Simple Formation of Poly(sodium 4-styrenesulfonate) Pattern on the Hydrophobic Substrate for the Control of Cell Adhesion via a Selective Ion Irradiation

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Abstract - In this study, the simple preparation of poly(sodium 4-styrenesulfonate) (PSS)-patterned substrate via a selective ion irradiation was investigated to manipulate cell adhesion. PSS thin films spin-coated onto the hydrophobic polystyrene (PS) was patterned through masked 150 keV proton irradiation followed by developing with deionized water. The characteristics of the resulting PSS-patterned surfaces were investigated by using microscope, surface profiler, FT-IR, XPS, and contact angle analyzer. These analytical results revealed that the resolved 100 μm PSS patterns were formed on the hydrophobic PS surface above the fluence of 1×10^{15} ions cm^{-2} and the chemical structure, composition, and wettability of the PSS patterns were dependant on a fluence. Moreover, the results of the *in-vitro* cell culture and proliferation assay exhibited that H1299 cells preferentially adhered and proliferated onto the more hydrophilic PSS part of the PSS-patterned PS and the well-aligned cell patterns was formed on the PSS-patterned PS particularly at the fluence of 1×10^{15} ions cm^{-2}.

Key words : Ion irradiation, Poly(sodium 4-styrenesulfonate), Cell micropatterns

INTRODUCTION

The arrangement of cells at the desired regions of an polymer substrate has attracted enormous interest since it is of great important for the biological applications aiming at the fundamental study of cell biology, tissue engineering, cell-based bioassays, and cell-based drug screening (Robertus *et al.* 2010; Thery 2010; Guillotin *et al.* 2011; Versaevel *et al.* 2012). To control the cell adhesion, the formation of micropatterns on substrates providing both the cell-favorable and -unfavorable regions has been typically performed by means of lithographic methods based on electron beams and UV light (Dalby *et al.* 2008; Arnold *et al.* 2009; Gong *et al.* 2013). However, these methods require multiple fabrication steps and a necessity of biologically unfavorable chemicals, even though they produce well-defined micropatterns on the substrate (Nakanishi *et al.* 2008; Chien *et al.* 2009). Accordingly, a more convenient and biologically desirable micropatterning method is necessary for the establishment of well-defined cell patterns on the substrate amenable to such biological applications.

An ion beam-based method is quite attractive for the generation of micropatterns on the polymer substrate because the liner energy transfer (LET) and straighter penetration trajectory of the ion beams compared to the other methods using electron beams and UV light (Dong and Bell 1999; Hasebe *et al.* 2012; Park *et al.* 2012). This method provides manifold benefits including simplicity, precise controllability, reliability, temperature-independence, and green chemistry without the use of harsh chemicals (Suzuki and Kusakabe 1993; Kondyurin *et al.* 2008). Thus, the ion beam-based micropat-

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terned substrates have been widely explored to control the adhesion and proliferation of cells with or without extracellular matrix molecules (ECM) (Hwang *et al.* 2010; Jung *et al.* 2010; Sommani *et al.* 2011).

Poly(sodium 4-styrenesulfonate) (PSS) is a polyelectrolyte possessing biocompatibility and good capability of thin film formation (Garg *et al.* 2005; Chiang *et al.* 2011). It has been widely used in diverse pharmaceutical fields. To our best knowledge, it is first to report the formation of negative PSS patterns on the hydrophobic substrate via a simple selective ion irradiation to regulate the cell behavior.

In this study, the fabrication of PSS-patterned polystyrene (PS) substrate for cell patterning was performed via the simple and biocompatible ion beam-based methods without any toxic chemicals. The condition for the formation of negative PSS patterns on the PS and the surface properties of the resulting PSS-patterned substrates were investigated. Moreover, the PSS-patterned PS substrates were estimated for their ability to form micropatterns of cell through an *in-vitro* cell culture.

MATERIALS AND METHODS

1. Materials

Poly(sodium 4-styrenesulfonate) (PSS, M.W. 200,000) and Non-biological PS petri dishes were purchased from Aldrich Chemical Company and SPL Life Science Company, respectively. All materials were used without any further purification. A customized metal mask (100 μm spaces and 300 μm pitches) was provided from Youngjin Astech Co., Ltd.

2. Formation of PSS patterns on NPS substrates

To form thin PSS films on PS substrates, the aqueous solution of PSS was prepared by dissolving 1.6 g of PSS in 19.6 g of distilled water, spin-coated on PS substrates at 4,000 rpm, and finally dried in a vacuum oven for 24 h. The resulting substrate was selectively irradiated by 200 keV H^+ ions through a 100 μm line-patterned mask at room temperature on a 300-keV ion implanter at the Advanced Radiation Technology Institute (ARTI, Republic of Korea) (Hwang *et al.* 2010). The fluence ranged from 5×10^{14} to 1×10^{16} ions cm^{-2} and the 1.0 μA cm^{-2} of the beam current density was kept to avoid the thermal effect of the ion irradiation. After that, to form the negative patterns of PSS, the selectively irradiated substrates were developed with deionized water, and then dried in an N_2 stream.

3. Characterization

The PSS-patterned PS substrate was observed with an optical microscope (Type 020-519, Leica, Germany) and using a 3D optical surface profiler (NanoSystem, Korea). The static contact angles based on the sessile drop method were performed on a Phoenix 300 contact angle analyzer (Surface Electro Optical Company, Korea). The analysis of surface chemical structure was carried out on an attenuated total reflectance Fourier transform infrared spectroscope (ATR-FT-IR, Varian 640, Australia) equipped with an ATR PIKE MIRacle accessory containing a ZnSe crystal. The surface chemical composition was measured using an X-ray photoelectron spectrometer (XPS, MultiLab 2000, ThermoElectron Co., England) with a monochromatic Mg-Kα radiation.

4. *In-vitro* cell culture on PSS-patterned PS substrate

Pre-confluent H1299 (human lung carcinoma cell) cells were detached through a trypsin-EDTA treatment, and then dispersed them into single cells. Before the cell culture, PSS-patterned PS substrates were sterilized with 70% ethanol solution. For the cell growth on PSS-patterned surfaces, cells with a density of 2.5×10^4 cells $well^{-1}$ were plated in a RPMI 1640 medium (Gibco) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and incubated in a humidified incubator at 37°C and 5% CO_2. For the duration of 3 days, the adhesion and patterned-growth of the cells were observed with an optical microscope (Type 020-519, Leica, Germany).

5. Cell proliferation assay

Cell proliferation was measured using a Cell Counting Kit-8 (CCK-8) assay (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's protocol (Wang *et al.* 2011). Briefly, H1299 cells were plated at a density of 1×10^4 cells $well^{-1}$ onto a PS and PSS surfaces irradiated at different fluences. After the certain incubation periods, the culturing media were daily exchanged with 1 ml of culture medium containing a 10% CCK-8 solution. After storing for 1 h under

the same condition, the absorbance of the CCK-8 solution was measured at 450 nm with a UV-Vis spectrophotometer (MQX 200 model, Bio-Tek Instruments, USA). The absorbance value for the respective sample at the certain incubation period stands for the relative proliferation rate of cells. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

The PSS-patterned PS substrate formed at various fluencies was observed using an optical microscope and the results are shown in Fig. 1. Well-defined negative stripe patterns of PSS patterns spaces were formed at the given fluences. On the other hand, PSS patterns were not generated at the fluence less than 1×10^{15} ions cm^{-2} (data not shown), indicating that the PSS was not properly crosslinked. Therefore, the fluences more than 1×10^{15} ions cm^{-2} are required to produce the PSS patterns in this system.

To measure the remaining thickness of the PSS patterns, the surface profiles of PSS-patterned PS substrates was investigated using a surface profiler. As presented in Fig. 2, the remaining thicknesses of the PSS patterns were increased from 92 nm to 129 nm with the increase in the fluence. This result can be ascribed to the fact that the PSS was more effectively crosslinked at higher fluence, and thus less removed by the developing process dissolving the uncrosslinked PSS (Ahn *et al.* 2010; Hwang *et al.* 2013).

To investigate the ion irradiation-induced changes in the chemical structure of PSS surfaces taking place under ion irradiation, ATR-FTIR analysis was performed, and the results are shown in Fig. 3. In the spectrum of non-irradiated

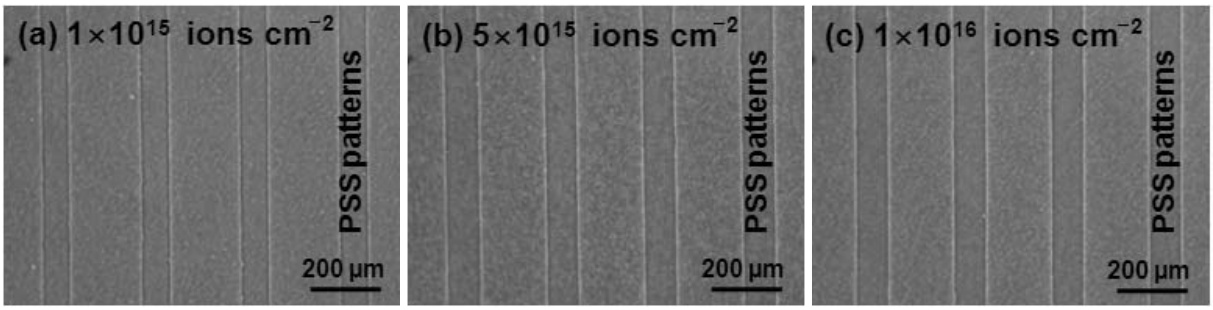

Fig. 1. Optical microscopic images of PSS patterns on PS substrates formed at fluences of 1×10^{15} (a), 5×10^{15} (b), and 1×10^{16} ions cm^{-2} (c).

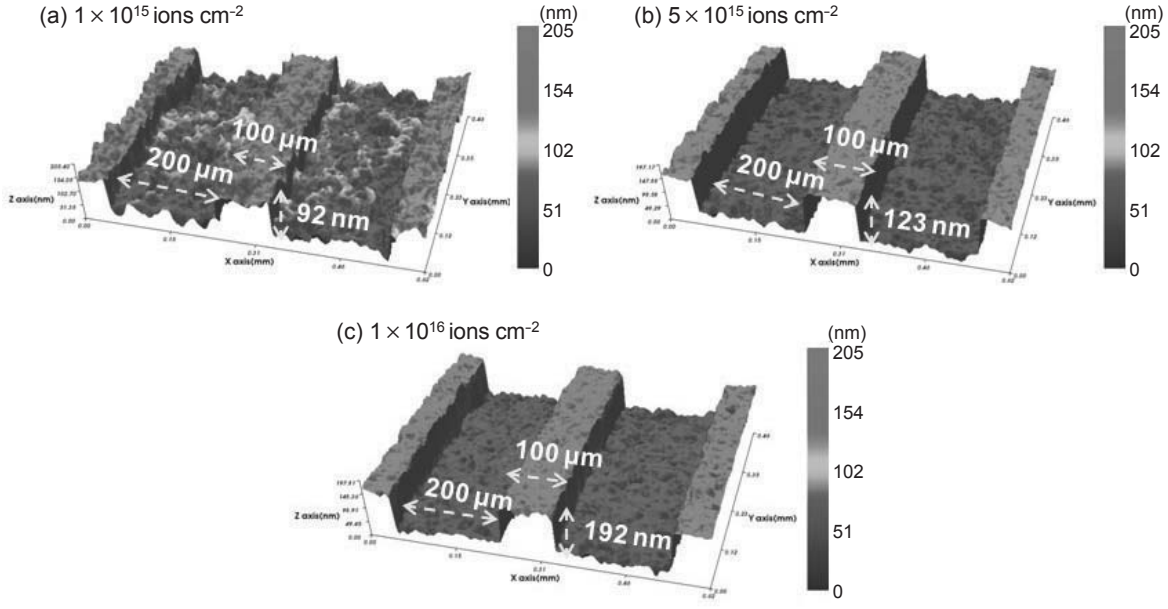

Fig. 2. 3D optical surface profiles of PSS-patterned PS substrates prepared at various fluences.

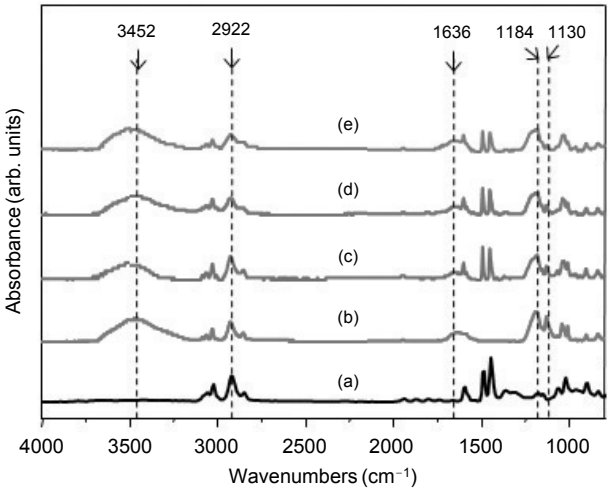

Fig. 3. ATR-FTIR spectra of the PS (a), non-irradiated PSS (b), and irradiated PSS films at fluences of 1×10^{15} (c), 5×10^{15} (d), and 1×10^{16} ions cm^{-2} (e).

PSS in Fig. 3(a) and (b), the respective OH stretching and scissor vibration of H_2O were observed at 3452 and 1636 cm^{-1} and the peaks corresponding to the asymmetric and symmetric vibration of SO_3 group were identified at 1184 and 1130 cm^{-1}, respectively, meaning the existence of PSS on the PS. As shown in Fig. 3(c-e), the irradiated PSS spectra was almost similar to that these of non-irradiated PSS, but the intensities of SO_3 peaks was gradually decreased with an increase in the fluence (Yang *et al.* 2002). This result indicates that the chemical structure of PSS was changed by ion irradiation.

The surface elemental ratio of the irradiated PSS at various fluences obtained from an XPS analysis is shown in Fig. 4. As shown in Fig. 4(a) and (b), both [O]/[C] and [S]/[C] elemental ratios of the irradiated PSS were both gradually decreased with the increase in the fluence in comparison to these of the non-irradiated PSS. Thus, this result implies that the PSS surface was converted to more hydrophobic carbon-abundant surface because of the occurrence of crosslinking and chain scission during the ion irradiation.

To investigate the ion irradiation effect on the wettability of PSS, the water contact angles of irradiated PSS surfaces at various fluences were measured, and the results are shown in Fig. 5. The contact angle of the PS substrate was around 86°. In the cases of the irradiated PSS, the contact angels were increased up to 73° with the increase in the fluence, which was smaller than that of the PS. This result indicates that the wettability of PSS surface was reduced due to the ion beam-induced formation of the carbon-abundant chemical environment, and yet is better than PS.

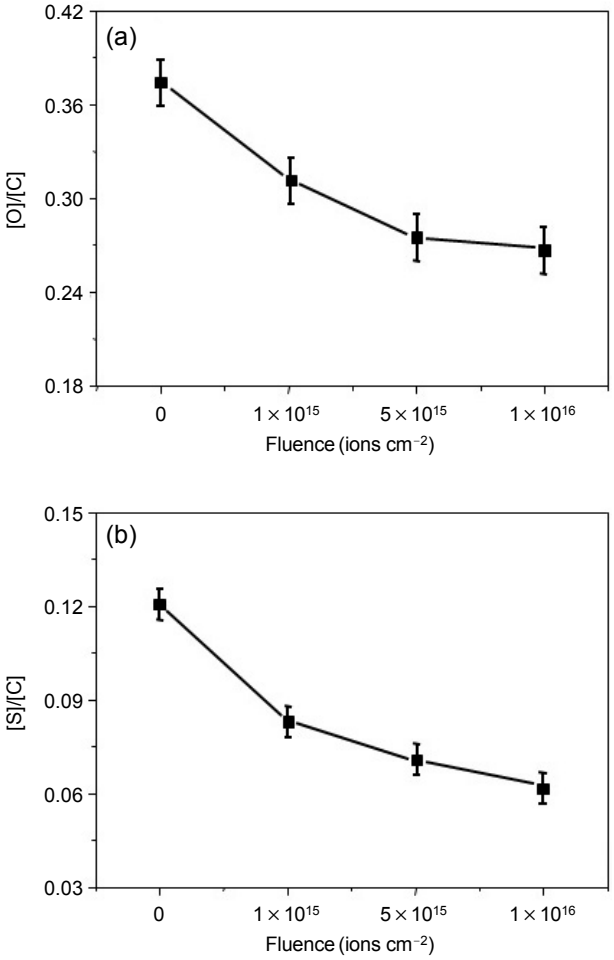

Fig. 4. [O]/[C] (a) and [S]/[C] atomic ratios (b) of non-irradiated and irradiated PSS films at various fluences.

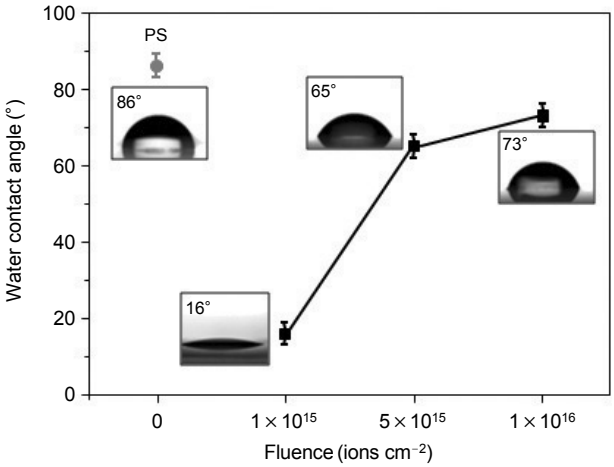

Fig. 5. The variation of water contact angles at various fluences.

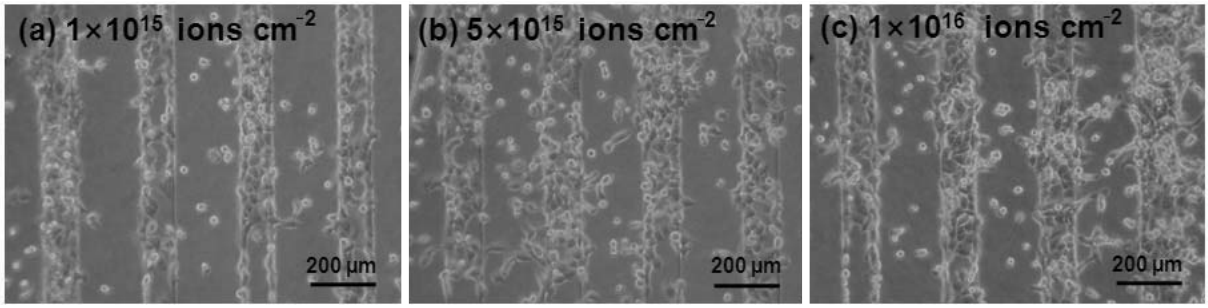

Fig. 6. Optical microscopic images of H1299 cells (d-f) on PSS-patterned PS substrates prepared at various fluences.

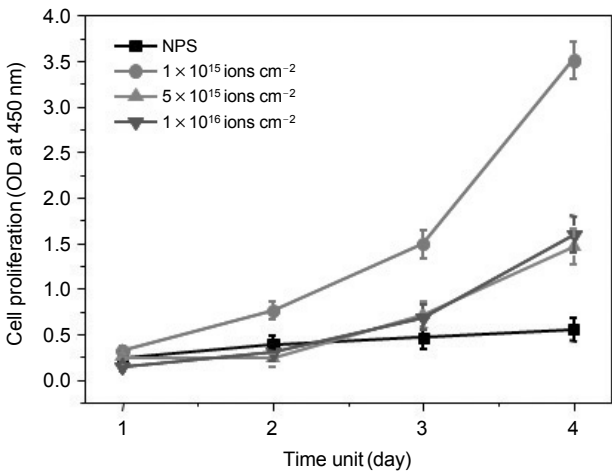

Fig. 7. Proliferation of H1299 cells cultured on PSS-coated PS substrates prepared at various fluences.

Human cancer cell line (H1299) was used as a model system to investigate the cellular response to PSS-patterned PS substrate. As shown in Fig. 6, the cells were dominantly adhered and proliferated onto the more hydrophilic PSS regions, resulting in the formation of well-defined 100 μm patterns of the cells on the PSS-patterned PS substrates. Particularly, the most resolved cell patterns were formed on PSS-patterned PS surface prepared at the fluence of 1×10^{15} ion cm^{-2}. This different cellular response could be ascribed to the difference in the hydrophilicity between the irradiated PSS.

To elucidate the difference in the cellular response between the PSS-patterned surfaces, the proliferation of H1299 cells on the wholly irradiated PSS surfaces at the same condition as the formation of PSS patterns was examined using a CCK-8 assay. As shown in Fig. 6, the proliferation rates of cells cultured on all the irradiated PSS were higher than that on the PS and the cell proliferation rate on the PSS irradiated at a fluence of 1×10^{15} ion cm^{-2} was highest, indicating that the more hydrophilic PSS surface provided a better growing environment for the cells. Therefore, the well-defined cell patterns was formed on the more hydrophilic PSS-patterned PS substrate.

CONCLUSION

The formation of hydrophilic PSS patterns on the PS surface to control cell adhesive behavior was successfully performed by selective ion irradiation. It was clearly seen from the optical microscopic images and surface profiles that the 100 μm PSS patterns were successfully formed on the PS substrate at the fluence above 1×10^{15} ions cm^{-2} and their remaining thickness was dependent on the fluence. Based on the results of ATR-FTIR, XPS, and contact angle measurement, it was confirmed that hydrophilic PSS thin layer was effectively formed on the PS surface by ion irradiation-induced crosslinking and the changes in their chemical structures and wettability was dependent on the ion fluence. Moreover, the results of the *in-vitro* cell culture and proliferation assay clearly demonstrated that the cells are found to be preferentially adhered onto adhere more hydrophilic PSS compared to PS surface, resulting in the formation of well-defined 100 μm cell micropatterns. This facile and biocompatible method can be helpful to enhancing our understanding of cell responses to cellular microenvironment.

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