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Design of biocompatible hydrogels with attention to structure of water surrounding polar groups in polymer chains

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We present structures of water surrounding polymer chains as a new factor for molecular design of biocompatible polymer hydrogels. We hypothesized that the design of polymers based on the structure of water is effective in controlling interaction between the hydrogels and the biocomponents such as proteins, DNA, and cells because intrinsic structures of water surrounding the biocomponents are an important factor for control and expression of biological reactions. To investigate standards for the design, we evaluated water structure in a hydrogel composed of 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer having a phosphorylcholine (PC) group as a polar group. From the results of spin-spin relaxation time (T_2) measurements by ^{1}H-pulsed NMR method, it was found that most of the water in the poly(MPC) hydrogel has slight interaction with the polymer chains (free water). This tendency was obtained even when the hydration degree of the hydrogel was decreased. The PC groups resulted in the high fraction of free water. We suggest that the high fraction of free water in hydrogels is a significant factor to have weak interaction with the biocomponents. So, it is necessary to give functional groups such as the PC groups in the design of the biocompatible hydrogels.
Key words: biocompatible hydrogel, water structure, MPC polymer, phosphorylcholine (PC) group, free water

1. INTRODUCTION

Polymer hydrogels attract considerable interest as soft materials which have structures and functions close to those of the tissues in living body because they have several properties such as absorption in large quantities of water, volume phase transition induced by external stimuli, and permeation of molecules. They are expected to be applied as micro-actuators, carriers in drug delivery systems, artificial organs, and devices for measuring the properties of biocomponents such as proteins, DNA, and cells. In design of the hydrogels with such performance, biocompatibility in the hydrogels is an essential factor. Equilibrium water content (EWC) in hydrogels has been considered to have contribution to the biocompatibility [1]. For example, hydrogels with high EWC have been often used for contact lenses and for matrices in tissue engineering. However, contact lenses with high EWC have the disadvantage of dehydration when they are placed on the eyes. In addition, it was reported that the hydrogels with high EWC were less compatible with tissues than those with low EWC [2].

Here, we present structures of water surrounding polymer chains as a new factor for the biocompatibility in the design of the hydrogels. In living body, the biocomponents have intrinsic structures of water in vicinity of them, and the intrinsic structures of water are an essential factor for control and expression of biological reactions [3-5]. In addition, cells and extracellular matrices retain quantities of water, and the water plays an important role in vital activities [6-8]. To investigate standards of the design of the hydrogels based on the structures of water surrounding polymer chains, we evaluated structures of water in the polymer hydrogel composed of 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer [9-11] having a phosphorylcholine (PC) group as a polar group. It is well known that a surface coated with the MPC polymer bearing the PC groups suppressed non-specific protein adsorption compared with surfaces of poly(2-hydroxyethyl methacrylate) (poly(HEMA)) and poly(*n*-butyl methacrylate (BMA)) [9,10]. Moreover, the adsorbed proteins at the surfaces of the MPC polymer were not denaturalized [11]. Thus, the structure of water in the poly(MPC) hydrogel is effective as standards for designs of the biocompatible hydrogels. We measured the spin-spin relaxation time (T_2), a parameter for evaluating molecular mobility of polymer chains and water in hydrogels [12-14], of the poly(MPC) hydrogel by ^{1}H-pulsed NMR method to investigate the structures of the water in the hydrogel. The structure of water in the poly(MPC) hydrogel was discussed in comparison with that in the poly(HEMA) hydrogel which has been widely used as a material of soft contact lenses.

2. EXPERIMENTAL

Materials

The monomer, MPC, was synthesized by a method from a previous report [15]. HEMA (97%) was purchased from Aldrich and used without further purification. Triethylene glycol dimethacrylate (TEGDMA) (Tokyo Kasei) as a cross-linker,

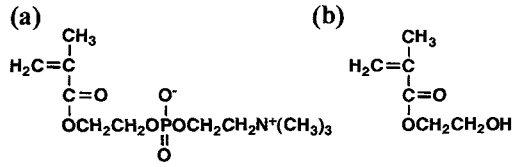

Figure 1. Chemical structure of (a) MPC (b) HEMA.

ammonium peroxodisulfate (APS) (Kanto Chemicals) as an initiator and *N, N, N', N'*-tetramethylethylenediamine (TMEDA) (Kanto Chemicals) as a catalyst were used without further purification. Distilled water was used for all sample preparations. Fig. 1 (a) and (b) show chemical structure of MPC and HEMA, respectively.

Preparation of poly(MPC) hydrogel

The poly(MPC) hydrogel was prepared by a method from a previous report [16]. MPC monomer aqueous solution (2.5 mol/L, 1.0 mL), the TEGDMA (7.2 mg) which is 1.0 mol% to the monomer, and the APS aqueous solution (0.22 mol/L, 60 μL) were placed on a Perti dish. After the solution was stirred until fully mixed, the TMEDA (20 μL) was added. The poly(HEMA) hydrogel was prepared by mixing the HEMA solution (300 μL), the TEGDMA (7.2 mg) which is 1.0 mol% to the monomer, and the APS aqueous solution (0.22 mol/L, 60 μL), and the TMEDA (20 μL). Both the hydrogels were transparent. EWC of the poly(MPC) hydrogel and poly(HEMA) hydrogel were 91.9 % and 45.6 %, respectively. We calculated water content by the following equation,

$$water\ content(\%) = \frac{W_s - W_d}{W_s} \times 100$$

where W_d and W_s are the weights of the dried and swollen gels, respectively.

^{1}H T_2 measurements by ^{1}H-pulsed NMR method

^{1}H pulsed NMR measurements were carried out by JNM-MU 25A (JEOL, Japan) with the resonant frequency of 25 MHz for the proton. The CPMG (Carr-Purcell-Meiboom-Gill) pulse sequence [17,18] was used for the T_2 measurements. The signal of the pulsed NMR is superposition of different decay curves, and the relaxation time and fraction for each component are obtained by decomposing the signal. The decay curves of signal intensity obtained by the CPMG method are expressed by the following multiexponential equation,

$$M(t) = M_0 \sum_i^N F_i \exp(-\frac{t}{T_{2,i}})$$

, where M(t) is a signal intensity at time t, M_0 is an initial signal intensity, F_i is a fraction of the *i*th component and $T_{2,i}$ is a T_2 of the *i*th component. The decomposition of the T_2 signal into components with different molecular mobility was carried out with nonlinear least-square method. In the T_2 measurements, the values for repetition time, the number of loop and scan times were 12.0 sec, 500, and 16, respectively.

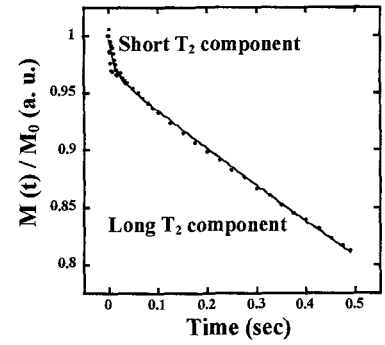

Figure 2. ^{1}H T_2 decay curve for the poly(MPC) hydrogel with EWC.

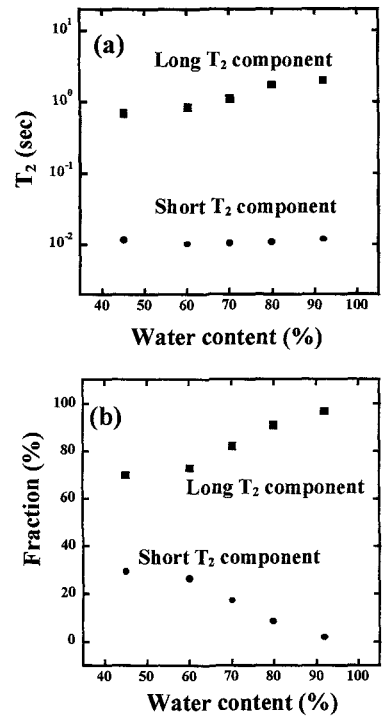

Figure 3. (a) T_2 values for each poly(MPC) hydrogel. (b) Fractions for each poly(MPC) hydrogel.

Temperature during the measurements was kept at 35 °C. All the T_2 measurements were carried under the same experimental conditions. The shown T_2 values and fractions were the average of six measurements.

3. RESULTS AND DISCUSSION

Fig. 2 shows the ^{1}H T_2 decay curve for the poly(MPC) hydrogel with EWC. The decay curve was decomposed into two components: short T_2 component and long T_2 component. To assign the two components, we prepared poly(MPC) hydrogels with water contents of 79.8, 70.0, 60.0, and 45.0 % by evaporating water contained in the poly(MPC) hydrogel with EWC. ^{1}H T_2 decay curves for each of the poly(MPC) hydrogels were also decomposed into two components. Fig. 3 (a) and (b) show the T_2 values and the fractions of the two components in the poly(MPC) hydrogels, respectively. From Fig. 3 (a), the T_2 values in the short T_2 component

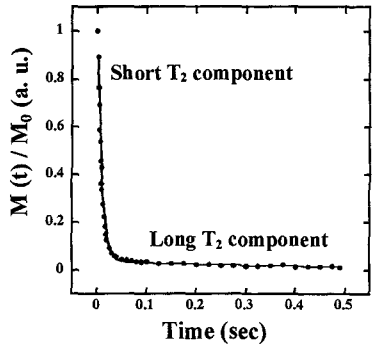

Figure 4. ^{1}H T_2 decay curve for the poly(HEMA) hydogel with EWC.

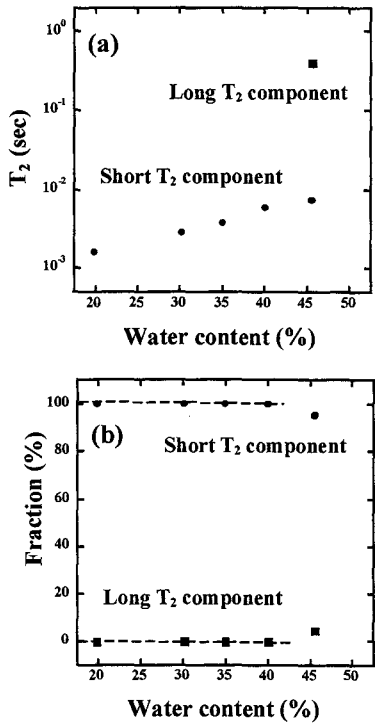

Figure 5. (a) T_2 values for each poly(HEMA) hydrogel. (b) Fractions for each poly(HEMA) hydrogel.

hardly changed in a variety of water contents in the hydrogels. On the other hand, the T_2 values in the long T_2 component decreased with decreasing the water content (increasing the polymer concentration in the hydrogels). In addition, in Fig. 3 (b), according to the decrease of the water content, the fractions of the short and long T_2 components increased and decreased, respectively. From these results, the short T_2 component was assigned to the MPC polymer chains. The short T_2 component should have a slight contribution of water which strongly interacts with the polymer chains, namely bound water. The long T_2 component corresponded to water which has slight interaction with polymer chains, namely free water. Most of the water in the poly(MPC) hydrogel was free water. This tendency

Table 1. Comparison of the T_2 values and fractions for the poly(MPC) and poly(HEMA) hydrogels.

Hydrogel	Water content (%)	Short T2 component		Long T2 component	
		T2 (msec)	fraction (%)	T2 (sec)	fraction (%)
MPC	91.9% (EWC)	11.95	3.0	2.00	97.0
MPC	45.0%	11.73	29.7	0.70	70.3
HEMA	45.6% (EWC)	7.50	95.5	0.40	4.5

was obtained even when the water content of the hydrogel was decreased.

Next, we measured the ^{1}H T_2 decay curve for the poly(HEMA) hydrogel with EWC. The decay curve is shown in Fig. 4. The decay curve was decomposed into two components: short T_2 component and long T_2 component. We also measured the decay curves for poly(HEMA) hydrogels with water contents of 40.1, 35.0, 30.2, and 19.9 % prepared by evaporating water in the hydrogel with EWC. The decay curves of the evaporated poly(HEMA) hydrogels were well fitted by single exponential curves. Fig. 5 (a) and (b) show the T_2 values and the fractions of the components in the poly(HEMA) hydrogels, respectively. The short T_2 component corresponded to HEMA polymer chains and bound water. The long T_2 component was assigned to free water. The fraction of the free water in the poly(HEMA) hydrogel with EWC was extremely small compared with that in the poly(MPC) hydrogel with EWC. In addition, no free water existed in the poly(HEMA) hydrogels with water contents below 40 %. These results show that most of the water in the poly(HEMA) hydrogel strongly interacted with the polymer chains. In addition, the T_2 values in the short T_2 component decreased with decreasing the water content (Fig. 5 (a)), although the T_2 values in the poly(MPC) hydrogels hardly changed (Fig. 4 (a)). We think that this is caused by the increase of the HEMA polymer chain concentration according to the decrease of amount of bound water. The increase of the HEMA polymer chain concentration led to the decrease of molecular mobility of the polymer chains and bound water. In the poly(MPC) hydrogels, the molecular mobility of the MPC polymer chains and bound water did not change because little bound water in the hydrogels was evaporated.

We compared the water structure in the poly(MPC) hydrogels with that in the poly(HEMA) hydrogels. Table 1 shows the T_2 values and fractions for the poly(MPC) hydrogels with EWC and with water content of 45.0 % and for the poly(HEMA) hydrogels with EWC. From Table 1, the fraction of free water in the poly(MPC) hydrogel with water content of 45.0 % was about 16 times compared with that in the poly(HEMA) hydrogel with EWC. This shows that the fraction of free water in the hydogels does not depend on the water contents. From the difference in the chemical structure between the MPC and HEMA, the fraction of free water is caused by structures of polar groups of polymer chains. That is to say, the PC groups in the MPC polymer chains result in the high fraction of

free water in the poly(MPC) hydrogels. We think that the high fraction of free water is due to hydrophobic hydration induced by the trimethylammonium groups which are the end groups in the PC groups. Water in the vicinity of tetraalkylammonium ions ($(C_nH_{2n+1})_4N^+$) is considered to have clathrate structures by hydrophobic hydration, and the structures provide weak interaction between the water and the ions [19-21]. Thus, water surrounding the trimethylammonium groups should have the clathrate structures, leading to the high fraction of free water around in the poly(MPC) hydrogels caused by slight interaction between the trimethylammonium groups and the water. On the other hand, the hydroxyl groups, which are the polar end groups of the HEMA polymer chains, form hydrogen bonds with water surrounding the polar end groups of the HEMA polymer chains [22-24]. The hydrogen bonds constrain molecular mobility of water surrounding the polar end groups of the HEMA polymer chains. This induces the high fraction of bound water in the poly(HEMA) hydrogels.

4. CONCLUSIONS

We clarified that understanding of water structure in polymer hydrogels is effective for molecular design of biocompatible hydrogels. It was found that the water in the poly(MPC) hydrogels has the much higher fraction of free water compared with that in the poly(HEMA) hydrogels. The high fraction of free water in the poly(MPC) hydrogels was caused by the PC groups in the MPC polymer chains. Because the surfaces coated with the MPC polymers suppressed non-specific protein adsorption and denaturalization of a small number of the adsorbed proteins compared with those with the HEMA polymers [9-11], we suggest that high fraction of free water in polymer hydrogels is a significant factor to have weak interaction between the hydrogels and the biocomponents such as proteins, DNA, and cells. In addition, we think that structures with zwitter-ions such as the PC groups also contribute to the weak interaction because zwitterionic polymers in aqueous solutions do not significantly disturb hydrogen-bond network structures of waters surrounding the polymers [25]. So, we believe that it is necessary to give function groups with hydrophobic groups, which induce hydrophobic hydration, and with zwitter-ions such as the PC groups for designing the biocompatible hydrogels.

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