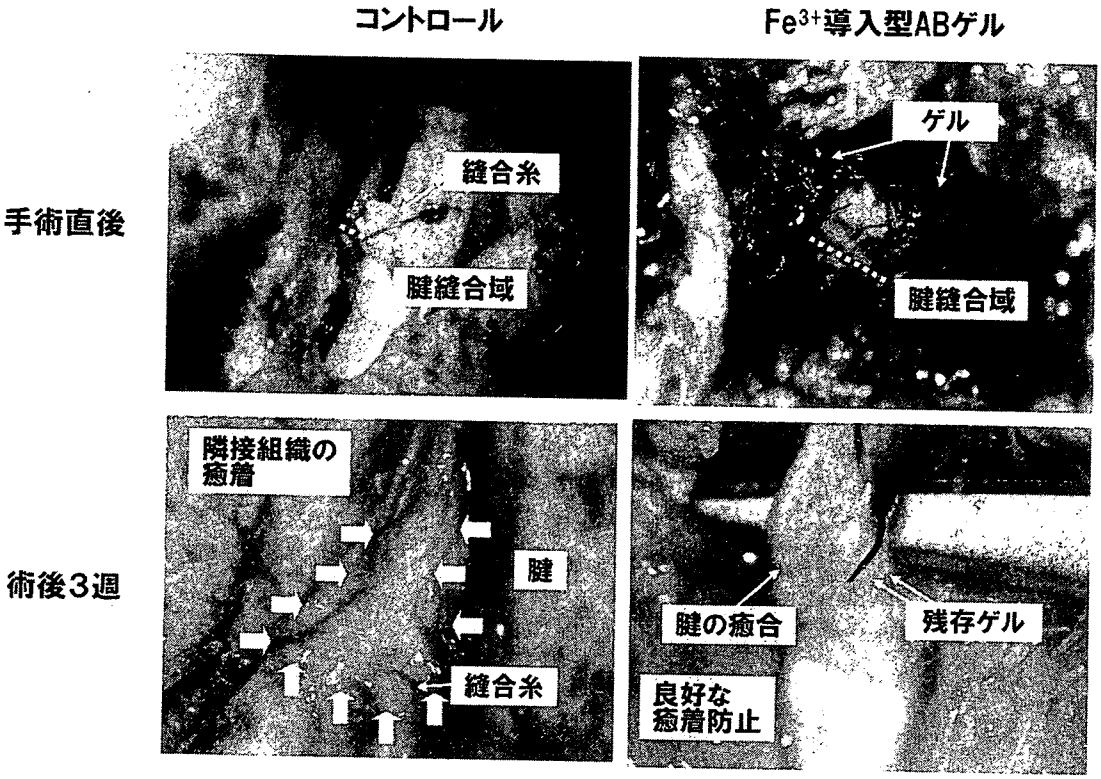


図7　腱癒合術直後および術後3週間の様子

vivo 評価を行った[10]。ABゲルで被覆した縫合部位の周辺組織に目立った炎症反応は惹起しておらず，また周囲と癒着することなく腱の癒合が確認できた（図7）。また術後3週間後においてもゲルの残存が確認できた（図7）。ゲルを使用しなかったコントロール群では，腱断裂部に新しく肉芽組織が形成し，腱の癒合とともに周辺組織との癒着が高度に進行していた。

これらの結果より，Fe^{3+}を含むABゲルはハイドロゲルという多孔質構造の特性から留置した組織において組織修復に必要なサイトカインなどの液成因子の透過を阻害せず，かつ周辺組織の侵潤を阻止するために，癒着を誘起しない画期的なソフトバイオデバイスとして機能することが実証された。

4　常温常圧で生体細胞の保持・輸送を実現するセルコンテナー

機能性細胞の素性が明確にされてきている現在，これらを用いた革新医療にかけられる期待が益々高まっている。革新医療の中でもとりわけ胚性幹（ES）細胞に代表される各種幹細胞を利用した再生医療や組織工学は次世代型医療のコンセプトを提示しており学術的にも社会的にもその研究成果に期待が寄せられている。

細胞を培養する基本要素は細胞，培養液，培養器材である。細胞は培養液中に浸漬された状態で器材表面という二次元系で培養に供されている。多くの細胞操作法は全てこの手法を踏襲しているものである。しかし一方で，期待されている革新医療を実現するためには培養環境を三次元化する必要があり，そのための基盤技術としてポリマーハイドロゲルにかけられている期待は大きい。新しい細胞工学を切り開くためのブレークスルーは第一に細胞培養環境の構築に掛かっているといって過言ではない。

ここでは細胞培養環境を常温・常圧で固体化して可逆的に細胞を保持する新しいソフトバイオデバイスであるセルコンテナーを紹介する。フェニルボロン酸は水中でアニオン構造をとることでポリビニルアルコール（PVA）に代表されるポリオール化合物と可逆的なコンプレックスを形成することが知られている。このゲル化機構はグルコースなどのジオール化合物の添加によって可逆的な交換反応を生起する特徴を持つ。フェニルボロン酸を利用したハイドロゲルはグルコース濃度応答性インスリン制御放出システムとして研究が進められてきた[11]。

筆者らは細胞操作への応用を目指して細胞親和性に優れたリン脂質ポリマーを基盤としたハイドロゲルを設計した[12]。ビニルフェニルボロン酸を一成分とした水溶性リン脂質ポリマー（PMBV，図８）はPVA水溶液と混合すると数十秒で自発的にゲル化する。

このゲル化に要する時間はそれぞれのポリマー濃度やその混合比によって制御することが可能である。このゲルは生理環境中でも形成可能であり，図９に示すように培養液自体を可逆的にゲル化することができた。

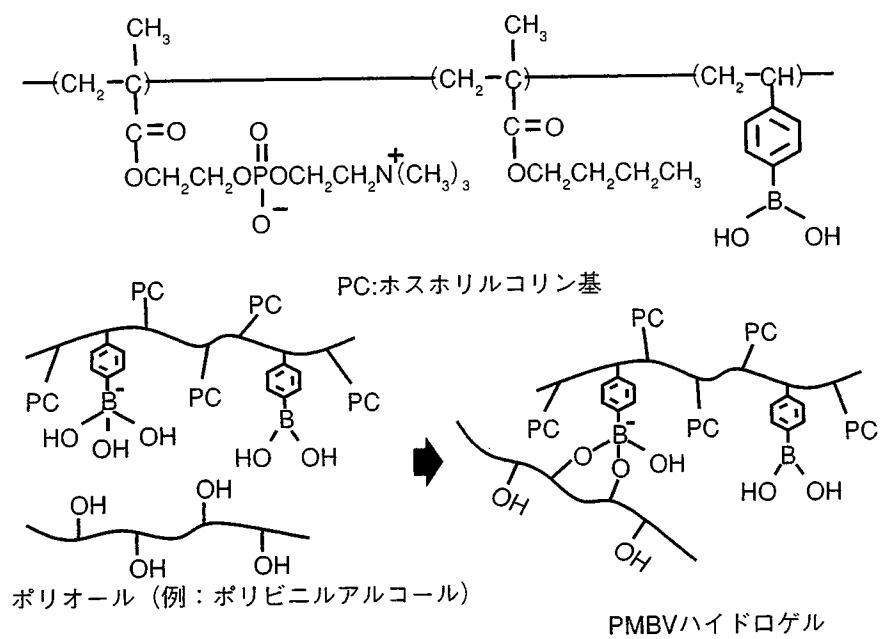


図８　PMBVの構造式およびポリオールとのゲル化様式

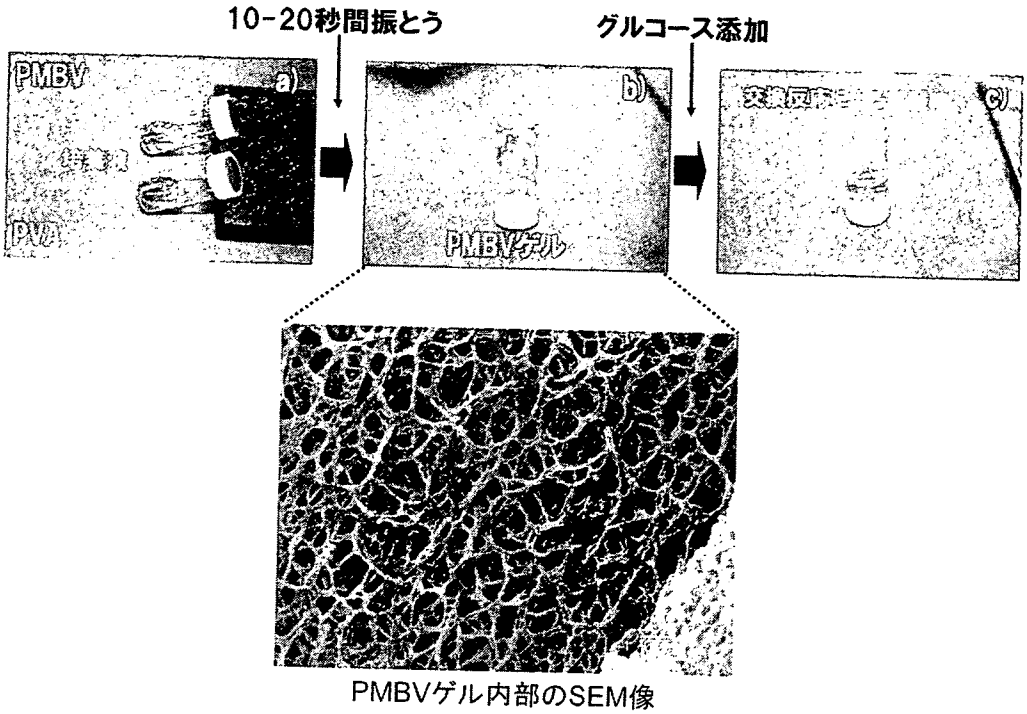


図9　培養液のハイドロゲル化の可逆特性およびハイドロゲル内部の SEM 像

またゲル内部は多孔質構造を有しており内部まで各種アミノ酸やグルコースなどの他，成長因子を行き渡らせることができる。このハイドロゲルを用いて接着性細胞の内包を試みた。細胞は均一にハイドロゲル内部に分散することができた。また，6日間にわたって播種初期の形態を保持し続けた（図10)。

培養（保持）過程における細胞増殖数を検討したところ，通常の培養環境やそれぞれのポリマー水溶液を含む培養液中では接着伸展に引き続く増殖が認められた。一方，PMBV ハイドロゲ

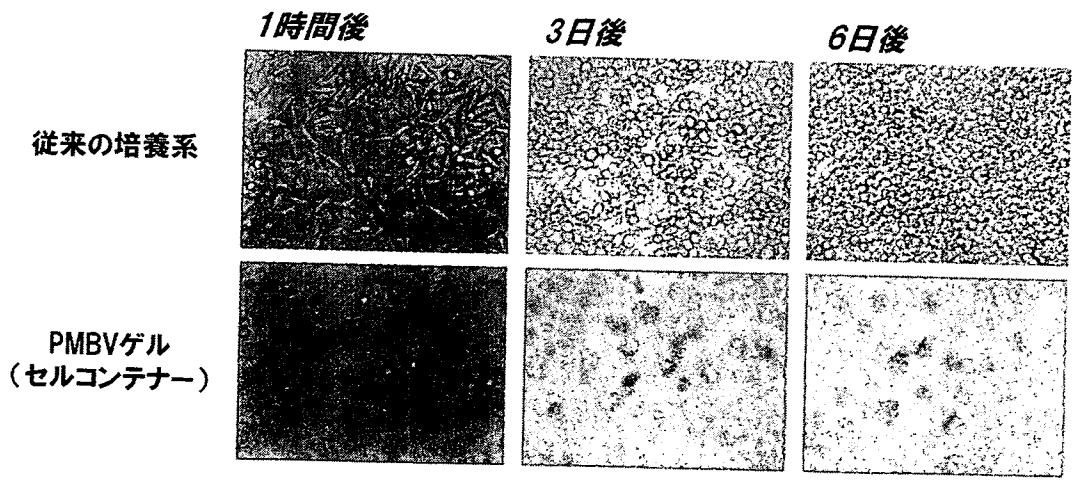


図10　従来の培養系と PMBV ゲル（セルコンテナー）内での接着性細胞の様子

ルでは明らかに増殖を抑止できることがわかった（図 11）。

1 週間にわたって細胞を保持していた PMBV ハイドロゲルを解離した後に，細胞を通常の培養環境に戻すと接着，伸展，増殖が認められた（図 12）。

つまり 1 週間の間，PMBV ハイドロゲルは細胞の基本的性質を消失させることなく細胞を維持していた。これは液体培地を基盤としていたこれまでの培養条件とは一線を画すものである。細胞を常温・常圧で保持することで達成できる応用例は数多い。例えば各種幹細胞や種々の初代細胞など凍結障害性が懸念される場合の保持輸送に利用することができる。また，ハイドロゲルである特性は生体内に酷似した環境の構築が急務とされている生体外での幹細胞培養環境の設計などの次世代型の革新医療において有用であると考えられる。この PMBV ハイドロゲルはセルコンテナーとして様々な細胞工学技術を開拓していくものと結論できる。

5　おわりに

本稿ではハイドロゲルのソフトバイオデバイスとしての機能に着目して概説した。ハイドロゲ

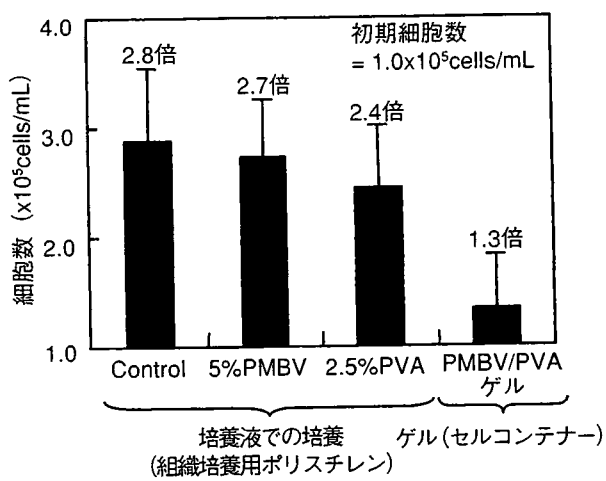
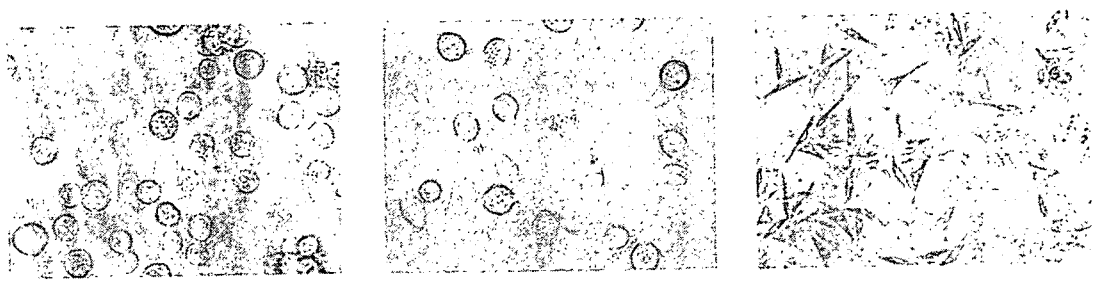


図 11　各種環境中での細胞増殖数



ゲル解離 5 分後　　ゲル解離 30 分後　　ゲル解離 3 時間後

図 12　ゲル解離後に回収した細胞の再培養時の様子

ルの医療分野への応用例は多岐にわたると考えられてきたが，これまでのゲルは，なによりも生体親和性に欠けていた。これを解決するポイントはハイドロゲルを構成するポリマーの分子設計にあることがわかる。つまり，生体成分との接触が必至な医療分野で利用するハイドロゲルには生体成分との非特異的相互作用を抑制する基盤ポリマーの分子設計を組み込んだハイドロゲル設計によって，多機能化を実現させなければならない。

本稿で紹介したハイドロゲルはいずれもポリマー分子の設計概念を組み込むことで，タンパク質や細胞，さらには生体組織といったあらゆるスケールの生体と調和できることを示すものである。次代のバイオエンジニアリングは分子—細胞—組織といった全くスケールの異なるステージが対象であり，これらのステージで横断的に機能できるマテリアルがハイドロゲルである。ソフトマテリアルとしての機能はもとより，ソフトバイオデバイスとしての有用性は今後益々注目に値するものである。先進医療分野においてソフトバイオデバイスとしてのハイドロゲルが一時代を築くことは確実である。

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Temporal and spatially controllable cell encapsulation using a water-soluble phospholipid polymer with phenylboronic acid moiety

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Abstract

Temporal and spatially controllable cell encapsulation based on a water-soluble phospholipid polymer is reported in this study. Phospholipid polymers, i.e., poly(2-methacryloyloxyethyl phosphorylcholine-*co*-*n*-butyl methacrylate-*co*-*p*-vinylphenylboronic acid) (PMBV), were synthesized. A series of hydrogels was prepared between the water-soluble PMBV and other water-soluble polymers having multi-valent alcoholic groups, such as poly(vinyl alcohol) (PVA). The PMBV/PVA hydrogels were formed not only in water, but also in a cell culture medium, and dissociated by the excess addition of low molecular weight di-valent hydroxyl compounds, such as D-glucose. The PMBV/PVA hydrogel was applied as a cell-container which has three-dimensional matrices for the reversible encapsulation of living cells without any response in it. Uniform cell seeding can be achieved using the hydrogels due to the homogenous gel formation of PMBV and PVA in the cell culture medium. Fibroblast cells were encapsulated in the PMBV/PVA hydrogel and maintained for 1 week. After dissociation of the PMBV/PVA hydrogel, the cells were seeded on conventional tissue culture polystyrene. The cells adhered and proliferated as usual on the plate. That is, the PMBV/PVA hydrogel will be useful as a cell-container, which can maintain the cells without any significant adverse effect on the entrapped cells.

Keywords: Phospholipid polymer; Cell engineering; Polymer complex; Reversible hydrogel; Cytocompatibility; Encapsulation

1. Introduction

Recent cell engineering has progressed toward regenerated medicine and cells. At such a time, cells with high functions should be obtained and treated by significantly developed nano- bio-technology. Polymer matrices for cell culture are very important for realizing these goals.

Three-dimensional (3-D) matrices for cell cultures have been prepared from poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers [1–3]. These classic biodegradable polymers are almost highly hydrophobic. Therefore, these 3-D matrices inhibited the penetration of the cell culture medium [4]. Also, the nutrients did not penetrate into these matrices. As a result, the seeded cells did not grow in the inner portion of the matrices. Several studies have developed 3-D matrix materials to solve these problems. Unfortunately, the resulting cell distribution in the polymer matrix is often not uniform, with most of the cells attached only on the surface.

Spontaneously gelation polymers can provide the desired size and shape for the seeded and encapsulated cells. Moreover, it will be useful that the cross-linking network can be dissociated by the addition of chemical or physical stimulations. Based on these requirements, recoverable hydrogels have been prepared by mixing two kinds of polymer aqueous solutions.

First, cross-linking of the polymer chains under physiological conditions should be necessary for this purpose in

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order to avoid any reduced activity of the entrapped cells and biomolecules. The cytocompatiblity and nutrient permeability of the hydrogel are important factors for the entrapped cells.

To obtain a cytocompatibility and nutrient permeability of the hydrogels, we focused on the cell membrane structure. We have reported that the 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers containing a phospholipid polar group in the side chain have an excellent biocompatibility due to inhibiting the non-specific interaction with biomolecules including serum proteins, platelets, and cells [5–7]. Also, the MPC polymer hydrogel membranes have a high gas permeability [8] and solute permeability [9]. We succeeded in the spontaneously gelation of water-soluble MPC polymers from their aqueous solutions by hydrogen bonding without any physical treatments [10–13]. The MPC polymer hydrogel prepared from an aqueous solution containing 5 wt% poly[MPC-*co*-*n*-butyl methacrylate (BMA)] and poly(MPC-*co*-methacrylic acid) could be dissociated by a change in pH. In this hydrogel, the internal pH of the hydrogel was too low (pH < 4) to entrap the cells. It is necessary to provide more compatible with the biological system, when the hydrogel is applied to the cell entrapment matrices.

In this study, we prepared a new reversible hydrogel system composed of MPC polymers which can encapsulate the cells and proteins by mild treatment with a high viability and activity. As the cross-linking mechanism between the MPC polymers in an aqueous medium, we modified the specific reaction between boronic acid and multi-valent alcoholic compounds.

The boronic acid in a tetrahedral anionic structure produces stable complexes with the alcohol compounds including PVA, glucose, sorbitol, etc. [14–26]. If the boronic acid moieties are introduced into the polymer chain and the polymer is reacted with PVA, the hydrogel may be formed in an aqueous medium. The hydrogel is reversibly dissociated by the addition of low-molecular-weight compounds such as D-glucose. This reaction mechanism is well known as the glucose concentration responsive hydrogel for application as glucose sensors [15–22]. The hydrogels containing the boronic acid moiety have attracted attention in the affinity chromatography of biological agents [23–26]. We propose a new cell maintenance system called the "cell-container" based on this hydrogel. This system will be useful for maintaining the cells with a high activity and allow their specific functions after being released from the hydrogel accompanied by dissociation of the hydrogel.

To achieve these objectives, we synthesized a cytocompatible water-soluble MPC polymer containing *p*-vinylphenylboronic acid (VPBA) units, that is, poly[MPC-*co*-BMA-*co*-VPBA] (PMBV). The characterization of the PMBV, formation and properties of the PMBV/PVA hydrogel, and behavior of the fibroblast cells in the hydrogel are reported. Finally, we demonstrate the usefulness of the PMBV/PVA hydrogel as the cell-container.

2. Materials and methods

2.1. Materials

MPC was synthesized by a previously reported method and used after recrystallization from acetonitrile [27]. BMA was purchased from Nakalai Tesque Co., Ltd. (Kyoto, Japan) and purified by distillation under a 30 mmHg reduced pressure/b.p. fraction of 60 °C. VPBA was kindly provided by Osaka Organic Chemical Industry, Ltd. (Osaka, Japan) and used without further purification. PVA (polymerization degree 1500, saponification value 86–90 mol%) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The other organic reagents and solvents were commercially available reagents of extra-pure grade and were used without further purification. The cell culture medium Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), and other substances for the cell cultures were purchased from Invitrogen Corporation, Grand Island, NY, USA.

2.2. Synthesis and characterization of the phospholipid polymer

The PMBV was synthesized by a conventional radical polymerization technique using α,α′-azoisobutyronitrile (AIBN) as the initiator. The synthesis procedure is briefly described as follows: the desired amount of MPC, BMA and VPBA were placed in a glass ampoule, AIBN was dissolved in the mixture (1.0 mmol/L), and the mixture was then diluted with ethanol to a 1 mol/L monomer concentration. Argon gas was bubbled into the solution for 5 min to eliminate the oxygen, and then the glass ampoule was sealed. The polymerization was performed at 60 °C for a specific time. After cooling the glass ampoule, the contents were poured into a large amount of a mixture of diethyl ether and chloroform (8/2 by volume) to eliminate any remaining monomer and precipitate the polymer. The precipitate was filtered off using a glass-filter and dried in vacuo. The chemical structure of the PMBV was confirmed by ^{1}H NMR (α-300, JEOL Co., Ltd., Tokyo, Japan) and FT-IR (FT-IR 615, Jasco Co., Ltd., Tokyo, Japan) measurements. The composition of each component in the PMBV was determined by the ^{1}H NMR measurement. The molecular weight of the polymers was measured by gel permeation chromatography (GPC, JASCO Co., Ltd., Tokyo, Japan). The mixture of methanol and water (7/3 by volume) containing 10 mmol/L of lithium bromide was used as an eluent for the GPC measurement, and well-defined poly(ethylene oxide) (PEO) was used as the standard samples for the calibration curve.

2.3. Preparation and basic characterization of the hydrogel

To confirm the gelation between the water-soluble PMBV and PVA, various compositions of these polymer solutions were prepared. The inner morphology of the PMBV gels was observed using a scanning electron microsope (SEM, SM-200, Topcon Co., Ltd., Tokyo, Japan). For the SEM observation, the PMBV/PVA hydrogels were prepared using distilled water, and the lyophilized samples were observed.

To estimate the spontaneous gelation time, a dynamic viscoelasticity measurement was performed using a rheometer (Rheolograph-Micro, Toyoseiki, Tokyo, Japan). Equal amounts (0.75 mL) of PMBV (5.0 wt%) and PVA (2.5 wt%) aqueous solutions were slowly injected into both sides of a vibration blade (surface area of the brade was 5 cm^2). Just after injection, these polymer solutions were mixed by automatic vibration (20 Hz). Changes in the storage modulus (G') and the loss modulus (G'') were recorded. The gelation time was estimated as the time when the G' value becomes greater than G'' [28].

2.4. Cell entrapment in the PMBV/PVA hydrogel

The mouse fibroblast cell line, L929, was used as the model cells. The L929 cells were routinely cultured in DMEM containing 10% FBS at 37 °C in a 5% CO_2 atmosphere. After trypsinization, the cell-density was adjusted to 5×10^3 cells/mL by a culture medium containing 5 wt% of

PMBV. The L929 suspension in the PMBV solution was mixed with 2.5 wt% of the PVA aqueous solution. The mixture was pipetted and gently shaken until the gel formation was visually confirmed. The shape and morphology of the encapsulated L929 cells were observed using a phase contrast microscope (BX60, OLYMPUS Co., Ltd., Tokyo, Japan). After 1 week, the PMBV/PVA hydrogel was dissociated by the addition of an excess amount of D-glucose, and the recovered L929 cells were then plated on the tissue culture polystyrene (TCPS, Asahi Technoglass Corp., Chiba, Japan).

2.5. Cell proliferation test

Measurement of the cell proliferation rate in the PMBV/PVA hydrogel was then performed. The L929 cells were resuspended in DMEM containing 10% FBS, and adjusted to 4.0×10^5 cells/mL. The cell suspension (500 μL) was mixed with an equal amount of 10% PMBV solution dissolved by DMEM. The PMBV solution containing the L929 cells was mixed with an equal amount of 2.5% PVA solution diluted by DMEM. The mixture was slowly pipetted and mixed in the 24-well multiplate until the gelation was confirmed. The PMBV/PVA hydrogel containing the L929 cells were prepared using this procedure. Likewise, the cells were seeded on the 24-well plate without the PVA solution (only 5% PMBV), PMBV solution (only 2.5% PVA), and without any polymers (only medium). After 3 days, the increase in the cell number under the various conditions was counted by a hemocytometer.

3. Results and discussion

3.1. Preparation of PMBV and formation of PMBV/PVA hydrogel

Water-soluble PMBV could be synthesized by a conventional radical polymerization. The polymerization homogenously proceeded in ethanol. The chemical structure and synthetic results of the PMBV are summarized in Fig. 1 and Table 1. The chemical structure of the formed polymer was determined by ^{1}H NMR. Fig. 2 shows the ^{1}H-NMR spectrum of PMBV. The NMR peaks completely assigned each component of the PMBV. The solubility in water of the PMBV depended on the MPC composition in the polymers. For example, when the monomer unit compositions of MPC, BMA, VPBA were 0.4, 0.5, 0.1, respectively, the obtained polymer did not dissolve in water.

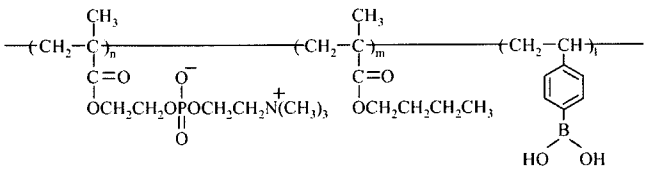

Fig. 1. Chemical structure of poly(MPC-*co*-BMA-*co*-VPBA) (PMBV).

The water-soluble PMBV was used to make a hydrogel with multi-valent hydroxyl group compounds such as PVA. When the polymer aqueous solutions contained PMBV and PVA, a hydrogel was formed within a short period after gentle shaking at room temperature. Fig. 3 shows a schematic representation of the covalently cross-linking mechanism between PMBV and PVA. The gelation mechanism was based on the covalent cross-linking between the phenylboronic acid moiety of PMBV and the hydroxyl groups of PVA. There are some reports about this phenomenon [29]. It is well known that the boronic acid is a protecting group for 1,2-diol compounds including saccharides in an organic synthesis [30]. The hydrogel formation between the phenylboronic acid moiety bearing a polymer and PVA has been investigated to develop a novel glucose-responsible drug delivery system [31–34]. That is, the hydrogel from PMBV and PVA is also reversibly dissociated by the addition of glucose into the system. Before mixing, both aqueous polymer solutions were low viscosity liquids. After mixing, the viscosity

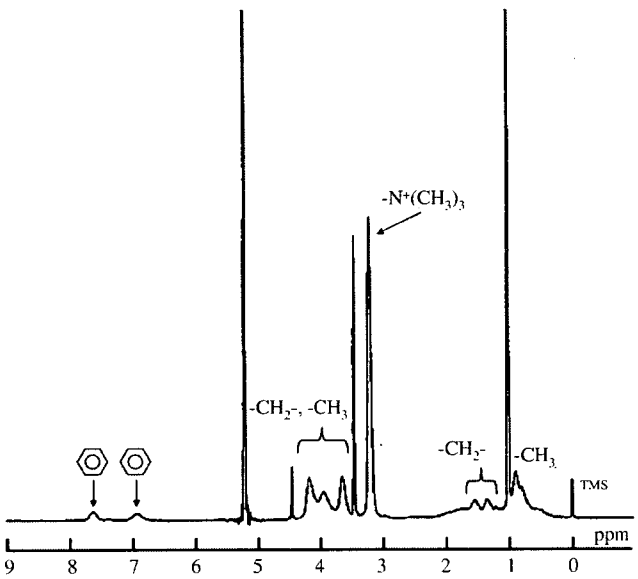

Fig. 2. ^{1}H NMR spectrum of water-soluble PMBV containing 60 mol% MPC unit.

Table 1
Synthetic results of PMBV

Abbreviation	In feed (mole fraction) MPC/BMA/VPBA	In copolymer (mole fraction) MPC/BMA/VPBA	Polymerization time (h)	Yield (%)	Solubility in water	Mw (10^{-4})	Mw/Mn
PMBV	0.6/0.3/0.1	0.6/0.3/0.1	6	71	+	5.4	2.6

[Monomer] = 1.0 mol/L. [AIBN] = 1 mmol/L. Polymerization temperature = 60 °C.
Solubility was determined by 1.0 mg/mL each polymer sample and described as soluble (+) and insoluble (−).

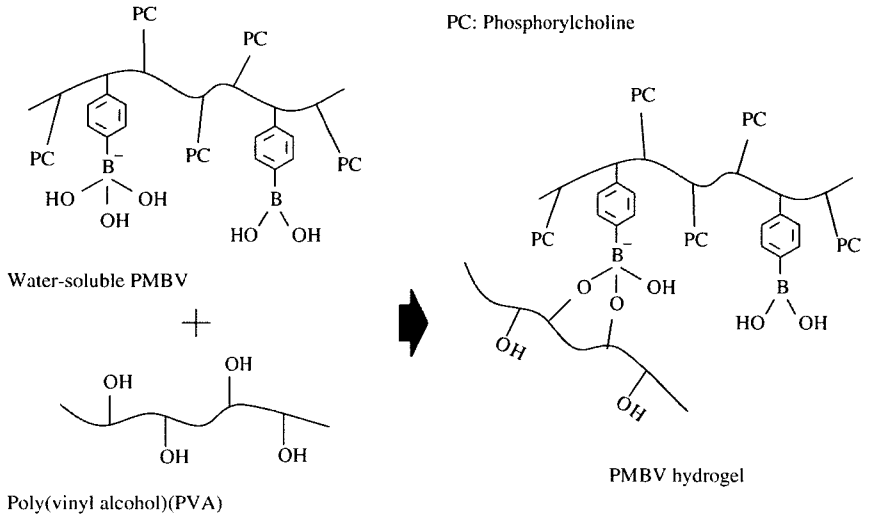

Fig. 3. Schematic representation of cross-linking mechanism between phenylboronic acid moiety in the PMBV and hydroxyl group in the PVA.

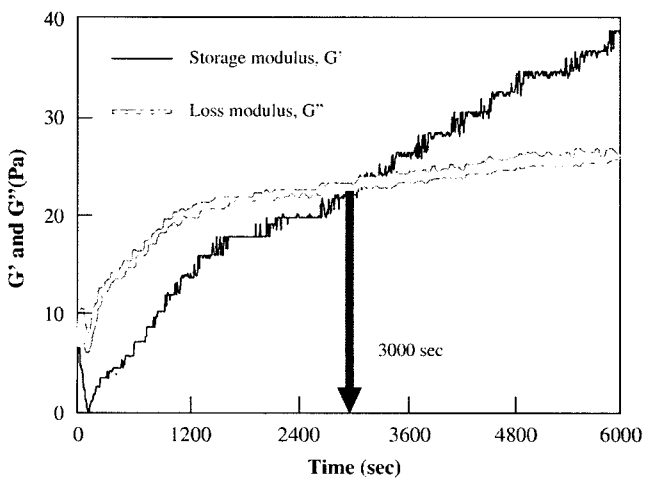

Fig. 4. Change in the storage modulus (G', black line) and loss modulus (G'', gray line) during gel formation process.

gradually increased. Finally, the mixture formed the hydrogel. After the addition of the other low molecular weight diol compound, such as D-glucose, the PMBV/PVA hydrogel gradually dissociated. The PMBV/PVA hydrogel has a reversibility between hydrogel formation and dissociation. The dynamic viscoelasticity measurement of the mixed solution was performed. Fig. 4 shows the typical result when the 5 wt% PMBV and 2.5 wt% PVA mixture were applied. The gelation was based on the diffusion of both polymer chains in the mixed solution. Therefore, the gelation times were relatively longer than that of the gentle shaking under these experiment conditions. It was obviously confirmed that the cross point between the storage modulus (G') and loss modulus (G") is at 3000 s. This result indicated that the PMBV and PVA mixture gradually formed the cross-linking network, and the mixture finally produced a hydrogel structure. The cross point between G' and G" was confirmed for the 2.5 wt% PMBV and 2.5 wt% PVA mixture. The composition ranges of PMBV and PVA for making a hydrogel are summarized in Table 2. Based on these results, it was revealed that the gelation depended on the polymer concentration. The PMBV/PVA hydrogel was realized even in the PBS and cell culture medium. It was considered that this gelation mechanism did not affect the ionic strength in the saline and medium.

Fig. 5 shows SEM images of the cross section of the PMBV/PVA hydrogel after lyophilization. Every hydrogel had a porous structure, because the water content of the hydrogel was above 93%. The pore size was almost 1 μm under the lyophilized condition. The higher water content and micropores in the hydrogel provide permeation of the bioactive molecules including proteins and hormones. This is a very good characteristic for culturing cells inside the hydrogel.

3.2. Cell maintenance in the PMBV/PVA hydrogel

To understand the behavior and state of the cells in the PMBV/PVA hydrogel in order to evaluate the performance of the PMBV/PVA hydrogel as the cell-container, the mouse fibroblast (L929) cells were cultured (encapsulated) in the PMBV/PVA hydrogel. A schematic illustration of the cell culture procedure is shown in Fig. 6. The PMBV was dissolved using DMEM containing 10% FBS. We have observed that the MPC polymers suppressed the nonspecific interaction with not only the various serum proteins, but also the cells [35]. Among these, the poly(MPC-*co*-BMA) could effectively suppress the L929 cell adhesion compared to that on TCPS due to the reduced cell adhesive protein adsorption. Indeed, the L929 cells adhered and proliferated as usual in the

Table 2
Gelation between PMBV and PVA solutions

PVA (wt%)	PMBV (wt%)				
	5.0	2.5	1.2	0.60	0.30
5.0	○	○	×	×	×
2.5	○	○	×	×	×
1.2	○	×	×	×	×
0.60	○	×	×	×	×
0.30	×	×	×	×	×

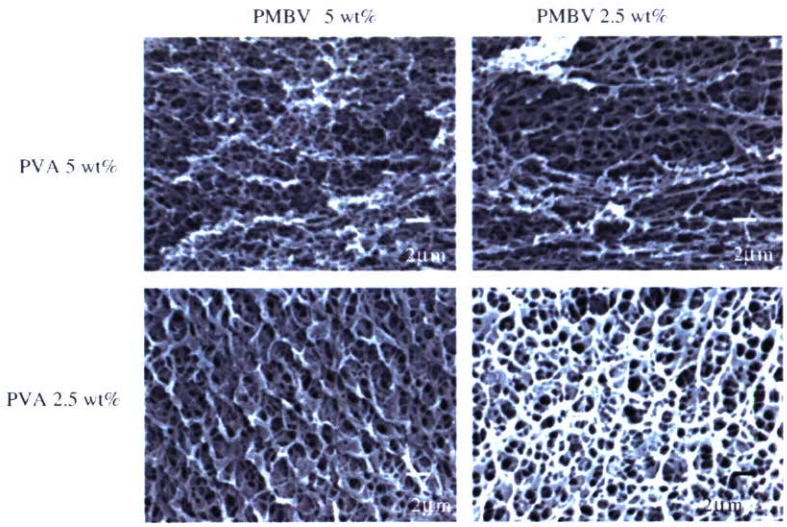

Fig. 5. SEM images of inner structure of PMBV gel (after lyophilization).

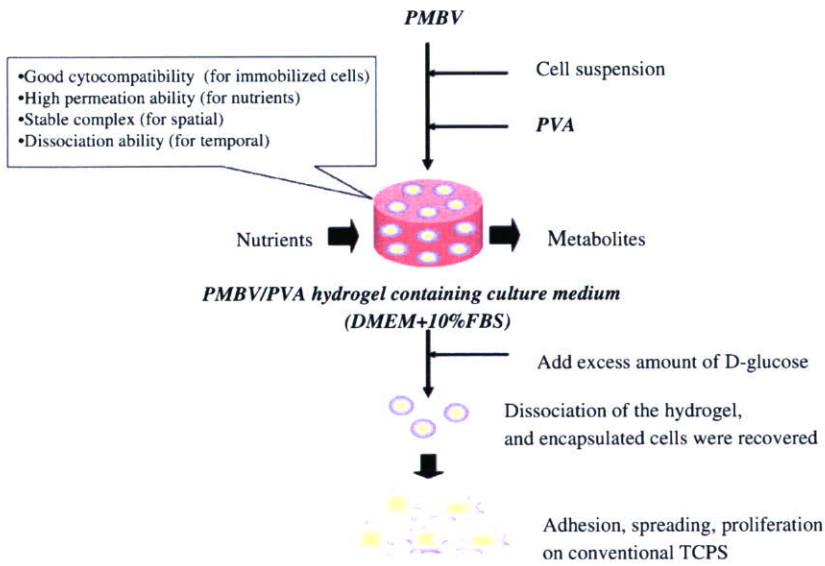

Fig. 6. Schematic illustration of cell culture (encapsulation) method used in this study.

5 wt% PMBV medium solution on the TCPS. The L929 cells were suspended in the 5 wt% PMBV medium solution. The L929 in the PMBV solution was mixed with the 5 wt% PVA aqueous solution. The gelation was confirmed after gentle shaking for 10 s at room temperature.

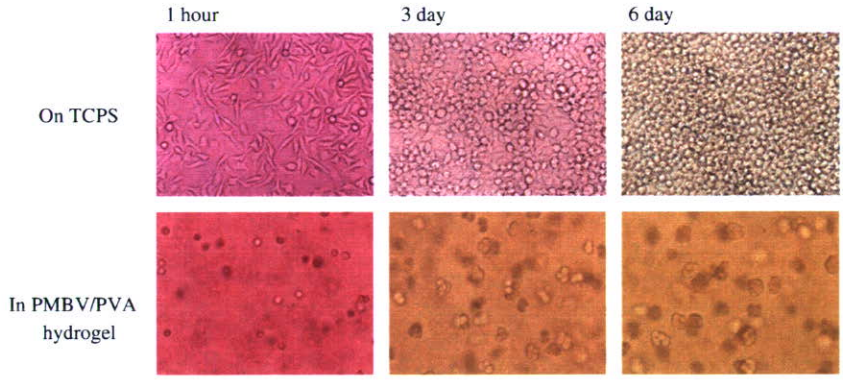

Fig. 7. Phase contrast microscope images of L929 cells on conventional TCPS and in the PMBV gel.

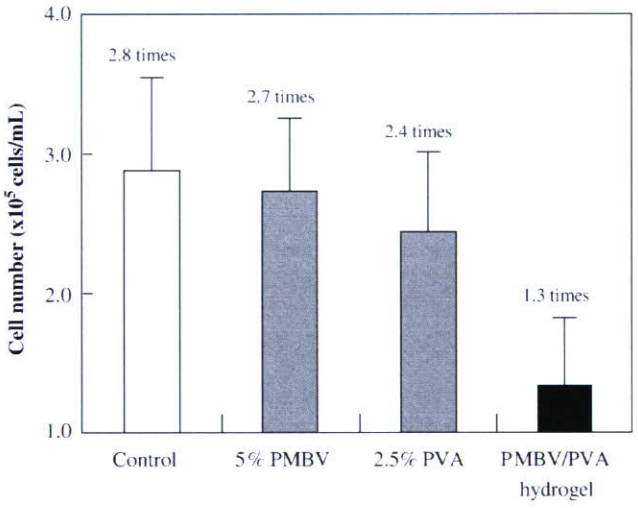

Fig. 8. Change in the cell number under the various conditions after 3 days ($n = 4$). DMEM + 10% FBS was used as the control.

Fig. 7 shows the phase contrast microscope images of the L929 cells in the PMBV/PVA hydrogel and on a TCPS. The cells adhered, spread and proliferated on the TCPS as usual. On the other hand, the cell morphology was circular and the cells did not spread out in the PMBV/PVA hydrogel during the culture period. The cell morphology correlates with the cellular activities and functions; a strong cell adhesion and spreading often favor proliferation while a round cell shape is required for cell-specific functions [36,37]. In noteworthy that the cells did not aggregate with each other in the hydrogel. When the cells were cultured on the MPC polymer surface, the seeded cells were locally aggregated with each other [38]. It was considered that the PMBV/PVA hydrogel immobilized the individual cells. Therefore, the cell culture in the gel is useful for investigating the individual cell-specific functions.

We evaluated the cell proliferation under the various culture conditions. Fig. 8 shows the cell number which was counted by a hemocytometer after dissociation or trypsin treatment ($n = 4$). For the liquid medium with 5% PMBV or 2.5% PVA, the cells were proliferated the same as the control sample (without any polymers). The increase ratio was 2.8, 2.7, and 2.4 times, respectively. Based on this result, both PMBV and PVA did not affect the cell proliferation. For the PMBV/PVA hydrogel, the cells hardly proliferated. The increased ratio was 1.3 times compared to the initial number. The cells were locally proliferated with a spherical shape.

After a 1-week culture without changing the fresh medium, the PMBV/PVA hydrogel was dissociated by the addition of an excess amount of D-glucose, and the cells that were recovered from the hydrogel were seeded on the TCPS. Fig. 9 shows the phase contrast microscope images of the L929 cells recovered and recultured on the TCPS. The L929 cells immediately adhered and proliferated after seeding on the TCPS. However, we do not have any quantitative data for the activity of the L929 cells inside the PMBV/PVA hydrogel at the present time. It is considered that the viability of the L929 cells was maintained even though they were in the PMBV/PVA hydrogel for 1 week. We should have quantitative results on the activation of the cells after being recovered from the PMBV/PVA hydrogel. However, it seems that the PMBV/PVA hydrogel had no adverse effects on the cells from the morphological observation and that is had a good cytocompatibility. When the cells were seeded in/on the conventional 3-D matrices made from PLA or PGA, the cells hardly penetrated into the matrices based on the hydrophobicity of the polymers, even if the materials have a pore structure. The cells were adhered only on the surface of these matrices. Moreover, these 3-D matrices were passively degraded by hydrolysis with time under physiological conditions. On the other hand, the PMBV/PVA hydrogel could homogeneously disperse the cells inside of the hydrogel and recovered them by the addition of natural chemical compounds such as D-glucose in a shorter time period. The cells encapsulated in the PMBV/PVA hydrogel maintain their morphology when they are in suspension with the cell culture medium. When the cells were recovered from the hydrogel, the cells demonstrated good adhesion to the solid substitute and proliferation on the surface.

Based on these results, we considered that the PMBV/PVA hydrogel could be applied as a new tool for cell

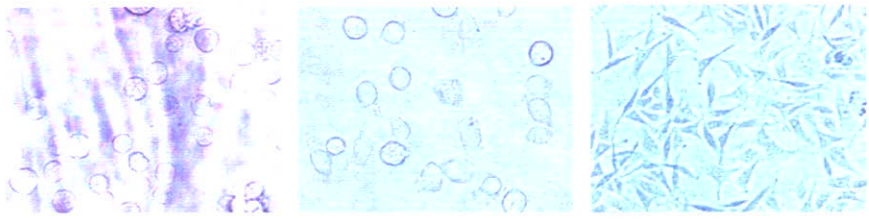

Fig. 9. Phase contrast microscope images of recovered L929 cells after dissociation of the PMBV gel.

engineering, that is, the cell-container. The cell-container provides a mild and suitable environment for cells just like a cell suspension. Treatment of the cells is made much easier by solidification with the PMBV/PVA hydrogel. The cells were also recovered from the hydrogel at any time.

4. Conclusion

We could prepare a phospholipid polymer bearing both MPC and *p*-vinylphenylboronic acid units, PMBV, for preparation of a cytocompatible hydrogel in an aqueous medium without any chemical and physical treatments. The gelation of PMBV spontaneously occurred after gentle mixing with PVA even in the presence of the cell culture medium. This was due to the formation of covalent cross-linking between PMBV and PVA. The cells could be encapsulated and uniformly distributed in the hydrogel. Since the viability of the encapsulated cells was good, the cells were recovered in good condition by the addition of glucose in the medium to dissociate the hydrogel. We concluded that the novel PMBV/PVA hydrogel system might be applied as a cell-container to encapsulate and transport the cells. Also, the hydrogel could be applied as a temporal and spatial controllable cell-containing hydrogel.

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Prevention of Tissue Adhesion by a Spontaneously Formed Phospholipid Polymer Hydrogel

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Abstract. We investigated phospholipid polymer hydrogels containing Fe^{3+} ions (PMA/PMB/Fe hydrogel) for their use as antiadhesive materials in the healing tissues. These hydrogels were prepared from the aqueous solutions of poly(2-methacryloyloxyethyl phosphorylcholine (MPC)-*co*-methacrylic acid) (PMA) and poly(MPC-*co*-*n*-butyl methacrylate) (PMB). The PMA/PMB hydrogel is formed by the intermolecular interactions between PMA and PMB, and it reversibly dissociates under physiological conditions. The addition of Fe^{3+} ions could control the gelation time and the dissociation time. Mechanical properties such as the gelation time and viscoelastic properties can be controlled by the $FeCl_3$ concentration. With regard to biocompatibility, no evidence of inflammation was observed *in vivo*. Therefore, the PMA/PMB/Fe hydrogel has a potential to be used as an antiadhesive material.

Introduction

Adhesion of tissues such as a tendon and intestines after an injury or surgery is a type of inflammatory reaction. It can cause difficulty in movement or pain, thereby decreasing the quality of life of a patient [1]. Although some efforts have been invested in developing antiadhesive materials for tissues, no effective material has yet been put to practical use. The existing antiadhesive materials serve as a physical barrier to prevent contact of the healing tissue with the surrounding normal tissues. However, these materials have some drawbacks [2]: (1) The permeability of bioactive molecules such as cytokines is so low that healing is delayed. (2) Certain degree of tissue adhesion may occur after an operation for the removal of non-biodegradable materials and during adsorption of biodegradable materials.

Therefore, we propose a spontaneously formed phospholipid polymer hydrogel as a novel anti-adhesive material. This hydrogel can be prepared under physiological conditions simply by mixing the aqueous solutions containing poly(2-methacryloyloxyethyl phosphorylcholine (MPC)-*co*-methacrylic acid) (PMA) and poly(MPC-*co*-*n*-butyl methacrylate) (PMB) (Fig. 1) [3]. The hydrogel is formed by molecular interactions such as hydrogen bonding and hydrophobic interactions, and it demonstrates physical properties that correspond to the polymer structure [4,5]. Thus, it can be dissociated by changing the surrounding conditions, namely, pH, ionic strength, temperature, etc. The *in vivo* injection test did not show toxicity of the constitutive polymers—PMA and PMB. Since the PMA/PMB hydrogel has more than 95 wt% aqueous medium, it is expected to be (1) porous to allow the permeation of humoral factors, (2) biocompatible in order to prevent an inflammatory reaction, and (3) biodegradable so that a special procedure for its removal is not required after the tissues heal. The PMA/PMB hydrogel is dissociated in a large amount of aqueous medium within a few hours, and it is expected to dissociate *in vivo* within a relatively short time. Biodegradability is an advantageous property for its medical use because it can control the release of content as the degradation and eliminate the need of surgery for its removal. However, since the dissociation time of the PMA/PMB hydrogel is short, its long-term application to tissues as an antiadhesive material

is not possible. Thus, we introduced another crosslinking mechanism, that is, ionic crosslinking between counter-cation and carboxylate anion in the PMA/PMB hydrogel for achieving stabilization. Although NaCl and $CaCl_2$ did not show the expected stabilization effect, $FeCl_3$ improved the stability of the PMA/PMB hydrogel in a large amount of aqueous medium [3].

In this study, we investigated the PMA/PMB hydrogel containing $FeCl_3$ for their use as an antiadhesive material in tissues. We examined the stabilization of the PMA/PMB hydrogel by $FeCl_3$ *in vitro* and *in vivo*. We also evaluated the performance of the PMA/PMB hydrogel as an antiadhesive material *in vivo*.

Materials and Methods

Materials. The phospholipid polymers, PMA (Mn = 2.7 × 10^5, Mw = 8.4 × 10^5, and MPC mole fraction = 0.3) and PMB (Mn = 1.1 × 10^5, Mw = 8.6 × 10^5, and MPC mole fraction = 0.8), were prepared from the corresponding monomer by radical polymerization [Figure 1][6]. For this study, these polymers were supplied by the NOF Corporation (Tokyo, Japan) as 5 wt% aqueous solutions. Iron (III) hexahydrate ($FeCl_3$) was purchased from Kanto Chemical Co.

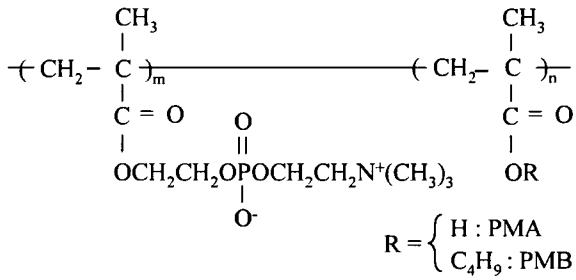

Fig. 1. Structure of PMA and PMB.

Hydrogel Preparation. Equal volumes of 5 wt% PMA and PMB aqueous solutions were taken in a microtubing and vigorously stirred for 10 s. After 10–20 s, the mixture of these MPC polymer solutions was spontaneously transformed into a hydrogel state. A hydrogel containing $FeCl_3$ (PMA/PMB/Fe hydrogel) was prepared by using PMB containing $FeCl_3$. The final concentration of $FeCl_3$ in a hydrogel is expressed by the number following the PMA/PMB/Fe hydrogel, for example, PMA/PMB/Fe hydrogel-71 implies a PMA/PMB hydrogel containing 71 mM of $FeCl_3$.

Stability of the PMA/PMB/Fe Hydrogel *in vitro and in vivo*. One gram of PMA/PMB/Fe hydrogel was put in a nylon mesh bag and immersed in 100 mL of phosphate buffered saline (PBS; 0.15 M, pH 7.1). The mesh bag was weighed at specific time intervals, and the weight of the remaining hydrogel was determined. For an *in vivo* test, a diffusion chamber (pore size, 0.3 μm) containing the PMA/PMB hydrogel or PMA/PMB/Fe hydrogel-71 was implanted subcutaneously into a mouse. After 3 weeks, the chamber was removed, and the hydrogel was observed by SEM.

Viscoelastic Properties of the Hydrogels. The aqueous solutions of PMA (0.75 mL) and PMB (0.75 mL) were injected slowly into both sides of a vibration blade (1.71 cm^2) in a liquid cell. Immediately after the injection, the blade was set in motion (vibrational amplitude, 200 μm; frequency, 20 Hz) to mix these polymer solutions in the cell in order to enable the PMA/PMB hydrogel formation. Changes in the elastic modulus (G') and the viscous modulus (G") were recorded using a rheometer (Rheolograph-Micro, Toyoseiki, Tokyo, Japan). The gelation time is defined as the time when G' becomes greater than G". The gelation time of the PMA/PMB/Fe hydrogel was also measured by using PMB containing $FeCl_3$ in the same manner. In addition, the viscoelastic properties of the hydrogel were investigated using the rheometer at a predetermined time after the hydrogel was prepared and kept at room temperature.

Results and Discussion

Fig. 2 shows the effect of $FeCl_3$ addition on the stability of the PMA/PMB/Fe hydrogel. The relative weight of PMA/PMB/Fe hydrogel-14 gradually decreased, and it completely dissociated within 6 h after its immersion in PBS. PMA/PMB/Fe hydrogel-28 also dissociated completely within 24 h, although PMA/PMB/Fe hydrogel-39 and PMA/PMB/Fe hydrogel-71 retained almost a constant weight after a slight initial decrease. Because a Fe^{3+} ion theoretically interacts with 3 carboxylate anions, the residual carboxylic acid groups exist in PMA/PMB/Fe hydrogel-14 and PMA/PMB/Fe hydrogel-28. The $FeCl_3$ concentration is adjusted to the theoretical ratio in

PMA/PMB/Fe hydrogel-39. Thus, PMA/PMB/Fe hydrogel-39 and PMA/PMB/Fe hydrogel-71 did not dissociate because of the higher $FeCl_3$ concentration.

The relative weight of PMA/PMB/Fe hydrogel-28 showed a slight initial increase, and then it steadily decreased. This suggests swelling of the hydrogel immediately after its immersion in PBS, followed by its dissociation. When the PMA/PMB/Fe hydrogel is immersed in PBS, swelling of the hydrogel and diffusion of $FeCl_3$ starts. Influx of water can cause ionization of the carboxylic acid groups and lead to electrostatic repulsion between the carboxylate anions. As a result, the polymer concentration and the crosslinking density decreases. Subsequently, the polymer networks collapse, i.e., dissociation of the hydrogel occurs. As seen in the yellowish PBS obtained after the immersion of the hydrogel, diffusion of the Fe^{3+} ions and swelling of PMA/PMB/Fe hydrogel-71 and PMA/PMB/Fe hydrogel-39 is possible. However, even during the swelling process, these hydrogels retained the polymer network and attained equilibrium due to a high density of Fe^{3+} crosslinking.

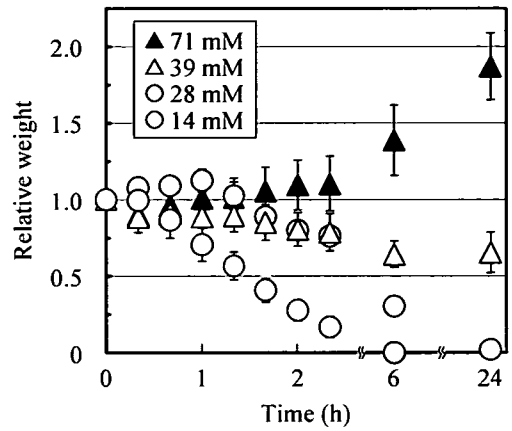

Fig. 2 Change in the weight of PMA/PMB hydrogel containing $FeCl_3$ immersed in PBS.

Although PMA/PMB/Fe hydrogel-71 was implanted subcutaneously, stabilization of the PMA/PMB hydrogel containing Fe^{3+} was observed. That is, while the PMA/PMB hydrogel was dissociating, PMA/PMB/Fe hydrogel-71 remained and maintained the hydrogel state even after 3 weeks. A three-dimensional network structure could be observed under SEM, and the results of viscoelastic measurements also indicated the defining characteristic of a hydrogel, that is, G' > G".

Table 1 shows the gelation time of the PMA/PMB hydrogel and the PMA/PMB/Fe hydrogel. The gelation time was longer for the PMA/PMB hydrogel than for the PMA/PMB/Fe hydrogel. Since the $FeCl_3$ solution has a low pH, ionization of the carboxylic acid groups in PMA can be suppressed by mixing PMA with PMB containing $FeCl_3$. Suppression of the carboxylic acid groups leads to hydrogen bond formation, resulting in the shortening of the gelation time of the PMA/PMB/Fe hydrogel when compared with that of the PMA/PMB hydrogel. Increase in the $FeCl_3$ concentration decreased the gelation time. This is because the pH of PMA/PMB/Fe hydrogel-142 was lower than that of PMA/PMB/Fe hydrogel-71.

Table 1 Gelation time of PMA/PMB hydrogel containing $FeCl_3$

	Gelation time (s)
PMA/PMB hydrogel	1007 ± 137
PMA/PMB/Fe hydrogel-71	605 ± 101
PMA/PMB/Fe hydrogel-142	468 ± 47

Thus, the addition of $FeCl_3$ significantly reduced the gelation time and the dissociation time of the PMA/PMB/Fe hydrogel; moreover, these parameters can be controlled by the $FeCl_3$ concentration.

The viscoelastic properties of the PMA/PMB hydrogel and the PMA/PMB/Fe hydrogel are shown in Fig. 3. With regard to the gelation time, agitation efficiency was so high that it took shorter time compared to the preparation method described in the gelation time measurement section. Vigorous stirring by a vortex mixer for 10 s is sufficient to prepare both PMA/PMB and PMA/PMB/Fe hydrogels. The mechanical strength of the PMA/PMB/Fe hydrogel immediately after its preparation (10 s) was so low that it appeared almost sol. Both G' and G" of PMA/PMB/Fe hydrogel-142 were lower than those of PMA/PMB/Fe hydrogel-71. G' and G" of the

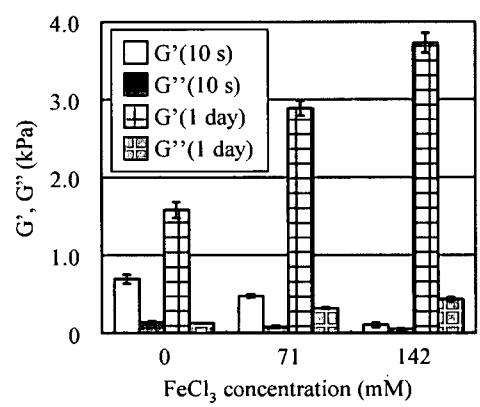

Fig. 3 Change in the elastic modulus (G') and the viscous modulus (G") of PMA/PMB hydrogel containing $FeCl_3$.

PMA/PMB hydrogel were higher than those of the PMA/PMB/Fe hydrogel. One day after the hydrogel preparation, increase in G' and G" was observed in all the hydrogels; G' of the PMA/PMB hydrogel was more than double its value immediately after the hydrogel preparation, while G" remained almost constant. It is noteworthy that G' of PMA/PMB/Fe hydrogel-71 increased 6-fold and that of PMA/PMB/Fe hydrogel-142 increased more than 60-fold after 1 day. G" of PMA/PMB/Fe hydrogel-71 also increased by approximately 4-fold and that of PMA/PMB/Fe hydrogel-142 increased more than 8-fold. Interestingly, among the 3 hydrogels, G' of PMA/PMB/Fe hydrogel-142 was the lowest immediately after the hydrogel preparation, but it was the highest after 1 day; this observation can be explained by the ionic crosslink formation.

During clinical application, the treated tissue will be covered by an antiadhesive agent and then sutured. Thus, the change in the mechanical properties during and after surgery would determine the clinical usefulness of an antiadhesive agent. A point worth noting is that immediately after its preparation, the PMA/PMB/Fe hydrogel is a weak gel; however, it improves its mechanical strength with time. This change in the mechanical properties of the PMA/PMB/Fe hydrogel can enable the hydrogel to attain a specific shape according to the application site and solidify after suturing; these features are a requisite for an antiadhesive material.

Furthermore, implantable antiadhesive materials should also be biocompatible in order to prevent the occurrence of any inflammatory reaction that would result in adhesions. Based on the results of the *in vivo* injection test, we have previously reported that PMA and PMB do not show notable adverse effects [5]. Although $FeCl_3$ is applied to dental materials to facilitate the adhesion of dental prosthesis to the dentin tissue, a report has shown the potential of $FeCl_3$ to cause oxidative stress on cells leading to the development of mutation [7]. The biocompatibility of the PMA/PMB/Fe hydrogel should be closely examined. We have been investigating the antiadhesive property of hydrogels and its effect on healing. No evidence of inflammation was observed in the tissues surrounding the hydrogel. We shall provide detailed reports of the results elsewhere.

Conclusions

A PMA/PMB hydrogel containing $FeCl_3$ (PMA/PMB/Fe hydrogel) shows that the mechanical properties of a hydrogel can be controlled by a combination of hydrogen bonding and ionic crosslinking. Severe inflammatory reaction was not observed in the tissues surrounding the hydrogel. Therefore, it can be concluded that the PMA/PMB/Fe hydrogel satisfies the basic requirements for an antiadhesive material.

Acknowledgement

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

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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

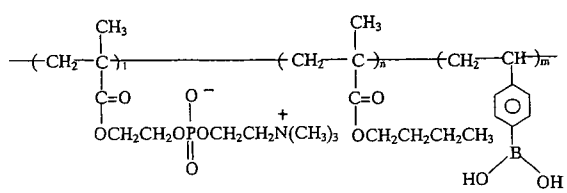

Fig. 1. Structure of PMBV60

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.

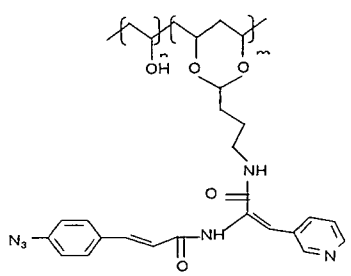

Fig. 2. Structure of photoreactive PVA (AWP)

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

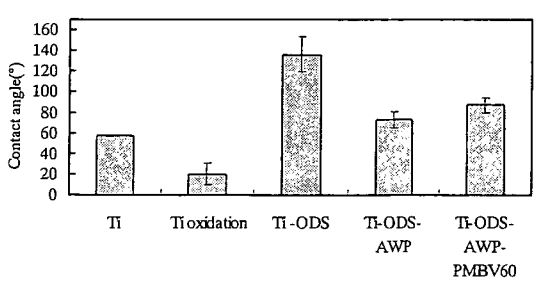

Fig. 3. The static contact angle measured according to changing titanium surfaces.

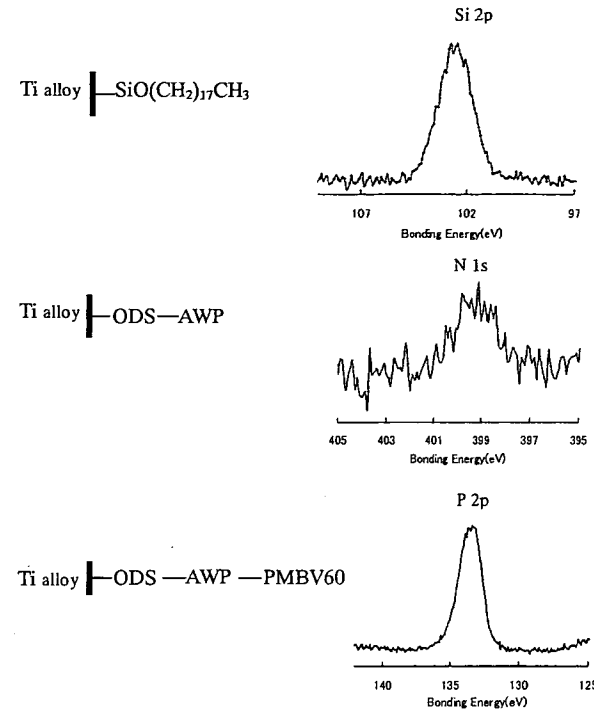

Fig. 4. XPS spectra showing the appearance of Si_{2p}, N_{1s}, P_{2p} peaks according to changing surfaces

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.

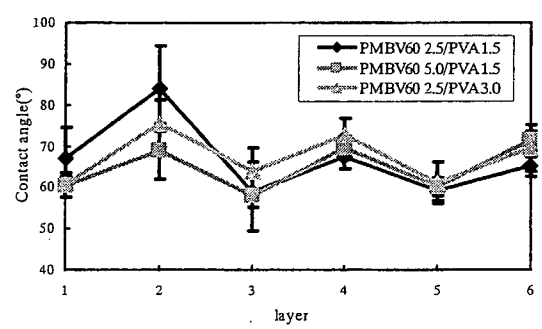

Fig. 5. The static contact angle measured from layer containing a different number of bonded of PMBV60 and PVA. Even number and odd number represents PMBV60 and PVA layer, respectively.