First Preparation of Biotinylated Gradient Polyethylene Surface to Bind Photoactive Caged Streptavidin

Moon Suk Kim,† Kwang Su Seo,‡ Gilson Khang,‡ and Hai Bang Lee*,†

Nanobiomaterials Laboratory, Korea Research Institute of Chemical Technology, P.O. Box 107, Yuseong, Daejeon 305-600, Korea, and Department of Polymer/Nano Science and Technology, Chonbuk National University, 664-14, Duckjin, Jeonju 561-756, Korea

Received December 17, 2004. In Final Form: February 2, 2005

A gradient polyethylene (PE) surface was created through corona treatment, in which the corona power increased along the 5 cm length of the PE. The gradient surface was treated with polyethyleneimine and then biotin. Fluorescein-conjugated streptavidin (SAV) caged within 5-carboxymethoxy-2-nitrobenzyl (CMNB) adsorbed onto the biotinylated gradient PE surface following molecular recognition principles. Photoirradiation decomposed the CMNB cage and allowed the fluorescein-conjugated SAV to fluoresce, the intensity of which increased gradually along the PE surface.

Introduction

Recent developments in the design of biocompatible materials have focused on biomimetic materials that are able to recognize biomolecules originating from the extracellular matrix (ECM) or specific cell responses.[1] Surface modification of biocompatible materials is one of the simplest methods to make biomimetic materials that possess biomolecule-recognizing properties.[2] Surface modification has been performed by employing physical or chemical immobilization of bioactive molecules and offers the potential to examine protein or cell behavior through binding with targetable receptor biomolecules that are present in biological systems.[3] Thus, the rapid and accurate recognition or binding of bioactive molecules at a specific location on biocompatible materials in vivo or in vitro presents a challenging and diverse set of constraints.

Surface modification has some limitations. Because surface modification has been performed on uniform surfaces, evaluation of the binding of bioactive molecules onto the biocompatible materials routinely requires several experiments.

A gradient surface is a surface on which a continuously varying chemical composition exists along its length. If bioactive molecules are introduced to a gradient surface, gradient-sensitive molecules can bind according to the gradient, and thus, gradient results can be gained through a single experiment.[4] Consequently, this method can be a useful tool for evaluating bioactive molecules on a surface. Several techniques for preparing chemical gradients on various substrates have been developed, and such gradients have been used for further experiments and applications such as investigating biomolecular interactions, cell-motility studies, or diagnostics.[5]

o-Nitrobenzyl (NB)-caged bioactive molecules appear to be an attractive candidate to turn on the bioactive molecules upon brief light exposure, as the NB cage rapidly decomposes upon photoirradiation.[6] NB-caged fluorescent labels on streptavidin (SAV) might have wide applications in the spatial imaging of biological systems, because the image gained clearly demonstrates the presence of caged substrates and proteins.[7]

Thus, a designed gradient surface that can reveal the presence of bound bioactive molecules in response to an external stimulus may be highly interesting. Relating the intensity or concentration of the bioactive molecules on the gradient surface to the chemical gradient would allow the gradient surface to become a useful method for further practical biomedical applications such as diagnostic investigation of biomolecular interactions. However, such analytical tools have thus far been difficult to prepare because of the challenge of creating spatial images that express only the molecular distribution on uniform surfaces.

The aim of our research was to develop a simple and generally applicable method for using gradients of biomolecules on a polyethylene (PE) surface at the millimeter−centimeter scale as an analytical tool for bio-

* Corresponding author. E-mail: hblee@krict.re.kr. Phone: 82-42-860-7220. Fax: 82-42-860-7228.

† Korea Research Institute of Chemical Technology.

‡ Chonbuk National University.

(1) (a) Shin, H.; Jo, S.; Mikos, A. G. *Biomaterials* **2003**, *24*, 4353. (b) Courtney, J. M.; Zhao, X. B.; Qian, H.; Sharma, A. *Perfusion* **2003**, *18*, 33. (c) Reyes, C. D.; Garcia, A. J. *J. Biomed. Mater. Res.* **2003**, *65*, 511.

(2) (a) Zhi, Z. L.; Morita, Y.; Hasan, Q.; Tamiya, E. *Anal. Chem.* **2003**, *75*, 4125. (b) Quirk, R. A.; Chan, W. C.; Davies, M. C.; Tendler, S. J.; Shakesheff, K. M. *Biomaterials* **2001**, *22*, 865.

(3) Ratner, B. D.; Castner, D. G. In *Surface Modification of Polymeric Biomaterials*; Ratner, B. D., Castner, D. G., Eds.; Plenum Press: New York, 1997.

(4) (a) Whittle, J. D.; Barton, D.; Alexander, M. R.; Short, R. D. *Chem. Commun.* **2003**, 1766. (b) Alexander, M. R.; Whittle, J. D.; Barton, D.; Short, R. G. *J. Mater. Chem.* **2004**, *14*, 408. (c) Dertinger, S. K.; Jiang, X.; Li, Z.; Murthy, V. N.; Whitesides. G. M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 12542. (d) Elwing, H. *Biomaterials* **1998**, *19*, 397. (e) Maruno, S.; Itoh, H.; Ban, S.; Iwata, H.; Ishikawa, T. *Biomaterials* **1991**, *12*, 225. (f) Karmali, A.; Drake, A. F.; Spencer, N. *Biochem. J.* **1983**, *211*, 617.

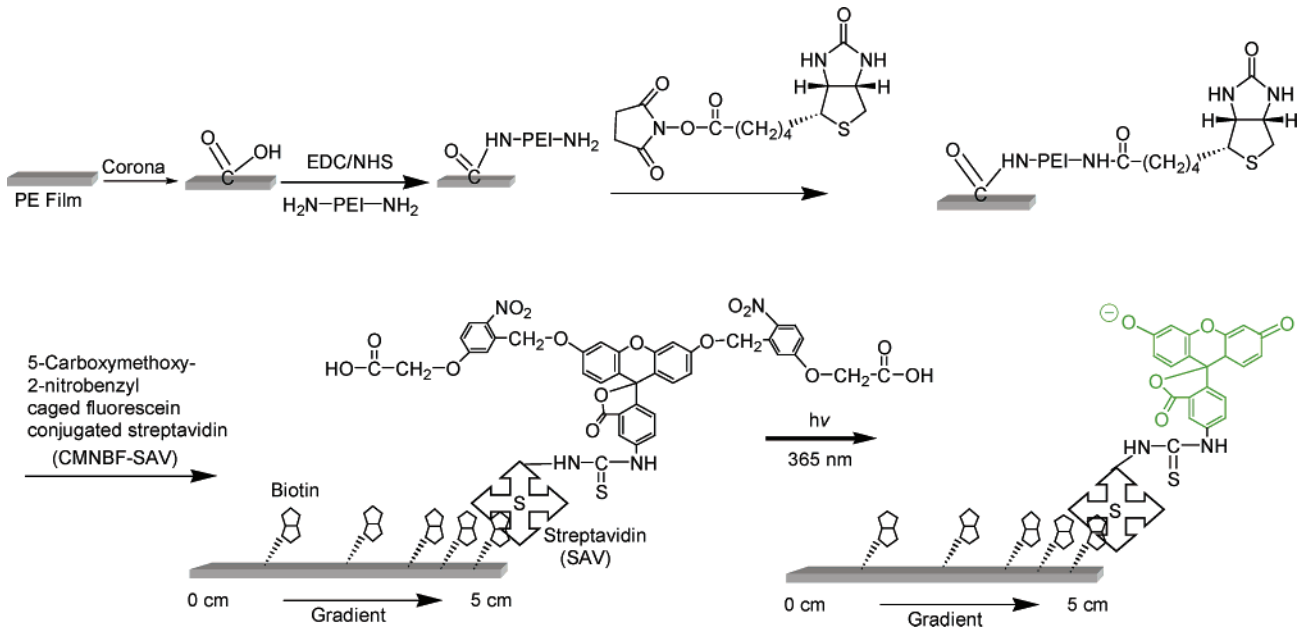
(5) (a) Morgenthaler, S.; Lee, S.; Zurcher, S.; Spencer, N. D. *Langmuir* **2003**, *19*, 10459. (b) Wijesundara, M. B. J.; Fuoco, E.; Hanley, L. *Langmuir* **2001**, *17*, 5721. (c) Jayaraman, S.; Hillier, A. C. *Langmuir* **2001**, *17*, 7857. (d) Jeon, N. L.; Dertinger, S. K. W.; Chiu, D. T.; Choi, I. S.; Stroock, A. D.; Whitesides, G. M. *Langmuir* **2000**, *16*, 8311. (e) Terrill, R. H.; Balss, K. M.; Zhang, Y.; Bohn, P. W. *J. Am. Chem. Soc.* **2000**, *122*, 988. (f) Hypolite, C. L.; McLernon, T. L.; Adams, D. N.; Chapman, K. E.; Herbert, C. B.; Huang, C. C.; Distefano, M. D.; Hu, W.-S. *Bioconjugate Chem.* **1997**, *8*. 658. (g) Liedberg, B.; Wirde, M.; Tao, Y.-T.; Tengvall, P.; Gelius, U. *Langmuir* **1997**, *13*, 5329. (h) Liedberg, B.; Tengvall, P. *Langmuir* **1995**, *11*, 3821.

(6) (a) Givens, R. S.; Kueper, L. W., III. *Chem. Rev.* **1993**, *93*, 55. (b) Olejnik, J.; Sonar, S.; Krzymanska-Olejnik, E.; Rothschild, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7590.

(7) (a) Nerbonne, J. M. *Curr. Opin. Neurobiol.* **1996**, *6*, 379. (b) Nerbonne, J. M.; Richard, S.; Nargeot, J.; Lester, H. *Nature* **1984**, *310*, 74. (c) Haugland, R. P. *Handbook of Fluorescent Probes and Research Products*, 9th ed.; Molecular Probes Inc.: Eugene, OR, 2003.

10.1021/la046868a CCC: $30.25
Published on Web 03/22/2005

Scheme 1. Chemical Modification of the PE Surface



medical applications. In this article, we represent the preparation of a gradient biotinylated PE surface, through modification with polyethyleneimine (PEI) and biotin after corona treatment of the PE. Onto this surface, we bound NB-caged, fluorescein-conjugated SAV, which does not fluoresce until photoactivated.

Experimental Section

Materials. A low-density polyethylene sheet (280 ± 20 μm thickness, Hanyang Chemical Co.), prepared without additives, was used as the substrate for the preparation of gradient surfaces. Biotin, *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide (EDC), and PEI (M_w 60 000 g mol^{-1}) were purchased from Sigma-Aldrich. 5-Carboxymethoxy-2-nitrobenzyl (CMNB)-caged, fluorescein-conjugated SAV (CMNBF-SAV) was purchased from Molecular Probes.

Surface Characterization. The water contact angle was measured using the sessile drop method at room temperature with an optical bench-type contact angle goniometer (model 100-0, Rame-Hart, Inc.) as a function of position along the longitudinal axis of the sample. One drop of purified water (3 μL) was deposited onto the PE surface by means of a microsyringe attached to the goniometer. The water contact angle was measured within 5 s. The contact angles of three specimens for the corona-treated and functional-group-grafted surfaces were individually measured at several different positions (at least 10 positions) for each specimen and then pooled to an average value.

To investigate the gradient changes of the corona-untreated and -treated PE surface, electron spectroscopy for chemical analysis (ESCA), Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR), and atomic force microscopy (AFM) experiments were undertaken. For ESCA, the PE film was cut perpendicularly to the direction of the gradient at regular (1.0 cm) intervals over the 5 cm length of the surface. These samples were subjected to ESCA analysis (Escalab MK II, V.G. Scientific Co.; Al Kα radiation source at 1487 eV, 300 W power at the anode) of the carbon 1s core level scan spectra for each section (analysis area ~5 mm^2). FTIR-ATR spectra were measured with a Magna-IR 550 (Nicolet) spectrometer. AFM measurements were carried out in the tapping mode with a Nanoscope IV instrument (Digital Instruments Inc.).

Preparation of Gradient Surfaces. The PE sheet was cut into 5 × 5 cm^2 pieces, ultrasonically cleaned twice in ethanol for 30 min each, and then dried at room temperature on a clean bench. The pieces were stored in a vacuum oven until use. The PE film was treated with a homemade radio frequency (rf) corona-discharge apparatus for the preparation of gradient surfaces. A knife-type electrode was connected to the rf generator. The power was increased gradually from 10 to 50 W at 100 kHz by the operation of an automatic motorization drive. The cleaned PE film was placed on the bed moving at a constant speed of 1.0 cm s^{-1} under an airflow (20 L min^{-1}). The electrode was 1.5 mm away from the moving bed. The PE film was treated for 5 s.

Surface Modification. *PEI-Modified PE Surface.* A corona-treated PE film was activated by placing it in a mixture of NHS (12 mg, 0.1 mmol) and EDC (19 mg, 0.1 mmol) in water (15 mL) and shaken at room temperature for 30 min. The film was washed several times with deionized water. A PEI (200 mg, 0.0033 mmol) solution was poured into the activated carboxylic PE film. The PE film was shaken at room temperature for 1 h. The PE film was washed several times with deionized water and dried on a clean bench.

Biotin-Modified ("Biotinylated Gradient") PE Surface. Biotin (30 mg, 0.12 mmol) was activated with NHS (14 mg, 0.12 mmol) and EDC (23 mg, 0.12 mmol) in dimethyl sulfoxide (3 mL). The activated biotin solution and deionized water (12 mL) were poured into the PEI-modified gradient PE surface and shaken for 1 h. The PE film was washed several times with water and dried at room temperature on a clean bench.

Binding of CMNBF-SAV with the Biotinylated Gradient PE Surface. CMNBF-SAV was dissolved in a Tris−HCl buffer at a

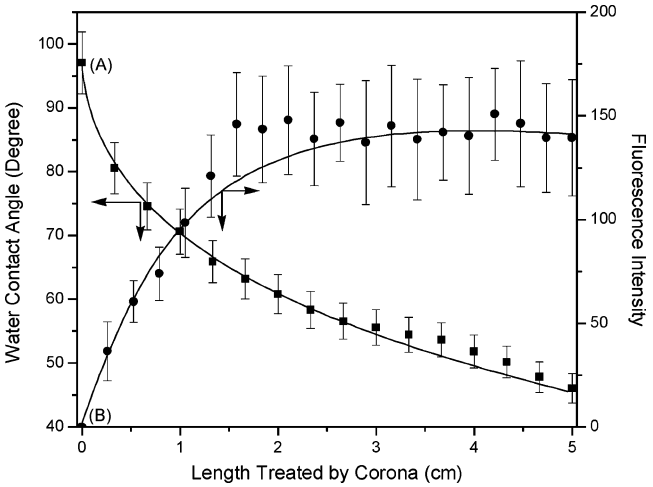


Figure 1. Plot of (A) water contact angle versus length treated by corona and (B) fluorescence intensity versus length treated by corona.

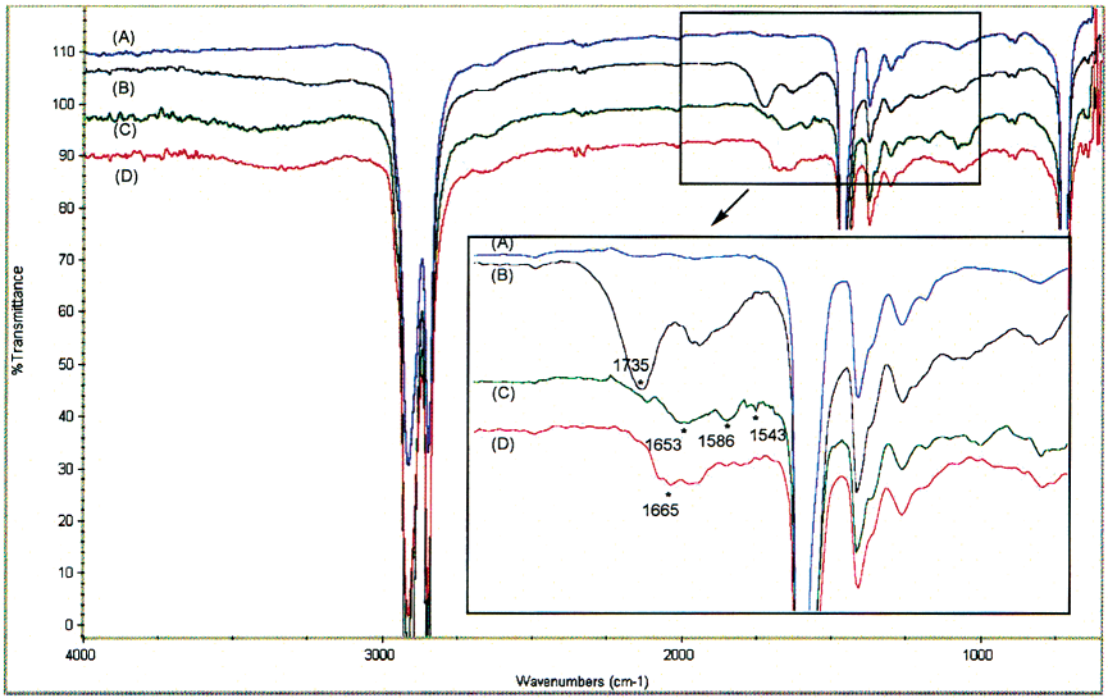


Figure 2. FTIR-ATR spectra at around a position of 4.5 cm of the (A) untreated PE surface, (B) corona-treated PE surface, (C) PEI-modified PE surface, and (D) biotinylated PE surface.

concentration of 1 μg mL^{-1} and stored in 0.5 mL Eppendorf tubes at −80 °C until needed. The CMNBF-SAV solution (1 mL) was poured onto the biotinylated gradient PE surfaces and shaken for 1 h. The PE film was washed with PBS several times and dried on a clean bench.

Photolysis was performed with an ENF-240C device (Spectronics Corp.) at a distance of 1 cm using 365 nm wavelength light (optical density 0.3 mW cm^{-2} at 15 cm) for up to 60 min. Images of the fluorescence were taken at several (at least 10) different positions of three specimens by using a fluorescence microscope at ~0.25 cm intervals and then pooled to an average value. The fluorescence intensity was determined using Adobe Photoshop 7.0 with standard image-editing procedures to generate a fluorescence intensity histogram of the image.

Results and Discussion

Corona-discharge treatment is one of the most useful methods for introducing oxygen-containing functional groups and/or roughness to polymer surfaces, because it can be very easily performed under atmospheric conditions.[8,9] Recently, we reported the preparation of a gradient surface, by rf corona discharge using knife-type electrodes, and its biomedical applications.[9] To expand the fundamental interest in gradient surfaces, we examined further applications in the fields of diagnostics for biomolecular interactions.

In this work, the chemical modifications after the corona-discharge treatment were carried out according to Scheme 1.[10] The corona-discharge treatment produced carbon radicals on the hydrocarbon backbone of the surface PE. Successive reaction with aerial oxygen formed several types of functional groups, such as hydroxyl, ether, ketones, aldehydes, carboxylic acid, and carboxylic esters. The functional group density gradually increased along the PE sample length, as the corona power increased.

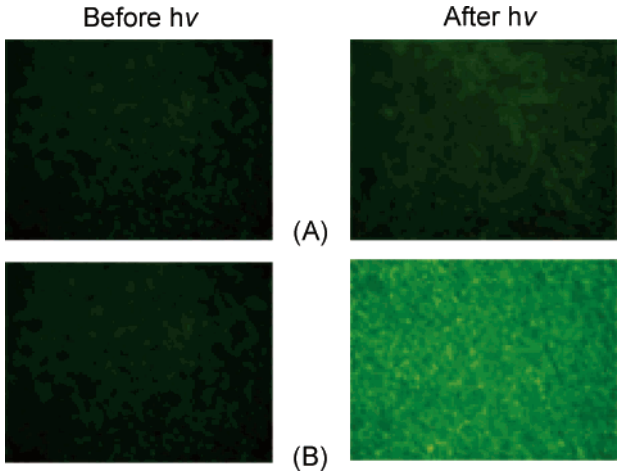


Figure 3. Fluorescence image changes of the PE surface at positions of (A) 0.25 and (B) 4.5 cm before and after photoirradiation for 10 min.

To confirm the formation of a gradient surface according to corona treatment, the surfaces of the untreated and treated PE films were examined by water contact angle, FTIR-ATR, AFM, and ESCA methods.

The water contact angles of the PE surface were measured at room temperature in a static condition. As shown in Figure 1A, the contact angle decreased gradually from 98−99° to 45−50° along the PE length, indicating the gradual wettability change as the surface changes from hydrophobic to more hydrophilic, due to the introduction of oxygen-based functional groups on the PE surface by corona treatment. A peak assignable to a C=O functional group was observed using FTIR-ATR at 1735 cm^{-1} after corona treatment (Figure 2). In addition, the intensity of this peak increased gradually along the PE length.[11]

(8) Ruardy, T. G.; Schakenraad, J. M.; van der Mei, H. C.; Busscher, H. J. *Surf. Sci. Rep.* **1997**, *29*, 1.

(9) (a) Lee, J. H.; Lee, S. J.; Khang, G.; Lee, H. B. *J. Colloid Interface Sci.* **2000**, *230*, 84. (b) Lee, J. H.; Lee, J. W.; Khang, G.; Lee, H. B. *Biomaterials* **1997**, *18*, 351.

(10) A schematic diagram of the corona-discharge apparatus is illustrated in Supporting Information Figure S1.

(11) FTIR-ATR spectra along the PE length are illustrated in Supporting Information Figure S2.

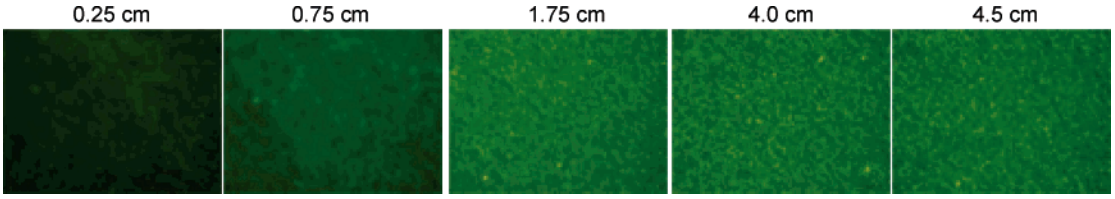


Figure 4. Fluorescence images of the gradient PE surface from 0 to 5 cm after photoirradiation.

AFM measurements showed the corona-treated PE surface increased in roughness, from 10 nm at a position of 0.5 cm to 100 nm at a position of 4.5 cm, after corona treatment.[12] This indicates that the PE surface changes in roughness after corona treatment and as the corona power increased along the PE length.

By ESCA, the untreated PE surface exhibited only a broad C_{1s} peak at a binding energy of 285 eV, while new peaks assignable to ketone or aldehyde groups, hydroxyl or ether groups, and carboxylic acid or ester groups after corona treatment were observed at 287.9, 286.5, and 289.1 eV, respectively. The peak heights increased gradually along the PE length.

These experiments confirmed that corona treatment introduced various functional groups to the PE surface, especially carboxylic acid groups which can serve as reaction sites.

The carboxylic acid on the PE surface was chemically modified using PEI. PEI on the gradient surface could serve as a flexible spacer, and it possesses multifunctional sites that could immobilize a large fraction of the biomolecules. The carboxylic group on the corona-treated PE surface was activated with NHS and EDC. The activated PE surface reacted with the amine group of PEI, resulting in the formation of an amide bond.

After PEI treatment, the peak in the FTIR spectrum at 1735 cm^{-1} assignable to the C=O functional group almost disappeared, while a new peak, assignable to PEI, appeared at 1586 cm^{-1}. In addition, peaks assignable to amide I and amide II were also observed at 1653 and 1543 cm^{-1}, respectively (Figure 2C).[13] The ESCA spectrum of the PEI-grafted gradient PE surface showed a new peak with a binding energy of 288.6 eV. In addition, a signal attributed to N_{1s} was observed at 399 eV in the survey scan spectrum,[14] confirming the attachment of PEI on the PE surface. The increase in height of this peak reflected an increase in nitrogen content along the PE length. AFM measurements indicated considerable recovery of surface roughness after this modification, probably due to retention of air or water.[3] Nevertheless, it was evident from the FTIR-ATR as well as ESCA analyses that the PEI modification occurred gradually along the PE surface.

To design a gradient PE surface with a model bioactive molecule that can selectively recognize or identify targetable biomolecules, we chose the avidin−biotin system on account of the exceptionally high binding affinity of avidin to biotin ($K_D \sim 10^{-15}$ M) and because the binding of SAV to immobilized biotin has already been extensively studied.[15]

The PEI-modified PE surface was treated with biotin, preactivated with NHS and EDC, to give a biotinylated PE surface. The biotinylated PE surface was characterized by FTIR-ATR (Figure 2D). The new signals assignable to the characteristic peaks due to amide stretching vibration of biotin appeared at around 1665 cm^{-1} after treatment with biotin.[16]

Biotin immobilized on the gradient surface could be expected to bind SAV according to its concentration and thus according to the position along the surface. A fundamental requirement to reveal the presence of such binding as a display tool is that the response occurs only when the surfaces are exposed to external stimulation.

CMNB-caged, fluorescein-conjugated SAV (CMNBF-SAV), which can turn on the fluorescence of the SAV molecules on the surface upon photoirradiation as external stimulation, was selected and examined for light exposure. The gradient biotinylated PE surface was incubated in a solution of CMNBF-SAV to give a SAV-bound gradient PE surface. The CMNBF-SAV on the gradient surface did not fluoresce until the cage was photolyzed. Photoirradiation of gradient biotinylated PE surfaces incubated with CMNBF-SAV was carried out using 365 nm light (0.3 mW cm^{-2}) for 0−60 min. Figure 3 shows the changes in the fluorescence images taken from positions of 0.25 and 4.5 cm along the PE surface before and after photoirradiation. No large difference is observed between the fluorescence microscopy images recorded at a position of 0.25 cm before and after photoirradiation. At a position of 4.5 cm, however, a strong fluorescence appears in the image recorded after 10 min of photoirradiation that was not observed in the image recorded before irradiation. The image from a position of 4.5 cm showed almost no change in fluorescence intensity upon further photoirradiation.

Fluorescence images taken along the surface are shown in Figure 4.[17] The fluorescence increases gradually along the PE surface. Figure 1B shows the fluorescence intensity versus the surface position, equivalent to the degree of corona treatment. Greater corona treatment correlates to a smaller water contact angle value and a stronger fluorescence intensity, which could explain the gradient PE surface. However, PEI, with a hyperbranched multifunctional structure, provides many biotin anchor groups on the same surface to bind to SAV. This, unfortunately, resulted in the saturation of fluorescence intensity after ~2 cm along the surface.

Conclusions

We prepared a gradient PE surface using an increasing corona treatment along the PE length. A biotinylated gradient PE surface was prepared through the reaction of PEI and biotin. CMNB-caged, fluorescein-conjugated SAV was bound to the surface through avidin−biotin binding. The so-designed gradient PE surface fluoresced after photoirradiation. We believed that these gradient

(12) AFM images at positions of 0.5 and 4.5 cm after corona treatment are illustrated in Supporting Information Figure S3.

(13) (a) Lee, P. H.; Sawan, S. P.; Modrusan, Z.; Arnold, L. J., Jr.; Reynolds, M. A. *Bioconjugate Chem.* **2002**, *13*, 97. (b) Forato, L. A.; Filho, R. B.; Colnago, L. A. *Anal. Biochem.* **1998**, *5*, 136.

(14) ESCA survey scan spectra are illustrated in Supporting Information Figure S4.

(15) (a) Green, N. *Biochem. J.* **1963**, *89*, 585. (b) Brandts, J. F.; Lin, L. N. *Biochemistry* **1990**, *29*, 6927.

(16) Polyak, B.; Geresh, S.; Marks, R. S. *Biomacromolecules* **2004**, *5*, 389−396.

(17) All fluorescence microscope images of the gradient PE surface after photoirradiation are illustrated in Supporting Information Figure S5.

PE surfaces might be applied as an analysis tool in the form of a latent image display, which shows no activity under ambient conditions but generates a display upon external stimulation.

Acknowledgment. This work was supported by KMOCIE (N11-A08-1402-05-1-3).

Supporting Information Available: Corona-discharge apparatus, FTIR-ATR spectra, AFM images, ESCA survey scan spectra, and fluorescence microscope images of the gradient PE surface. This material is available free of charge via the Internet at http://pubs.acs.org.

LA046868A