CHEMISTRY OF MATERIALS
Article

Chem. Mater. **2010,** *22,* 2923–2929 2923
DOI:10.1021/cm1002302

Fabrication of Surface-Modified Hydrogels with Polyion Complex for Controlled Release

Yukie Takemoto,[†] Hiroharu Ajiro,[†,‡] Taka-aki Asoh,[†] and Mitsuru Akashi*[,†,‡]

[†]*Department of Applied Chemistry, Graduate School of Engineering, Osaka University, 2-1 Yamada-oka, Suita, Osaka 565-0871, Japan, and* [‡]*The Center for Advanced Medical Engineering and Informatics, Osaka University, 2-2 Yamada-oka, Suita, Osaka, 565-0871, Japan*

Received January 25, 2010. Revised Manuscript Received March 24, 2010

Surface-modified hydrogels with a polyion complex composed of poly(vinylamine) (PVAm) and poly(acrylic acid) (PAAc) were prepared in order to control the release of drug molecules without a volume change of the hydrogel. We first prepared a poly(*N*-vinylacetamide)-*co*-poly(*N*-vinylformamide) (poly(NVA-*co*-NVF)) hydrogel, and then used a hydrolysis reaction to produce a cationic PVAm layer on the surface of the hydrogel. The polymerization of AAc to the surface-cationized hydrogel resulted in a hydrogel that possesses a polyion complex (PIC) of PVAm and PAAc only on the surface. This surface-PIC hydrogel (sPIC gel) could suppress the release of a model drug (Fluorescein isothiocyanate labeled Dextran, M_w = 9500) under neutral pH conditions because of the tight PIC surface layer, and repeatedly controlled the drug release against the pH conditions depending on the formation and dissociation of PIC. Controlled release was achieved without a large volume change, because the PIC layer was thin enough to maintain the original size of the hydrogel. Furthermore, the sPIC gel retained a larger amount of model drug as compared to the PIC gel, which possesses the polyion complex from the surface to the inside of the hydrogel. Consequently, the surface-modified hydrogel with PIC, that is sPIC gel, is useful for controlled drug delivery systems that require a constant volume and large drug loading.

Introduction

Controlled release systems with polymeric micelles,[1] nanoparticles,[2] capsules,[3] and hydrogels[4] have attracted much attention because of their potential applications in many applied scientific fields, including pharmaceuticals, medicine, agriculture, and materials science. One of the most suitable materials for controlled release systems is hydrogel, which has a high water content and the capacity for loading the water-soluble drug molecules. Over the last few decades, many studies on hydrogels for sustained release,[5,6] accelerated release speed,[7] pulsatile release systems,[8−10] and drug release in response to specific chemical agents[8] have been reported. Almost all controlled release systems are based on a large volume phase transition, such as squeezing the hydrogel to release a drug molecules[11,12] or expanding the polymer network to allow the diffusion of a drug molecule.[13] However, controlled release based on a large volume change would become a problem when the hydrogel is embedded in a small space such as the defective tissues or in the soil for water retention, because it produces a physical gap between the hydrogel and the exterior. Additionally, a large volume change could involve alterations in the original elasticity and solvent retention capacity of the hydrogel. Thus, a large volume change is not indispensable for controlled release, and a constant volume would actually be preferable.

Probing the key factors that decide the release and suppression of incorporated drug molecules, the interface between the hydrogel and its exterior was discovered to be important. In other words, a hydrogel with a functional surface which works as a switch has the potential to control the release of drug molecules. In fact, some researchers have performed additional surface modifications to hydrogels in recent years. For example, Kiser et al. described lipid bilayer coated microgels which

*Corresponding author. Tel: +81-6-6879-7356. Fax: +81-6-6879-7359. E-mail: akashi@chem.eng.osaka-u.ac.jp.

(1) Kataoka, K.; Harada, A.; Nagasaki, Y. *Adv. Drug. Delivery Rev.* **2001**, *47*, 113.
(2) Otsuka, H.; Nagasaki, Y.; Kataoka, K. *Adv. Drug Delivery Rev.* **2003**, *55*, 403.
(3) Johnston, A. P.R.; Cortez, C.; Alexandra, A. S.; Angelatos, S.; Caruso, F. *Curr. Opin. Colloid Interface Sci.* **2006**, *11*, 203.
(4) Qiu, Y; Park, K. *Adv. Drug Delivery Rev.* **2001**, *53*, 321.
(5) Tabata, Y.; Hijikata, S.; Ikada, Y. *J. Controlled Release* **1994**, *31*, 189.
(6) Fukunaka, Y.; Iwanaga, K.; Morimoto, K.; Kakemi, M.; Tabata, Y. *J. Controlled Release* **2002**, *80*, 333.
(7) Asoh, T.; Kaneko, T.; Matsusaki, M.; Akashi, M. *Macromol. Biosci.* **2006**, *6*, 959.
(8) Kataoka, K.; Miyazaki, H.; Bunya, M.; Okano, T.; Sakurai, Y. *J. Am. Chem. Soc.* **1998**, *120*, 12694.
(9) Yoshida, R.; Sakai, K.; Okano, T.; Sakurai, Y. *Ind. Eng. Chem. Res.* **1992**, *31*, 2339.
(10) Yoshida, R.; Okano, T.; Sakurai, Y.; Sakai, K. *J. Biomater. Sci. Polymer Edn.* **1994**, *6*, 585.
(11) Kaneko, Y.; Yoshida, R.; Sakai, K.; Sakurai, Y.; Okano, T. *J. Membr. Sci.* **1995**, *101*, 13.
(12) Hoffman, A. S.; Afrassiabi, A.; Dong, L. C. *J. Controlled Release* **1986**, *4*, 213.
(13) Dong, L. C.; Hoffman, A. *J. Controlled Release* **1991**, *15*, 141.

Published on Web 04/02/2010

mimic secretory granules[14] and Iskakov et al. employed an alginate gel coated with carboxy-*n*-propylacrylamide to retard the onset release time.[15] Our group has also demonstrated that a surface-modified hydrogel, such as a hydroxyapatite hydrogel coated with $CaCO_3$[16] and alginate hydrogels coated with a multi layer polymer film by chitosan/dextran,[17] can slow the release rate.

The aforementioned hydrogels, however, gradually lose their coating layer during the release period, which means they are unsuitable for repeated use. To overcome the deteriorating control, it is necessary to develop a heterogeneous structure not outside of the hydrogel but rather inside it. In other words, the original hydrogel and the switching layer should be one continuous structure. The fabrications of a heterogeneous structure inside the hydrogel such as multilayer or a core−shell structure has been recently reported,[18−22] although the polymer species were limited. Furthermore, application for controlled release with an inner structured hydrogel, which has a continuous polymer network, has rarely been accomplished.

In this study, we designed a surface-modified hydrogel with a polyion complex (PIC) composed of poly(vinylamine) (PVAm) and poly(acrylic acid) (PAAc), and possessing a continuous polymer network. First, we prepared a copolymer hydrogel composed of poly(*N*-vinylacetamide) (PNVA) and poly(*N*-vinylformamide) (PNVF).[23−25] These polymers are hydrophilic and nonionic, and their amide groups are easily hydrolyzed with acidic or basic aqueous solutions to produce cationic PVAm,[26] which can form a PIC with anionic PAAc.[27] After cationization of the hydrogel surface, anionic AAc was polymerized into the surface-cationized poly (NVA-*co*-NVF) hydrogel to obtain a surface-modified hydrogel with a PIC composed of PVAm and PAAc. Here, we successfully prepared a surface-modified hydrogel with a PIC (surface-PIC gel: sPIC gel), which comprised two segments: a stable swollen segment composed of poly(NVA-*co*-NVF) and PAAc for drug release, and a stimuli responsive layer composed of PIC for controlled drug release. The poly(*N*-vinylamide) network and PAAc network have interpenetrating polymer networks (IPN),[25,28] and thus the two segments are completely covalently bound. We therefore conclude that the sPIC gel makes it possible to achieve the repeated and controlled release of incorporated drug molecules without a large volume change.

Experimental Section

Materials. *N*-Vinylacetamide (NVA) was purchased from Showa Denko (Japan) and was recrystallized from toluene/cyclohexane (1/3) and dried under a vacuum at room temperature. *N*-vinylformamide (NVF) was purchased from Sigma Aldrich (Japan) and was purified by distillation. Fluorescein isothiocyanate labeled dextran (FITC-Dex) was purchased from Sigma Aldrich (Japan). Fluorescein isothiocyanate (FITC) was purchased from Thermo Scientific (Japan). Acrylic acid (AAc) was purchased from Wako Pure Chemical Industries (Japan), purified by distillation, and then neutralized with 5 N NaOH aq. (27 vol%). *N,N*-methylenebisacrylamide (MBAAm), 2,2′-azobis(2-methylpropionamidine)dihydrochloride (V-50), 2-propanol, and potassium hydroxide (KOH) were purchased from Wako Pure Chemical Industries (Japan) and were used as received. *N,N*-5-oxanonamethyene-bis-*N*-vinylacetamide (5ON-bis-NVA) was used as a cross-linker for poly(NVA-*co*-NVF), and was prepared according to a previously reported method[25] (see the Supporting Information). Ammonium persulfate (APS) and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) were purchased from Nakarai Tesque (Japan), and were used without further purification. The various pH solutions (ionic strength (I) = 0.1 M) were prepared with 0.01 M NaOH aqueous solution, 0.01 M HCl aqueous solution, and NaCl purchased from Wako Pure Chemical Industries (Japan).

Preparation of sPIC Gels. First, a poly(NVA-*co*-NVF) hydrogel was prepared. Pregel aqueous mixtures of monomer NVA (0.2 M) and NVF (0.8 M), the cross-linker 5ON-bis-NVA (3 mol % to monomer), N_2 bubbled ultrapure water (8 mL), and the initiator V-50 (1 mol % to monomer) were injected into glass tubes of 3.3 mm internal diameter. After polymerization for 4 h at 55 °C, the obtained hydrogel was adequately rinsed with ultrapure water.

Second, the hydrolysis of the amide groups of poly(NVA-*co*-NVF) hydrogel was performed in order to produce amino groups. Poly(NVA-*co*-NVF) hydrogels cut into a cylindrical shape of 10 mm length and 4.4 mm diameter were immersed into a 2-propanol solution to be shrunken. Hydrolysis of the amide groups was then carried out on the shrunken gels in KOH/2-propanol (5 wt %) at 80 °C for the required time. After hydrolysis, the resulting gels were rinsed with aqueous buffer solution (pH 7.4).

Finally, anionic AAc was polymerized into the surface cationized hydrogel. Pregel aqueous mixtures of the monomer sodium acrylate solution (0.25 M), the cross-linker MBAAm (5 mol % to monomer), the initiator APS (0.5 mol % to monomer), hydrolyzed poly(NVA-*co*-NVF) hydrogels and N_2 bubbled 0.01 M HCl aq. (to 16 mL) were kept at 4 °C for 12 h to immerse the hydrogels into the monomer solution. TEMED (6.9 μL) was added to the monomer solution at 1 h before polymerization, and then the polymerization was carried out for 8 h at 37 °C. The obtained hydrogels were rinsed with a large amount of 0.1 M NaCl aqueous solution.

FITC-Labeling of Amino Groups of the Hydrolyzed Poly(NVA-*co*-NVF) Hydrogel. The hydrolyzed gels were sliced vertically, and immersed into a FITC solution (7.5 μg/mL,

(14) Kiser, P. F.; Wilson, G.; Needham, D. *Nature* **1998**, *394*, 459.
(15) Iskakov, R. M.; Kikuchi, A.; Okano, T. *J. Controlled Release* **2002**, *80*, 57.
(16) Ogomi, D.; Serizawa, T.; Akashi, M. *J. Controlled Release* **2005**, *103*, 315.
(17) Matsusaki, M.; Sakaguchi, H.; Serizawa, T.; Akashi, M. *J. Biomater. Sci., Polym. Ed.* **2007**, *18*, 775.
(18) Ladet, S.; David, L.; Domard, A. *Nature* **2008**, *452*, 76.
(19) Dai, H.; Li, X.; Long, Y.; Wu, J.; Liang, S.; Zhang, X.; Zhao, N.; Xu, J. *Soft Matter* **2009**, *5*, 1987.
(20) Jones, C. D.; Lyon, A. *Macromolecules* **2000**, *33*, 8301.
(21) Berndt, I.; Pedersen, J. S.; Richtering, W. *Angew. Chem., Int. Ed.* **2006**, *45*, 1737.
(22) Iizawa, T.; Matsuura, Y.; Onohara, Y. *Polymer* **2005**, *46*, 8098.
(23) Akashi, M.; Yashima, E.; Yamashita, T.; Miyauchi, N.; Sugita, S.; Marumo, K. *J. Polym. Sci., Part A* **1990**, *28*, 3487.
(24) Akashi, M.; Saihata, S.; Yashima, E.; Sugita, S.; Marumo, K. *J. Polym. Sci., Part A* **1993**, *31*, 1153.
(25) Ajiro, H.; Takemoto, Y.; Akashi, M. *Chem. Lett.* **2009**, *38*, 368.
(26) Yamamoto, K.; Imamura, Y.; Nagatomo, E.; Serizawa, T.; Muraoka, Y.; Akashi, M. *J. Appl. Polym. Sci.* **2003**, *89*, 1277.
(27) Ajiro, H.; Takemoto, Y.; Asoh, T.; Akashi, M. *Polymer* **2009**, *50*, 3503.
(28) Asoh, T.; Akashi, M. *Chem. Commun.* **2009**, 3548.

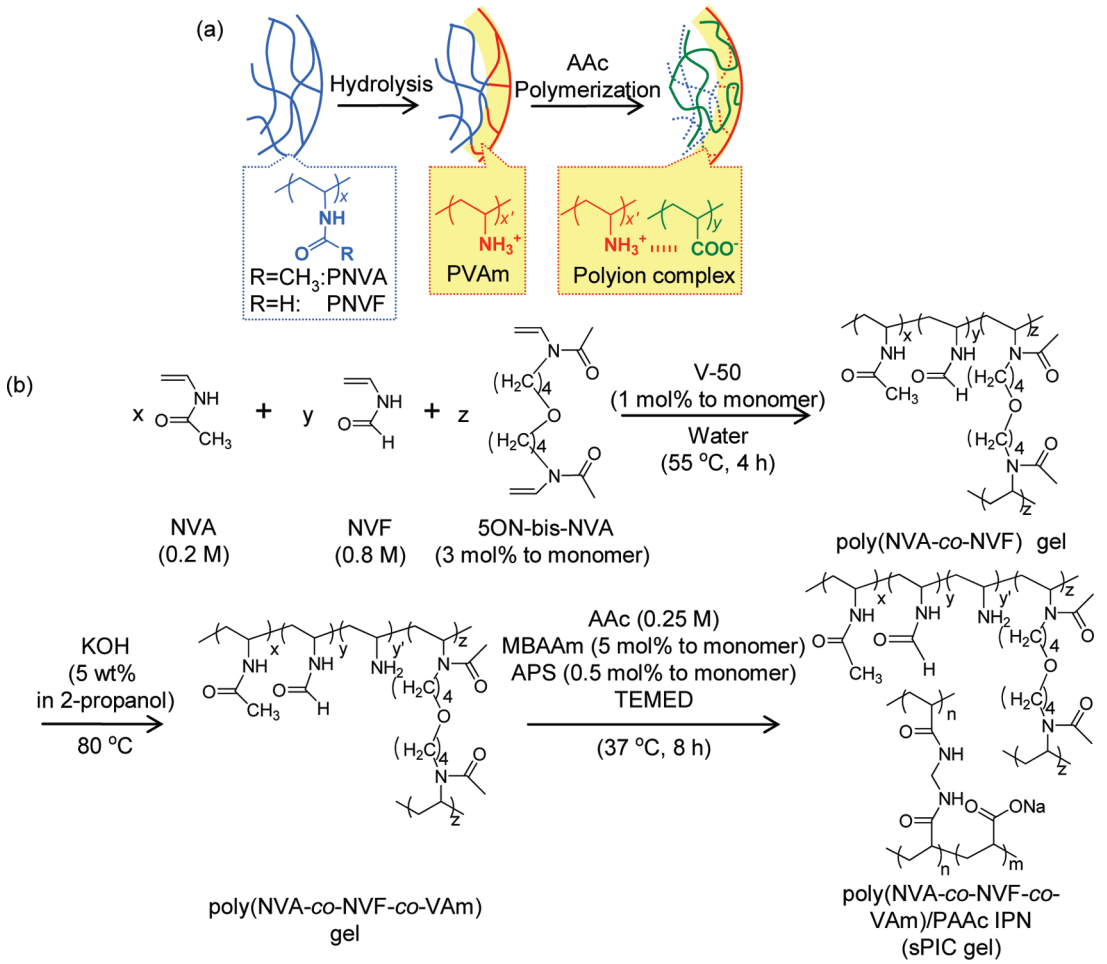

Figure 1. Preparation of a surface polyion complex hydrogel (sPIC gel). (a) Schematic illustration of preparing an sPIC gel in three steps. (1) Preparation of a poly(NVA-*co*-NVF) hydrogel. (2) Hydrolysis of the amino group in poly(NVA-*co*-NVF) (3) AAc polymerization to obtain an sPIC gel. (b) Scheme of preparation of sPIC gel.

$NaCO_3/NaHCO_3$ buffer (pH 9.5), 2 mL) for 12 h. After rinsing with buffer solution, a cross-section of the gel was observed with confocal fluorescence microscopy (OLYMPUS, IX81S1F-3, Japan).

Scanning Electron Microscopy (SEM). The morphologies of a cross section of the sPIC gel and non-sPIC gel (poly(NVA-*co*-NVF)/PAAc hydrogel) were observed by FE-SEM (JEOL, JSM-6701F, Japan) at 5−10 kV. The freeze-dried gel fixed on metallic supports with carbon tape was then coated with osmic acid.

Drug Release Experiment. FITC-Dex (M_w = 9500, λ_{ex} = 490 nm, λ_{em} = 517 nm) was used as a model drug molecule. Cylindrical-shaped sPIC hydrogels prepared with various hydrolysis time were immersed into a FITC-Dextran solution of high ionic strength (0.5 g/L in 2 M NaCl aq.; PIC dissociation conditions) and subsequently a FITC-solution of low ionic strength (0.5 g/L in 0.1 M NaCl aq.; PIC formation conditions). The drug loaded hydrogels were immersed into various pH aqueous solution with ionic strengths = 0.1 M, in which the supernatants were measured by a fluorescence spectrophotometer (JASCO, FP-6500, Japan). The release percentages were determined and compared to the total amount of FITC-Dex released at pH 2 (PIC dissociation condition) as a standard. The amount of the released FITC-Dex was decided by the calibration curve obtained from the fluorescence intensity of the FITC-Dex aqueous solution with various concentration.

Results and Discussion

Preparation of Surface Polyion Complex Gel (sPIC Gel). The sPIC gel was prepared in 3 steps as follows: (1) preparation of poly(NVA-*co*-NVF) hydrogel, (2) hydrolysis of the amide groups on the surface of poly(NVA-*co*-NVF) hydrogel, and (3) polymerization of AAc in the hydrolyzed poly(NVA-*co*-NVF) hydrogel (Figure 1). First, the poly(NVA-*co*-NVF) hydrogel was prepared by the free-radical polymerization of NVA, NVF, and the cross-linker in a glass tube. The obtained hydrogels were transparent, and were cut into a cylindrical shape with lengths and diameters of 10 mm and 4.4 ± 0.1 mm, respectively. Second, poly(NVA-*co*-NVF) hydrogels which were preshrunk in 2-propanol were immersed into a KOH/2-propanol solution (5 wt %) at 80 °C for the required time to hydrolyze the amide group of poly(NVA-*co*-NVF). Because the cross-linker of poly(NVA-*co*-NVF) is not cleavable by hydrolysis reaction, the poly(NVA-*co*-NVF) hydrogel can maintain its gel state during the hydrolysis reaction. Before hydrolysis, the poly(NVA-*co*-NVF) hydrogel was opaque and shrunken in 2-propanol, because poly(NVA-*co*-NVF) was insoluble in 2-propanol. During the hydrolysis reaction, the shrunken poly(NVA-*co*-NVF) gel gradually swelled

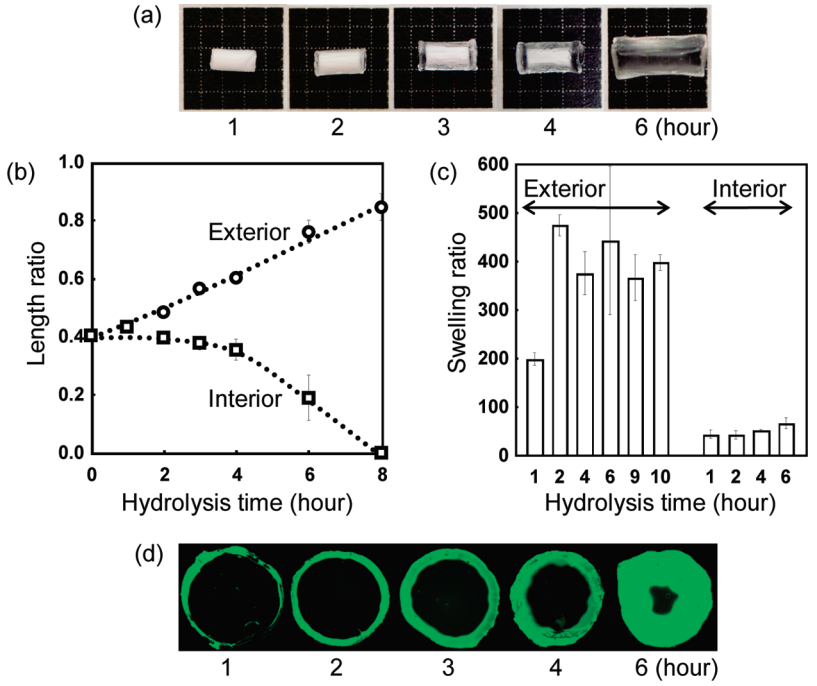

Figure 2. Surface hydrolysis of a poly(NVA-*co*-NVF) gel shrunken with 2-propanol. (a) Images of gels after hydrolysis. (b) Volume change during hydrolysis. The entire size (exterior, open circle) and shrunken part (interior, open square) were measured and the length ratio was calculated. Length ratio = D/D_0, where D is the diameter of the hydrolyzed gel and D_0 is the diameter of the poly(NVA-*co*-NVF) hydrogel (4.4 mm), respectively. (c) Swelling ratio of the outer and inner parts of a hydrolyzed poly(NVA-*co*-NVF) hydrogel. Swelling ratio = $(W_s - W_d)/W_d$, where W_s and W_d are the weight of the swollen and dried hydrogels, respectively. (d) FITC-labeled poly(NVA-*co*-NVF-*co*-VAm) hydrogel. The scale bar in parts a and d is 2 mm.

from its surface outward to create a transparent exterior and an opaque shrunken core (Figure 2a). The transparent exterior is expected to contain PVAm, because the linear poly(NVA-*co*-VAm) is more soluble in KOH/2-propanol as compared to linear poly(NVA-*co*-NVF). The diameters of swollen and shrunken segments were measured and length ratio was calculated. The length ratio was defined as D/D_0, where D was the diameter of each segment and D_0 was the diameter of poly(NVA-co-NVF) hydrogel (4.4 mm). The swollen segment became larger (Figure 2b, exterior), whereas the shrunken core became smaller with increasing hydrolysis time (Figure 2b, interior). This difference between the outside and inside implies that the thickness of the PVAm layer, which became larger depending on the hydrolysis time (Table 1).

To confirm the presence of the amino moiety, the equilibrium swelling ratio, hydrolysis ratio calculated by elemental analysis, and the labeling of amino groups with fluorescein isothiocyanate (FITC) were all examined. The swollen and shrunken segments in Figure 2a were cut off and reswelled in ultrapure water to estimate the swelling ratio. Figure 2c shows the equilibrium swelling ratio (SR) of each segment. The SR of the originally swollen segment was about 300, whereas that of the originally shrunken segment was about 20, showing dramatically different values. Because electrolyte polymers such as PVAm show a large swelling ratio due to electrostatic repulsion and osmotic pressure, this result indicates the presence of cationic PVAm on the outside and nonionic poly(NVA-*co*-NVF) inside the core of the hydrogel.

The hydrolysis ratio of the amide group in the swollen exterior was quantitatively estimated by elemental analysis (see Table S1 in the Supporting Information). With increasing hydrolysis time, the hydrolysis ratio became higher to produce more PVAm, which forms a polyion complex with PAAc, as shown in a previous report.[27]

Table 1. Thickness of the PVAm Layers and a PIC Layer Depending on the Hydrolysis Time

entry	hydrolysis time (h)	PVAm segment[a] (in 2-propanol) (mm)	PVAm segment[b] (in buffer solution) (mm)	PIC layer[c] (in buffer solution) (mm)
1	1	0	0.51 ± 0.03	0.49 ± 0.06
2	2	0.38 ± 0.06	0.68 ± 0.01	0.48 ± 0.07
3	3	0.53 ± 0.08	1.08 ± 0.03	0.75 ± 0.09
4	4	1.09 ± 0.14	1.46 ± 0.10	1.04 ± 0.05

[a] Estimated from the difference of the swollen segment and the shrunken core in Figure 2b. [b] The thickness of the green fluorescent segment was measured in Figure 2d. [c] The thickness of the green fluorescent segment was estimated by a line scan of images in Figure 3d.

To visually confirm the amino group, we vertically sliced the hydrolyzed poly(NVA-*co*-NVF) hydrogel and labeled new cross-sections with FITC, which reacts with amino groups. As shown in Figure 2d, the circumference of the cross-section showed the green fluorescence of FITC, whereas the center remained dark. Furthermore, the FITC-labeled segment became thicker depending on the hydrolysis time, suggesting that the thickness of the amine segment is controllable (Table 1). Thus, performing the hydrolysis reaction on the shrunken gel in 2-propanol, we successfully carried out the hydrolysis reaction to produce cationic PVAm only on the surface of the hydrogel, leaving the center as nonionic poly(NVA-*co*-NVF).

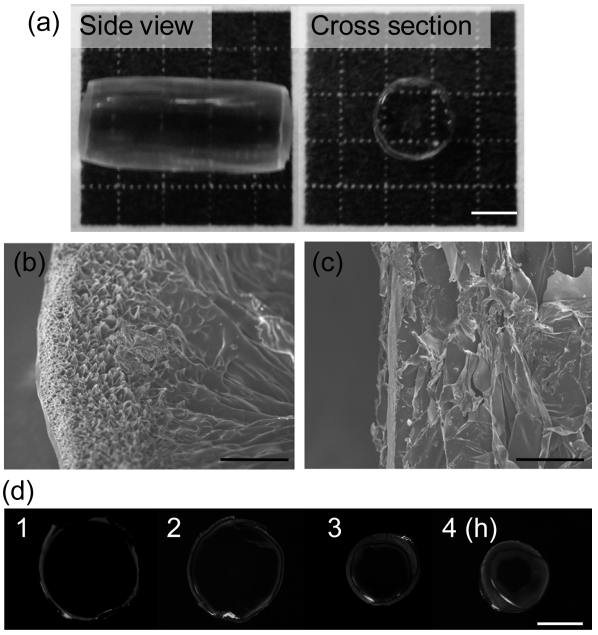

Figure 3. Surface polyion complex hydrogel (sPIC hydrogel). (a) Side view (left) and cross-section (right) of an sPIC hydrogel. (b) SEM image of a freeze-dried sPIC gel. (c) SEM image of a poly(NVA-*co*-NVF)/PAAc IPN gel (non-sPIC gel). (d) Cross-section of an sPIC gel with a FITC-labeled amino group. The scale bar in part a is 2 mm, that in parts b and c is 50 mm, and that in part d is 5 mm.

Next, AAc and the cross-linker were polymerized in the surface-cationized hydrogel to obtain a surface polyion complex hydrogel (sPIC gel). Figure 3a shows photographic images of an sPIC gel from the side view and cross section. From the side view, the surface of the gel is dense and opaque because of PIC formation. From the cross sectional view, the interior of the gel is transparent, indicating that there was no complexation. The cross-section of the freeze-dried sPIC gel was observed by scanning electron microscopy (SEM) (Figure 3b). When compared with the SEM image of the poly(NVA-*co*-NVF)/PAAc gel (Figure 3c), the sPIC gel has a graded mesh structure with much a denser network close to the surface. The structure of the surface side (left side) was denser than the inner side (right side) in Figure 3b. The SEM images also show polyion complex formation on the surface of the poly(NVA-*co*-NVF) hydrogel.

As shown in Figure 2 and Table 1, the thickness of the PVAm segment is controllable by the hydrolysis time. Therefore, the thickness of the PIC segment of the sPIC gels is also expected to change. In order to check the thickness of the PIC, the FITC-labeling of the PIC layer was performed as mentioned in the Experimental Section. After labeling the amino group with FITC, AAc polymerizations were performed in those hydrogels to obtain sPIC gels with the PIC layer labeled with FITC. The cross-section of each cylindrical shaped hydrogel was observed by fluorescence microscopy to show green fluorescence outside of the gel (Figure 3d). We conducted a line scan of those images, and estimated the length of the fluorescent segment (Table 1). The PIC layer was thinner than the PVAm segment, which means that PVAm segment shrunk due to the PIC formation after AAc polymerization. The thickness of the green fluorescent segment became thicker with increasing hydrolysis time. With a hydrolysis reaction from 1 to 4 h, the thickness of the labeled layer increased from 0.5 to 1 mm. In spite of almost the same value in the fluorescence microscope observation (Table 1, entries 1 and 2), the structure of PIC layer prepared via 2 h hydrolysis is assumed to be tighter because the amount of amino groups for PIC formation[27] (see Table S1 in the Supporting Information) and the thickness of PVAm layer became larger with increasing the hydrolysis time. Because the amino groups are present in the fluorescence-labeled segment to form PIC with PAAc, the fluorescent segment implies PIC segment. Thus, the thickness of the PIC layer was controllable by the hydrolysis reaction time. sPIC gels with various thicknesses of PIC continuous with the original hydrogel network were successfully prepared.

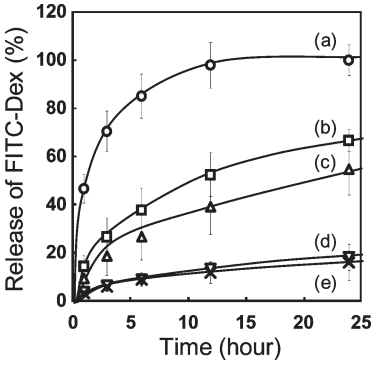

Figure 4. Release of FITC-Dextran from an sPIC gel with various PIC thicknesses (prepared by various hydrolysis times). The open circles, squares, triangles, reverse triangles, and crosses indicate a 0 (0 h), 0.5 (1 h), 0.5 (2 h), 0.7 (3 h), and 1 mm (4 h) thickness of the PIC layer, respectively.

Suppression of FITC-Dex Release by The PIC Layer Fabricated on the Hydrogel Surface. Drug release experiments on the sPIC gels obtained with PIC layers of various thicknesses were performed using FITC labeled Dextran (FITC-Dex, M_w = 9500) as a model drug molecule. Figure 4 shows the release profile of FITC-Dex from sPIC gels of various PIC thicknesses at pH 7.4. With an increase in the PIC thickness, the amount of FITC-Dex released was suppressed, while all of the FITC-Dex was released from the hydrogel without a PIC segment (hydrolysis time = 0 h, Figure 4a). It was shown that an sPIC gel with a PIC thickness greater than 0.75 mm (Figure 4d,e; hydrolysis time >3 h) can effectively suppress the release of FITC-Dex.

It is considered that this release suppression was caused not by electrostatic interaction but by the size of the drug molecule and the mesh size of the polymer network, because nonionic FITC-Dex does not interact with PIC composed of PVAm and PAAc at any pH value, including pH 7.4. When an sPIC gel was immersed in FITC-Dex solution for the incorporation of drug molecules, the ionic strength (I) of the FITC-Dex solution was high enough (I = 2 M) to dissociate the PIC. In the PIC dissociated state, FITC-Dex could penetrate the PIC layer for incorporation into the sPIC gel. At a lower ionic

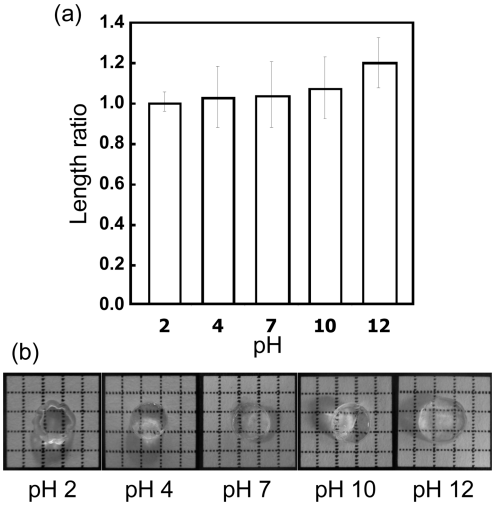

Figure 5. Volume of sPIC gels under various pH conditions (n = 3). (a) Length ratio of a cylindrical shaped sPIC gel. Length ratio = D/D_0, where D is the diameter of the sPIC gel and D_0 is the diameter of the poly-(NVA-*co*-NVF) hydrogel, respectively. (b) Images of the cross-section of an sPIC gel.

strength (I = 0.1 M), the cationic PVAm and anionic PAAc formed a PIC to aggregate the polymer network, resulting in minimizing the mesh size of the hydrogel. Therefore, the FITC-Dex could not be released smoothly because of the small mesh size. Thus, a PIC layer fabricated on the surface of a hydrogel with 3 h of hydrolysis reaction could effectively suppress FITC-Dex release. In short, the suppression ratio of FITC-Dex was controllable by the PIC thickness, which is easily controllable by the hydrolysis time. The release of FITC-Dex was effectively suppressed by sPIC gels with a 0.75 mm PIC thickness prepared by 3 h of hydrolysis.

Volume Change of sPIC Gels under Various pHConditions. The volume change of sPIC gels depending on the pH was investigated for controlled release with a stable volume. The length ratio of cylindrical shaped sPIC gels under various pH solutions are shown in Figure 5. The length ratios of the sPIC gels were almost constant at any pH. Furthermore, the SR, which was calculated from the weight of the gel was quite stable (SR = 18 ± 3). For PIC gels, which formed a polyion complex with PVAm and PAAc from the surface to the inside of the hydrogel as a negative control, the length ratio almost doubled at pH 2 and 12 as compared to pH 4−10 (see Figure S1 in the Supporting Information). The SR of the PIC gel changed enormously depending on the pH, and shrank to less than 5 at neutral pH, but increased to more than 20 under acidic and basic conditions.

This result can be explained by PIC formation and dissociation depending on the pH. In a previous study, we reported that PVAm and PAAc formed a polyion complex over the range of pH 4 to 10, but dissociated at pH 2 or pH 12 because the pK_a values of PVAm and PAAc are about 10 and 4, respectively.[27] The volume of the PIC gel was dramatically changed under various pH conditions because PIC formation occurred from the surface to the interior of these hydrogels. However, the fabrication of a PIC layer on the hydrogel surface allowed it to keep the volume of the hydrogel constant, because a PIC layer of submicrometer thickness is too thin to alter the total volume of the hydrogel.

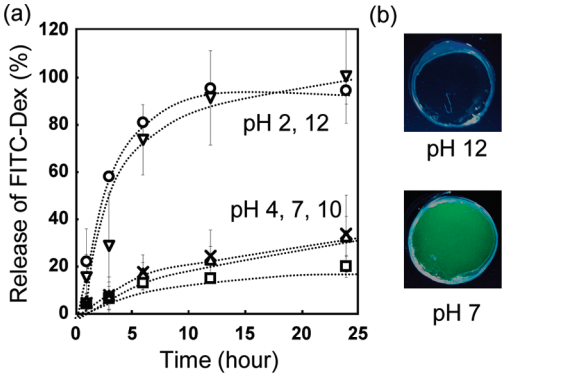

Figure 6. (a) Release of FITC-Dextran at various pH conditions from an sPIC gel prepared with a 0.7 mm PIC thickness (n = 3). The open circles, squares, triangles, crosses, and reverse triangles indicate pH 2, 4, 7, 10, and 12, respectively. (b) The cross-ections of an sPIC gel after releasing FITC-Dex for 24 h in pH 12 and 7 aqueous solutions.

Controlled release with a stimuli-responsive gel usually requires a large volume change like a PIC gel. The sPIC gel, however, does not need this large volume change because it has an intelligent switchable layer on the surface. Furthermore, the interior space of the sPIC gel has the potential to sequester drug molecules effectively.

pH Responsive Release of FITC-Dex. pH responsive controlled release is required for biomedical and environmental applications. The sPIC gel has the potential ability for pH responsive release due to the PIC formation and dissociation fabricated on the surface of the hydrogel. FITC-Dex was released in various pH aqueous solutions from an sPIC gel with a PIC thickness of 0.75 mm, prepared by 3 h of hydrolysis, which can effectively suppress drug release (Figure 4d). Figure 6 shows the release profiles of FITC-Dex from an sPIC gel under various pH conditions. FITC-Dextran was released completely at pH 2 and 12, but it was suppressed over the range of pH 4 to 10. After releasing for 24 h at pH 7, the remaining FITC-Dex within the hydrogel was confirmed by fluorescence microscopy (Figure 6b).

This result can be explained by a pH responsive volume change due to PIC formation and dissociation. As a negative control, a PIC gel which contained PVAm and PAAc from the surface to the interior of the hydrogel was tested in the releasing experiment. FITC-Dex was released at pH 2 and 12, but was suppressed over the range of pH 4 to 10 in the same manner as the sPIC gel (see Figure S2 in the Supporting Information). As mentioned above, the PIC gel changes its volume to cause the mesh size to become narrower or to expand. At PIC formation conditions (pH 4−10), the polymer network is dense and stops the release of FITC-Dex. At PIC dissociated conditions (pH 2 or 12), the polymer networks are expanded to allow the release of FITC-Dex. In the case of the PIC gel, this is due to the large volume change induced by PIC formation and dissociation. However, when the sPIC gel was employed, the PIC should be formed on the surface of the hydrogel, and a very small volume change occurred on

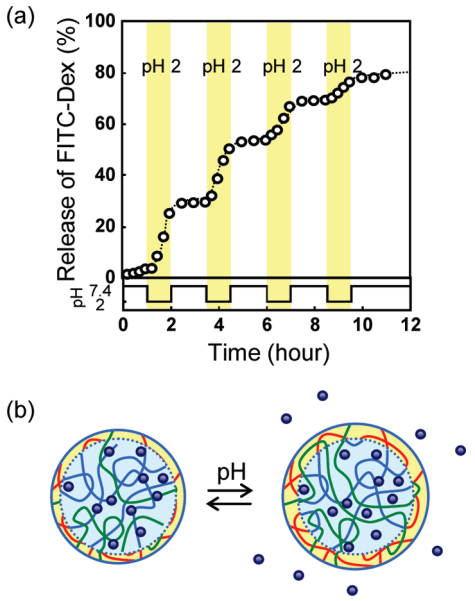

Figure 7. (a) Pulsatile release of FITC-Dextran from an sPIC gel prepared with 3 h of hydrolysis in pH 2 and pH 7.4 aqueous solutions. (b) Schematic illustration of controlled release of drug molecules from the sPIC gel.

the surface of the hydrogel to control the release of FITC-Dex. Thus, the PIC layer could work as a switching region for controlled drug release. In addition to controlled release with a stable volume, the sPIC gel can retain more drug molecules as compared to a PIC gel. The amount of FITC-Dex released from an sPIC gel and PIC gel is about $22 \pm 6\ \mu g$ (0.3 wt % to dry sPIC gel) and $12 \pm 3\ \mu g$ (0.17 wt % to dry PIC gel), respectively. Furthermore, sPIC gel can release more FITC-Dex when the hydrogel is immersed into FITC-Dex solution with higher concentrations for a drug loading. The final amount of FITC-Dex released was 15 ± 1, 22 ± 7, and $39 \pm 15\ \mu g$ when concentrations of the FITC-Dex solutions for incorporation of drug molecules are 0.25, 0.5, and 1 g/L, respectively. Thus, the swollen segment of the sPIC gel can retain drug molecules effectively, whereas shrunken PIC gel has a disadvantage in sequestering the drug molecule.

As a result, a pH-responsive controlled release of FITC-Dex was achieved with an sPIC gel and the amount of the FITC-Dex released from the sPIC gel was larger than that from a PIC gel, which is an example of commonly used stimuli-responsive hydrogel.

Repeatedly Controlled Release from the sPIC Gel. As mentioned in the introduction, a surface modified hydrogel for controlled release is usually prepared by depositing other polymers or minerals onto the hydrogel, which should be dissolved during the release of the incorporated molecules. This means that the incorporated molecules cannot be stopped once the surface layer has dissolved. However, the sPIC gel could be used for repeated controlled release in an on−off state, because the sPIC gel has a continuous network between the inner drug retention segment and the surface switching segment. Figure 7a shows the results of the pulsatile release of FITC-Dex from the sPIC gel upon changing the pH conditions between 7.4 and 2. The rapid release of FITC-Dex from the sPIC gel at pH 2 was effectively shut off upon changing the pH to 7.4, and was repeatedly controllable. The sharp response of this ON−OFF releasing is attributed to the fact that the PIC layer was very thin, and took a very short time to accomplish its switching function when the charge changed on PVAm. During repeatedly controlled release cycles, the volume of the hydrogel did not change, as shown in Figure 5. Thus, the sPIC gel can precisely control the release of the incorporated molecules without depending on a volume change (Figure 7b).

Conclusion

The sPIC gel, a surface-modified hydrogel with a PIC layer, was successfully prepared via hydrolysis of the surface of poly(NVA-*co*-NVF) gel, with the subsequent polymerization of AAc. The thickness of the PIC was easily controllable by the hydrolysis time. A PIC layer of submicrometer thickness can suppress the release of FITC-Dex as a model drug molecule. The pH-responsive release of FITC-Dex from an sPIC gel was accomplished without a large volume change of the hydrogel. The sPIC gel has a continuous polymer network, and possesses different functions between the surface and the interior of the hydrogel; the surface segment is designed for the controlled release of drug molecules, and the interior of the gel is designed for stable drug retention. This well-designed hydrogel is expected to be useful as a novel drug release system for tissue engineering and green businesses.

Acknowledgment. This study was partly supported by a Grant-in-Aid for Young Scientists (B) No. 21750220 from the Ministry of Education, Culture, Sports, Science and Technology (MEXT). The authors thank Drs. T. Kida, M. Matsusaki, J. Watanabe, and T. Akagi for fruitful discussion.

Supporting Information Available: Synthetic schemes for the cross-linker (5ON-bis-NVA); elemental analysis of hydrolyzed poly(NVA-*co*-NVF) hydrogels; length ratio of cylindrical-shaped PIC gel under various pH conditions; releasing profile of FITC-Dex from a PIC gel; cross-section of the sPIC gel after releasing FITC-Dex (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.