

Phospholipid Polymer Hydrogel Formed by the Photodimerization of Cinnamoyl Groups in the Polymer Side Chain

Jun Yamaguchi, Junji Watanabe, Madoka Takai, Kazuhiko Ishihara

Department of Materials Engineering, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Tokyo 113-8656, Japan

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ABSTRACT: We synthesized new polymers with both photocrosslinkability and biocompatibility by a random copolymerization of 4-(4-methoxycinnamoyl)phenyl methacrylate and 2-methacryloyloxyethyl phosphorylcholine. These polymers were used as prepolymers to make a hydrogel by photoirradiation. Gelation began with 5 s of photoirradiation and reached an equilibrium state after 360 s of photoirradiation. The absorption maximum at 347 nm, attributed to the cinnamoyl group, disappeared with the photoirradiation time. That is, dimerization between cinnamoyl groups in the polymer proceeded and formed a hydrogel. The equilibrium water

concentration of the hydrogels was more than 90%. Moreover, we succeeded in making a microshape hydrogel on glass by photoirradiation through a photomask. The window shape of the photomask was transferred to the hydrogel that was formed. We concluded that these photocrosslinkable polymers could be useful in preparing microfluidic devices for separating or immobilizing biomolecules and cells. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 104: 44–50, 2007

Key words: biocompatibility; gelation; hydrogels; photopolymerization; phospholipid polymer

INTRODUCTION

The photochemical reactions of polymers have attracted strong attention not only for photoresist applications in electronics but also for the photofabrication of microfluidic devices in modern bioengineering^{1–3} because photochemical reactions have several advantages, such as rapidity and homogeneity at room temperature. Moreover, microfabrication by photochemical reactions can be carried out more efficiently than normal chemical and physical techniques.

Recently, through the use of photocrosslinking reactions between polymer chains, several microstructures have been constructed in the microfluidics on a biochip, such as gate, valve, salt-bridge, and immobilized biocomponents including proteins, DNA, and cells. The polymers generally used for these purposes are polysaccharide, polypeptide, and hydrophilic synthetic polymers.^{4–10} Most are used in a photopolymerization system composed of a monomer, a crosslinker, and an initiator for radical generation by photoirradiation. Jiménez et al.¹¹ reported the photopolymerization

of acrylamide for making enzymatic microsensors. It is a successful method; however, sometimes the remaining monomer and photoinitiator disturb enzymatic reactions, and radicals generated by photopolymerization attack the enzyme and induce a significant reduction of the activity of the enzyme immobilized in the polymer matrix. Thus, polymer-type photocrosslinkers have been researched. Some photochemical reactive groups should be introduced into the polymer chains. Although there are many photochemical reactions, photoinduced dimerization is suitable for making crosslinking points in biomedical hydrogels.^{5,12–14} Because no byproduct is formed during the reaction, biomolecules immobilized in the hydrogels may maintain their activity. Poly(vinyl alcohol) with stilbazolium groups (PVA–SbQ), known as a photocrosslinkable polymer, has been put to practical use in the immobilization of enzymes.¹² PVA–SbQ is a hydrophilic polymer and forms a hydrogel by photoirradiation. In general, poly(vinyl alcohol) (PVA) is believed to be a biocompatible polymer, but considerable protein adsorption has been observed at the surface, and clot formation is induced shortly. That is, when the surface of a biosensor is covered with a PVA membrane and the sensor is implanted subcutaneously, the sensor output decreases with time dramatically because of protein adsorption.¹³ Moreover, poly(*N*-isopropylacrylamide) with stilbazolium has been reported.¹⁴ In the case of microfabrication in microfluidics on biochips, the condition may be much severer because

Correspondence to: K. Ishihara (ishihara@mpc.t.u-tokyo.ac.jp).

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the concentrations of the targeted biological components are quite low in the microfluidics. That is, inhibiting nonspecific adsorption of these biocomponents should be required of the surface. Fortunately, in our current research on biocompatible polymers, polymers bearing phosphorylcholine groups showed excellent performance with respect to protein-adsorption resistance and cell-adhesion inhibition.^{15–20} These polymers are composed of 2-methacryloyloxyethyl phosphorylcholine (MPC) and other vinyl compounds. The MPC polymers are extremely hydrophilic, and a hydrogel structure can be expected with suitable crosslinkers. In this study, to obtain a hydrogel by photoirradiation, we synthesized polymers composed of MPC and 4-(4-methoxycinnamoyl) phenyl methacrylate (MOCPMA). The MOCPMA unit was expected to form crosslinking points through the photodimerization process of cinnamoyl groups.²¹ We report the photoreaction of the MPC polymer in the dry state and some characteristics of the hydrogel formed by the crosslinked MPC polymer.

EXPERIMENTAL

Materials

MPC was synthesized by a method previously reported.²² 4-Hydroxyacetophenone, 4-methoxybenzaldehyde, and 2,2'-azobisisobutyronitrile (AIBN) were purchased from Kanto Chemical (Tokyo, Japan). Methacryloyl chloride was purchased from Wako Pure Chemical (Osaka, Japan). Other organic reagents and solvents were purified by the usual method.

Synthesis of the polymers

MOCPMA was synthesized with the method previously described by Rami Reddy et al.²³ The chemical structure of MOCPMA was confirmed with ¹H-NMR (α -300, JEOL, Tokyo, Japan). Random copolymers of MPC and MOCPMA {poly[2-methacryloyloxyethyl phosphorylcholine-co-4-(4-methoxycinnamoyl) phenyl methacrylate] (PMMC)} (Fig. 1) were synthesized by copolymerization in an ethanol (EtOH)/ethyl methyl ketone mixture with AIBN as the initiator (Fig. 1). The concentrations of the monomer and AIBN were 0.2 and 2 mmol/L, respectively. The polymerization was carried out with stirring at 70°C for 2 h in an argon atmosphere. PMMC was purified by precipitation in a diethyl ether/chloroform mixture (50/50 v/v). The polymers

were characterized with ¹H-NMR spectroscopy, gel permeation chromatography (GPC; Jasco, Tokyo, Japan), and solubility testing. The GPC measurements were carried out with a mixture of methanol and water (7/3) containing 10 mmol/L lithium bromide as an eluent at a flow rate of 0.5 mL/min (SB-804 HQ column, Shodex, Tokyo, Japan). A poly(ethylene glycol) standard was used to determine the molecular weight.

Photoreaction of the polymers

The photoreactivity of the copolymers was evaluated by ultraviolet–visible spectroscopy (V-560, Jasco) and Fourier transform infrared spectroscopy (FTIR-615, Jasco). The photoirradiation was carried out at room temperature with an ultrahigh-pressure mercury lamp (UVL-400HA, Riko, Chiba, Japan; wavelength = 360 ± 50 nm). The UV-light intensity at the reaction site was approximately 3.5 × 10⁴ lx according to a light meter (LX-105, Custom Co., Tokyo, Japan). Polymer solutions (20 wt %) in EtOH were cast onto mold plates. After the polymer solutions were dried, photoirradiation was carried out between 10 and 3600 s. The photocuring yield was defined as follows:

$$\text{Photocuring yield (wt \%)} = W_g/W_0 \times 100 \quad (1)$$

where W_g and W_0 are the weights of the insoluble part and initial polymer, respectively. After irradiation, the polymers were immersed in water, and hydrogels were obtained.

The EtOH solution containing 20 wt % PMMC90 was spin-coated (500 rpm for 5 s and 2000 rpm for 20 s) onto a glass substrate. After drying, it was exposed to light for 60 s through a photomask with a circular structure pattern (0.3 mm in diameter) and a cross structure pattern (1.4 mm long) at room temperature. Then, the polymer on the substrate was washed with EtOH to remove the unreacted polymer.

Measurement of the equilibrium water content (EWC)

Hydrogels obtained by photoirradiation were used for EWC measurements. EWC was defined as follows:

$$\text{EWC (wt \%)} = [(W_b - W_a)/W_b] \times 100 \quad (2)$$

where W_a and W_b are the weights of the dry hydrogel and hydrated hydrogel, respectively.

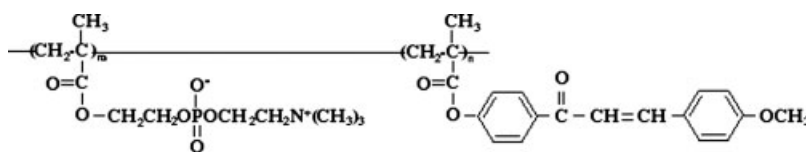


Figure 1 Chemical structure of PMMC.

TABLE I
Characterization of PMMC

Sample	Feeding ratio (mol %)		Copolymer composition (mol %) ^a		$M_w (\times 10^{-4})^b$	Yield (%)	Solubility ^c	
	MPC	MOCPMA	MPC	MOCPMA			EtOH	Water
PMMC90	90	10	91	9	2.9	79	Yes	Yes
PMMC95	95	5	96	4	3.1	66	Yes	Yes
PMMC97	97	3	97	3	5.3	73	Yes	Yes

Monomer concentration = 0.2 mol/L; [AIBN] = 2 mmol/L; polymerization time = 2 h; polymerization temperature = 70°C.

^a Determined by ¹H-NMR.

^b Weight-average molecular weight determined by GPC in 3/7 water/methanol with a poly(ethylene glycol) standards.

^c Determined at a polymer concentration of 1 mg/mL.

RESULTS

Synthesis of PMMC

Random PMMC copolymers were synthesized by free-radical copolymerization with AIBN as the initiator. The results of the polymerization and characteristics of the polymers are summarized in Table I. The polymerization proceeded homogeneously. Varying the composition of MOCPMA in the feed controlled the number of MOCPMA units in the copolymers. Every polymer that was obtained was soluble in EtOH and water.

Photochemical reaction of PMMC

We evaluated the photoreactivity of the polymers by monitoring the change in the UV absorption at 345 nm and the IR spectra at 1600 cm⁻¹ of PMMC90 spin-coated onto a glass substrate with an EtOH solution containing 20 wt % PMMC90. Figure 2 shows the changes in the UV absorption spectra of PMMC90 spin-coated onto the glass with various photoirradiation times. The absorption maximum was observed at 345 nm and originated from a double bond in a cinnamoyl group. The absorbance at 345 nm decreased with an increase in the irradiation time. Figure 3

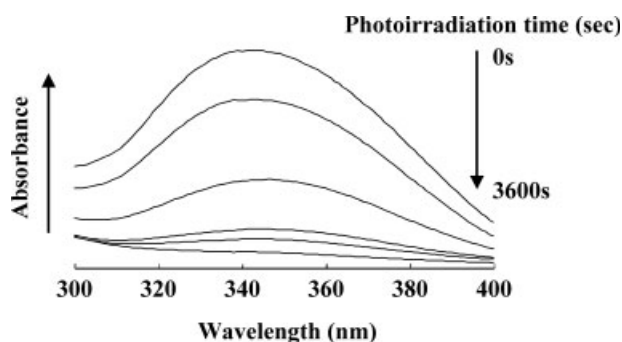


Figure 2 Changes in the UV absorption spectra of PMMC90 spin-coated on a glass substrate with various photoirradiation times.

shows the changes in the IR spectra of PMMC90 spin-coated onto the glass substrate with various photoirradiation times. IR adsorption at 1600 cm⁻¹ (C=C) in a cinnamoyl group was observed. The adsorption peak at 1600 cm⁻¹ decreased with an increase in the irradiation time in good agreement with the measurement results of UV absorption. The dimerization content of PMMC determined by the absorption wavelength near 345 nm is shown in Figure 4. The cinnamoyl groups in the polymer easily made photodimerization up to 80% within 300 s, and it rose above 90% with 600 s of photoirradiation.

Formation of the PMMC hydrogels

Figure 5 shows the photocuring yield of the polymers after they dried from a solution of EtOH. Polymer hydrogels were prepared successfully by photoirradiation of the copolymers. However, the hydrogel yield

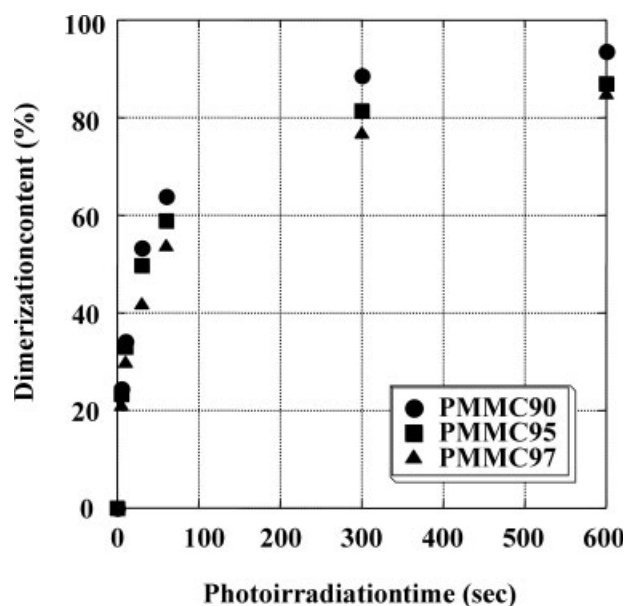


Figure 3 Dimerization content of PMMC determined by the UV absorption wavelength near 345 nm.

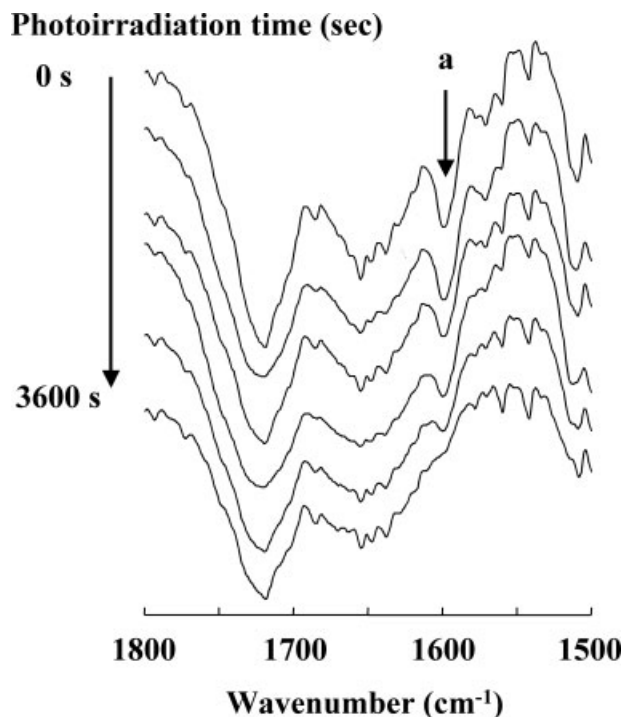


Figure 4 Changes in the IR spectra of PMMC90 spin-coated on a glass substrate with various photoirradiation times. The IR adsorption at 1600 cm^{-1} (C=C) in a cinnamoyl group is labeled "a".

by photoirradiation depended on the number of cinnamoyl groups in the polymer; that is, PMMCs with more cinnamoyl groups showed higher photocuring yields.

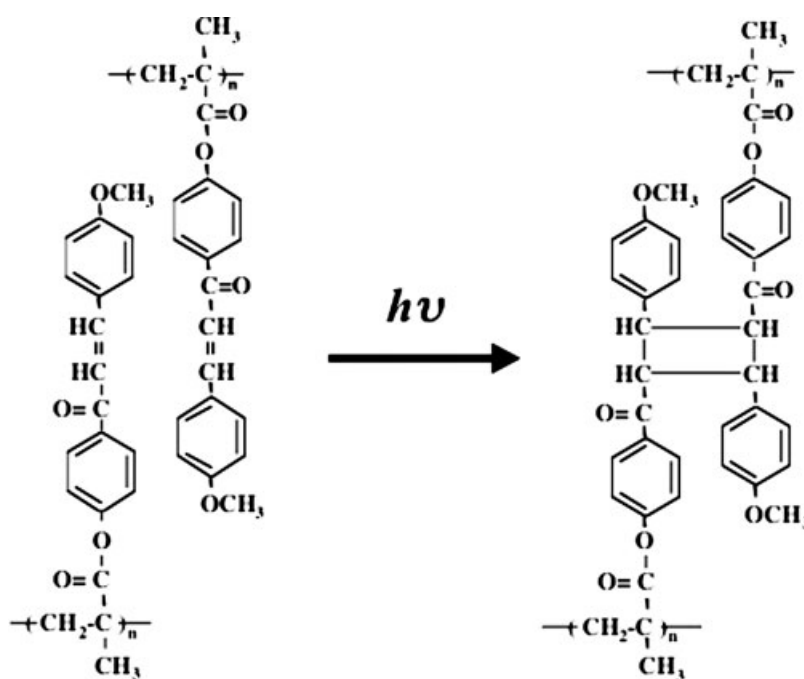


Figure 5 Mechanism of crosslinking of the cinnamoyl group by photoirradiation.

Figure 6 shows the EWCs of the obtained hydrogels. The EWCs of the hydrogels slightly depended on the number of cinnamoyl groups, but every polymer had an EWC greater than 90%.

Figure 7 shows pictures of a hydrogel prepared from PMMC90 with a photomask during photoirradiation. The microfabricated PMMC90 gel, having two shapes (circle and cross), was observed under an inverted optical microscope. The thickness of the PMMC90 gel was $1.6\text{ }\mu\text{m}$, and it did not change before and after photoirradiation. The pattern of the photomask was transferred to the hydrogel with high fidelity. Figure 8 shows optical microscopy pictures of a polymer gel formed by photoirradiation before and after immersion in water. The diameter of the hydrogel increased 50% when it reached an equilibrium state (Fig. 9). After the hydrogel reached equilibrium swelling, many defects were observed, but the shape was not changed.

DISCUSSION

The microfabrication of a hydrogel in microfluidics recently became a significant method for developing novel microfluidic devices such as bioseparators for proteins and high-performance diagnostic devices on a chip. For this purpose, the hydrogel should be prepared *in situ* in the microfluidics on the chip and have excellent biocompatibility to prevent nonspecific adsorption of biocomponents such as proteins and cells. We reported that hydrogels bearing phosphorylcholine groups show protein-adsorption resistance

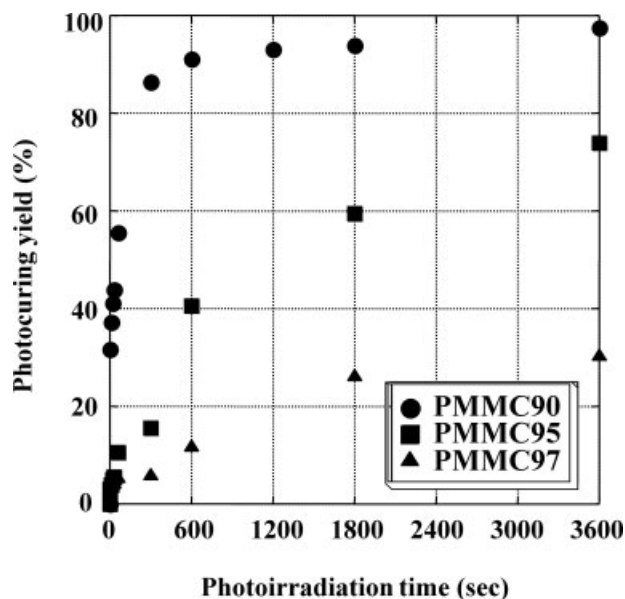


Figure 6 Photocuring yields of PMMC90, PMMC95, and PMMC97 with various photoirradiation times.

and cell-adhesion inhibition.^{13–20,22} That is, if we could make a hydrogel with polymers bearing phosphorylcholine group *in situ* by physical signals, it would be very useful for developing biodevices. With these considerations in mind, photocrosslinkable phospholipid polymers were synthesized, and hydrogels were prepared from them.

The photodimerization of cinnamoyl groups is generally known and is introduced into a polymer chain to form crosslinking points by photoirradiation. We prepared PMMC by a conventional radical polymerization of the corresponding monomers. Because the concentration of the MPC units was more than 90%, the obtained polymers were water-soluble. The photodimerization of the cinnamoyl groups in the polymer proceeded well by photoirradiation, and 10 min of irradiation made equilibrium up to 90% dimerization even in the solid state. This result showed that the cinnamoyl groups gathered together and reacted between two molecules easily. On the other hand, the hydrogel yield strongly depended on the composition of the polymers.

The PMMC with less cinnamoyl had only a 30% yield of the hydrogel. The increase in the number of cinnamoyl groups improved the hydrogel yield dramatically; that is, PMMC90 could form a hydrogel with more than a 95% yield. It is very difficult to distinguish intermolecular and intramolecular reactions. However, the possibility of intermolecular crosslinking by dimerization might increase with an increase in the number of cinnamoyl groups in the polymer. This is due to the dimerization of the cinnamoyl groups induced intramolecularly. The hydrogel that formed was homogeneously swollen in water and was trans-

parent. The EWC of the PMMC hydrogel was between 91 and 96% because of the extreme hydrophilicity of the MPC units. Protein adsorption on the MPC polymer has been researched not only by us but also by other research groups.^{13–20,22,24–28} Every study has revealed that MPC polymers can reduce protein adsorption in comparison with conventional polymers. This is related to the water structure of the hydrogels. Because of the lack of mechanical properties of the PMMC hydrogel, we could not evaluate the protein adsorption and permeability of the solute through the PMMC hydrogel. However, this led us to strongly speculate that the protein adsorption on the PMMC hydrogel might be slight. Also, the EWC of the PMMC hydrogel was over 90%. Because the permeability of the solute through the hydrogel depended on the water content, it can be said that the PMMC hydrogel could render good permeability of the solute. In our previous report,²² an MPC polymer with hydrophobic units, poly(MPC-*co*-*n*-butyl methacrylate), took a hydrogel structure with an EWC value of about 40%; the protein (molecular weight = 6×10^4), bovine serum albumin, could permeate the hydrogel membrane. Direct observations will be made in the near future after we improve the PMMC hydrogels. The shape of the hydrogel that was formed from PMMC was controlled with a photomask. We tried to make two different shapes with the hydrogels, as shown in Figure 7. The size and shape of the photomask were transferred to the hydrogel very well in both cases. That is, fabrication of a hydrogel in the microfluidics on a chip could be achieved by photoirradiation. Moreover, PMMC was able to form the hydrogel quickly in comparison with poly(*N*-isopropylacrylamide) with stilbazolium.⁷ On the other hand, as shown in Figure 8, many defects were observed after the hydrogel reached equilibrium swelling. This was thought to generate osmotic pressure in the hydrogel during swelling. The mechanical strength of the PMMC hydrogel is too low for the hydrogel to be used as a matrix for the immobilization of biomolecules at this time; we are now improving the hydrogel by changing the molecular weight and mo-

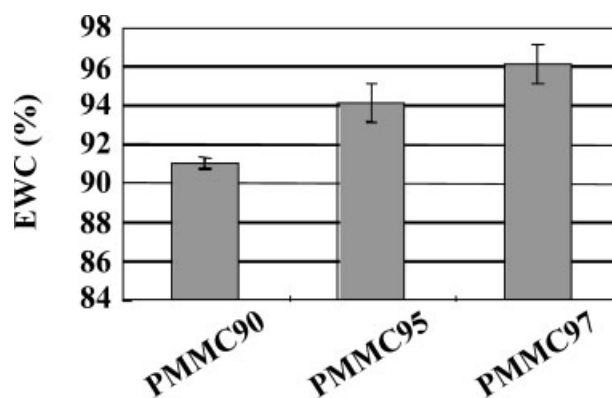


Figure 7 EWC of PMMC gels.

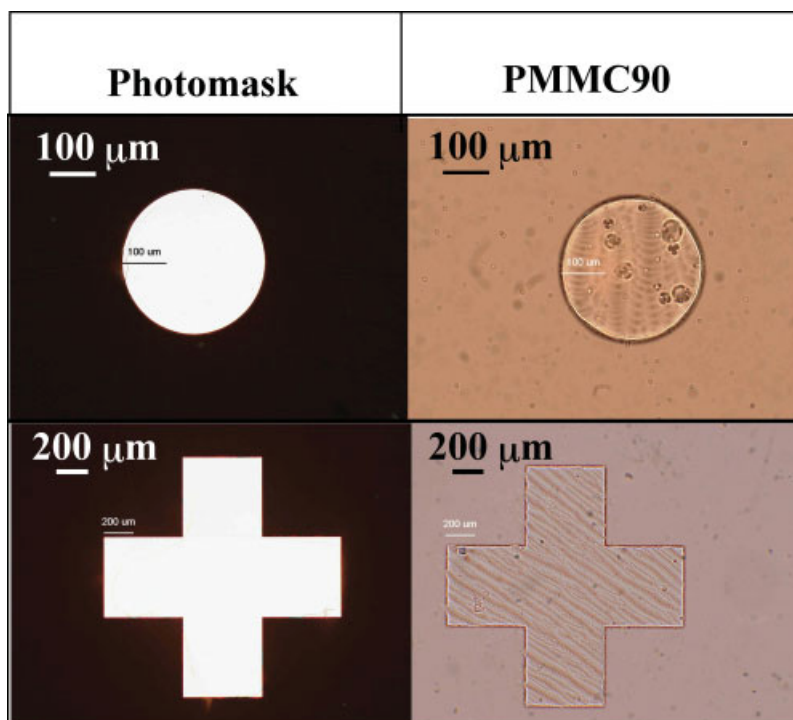


Figure 8 Microfabricated PMMC90gel having two shapes (circle and cross) observed under an inverted light microscope. The microfabricated PMMC90 gels were cured by photoirradiation (60 s, 320 W). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

lecular structure of the photocrosslinkable phospholipid polymer.

CONCLUSIONS

New photocrosslinkable polymers with both phosphorylcholine and cinnamoyl groups were success-

fully synthesized. The polymers were used as prepolymers to make hydrogels by photoirradiation. The photoreaction of cinnamoyl groups began with 5 s of photoirradiation time and reached an equilibrium state after 360 s. It was thought that a crosslinking bond was formed by the dimerization of cinnamoyl groups in the polymer upon photoirradiation. The

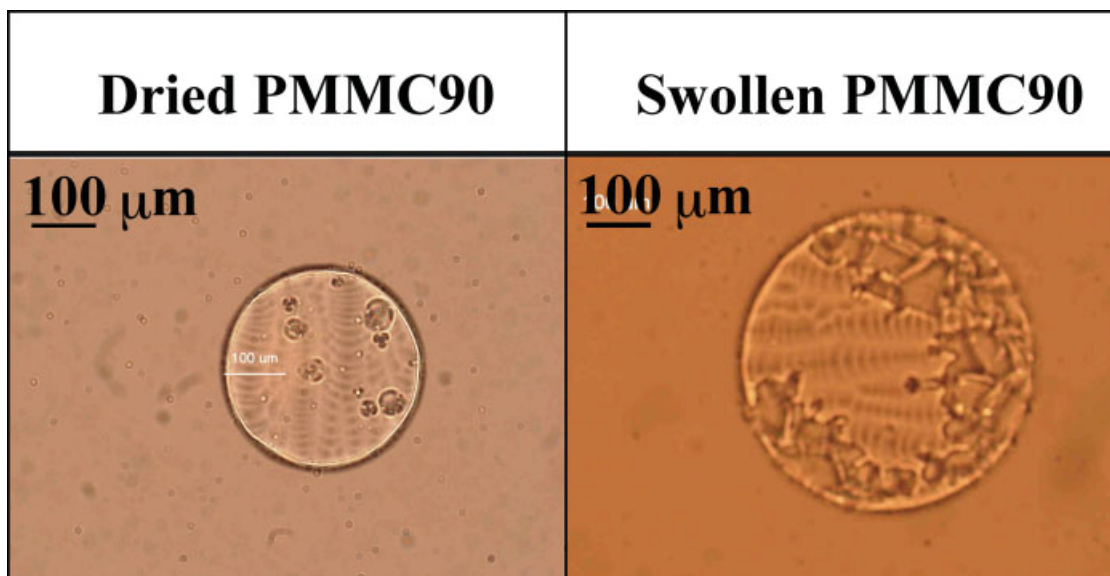


Figure 9 Swelling behavior of the microfabricated PMMC90 gel (circular shape) in water. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

EWC of the hydrogels was more than 90%. Moreover, we succeeded in microfabricating the hydrogel on a glass substrate. The pattern of the photomask was transferred to the hydrogel with high fidelity. It was determined that the microfabricated PMMC90 gel was swollen by water as well as the PMMC90 gel synthesized with mold plates. If the mechanical properties can be improved and the biocompatibility of the hydrogel can be confirmed in the future, we expect that the polymer can be applied to both making a microgate for separation and immobilizing biological components at a specific position in a microfluidic device.

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