Fabrication of Poly(diallyldimethylammonium chloride) - Patterned Substrates for Patterning of Single Strand DNA Using Ion Implantation

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Abstract - In this study, a convenient method for the selective immobilization of single strand DNA (ssDNA) on a polymer surface was described. A positively charged polyelectrolyte, poly(diallyldimethylammonium chloride) (PDDA), was spin-coated on a tissue culture petridish and the micropatterns of the PDDA were formed by selective ion implantation through a pattern mask. The surface property of the implanted PDDA was investigated by using a surface profiler and FT-IR spectrometer. Cy3-labeled ssDNA was selectively immobilized on the PDDA patterns through ionic interaction and thus, well-defined ssDNA patterns were obtained.

Key words : Ion implantation, Polyelectrolyte, Poly(diallyldimethylammonium chloride), Single strand DNA

INTRODUCTION

Patterning of biologically active molecules such as DNA, proteins, and cells on substrates is important for biological applications such as biosensors, microarrays, fundamental biological studies, and medical diagnostics (DeRisi *et al*. 1996; Brown and Botstein 1999; Wang 2000). Several surface patterning methods, such as photolithography, soft lithography, electron beam lithography, photochemical technique, plasma treatment, ion implantation, and laser lithography, have been developed to pattern biomolecules on various substrates (Kane *et al*. 1999; Falconnet *et al*. 2006). Among them, ion implantation is particularly attractive owing to its effectiveness, and environmentally clean compared with other techniques. In addition, its process is very controllable, reproducible, and it is also a low temperature process (Venkatesan *et al*. 1984; Dong and Bell 1999; Jagielski *et al*. 2006).

The development of DNA-based biosensors has attracted recent research efforts because of the interesting electrical conducting properties and unique three dimensional structures of DNA. DNA molecules are negatively charged polyelectrolytes and can interact with positively charged polyelectrolytes based on the electrostatic attraction forces. Therefore, positive charged polymers are considered to offer an efficient way for DNA immobilization (Chang *et al*. 2008; Li *et al*. 2008). Poly(diallyldimethylammonium chloride) (PDDA) has the backbone of five-membered cyclic ring units and hydrophilic quaternary ammonium groups (Jing and Hongfei 2001; Marcelo *et al*. 2005; Zheng and Wang 2010). This unique structure provides this polymer with high water solubility and strong cationic electrolytic properties that can be easily interacted with anionic DNA molecules.

In this study, we report on a facile method for the selective immobilization of DNA on a polymer surface using ion

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implantation. Micropatterns of PDDA were fabricated by spin-coating of PDDA solutions on a tissue culture petridish (TCPS) followed by ion implantation through a square-shaped mask. The thickness of PDDA micropatterns were measured by an alpha-step surface profiler and the surface properties of the micropatterns were investigated by FT-IR spectroscopy. Micropatterns of Cy3-labeled single strand DNA immobilized on PDDA-patterned substrates were observed with a fluorescence microscope.

MATERIALS AND METHODS

Micropatterning of PDDA by ion implantation

Poly(diallyldimethylammonium chloride) (PDDA, Mw: 400,000 ~ 500,000, 20 wt% in H_2O) was purchased from Aldrich Chemical Company, which was diluted with distilled water to make 5 wt% solution. Tissue culture petridish (TCPS) purchased from SPL life science was used as a substrate without any further purification. In order to form PDDA patterns, a customized pattern mask was supplied by Youngjin Astech Co., Ltd. Thin PDDA films (thickness: 300 nm) were prepared by spin-coating a 5 wt% PDDA solution onto TCPS. Afterwards, the samples were dried in a vacuum oven for 24 hours. The prepared PDDA films were implantated with 150 keV H^+ ions through a pattern mask at room temperature. Ion implantation was executed by using 300-keV ion implanter in the Advanced Radiation Technology institution (ARTI), Korea. The ion fluence ranged from 5×10^{14} to 2.5×10^{15} ions cm^{-2} and the ion current density was less than 1.0 μA cm^{-2} to prevent a thermal effect on the samples. The pressure in the implanter's target changer was 10^{-5} to 10^{-6} Torr. The implanted samples were then developed with distilled water for 1 min to obtain the micropatterns of the PDDA and then dried in a N_2 stream.

DNA immobilization

Oligonucleotide that was labeled with Cy3 at the 3′ position with the sequence 5′-TGAGCTTGACAAAGTGGTCG-Cy3-3′ was used to immobilize on the PDDA micropatterns. To immobilize the DNA onto the PDDA micropatterns, 50 μg ml^{-1} of DNA was applied over the PDDA-patterned substrates and allowed to react for 2 h. Afterward, the samples were washed thoroughly with deionized water. The images of DNA micropatterns were obtained using a fluorescence microscope (Olympus BX61).

Surface characterization

The micropatterns of the PDDA on the TCPS were examined by an optical microscope. The thickness of the PDDA patterns was measured with an AS-500 surface profiler (KLA Tencor, USA). The ion implantation effects on the chemical structure of the pristine and irradiated PDDA surfaces were investigated by using an attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR, Tensor37, Bruker Co., USA).

RESULTS AND DISCUSSION

Fig. 1 shows the schematic representation for the DNA immobilization on PDDA-patterned TCPS surface. Thin PDDA films were prepared by spin-coating, and the micropatterns of PDDA were formed by ion implantation through a pattern mask. Single strand DNA (ssDNA) was immobilized on the PDDA-patterned TCPS for the patterning of ssDNA.

Micropatterning of PDDA on TCPS substrates was performed by ion implantation through a pattern mask at various fluences and the optical images of the formed PDDA micropatterns are shown in Fig. 2. The micropatterns of the PDDA were generated on the TCPS surface above a fluence of 5×10^{14} ions cm^{-2}. However, the PDDA patterns were not formed at a lower fluence than 5×10^{14} ions cm^{-2}. This result indicates that PDDA was effectively cross-linked by

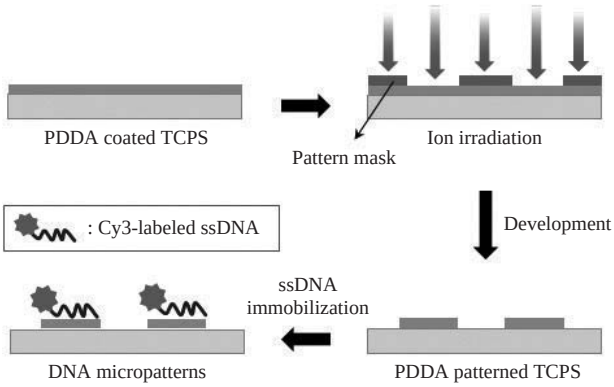

Fig. 1. Schematic diagram showing the preparation of PDDA patterned TCPS surface and the micropatterning of ssDNA.

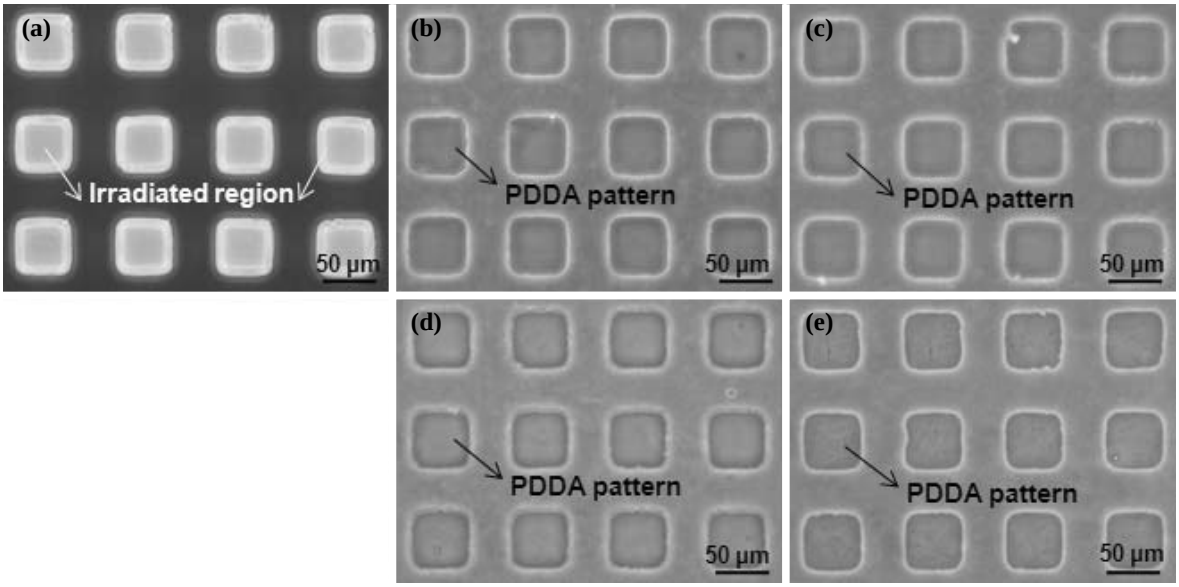

Fig. 2. Optical microscopic images of the used pattern mask (a) and the PDDA micropatterns on the tissue culture petridishs formed by ion implantation at fluences of 5×10^{14} (b), 7.5×10^{14} (c), 1×10^{15} (d), and 2.5×10^{15} ions cm^{-2} (e).

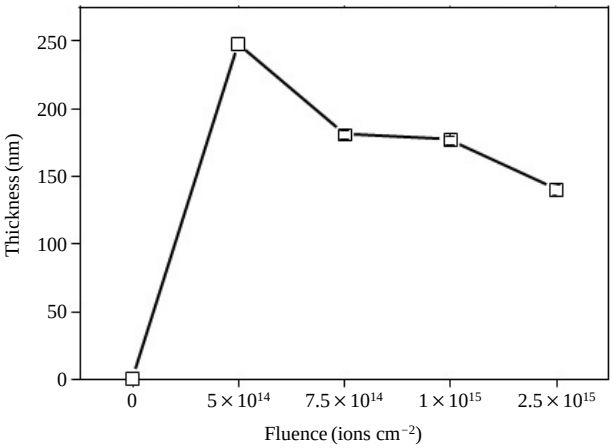

Fig. 3. The remaining thickness of the PDDA as a function of the fluence.

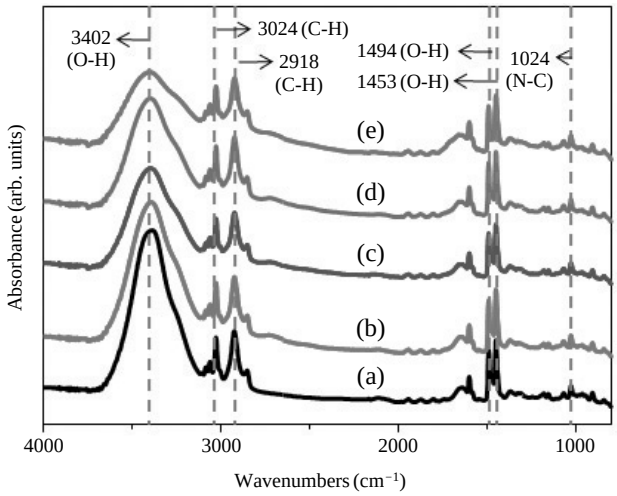

Fig. 4. ATR-FTIR spectra of the pristine PDDA (a) and implanted PDDA at fluences of 5×10^{14} (b), 7.5×10^{14} (c), 1×10^{15} (d), and 2.5×10^{15} ions cm^{-2} (e).

ion implantation above a fluence of 5×10^{14} ions cm^{-2}.

The remaining thickness of the PDDA micropatterns decreased with increasing the ion fluence (Fig. 3). The decrease in the thickness of the PDDA micropatterns can be attributed to the fact that gaseous species such as H_2 as generated by ion implatation-induced bond cleavage in PDDA were liberated. Therefore, the thickness of the PDDA micropatterns decreased with an increasing fluence (Hwang *et al.* 2010).

The ATR-FTIR spectra of the control and implanted PDDA are shown in Fig. 4. The characteristic absorbtion bands of the pristine PDDA around 3,000 and 1,500 cm^{-1} correspond to the CH_2 or CH_3 stretching and bending vibrations, respectively. In addition, PDDA spectra show distinctive peaks at about 1,000 cm^{-1}, which attributed to the presence of the quaternary amine group. After ion implantation, the intensities of these peaks were not substantially changed with an increasing fluence and no newly generated peaks appeared. This result means that PDDA was successfully crosslinked by ion implantation without a significant chemi-

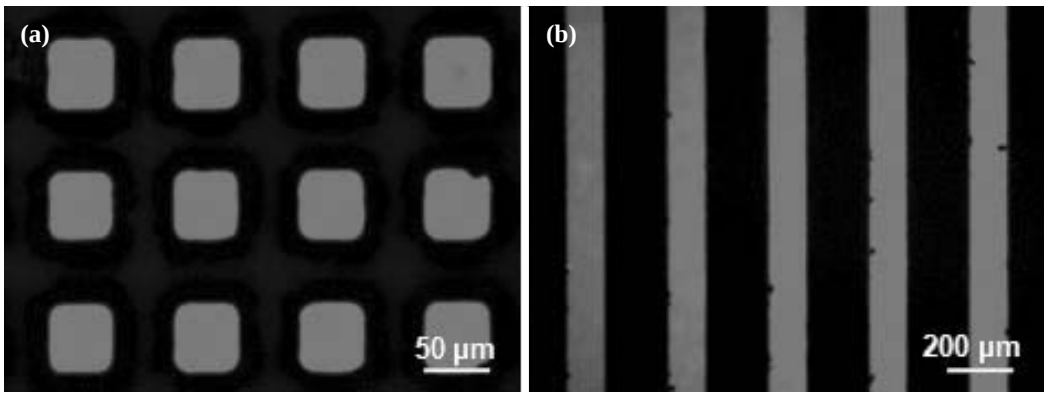

Fig. 5. Fluorescence microscopic images of the micropatterns of Cy3-labeled ssDNA on the PDDA-patterned substrates prepared at a fluence of 5×10^{14} ions cm^{-2}: (a) square patterns of ssDNA and (b) line patterns of ssDNA.

cal change.

To demonstrate the applicability of the PDDA-patterned substrates for the micropatterning of DNA, the PDDA-patterned substrates prepared at a fluence of 5×10^{14} ions cm^{-2} was used for the immobilization of single strand DNA (ssDNA). After incubation of Cy3-labeled ssDNA on the PDDA-patterned substrates, well defined micropatterns of the ssDNA were obtained. Fig. 5 shows the fluorescence microscopic images of the resulting micropatterns of the ssDNA. As shown in Fig. 5, the Cy3-labeled ssDNA was successfully immobilized on the surface of the PDDA micropatterns. The selective immobilization of the ssDNA on the PDDA micropatterns are attributed to the ionic interaction between the positive charges of PDDA and negative charges in DNA.

CONCLUSION

The formation of the PDDA micropatterns on the TCPS to immobilize ssDNA was successfully performed by ion implantation. The results of the remaining thickness measurements and ATR-FTIR analysis showed that the PDDA was effectively crosslinked by ion implantation without a significant chemical change. The ssDNA was selectively adsorbed and immobilized only onto the PDDA micropatterns. It is owing to the ionic interaction between the positively charged PDDA and negatively charged ssDNA. The development of DNA patterning technique based on ion implantation method can be used for a variety of biological applications such as DNA chips, gene therapy, and biosensors.

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