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Photocrosslinkable and Biocompatible Phospholipid Polymers for Making Microhydrogel in Microfluidic Devices

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ABSTRACT

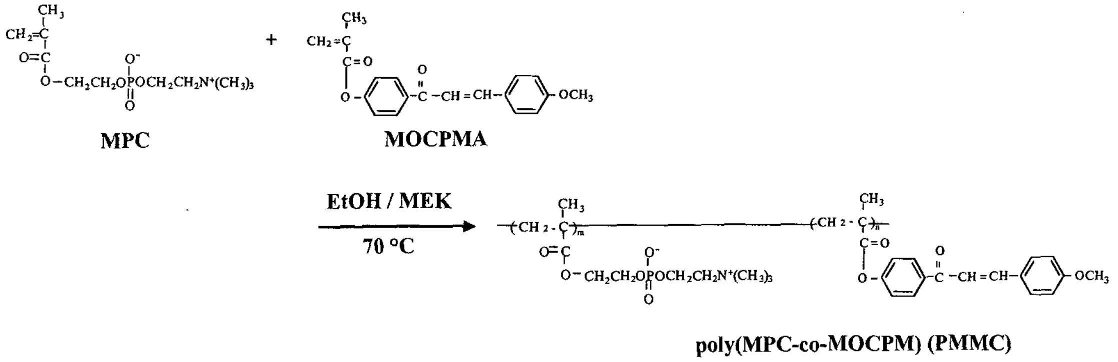
Development of soft polymer materials, such as hydrogel formed by a microfabrication technique using a photochemical reaction has been focused on a biochip application. The biocompatible properties, such as prevention of protein adsorption and cell adhesion towards the surface are also required to the polymer materials because the biochip is one of microfluidic devices handled with mainly biological components. We synthesized new photocrosslinkable polymers composed of 4-(4-methoxycinnamoyl)phenyl methacrylate and 2-methacryloyloxyethyl phosphorylcholine(MPC). These polymers were used as a prepolymer to make a microhydrogel by photocuring. A gelation began by 5 s photoirradiation and it reached equilibrium state after 360 s photoirradiation. The absorption maximum was observed at 347 nm, which is originated by a double bond in a cinnamoyl group, disappears by an increase in the photoirradiation time. It was considered that a crosslinking bond was formed by dimerization between cinnamoyl groups in the polymer by a photoirradiation. The equilibrium water content of the hydrogels was about more than 90% due to extremely hydrophilicity of the MPC units. Moreover, we succeeded to prepare the hydrogel in a small area (0.5 mm in diameter) on a glass substrate. Since the hydrogel formed in the micro-space, it is thought that the polymer can be used as construction components of biochip.

Key words: biocompatibility, photocrosslinking phospholipid polymer, hydrogel, microfuluidic device

1. INTRODUCTION

A microfabrication technology based on photochemical reaction of polymer materials is used in many fields focusing on processing micro-3D structure of plastic and semiconductor devices. This is partly because the photochemical reactions have several advantages such as being rapid and homogeneous reactions proceed at room temperature. Moreover, microstructured fabrication by photochemical reaction can be carried out more efficiently in comparing with normal chemical and physical techniques.

Recently, by using the suitable properties of photochemical reactions such as photocrosslinking, an application of fabrication technology has been extended widely in biotechnology, for instance components in microfluidic devices for bioseparation [1, 2], and three-dimensional scaffold for a tissue engineering [3]. Especially on the microfluidic structures in a biochip, such as microhydrogel-gate, -valve, gel-salt-bridge, and immobilized enzyme membrane for biosensor, biocompatible properties are strongly required because it is one of the microfluidic devices handled with biological components, that is, proteins, DNA, and cells. The polymeric materials, in general, use in this purpose are polysaccharide, polypeptide and hydrophilic synthetic polymers. However, since the amount of target biological components is quite low in the microfluidics, much better capability in the biocompatibility of the polymeric materials in viewpoint of biocompatibility should be required. Fortunately, in our current research for biocompatible polymeric materials, polymers with phosphorylcholine group show excellent performance of protein adsorption resistance and cell adhesion inhibition [4]. These polymers are composed of 2-methacryloyloxyethyl phosphorylcholine (MPC) and other vinyl compounds. From this, we synthesized photocrossliknable polymers from the MPC and 4-(4-methoxycinnamoyl) phenyl methacrylate (MOCPMA). The MOCPMA unit was crosslinkable by a dimerization of cinnamoyl groups upon photoirradiation [5]. Moreover, it is unquestionable because there is an example of using as biomaterial [6], too for the biocompatibility. Since the MPC polymers having both photocrosslinkable and hydrophilic natures, desirable microhydrogel is expected to be formed by photoirradiation process.



Scheme 1: Synthesis of poly(MPC-co-MOCPMA) (PMMC).

2. MATERIALS and METHODS

2.1 Materials

The MPC was synthesized by the method previously reported [7]. 4-Hydroxyacetophenone, 4-methoxybenzaldehyde, and 2,2'-azobisisobutyronitrile (AIBN) were purchased from Kanto Kagaku (Tokyo, Japan), respectively, and were used as received. Methacryloyl chloride was purchased from Wako Pure Chemical (Osaka, Japan). Other organic reagents and solvents were purified by the usual method.

2.2 Synthesis of polymers

4-(4-Methoxycinnamoyl)phenyl methacrylate (MOCPMA) was synthesized as previously described by Reddy et al. [5]. Random copolymers of MPC and MOCPMA were synthesized by copolymerization in ethanol (EtOH)/methyl ethyl ketone (MEK) mixture, using 2,2'-azobisisobutyronitrile (AIBN) as an initiator. The initiator concentration was 1 mol% of the total monomers. The concentration of monomer to the solvent was 0.2 mol/L. The polymerization was carried out with stirring at 70 °C for 2 h in argon atmosphere. The polymers obtained were precipitated from large excess of diethyl ether/chloroform (50/50 in volume). The polymers were characterized by using ^{1}H-NMR spectroscopy and GPC.

2.3 Photoreactivity measurements

The photoreactivity of the polymers was evaluated by monitoring the change of UV absorption at 347 nm of a 0.01 wt% polymer solution in EtOH or 0.15 mol/L phosphate-buffered saline (PBS), upon photoirradiation.

2.4 Photocuring behavior

Polymer solutions of 20 wt% in the EtOH or the 0.15 mol/L PBS were cast on mold plates. After drying the polymer solutions, photoirradiation was carried out in the range of 10 ~ 3600 s. The photocuring yield was defined as:

$$\text{Photocuring yield (wt\%)} = Wg/W_0 \times 100,$$

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

2.5 Dimerization content

The dimerization content of the PMMC90 was calculated from a decrease of UV absorbance at 347 nm to time as follow:

$$\text{Dimerization content (\%)} = (H_0 - H_x) / H_0 \times 100$$

where H_x and H_0 are the peak heights of the absorption wavelength near 347 nm on various photoirradiation times (x=5~600 s) and the one

Table 1: Characterization of PMMC

	Feeding ratio (mol%)		Copolymer composition a) (mol%)		Polymerization solvent (vol%)					
	MPC	MOCPMA	MPC	MOCPMA	EtOH	MEK	M_w b) ($\times 10^{-4}$)	M_n b) ($\times 10^{-4}$)	M_w/M_n b)	Yield (%)
PMMC70	70	30	76	24	50	50	6.0	3.2	1.9	62
PMMC80	80	20	83	17	60	40	5.4	2.6	2.1	65
PMMC90	90	10	91	9	70	30	2.9	1.2	2.4	79
PMMC95	95	5	96	4	70	30	3.1	1.4	2.2	66
PMMC97	97	3	97	3	70	30	5.3	2.4	2.2	73

[M] = 0.2mol/L, [AIBN] = 2mmol/L
Polymerization time = 2h
Polymerization temperature = 70°C

a)Determined by ^{1}H-NMR.
b)Determined by GPC in water/methanol = 3 / 7, poly(ethylene glychol) standard.

on 0 s, respectively.

2.6 Equilibrium water content (EWC)

Hydrogels obtained by photoirradiation for 3600 s were used for EWC measurement. EWC was defined as the following equation:

$$\mathrm{EWC\ (wt\%)} = \{(W_b-W_a)/W_b\} \times 100$$

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

3. RESULTS AND DISCUSSION

3.1 Synthesis of PMMC

The copolymers (PMMC) were synthesized. The results of polymerization and molecular characteristics of the polymers are summarized in Table 1. The polymerization proceeded homogeneously. The last two numbers in the end of the name of all the copolymers indicates the content of MPC unit (mol%) in feed. The compositions of MOCPMA unit in the copolymers are controllable with the composition of MOCPMA in feed. The polymers obtained were soluble in water.

3.2 Photochemical reaction of PMMC

Fig. 1 shows the photochemical reaction of PMMC90 in EtOH. The absorption maximum was observed at 347 nm, which is originated by a double bond in a cinnamoyl group. The absorbance at 347 nm was decreased with an increase in the irradiation time. It is considered that a crosslinking was formed of a dimerization of cinnamoyl groups by a photoirradiation as shown in Scheme 2. The similar result was obtained in the PBS.

Fig. 2 shows the photocuring yield of the three kind of polymers: PMMC90, PMMC95, and PMMC97 after drying from the solution of EtOH or PBS. Polymer hydrogels were prepared successfully by photoirradiation on the copolymers. The PMMCs with more photocrosslinkable units show more photocuring yields. By comparison with the solvents of polymer solution, the photocuring yields of PMMC after drying from the solution of PBS are lower than those from EtOH solution. However, it can be seen from Fig. 3 that the dimerization content of the PMMC90 dissolved in PBS is higher than that in EtOH. It is considered that the PMMC90 aggregates in PBS due to its amphiphilic nature. The hydrophobic MOCPMA units could gather in the polymer aggregate. Thus, in PBS, intramolecular crosslinking become much easier than intermolecular crosslinking.

3.3 Properties of PMMC hydrogel

Fig. 4 shows the equilibrium water contents (EWC) of the obtained hydrogels. The EWCs were more than 90%. The PMMCs with less photocrosslinkable units show higher EWC. We could not find the effect of the solvent of the polymer solution on the EWCs.

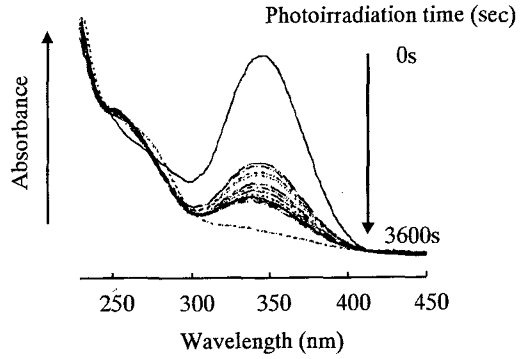
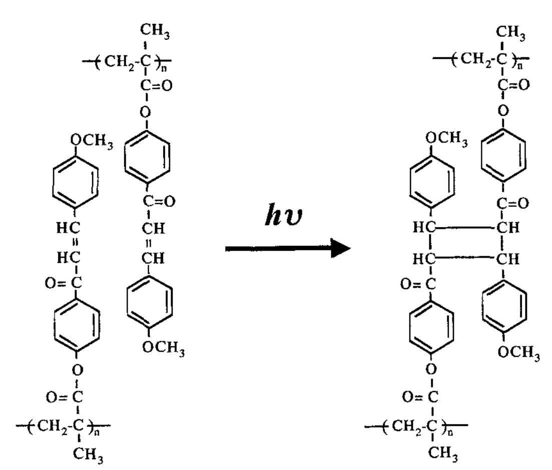


Figure 1: The changes in the UV absorption spectra of 0.01 wt% PMMC90 in EtOH with various photoirradiation time.



Scheme 2: Crosslinking of cinnamoyl group by photoirradiation.

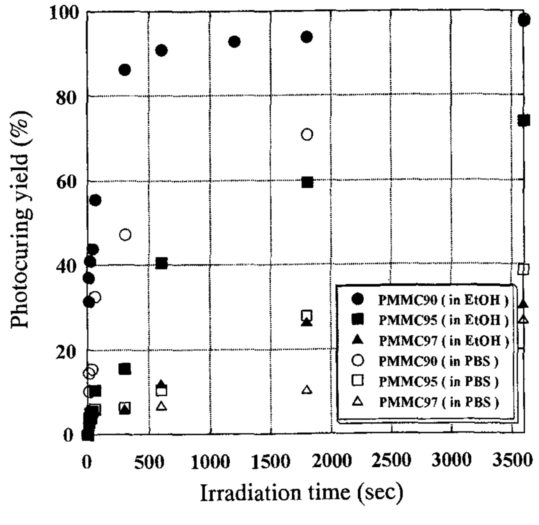


Figure 2: The photocuring yield of PMMC90, PMMC95 and PMMC97 after drying up the EtOH or the PBS.

The EtOH solution containing 20 wt% PMMC90 was spincoated on the glass substrate. After drying up, it was exposed with light for 60 s through a photomask at room temperature. The photomask has a circle structure pattern (0.5 mm in diameter). It has been prepared the microhydrogel of 0.5 mm in the diameter on a glass substrate. The pattern of the photomask was transferred to the hydrogel with high fidelity.

The polymer hydrogel obtained by photoirradiation mainly constructed of MPC unit. It is thought that it is excellent in the biocompatibility because the MPC polymers are well-known biocompatible polymers [8-11]. The protein adsorption and cell adhesion will be investigated on the polymer hydrogel to prove the possibility for using the hydrogel at contact interface against the biological components.

4. CONCLUSION

New photocrosslinkable polymers having both phosphorylcholine and cinnamoyl groups have been synthesized successfully. The polymer was used as a prepolymer to make a microhydrogel by photoirradiation. Gelation began by 5 s photoirradiation and it reached equilibrium state after 360 s irradiation. The absorption at 347 nm, which is originated by a double bond in the cinnamoyl group, disappeared by an increase in the irradiation time. It was considered that a crosslinking bond was formed of a dimerization of cinnamoyl groups in the polymer upon photoirradiation. The photocuring yields of the polymer depended on the solvent of polymer solution before photoirradiation. That is, it is considered that solubilizing state of the polymer in solution reflected to the ability of gelation. The EWC of the hydrogels were more than 90%. Moreover, we succeeded to prepare the hydrogel in small area (0.5 mm in diameter) on a glass substrate. We are expecting that the polymer can be applied for both making a microgate and immobilizing biological components at a specific position in a microfluidics device.

5. ACKNOWLEDGEMENTS

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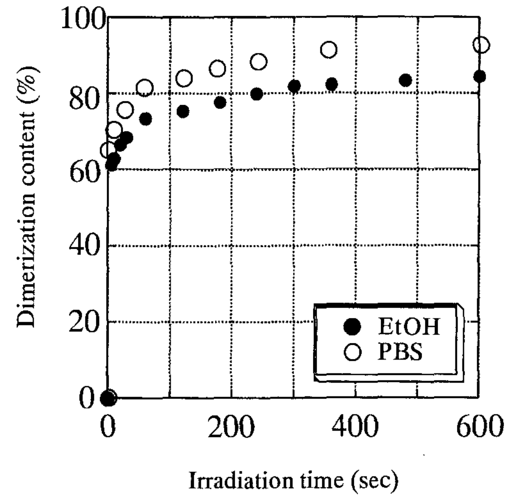

Figure 3: The dimerization content of PMMC90 determined by the absorption wavelength near 347 nm.

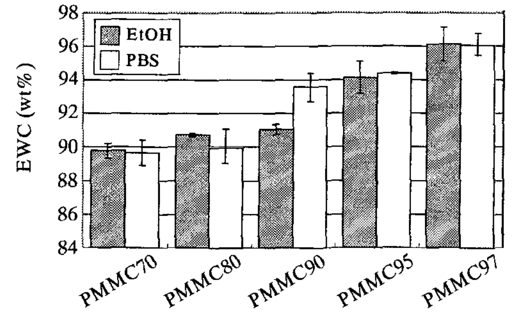

Figure 4: The EWCs of PMMCs after drying up EtOH or PBS.

6. REFERENCES

[1] D. J. Beebe, J. M.Bauer, Q. Yu, R. H. Liu, C. Devadoss, B. H. Jo, *Nature*, **404**, 588-590 (2000).

[2] Y. Takamura, H. Onoda, H. Inokuchi, S. Adachi, A. Oki, Y. Horiike, *Electrophoresis*, **24**, 185-192 (2003).

[3] V. M. Nivasu, T. T. Reddy; S. Tammishetti, *Biomaterials*, **25**, 3283-3291 (2004).

[4] K. Ishihara, *Trend Polym Sci*, **5**, 401-407 (1997).

[5] A. V. Rami Reddy, K. Subramanian, A. V. Sesha Sainath, *J Appl Polym Sci*, **70**. 2111-2120 (1998).

[6] C. Quek, J. Li, T. Sun, M. L. Hou Chan, H. Mao, L. M. Gan, K. W. Leong, H. Yu, *Biomaterials*, **25**, 3531-3540 (2004).

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

[8] K. Ishihara, N. P. Ziats, B. P. Tierney, N. Nakabayashi, J. M. Anderson, *J. Biomed. Mater. Res.*, **25**, 1397-1407 (1991).

[9] K. Ishihara, H. Oshida, T. Ueda, Y. Endo, N. Nakabayashi, *J. Biomed. Mater. Res.*, **26**, 1543-1552 (1992).

[10] T. Yoneyama, K. Ishihara, N. Nakabayashi, M. Ito, Y. Mishima, *J. Biomed. Mater. Res.*, **41**, 15-20 (1998).

[11] T. Moro, Y. Takatori, K. Ishihara, T. Konno, Y. Takigawa, T. Matsushita, U. Chung, K. Nakamura, H. Kawaguchi, *Nature Materials*, **3**, 829-836 (2004).

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