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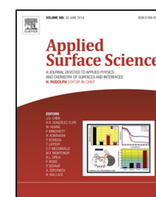

Simple and non-toxic fabrication of poly(vinyl alcohol)-patterned polymer surface for the formation of cell patterns

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ABSTRACT

In this study, a facile and non-toxic method for the formation of cell-adhesive poly(vinyl alcohol) (PVA) patterns on the surface of a non-biological polystyrene substrate (NPS) is developed to control cellular micro-organization. PVA thin films spin-coated onto the NPS are selectively irradiated with 150 keV H^+ ions through a pattern mask and developed with deionized water to form negative-type PVA patterns. Well-defined stripe patterns of PVA with a width of 100 μm are created on the NPS at a higher fluence than 5×10^{15} ions/cm^2, and their surface chemical compositions are changed by ion irradiation without any significant morphological change. Based on the results of the protein adsorption test and *in vitro* cell culture, cancer cells are preferentially adhered and proliferated onto the more hydrophilic PVA regions of the PVA-patterned NPS, resulting in well-defined cell patterns.

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1. Introduction

Cell patterning on an artificial substrate has received a great amount of attention as an essential prerequisite for a variety of biological applications such as the fundamental study of cell biology, tissue engineering, cell-based bioassays, and cell-based drug screening [1–7]. To facilitate the formation of cell patterns, a variety of surface patterning techniques, including inkjet printing, photolithography, and micro-contact printing, has been extensively explored [8–10]. However, although they provide resolved patterns on the surface of a substrate, they have drawbacks, such as multiple steps and non-biocompatible processes with a necessity of toxic chemicals to form the patterned surfaces [11–13]. Therefore, a simpler and more biocompatible surface patterning method is required to prepare patterned surfaces for cell patterning.

An ion beam-based patterning technique is a powerful surface patterning method for the formation of cell patterns. It offers several advantages including convenient and precise controllability, reliability, temperature-independent processing, and non-toxic processing without the use of harsh chemicals owing to the greater liner energy transfer (LET) and straighter penetration trajectory of the ion beams in comparison to other techniques based on electron beams, UV light, γ-rays, and X-rays [14–18]. Thus, microstructures formed by ion irradiation have been widely used to spatially control the adhesion and proliferation of cells [19–21].

Poly(vinyl alcohol) (PVA) has been used in the biomedical field because of its water solubility, biocompatibility, optical transparency, and good capability of thin film formation [22–29]. Despite these benefits, it has not been extensively used as a cell guiding material for patterned cell culture because the patterns of PVA are difficult to form by conventional photolithography without biologically-undesirable chemicals, such as photoacid generators and a developer [30–32]. Thus, the fabrication of PVA-patterned platforms for the formation of cell patterns by an eco-friendly and biocompatible ion beam-based technique without any toxic chemicals has not been previously studied. To the best of our knowledge, this is the first report on the formation of PVA patterns on a polymer substrate using a simple and biocompatible ion beam-based technique.

In this study, ion beam-based patterning of cell-adhesive PVA on a non-biological surface was carried out to control cellular behaviors. This technique offers several advantages including easy and precise controllability, temperature-independence, reliability, and non-toxicity without the need of any harsh chemicals. The ion beam-based patterning of PVA on a non-biological surface was investigated under various conditions to form negative-type PVA

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patterns. The surface properties of the resulting PVA patterns were investigated in terms of the thickness, wettability, chemical composition and structure. Furthermore, selective cell adhesion on a PVA-patterned non-biological surface was investigated by means of an *in-vitro* cell culture test.

2. Experimental

2.1. Materials

PVA (weight average molecular weight: 89,000–98,000, degree of hydrolysis: >99%) was purchased from Aldrich Chemical Company. Non-biological polystyrene (NPS) petri dishes supplied from SPL Life Science Company were used as a substrate without any further purification. To form the PVA patterns, a customized metal mask (100 μm spaces and 300 μm pitches) was obtained from Youngjin Astech Co., Ltd.

2.2. Patterning of PVA on NPS substrates

Thin PVA films on NPS substrates were formed by spin-coating a 7 wt% PVA solution in distilled water and drying in a vacuum oven for 24 h. The PVA films formed on the NPS were selectively irradiated with 200 keV H^+ ions at fluences ranging from 3×10^{15} to 9×10^{15} ions/cm^2 through a pattern mask at room temperature. Ion irradiation was carried out by using a 300-keV ion implanter at the Advanced Radiation Technology Institute (ARTI, Republic of Korea) [33]. The ion current density was kept at approximately 1.0 μA/cm^2 to prevent the thermal effect. The working pressure of the implanter's target was kept under 10^{-5}–10^{-6} Torr. Afterwards, to generate the PVA patterns, the resulting substrates were developed with a deionized hot water (70 °C) and then dried in an N_2 stream.

2.3. Surface characterization of PVA-patterned NPS substrates

The surface morphology and profiles of PVA-patterned NPS substrates were analyzed using an optical microscope (Type 020-519, Leica, Germany) and a 3D optical surface profiler (NanoSystem, Korea), and an atomic force microscope (AFM, XE-100, Park system, Korea), respectively. The contact angles of the non-irradiated and irradiated PVA were measured using a contact angle analyzer (Phoenix 300, Surface Electro Optical Company, Korea). A deionized water droplet (4 μl) was dropped carefully onto the surface at room temperature. The average contact angle was obtained by five measurements. The chemical structure of the non-irradiated and irradiated PVA was investigated using an attenuated total reflectance Fourier transform infrared spectrometer (ATR-FTIR, Tensor 37, Bruker Co., USA). The change in the chemical composition of PVA before and after ion irradiation was analyzed using an X-ray photoelectron spectrometer (XPS, MultiLab 2000, ThermoElectron Co., England) employing MgK_α radiation. The applied power was 14.5 keV and 20 mA, and the base pressure in the analysis chamber was less than 10^{-9} mbar.

2.4. Pattern stability test

The stability test of the PVA-patterned NPS substrates was performed by measuring the thickness of PVA patterns before and after incubation in phosphate buffered saline (PBS, pH 7.4, Life Technologies) solutions. The PVA-patterned NPS substrates were immersed in the PBS solutions, and subsequently incubated at 37 °C and 5% CO_2 in a humidified incubator. After incubation for 15 days, the thicknesses of the PVA patterns were measured by a 3D optical surface profiler.

2.5. Protein adsorption test

The protein adsorption test was performed with FITC-labeled bovine serum albumin proteins (BSA-FITC, Sigma Aldrich) reported in the literature [34]. A 200 μl of BSA-FITC in a PBS solution with a concentration of 1 mg/ml was fully covered on the PVA-patterned NPS substrates, and successively incubated at 37 °C and 5% CO_2 in a humidified incubator for 1 h. After washing with distilled water several times, the adsorption of BSA-FITC on the PVA-patterned NPS substrates was observed with a fluorescence microscope (DMI4000 B, LEICA). The representative plot profiles of the adsorbed BSA-FITC were drawn with the ImageJ software from their fluorescence images.

2.6. In vitro cell culture

Pre-confluent H1299 (human lung carcinoma cell), HeLa (human cervical cancer cell), and NIH3T3 (mouse fibroblast cell) cells were detached by trypsin-EDTA and then pipetted several times to disperse them into single cells. Prior to the cell culture, PVA-patterned NPS dishes were sterilized with 70% ethanol. Cells with a density of 2.5×10^4 cells/ml were seeded on PVA-patterned NPS substrates and kept in a RPMI 1640 medium (Gibco) for H1299 and in a Dulbecco's modified eagle medium (DMEM, Gibco) for HeLa and NIH3T3 containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C and 5% CO_2 in a humidified incubator. After 72 h, the adhesion and growth behavior of the cells were observed with an optical microscope (Type 020-519, Leica, Germany).

2.7. Cell proliferation assay and viability test

Cell proliferation was measured with a CCK-8 assay kit (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's protocol [35]. Briefly, H1299 cells with a density of 1×10^4 cells/ml were seeded onto the normal polystyrene (NPS) and thin

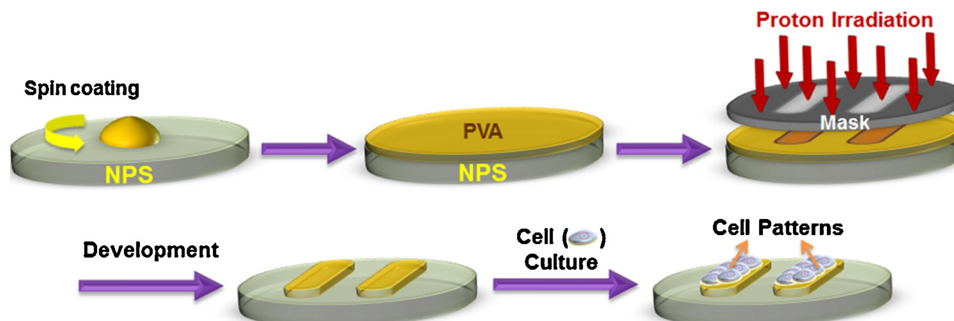

Fig. 1. Schematic illustration of ion beam-based patterning of PVA on a non-biological substrate to control cellular micro-organization.

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