Construction of Protein-Resistant pOEGMA Films by Helicon Plasma-Enhanced Chemical Vapor Deposition

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Abstract
This paper describes the formation of protein-resistant, poly(ethylene glycol) methyl ether methacrylate (pOEGMA) thin films by helicon plasma-enhanced chemical vapor deposition (helicon-PECVD). pOEGMA was successfully grafted onto a silicon substrate, as a model substrate, without any additional surface initiators, by plasma polymerization of OEGMA. The resulting pOEGMA films were characterized by ellipsometry, FT-IR spectroscopy, X-ray photoelectron spectroscopy and contact angle goniometry. To investigate the protein-resistant property of the pOEGMA films, four different proteins, bovine serum albumin, fibrinogen, lysozyme and ribonuclease A, were tested as model proteins for ellipsometric measurements. The ellipsometric thickness change for all the model proteins was less than 3 Å, indicating that the formed pOEGMA films are protein-resistant.
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1. Introduction

The construction of protein- and cell-resistant surfaces is a crucial requirement for medical or analytical devices that involve the intimate contact with biological fluids [1], e.g., biosensors [2–5], chip-based diagnostic devices [6], affinity chromatography columns [7, 8] and biomaterials used for implants and tissue engineering [9–11]. A simple but versatile strategy to reduce biofouling adsorption

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is surface coating with non-biofouling poly(ethylene glycol) (PEG) [12–15]. The non-biofouling character of PEG is attributed to very high levels of hydration of the polymer chains, as well as conformational flexibility of PEG [16, 17]. In addition, PEG shows good biocompatibility, low toxicity, non-immunogenicity and high water solubility. Methods reported for coating substrates with PEG include physisorption [18–21], chemisorption [13, 22, 23], covalent grafting [14, 24] and plasma polymerization of oligo(ethylene glycol) (OEG) precursors [25]. The use of plasma for material processing has gained acceptance in various areas, from metallurgy to manufacturing of computer chips, and covers a wide variety of materials, including metals, semiconductors and polymers. The use of plasma has some advantages, including operation at relatively low temperatures [26]. The high density plasmas have been generated by inductively coupled plasma (ICP), electron cyclotron resonance (ECR) and helicon plasma [18, 27]. Most applications of plasma-based or plasma-enhanced chemical processing focus on surface treatment/modification, coating, film deposition and etching. Badyal and co-workers have intensively utilized plasma polymerization for surface coating, and various precursors have been used, such as glycidyl methacrylate [28], maleic anhydride [29], 2-cyanoethyl acrylate [30], 2-hydroxyethyl methacrylate [31], ethylene glycol dimethacrylate [32], cyclic methacrylic anhydride [32], acrylic acid [33], $1H,1H,2H,2H$-perfluorooctyl acrylate [34], *N*-acryloylsarcosine methyl ester [35], *N*-isopropylacrylamide [36], furfuryl methacrylate [26] and allylmercaptan [37]. In particular, it was reported that surfaces coated by plasma polymerization of *N*-acryloylsarcosine methyl ester and *N*-isopropylacrylamide showed protein-resistant ability [35, 36]. We have been interested in the construction of non-biofouling surfaces [38, 39] and herein applied helicon plasma-enhanced chemical vapor deposition (helicon-PECVD) for the formation of poly(ethylene glycol) methyl ether methacrylate (pOEGMA) thin films. After the formation, the protein-resistant property of the pOEGMA films was investigated by using model proteins such as bovine serum albumin (BSA), fibrinogen, lysozyme and ribonuclease A (RNase A).

2. Materials and Methods

2.1. Materials

Absolute ethanol (EtOH, 99.9+%, Merck), bovine serum albumin (BSA, Sigma), fibrinogen (Sigma), ribonuclease A (RNase A, Sigma), lysozyme (Sigma), phosphate buffered saline (PBS, Sigma) were used as received. Poly(ethylene glycol) methyl ether methacrylate (OEGMA, M_n approx. 475, Aldrich) was passed through a basic activated alumina column to remove inhibitors. The bare silicon oxide surfaces were sonicated for 5 min in absolute ethanolic solution, followed by completely rinsing with ethanol and then purging under argon gas.

2.2. Helicon Plasma-Enhanced Chemical Vapor Deposition (Helicon-PECVD)

The helicon-PECVD of OEGMA onto a silicon wafer was performed by using a home-made instrument composed of a source chamber (Pyrex tube), a processing

chamber, RF power, Nagoya type-III antenna and Helmholtz coil. RF power was generated with the output of 13.56 MHz, and the source chamber was 10 cm in diameter and 50 cm in length. OEGMA was put in the bubbler and evaporated at 100°C. The evaporated OEGMA, which was mixed with Ar carrier gas at a flow rate of 2000 sccm, was put in the source chamber. Total pressure in the chamber was kept at 0.1 Torr. A silicon wafer was put on the substrate holder inside the chamber. Helicon plasma (approx. 10^{10} cm^{-3}) was generated by a RF power of 500 W and magnetic field of 400 G. The deposition was performed for 50 min. After the deposition process, the resulting pOEGMA films were characterized by various techniques, such as Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS), contact angle goniometry and ellipsometry.

2.3. Protein Adsorption Experiments

The protein-resistant effect of pOEGMA film was tested with BSA, fibrinogen, lysozyme and RNase A as model proteins in phosphate-buffered saline (PBS, pH 7.4) of 1 mg/ml. After immersing the films in the buffered solution of proteins for 2 h at room temperature, the substrates were washed with PBS buffer solution and the distilled water under purging argon gas. The change in ellipsometric thickness was measured by ellipsometry.

2.4. Measurements

2.4.1. Polarized Infrared External Reflectance Spectroscopy (PIERS)
Polarized infrared external reflectance spectroscopy (PIERS) spectra were obtained in a single reflection mode using a dry N_2-purged Thermo Nicolet Nexus FT-IR spectrophotometer equipped with the smart SAGA (smart apertured grazing angle) accessory. The p-polarized light was incident at 80° relative to the surface normal of the substrate and a narrow band mercury-cadmium-telluride (MCT) detector cooled with liquid nitrogen was used to detect the reflected light. We averaged 16 000 scans to yield the spectrum at a resolution of 4 cm^{-1} and reported in the absorption mode relative to a clean silicon surface.

2.4.2. X-ray Photoelectron Spectroscopy (XPS)
The X-ray photoelectron spectroscopy (XPS) study was performed with a VG-Scientific ESCALAB 250 spectrometer (UK) with monochromatized Al K$_\alpha$ X-ray source. Emitted photoelectrons were detected by a multi-channel detector at a take off angle of 90° relative to the surface. During the measurements, the base pressure was 10^{-9}–10^{-10} Torr. Survey spectra were obtained at a resolution of 1 eV from 3 scans and high-resolution spectra were acquired at a resolution of 0.05 eV from 5–20 scans. Each XPS spectrum was referenced to the C_{1s} hydrocarbon peak centered at 285.0 eV and fitted using Marquardt minimization computer software to a liner background with equal full-width at half-maximum (fwhm) Gaussian components.

2.4.3. Contact Angle Goniometry

A Phoenix 300 apparatus (Surface Electro Optics) equipped with a video camera was used to measure the static contact angles on water drops of approx. 3 µl in volume. Reported values represent averages of at least four independent measurements.
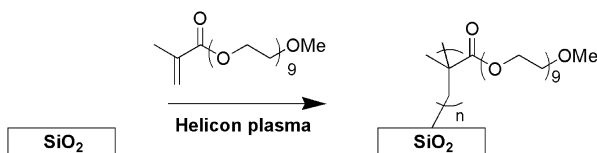
2.4.4. Ellipsometry

Plasma polymer film thickness was measured using a Gaertner L116s ellipsometer (Gaertner Scientific) equipped with a He–Ne Laser (632.8 nm) at a 70° angle of incidence. A refractive index of 1.46 was used for all films.

3. Results and Discussion

Many attempts have been made to immobilize biological molecules, such as proteins [40] and DNA [41, 42], onto the plasma polymerized non-biofouling polymer used to inhibit non-specific interactions. In immobilization of biological molecules it is important to consider signal-to-background. Namely, we have to increase the detection limit of desirable materials by blocking and reducing a non-specific binding of unwanted materials. Therefore, we used OEGMA among materials with the protein-resistant effect in order to minimize/reduce this non-specific interaction in our experiment.

3.1. Plasma Polymerization of OEGMA

We performed helicon plasma polymerization of OEGMA onto a silicon wafer without general constituents required in an aquous polymerization, such as surface initiatiors, solvent, catalyst and ligand. This plasma deposition approach permitted a tightly adherent, non-fouling layer to be coated on the silicon substrate in a one-step, gas-phase (dry) process. Badyal and co-workers have already coated various precursors with methacrylate [28, 31, 32] or acrylate [26, 30, 34] groups using various plasma devices, except for helicon plasma socure onto substrates. The helicon plasma-enhanced chemical vapor deposition has attractive features for film deposition because uniform plasma with large area and high efficiency can be generated [43]. The low contaminated [44] and good cross-linked [45] film can be made due to ion flux with low temperature processing. Ar gas carries evaporable OEGMA into the reaction chamber and then the polymerization of OEGMA is initiated on the substrate by plasma generated by the helicon source (Fig. 1).

Figure 1. Schematic representation of the polymerization process.

3.2. Characterization

The resulting pOEGMA films were characterized by FT-IR spectroscopy, X-ray photoelectron spectroscopy (XPS), ellipsometry and contact angle goniometry. Figure 2 shows the IR spectrum of pOEGMA films after the helicon-PECVD process. We observed peaks at 3204 cm^{-1} (–CH$_3$ stretching), 1763 cm^{-1} (C=O, stretching), 1499 cm^{-1} (CH$_2$ scissoring), 1309 cm^{-1} (CH$_2$ wagging), 1138 cm^{-1} (C–O and C–C stretching), 1058 cm^{-1} (C–O, C–C stretching and CH$_2$ rocking), 940 cm^{-1} (C–C stretching and CH$_2$ rocking) and 841 cm^{-1} (C–O, C–C stretching and CH$_2$ rocking) [46], in addition to a broad peak in the range of 1100–700 cm^{-1} and peaks at 710 cm^{-1} and 587 cm^{-1}, which were probably due to SiO$_2$ substrate [47]. The XPS spectra of wide scan and C$_{1s}$ level are shown in Fig. 3. The intense peaks of C$_{1s}$, O$_{1s}$ and Si$_{2p}$ (Si$_{2s}$) were detected at 284, 531 and 96 eV (148 eV), respectively. The C$_{1s}$ region shows peaks at 284.0 (CH$_x$), 285.6 (C–OR) and 288.2 eV (COOR).
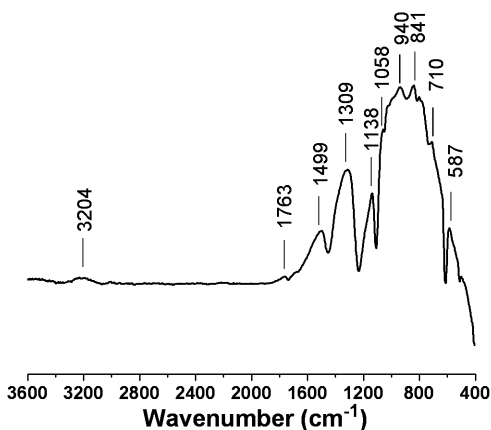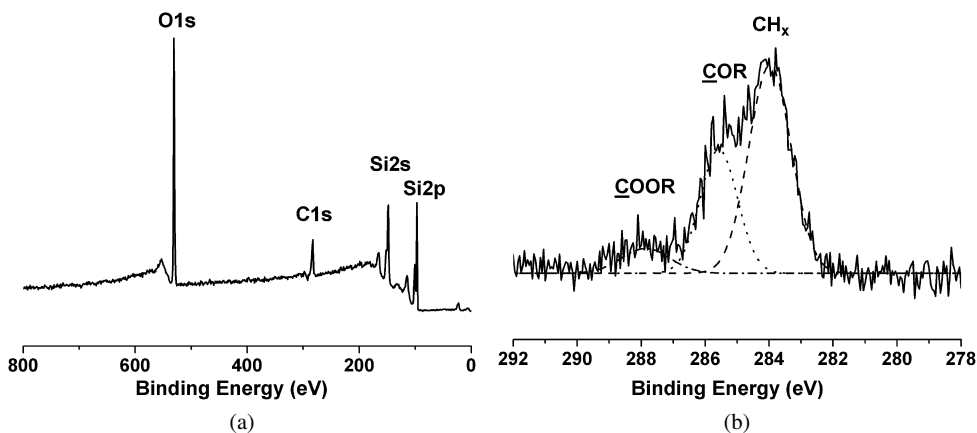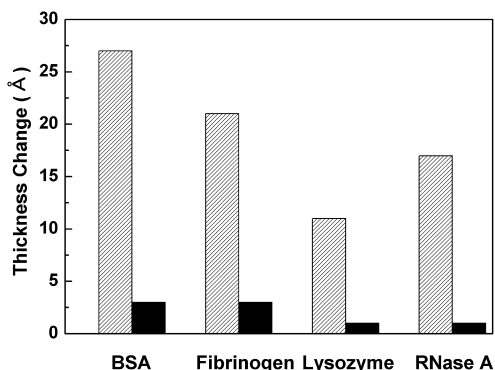

Figure 2. IR spectrum of pOEGMA films.

Figure 3. XPS spectra of pOEGMA films.

Figure 4. Protein adsorption experiments. Change of the ellipsometric thickness on bare silicon wafers (hatched bars) and pOEGMA films (filled bars) for proteins adsorption experiments. Model proteins are BSA, fibrinogen, lysozyme and RNase A, respectively.

The differences in the binding energy of CH_x and COOR from C–OR were 1.6 and 2.6 eV, respectively [46]. Mean ellipsometric thickness and static contact angle of pOEGMA films were 31 Å and 49°, respectively.

3.3. Protein Adsorption Experiments: Protein-Resistant Properties

Protein-repellent or non-biofouling surfaces are the basic consideration in establishing the biocompatibility and blood compatibility of blood-contacting devices such as catheters, dialyzers, vascular grafts, blood containers, etc. We know that the incorporation of PEG molecules on any substrate surface generally results in a reduction/elimination of biomolecules and bio-species such as protein and cell on the PEG-modified surface [38, 39, 46]. Here, we performed ellipsometric measurement to observe the reduction of the non-specific adsorption onto the OEGMA films polymerized by helicon-PECVD. Ellipsometric measurement was used to determine the thickness before and after protein adsorption on pOEGMA films. As a control, a bare silicon wafer was used: thickness increase was on average 27, 21, 11 and 17 Å for BSA, fibrinogen, lysozyme and RNase A, respectively. After helicon-PECVD of pOEGMA, the ellipsometric thickness increase was determined to be 3, 3, 1 and 1 Å for each protein (Fig. 4). The amounts of non-specific adsorption with less than 3 Å for the four model proteins are smaller values than those previously reported on pOEGMA films coated by atom transfer radical polymerization [38]. It is indicated that pOEGMA films coating by helicon-PECVD results in a protein-resistant surface. If biomacromolecules, like cells, would be contacted with the pOEGMA films with protein-resistant properties, we expect that they have low affinity with the films and will anticipate non-biofouling properties in the event.

4. Conclusions

In this study, we demonstrated that helicon plasma-enhanced chemical vapor deposition (helicon-PECVD) was a promising method for coating surfaces with protein-

resistant pOEGMA. Helicon-PECVD of OEGMA provides a one-step solventless route for prodcuing well-defined ethylene glycol-functionalized solid surfaces. It is comparable to the previously reported systems: the adsorption of protein was less than 3 Å onto the coated surface for all the model proteins. Overall, the results obtained from the present work provide additional support for the utility of one-step plasma process to reduce biological fouling of surfaces *via* deposition of pOEGMA surface units. These coatings will be useful for applications that can benefit from a reduction in the adventitious adsorption of proteins, ranging from biomedical implants to clinical diagnostics on solid support and protein arrays.

References

1. E. F. Leonard, V. T. Turitto and L. Vroman (Eds). *Annals of the New York Academy of Sciences*, Vol. 516. New York Academy of Sciences, New York, NY (1987).
2. P. B. Lupa, L. J. Sokoll and D. W. Chan, *Clin. Chim. Acta* **314**, 1 (2001).
3. J. J. Ramsden, *J. Mol. Recognit.* **10**, 109 (1997).
4. K. R. Rogers, *Mol. Biotechnol.* **14**, 109 (2000).
5. T. Wink, S. J. van Zuilen, A. Bult and W. P. van Bennekom, *Analyst* **122**, R43 (1997).
6. R. C. McGlenmen, *Clin. Chem.* **47**, 393 (2001).
7. M. V. Novotny, *Methods Enzymol.* **270**, 101 (1996).
8. M. V. Novotny, *J. Chromatogr. B* **689**, 55 (1997).
9. P. Ducheyne and Q. Qiu, *Biomaterials* **20**, 2287 (1999).
10. R. Bizios, *Biotechnol. Bioeng.* **43**, 582 (1994).
11. L. P. Tang and J. W. Eaton, *Am. J. Clin. Pathol.* **103**, 466 (1995).
12. R. Israels, F. A. M. Leermakers and G. J. Fleer, *Macromolecules* **28**, 1626 (1995).
13. K. L. Prime and G. M. Whitesides, *J. Am. Chem. Soc.* **115**, 10714 (1993).
14. W. R. Gombotz, G. H. Wang, T. A. Horbett and A. S. Hoffman, *J. Biomed. Mater. Res.* **25**, 1547 (1991).
15. J. M. Harris, *Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications*. Plenum Press, New York, NY (1992).
16. J. H. Lee and J. D. Andrade, in: *Polymer Surface Dynamics*, J. D. Andrade (Ed.), p. 119. Plenum, New York, NY (1988).
17. H. Chen, Z. Zhang, Y. Chen, M. A. Brook and H. Sheardown, *Biomaterials* **26**, 2391 (2005).
18. S. Herrwerth, W. Eck, S. Reinhart and M. Grunze, *J. Am. Chem. Soc.* **125**, 9395 (2003).
19. J. H. Lee, J. Kpecek and J. D. Andrade, *J. Biomed. Mater. Res.* **23**, 351 (1989).
20. D. L. Elbert and J. A. Hubbell, *J. Biomed. Mater. Res.* **42**, 55 (1998).

21. V. A. Liu, W. E. Jastromb and S. N. Bhataia, *J. Biomed. Mater. Res.* **60**, 126 (2002).
22. N. Xia, Y. H. Hu, D. W. Graiger and D. G. Castner, *Langmuir* **18**, 3255 (2002).
23. J. P. Bearinger, S. Terrettaz, R. Michel, N. Tirelli, H. Vogel, M. Textor and J. A. Hubbell, *Nature Mater.* **2**, 259 (2003).
24. G. R. Llanos and M. V. Sefton, *J. Biomater. Sci. Polymer Edn* **4**, 381 (1993).
25. G. P. Lopez, B. D. Ratner, C. D. Tidwell, C. L. Haycox, R. J. Rapoza and T. A. Horbett, *J. Biomed. Mater. Res.* **26**, 415 (1992).
26. C. Tarducci, J. P. S. Badyal, S. A. Brewer and C. Willis, *Chem. Commun*. **3**, 406 (2005).
27. M. Malmsten, K. Emoto and J. M. Van Alstine, *J. Colloid Interface Sci.* **202**, 507 (1998).
28. C. Tarducci, E. J. Kinmond, J. P. S. Badyal, S. A. Brewer and C. Willis, *Chem. Mater.* **12**, 1884 (2000).
29. S. A. Evenson, C. A. Fail and J. P. S. Badyal, *Chem. Mater.* **12**, 3038 (2000).
30. C. Tarducci, W. C. E. Schofield, J. P. S. Badyal, S. A. Brewer and C. Willis, *Chem. Mater.* **13**, 1800 (2001).
31. C. Tarducci, W. C. E. Schofield, J. P. S. Badyal, S. A. Brewer and C. Willis, *Chem. Mater.* **14**, 2541 (2002).
32. C. Tarducci, W. C. E. Schofield, J. P. S. Badyal, S. A. Brewer and C. Willis, *Macromolecules* **35**, 8724 (2002).
33. L. J. Ward, W. C. E. Schofield, J. P. S. Badyal, A. J. Goodwin and P. J. Merlin, *Chem. Mater.* **15**, 1466 (2003).
34. D. O. H. Teare, C. G. Spanos, P. Ridley, E. J. Kinmond, V. Roucoules, J. P. S. Badyal, S. A. Brewer, S. Coulson and C. Willis, *Chem. Mater.* **14**, 4566 (2002).
35. D. O. H. Teare, W. C. E. Schofield, R. P. Garrod and J. P. S. Badyal, *J. Phys. Chem. B* **109**, 20923 (2005).
36. D. O. H. Teare, D. C. Barwick, W. C. E. Schofield, R. P. Garrod, A. Beeby and J. P. S. Badyal, *J. Phys. Chem. B* **109**, 22407 (2005).
37. W. C. E. Schofield, J. McGettrick, T. J. Bradley, J. P. S. Badyal and S. Przyborski, *J. Am. Chem. Soc.* **128**, 2280 (2006).
38. B. S. Lee, J. K. Lee, W.-J. Kim, Y. H. Jung, S. J. Sim, J. Lee and I. S. Choi, *Biomacromolecules* **8**, 744 (2007).
39. W. K. Cho, B. Kong and I. S. Choi, *Langmuir* **23**, 5678 (2007).
40. B. D. Ratner, *J. Mol. Recogn.* **9**, 617 (1996).
41. L. Chu, W. Knoll and R. Förch, *Plasma Process. Polym*. **3**, 498 (2006).
42. H. Miyachi, K. Ikebukuro, K. Yano, H. Aburatani and I. Karube, *Biosens. Bioelectr.* **20**, 184 (2004).
43. F. Zhang, E. T. Kang, K. G. Neoh, P. Wang and K. L. Tan, *Biomaterials* **22**, 1541 (2001).
44. S. Gorbatkin and L. Berry, *J. Vac. Sci. Technol.* **10**, 304 (1992).
45. K. Seaward, J. Turner, K. Nauka and A. Nel, *J. Vac. Sci. Technol. B* **13**, 118 (1995).
46. H. Ma, M. Wells, T. P. Beebe Jr. and A. Chilkoti, *Adv. Funct. Mater.* **16**, 640 (2006).
47. A. J. M. de Man and R. A. van Santen, *Zeolites* **12**, 269 (1992).