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Multiscale engineered hierarchical structures with precisely controlled sizes for bio-inspired cell culture

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ABSTRACT

We present a simple method of fabricating bio-inspired multiscale structure by combining replica molding and surface wrinkling. With this technique, we demonstrates that our fabrication method is manageable to fabricate various types of substrate with various orientations of nanopatterns on microwrinkle structure, resulting perfectly conform the wrinkling phenomenon. Finally we have explored cells behavior on multiscale substrate.

1. Introduction

Providing cells with an *in vivo*-like environment is one of the most critical issues impeding the progress of regenerative medicine toward a clinical phase [1–3]. *In vivo* tissues mainly consist of cells and extracellular matrix (ECM), which forms the extracellular environment, and the interaction between the cells and the ECM regulates the fate and function of the cells. While several factors such as biochemical and mechanical cues affect cells, topographical cues have been found to play a pivotal role in directing the function and behavior of cells. Cells are exposed to multiscale topographical environments of the ECM, for which the dimensions span micrometers to nanometers [4–13]. Therefore, a clinically reliable artificial ECM that can be used for tissue transplantation must possess multiscale structures that can provide cells with topographical cues similar to that of a natural ECM.

An extensive effort has been made through interdisciplinary research to obtain an *in vivo*-like artificial ECM [14–16]. However, most of the preceding results are partially limited in their similarity to a natural ECM due to technical difficulties in fabricating a multiscale structure. A natural ECM consists of nanofibrils, which are aligned in a specific direction, as well as microfibrils, and these constitute a complex matrix in a hierarchically organized fashion. Although the importance of the effects of nanofibrils on the function and behavior of cells is widely studied, the effects of microfibrils remain poorly understood. Most of the preceding studies on artificial ECMs focus on the effects of nanotopography using a single-scale substrate with nanoscale structures only, which does not fully reflect the morphological features of a natural ECM. Therefore, these substrates are restricted in their applicability as *in vivo*-like topographical cues to cells in a synthetic environment.

Here, we propose a simple method of fabricating an ECM-mimicking multiscale substrate that is based on capillary force lithography (CFL) and the wrinkling effect of polymeric materials. Nanogrooves were fabricated by CFL using poly(ethylene glycol) diacrylate (PEGDA) on an oxidized surface of poly(demethylsiloxane) (PDMS), and this was mechanically compressed in a uniaxial direction to induce the wrinkling effect at the microscale. Strong bonding between the nanostructure and the PDMS surface as well as a relatively low modulus of PEGDA compared to high modulus of surface treated PDMS enables the preformed nanogrooves to perfectly conform to the micro-sized wrinkles of the PDMS substrate. This results in a microwavy nanopatterned substrate, which is used as a mother mold from which ECM-mimicking multiscale substrates are replicated. Our method is versatile and tunable in that the geometry of nanostructures can be altered easily by selecting a different master mold, and the wavelength of the microwrinkles can be varied by adjusting the compressive strain.

2. Materials and methods

2.1. Preparation of the poly(urethane acrylate) (PUA) mold

The silicon masters with various hexagonal arrays of

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nanopillars were prepared by photolithography and subsequent reactive ion etching (RIE). The drops of UV-curable PUA precursor (0.1–0.5 ml), purchased from the Minuta Tech, Korea, were dispensed on the master and a polyethylene terephthalate (PET) film (50 μm) was brought into contact with the liquid drop for it to be used as a supporting backplane. Subsequently, the assembly was exposed to UV light (λ=200–400 nm, dose=100 mJ cm^{-2}) for 20 s. After UV curing, PUA mold was removed from the silicon master and further cured for 10 h for complete annihilation of active acrylate groups on the surface.

2.2. Design and fabrication of multiscale tissue like substrate

PDMS pre-polymer (Sylgard 184 Silicon elastomer, Dow corning) was mixed with 10% curing agent, poured into a petri dish until enough thickness, and baked at 80 °C for at least four hours for perfect curing without any residues. After peeled off from the petri dish, it was exposed to ultraviolet/ozone (UV/O) radiation for 5 min. Thereafter, a small quantity of adhesion promoter TMSPMA (10% v/v) was poured onto the UV/O-treated PDMS sheet and spread evenly using a spin coater set as 3000 RPM, following which the sheet was baked for 1 h to dry the promoter. Then, a few droplets of PEG-DA (Sigma-Aldrich) precursor solution were dropped and covered with the prepared PUA mother mold. The assembly was pressed at 10 bar for one hour. After UV curing for five minutes, the mother mold was peeled off carefully using a tweezers. And then, based on the study of wrinkle phenomena, the compressive strain was applied using custom-designed strain apparatus (Step 1 in Fig. 1a). PUA precursor was drop-dispensed and UV-cured to be used as self-replica mold with hierarchical wrinkling patterned (Step 2 in Fig. 1a). Finally, additional UV-curing was performed for more than 10 h.

2.3. Culturing of NIH3T3 fibroblast cells

NIH3T3 fibroblast cells were grown in DMEM with 10% FBS and 1% penicillin-streptomycin (Gibco, Milan, Italy) at 37 °C in a 5% CO_2 atmosphere. The quantitative analysis was analyzed using a custom-written MATLAB script using the images by obtained.

2.4. Quantification of morphology of NIH3T3 fibroblast cells

The cells were cultured for 14 h. The quantitative analysis for cell orientation was analyzed using phase contrast images of NIH3T3 fibroblast cells cultured single- or multiscale patterned substrates and a custom-written MATLAB script. One hundred cells were used for quantification.

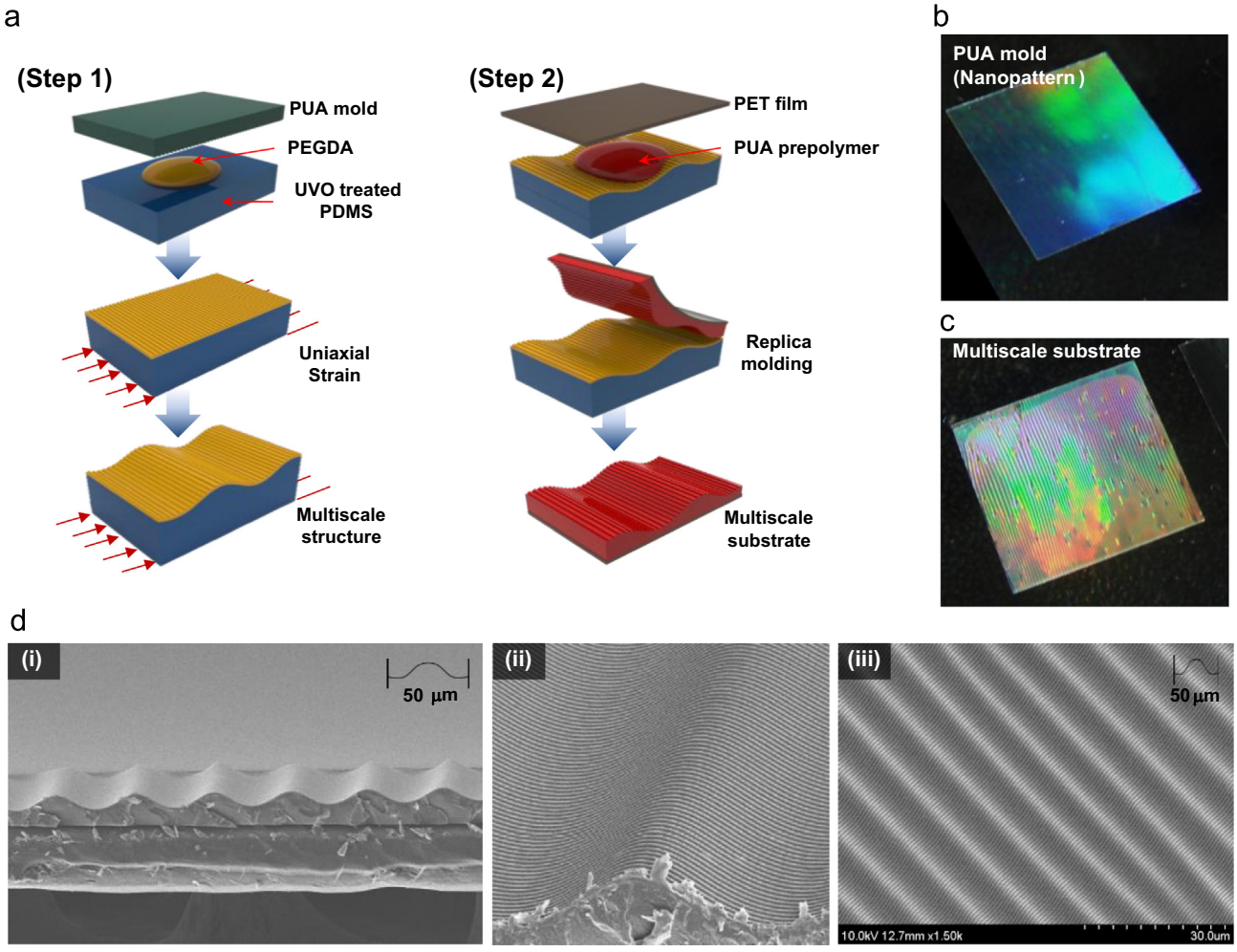

Fig. 1. (a) Schematic description of the fabrication process of the multiscale hierarchical substrate. The fabrication process is mainly comprised of two steps: the preparation of a mother mold (step 1) and the replication of multiscale patterns (step 2). (b and c) Photographs of the visual difference in the color distribution between a nanopatterned substrate and the fabricated multiscale hierarchical substrate. The existence of the microstructures cause a larger deflection of the incident light, resulting in a more distorted color distribution. (d) SEM images of an extracellular matrix (ECM)-mimicking multiscale substrate depicting both fine and regular hierarchical structures.