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Ordered cylindrical micropatterned Petri dishes used as scaffolds for cell growth

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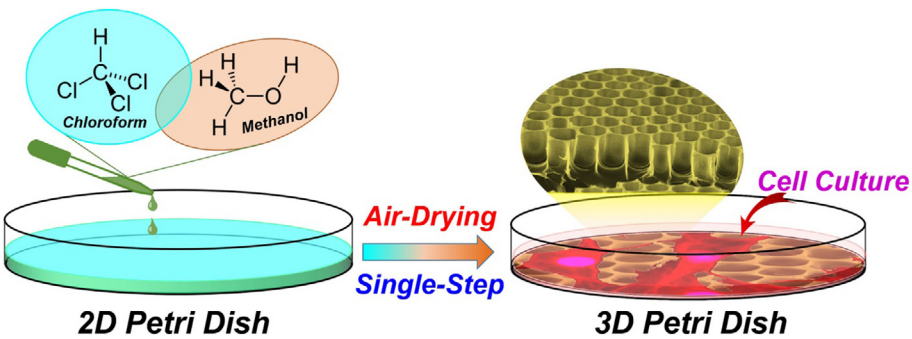
GRAPHICAL ABSTRACT



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ABSTRACT

Three-dimensional (3D) culture dish patterned with a microwell structure demonstrates a great application potential in biotechnology. This study reports on the easy fabrication of an ordered customizable honeycomb microwell array on the surface of polymer substrates including the commercial Petri dish to create a biological platform for cell culture. The fabrication method is based on a very simple solvent dip-coating technique and the methanol accumulation-induced phase separation in which a binary mixture of chloroform and methanol is used to induce a ternary solution and to guarantee the formation of the ordered pore array on the substrate. The surface topology of the honeycomb substrate is manipulated through varying the experimental conditions; notably, the obtained honeycomb structure is part of the substrate, which reveals an increase in the structure's stability for the practical applications. Honeycomb-structured Petri dish fabricated using this method is applied as a scaffold for cell growth to demonstrate its potential in biomedical applications.

1. Introduction

Over the past few decades, control of surface morphologies has become a key issue in materials science and surface chemistry [1,2]. In particular, 3D honeycomb-patterned substrates with controllable pore arrays have attracted significant attention in the field of tissue engineering and artificial organs of human body because they can offer biological environments where cells behave as they do in vivo [3–5]. In general, honeycomb porous structures can be fabricated using several microfabrication technologies such as lithography-based techniques, phase separation, emulsion, colloid crystals, and breath figures (BFs) [6–11]. Among these, BFs have been the most widely applied method due to their versatility

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and cost effectiveness [12–14]. Note that the traditional BF is normally used to fabricate the films with microporous structures because BFs rely on the arrangement of templating droplets on a polymer solution on substrates such as silicon wafers, glasses, mica, metals, plastics, and water. Consequently, the need for an external polymer solution severely limits the application of BFs to the polymer substrates, and it also reduces the application potential of the honeycomb structure. Moreover, the physical contact between the honeycomb film and the solid substrate causes the low stability of honeycomb film due to degradation of interfacial adhesion during practical applications. To date, significant efforts have been devoted to fabricating honeycomb porous films using the BF method, whereas direct fabrication of honeycomb patterns on substrates remains a significant challenge that has seldom been addressed.

Recently, Farbod reported a direct breath figure (DBF) method for creating a honeycomb structure that is part of the polymer substrate [15]. However, the structure generated by DBF was irregular and not ordered because tetrahydrofuran used as a water-miscible solvent was not favorable for resulting in ordered structure [13]. In order to improve the quality of the honeycomb structures formed on the substrate, Shen and his group developed the semi-direct breath figure (sDBF) method, which is based on the combination of DBF and normal BF [16]. Although ordered honeycomb patterns can be created on Petri dish surfaces using sDBF, the requirement of very humid conditions results in a more complicated implementation and the surface disturbance induced by the humidity gradient lowers the reproducibility associated with the difficulty in controlling the honeycomb pore arrays. For example, the inverse effect of a humidity disturbance on the formation of the honeycomb film using a dynamic method was observed in an experiment (SI, Fig. S1). It was clearly demonstrated that sDBF is very sensitive to humidity through which the ordered honeycomb structure was only achieved in a narrow range of high humidity (approximately 75%). In addition, sDBF drop-casted the solvent on the substrate surface in order to induce a solution layer. However, it should be emphasized that drop-casting is not compatible for mass production; in particular, it may suffer from a significant challenge to produce a uniform coating on non-flat surfaces. These limitations are critical hurdles to the industrial enlargement and application potential of the honeycomb substrate.

In microbiology laboratories, Petri dishes are common and essential pieces of equipment because they are used for culturing cells, which has become an important experimental process for numerous biological processes such as tissue engineering, gene testing, and drug testing in vitro. Commercial Petri dishes have flat surfaces; therefore, they do not provide a real 3D environment similar to that in vivo. Recently, the presence of the 3D cell culture plate has taken a big advantage for researchers to mimic tissue environment. However, the price of 3D cell culture plate is quite expensive. Therefore, the fabrication of 3D Petri dish in a simple and economical way is not only a significant challenge, but also a quite necessity in the development of biochemical and biomedical materials.

In order to address these issues, a very simple, cost effective, scalable, and single-step method is developed to directly fabricate ordered honeycomb patterns on polymer substrates in normal air. In this approach, a mixture of chloroform and methanol was used to induce a ternary solution and to guarantee the formation of ordered honeycomb structures through methanol accumulation-induced phase separation followed by the self-assembly of non-solvent droplets. Importantly, for the first time, a uniformly ordered honeycomb cylindrical cavity array with a large depth-to-diameter aspect ratio was prepared on complex substrate surfaces without active surface agents required. The fibroblast NIH3T3 cells were grown on the as-prepared 3D Petri to evaluate the proliferation as evidence for application potential in cell culture and tissue engineering.

2. Experimental section

2.1. Materials

Commercial Petri dishes (polystyrene; Mw = approx. 220,000; 3.5 cm in diameter) were received from Sigma-Aldrich (USA). The substrates were cut into rectangular plates (5×3 cm^2) and washed with distilled water in an ultrasonic bath followed by drying in a nitrogen gas stream prior to use. Chloroform (ChL; anhydrous, with amylenes as stabilizers, ≥99%) and methanol (MeOH; anhydrous, 99.8%) were purchased from Sigma-Aldrich (USA) and were used as received. Propidium iodide (PI), paraformaldehyde, tris(hydroxy methyl)aminomethane (Tris), and disodium salt solution (EDTA) were purchased from Sigma-Aldrich (Seoul, Korea). bisBenzimide H 33342 trihydrochloride (Hoechst 33342) was obtained from Invitrogen (Seoul, Korea). Fetal bovine serum (FBS), 100X antibiotic-antimycotic agent, and Dulbecco's modified Eagle's medium (DMEM) were bought from GIBCO (USA). The NIH3T3 cell line was obtained from Korea Cell Line Bank (Korea).

2.2. Preparation of honeycomb-patterned substrates

The binary mixture of chloroform and methanol was prepared through mixing two liquids. The composition of the mixture was varied using different volumes of methanol and chloroform. The honeycomb substrates were fabricated based on a solvent-immersion phase separation method using a dip-coater (E-flex, Seoul, Korea). Briefly, the substrate was dipped into the mixture solvent for a specified time, and then it was withdrawn and left in air to dry. The honeycomb structure was spontaneously formed after complete evaporation of the solvents. A humidity and temperature controller (TH-TG-1000, Jeio Tech, Korea) was used to control the ambient conditions.

2.3. Crosslinking of the honeycomb substrate

The crosslinking process was performed in an Oriel Flood Exposure System (Model 92521, Newport Corporation, USA) in air. The substrate was exposed to 220–260 nm UV light with a power density of 19.0 mW/cm^2 for 6 h. The distance between the UV source and the substrate surface was 5 cm. The solvent annealing was performed through placing the substrate into the saturated chloroform vapor for 5 min.

2.4. Cultivation of the NIH3T3 cells on the honeycomb substrate

NIH3T3 cells were selected in order to examine the cell growth behavior on flat and honeycomb-structured Petri dishes. All samples were first irradiated with UV light for 5 min and then washed twice with DPBS. Subsequently, the samples were placed into 24-well plates (for SEM observation) and 6-well plates (for cell counting characterization) with 1 ml and 5 ml of NIH3T3 cell solution per well (10^5 cells/ml) containing 89% DMEM, 10% FBS, and 1% antibiotic; then, these were incubated at 37 °C with 5% CO_2. After the specified incubation time, the substrate was removed from the medium and washed twice with DPBS followed by fixing with 4% formaldehyde for 30 min. Subsequently, the substrate was washed with DPBS again before successive dehydration of the cells using graded concentrations of ethanol subscript at 50% ethanol for 15 min, 75% ethanol for 15 min, 95% ethanol for 15 min (twice), and 100% ethanol for 30 min (twice) [17,18]. The samples were held in a fume hood for air drying before the SEM observations.