Higher Water Intrusion Property on Novel Porous Matrix Composed of Bioinspired Polymer Stereocomplex for Tissue Engineering

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(Received November 11, 2002; CL-020952)

A porous matrix composed of bioinspired phospholipid polymers with enantiomeric poly(lactic acid) segments in side chain, were prepared by formation of stereocomplex between them and characterized for use as new cell culture matrix.

Tissue engineering is an interdisciplinary science that utilizes basic principles from materials engineering and life sciences to create tissues from polymer matrices and cellular components. Langer et al. reported three-dimensional tissue reconstruction by using porous polymer matrix.[1,2] Required properties of the polymer matrix may involve: (i) cytocompatibility, (ii) hydrolytic degradation, and (iii) cell invasion and cell culture medium intrusion into the matrix. Most of the researcheres concerning polymer matrix utilize poly(L-lactic acid) (PLLA) and poly(D,L-lactic acid-*co*-glycolic acid) (PLGA) as synthetic polymer and collagen as natural polymer. The collagen involves serious immunogenicity by animal origins. From this, synthetic polymers such as PLLA and PLGA are favorable materials as cell culture matrix.[3,4] However, wettability is not sufficient to aim at cell culture, for example the medium intrusion is quite low due to the hydrophobicity of the PLLA matrix. Such a poor wettability of the matrix seriously inhibited three-dimensional tissue reconstruction. To improve the wettability, a novel material design is necessary for preparation of the matrix.

In our recent studies, a novel bioinspired polymer aiming at cell culture was designed and synthesized by using 2-methacryloyloxyethyl phosphorylcholine (MPC),[5] poly(D,L-lactic acid) (PLA) macromonomer, and *n*-butyl methacrylate (BMA).[6] Most favorable characteristics of the polymer will involve cytocompatibility in terms of suppression of secretion of inflammatory cytokines.[7,8] The polymer-coated surface shows fibroblast cells adhesion corresponding to the PLA macromonomer content in the polymer. Furthermore, cell morphology would be rounded with increasing MPC content in the polymer. We have already reported the bioinspired polymers with enantiomeric poly(L-(D)-lactic acid) (PL(D)LA) macromonomer.[9,10] The enantiomeric polymer segments, PLLA and PDLA, would be utilized for preparation of a porous matrix by formation of stereocomplex. The advantage of the matrix by polymer stereocomplex would be expected to have the following characteristics: (i) easy preparation of porous matrix and adequate cell adhesion on the crystalline region by stereo-complexation and (ii) quick medium intrusion by MPC unit, (iii) adequate cell invasion into the porous matrix, and (iv) cytocompatibility by MPC unit. We now report the mobility of the segment in the polymer by dynamic contact angle by water and water intrusion into the porous matrix. The matrix can be advantageous in the design of implantable materials for tissue engineering.

PL(D)LA macromonomers were synthesized by our previous method.[9] The degree of polymerization in the PLLA and PDLA macromonomers were determined to be 23 and 27 by ^{1}H NMR, respectively. The macromonomer was copolymerized with MPC and BMA. The obtained polymers were summarized in Table 1. MPC content in the each polymer was 16 mol%, and macromonomer contents were 12 mol% (PMBLLA) and 16 mol% (PMBDLA). The number average molecular weight (*M*n) was estimated to be 1.1×10^5 (PMBLLA) and 1.2×10^5 (PMBDLA). The bioinspired polymer was dissolved by chloroform (1 w/v%). To evaluate side chain mobility, phosphorylcholine group and PL(D)LA segment, quartz glass was coated using chloroform solution. PLGA was also coated as a control. Dynamic contact angle analysis was carried out using distilled water at 25 °C (Table 2). After polymer coating, advancing contact angle showed 79.0–82.0 degree. In the case of bioinspired polymers, receding contact angle decreased in the range of 23.5–25.9 degrees. The hysteresis was larger than that of PLGA. This result indicates that the polymer-coated surface would spontaneously rearrange hydrophilic-hydrophobic component to reduce surface free energy by exposure to different external environment. The phosphorylcholine groups are the only hydrophilic unit in the polymer. In our previous study, the rearrangement was evaluated by X-ray photoelectron spectroscopy (XPS).[6] Before contacting with water, N_{1s} and P_{2p} core level spectra attributed to phosphorylcholine group were weak. However, these N_{1s} and P_{2p} spectra were clearly observed after contacting with water. Taking these results into account, the bioinspired polymer surface would easily rearrange by contacting with water.

Table 1. Composition of MPC polymers for stereo-complexation

Code	Polymer composition/mol%[a]			Mn[b]
	MPC	BMA	Macromonomer	
PMBLLA	16	72	12	1.1×10^5
PMBDLA	16	68	16	1.2×10^5

[a]Determined by ^{1}H NMR. [b]With poly(styrene) standard.

Table 2. Dynamic contact angle analysis

Code	Contact angle/degree		Hysteresis/degree
	Advancing	Receding	
PMBLLA	80.7 ± 2.4	54.8 ± 1.7	25.9 ± 1.9
PMBDLA	82.0 ± 0.2	58.5 ± 1.2	23.5 ± 1.2
PLGA	79.0 ± 0.8	64.5 ± 0.6	14.4 ± 1.4
Quartz	64.5 ± 1.3	56.1 ± 1.2	8.9 ± 0.4

$n = 3$, Mean value and standard deviation were indicated.

We have already reported bioinspired polymer matrix composed of PMBLLA and PMBDLA by formation of stereocomplex between PLLA and PDLA segments.[9] Our next concern of the polymer matrix is enhancement of water intrusion into the matrix. Driving force of the water intrusion would considered to be rearrangement of the phosphorylcholine groups by contacting with water. And, solution property by ^{1}H NMR was then investigated by

changing solvent composition (Figure 1).[11] $CDCl_3$ and $CDCl_3$/CD_3OD = 1/2 (v/v) mixed solvent were selected for ^1H NMR study. Figure 1(a) shows PMBLLA spectrum in $CDCl_3$. A broad signal attributed to the phosphorylcholine group was observed on 3.32–3.40 ppm. A sharp signal by methyl group by PLLA was observed on 1.50–1.54 ppm. Winnik et al. reported that broad signal of ^1H NMR indicates restriction of the motion of the corresponding protons.[12] 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 mixed solvent. Methyl group in PLLA was also observed as a sharp signal. This result indicates that the phosphorylcholine groups are free to move in the solution. In the mixed solvent, the phosphorylcholine groups may locate the pathway for water intrusion after stereo-complexation.

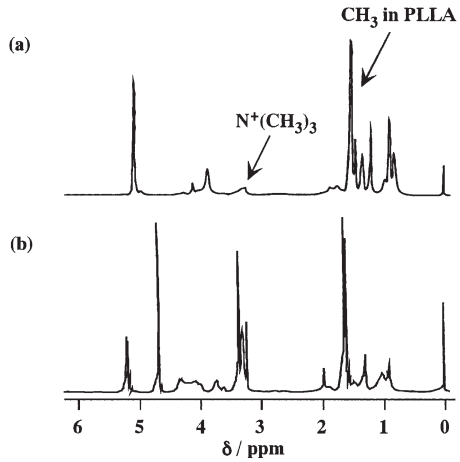

Figure 1. ^1H NMR spectra of PMBLLA in different solvent; (a) $CDCl_3$ and (b) $CDCl_3$/CD_3OD = 1/2 (v/v).

Stereo-complexation by mixing PMBLLA and PMBDLA polymers was carried out by using $CHCl_3$/CH_3OH mixed solvent. Twenty wt% of PMBLLA (PMBDLA) $CHCl_3$/CH_3OH = 1/2 solutions were prepared for the stereocomplexation. Detailed procedures to prepare porous matrix was previously reported.[9] A porous matrix was prepared by sodium chloride leaching technique. The salt was fully leaked out by using sufficient distilled water. Surface morphology of the matrix was flat due to the Teflon spacer. The pore size was found to be ca. 200 μm and was in good agreement with the size of sodium chloride as a template. The formed pores had penetrated into the scaffold and the pores dispersed in the matrix. Thermal property (by differential scanning calorimetry) and crystalline structure (by wide-angle X-ray diffraction) were also characterized by the method of previous report, and stereo-complexation was confirmed.[9] The water intrusion was evaluated by using static contact angle apparatus. The porous matrix was firstly lyophilized, and then set on the stage. Direct methods of measuring contact angles involve measurement on a drop resting on a porous matrix surface. This is referred to as the sessile drop method. The 10 μL of pure water droplet was introduced on the surface by using micro-syringe. The water droplet was completely absorbed in the porous polymer matrix for a few minutes. However, the effect of vaporization of the water droplet is not negligible by contact angle measurement for a long period. Therefore, the contact angle measurement was carried out within 2 minutes. The water intrusion was characterized as change in the contact angle, which is monitored in 6 s interval. Figure 2 shows the change in the contact angle. In the case of the bioinspired polymer matrix formed by stereocomplexation, the contact angle quickly decreased about 40 degrees. At the first period, it is suggested that the obtained contact angle (108 degrees) was caused by crystalline region by stereo-complexation. On the other hand, any significant contact angle decrease was not observed on the conventional PLGA matrix, which was also prepared by sodium chloride leaching technique. This result suggests that water intrusion was enhanced by MPC unit. It is quite important property for the porous matrix aiming at three-dimensional tissue reconstruction.

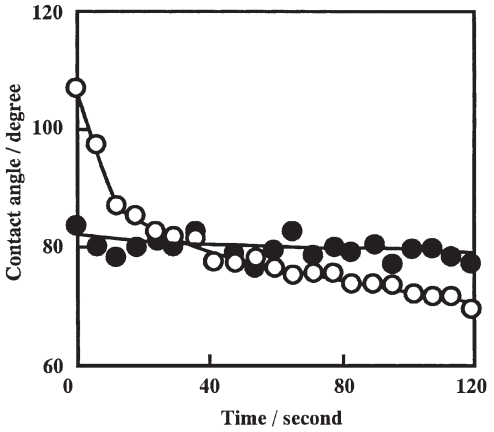

Figure 2. Static contact angle by water on porous matrix; (○) MPC polymer matrix and (●) PLGA matrix.

In conclusion, we synthesized a porous matrix with the bioinspired polymers having phosphorylcholine groups. The porous matrix has a highly affinity to the water in comparison with the conventional PLGA. The control of water intrusion into the porous matrix will be necessary for development of tissue engineering matrix. The degree of PLLA and PDLA polymerization and their content in the polymer are now in progress in order to control the degradation time.

References and Notes

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11 a) ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 0.85 (t, 3H, CH_3 for *n*-dodecanol), 0.96 (s, 3H, α-methyl), 1.21 (m, 2H x10, CH_2 for *n*-dodecanol), 1.50–1.54 (m, 3H x23, CH_3 for PLLA), 1.88 (broad, 2H, CH_2 for polymer backbone), 3.32–3.40 (m, 3H x3, CH_3 for choline), 3.88–4.13 (m, 2H x2, CH_2 for side chain), 4.03–4.06 (m, 2H, $COOCH_2$ in terminal of *n*-dodecanol), 5.05–5.18 (m, 1H x23, CH for PLLA). b) ^1H NMR ($CDCl_3$/CD_3OD = 1/2 (v/v), 300 MHz, ppm): δ 0.90 (t, 3H, CH_3 for *n*-dodecanol), 1.00 (s, 3H, α-methyl), 1.28 (m, 2H x10, CH_2 for *n*-dodecanol), 1.60–1.62 (m, 3H x23, CH_3 for PLLA), 1.96 (broad, 2H, CH_2 for polymer backbone), 3.30 (s, 3H x3, CH_3 for choline), 3.65–3.84 (m, 2H x2, CH_2 for side chain), 4.02 (m, 2H, $COOCH_2$ in terminal of *n*-dodecanol), 5.18–5.22 (m, 1H x23, CH for PLLA).
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