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Short communication

Patterning of biomolecules on a poly(ε-caprolactone) film surface functionalized by ion implantation

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ABSTRACT

Biomolecule patterning is important due to its potential applications in biodevices, tissue engineering, and drug delivery. In this study, we developed a new method for a biomolecular patterning on poly(ε-caprolactone) (PCL) films based on ion implantation. Ion implantation on a PCL film surface resulted in the formation of carboxylic acid groups. The generated carboxylic acid groups were used for the covalent immobilization of amine-functionalized p-DNA, followed by hybridization with fluorescently tagged c-DNA. Biotin-amine was also covalently immobilized on the carboxylic acid generated PCL surfaces. Successful biotin-specific binding of streptavidin further confirmed the potential of this strategy for patterning of various biomolecules.

1. Introduction

Patterning of biologically active molecules such as proteins, DNAs and cells onto polymers is important for various biomedical applications such as tissue engineering, drug delivery and screening, biosensors, and the fabrication of microarrays [1–7].

Several micro- to nano-fabrication techniques such as photolithography, soft lithography, electron beam lithography, laser photoablation, ion implantation, self-assembled monolayers, and scanning probe lithography, have been developed to pattern biomolecules onto polymer surfaces [1–14].

Poly(ε-caprolactone) (PCL), a biodegradable polyester, has received a great deal of attention for its multipurpose applications such as drug delivery devices, adhesion barriers, scaffolds for tissue engineering, biomaterials, biocompatible materials, biosensors, cell chips, etc. [15]. However, the hydrophobicity and poor physiological activity of a PCL surface have limited their biomedical applications. Therefore, a PCL must undergo a surface modification to improve its hydrophilicity, cell adhesion, and biocompatibility via introducing functional groups onto its surface [16–17]. A PCL surface can be functionalized by wet chemical treatment, plasma treatment, ion implantation, enzymatic hydrolysis, adsorption of cell adhesive proteins, etc. Among them, ion implantation has numerous advantages for a modification of a PCL surface to improve its functionality and biocompatibility. It can induce the generation of functional groups selectively such as carboxylic acid and hydroxyl groups on a PCL surface without the use of any chemicals. In addition, it is a surface-specific method without detrimentally affecting the bulk properties; and its process is controllable, reproducible, clean, and it is also a low temperature process [18–21].

The general methods for immobilizing biomolecules to a polymer surface are physical adsorption by electrostatic forces or by hydrophobic interactions, physical entrapment, specific affinity interactions such as receptor/ligand or antigen/antibody, and covalent bond. Among them, an immobilization by covalent bond can be attractive owing to it providing the most stable bond between a biomolecule and a functionalized polymer surface [22–24].

In this communication, we report on a simple and efficient surface functionalization method for a covalent immobilization of biomolecules on a PCL surface by ion implantation. The major advantage of this method is that it requires no harmful chemicals to generate functional groups, thereby making it suitable for biological applications. For this, PCL films were ion-implanted through a pattern mask for a selective functionalization with carboxylic acid groups in the ion-implanted regions. These carboxylic acid groups were further used to covalently immobilize various biomolecules such as proteins and DNAs on the ion-implanted PCL surfaces.

2. Experimental

2.1. Materials

Poly(ε-caprolactone) (PCL, Mw: 80,000, Aldrich), methylene chloride (CH_2Cl_2), 1-ethyl-3-(3-dimethylaminopropyl)carbodii-

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mide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were purchased from Aldrich Company and used as-received. All the oligonucleotides used in this study were purchased from Genotech Company (Korea). The oligonucleotide that has an amino group at its 3′ position with the sequence 5′-CGACCACTTTGTCAAGCTCA-NH_2-3′ was used as probe-DNA (p-DNA) and the oligonucleotide that has been labeled with Cy 5 at the 3′ position with the sequence 5′-TGAGCTTGACAAAGTGGTCG-Cy 5-3′ was used as a complementary-DNA (c-DNA). (+)-Biotinyl-3,6,9-trioxaundecanediamine (biotin-amine) and fluorescein isothiocyanate-tagged streptavidin (SAv-FITC) were purchased from Pierce Company (USA).

2.2. Surface functionalization by ion implantation

PCL films were obtained by casting of a 6 wt% PCL solution in CH_2Cl_2 on glass substrates and drying in air at room temperature for a slow evaporation. The dried films were further dried in a vacuum oven for 24 h. The thickness of the resulting PCL films was around 60 μm. The prepared PCL films were implanted through a pattern mask at room temperature with 100 keV Ne^+ ions at fluences between 1×10^{14} and 1×10^{16} ions/cm^2. The pressure in the implanter's target chamber was 10^{-5} to 10^{-6} Torr, and the ion beam current density was kept about 0.5 μA/cm^2 to prevent a detrimental thermal effect on the PCL surface. The ion-implanted PCL films were placed in air for about 1 day to be oxidized.

2.3. Characterization of ion implanted PLC surface

The changes in the chemical structure of the PCL surface after ion implantation were investigated by using an attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR, Bruker, Tensor 37).

The surface chemical composition of the control PCL and ion-implanted PCL films were investigated by using an X-ray photoelectron spectrometer (XPS, MultiLab 2000, ThermoElectron Corporation, England) with Mg Kα X-ray source. The applied power was 14.5 keV and 20 mA, and the base pressure of the analysis chamber was less than 10^{-9} mbar. All the binding energies were referenced to the C 1s neutral carbon peak at 285.0 eV. The surface elemental compositions were determined from the peak areas. To measure the amount of carboxylic acid groups generated on the ion-implanted PCL surfaces, the control and ion-implanted PCL films were immersed in a 5% aqueous solution of silver nitrate (TCI Chemicals) for 24 h in the dark at room temperature for a complete complexation between the carboxylic acids and silver ions. Afterwards, the films were washed with deionized water and then dried in a vacuum oven at 45 °C. The resulting films were investigated by using XPS analysis and the Ag contents on the control and ion-implanted PCL surfaces were obtained from the Ag 3d peak in the XPS spectra.

2.4. DNA immobilization

To immobilize the p-DNA onto the ion-implanted regions, a solution containing 15 mM NHS, 45 mM EDC and 50 μg/mL of the p-DNA was applied over the ion-implanted PCL films. After a reaction for 6 h, the films were washed thoroughly with a copious amount of deionized water and used for the hybridization with c-DNA. The p-DNA immobilized substrates were incubated in 10 μL of a hybridization solution containing c-DNA at the same concentration as p-DNA, 6 × saline/sodium phosphate/EDTA (SSPE) (pH 7.4) and 20% (v/v) formamide. After hybridization for 6 h, the films were washed well with 3 × SSPE for 5 min, 2 × SSPE for 5 min and finally with 1 × SSPE for 5 min and then the micropatterns of the DNA were characterized by a fluorescence microscope. The fluorescence intensity was determined with ImageJ software (the National Institute of Health, Bethesda, MD, USA) from the original images.

2.5. Protein immobilization

Immobilization of biotin on the ion-implanted PCL film was carried out in a similar procedure to that of p-DNA. The ion-implanted PCL films were immersed in a solution containing 15 mM NHS, 45 mM EDC and 10 mM biotin-amine for 6 h at room temperature. After this time, the films were washed well with deionized water. The prepared biotin-immobilized PCL films were subsequently

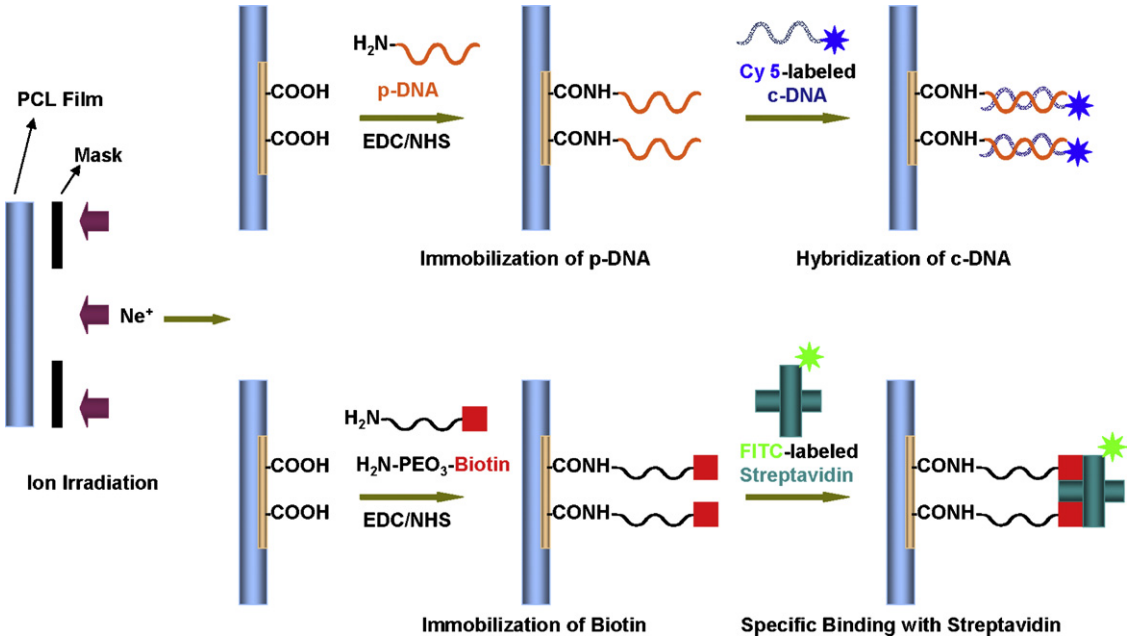

Fig. 1. Schematic representation of biomolecule immobilization on a PCL film surface functionalized by ion implantation.