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Water Intrusion Property on Novel Porous Matrix Composed of Bioinspired Polymer Stereocomplex

Junji Watanabe and Kazuhiko Ishihara*
Department of Materials Engineering, School of Engineering, The University of Tokyo
Fax: 81-3-5841-8647, e-mail: ishihara@bmw.t.u-tokyo.ac.jp

A novel polymer matrix was designed for tissue regeneration. The favorable characteristics of the matrix involve; (i) cytocompatibility by phospholipid polymer, (ii) complete dissolution after hydrolysis, and (iii) easy water intrusion into the matrix. A bioinspired polymer was prepared by 2-methacryloyloxyethyl phosphorylcholine (MPC), *n*-butyl methacrylate, and enantiomeric oligo lactic acid macromonomers. The polymer could form thin membrane and the surface showed spontaneous chain rearrangement after contacting water. The mobility of the polymer chain was caused by MPC unit. The matrix was prepared by stereocomplex formation between enantiomeric oligo lactic acid segments. The matrix has inter-connecting structure which was formed by aggregation of micro particles. The obtained matrix showed dissociation of stereocomplex under physiological conditions, and the polymer backbone would dissolve after hydrolysis of the oligo lactic acid segment. Furthermore, the excellent water intrusion property was evaluated by using static contact angle measurement. In the case of porous polymer stereocomplex, a water droplet easily intruded into the matrix. However, the water intrusion could not observe on a conventional porous poly(D,L-lactic acid-co-glycolic acid) due to the hydrophobicity. The novel polymer matrix would be promising materials for tissue regeneration.
Key words: phospholipid polymer, oligo lactic acid, water intrusion, porous matrix, tissue regeneration

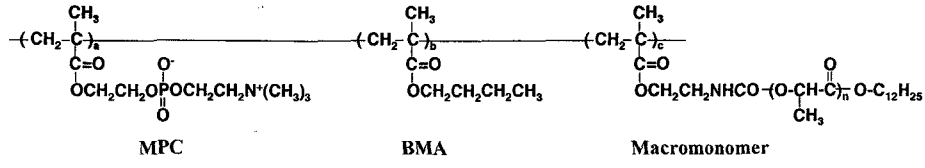
1. INTRODUCTION

Tissue regeneration is focused on as a novel medical treatment by medical doctors and engineering researchers. Recent new material for tissue regeneration was reported by Hubbell *et al.* as follows: degradation profile on the matrix is synchronized with the cell proliferation [1]. The requirements on tissue regeneration involve; (i) cells, (ii) cytokines, (iii) matrix. In particular, matrix is the most important for cell support. Cytocompatibility, complete degradation, and cell invasion are required for design of the matrix. From the viewpoint, we have proceeded the new approach to prepare the matrix by using bioinspired phospholipid polymer. The bioinspired polymers were well known excellent blood- and bio-compatible materials [2-3]. We target on the compatibility to prepare cell culture matrix. In our previous study, novel bioinspired phospholipid polymers composed of enantiomeric oligo L-(D-)lactic acid macromonomer, 2-methacryloyloxyethyl phosphorylcholine (MPC), and *n*-butyl methacrylate (BMA) were prepared for the porous polymer matrix [4-5]. One of the most favorable characteristics of the polymer is enhancement of cell adhesion on the bioinspired phospholipid polymer surface. The oligo lactic acid segment would also hydrolyze under physiological condition, and then the polymer backbone would completely dissolve. Furthermore, the oligo lactic acid segment could form stereocomplex each other for enhancement of mechanical property as cell culture matrix. These excellent characteristics would be leading materials in the field of tissue regeneration. In this study, cytocompatible phospholipid polymers with enantiomeric oligo lactic acid segments were prepared. The polymer-coated surface was characterized in terms of the spontaneously rearrangement of the phospholipid polar group and cell adhesion. Furthermore, a porous polymer matrix (stereocomplex) was prepared by using the phospholipid polymers, and the water intrusion and cell invasion were then evaluated.

2.MATERIALS AND METHOD

2.1 Maerials

L-Lactide and D-lactide were kindly supplied by Dainippon Ink and Chemicals, Inc. (Tokyo, Japan) and were recrystalized from ethyl acetate. *n*-Butyl methacrylate (BMA), *n*-dodecyl alcohol, stannous octoate, and dibutyltin dilaurate were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan), and BMA was distilled at 50 °C (20 mmHg). 2-Isocyanate ethyl methacrylate (Showa Denko Co., Tokyo, Japan) were distilled at reduced pressure (60 °C/ 2.5 mmHg). 2-Methacryloyloxyethyl phosphorylcholine (MPC) was synthesized and purified by a method from a previous report [6]. The other reagents were commercially available and used without further purification.



Scheme 1. Chemical structure of bioinspired polymer for stereocomplex.

2.2 Synthesis of bioinspired polymers

The bioinspired polymers (Scheme 1) with oligo lactic acid segment were copolymerized by using MPC, BMA, and enantiomeric oligo lactic acid macromonomer, which were reported in detail in our previous paper [4].

2.3 Characterization of polymer-coated surface

The obtained polymers were dissolved in chloroform in 1 w/v%, and be coated onto poly(ethylene terephthalate) (PET) film by dip coating process. The coating film was dried *in vacuo*, and utilized as DRY surface. For the purpose of WET surface, the coating film was immersed into distilled water for over night, and then lyophilized. Poly(D,L-lactic acid-co-glycolic acid) (PLGA, Aldrich Chem. Co., WI, USA) was used as conventional materials.

The surface properties were characterized in terms of elemental analysis and mobility factor. X-ray photoelectron spectroscopy (XPS, AXIS-HSi, Shimadzu/KRATOS, Kyoto, Japan) with MgKα was carried out. The releasing angle of the photoelectron for each element was fixed at 90 degrees. Dynamic contact angle by water was measured by using Wilhelmy plate method (DCA, DCA-100, Orientec Co., Ltd., Tokyo, Japan). The crosshead speed was 10 mm/min, and the measurement was carried out four times. The mobility factor (Mf) was calculated from advancing contact angle (θ_A) and receding contact angle (θ_R) as follows.

$$Mf = (\theta_A - \theta_R)/ \theta_A$$

2.4 Stereocomplex formation by enantiomeric oligo lactic acid segments

A configuration of the bioinspired polymer was evaluated by using ^{1}H-NMR. The polymer was dissolved in $CDCl_3$ or $CDCl_3$: CD_3OD = 1:2 (v/v). The stereocomplex was formed by mixing 20 wt% of the polymers with L-form and D-form oligo lactic acid. NaCl particles (200 μm cube) were simultaneously incorporated into the stereocomplex as a template. After the molding process, the polymer matrix was immersed into distilled water to dissolve NaCl. Then, porous structure was formed.

2.5 Characterization of porous matrix

The obtained stereocomplex was characterized by using scanning electron microscopy (SEM, SM-200, Topcon, Tokyo, Japan) and static contact angle (SCA, CA-W, Kyowa Interface Science Co., Ltd., Saitama, Japan) by water. Mouse fibroblast (L-929) cells were used and were routinely cultured in Eagle's Minimum Essential Medium (E-MEM, Nissui, Tokyo Japan), supplemented with 10 % fetal bovine serum (FBS, Gibco, NY, USA) at 37 °C in a 5 % CO_2 atmosphere. After treatment with 0.25 % trypsin, the cell density was adjusted to $5x10^5$ cells/mL for the porous matrix. After 5 days, cell invasion into the matrix was characterized by using scanning electron microscopy, after the glutaraldehyde fixation and staining with OsO_4.

3. RESULTS AND DISCUSSION

3.1 Synthetic results of polymers

The bioinspired polymers with oligo lactic acid segment were quantitatively synthesized as shown in Table I. The obtained polymers were abbreviated as PMBLLA (L-form) and PMBDLA (D-form). The MPC unit mole fraction was 0.16 in the polymer, and 0.12-0.16 of macromonomer was incorporated. The repeating unit of lactic acid was 23 (L-form) and 27 (D-form), which was determined by ^{1}H-NMR.

Table I. Synthetic results of bioinspired polymers

Abb.	Mole fraction in polymer*			Yield (%)
	MPC	BMA	Macromonomer	
PMBLLA	0.16	0.68	0.16	55
PMBDLA	0.16	0.72	0.12	69

[Monomer]=0.5 mol/L, [AIBN]=2.5 mmol/L, Solvent: THF/EtOH=1/1, Polymerization temperature: 60 °C, Polymerization time: 24 h.
*Determined by ^{1}H-NMR.

3.2 Surface properties on bioinspired polymers

The thickness of the polymer-coated surface was considered to be 100 nm. The depth of the XPS measurement was roughly 10 nm, when the releasing angle of the photoelectron was fixed at 90°. Figure 1 shows XPS chart of PMBLLA in DRY and WET state. The spectra attributed to the MPC and macromonomer unit were obtained at 400 eV (N_{1s} core level, urethane bonds in macromonomer), 402 eV (N_{1s} core level, choline methyl groups in MPC), and 134 eV (P_{2p} core level, phosphate group in MPC) in DRY state. Generally, phosphorylcholine polar group could not observed on MPC polymer coating surface in DRY state because of reduction of surface free energy. The phosphorylcholine groups were spontaneously rearranged to contact with water molecules in WET condition [7]. However, the PMBLLA and PMBDLA surface showed both choline methyl group and phosphate group in

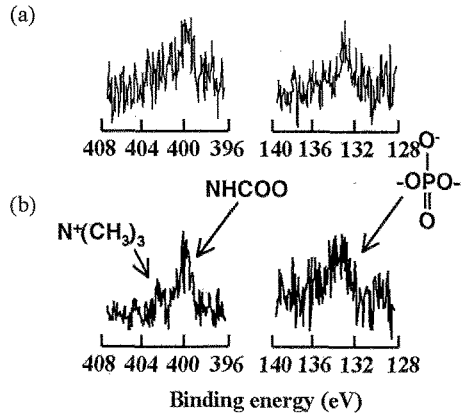


Figure 1. XPS chart of bioinspired polymer-coated surface; (a) DRY condition and (b) WET condition.

DRY state. This result indicated that the effect of MPC unit and oligo lactic acid segment would be observed at primary period. In the case of WET condition, similar spectra were observed. This result indicated that the oligo lactic acid segment was located on the surface in spite of WET circumstance.

The mobility of the polymer chain was evaluated by the mobility factor (Mf) as shown in Figure 2. In the case of bioinspired polymers, Mf was roughly 0.3 on both polymer-coated surface. On the other hand, 0.15 of Mf was calculated on conventional PLGA, indicating poor mobility of polymer chain. The Mf (0.3) was relatively high level, when the polyethylene (PE) surface was examined by using DCA, 0.1 of Mf was calculated. Furthermore, when highly hydrophilic polymer, poly(MPC), was blended with PE as a polymer alloy, the Mf increased at 0.25 [8]. From this report, the polymer alloy surface showed completely suppressed protein adsorption and platelet adhesion, indicating effect of MPC unit. Taking this result into account, the obtained bioinspired polymer surface, particularly interface, would show unique properties; (i) cytocompatibility by MPC unit, (ii) highly hydrophilicity by MPC unit, and (iii) adequate hydrophobicity for cell adhesion by oligo lactic acid segments.

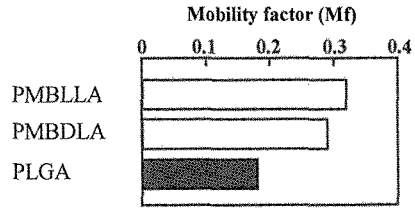


Figure 2. Mobility factor on polymer-coated surfaces by dynamic contact angle measurement.

3.3 Characterization of stereocomplex

We have already reported bioinspired polymer matrix composed of PMBLLA and PMBDLA by formation of stereocomplex as preliminary study [4-5]. Our next concern of the polymer matrix is enhancement of water intrusion into the matrix, indicating cell invasion. Driving force of the water intrusion would considered to be rearrangement of the phosphorylcholine groups by contacting water. We have already examined the polymer chain rearrangement by using Mf. For the effective stereocomplex formation, polymer configuration is important for the stereocomplexation and mechanical properties. The polymer configuration was estimated by ^{1}H-NMR measurement (Figure 3). $CDCl_3$ and $CDCl_3$:CD_3OD=1:2 (v/v) mixed solvent were used. In the case of $CDCl_3$, a broad signal attributed to the phosphorylcholine group was observed on 3.3 ppm. A sharp signal by methyl group by oligo lactic acid segments was observed on 1.5 ppm. Winnik *et al.* reported that broad signal of ^{1}H-NMR indicates restriction of the motion of the corresponding protons [9]. From this report, it is suggested that the phosphorylcholine groups aggregate in $CDCl_3$. On the other hand, sharp signal corresponding to the phosphorylcholine groups were observed in $CDCl_3$:CD_3OD=1:2 mixed solvent. Methyl group in oligo lactic acid segment was also observed as a sharp signal. This result indicates that the phosphorylcholine groups and oligo lactic acid segment are free to move in the solution. From this, stereocomplex formation was examined by using $CHCl_3$:CH_3OH=1:2 mixed solvent.

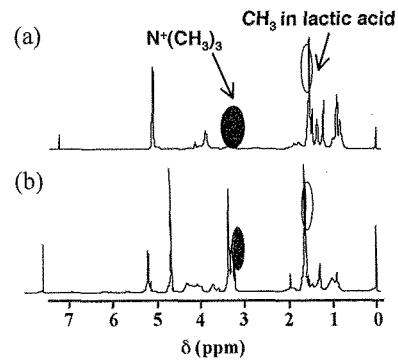


Figure 3. Polymer configuration of phospholipid polymer: (a) in $CDCl_3$ and (b) in $CDCl_3$: CD_3OD=1:2.

The obtained polymer stereocomplex was shown in Figure 4. The stereocomplex was freely molding by using spacer. From the SEM picture, the porous structure was clearly observed, 200 μm cubic space was formed by NaCl leaching technique. Inter-connecting capillary were utilized as the pathway for water intrusion and cell invasion.

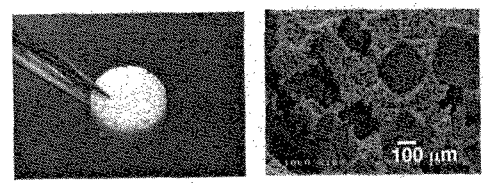


Figure 4. Pictures of porous polymer stereocomplex.

3.4 Water intrusion property on porous matrix

The water intrusion was evaluated by using SCA apparatus. If the water droplet was placed onto the porous polymer film, we can see the change of water contact angle. The contact angle by water would decrease, when the droplet permeated into the matrix. In this study, the water contact angle was measured for 2 minutes, which period is complete negligible to the effect of vaporization of water droplet. In the case of bioinspired porous matrix, the contact angle quickly decreased within 40 seconds, indicating highly water permeability (Figure 5). After 5 minutes, the water droplet was completely absorbed into the matrix. On the other hand, any significant contact angle change was not observed on the conventional PLGA porous matrix, which was also prepared by NaCl leaching technique. This result suggests that water intrusion was effectively enhanced by MPC unit. It is quite important property for the water intrusion and cell invasion into the bioinspired polymer matrix.

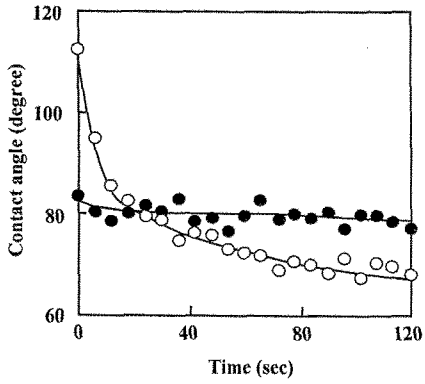


Figure 5. Water intrusion property on porous matrix; (○) stereocomplex, and (●) PLGA.

3.5 Cell invasion into porous matrix

Cell invasion was evaluated by using bioinspired polymer matrix as shown in Figure 6. First of all, we have already checked time dependence for cell invasion. After 1 day cell culture, the fibroblast cells adhered on the only surface, cell invasion was not observed in the matrix. This result indicated that 1 day cell culture was not enough to invade cells into the matrix, and the cell suspension could not spontaneously invade into the matrix by pathway of micro capillary. However, a lot of cell invasion was observed in the matrix after 5 days cell culture. The morphology of adherent cells was spread, and the morphology showed good cell proliferation. These results indicated that the bioinspired polymer matrix showed dual function; cell medium intrusion and cell invasion.

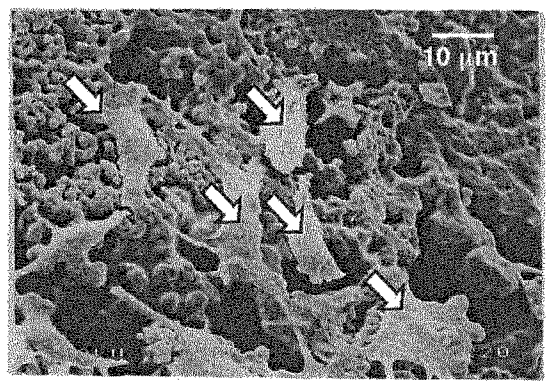


Figure 6. SEM picture of cell invasion into porous polymer stereocomplex.

4. CONCLUSIONS

The novel bioinspired polymers composed of MPC, BMA, and enantiomeric oligo lactic acid macromonomers were synthesized. The polymers were spontaneously forming stereocomplex between oligo lactic acid segments. The most attractive characteristics as matrix for tissue regeneration were highly water intrusion property and cell invasion into the matrix. The matrix showed adequate hydrophobicity for cell adhesion, and excellent polymer chain rearrangement was observed by MPC unit. These excellent properties would be promising matrix for 3-D cell culture.

5. REFERENCES

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