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Manipulation of cell mechanotaxis by designing curvature of the elasticity boundary on hydrogel matrix

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ABSTRACT

Directional cell migration induced by the stiffness gradient of cell culture substrates is known as a subset of the mechanical-cue-induced taxis, so-called mechanotaxis, typically durotaxis toward hard region. To establish the general conditions of biomaterials to manipulate the mechanotaxis, the effect of the shape of the elasticity transition boundary between hard and soft regions of a substrate on mechanotaxis should be systematically determined as well as the conditions of elasticity gradient strength. Here, as a simplified factor of expressing variations in the shape of the elasticity boundary in living tissues, we focus on the curvature of the elasticity boundary. Mask-free photolithographic microelasticity patterning of photocurable gelatin gel was employed to systematically prepare elasticity boundaries with various curvatures, and the efficiency of mechanotaxis of fibroblast cells around each curved boundary was examined. Highly efficient usual durotaxis was induced on a convex boundary with 100 μm in radius and on a concave boundary with 750 μm in radius of curvature. Interestingly, biased migration toward soft regions of the gel, i.e., inverse durotaxis, was first observed for concave boundaries with 50 μm or 100 μm in radius of curvature, which was named as "negative mechanotaxis". The curvature of the elasticity boundary was found to markedly affect the efficiency of induction and the direction of mechanotaxis. The mechanism responsible for this phenomenon and the implication for the curvature effect in *in vivo* systems are discussed.

1. Introduction

Directional migration of cells plays an essential role in a number of physiological and pathological processes in living tissues, including morphogenesis [1–4], inflammation [5,6], wound healing [7,8] and tumor metastasis [9,10]. Such taxis behaviors are a result of the intrinsic ability of cells to respond to gradients of various extracellular stimulants, including soluble chemical factors and the physical condition of extracellular matrices. For example, chemotaxis induced by chemoattractants or chemorepellents [11], galvanotaxis induced by changes in the electric field gradient [12], haptotaxis induced by surface-immobilized haptoattractants [13], and durotaxis/mechanotaxis induced by the stiffness gradient on extracellular matrices [14–16] are employed to manage tissue kinetics.

While the control of cellular migration is primarily governed by biochemical factors [17–19], biomechanical factors are also involved in taxis. Cellular motility is a fundamental biomechanical process that involves the architectural dynamics of the cytoskeleton and organized dynamics of intracellular stress generated by the cytoskeleton and cell membrane [20,21]. Movement of cells is essentially determined by their interactions with extracellular matrices or culture substrates, whereby focal adhesions link the cytoskeleton to the surroundings at the adhesion interface. Therefore, matrix mechanics can affect cell motility and regulate the direction of movement, as observed during mechanotaxis [14–16]. Systematically altering the stiffness gradient of the extracellular matrix and examining the effect on mechanotaxis would not only provide essential information regarding the mechanical principle of cell motility in living tissues, but also become important guides to design the biomaterials to manipulate migration and localization of cells.

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We have previously reported essential requirement for substrate design to induce mechanotaxis, which is the forced generation of cell polarity over the elasticity boundary with a certain threshold strength of elasticity gradient inside single cellular adhered area [22–24]. Other groups have also reported conditions required to induce mechanotaxis [25,26]. To date, these induction conditions have only been determined for a uniaxial elasticity gradient positioned perpendicular to the typical straight elasticity transition boundary; however, the occurrence of such a configuration is rare in living tissues because the stiffness of the extracellular milieu is distributed heterogeneously. The use of more realistic stiffness gradients and nonlinear elasticity boundaries are required to gain a more complete understanding of the conditions required to manipulate mechanotaxis.

Although most living organs are composed of three-dimensionally curved tissues with different degrees of curvatures, here we focus on the effect of curvature of the two-dimensional elasticity boundary on mechanotaxis in order to get a first basic insight to the curvature-dependent cell movements. Using a custom-made, mask-free photolithography system [27] for microelasticity patterning of photocurable gelatins established by our group previously [22–24,28], we systematically prepared elasticity boundaries of various curvatures and analyzed the effects on the induction of mechanotaxis of fibroblast cells. The convex and concave boundaries were defined as the form of the boundary toward the hard region and the radius of curvature was termed R (curvature: R^{-1}). Migration of cells toward the hard region (usual durotaxis, termed "positive mechanotaxis") was enhanced on convex boundaries with an R smaller than 250 μm and on concave boundaries with an R larger than 750 μm. Interestingly, cell migrations toward the soft region (inverse durotaxis, termed "negative mechanotaxis") were observed on concave boundaries with Rs of 50 or 100 μm. The mechanism involved in the observed phenomenon of curvature-dependent regulation of mechanotaxis is discussed in relation to the distribution of inner bending stress in the curved gel matrix.

2. Methods

2.1. Preparation of the photocurable sol solution

Photocurable styrenated gelatin (StG) was used for photolithographic microelasticity patterning of the gel, which was synthesized according to the method previously reported by our group [22,23]. A photocurable sol solution of StG was prepared as follows: (i) StG (30 wt %) and sulfonyl camphorquinone (3 wt % of gelatin; Toronto Research Chemicals, ON, Canada) were dissolved in phosphate buffered saline (PBS). (ii) The mixed solution was centrifuged (MX-301; TOMY, Tokyo, Japan) at 17,800× g for 1 h (iii) The clear sol solution was aspirated for 1 h to exclude dissolved oxygen, conditioned for 10 min using a MX-201 deforming agitator (THINKY, Tokyo, Japan), and then stored at −80 °C. The sol solution was warmed at 45 °C prior to use for photolithography.

2.2. Preparation of the supporting glass substrates used to fix gel samples

Vinyl-silanized glass substrates (vinyl-glass) were prepared to chemically fix the photocured StG gels according to the following procedure: (i) Glass substrates (0.12–0.17 mm thickness, 18 mm diameter; Matsunami Glass, Osaka, Japan) were immersed in piranha solution (conc. H_2SO_4: 30% H_2O_2 = 7:3) at 80 °C for 1 h (ii) After sequential rinsing with distilled water, ethanol and toluene, the glass substrates were immersed in a 5% (v/v) toluene solution of vinyl-trimethoxysilane (Tokyo Chemical Industry, Tokyo, Japan), and shaken for 3 days at room temperature. (iii) After sequential rinsing with toluene, acetone, ethanol, and distilled water, the glass substrates were air dried at 100 °C for 10 min.

2.3. Photolithographic microelasticity patterning of the gelatinous gel

A 30 μl sample of the StG sol solution was spread between vinyl-glass and a normal glass substrate coated with poly(N-isopropylacrylamide) (PNIPAAm, Sigma Aldrich, St. Louis, Missouri), and then placed on a hot plate set to 45 °C. The following steps were then performed in a N_2 chamber. First, a soft base gel was prepared by irradiation of the entire sample with visible light (400 mW/cm^2 at 488 nm) (light source: MME-250; Moritex, Saitama, Japan). Next, hard regions were prepared by local irradiation of the base gel using a set irradiation pattern and a custom-built, mask-free, reduction-projection-type photolithographic system [27]. The patterns of concentric circles with Rs of 250, 500, 750, and 1000 μm, and semicircles with Rs of 50 and 100 μm were irradiated on the gel using an EB-1770W liquid crystal display projector (SEIKO EPSON, Nagano, Japan). To equalize the intensity of light on the irradiated areas, the degree of light transmission over the designed pattern was gradationally decreased by 80–85% toward the center. Finally, the gels were detached from the PNIPAAm-coated normal glass substrate and washed thoroughly with PBS at 28 °C to remove the adsorbed PNIPAAm.

2.4. Measurement of the surface elasticity distribution around the elasticity boundary

The surface elasticity of the photocured StG gel was determined by nanoindentation analysis. Force-indentation curves were measured for the gel surface (in PBS) using an atomic force microscope (NVB100, Olympus Optical, Tokyo, Japan) in combination with the Nanoscope IIIa controller and software (Veeco Instruments, Santa Barbara, California). A commercial (OMCL-TR400PSAHW) silicon-nitride cantilever with a pyramidal tip and a nominal spring constant of 0.02 N/m (Olympus Optical) was also used. Young's moduli of the surface were evaluated from force-indentation curves by nonlinear least-square fitting to the Hertz model in the case of a conical indenter (semivertical α: 30°; Poisson ratio μ: 0.5) [29–31].

$$F = \frac{2E \tan \alpha}{\pi(1-\mu^2)}\delta^2 \quad (1)$$

2.5. Confocal microscopy

For analysis of the surface topography, the gels were stained by absorption of fluorescein-conjugated albumin, and then cross-sectional imaging was performed using a confocal laser scanning microscope (LSM510META, Carl Zeiss, Oberkochen, Germany).

2.6. Cell culture

The mouse 3T3-Swiss albino fibroblast cell line (Dainippon Pharmaceutical, Osaka, Japan) was cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, New York) supplemented with 10% fetal bovine serum (FBS; Gibco BRL), 50 units/ml penicillin, and 50 μg/ml streptomycin. Cells were grown on tissue culture grade polystyrene dishes at 37 °C in a humidified atmosphere containing 5% CO_2.

2.7. Time-lapse observation of cell migration

The migratory behavior of cells on the microelastically-patterned gel was monitored using an automated all-in-one microscope with a temperature- and humidity-controlled cell chamber (BIO REVO BZ-9000; Keyence Corporation, Osaka, Japan). Prior to the time-lapse observations, cells were seeded onto the gel sample at a density of 1.5 × 10^3 cells/cm^2 and cultured in DMEM containing 10% FBS for 8 h under 5% CO_2. The DMEM was then replaced by Leibovitz's L-15 medium (Gibco BRL) containing 10% FBS to adapt the cultured cells to prolonged observation under ambient air conditions. Phase-contrast images of cells were captured every 15 min for 24 h. Moving trajectories of 22–58 isolated cells on 3–6 gel samples were determined using ImageJ software (ImageJ 1.46r, http://rsbweb.nih.gov/ij/).

3. Results

3.1. Preparation of microelastically-patterned gels with different curvatures of elasticity boundary

To investigate the effect of the curvature of the elasticity boundary on mechanotaxis, mask-free lithography was used to generate curved elasticity boundaries on hydrogels of StG. A series of convex and concave boundaries with targeted Rs of 50, 100, 250, 500, 750, and 1000 μm were prepared. The gradient strength over the hard/soft boundaries were designed to be 200–300 kPa/50 μm on the softer base gel with several tens kPa (photoirradiation time: 85 s for soft region, 235 s for hard region), which is a little smaller than the strength required to induce strong mechanotaxis of fibroblast cells established in our previous study, i.e., 300–400 kPa/50 μm [23,24]. The present condition of gradient strength was applied to sensitively detect the enhanced or reduced effect for induction of mechanotaxis in the curved elasticity boundaries, compared to the control condition of linear boundary. If the condition of gradient strength was set to induce strong mechanotaxis, which means over 90% cells move to hard region, it should become difficult to detect the enhanced mechanotaxis contributed by the