM cantilevers with ~10 nm radius tips were also used for force measurements. The AFM cantilever spring constant was first calibrated on a silicon wafer. Force-indentation measurements were taken at 1 μm/s. Prior to measuring, tips were

ID	Title	Pages
94	Guiding cell migration with microscale stiffness patterns and undulated surfaces	10