Functionality of Polymeric Micelle Hydrogels with Organized Three-Dimensional Architecture on Surfaces

Kazunori Emoto,[†,‡] Michihiro Iijima,[†] Yukio Nagasaki,[†] and Kazunori Kataoka*,[‡]

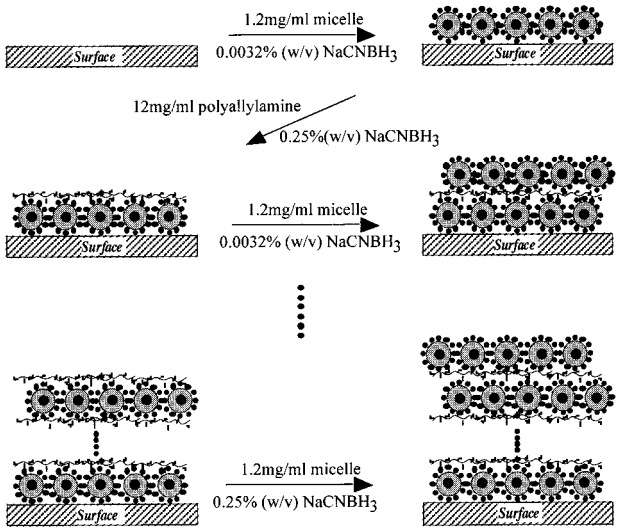
Department of Materials Science and Technology Science University of Tokyo, 2641 Yamazaki, Noda Chiba, 278-5180 Japan
Department of Materials Science Graduate School of Engineering The University of Tokyo 7-3-1 Hongo, Bunkyo-ku Tokyo, 113-8656 Japan

Received October 27, 1999

A variety of surfaces have been modified with hydrophilic polymers and hydrogels for biomedical and biotechnical applications. The modification reduces nonspecific adsorption of biomolecules such as proteins and allows the immobilization of bioactive molecules without loss of activity. We prepared a thin layer of hydrogel possessing a highly ordered three-dimensional architecture as shown in Scheme 1, and explored its applicability as a biofunctional material. The hydrogels consist of laminated layers of stabilized reactive polymeric micelles from a block copolymer of poly(ethylene glycol)−poly(D,L-lactide) (PEG−PLA) and polyallylamine.[1,2] The PEG−PLA bearing an acetal group at the PEG-end and a methacryloyl group at the PLA-end was prepared by anionic ring-opening polymerization of corresponding monomers as reported in our previous publication.[1] The micelle prepared by dialysis was stabilized by polymerizing the methacryloyl group in the core and made reactive by the hydrolysis of the acetal group on the surface of the micelle.[1] The resultant aldehyde-bearing micelle of 30−35 nm in diameter was coated on the aminated surface at 22 °C in the presence of $NaCNBH_3$.[2] Since the micelle bears aldehyde groups on its surface, it reacts with an amine on the substrate to form a Schiff base that can be reduced into a stable secondary amine by $NaCNBH_3$. Due to the polymerization of the PLA-end located in the core, the micellar structure was maintained, while nonpolymerized micelles disrupted when they covered the substrate.[2] A multilayer structure was then formed by the alternate coatings of polyallylamine (PAlAm) and the micelles.[3] This procedure is presented in Scheme 1. Following the formation of a single layer of the micelles on the substrate, amino groups were introduced on the top of the micellar layer by tethering PAlAm onto the surface-attached micelles for additional attachment of the second layer of the micelles. By repeating these alternate coatings of micelles and PAlAm in the presence of $NaCNBH_3$, micellar multilayers can be formed on the substrate. The resultant multilayer is a thin hydrogel possessing layer-by-layer structure, and the thickness of the layer can be controlled by the number of coatings, which is peculiar to this method.[3]

Hydrogels for biomedical, bioanalytical, and biotechnical applications should have a minimal nonspecific interaction with biological systems, induce no immunogenic reactions, and in some applications, need to hold biologically active molecules such as proteins in their native form.[4−13] The outermost layer of the

Scheme 1



present micellar gel is essentially covered with PEG, and indeed, the monolayer coating of micelles was shown to remarkably reduce protein adsorption.[14] Aminated polypropylene (PP) films coated with micelles and PAlAm alternately were exposed to 45 μg/mL of BSA solution in phosphate buffered saline (PBS) at pH 7.4 and 22 °C for 60 min. After the films were rinsed, the adsorbed BSA was eluted with sodium dodecyl sulfate, and the Cu^+ in the BSA was complexed with bicinchoninic acid (BCA).[15] The amount of adsorbed BSA was determined by the UV absorbance of the complex, and is presented in Figure 1. Obviously, the gel surface with micelles at the top layer significantly reduced the protein adsorption. This is comparable to a surface coated with a high density of PEG.[14] On the other hand, BSA adsorbed appreciably when the PAlAm was exposed on the gel surface.

Adsorption of plasma proteins such as BSA correlates with the electrokinetic properties of the surface.[16] The ζ-potential measured in 7.5 mM NaCl solution at 25 °C and pH 7.4 corresponds nicely with BSA adsorption as shown in Figure 1. When the micellar layer was at the top, the surface exhibited a small ζ-potential, indicative of the masking of surface charge. It should be noted that the micellar coating showed a small receding contact angle, indicative of its low tendency to dehydrate,[2] which resulted in an increased wettability by the micellar coating and

[†] Science University of Tokyo.
[‡] The University of Tokyo.

(1) Iijima, M.; Okada, T.; Nagasaki, Y.; Kato, M.; Kataoka, K. *Macromolecules* **1999**, *32*, 1140.
(2) Emoto, K.; Nagasaki, Y.; Kataoka, K. *Langmuir* **1999**, *15*, 5212.
(3) Emoto, K.; Nagasaki, Y.; Kataoka, K. *Langmuir*, manuscript submitted.
(4) Espadas-Torre, C.; Meyerhoff, M. E. *Anal. Chem.* **1995**, *67*, 3108.
(5) Platé, N. A.; Chupov, V. V. *Polym. Sci.* **1994**, *36*, 1568.
(6) Nakayama, Y.; Nakamata, K.; Hirano, Y.; Goto, K.; Matsuda, T. *Langmuir* **1998**, *14*, 3909.
(7) Nakayama, Y.; Takatsuka, M.; Matsuda, T. *Langmuir* **1999**, *15*, 1667.
(8) Rhee, W.; Rosenblatt, J.; Castro, M.; Schroeder, J.; Rao, P. R.; Hartner, C.; Berg, R. A. In *Poly(ethylene glycol): Chemistry and biological Applications*; Harris, J, M., Zalipsky, S., Eds.; ACS Symposium Series; American Chemical Society: Washington DC, 1997; p 420.
(9) Zhao, X.; Harris, J. M. In *Poly(ethylene glycol): Chemistry and biological Applications*; Harris, J, M., Zalipsky, S., Eds.; ACS Symposium Series; American Chemical Society: Washington DC, 1997; p 458.
(10) Han, D. K.; Hubbell, J. A. *Macromolecules* **1997**, *30*, 6077.
(11) Chen, G.; Hoffman, A. S.; Kabra, B.; Randeri, K. In *Poly(ethylene glycol): Chemistry and biological Applications*: Harris, J, M., Zalipsky, S., Eds.; ACS Symposium Series; American Chemical Society: Washington DC, 1997; p 441.
(12) Jeong, B.; Bae, Y.-H.; Lee, D.-S.; Kim, S.-W. *Nature* **1997**, *388*, 28, 860.
(13) Shiroya, T.; Yasui, M.; Fujimoto, K.; Kawaguchi, H. *Colloids Surf., B* **1995**, *4*, 275.
(14) Emoto, K.; Iijima, M.; Nagasaki, Y.; Kato, M.; Kataoka, K. *Colloids Surf., B* **2000**, in press.
(15) Smith, P. K.; Krohn, R. I.; Hermanson, G. T.; Mallia, A. K.; Gartner, F. H.; Provenzano, M. D.; Fujimoto, E. K.; Goeke, N. M.; Olson, B. J.; Klenk, D. C. *Anal. Biochem.* **1985**, *150*, 76.
(16) Norde, W.; Lyklema, J. *J. Colloid Interface Sci.* **1978**, *64*, 277.

10.1021/ja993821g CCC: $19.00
Published on Web 03/04/2000

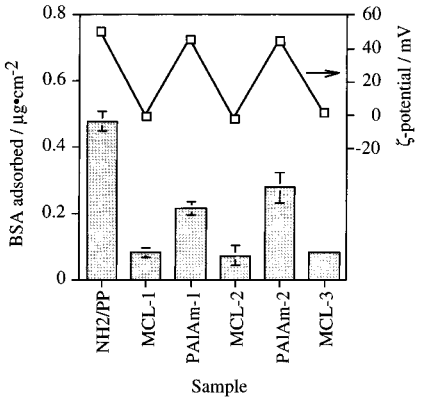


Figure 1. Adsorption of BSA from a 45 mg/mL solution in PBS at pH 7.4 to the coatings of micelle (MCL) and polyallylamine (PAlAm). The substrate was aminated polypropylene. The adsorption of BSA is correlated to the ζ-potential of the samples in 7.5 mM NaCl at pH ~7.4 and 25 °C.

therefore reduction of protein adsorption. When the PAlAm covers the outermost layer, the surface has a positive charge, as demonstrated by the ζ-potential, and becomes less hydrophilic than the surface with a micellar top layer.[3,17] Consequently, due to hydrophobic and electrostatic effects, negatively charged BSA is appreciably adsorbed to the PAlAm surface. The results of BSA adsorption and the ζ-potential indicate that micellar and PAlAm layers formed discretely in an alternate manner at least up to three layers.

The micellar gel has another unique property that is not typical of conventional hydrogels. Each micelle in the gel possesses a hydrophobic core with a narrow size distribution in the range of ~30 nm. Apparently the micellar core can hold hydrophobic reagents[18−21] and therefore, is expected to encapsulate and release hydrophobic drugs in a controlled manner. As a model drug, pyrene was incorporated into the micelle by mixing pyrene with the micellar solution at 60 °C for 4 h, following the procedure by Kwon et al.[21] The pyrene-incorporated micelles were coated on an aminated glass slide in the aforementioned manner. The sample was then exposed to an excess amount of Mili-Q water. By measuring the fluorescence at $\lambda_{ex} = 336$ nm, the release of pyrene from the micelle-coated surface was monitored. The peak intensity ratio of I ([0,0] band) to III ([0,2] band) of the emission spectrum obtained for the pyrene/micelle solution and the glass slides coated with the pyrene-loaded micelles was about 1.4, indicating that pyrene was localized in the hydrophobic core of the micelle.[21] The plot of the fluorescence intensity at $\lambda_{em} = 363$ nm of the micelle-coated glass slide with exposure time to water is presented in Figure 2. Note that the fluorescence from the glass slide itself at this wavelength was negligible compared with that of the sample. The initial amount of pyrene as well as the rate of release were dependent on the number of micelle coatings; as the number of coatings increased, the initial fluorescence was higher, and release-rate evaluated from the decline in the fluorescence intensity was slower. The formation of a multilayered micelle structure with thickness increased by the alternate coating process resulted in an increased retention capacity of pyrene in the gel layer and consequently lowered the release rate.

Repetitive loading and release of pyrene were also successfully accomplished with the micelle-gel layer. After the hexapoid coatings of pyrene-free micelle, the samples were exposed to the

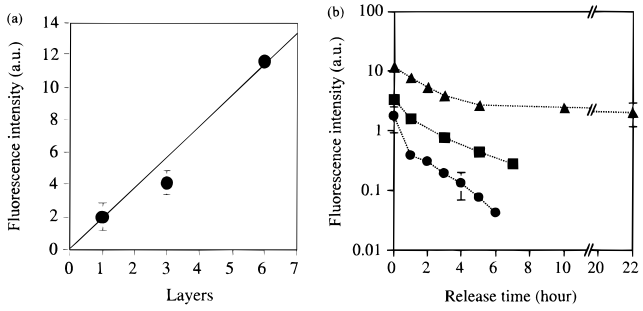


Figure 2. Dependence of the number of coatings on (a) the initital fluorescene intensity and (b) the decrease in fluorecsent intensity of aminated glass coated with (●) single, (■) triple, and (▲) hexapoid layer of micelle with exposure to water.

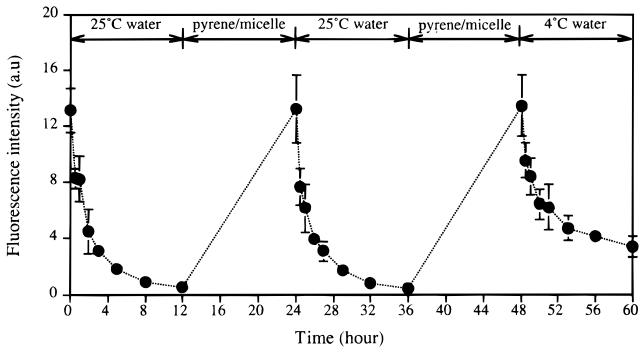


Figure 3. Change in fluorescence intensity of aminated glass coated with hexapoid layer of micelle after exposure to the pyrene-loaded micelle solution. The solutions used for the sample exposure were changed every 12 h as indicated in the upper part of the figure.

micelle solution containing pyrene for 12 h to transfer pyrene from solution to the gel phase. As in Figure 3, the initial fluorescence intensity of pyrene-loaded micellar layer coincides with that of the layer coated with pyrene-preloaded micelles. Pyrene was also released from the gel in the same manner as the coatings of pyrene-preloaded micelles. The loading and release of pyrene can be repeated reproducibly. The third cycle of the pyrene release carried out at 4 °C showed slower decline of fluorescence due to the reduced diffusion of pyrene out of the micelle to water. Since this phenomenon is partially related to the change in the mobility of the PLA segment with temperature, a variation in the release rate may be obtained by the use of block copolymers with a hydrophobic segment that is different from PLA. Observations with scanning probe microscopy showed little degradation of the micellar coatings even if the sample was stored in water at 22 °C for 7 days.[3] The layer of alternate coating is stable, and repetitive use might induce no change in the structure and conformation of the micelle−PAlAm layer.

The thin hydrogel layer made by alternate coating of micelles and PAlAm exhibited nonfouling properties and worked as the reservoir for hydrophobic reagents. The loading capacity and the release-rate of a drug can be controlled by the number of coatings. The thickness of the gel layer is easy to control; each increase in the number of coatings resulted in an increase in the thickness by 30−40 nm. Diffusion and permeation of solute molecules are expected to be regulated with this thin layer of laminated micelles. In the present investigation, the thin micelle layer was prepared on a flat surface. However, the coating can be applied to a variety of surfaces with different shapes and curvatures including microparticles. These surfaces covered with micellar gel should have a nonfouling property, may exhibit little adverse effect on the biological environment, and can be used in diverse fields of medicine and biology to construct high-performance medical devices and drug delivery systems.

JA993821G

(17) Elbert, D. L.; Herbert, C. B.; Hubbell, J. A. *Langmuir* **1999**, *15*, 5355.
(18) Yokoyama, M.; Miyauchi, M.; Yamada, N.; Okano, T.; Sakurai, Y.; Kataoka, K.; Inoue, S. *J. Controlled Release* **1990**, *11*, 269.
(19) Yokoyama, M.; Miyauchi, M.; Yamada, N.; Okano, T.; Sakurai, Y.; Kataoka, K.; Inoue, S. *Cancer Res.* **1990**, *50*, 1693.
(20) Yokoyama, M.; Okano, T.; Sakurai, Y.; Kataoka, K. *J. Controlled Release* **1994**, *32*, 269.
(21) Kwon, G. S.; Suwa, S.; Yokoyama, M.; Okano, T.; Sakurai, Y.; Kataoka, K. *J. Controlled Release* **1994**, *29*, 17.