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Multiscale patterned transplantable stem cell patches for bone tissue regeneration

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ABSTRACT

Stem cell-based therapy has been proposed as an enabling alternative not only for the treatment of diseases but also for the regeneration of tissues beyond complex surgical treatments or tissue transplantation. In this study, we approached a conceptual platform that can integrate stem cells into a multiscale patterned substrate for bone regeneration. Inspired by human bone tissue, we developed hierarchically micro- and nanopatterned transplantable patches as synthetic extracellular matrices by employing capillary force lithography in combination with a surface micro-wrinkling method using a poly(lactic-co-glycolic acid) (PLGA) polymer. The multiscale patterned PLGA patches were highly flexible and showed higher tissue adhesion to the underlying tissue than did the single nanopatterned patches. In response to the anisotropically multiscale patterned topography, the adhesion and differentiation of human mesenchymal stem cells (hMSCs) were sensitively controlled. Furthermore, the stem cell patch composed of hMSCs and transplantable PLGA substrate promoted bone regeneration *in vivo* when both the micro- and nanotopography of the substrate surfaces were synergistically combined. Thus, our study concludes that multiscale patterned transplantable stem cell patches may have a great potential for bone regeneration as well as for various regenerative medicine approaches.

1. Introduction

Stem cells are capable of differentiating into various types of cells, offering opportunities and alternatives not only for the treatment of diseases but also for the regeneration of tissues and organs beyond complex surgical treatments or tissue/organ transplantation [1–5]. Therefore, developing a viable and practical stem cell-based therapy for clinical applications is important. The most commonly proposed stem cell therapy in current clinical trials is to inject autologous stem cells isolated from a patient into the targeted area after expanding sufficient numbers of stem cells *in vitro* [3–5]. However, this strategy has critical limitations [1–5]: (i) the efficiency of transplanted stem cells located in the targeted area is very low (i.e. cells could easily be washed from the targeted tissues), and (ii) the transplanted stem cells often fail to maintain their viability and function in the host tissues, which has resulted in only limited success in restoring the damaged tissues and organ, especially in large scale tissue repair or regeneration. Consequently, an efficient platform that could help transplanted stem cells to integrate into host tissues effectively is urgently needed in order to achieve the goal of tissue repair and regeneration.

The construction of synthetic extracellular matrices (ECMs) inspired by tissue-specific niches for programmed stem cell fate and response, such as proliferation and differentiation, is a topic of interest in the field of tissue regeneration [6,7]. For example, inspired by

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the highly oriented topographical features of natural ECMs in various tissues, including bone, tooth, nerve, skin, muscle, and heart, previous studies have developed nanogrooved matrices using nanofabrication technologies, demonstrating a critical role for topographical cues in the controlled structure and functions of stem cells [8–14]. Importantly, the nanotopographical cues could reportedly promote the differentiation of stem cells; for example, nanogrooved matrices enhanced the osteo- or neurogenesis of human mesenchymal stem cells (hMSCs) compared to a flat substrate or microgrooved matrices [8,9,12]. In conjunction with the significant findings mentioned above, a platform that can integrate stem cells into synthetic ECMs (called a 'stem cell patch') has been newly proposed as an approach that facilitates the repair or regeneration of tissues [15,16].

Despite growing interest in the use of synthetic ECMs for stem cell therapy for clinical applications, further mimicking the complexity of ECMs remains a pressing challenge to improve such platforms. Most studies have examined simple topographical designs for fabricating synthetic ECMs (i.e. single-scale micro- or nanotopography), partly because of limited fabrication techniques. However, native ECMs are composed of complex and hierarchical structures with various sizes ranging from nanometers to micrometers [8,17,18]. For example, individual collagen fibrils in ECMs tend to be approximately a few hundred nanometers in size, whereas actual collagen fibers formed by multiple fibrils are a few tens of micrometers in size [19]. Thus, the hierarchically micro- and nanotopographical cues from the ECM structures where cells populate *in vivo* may be an essential element for regulating or improving stem cell fate and function, which may be an important factor to consider for the design and manipulation of synthetic ECMs. In addition, the enhanced tissue bonding ability of synthetic ECMs may provide another opportunity for improving stem cell-based therapy, i.e. it may allow precise tissue adaptation and bonding to the underlying tissue, which could eventually promote topography-induced spontaneous tissue regeneration as well as enhance the efficiency of transplanted stem cells in the targeted area.

Here, we propose the rational design and manipulation of transplantable substrates as a synthetic ECM to meet the challenges mentioned above for advanced stem cell therapy. As a key design criterion for more physiologically relevant ECMs, hierarchically micro- and nanopatterned multiscale topographies with precisely controlled sizes were developed by employing capillary force lithography in combination with a surface micro-wrinkling method using a Food and Drug Administration (FDA)-approved poly(lactic-co-glycolic acid) (PLGA) polymer due to its biocompatibility and biodegradability. Using these platforms, we investigated the influence of multiscale hierarchical topography on the adhesion, proliferation, and differentiation of stem cells. Furthermore, multiscale patterned transplantable stem cell patches were proposed as an approach for bone regeneration.

2. Materials and methods

2.1. Preparation and observation of ex vivo human bone tissue

Bone tissue was obtained from a patient during dental surgery under the approval of the Institutional Review Board of Seoul National University (Seoul, Korea). The tissue was fixed overnight with a solution containing 2% glutaraldehyde, 0.1 M sodium cacodylate, and 3 mM calcium chloride (pH 7.4) at 4 °C. The tissue was rinsed three times with PBS. The specimen was perfused with 1% osmium tetroxide and placed on a tissue rotator for 30 min. The sample was then rinsed three times in PBS. Subsequently, the sample was serially dehydrated in 50%, 70%, 90%, 95%, and 100% acetone. The sample was treated with hexamethyldisilazane (HMDS), air dried, and placed on a stub for sputter-coating with gold. The tissue was then observed with a FESEM (JEOL, JSM-5410LV, Japan).

2.2. Fabrication of PUA mother mold

Fig. S1 shows the schematic procedure for fabricating a multiscale patterned poly(urethane acrylate) (PUA) mother mold (350 nm ridge and groove width). A droplet of UV-curable PUA (Minuta Tech., Korea) precursor solution with photo-initiator was dropped on the silicon master mold, on which nanosized linear grooves (350 nm) were etched using conventional photolithography and reactive ion etching. The mold was then uniformly covered with a transparent poly(ethylene terephthalate) (PET) film utilizing capillary force. After the master was exposed to UV light ($\lambda = 250–400$ nm, 100 mJ/cm^2) for 15 s, the cured PUA replica was peeled off from the master mold using tweezers and again exposed to UV light for 10 h to completely annihilate any residual reactive acrylate groups.

2.3. Design and fabrication of multiscale PUA mold

First, PDMS pre-polymer (Sylgard 184 Silicon elastomer, Dow corning) was mixed with 10% curing agent, poured into a petri dish to a sufficient thickness (~1 cm), and baked at 70 °C for at least 2 h to ensure curing without any residue. The polymer was then peeled off from the petri dish and oxidized with UV/ozone treatment system (Yuil Ultra Violet system, Korea) in 5 min and 15 min for 30 μm and 100 μm wavelength respectively. To ensure uniform oxidation, the distance from the UV lamp was constant at 5 cm. The dose of UV/ozone was 15 mW/cm^2 (measured at a 10 mm distance) at wavelengths of 185 and 254 nm. Thereafter, a small quantity of 10% (w/v) adhesion promoter TMSPMA (100 μL) was drop-dispensed onto the UV/ozone-treated PDMS sheet and spread evenly using a spin coater set to 3000 RPM, after which the sheet was baked for 1 h to dry the promoter (Fig S1; Step 1). Subsequently, 100 μL of PEG-DA (Sigma–Aldrich) precursor solution was drop-dispensed and covered with the prepared PUA mother mold (350 nm ridge and groove width). The assembly was pressed at 0.7 bar for 1 h. After UV curing for 5 min, the PUA mother mold was carefully peeled off using tweezers (Fig S1; Step 2). Second, based on the study of wrinkle phenomena, 100% compressive strain was then applied using a custom-designed strain apparatus resulting 30 μm (5 min UV/ozone) or 100 (15 min UV/ozone) μm wavelength. To replicate multiscale structures (350 nm/30 μm and 350 nm/100 μm) on PDMS, a PUA precursor was drop-dispensed and UV-cured for use as a self-replicating mold (Fig S1; Step 3). Finally, additional UV-curing was performed for more than 10 h to remove any non-cured acrylate groups.

2.4. Fabrication of multiscale patterned PLGA substrates

A 3% (w/v) solution of PLGA prepared in chloroform (200 μl) was drop-dispensed onto the PDMS block, and a flat PDMS upper block was placed to obtained a smooth PLGA film layer and remove the solvent. Subsequently, the PDMS block was preheated on a hot plate to remove additional solvent. A multiscale patterned PUA mother mold (350 nm ridge and groove width on a 30 μm or 100 μm wavelength) was then placed on the preheated PLGA film and embossed into the molten PLGA by applying constant pressure (~0.7 bar) while heating at 100 °C for 10 min (glass transition temperature (T_g) of PLGA = 59 °C). After the thermal imprinting process, the assembly of the PDMS block and mother mold was cooled to room temperature, and the mold was peeled off the substrate resulting multiscale patterned PLGA substrates with 350 nm ridge and groove width on a 30 μm or 100 μm wavelength.

2.5. Surface property of multiscale patterned PLGA substrates

The static contact angles (CAs) of liquids used in the experiment were measured using DSA100 goniometer (Kruss, Germany). For each measurement, 10 μl of liquid was drop-dispensed on the surface over a time span of 1 min. The measurement was averaged over at least ten different locations for each sample conditions.

High-resolution scanning electron microscopy (SEM) images of the nanometer sized PUA and PLGA structures were obtained using a HITACHI S-4800 microscope (Hitachi, Japan) at an acceleration voltage of 10.0 kV and an average working distance of 11.4 mm. To avoid charging effects, the substrates were sputter-coated with Au to a thickness of 3 nm prior to the measurements.

2.6. Measurement of adhesion force with porcine small intestine

The macroscopic normal and shear adhesion forces of multiscale PLGA patches against the porcine small intestine surface were measured using custom-built equipment. Prior to the adhesion test, fresh porcine intestine tissue was rinsed several times with DI water and then placed onto a gel ice pack to prevent spoiling. A PLGA patch (area: 1 × 1 cm^2) was then attached to the surface of the small intestine under a preload of ~0.5 N/cm^2, and the adhesion force was measured by gradually increasing the pulling weight until adhesion force failure occurred. To ensure statistical significance, the adhesion force was measured 30 times for each sample under identical conditions at a relative humidity of 40% and ambient temperature of 25 °C, and the averaged data were used throughout the experiment.

2.7. Isolation and culturing of hMSCs

The experimental protocol was approved by the Institutional Review Board of the Seoul National University (Seoul, Korea), and the detailed method to isolate hMSCs has already been reported by our group [20]. The bone segments were digested in a solution of 3 mg/ml collagenase type I and 4 mg/mL dispase for 1 h at 37 °C. Single-cell suspensions were obtained by passing the cells through a 70 μm