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Sinusoidal wavy surfaces for curvature-guided migration of T lymphocytes

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ABSTRACT

Micro/nanofabricated surfaces have been widely used for the study of topography-guided migration of cells. While the current studies mostly utilized micro/nanostructures containing sharp edges, internal tissues guiding migration of cells such as blood and lymphatic vessels, bone cavities, perivascular tracks have smooth microscale topographical structures. To overcome these limitations, we fabricated sinusoidal wavy surfaces with various wavelengths by deep X-ray lithography enabling precise and simultaneous control of amplitudes and wavelengths. Using these surfaces, we systematically studied curvature-guided migration of T cells. The majority of T cells migrated along the concave surfaces of sinusoidal wavy structures and as wavelength increased (or curvature decreased), preference to concave surfaces decreased. Integrin-mediated adhesion augmented the tendency of T cells crawling along grooves of highly curved wavy surfaces. To understand mechanisms of curvature-guided migration of T cells, T cells were treated with small molecule drugs such as blebbistatin and CK636, inhibiting myosin II activity and lamellipodia formation, respectively. While lamellipodia-inhibited T cells frequently crossed ridges, myosin II-inhibited T cells were mostly confined within concave surfaces. These results suggest that lamellipodia regulate local actin polymerization in response to surface curvature to maintain T cells within concave surfaces while myosin II-mediated contractile forces push T cells out of concave surfaces to make T cells less sensitive to surface curvature.

1. Introduction

Migration of cells guided by various micro/nanoscale topographical structures *in vivo* has been observed by intravital microscopy during immune surveillances/responses [1,2] and cancer invasion/metastasis [3,4], but the mechanisms by which such structures guide migration of cells have not been completely understood [5,6]. Micro/nanofabricated surfaces containing well-defined topographical structures have been useful for the study of topography-guided migration of cells [7–10]. Most of these microfabricated structures had sharp edges [7–9], but tissue structures known to guide migration of cells such as blood and lymphatic vessels, bone cavities and perivascular tracks have smooth microscale topography [11–13].

Various methods such as laser interference lithography [14,15] and spontaneous buckling [16,17] or wrinkling of elastomers [18,19] have been applied to fabricate sinusoidal wavy structures, but precise and simultaneous control of wavelength λ and of amplitude A of wavy structures has been challenging. To overcome these limitations, we used deep X-ray lithography (DXRL) based on synchrotron radiation to fabricate sinusoidal wavy structures with $\lambda = 20$, 40, 80, and 160 μm and $A = 10$ μm. DXRL allows fabrication of features with height up to several millimeters with excellent sidewall quality [20,21], so sinusoidal wavy structures with height ~1 mm were fabricated. By orienting vertically-fabricated sinusoidal wavy structures horizontally and replicating them using polymers, we generated sinusoidal wavy topographical surfaces with

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precisely-controlled λ and fixed A. Using these surfaces, we systematically studied curvature-guided migration of T cells. T cells are immune cells that migrate virtually everywhere in the body to orchestrate cell-mediated immune responses; thus understanding motility of T cells under complex microenvironments is essential for designing therapeutic strategies against various immune-related diseases [22–24].

2. Materials and methods

2.1. Fabrication of sinusoidal wavy surfaces

To fabricate substrates containing sinusoidal waves, deep X-ray LIGA (German acronym for lithographie, galvanoformung, abformung, or lithography, electroforming, and molding) process was used as previously described [25,26]. Briefly, a 1.1-mm-thick poly(methyl methacrylate) (PMMA) template with step-gradient periods of 20, 40, 80, and 160 μm with an amplitude of 10 μm was prepared by deep X-ray lithography (Fig. 2A(i)). An X-ray mask was fabricated by standard UV-photolithography followed by gold electroplating (gold thickness: ~18 μm) to selectively block the synchrotron X-ray beam. An 1.1-mm-thick PMMA sheet (X-ray photoresist) bonded to an electrically conductive plate was exposed to X-rays in the LIGA beam line at the Pohang Light Source (PLS) through the X-ray mask (total dose 3.5 kJ cm^{-3}) and developed using a GG developer. Then, electroforming was conducted in a nickel sulfamate bath to create a nickel mold insert using the PMMA template (current density 8.5 mA cm^{-2}) (Fig. 2A(ii)). After the electroforming, both sides of the electroformed part were precisely polished, resulting in a nickel mold insert with a thickness of 0.9 mm (Fig. 2A(iii)). The mold insert and jig blocks were assembled to secure sufficient area for hot embossing (Fig. 2A(iv)). Flat polystyrene (Dow Chemical) plates (30 mm × 30 mm) prepared by cutting injection-molded plates were embossed at 130 °C and 3 MPa for 3 min (Fig. 2A(v)). The hot embossed PS master was replicated with poly(urethane acrylate) (PUA) by capillary force lithography to obtain transparent patterns on glass for the observation of cell dynamics. PUA precursor solution (Minuta Tech, Korea) was drop-dispensed on the PS master, covered with a transparent glass coated with adhesive agent, and cured by 30 s of UV exposure (λ_{UV} = 250–400 nm, 100 mJ cm^{-2}, Minuta Tech., Korea). The UV-cured PUA pattern was peeled from the PS master and further exposed to UV light for 2 h to annihilate reactive acrylate groups.

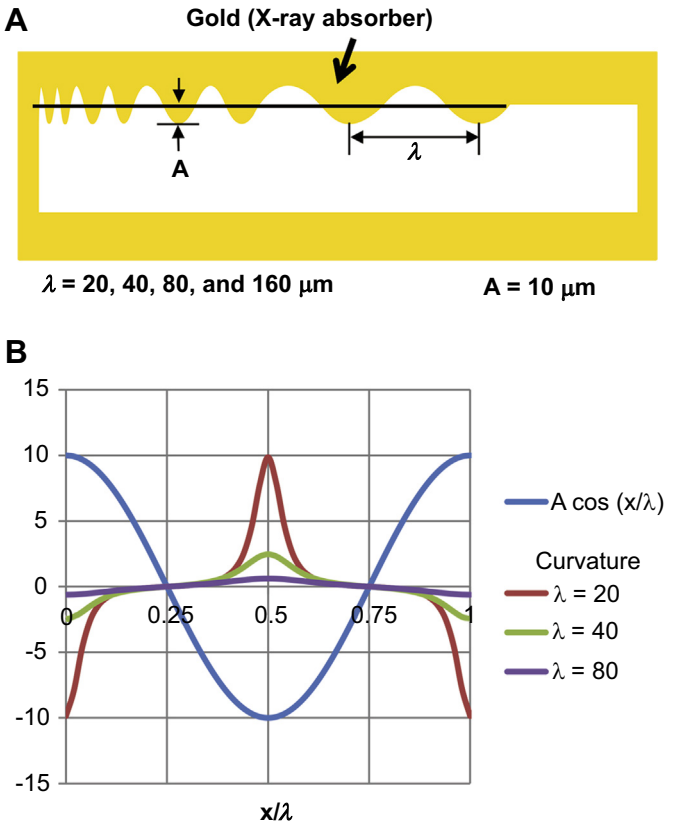

Fig. 1. Design of sinusoidal wavy structures with various wavelengths. (A) Schematic diagram of a gold X-ray mask containing various sinusoidal wavy patterns. (B) Superimposed sinusoidal wave with normalized distances (x/λ) and curvatures of sinusoidal waves with various wavelengths (λ = 20, 40, 80 μm).

2.2. T cell preparation and culture

DO11.10 T cell blasts were prepared from the spleens and lymph nodes of DO11.10 T cell receptor (TCR) transgenic mice (Jackson Laboratories). DO11.10 TCR transgenic mice were bred in the POSTECH Biotech Center (PBC), and experiments using mice were approved by the Institutional Animal Care and Use Committee at PBC. Cells from spleens and lymph nodes of DO11.10 TCR transgenic mice were harvested and stimulated with 1 μg ml^{-1} of OVA323–339 peptide (ISQAVHAA-HAEINEAGR, Peptron, Inc., Korea). Stimulated DO11.10 blasts were grown in RPMI 1640 (Invitrogen) with 10% of FBS (Gibco), 100 U ml^{-1} of penicillin, 100 mg ml^{-1} of streptomycin (Invitrogen), and 0.1% beta-mercaptoethanol (Sigma); 1–2 U ml^{-1} of IL-2 was added on the 2nd day of stimulation. Cells from the 5th day of stimulation were used in all experiments. For drug treatment, cells were incubated with blebbistatin (50 μM, Sigma) and CK636 (100 μM, Sigma) for 1 h, rinsed extensively, suspended in culture medium and used immediately.

2.3. Live cell imaging

Substrates were coated with ICAM-1 (2 or 10 μg ml^{-1}, PeproTech) or casein (160 nM, Sigma) for 1.5 h and loaded in Chamlide chambers (Live Cell Instrument, Korea) maintaining 37 °C and 5% CO_2 (Live Cell Instrument, Korea). DO11.10 T blasts in culture medium were added to the chamber, and time-lapse images (15-s intervals for 20 min) of their dynamics on the substrates were acquired using an automatically-controlled microscope with Axiovision 4.6 (Carl Zeiss). The acquired images were analyzed and processed using Methamorph (Universal Imaging, Molecular Devices) or ImageJ (NIH).

3. Results and discussion

3.1. Design and fabrication of sinusoidal wavy surfaces with various wavelengths

To fabricate sinusoidal wavy structures with various λ, X-ray lithography, electroforming and molding (German acronym LIGA) processes were used [25,26]. An X-ray mask containing sinusoidal wavy patterns with λ = 20, 40, 80 and 160 μm and A = 10 μm (Fig. 1A) was prepared by standard photolithography and gold electroplating. The curvature of a sinusoidal wave A $cos 2\pi/\lambda x$, is $-\left(\frac{2\pi}{\lambda}\right)^2 A\cos\frac{2\pi}{\lambda}x \Big/ \left(1+\left(\left(\frac{2\pi}{\lambda}\right)A\sin\frac{2\pi}{\lambda}\right)^2\right)^{3/2}$. Since curvature is a function of both λ and A, varying λ over a large range of number with a fixed A should be sufficient to systematically examine the effect of curvature on the motility of T cells. With a normalized distance x/λ, sinusoidal waves with various λ can be superimposed onto a single curve while curvatures are still strongly influenced by λ (Fig. 1B). The amplitude and half of the minimum λ were determined to be close to the typical diameter of a T cell [27,28]. As a result, T cells on surfaces with λ = 20 μm would experience large curvature changes. As λ of the sinusoidal wavy surfaces increases, curvature changes experienced by a T cell decrease. Depending on their positions, T cells on sinusoidal surfaces may contact concave (positive curvature) or convex (negative curvature) surfaces. Therefore, the effect of surface curvature on the migration of T cells can be studied systematically in this experimental setting.

Using the X-ray mask containing various sinusoidal wavy patterns, DXRL was performed: A poly(methyl methacrylate) (PMMA) sheet with 1.1-mm thickness bonded to an electrically conductive plate was exposed to synchrotron X-ray radiation through the mask and developed to remove X-ray-irradiated regions (Fig. 2A(i)). The developed regions were filled with nickel by electroforming to generate a nickel mold insert (Fig. 2A(ii)). The electroformed nickel part was polished and then released from the PMMA template to form a 0.9-mm-thick nickel mold insert (Fig. 2A(iii)). The polished mold insert and jig blocks were assembled to form a mold with a sufficient surface area for hot embossing (Fig. 2A(iv)). By performing hot embossing with a polystyrene (PS) plate (Fig. 2A(v)), a PS template containing various wavelengths of sinusoidal wavy