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Patterned Immobilization of Biomolecules by Using Ion Irradiation-Induced Graft Polymerization

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ABSTRACT: A new method for biomolecular patterning based on ion irradiation-induced graft polymerization was demonstrated in this study. Ion irradiation on a polymer surface resulted in the formation of active species, which was further used for surface-initiated graft polymerization of acrylic acid. The results of the grafting study revealed that the surface graft polymerization using 20 vol % of acrylic acid on the poly(tetrafluoroethylene) (PTFE) film irradiated at the fluence of 1×10^{15} ions/cm² for 12 h was the optimum graft polymerization condition to achieve the maximum grafting degree. The results of the fluorescence microscopy also revealed that the optimum fluence to achieve the maximum fluorescence intensity was 1×10^{15} ions/cm². The grafting of acrylic acid on the PTFE surfaces was confirmed by a fluorescence labeling method. The grafted PTFE films were used for the immobilization of amine-functionalized p-DNA, followed by hybridization with fluorescently tagged c-DNA. Biotin-amine was also immobilized on the acrylic acid grafted PTFE surfaces. Successful biotin-specific binding of streptavidin further confirmed the potential of this strategy for patterning of various biomolecules.

Keywords: Ion irradiation; graft polymerization; biomolecule; patterning

INTRODUCTION

Patterning of biomolecules onto solid surfaces such as glass, silicon and polymers, is essential to a variety of applications including biosensors, tissue engineering and fundamental biological studies.¹⁻³ Most industrial polymers have drawn great attention for these applications due to their mechanical, thermal, and chemical stability, and low production cost as well as their excellent processing properties. However, many of the polymers' surface properties such as biocompatibility are not suitable for their biological applications. Therefore, surface modification of polymers has been widely studied for those applications using physical and chemical processes.

Surface grafting is one of the attractive methodologies due to its many advantages including an easy and controllable introduction of grafted chains with a high density and an exact localization of grafted chains to a surface without affecting the bulk properties.⁴⁻⁷ Surface grafting can be done by several methods including UV radiation, use of chemical initiator, plasma treatment and high energy radiation (γ -rays, ion beams, or electron beams). Among them, high energy radiation-induced graft polymerization is a well-established technique that does not require any initiators. A well known effect of a polymer irradiation with high energy radiation is the creation of active species such as radicals and peroxides which can induce graft polymerization of functional

monomers.^{8,9}

Several micro- to nano-fabrication techniques such as photolithography, laser photoablation, soft lithography and electron beam lithography have been used to pattern biomolecules on various polymeric substrates.^{1-7,10-16} Recently, patterned grafting of functional monomers on flexible polymeric substrates using electron beams, X-rays and extreme UV has been reported.¹⁷⁻²⁰ However, biomolecular patterning on a polymeric substrate via ion irradiation-induced graft polymerization has rarely been carried out so far.^{21,22} Ion irradiation has several advantages: (i) modification is surface-specific without detrimentally affecting the bulk properties; (ii) it is a controllable, reproducible, clean and low temperature process; and (iii) the projected depth and ion fluence of the irradiated region can be accurately selected.²³⁻²⁵

The major methods for immobilizing biomolecules onto a polymer surface are physical adsorption by electrostatic forces on charged surfaces or by hydrophobic interactions, physical entrapment, receptor/ligand pairing (molecular recognition), and covalent immobilization. Among them, covalent immobilization is a robust approach that offers several advantages by providing the most stable bond between a biomolecule and a functionalized polymer surface.^{4-7,26,27}

In this study, we developed a new surface patterning method which is capable of

creating desired patterns via ion irradiation-induced graft polymerization. This method could be useful in bio-electronics, bio-mimetic material fabrications, cell growth control and drug delivery. A variety of desired functional groups including carboxylic acid, amine, alcohol, aldehyde, etc., can be patterned on the surface of polymers using this method. These functional groups can be further used to covalently immobilize various biomolecules. The surface functionalization and surface property were investigated by using X-ray photoelectron spectroscopy (XPS), attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and fluorescence microscopy.

EXPERIMENTAL

Materials

Poly(tetrafluoroethylene) (PTFE) films (200 μm thickness) were purchased from Hanmi Rubber and Plastics Company. Acrylic acid, Mohr's salt $((\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2)$, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), Toluidine Blue O (TBO), and N-hydroxysuccinimide (NHS) were purchased from Aldrich Company and used as received. Fluoresceinamine (FA), N,N'-dicyclohexyl carbodiimide (DCC), and N,N'-dimethylformamide (DMF) were supplied from TCI Company (Japan). Other chemicals

are reagent grade and used without further purification. 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-NH₂-3' was used as probe-DNA (p-DNA) and the oligonucleotide that has been labeled with Cy5 at the 3' position with the sequence 5'-TGAGCTTGACAAAGTGGTCG-Cy5-3' 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).

Ion Irradiation-Induced Graft Polymerization

PTFE films were washed with ethanol and dried under a vacuum prior to use. Ion irradiation was executed by using a 300-keV ion implanter in the Advanced Radiation Technology Institute (ARTI), Korea. The films were irradiated through a pattern mask (SUS, 40 μm square space) at room temperature with 150 keV Ar ions at fluences ranging from 1×10^{14} to 1×10^{16} ions/cm². The pressure in the implanter's target chamber was 10^{-5} to 10^{-6} Torr and the ion beam current density was kept at about 0.5 $\mu\text{A}/\text{cm}^2$ to prevent a thermal effect on a specimen. The irradiated films were kept in air

for a day at room temperature.

For graft polymerization, the irradiated films were positioned in polymerization tubes containing an aqueous solution of 10 to 80 vol % acrylic acid, 0.2 M H₂SO₄ and 0.1wt % Mohr's salt, and then were purged with nitrogen gas for 30 min to remove oxygen. The tubes were placed in a constant temperature water bath at 65 °C for 3 to 48 h. After grafting reaction, the films were thoroughly washed with water to completely remove the homopolymer and the unreacted monomer. The poly(acrylic acid) (PAA)-grafted PTFE films (PTFE-g-PAA) were then dried under a vacuum at 50 °C.

Surface Characterization

The grafting degree of the grafted PAA onto the PTFE films was measured by the colorimetric method with a TBO staining method reported in the literature.^{28,29} The grafted films of 3 x 3 cm² were immersed in a 0.5 mM TBO solution prepared at pH 10 and then constantly agitated for 6 h at room temperature for an electrostatic complexation between the carboxyl acids on the surface of the PTFE-g-PAA films and the TBO. Afterward, the TBO-stained films were thoroughly washed with an excess amount of sodium hydroxide solution (pH 10) to eliminate the free TBO molecules adhering to the surface and then dried in a vacuum oven at 40 °C. The TBO molecules

complexed on the PTFE-g-PAA films were desorbed in 50 % acetic acid solutions and then the optical densities of the resulting solutions were measured at 633 nm using a UV-Vis spectrophotometer (MQX 200 model, Bio-Tek Instruments, USA). Based on the assumption that the concentration of the carboxylic acids on the surface of the PTFE-g-PAA films had combined with the TBO molecules, a calibration curve of the optical density versus the TBO concentration was generated and then with reference to this curve, the grafting degree of PAA grafted on the PTFE surface was calculated.

The chemical structure of the control, irradiated and grafted PTFE film surfaces were examined by using an attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR, Tensor 37, Bruker Co., USA). The changes in the chemical composition of the PTFE surface after ion irradiation and graft polymerization were investigated by using an X-ray photoelectron spectrometer (XPS, MultiLab 2000, ThermoElectron Corporation, England) by employing Mg-K α radiation. The contact angle measurement of the control, irradiated and grafted PTFE films was carried out by a sessile drop method using a Phoenix 300 contact angle analyzer (Surface Electro Optics Co., Korea). Each value of the contact angle was taken as an average value measured from five different samples fabricated under the same experimental conditions.

Fluoresceinamine Immobilization

The PAA-grafted PTFE films were labeled with FA using a well-known procedure.³⁰

The grafted films were soaked in a solution of DCC (95.04 mg) dissolved in DMF (4 ml) and then incubated for 2 h at room temperature. Subsequently, a freshly prepared solution of FA (79.99 mg) in DMF (4 ml) was added to the above solution containing the grafted films. The reaction was carried out in darkness at room temperature for 12 h. Then, the FA-labeled PTFE films were washed thoroughly with DMF.

DNA Immobilization

Biomolecules such as DNA and protein were immobilized on the PAA-grafted PTFE films by a similar method published in our previous papers.^{31,32} To immobilize the p-DNA onto the PAA-grafted regions, a solution containing 15 mM NHS, 45 mM EDC and 50 µg/mL of the p-DNA was applied over the PAA-grafted PTFE films and allowed to react overnight. Afterward, the films were washed thoroughly with copious amounts of deionized water and used for hybridization with c-DNA. For this, the p-DNA immobilized films were incubated with 5 µL of c-DNA in a hybridization solution containing 6 x saline/sodium phosphate/EDTA (SSPE) (0.9 M NaCl, 10 mM NaH₂PO₄ in H₂O, 1 mM EDTA, pH 7.4) and 20 % (v/v) formamide. Hybridization was carried out

at 35 °C for 6 h. After this time, the films were washed well with 3 x SSPE for 5 min, 2 x SSPE for 5 min and finally with 1 x SSPE for 5 min.

Protein Immobilization

Immobilization of biotin on the PAA-grafted PTFE films was done in a similar manner to that of the p-DNA. The PAA-grafted PTFE films were immersed in a solution containing 15 mM NHS, 45 mM EDC and 10 mM biotin-amine overnight at room temperature. The films were then rinsed well with copious amounts of deionized water. The prepared biotin-immobilized PTFE films were subsequently incubated with SAV-FITC (0.1 mg/mL) in a phosphate-buffered saline (PBS, pH 7.4) containing 0.1% (w/v) BSA and 0.0 2% (v/v) Tween 20 at room temperature. After 60 min, the films were removed, washed several times with PBS and deionized water, and dried.

Fluorescence Microscopy

For fluorescence microscopy, the prepared samples were mounted on the glass slides and the fluorescence images were obtained using an Olympus BX61 fluorescence microscope. The graphs for fluorescence intensity were drawn with the ImageJ software from the original images without further treatment.

RESULTS AND DISCUSSION

Ion Irradiation-Induced Graft Polymerization

The schematic representation of the patterning process used in this study is shown in Scheme 1. The PTFE films were irradiated through a pattern mask with Ar ions at an energy level of 150 keV in order to selectively generate the active species such as radicals, peroxides, or hydroperoxides (Supporting Information). Acrylic acid was then selectively graft-polymerized onto the irradiated regions of the PTFE surface. These PAA-grafted regions were utilized for further immobilization of biomolecules.

<Scheme 1>

The surface-initiated graft polymerization of acrylic acid on the PTFE film was carried out under various conditions to optimize the surface graft polymerization. The grafting degree of PAA grafted onto the PTFE surface was measured by a TBO staining-based colorimetric method. Figure 1 shows the effect of fluence on the grafting degree. The grafting degree increased up to $4.2 \mu\text{g}/\text{cm}^2$ with increasing fluence up to 1×10^{15} ions/ cm^2 , above which it decreased. This result could be explained by the fact that the ion irradiation at a fluence up to 1×10^{15} ions/ cm^2 properly generated the active species

such as peroxide or hydroperoxide on the surface due to oxidation caused by chemical reaction between the generated radicals during irradiation and the oxygen in the air after being exposed to the air, but carbonization by defluorination predominantly occurred at a higher fluence.³³

<Figure 1>

Figure 2 shows the effect of acrylic acid concentration on the grafting degree. The grafting degree showed a characteristic behavior with increasing acrylic acid concentration. The grafting degree increased with increasing concentration of acrylic acid up to 20 vol % beyond which it slightly reduced. The greater the monomer concentration, the more monomers will diffuse into the grafting site. However, the already-grafted chains decrease the mobility of the active grafting sites. Therefore, the amount of homopolymers increases with monomer concentration. The viscous solution prevents diffusion of acrylic acid to a graft site and thereby decreasing the concentration of acrylic acid available for the grafting reaction, resulting in reduction of the grafting degree in this system.

<Figure 2>

The influence of grafting reaction time on the grafting degree was also investigated and the results are shown in Figure 3. It can be seen that the grafting degree initially increased with increasing grafting reaction time and then reached a plateau after 12 h. This result can be interpreted as follows.³⁴ With increasing reaction time up to 12 h, the graft polymerization of acrylic acid initiated by the radicals generated from the active species such as peroxide and hydrogen peroxide on the irradiated PTFE surface was predominant, resulting in an increase in the grafting degree. However, for the reaction time above 12 h, the grafting degree was not much improved because all the formed peroxides on the PTFE surface were almost consumed and the monomers were almost polymerized after the certain grafting time. From these data, it is evident that the surface graft polymerization with 20 vol % acrylic acid on the PTFE irradiated at a fluence of 1×10^{15} ions/cm² for 12 h provided the maximum grafting degree in this system.

<Figure 3>

The chemical structural changes in the PTFE surface after irradiation and graft

polymerization were investigated by ATR-FTIR analysis. Figure 4 shows the ATR-FTIR spectra of the control, irradiated PTFE films at a fluence of 1×10^{15} ions/cm² and the PTFE-g-PAA film with the grafting degree of 4.2 $\mu\text{g}/\text{cm}^2$. As shown in Figure 4a, the characteristic peaks of the control PTFE were identified at 1161 and 1241 cm⁻¹ corresponding to the stretching vibration of -CF₂. After ion irradiation, the absorption bands corresponding to hydroxyl or hydroperoxide and carbonyl groups were observed at 3450 and 1720 cm⁻¹ in Figure 4b, respectively, indicating that the PTFE surface was effectively activated by oxidation and defluorination caused by ion irradiation.³⁵ ATR-FTIR spectrum of the PAA-grafted PTFE film in Figure 4c shows that new characteristic peaks of the carbonyl and hydroxyl groups arising from the carboxylic acid groups of the PAA chains appeared at 3140 and 1710 cm⁻¹, respectively.

<Figure 4>

In order to further confirm the chemical changes of the PTFE surface after ion irradiation and graft polymerization in detail, XPS analysis was performed and the results are presented in Figure 5-7. Figure 5 shows the C1s spectra of the control and irradiated PTFE films at fluences of 1×10^{14} , 1×10^{15} , and 1×10^{16} ions/cm². As seen in

Figure 5a, the CF_2 and C-C peaks of the control PTFE film appeared at 292.1 and 285.0 eV, respectively.³³ In case of the irradiated films, the generation of new peaks such as CF_3 , CF, C-O, C=O and (C=O)-O were observed in Figure 5b-5d. These changes could be induced by oxidation and defluorination caused by the ion irradiation. After surface graft polymerization of acrylic acid, obvious changes in the chemical bonds of the irradiated surfaces are observed in Figure 6 in comparison with the irradiated PTFE films. As shown in Figure 6b-6d, for the PAA-grafted films prepared at fluences from 1×10^{14} to 1×10^{16} ions/cm², most of the chemical bonds generated after ion irradiation almost disappeared and new C-C and COOH carbon peaks corresponding to acrylic acid appeared at 285 and 289.1 eV. Also, the peaks corresponding to the CF_2 and CF peaks with the drastically-reduced intensities were observed in the XPS spectra of the PAA-grafted PTFE surface compared to that of the irradiated PTFE at the same fluence.³⁶ Furthermore, it can be seen from Figure 7 that the $[\text{F}]/[\text{C}]$ atomic ratio of the PAA-grafted PTFE dramatically decreased with increasing fluence when compared to the irradiated PTFE, while the $[\text{O}]/[\text{C}]$ atomic ratio considerably increased for the same samples. The changes in the $[\text{F}]/[\text{C}]$ and $[\text{O}]/[\text{C}]$ atomic ratios of the PTFE depended on the grafting degree. Accordingly, these results clearly proved that the PAA was successfully grafted onto the PTFE surface.

<Figure 5>

<Figure 6>

<Figure 7>

The effect of ion irradiation and graft polymerization on the wettability of the PTFE surface as a function of fluence was investigated by water contact angle measurement and shown in Figure 8. The contact angle of the control PTFE film was 105° , which shows that the surface of the PTFE is hydrophobic. With an increase in the fluence, the contact angle of the irradiated PTFE gradually decreased up to 68° at a fluence of 1×10^{16} ions/cm². In the case of the PAA-grafted PTFE films, the contact angle decreased to 51° at a fluence of 1×10^{15} ions/cm², beyond which it increased due to the lower grafting degree. Therefore, the wettability of the PTFE surface was improved by the ion irradiation and it was more enhanced by the graft polymerization due to the incorporation of more hydrophilic PAA on the surface.

<Figure 8>

FA Immobilization on the Micropatterns of PAA Grafted on the PTFE Surface

The micropatterns of PAA formed on the PTFE surface were identified by a fluorescent labeling method based on a DCC coupling by which the amino groups of FA should react with carboxylic acid groups via amide bond formation and the hydroxyl groups of FA should be mostly inert under these reaction conditions due to the use of DMF and water as solvents.³⁷ Figure 9 shows the fluorescence patterns of FA on the PAA-grafted PTFE surface prepared under different conditions. The fluorescent squares in the images correspond to the PAA-grafted areas on the PTFE surface. As shown in Figure 9a-9c, the resolved 40 μm square patterns of FA on the selectively PAA-grafted PTFE surface showed a tendency toward dependency on the grafting degree. Therefore, the most resolved 40 μm square patterns of FA were clearly observed on the patterns of the PAA-grafted PTFE surface with the highest grafting degree obtained at a fluence of 1×10^{15} ions/ cm^2 (Supporting Information).

<Figure 9>

DNA Patterning

PTFE has an advantage to be used for the biomolecular patterning study as it possesses

less adhesion to biomolecules and therefore it could be possible to obtain minimal background signal. To show the applicability of our approach for biomolecular patterning, the micropatterns of PAA on the PTFE surfaces prepared at different fluences were utilized for the immobilization of the p-DNA followed by hybridization with c-DNA. The p-DNA has an amine functional group at its 3' position, which is linked to the carboxylic groups present on the PAA-grafted PTFE surfaces by amide bond formation using EDC/NHS coupling chemistry.^{31,32} Figure 10 shows the fluorescence images of the Cy5 labeled c-DNA hybridized to the covalently immobilized p-DNA on the PAA patterns, which clearly proves the selectivity and functionality of the immobilized p-DNA with a minimal background noise (Supporting Information). The DNA patterning on the PAA-grafted PTFE showed a similar tendency to the FA labeling. Well-defined 40 μm square patterns of the DNA was obtained on the PAA-grafted PTFE surface with the highest grafting degree obtained at a fluence of 1×10^{15} ions/cm².

<Figure 10>

Streptavidin Patterning

For streptavidin patterning, biotin-amine was immobilized initially in the PAA-grafted regions of the PTFE surface using the EDC/NHS coupling reaction similar to the DNA patterning.^{31,32} The biotin-amine was immobilized specifically in the PAA-grafted regions, whereas the non-implanted regions did not react with the biotin-amine. Afterward, these biotin-immobilized PTFE films were immersed in a SAV-FITC solution that resulted in the specific binding of the SAV-FITC on the selectively immobilized biotin-amine regions due to the biotin/streptavidin specific binding. Figure 11 shows the resolved patterns of the fluorescently-labeled streptavidin on the biotin micropattern fabricated on PTFE surfaces under different conditions. Similar to the results of the FA labeling and the DNA patterning, well-defined streptavidin patterns were formed on the PAA-grafted PTFE surface obtained at a fluence of 1×10^{15} ions/cm² due to the maximum grafting degree of the PAA on the PTFE. The successful DNA and streptavidin patterning with a minimal background noise proves the potential of this patterning method for biomolecular applications (Supporting Information).

<Figure 11>

CONCLUSIONS

In this study, we have demonstrated an efficient method for biomolecular patterning based on ion irradiation-induced graft polymerization. The results of the IR, XPS and contact angle measurement confirmed that the surface graft polymerization of acrylic acid was successfully performed on the irradiated PTFE. The grafting degree was dependant on the fluence, monomer concentration and grafting reaction time. The surface graft polymerization using 20 vol % acrylic acid on the irradiated PTFE films at a fluence of 1×10^{15} ions/cm² for 12 h was the optimum graft polymerization condition to achieve the maximum grafting degree. Well-defined 40 μ m patterns of the PAA on the PTFE were confirmed by the FA coupling reaction. The PTFE-g-PAA sample prepared at a fluence of 1×10^{15} ions/cm² was found to be suitable for the immobilization of DNA and streptavidin. Using this method, various functional groups can be introduced onto polymer surfaces. These functional groups can be further used to covalently immobilize various biomolecules such as enzymes, proteins, and DNAs.

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Additional Supporting Information may be found in the online version of this article.

REFERENCES AND NOTES

1. Blawas, A. S.; Reichert, W. M. *Biomaterials* 1998, 19, 595-609.
2. Kane, R. S.; Takayama, S.; Ostuni, E.; Ingber, D. E.; Whitesides, G. M. *Biomaterials* 1999, 20, 2363-2376.
3. Senaratne, W.; Andruzzi, L.; Ober, C. K. *Biomacromolecules* 2005, 6, 2427-2448.
4. Goddard, J. M.; Hotchkiss J. H. *Prog Polym Sci* 2007, 32, 698-725.
5. Hook, A. L.; Thissen, H.; Voelcker, N. H. *Trends Biotechnol* 2006, 24, 471-477.
6. Kato, K.; Uchida, E.; Kang, E. T.; Uyama, Y.; Ikada, Y. *Prog Polym Sci* 2003, 28, 209-259.
7. Desai S. M.; Singh R. P. *Adv Polym Sci* 2004, 169, 231-293.
8. Nasef, M. M.; Hegazy, E. A. *Prog Polym Sci* 2004, 29, 499-561.
9. Chen, J.; Asano, M.; Maekawa, Y.; Yoshida, M. *J Polym Sci Part A: Polym*

Chem 2008, 46, 5559-5567.

10. Karp, J. M.; Yoon, Y.; Geng, W.; Cannizarro, C.; Yan, K.; Kohane, D. S.; Vunjak-Novakovic, G.; Langer, R. S.; Radisic, M. *Biomaterials* 2006, 27, 4755-4764.
11. Yang, P.; Zhang, X.; Yang, B.; Zhao, H.; Chen J.; Yang, W. *Adv Funct Mater* 2005, 15, 1415-1425.
12. Pan, F.; Wang, P.; Lee, K.; Wu, A.; Turro N. J.; Koberstein, J. T. *Langmuir* 2005, 21, 3605-3612.
13. Vaida, C.; Mela, P.; Keul, H.; Moller, M. *J Polym Sci Part A: Polym Chem* 2008, 46, 6789-6800.
14. Co, C. C.; Wang, Y.; Ho, C. J. *J Am Chem Soc* 2005, 127, 1598-1599.
15. Hyun, J.; Chilkoti, A. *J Am Chem Soc* 2001, 123, 6943-6944.
16. Hatakeyama, H.; Kikuchi, A.; Yamato, M.; Okano, T. *Biomaterials* 2007, 28, 3632-3643.
17. Brack, H. P.; Padeste, C.; Slaski, M.; Alkan, S.; Solak, H. H. *J Am Chem Soc* 2004, 126, 1004-1005.
18. Gursel, S. A.; Padeste, C.; Solak, H. H.; Scherer, G. G. *Nucl Instr and Meth B* 2005, 236, 449-455.

19. Padeste, C.; Solak, H. H.; Brack, H. P.; Slaski, M.; Gursel, S. A.; Scherer, G. G. J Vac Sci Technol B 2004, 22, 3191-3195.
20. Padeste, C.; Farquet, P.; Solak, H. H. Microelectron Eng 2006, 83, 1265-1268.
21. Kim, D.-K.; Choi, Y. M.; Jung, C.-H.; Kwon, H.-J.; Choi, J.-H.; Nho, Y.-C.; Seo, D.-H; Ganesan, R. Polym Adv Technol 2008, In press.
22. Padeste, C.; Farquest, P.; Potzner, C.; Solak, H. H. J Biomater Sci Ploymer Edn 2006, 17, 1285-1300.
23. Clough, R. L. Nucl Instr and Meth B 2001, 185, 8-33.
24. Massa, G.; Mazzei, R.; Bermudez, G. G.; Filevich, A.; Smolko, E. Nucl Instr and Meth B 2005, 236, 272-276.
25. Betz, N.; Begue, J.; Goncalves, M.; Gionnet, K.; Deleris G.; Le Moel, A. Nucl Instr and Meth B 2003, 208, 434-441.
26. Lutz J.; Borner, H. G. Prog Polym Sci 2008, 33, 1-39.
27. Hersel, U.; Dahmen, C.; Kessler, H. Biomaterials 2003, 24, 4385-4415.
28. Ying, L.; Yin, C.; Zho, R. X.;. Lenong, K. W; Mao, H. Q.; Kang, E. T.; Neoh, K. G. Biomacromolecules 2003, 4, 157-165.
29. Sano, S.; Kato, K.; Ikada, Y. Biomaterials 1993, 14, 817-822.
30. Ito, Y.; Heydari, M.; Hashimoto, A.; Konno, T.; Hirasawa, A.; Hori, S.; Kurita

- K.; Nakajima, A. *Langmuir* 2007, 23, 1845-1850.
31. Ganesan, R.; Yoo, S. Y.; Choi, J.-H.; Lee, S. Y.; Kim, J.-B. *J Mater Chem* 2008, 18, 703-709.
32. Kim, J.-B.; Ganesan, R.; Yoo, S. Y.; Choi, J.-H.; Lee S. Y. *Macromol Rapid Commun* 2006, 27, 1442-1445.
33. Colwell, J. M.; Wentrup-Byrne, E.; Bell, J. M.; Wielunski, L. S. *Surf Coat Technol* 2003, 168, 216-222.
34. Dong, Z.; Liu, Z.; Han, B.; He, J.; Jiang, T.; Yang, G. *J Mater Chem* 2002, 12, 3565-3569.
35. Clochard, M. C.; Begue, J.; Lafon, A.; Caldemaïson, D.; Bittencourt, C.; Pireaux, J. J.; Betz, N. *Polymer* 2004, 45, 8683-8694.
36. Steffens, G. C.; Nothdurf, L.; Buse, G.; Thissen, H.; Hoker, H.; Klee, D. *Biomaterials* 2002, 23, 3523-3531.
37. Qiu, X.; Liu, G. *Polymer* 2004, 45, 7203-7211.

Figure Legends

Scheme 1. Schematic representation of protein/DNA patterning by ion irradiation-induced graft polymerization.

Figure 1. The effect of fluence on the grafting degree: [acrylic acid] = 20 vol % and grafting reaction time = 12 h.

Figure 2. The effect of acrylic acid concentration on the grafting degree: the fluence = 1×10^{15} ions/cm² and reaction time = 12 h.

Figure 3. The effect of grafting reaction time on the grafting degree: the fluence = 1×10^{15} ions/cm² and [acrylic acid] = 20 vol %.

Figure 4. FTIR-spectra of the control (a), implanted (b), and PAA-grafted PTFE films (c).

Figure 5. Cls core-level spectra of the control (a) and PTFE films irradiated at fluences of 1×10^{14} (b), 1×10^{15} (c), and 1×10^{16} ions/cm² (d).

Figure 6. Cls core-level spectra of the control (a) and the PAA-grafted PTFE films prepared with the irradiated films at fluences of 1×10^{14} (b), 1×10^{15} (c), and 1×10^{16} ions/cm² (d).

Figure 7. [F]/[C] and [O]/[C] ratio of the irradiated and PAA-grafted PTFE films as a function of the fluence.

Figure 8. Contact angles of the control, irradiated, and PAA-grafted PTFE films as a function of the fluence.

Figure 9. Fluorescence micrographs of FA-labeled PTFE films prepared with the selectively PAA-grafted films at different fluences.

Figure 10. Fluorescence micrographs of the Cy5-labeled c-DNA (a-c) and nc-DNA (d-f) hybridized to p-DNA immobilized on PAA-grafted PTFE films at different fluences

Figure 11. Fluorescence micrographs of SAv-FITC bound (a-c) and biotin-preincubated SAv-FITC (d-f) bound to biotin on the selectively PAA-grafted PTFE films at different fluences.

Graphical Abstract

(Scheme 1)

An efficient method for biomolecular patterning based on ion irradiation-induced graft polymerization has been demonstrated. Ion irradiation resulted in the formation of active species on the polymer surface, which in turn was utilized for graft polymerization of acrylic acid. Polymerization conditions were optimized to yield maximum grafting degree of poly(acrylic acid) onto the PTFE. The application of this platform for biomolecular patterning has been successfully demonstrated by patterning DNA and streptavidin on the poly(acrylic acid)-grafted PTFE substrates. This method is capable of grafting various functional groups such as amide and alcohol onto a variety of polymer substrates.

Scheme 1.

Figure 1

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figure 8.

Figure 9.

Figure 10.

Figure 11.