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Research paper

Fabrication of polystyrene-based multi-well screening platform for micrometer-scale surface topographies promoting stem cell functions

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ABSTRACT

The study of surface topographic effect on stem cell function is getting attention these days due to its potential clinical applications such as regenerating injured tissues and drug screening. In this study, we suggest a simple and rapid fabrication method for a novel multi-well screening platform which contains a polystyrene (PS) bottom plate possessing different micrometer-scale surface topographies fabricated by the hot embossing process utilizing a polydimethylsiloxane (PDMS) mold. To obtain high replication quality with the reduced geometric deformation of surface topography, the processing condition for replication of the PS bottom plate was optimized through the design of experiments. The fabricated PS bottom plate is chemically bonded with a polymethyl methacrylate (PMMA) partition whose function is (1) isolating one type of surface topographies into an individual well and (2) preventing cross-contamination of soluble factors from a cell culture medium, resulting in a total fabrication time for one screening platform ~10 min. Given that the present platform is based on PS, which is the most widely used material in cell-based studies for the past decades, it is advantageous to compare the results from the present platform with the previously validated results performed in the conventional PS cell-culture wares. The effects of various micropillar arrays on the proliferation of human adipose-derived stem cells were examined using the present multi-well screening platform. Through those fabrication and stem cell-based experiments, it was shown that there is a possibility of considering the suggested fabrication method to be used for fabricating a standard screening platform for effective surface topography which promotes stem cell functions.

1. Introduction

As surface topographies are well known biophysical factors for regulating cell behaviors, there have been many efforts to investigate the relationship between the cell behaviors and the surface topography to determine an optimal surface topography for the specific regulation of cell functions such as cell adhesion, proliferation, migration and differentiation [1–7]. Therefore, development of a rapid screening system, in which the effects of various surface topographies on cell behaviors can be investigated at once, has drawn increasing attention from the researchers. Lovmand et al. introduced BioSurface Structure Array (BSSA), which is composed of several kinds of micropillar arrays, to study proliferation and differentiation of murine osteoblastic cell line (MC3T3-E1 cells) [8]. Moe et al. fabricated Multi-ARchitecture Chip (MARC) containing various kinds of surface topographies in micrometer- and nanometer-scales with structures such as grating, pore, pillar and hierarchy. They investigated the effects of those surface topographies on differentiation of primary murine neural progenitor cells [9]. Very recently, Hu et al. demonstrated an Integrated Mechanobiology Platform (IMP) containing nanofabricated trench-grid surfaces to examine the complex works of antibodies and surface topographies on T cell behaviors like activation and proliferation [10]. So far, although numerous types of screening platforms were suggested, it is hard to isolate the sole effect of surface topography on cell behaviors due to different types of substrate materials which have all different properties such as stiffness and surface chemistry. In this regard, it is of importance to eliminate other factors than surface topographic effect by standardization of the substrate material. Given that the polystyrene (PS) cell-culture wares, which were first introduced around the 1960s, have been the most widely used and validated as cell-culture platforms for the past decades due to its low material cost, good mechanical and chemical stability and biocompatibility, PS could be considered as the best candidate for a substrate material regarding the above-mentioned standardization [11].

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Our group previously reported a polydimethylsiloxane (PDMS) mold-based hot embossing technique for replication of MicroPattern Array with Gradient Size (μPAGS) PS surfaces [12]. Though a micropillar-arrayed surface was found to promote the proliferation of adipose-derived stem cells compared with a microwell-arrayed surface, we could not distinguish the effective pattern size due to not isolating the sole effect of surface topography from the continuous gradient micropillar pattern on μPAGS in a same cell culture chamber. In this study, we suggest a simple and rapid fabrication method of a PS-based multi-well screening platform, where the various micrometer-scale surface topographies were isolated by partition for investigating the sole effect of surface topography, via the hot embossing technique with a PDMS mold. Since there is no limitation on the types and shapes of the micrometer-scale topographies (e.g., pillars, pores, gratings, etc.), the circular cross-sectional micropillar arrays, which were found to enhance cell proliferation in our previous work, were exclusively utilized [12]. The design of experiments was carried out based on the Taguchi method with the replication quality and the geometric deformation of surface topographies. The PS bottom plate replicated under the optimal processing condition was combined with a polymethyl methacrylate (PMMA) partition to finally realize a PS-based multi-well screening platform in which each topography is isolated into an individual well to prevent cross-contamination of soluble factors. The fabricated multi-well screening platform was then applied to study of surface topographic effects on stem cell function, especially, proliferation of human adipose-derived stem cells (hASCs), which demonstrates the screening ability of the present platform.

2. Materials and methods

2.1. Fabrication of PS-based multi-well screening platform

Fig. 1 illustrates the entire fabrication process of a PS-based multi-well screening platform. It can be divided into three steps: (1) fabrication of a PDMS mold by PDMS replica molding against a SU-8 master, which was obtained through UV–photolithography (Fig. 1a), (2) replication of a PS bottom plate by hot embossing with the PDMS mold (Fig. 1b) and (3) chemical bonding of the PS bottom plate with a PMMA partition (Fig. 1c).

2.1.1. Fabrication of PDMS mold

In this study, various circular cross-sectional micropillar arrays with a diameter of 2 μm and a height of 1 μm were utilized as the micrometer-scale surface topographies to be investigated. The surface topographies of the micropillar arrays were designed varying the type of array (square [S]/hexagon [H]/random [R]) and interpillar spacing (2/4/8 μm) with control (flat), resulting in total 10 different patterns (denoted as S2, S4, S8, H2, H4, H8, R2, R4, R8 and Con, see Supplementary information).

Each micropillar array was located in a pre-defined section of the square area of 1 cm^2 and occupied three sections for allowing triplicate cell experiments within a single wafer, which results in total 30 culture regions on the same platform. A SU-8 master, which is to possess the designed surface topographies, was fabricated through UV-photolithography using negative photoresist SU-8 2 (Microchem Corp.) at a spin-speed of 2800 rpm and an exposure dose of 100 mJ cm^{-2} according to our previous paper [12]. Then, a PDMS mold was replicated by PDMS replica molding against the SU-8 master with PDMS pre-polymer (Sylgard 184, Dow Corning). PDMS was selected for a mold material during hot embossing due to its easy prototyping and elastomeric properties which create conformal contact to a PS surface, resulting in a relatively high replication quality of micrometer-scale surface topographies [12]. The pre-polymer was mixed with curing agent in a weight ratio of 10:1, and poured over the SU-8 master. The pre-polymer mixture was then degassed in vacuum and cured in a dry oven at 65 °C for 4 h. The fabricated PDMS mold having micropore arrays, which is to be used as cavities for the micropillar arrays during hot embossing, was peeled off from the master.

2.1.2. Replication of PS bottom plate with micropillar arrays

Flat PS (Solarene G-144, Hyundai Engineering Plastics) substrates were prepared by virtue of conventional injection molding process with a mold having a mirror-like surface [6]. To determine an optimal condition for replication of micropillar arrays with minimized geometric deformation of topographies, the design of experiments was carried out based on Taguchi method varying the processing parameters such as embossing temperature (T_E), embossing pressure (P_E) and embossing time (t_E). The hot embossing-based replication process for the PS bottom plate possessing various micrometer-scale topographies is as follows: (1) allocating the PDMS mold and PS substrate between hot embossing platens, (2) heating up both the PDMS mold and PS substrate to an embossing temperature of T_E, (3) pressing the PDMS mold and PS substrate under an embossing pressure of P_E for an embossing time of t_E, (4) cooling both the PDMS mold and PS substrate down to 27 °C and (5) detaching the replicated PS bottom plate from the PDMS mold.

2.1.3. Chemical bonding of PS bottom plate and PMMA partition

A PMMA partition was prepared by laser cutting of a PMMA sheet with a thickness of 10 mm using a laser cutting machine (IS 640, INNOSTA) at the cutting condition of a laser power of 100 W and a cutting speed of 0.2 mm/s. PMMA was adopted as partition material due to its good machinability within the laser cutting and biocompatibility (see Supplementary information). The PS bottom plate and PMMA partition were chemically bonded with the help of γ-

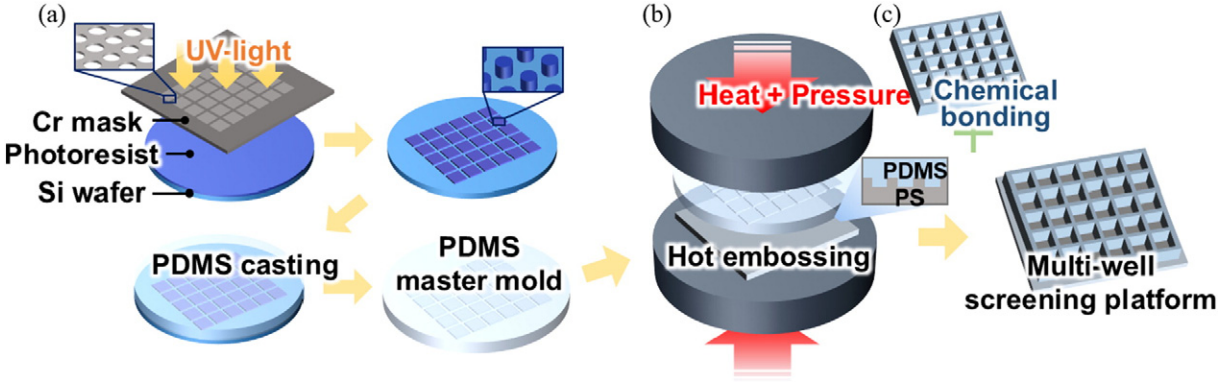

Fig. 1. The schematics of multi-well screening platform fabrication procedure. (a) PDMS mold fabrication. (b) Replication of micrometer-scale topographies into the PS bottom plate with PDMS mold. (c) Chemical bonding of the PS bottom plate with a PMMA partition.