Transactions of the Materials Research Society of Japan 32[4] 1243-1246 (2007)

Biocompatible Phospholipid Polymer Hydrogel Layer on Metal Surface for Releasing Bioactive Agents

Jiyeon Choi, Tomohiro Konno, Madoka Takai and Kazuhiko Ishihara*
Department of Materials Engineering, School of Engineering, and Center for NanoBio Integration, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
Fax: 81-3-5841-8647, e-mail: ishihara@mpc.t.u-tokyo.ac.jp

We have prepared new type biocompatible, water-soluble phospholipid polymer composed of phosphorylcholine units and phenyl boronic acid units. This polymer could make polymer hydrogel multilayer combined with polyvinyl alcohol (PVA). By using photo-reactive PVA and silanized titanium, durable chemical bonding between titanium and the polymer hydrogel was achieved by UV-irradiation. The final material of multilayered polymer hydrogel on titanium was constructed by utilizing the layer-by-layer method through ester complex of boronic acid and diol. Furthermore, the release study of anticancer agent paclitaxel dissolved in a certain interlayer of polymer hydrogel was performed. As the surfaces had changed from bare to modified titanium, the contact angles have changed depending on the surface properties. According to changing surfaces, the characteristic signals of X-ray photoelectron spectroscopy were also observed. And the contact angles reproducibly have alternated as the outermost layer is changed from PVA to PMBV60. As the locations of PTX layer in the polymer hydrogel multilayer changed, the releasing profile toward the time of PTX could be controlled.

Key words: MPC polymer, in situ hydrogel, layer-by-layer method, surface modification, drug release

1. INTRODUCTION

Titanium and its alloys have high mechanical strength and good biocompatibility such as resistance to chemical attack. As a result, they enjoy widespread use as surgical implants that are hip prostheses, dental implants and stents [1, 2].

In particular, a stent is a small, expandable wire mesh in a hollow cylinder form that is used in the treatment of coronary artery disease for maintaining vessel open. However, stent implantation tends to cause inflammatory response and crucial injury to the blood vessel giving rise to neointimal proliferation, known as in-stent restenosis [3]. In order to improve the biocompatibility of stents, drug-eluting stents (DES), which are covered with polymer matrix enabling single or multiple bioactive agents to release in a controlled manner into blood vessels after implantation, have been developed through a combination of understanding the biology of restenosis. DES has been accepted to be quite effective and promising treatment methods for preventing restenosis.

Various drugs are used for inhibiting inflammation and neointimal formation after stent implantation. Paclitaxel is one of the drugs for pharmacological intervention in in-stent restenosis. It binds to the beta tubuline subunit of microtubules and antagonizes their disassembly. Also, it inhibits smooth muscle cell migration and proliferation [4].

Since first introduced the method of sequential adsorption of charged polymers by Decher in 1991 [5], building up of organic multilayer films in a layer-by-layer method has attracted a great deal of attention. Electrostatic force has been used as the main driving force for constructing multilayer films. However, hydrogen bond and covalent bond are also used extensively to induce ordering polymers [6-8]. Specifically, this study employs the idea of covalent bonding-driven self-assembly to produce polymer hydrogel multilayer on titanium alloy substrates. The polymer we have adopted is the phospholipid polymer (PMBV) containing 2-methacryloyloxyethyl-phosphorylcholine (MPC), *n*-butyl methacrylate (BMA) and 4-phenylboronic acid unit. The MPC polymers inhibit protein adsorption and cell adhesion when they contact human whole blood without an anticoagulant. Therefore, it is widely used in biomedical field [9-11]. On the other hand, phenylboronic acid is known to the rapid formation of a cyclic boronic ester with cis-diol [12]. These diol complexes include carbohydrates such as glucose, catechol derivatives such as dopamine, and some polymers such as polyvinyl alcohol (PVA) and so on [13]. Interpolymer complexation of polymer comprising of boronic acid with PVA was reported to form a hydrogel due to the covalent linkage in both constituent polymers [14, 15]. It is expected that layer-by-layer deposition method enables the combination of PMBV and PVA to produce polymer hydrogel multilayer, which is able to utilize controlled drug delivery system.

2. MATERIALS AND METHODS

2. 1. Materials

MPC was synthesized by previous reported method [16]. *n*-butyl methacrylate (BMA) was purchased from

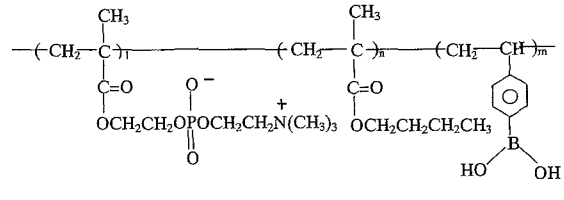

Fig. 1. Structure of PMBV60

Table 1. Synthesis result of PMBV60

Abb.	Monomer unit composition in polymer (mol%)			Molecular weight	Yield (%)
	MPC	BMA	VPBA	Mw(x10^4)	
PMBV60	57	25	18	6.5	70

Nacalai Tesque Co. Ltd. (Tokyo, Japan). 4-Phenylboronic acid (VPBA) was purchased from Sigma Aldrich Co. 2, 2'-Azobisisobutyronitrile (AIBN) were purchased from Kanto Chemical Co. Poly(vinyl alcohol) (PVA, Dn=1500) and paclitaxel (PTX) were purchased from Wako Pure Chemical Industries, Ltd. Octadecyltriethoxysilane (ODS) was purchased from ShinEtsu Chemical Co Ltd. Photoreactive polyvinyl alcohol (AWP, Azide-unit pendent water soluble photopolymer) was purchased from Toyo Gosei Co. Ltd., Japan. The structure of AWP indicates Fig. 2. The molecular weight of PMBV60 polymer was determined by gel-permeation chromatography (GPC). Poly (ethylene oxide) standards were purchased from Tosoh (Tokyo, Japan) and used without further purification. All other reagents were of extra-pure reagent grade.

2. 2. Synthesis of PMBV60

The synthesis of PMBV60 was executed by the conventional radical polymerization of the corresponding monomers as follows: the desired amount of MPC, BMA and VPBA was dissolved in ethanol in an ampoule. The total concentration of monomer was adjusted to 1 mol/L. The AIBN as an initiator was added to the ampoule at the concentration of 1 mmol/L. Argon gas was bubbled into the solution for 10 min to eliminate oxygen and then the ampoule was sealed. The polymerization was carried out at 60 °C for 2.5 h. After cooling, the content was poured into a large amount of diethylether and chloroform (8:2 by volume) to remove any unreacted monomers and to yield the PMBV60. The precipitant was collected and dried *in vacuo*. The structure of the copolymer was confirmed with 1H-NMR (α-300, JEOL, Tokyo, Japan) and Fourier transform infrared spectrometer (FT-IR) (FT/IR-615, JASCO, Tokyo, Japan). The molecular weight was determined by GPC. The chemical structure of PMBV60 polymer is shown in Fig. 1.

2. 3. Preparation of Ti surfaces and silanization

Square Ti samples approximately 10 x 10 mm were prepared from Titanium sheet (0.5 mm thick). These have been sonicated for 15 min in acetone and then in ethanol by the same manner. After drying in air, the samples were immersed in a 3:1(v/v) concentrated H_2SO_4 and 30 % H_2O_2 mixture for 1 h at 25 °C. The samples were rinsed three times with distilled water and dried in an oven at 60 °C. Silanization was immediately carried out after treating the plates in this fashion.

Monolayer of octadecyltriethoxysilane (ODS) was carried out in anhydrous toluene with 10 mM ODS for 5 hours at 80°C. Then, the Ti samples were rinsed in toluene three times and dried *in vacuo*.

2. 4. Photoreactive PVA coating and Preparation of polymeric hydrogel multilayer assemblies

The silanized Ti sample was immersed in an aqueous solution of AWP 1.0 wt% for 15 minutes and dried in an oven at 60°C. Subsequently, the sample was irradiated with ultraviolet light using an UV Spot Light Source L5662 (USHIO Co. Ltd.) for 40 sec.

Then, the AWP coated samples were employed in multilayer construction. The preparation of multilayer assemblies based on the solution-dipping method was achieved by dipping alternately in prescribed PMBV60 aqueous solution and then in prescribed PVA aqueous solution (each time dipped for 15 min). The samples were rinsed with distilled water (each time for 1 min) between these two steps and dried in an oven at 60°C.

2. 5. Drug loading and release

One mg of PTX was dissolved in 1 ml of ethanol and the PTX solution was added to 1 ml of prescribed concentration of PMBV60. Then, the ethanol was removed under the reduced pressure. The same procedure was adopted when constructing polymeric hydrogel multilayer containing PTX by solution-dipping method. Drug release experiments were performed as follows: Samples were submerged in 3 ml of phosphate buffered saline (PBS, pH 7.4) containing 0.1 % (v/v) Tween 20. At defined time intervals the buffer was removed and 0.5 ml of fresh medium was added to the samples. And the release of the drug was monitored using high performance liquid chromatography (HPLC) with a UV detector. HPLC analysis of PTX was performed using a HPLC Tosoh system (mobile phase 50:50 acetonitrile:water 0.5 ml/min, 20 μl injection, C18 column with detection at 229 nm)

2. 6. Surface analysis

The static contact angle of water on the prepared surfaces was measured using the sessile drop method at room temperature (21 °C) using a contact angle goniometer (Erma G-1). Five measurements were made on each sample. X-ray photoelectron spectroscopy (XPS) was performed using AXIS-HSi (Shimadzu/KRATOS, Kyoto, Japan) and XPS data was collected at take-off angel of 30° in dry state.

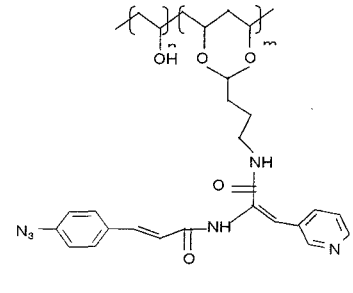

Fig. 2. Structure of photoreactive PVA (AWP)

3. RESULTS AND DISCUSSION

Fig. 3 indicates the contact angle of bared and modified titanium surfaces. The contact angle depends on the surface functional groups. Subsequently, it is used to confirm the change of surface properties. After oxidation in H_2SO_4 / H_2O_2 (Ti oxidation) , a significant decrease in contact angle was observed. However, the silane treatment (Ti-ODS) resulted in much more hydrophobic surface than the oxidazied titanium surface. The contact angle increased from 20° to 130°. It stands to a reason that the silanization produced the surface covered with hydrophobic alkyl chains. Bonding AWP (Ti-ODS-AWP) to the silanized surface through UV-irradiation, the contact angle decreased [17]. And PMBV60 was coated to the next step. Comparatively hydrophobic PMBV60 in comparison with PVA made the contact angle increase a little.

Various titanium surfaces, which are silanized titanium, titanium treated by AWP and titanium coated PMBV60, were analyzed by XPS. Fig. 4 exhibits the XPS spectra systematically. After the silanization of titanium surface was carried out, the peak of Si was observed indicating the presence of silane at a region of 102 eV. By using UV-irradiation for 40 sec, AWP containing azide group ($-N_3$) has bonded to the surface of silanized titanium. The azide group releases N_2 under UV-irradiation and is converted into highly reactive nitrene group which is expected to interact with –C–H on the silanized titanium surface [18]. Peak attributed to nitrogen of AWP was observed at 399 eV. However, after the sample was treated by PMBV60 solution through dip coating method, the phosphorus peak was introduced at a region of 133 eV. That is, the phosphorylcholine groups in the MPC unit were located at the surface. From these results, it is thought that the silanization and the treatment of PVA using UV-irradiation were achieved successfully.

To determine surface wettability changes in a layer-by-layer manner, samples having from 1 to 6 layers of the PMBV60 and PVA bilayer combination were built up and contact angle measured. Three combinations of PMBV60(2.5 wt%)-PVA(1.5 wt%), PMBV60(5.0 wt%)-PVA(1.5 wt%) and PMBV60 (2.5 wt%)-PVA(3.0 wt%) were evaluated. Fig. 5 shows the results of the static contact angle of water determined by sessile drop method. In this case, samples with an even number of layers have PMBV60, whereas samples

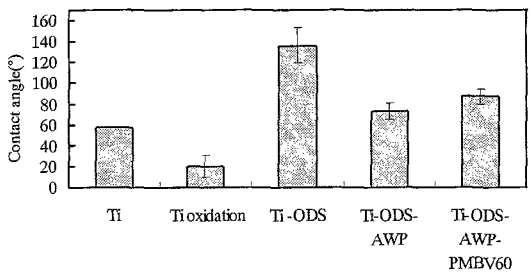

Fig. 3. The static contact angle measured according to changing titanium surfaces.

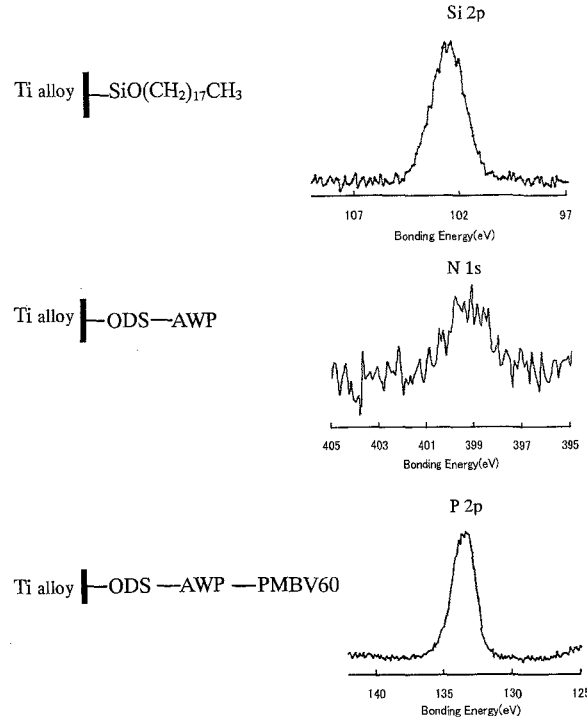

Fig. 4. XPS spectra showing the appearance of Si_{2p}, N_{1s}, P_{2p} peaks according to changing surfaces

with an odd number of layers have PVA. This figure indicates that the contact angle reproducibly alternates as the outermost layer is changed from PVA to PMBV60. It is thought that the outermost layer distinctly influences the wettability of the surface.

The solubility of hydrophobic drug, PTX, was high in PMBV60 solution, but not in PVA solution (not shown data). From this result, we could make hydrogel multilayer containing PTX. Among the 6 layers, PTX was loaded in PMBV60 layer located in the middle of multilayer. The results of drug release are illustrated in Fig. 6, which is expressed as cumulative concentration vs. time.

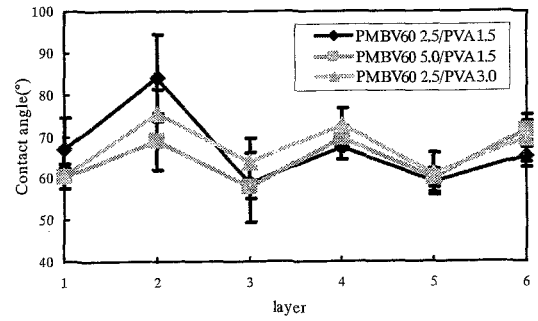

Fig. 5. The static contact angle measured from layer containing a different number of bonded of PMBV60 and PVA. Even number and odd number represents PMBV60 and PVA layer, respectively.

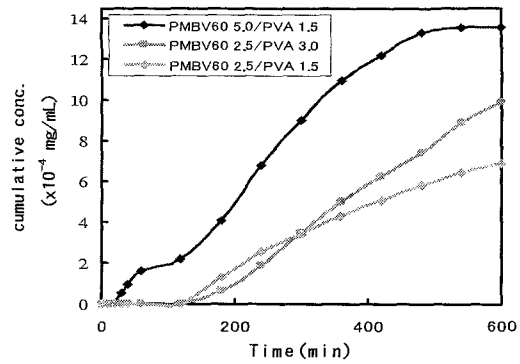

Fig. 6. Release profiles of PTX at the same location (middle in multilayer) from different polymer concentrations in PBS+0.1 % Tween 20

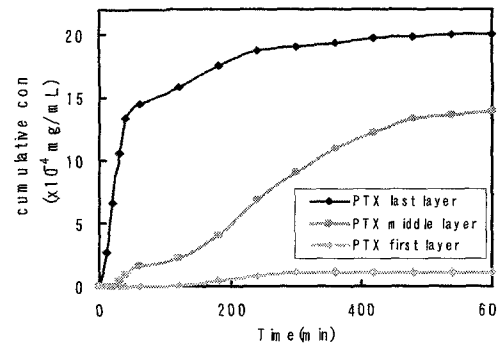

Fig. 7. Release profiles of PTX at different location from polymeric hydrogel multilayer of PMBV60(5.0 wt%) and PVA(1.5 wt%) in PBS+0.1 % Tween 20. Number of multilayer is the same as 6.

In device PMBV60(2.5 wt%)/PVA(3.0 wt%), the profile of releasing is linear, that is, the concentration of releasing PTX is constant. According to the polymer concentration, the release profile indicated various features.

To determine when the releasing time of PTX starts from the different location, three samples having different location of PTX in the PMBV60 layer were built up and the releasing experiments were performed. The 6 layered multilayer having PTX at difference location is indicated in Fig. 7. The PTX in the last layer (upper side) started releasing from 10 min. However, in case of middle layer, it was released from 30 min, whereas the PTX of the bottom of the multilayer started releasing from 180 min. It was confirmed that the location of layer with PTX allowed the difference of releasing timing. Through the observations made in this study, the thickness of layer is supposed to play an important role to control of the releasing timing of PTX.

4. CONCLUSION

The synthesis of water-soluble biocompatible phospholipid polymer(PMBV60) comprising MPC, BMA, and phenylboronic acid was achieved through a conventional radical polymerization. Moreover, the silanization allowed titanium surface to bond AWP containing azide group. The PMBV60 and PVA enabled to construct multilayer hydrogel system through boronate complex on the silanized titanium surface. Polymer hydrogel multilayer on the titanium was constructed conveniently and could load hydrophobic anticancer drug, PTX, in the hydrogel. As the locations of PTX layer in the polymer hydrogel multilayer changed, the releasing profile toward the time of PTX could be controlled. More detail works about the polymer multilayer hydrogel and the release of PTX are underway.

5. REFERENCES

[1] J. Schwartz, M. J. Avaltroni, M. P. Danahy, B. N. Silverman, E. L. Hanson, J. E. Schwarzbauer, K.S. Midwood, E.S. Gawalt *Maters Sci.Engineer.* C 23 (2003) 395-400

[2] Q.Liu and J.Ding Francis K. Mante, Stephanie L. Wunder and George R. Baran, *Biomaterials* 23 (2002) 3103-3111

[3] G. Acharya, K. Park *Adv. Drug Delivery Rev.* 58 (2006) 386-401

[4] H. M. Burt, W. L. Hunter, *Adv. Drug Delivery Rev.* 58 (2006) 350-357

[5] G. Decher, J. D. Hong, J. Schmitt, *Thin Solid Films* 210/211(1992) 831-835

[6] Stockton, Rubner, M. F. *Macromolecules* 30 (1997), 2717

[7] J. F. Quinn, F. Caruso, *Adv. Funct. Mater.* 16 (2006) 1179-1186

[8] S. A. Sukhishvili, S. Granick, *Macromolecules* 35 (2002) 301-310

[9] K. Ishihara, S. Ohata, T. Yoshikawa, N. Nakabayashi, *Journal of Biomed. Mater. Res.* 65A (2003) 210

[10] T. Ueda, H. Oshida, K. Kurita, K. Ishihara, N. Nakabayashi, *Polymer. J.* 24(1992) 1259

[11] T. Moro, Y. Takatori, K. Ishihara, T. Konno, Y. Takigawa, T. Matushita, U. I. Chung, K. Nakamura, H. Kawaguchi, *Nat. Mater.* 3(2004) 829

[12] J. Yan, G. Springsteen, S. Deeter, B. Wang, *Tetrahedron* 60 (2004) 11205-11209

[13] Y. Ma, L. Qian, H. Huang, X. Yang, *Journal of Colloid and Interface Sci.* 295 (2006) 583-588

[14] S. Kitano, Y. Sakurai et.al., *Polym. Adv. Techno.* 2 (1991), 261-264

[15] P.R. Hari, K. Sreenivasan, *Journal of Applied Polymer Science* 82 (2001) 143-149

[16] K. Ishihara, N. Nakabayashi, *Polym. J.*, 22 (1990) 355-360

[17] Q. Liu, J. Ding, F. K. Mante, S. L. Wunder, G.R. Baran, *Biomaterials* 23(2002) 3103-3111

[18] C. Mao, J. Shen, *Colloids and Surfaces B; Biointerfaces* 38 (2004) 47-53

(Received December 10, 2006;Accepted June 16, 2007)