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Protein Absorption and Characterization of Hydrogel Polymer Containing 2-Methacryloyloxyethyl Phosphorylcholine as Additive

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Abstract

This study evaluated the physical and optical characteristics of hydrophilic ophthalmic polymer with addition of 2-methacryloyloxyethyl phosphorylcholine in the basic hydrogel ophthalmic lens material, and in particular, the utility of 2-methacryloyloxyethyl phosphorylcholine as an ophthalmic contact lens material for ophthalmologic devices was investigated. In this study 2-methacryloyloxyethyl phosphorylcholine were used as additives. For the preparation of hydrogel lens 2-hydroxyethyl methacrylate, methyl methacrylate, acrylic acid and a cross-linker EGDMA were copolymerized in the presence of AIBN as an initiator. The physical properties of the produced polymers were measured as followings. The refractive index of 1.433~1.393, water content of 35.95~53.16%, contact angle of 70.62~51.24°, UV-B transmittance of 81.2~82.4%, UV-B transmittance of 81.2~82.4% and visible transmittance of 91.4~92.2% were obtained. Also, in case of protein absorption, the measurement showed that absorbance of Reference and MPC-10 sample was 0.2598 and 0.2250 respectively. Based on the results of this study, ophthalmic lens material containing 2-methacryloyloxyethyl phosphorylcholine is expected to be used usefully as a material for high wettability and inhibitor of protein adsorption for ophthalmic hydrogel lens.

Keywords: 2-Methacryloyloxyethyl Phosphorylcholine(MPC), Protein Absorption, Wettability, Ophthalmic Lens

1. Introduction

The performance of medical materials is adversely affected when protein is deposited therein as this causes adhesion of microorganisms and side effects. To control this phenomenon, studies have been actively conducted in the field of chemical analysis and environmental biology. Additionally, in the case of medical and optical hydrophilic lenses, deposits of tear protein cause many problems, such as blurred vision, hyperemia, papillary conjunctivitis, decrease of wearing sensation, and decrease of oxygen permeability[1-3].

As optical and medical hydrophilic lenses are used directly on the eyes, the perception degradation, corneal edema through hypoxia, and tear film confusion and evaporation rate through the destruction of the lipid layer may increase[4] . In addition, with respect to the wettability of the lenses, the dry-eye phenomenon due to the loss of corneal sensation, etc. may appear[5]. To solve these problems, studies on the application of hydrophilic medical lenses utilizing a functional monomer such as one with a high wettability, sun protection, and high oxygen permeability have been actively conducted in recent years[6-9].

Phosphorylcholine is also called “glycerophosphatid” and consists of phosphoglycerides, having a lipid bilayer structure that is the same as the membrane structure. The 2-methacryloyloxyethyl phosphorylcholine that was used in this study belongs to the phosphorylcholine group. As it has a lipid bilayer structure that is the same as the membrane structure, the adhesion of microorganisms is weakened, and consequently, the surface contamination is reduced[10]. In addition, the phosphorylcholine group consists of macromolecules and can minimize the adhesion of microorganisms on the microorganism-contacting surface when used to coat such surface[11]. Due to its weak coupling force on account of its chemical instability, however, it is used in the form of a macromolecule after copolymerization with other monomers[12]. In this study, the physical and optical properties of phosphorylcholine and the degree

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of protein deposition on it were measured and analyzed for the lenses produced after copolymerizing hydrogel ocular lenses that have a protein deposition function and high wettability, using 2-methacryloyloxyethyl phosphorylcholine as an additive to the basic hydrogel lens material.

2. Experimental Method

2.1. Materials

For the materials that are mainly used in hydrogel ocular lenses, HEMA (2-hydroxyethylmethacrylate) and AA (acrylic acid), and an initiator, AIBN (azobisiobutyronitirle), all products of JUNSEI were used, and for the cross-linking agent, EGDMA (ethylene glycol dimethacrylate), a product of SIGMA-ALDRICH was used. Additionally, MMA (methyl methacrylate) was purchased from Crown Guaranteed Reagents for use in this study. For MPC (2-methacryloyloxyethyl phosphorylcholine), which was used as an additive for wettability and for the prevention of protein absorption, a product of SIGMA-ALDRICH was used.

2.2. Polymerization

Hydrogel lenses with high wettability were produced having HEMA, MMA, AA, and EGDMA as well as an initiator, AIBN, as a basic combination, for hydrogel lens copolymerization. The additive, 2-methacryloyloxyethyl phosphorylcholine, was added at a 1-10% ratio, and copolymerization was done by way of thermal polymerization through an oven, using the cast mold method after 30 mins natural stirring at room temperature following 3 mins stirring using a stirrer (Vortex GENIE 2, Scientific Industries, USA). In addition, the prepared samples were hydrated in a saline solution containing 0.9% sodium chloride for about 24 hours at room temperature, and then their physical properties, including their refractive index, water content, contact angle, and light transmittance, and the degree of protein deposition were evaluated. For the experiment, the basic combination was set to Ref., and the samples in the 2-methacryloyloxyethyl phosphorylcholine that were added at a 1-10% ratio to the basic combination were named “MPC-1,” “MPC-3,” “MPC-5,” “MPC-7,” and “MPC-10.” The blending ratios of the high wettability hydrogel lens samples are presented in Table 1.

3. Instruments and Analysis

3.1. Physical Properties

The samples that were used in the experiment were hydrated in a saline solution containing 0.9% sodium chloride for 24 hours at room temperature to be in a state of equilibrium. To measure the refractive index of the prepared lenses, the gravimetric method was used with ISO 18369-4:2006 (Ophthalmic optics-Contact lens-Part 4: Physicochemical properties of contact lens materials) as the reference. After the measurement of the light transmittance of the lenses using Topcon’s TM-2, the results were analyzed with TM-1 PC ver. 1.30. Additionally, for the wettability of the prepared lenses, the contact angle was measured using P-Mini of SEO, and was then evaluated.

3.2. Protein Deposit

After the making of artificial tears at the composition ratio of 1.08 g albumin, 0.36 g lysozyme, 0.36 g mucin, 0.36 g globulin, and 0.002 g $CaCl_2$ in a 200 mL phosphate buffer solution (pH 7.4, 0.01M), the samples were made to absorb the artificial tears at 37°C and 5 RPM for 24 hours. The samples in which protein was deposited for an absorption experiment were heated in a sodium dodecyl sulfate (SDS: 2% (wt/vol)) solution at 95°C for 15 minutes. After being cooled down at room

Table 1. Percent composition of samples Unit: %

Sample	HEMA	AA	MMA	EGDMA	MPC[a)]
Ref	93.90	4.69	0.94	0.47	-
MPC-1	93.02	4.65	0.93	0.47	0.93
MPC-3	91.32	4.57	0.91	0.46	2.74
MPC-5	89.69	4.48	0.90	0.45	4.48
MPC-7	88.11	4.41	0.88	0.44	6.17
MPC-10	85.84	4.29	0.86	0.43	8.58

a): 2-methacryloyloxyethyl phosphorylcholine temperature, they were freeze-dried and stirred in 3 mL distilled water, and then the absorbance in the 280 nm ultraviolet region was measured.

4. Results and Discussion

4.1. Polymerization

As a result of the copolymerization of the MPC combination samples in which Ref. not containing 2- methacryloyloxyethyl phosphorylcholine and 2-methacryloyloxyethyl phosphorylcholine at each ratio were added, transparent hydrogel lenses were produced for all the samples. They were used for the analysis and evaluation of the physical and optical properties of the prepared samples, and for the protein deposition experiments after hydration for about 24 hours in a saline solution. As for the surface analysis of the lenses, in the SEM measurements, smooth lens surfaces were confirmed. The sample to which 10% MPC was added showed a smoother surface than the Ref. not containing MPC. This was assessed to have been the effect of MPC with a lipid bilayer structure that is the same as the membrane structure[13]. The SEM analysis results of the prepared Ref. and MPC-10 samples are presented in Fig. 1.

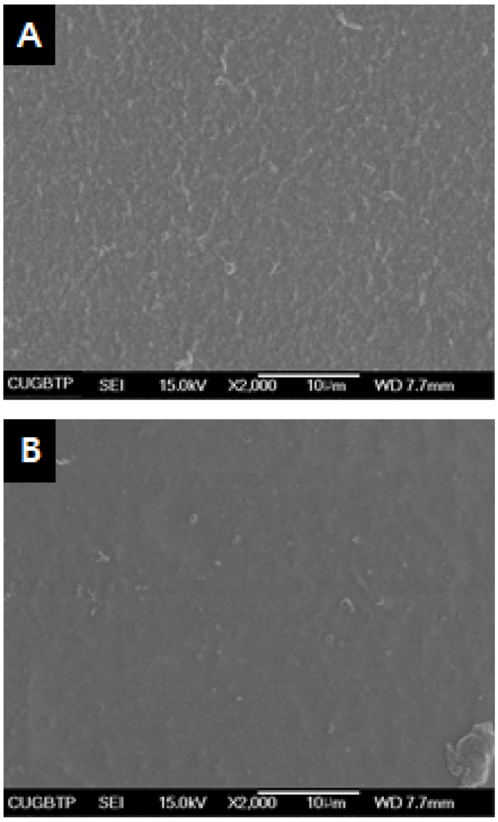

Fig. 1. SEM image of contact lens sample [(A) Ref. (B) MPC-10].

4.2. Refractive Index

As for the refractive index of the prepared hydrophilic lenses, the average refractive index of Ref. not containing 2-methacryloyloxyethyl phosphorylcholine was 1.433. For the MPC combinations to which 2-methacryloyloxyethyl phosphorylcholine was added at each of the aforementioned ratios, the refractive index of MPC-1 was 1.429, that of MPC-3 was 1.419, that of MPC-5 was 1.409, that of MPC-7 was 1.401, and that of MPC-10 was 1.393. As the proportion of MPC increased, the refractive index tended to decrease.

4.3. Water Content

As for the water content of the prepared hydrophilic lenses, the average water content of Ref. not containing 2-methacryloyloxyethyl phosphorylcholine was 35.95%. For the MPC combinations to which 2-methacryloyloxyethyl phosphorylcholine was added at each of the aforementioned ratios, the water content of MPC-1 was 38.05%, that of MPC-3 was 42.65%, that of MPC-5 was 45.80%, that of MPC-7 was 49.10%, and that of MPC-10 was 53.06%. As the proportion of MPC increased, the refractive index tended to increase.

In the study conducted by Kim et al. titled "Evaluation of the physical properties of soft contact lens with

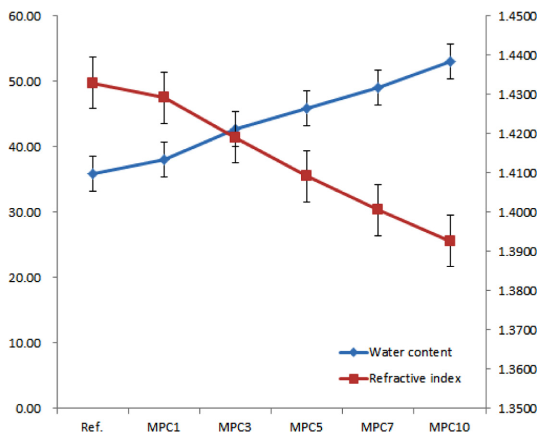

Fig. 2. Water content and refractive index of sample.

added pyridine substituted by halogen and carboxylic acid”[7], as the water content increased, the refractive index decreased. Compared to the refractive index of HEMA used as a major material of hydrogel lenses, which is 1.453, the water content of water is 1.333, lower than that of HEMA. This was assessed to be an effect of the reduction of the refractive index according to the increase of the water content of hydrogel lenses. In this study, an inverse proportional relationship between the refractive index and the water content was confirmed. A graph showing the changes in the water content and refractive index according to the amount of additive added is presented in Fig. 2.

4.4. Optical Transmittance

As for the light transmittance of the prepared hydrophilic lenses, that of Ref. was 81.8% for UV-B and 87.2% for UV-A. In the case of visible light, the transmittance was 91.4% in the visible-light region, which is a satisfactory value for the general hydrogel contact lenses. High transmittance was also shown in the UV-B, UV-A, and UV regions, indicating that they did not have a UV barrier. As for the light transmittance of the MPC combinations to which 2-methacryloyloxyethyl phosphorylcholine was added at each of the aforementioned ratios, in the UV-B, UV-A, and UV regions, the light transmittance values of MPC-1 were found to be 82.4, 88.0, and 92.2%, respectively; those of MPC-3 were 81.2, 86.4, and 91.6%; those of MPC-5 were 82.4, 86.2, and 92.2%; those of MPC-7 were 81.6, 86.2, and 91.60%; and those of MPC-10 were 81.8, 86.0, and 91.6%. In the visible-light and UV regions, high transmittance appeared when MPC was added, showing a transparent lens without a UV blocking ability. The obtained light transmittances values of Ref. and MPC-10 are compared in Fig. 3.

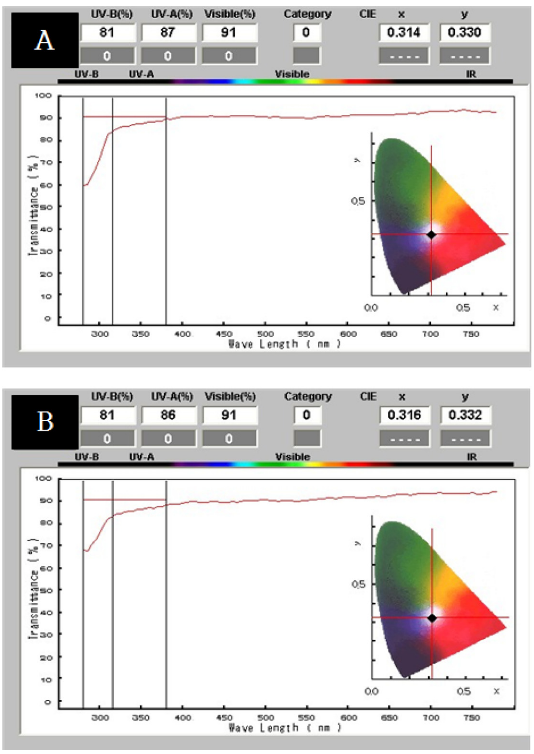

Fig. 3. Optical transmittance of samples [(A) Ref. (B) MPC-10].

4.5. Wettability

As for the contact angle of the samples that were prepared for the evaluation of the wettability of hydrogel contact lenses including 2-methacryloyloxyethyl phosphorylcholine, the average contact angle of Ref. not containing 2-methacryloyloxyethyl phosphorylcholine was found to be 70.62°. In the case of the MPC combinations to which 2-methacryloyloxyethyl phosphorylcholine was added at each of the aforementioned ratios, the average contact angle of MPC-1 was 67.92°, that of MPC-3 was 64.65°, that of MPC-5 was 61.08°, that of MPC-7 was 57.45°, and that of MPC-10 was 51.24°. It was confirmed that when the proportion of 2-methacryloyloxyethyl phosphorylcholine increased, the contact angle became lower. Thus, as the addition amount of 2-methacryloyloxyethyl phosphorylcholine increased, the wettability increased. In general, it has been reported that if the water content increases, the wettability also increases based on the nature of hydrogel lenses.[7] In this study, as the addition amount of 2-methacryloyloxyethyl phosphorylcholine increased, the water content also increased, and thus, the wettability also tended to increase. The obtained contact angles of Ref. and MPC-10 are compared in Fig. 4.

4.6. Protein Absorption

The measurement of the absorbance at 280 nm for the samples that were prepared for the evaluation of the degree of protein deposition of hydrogel contact lenses containing 2-methacryloyloxyethyl phosphorylcholine yielded the following results: 0.2598 for the Ref. com-

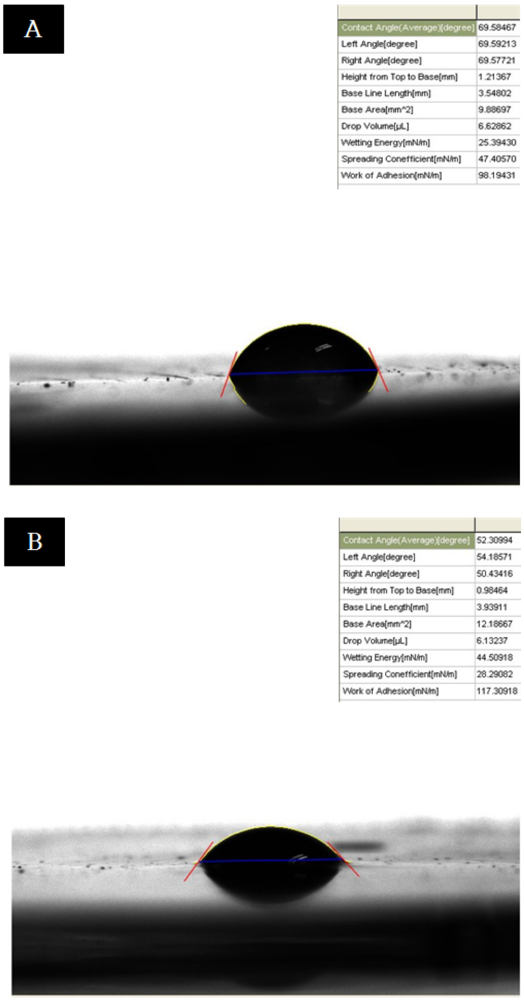

Fig. 4. Contact angle of samples [(A) Ref. (B) MPC-10].

bination and 0.2250 when 10% 2-methacryloyloxyethyl phosphorylcholine was added, which was lower than that of the Ref. combination. These results mean that the protein deposition tended to decrease when MPC was added.

The 2-methacryloyloxyethyl phosphorylcholine that was used in this study belongs to the phosphorylcholine group. According to the study conducted by Kim *et al.*[13], as it has a lipid bilayer structure that is the same as the membrane structure, the adhesion of microorganisms is weakened, and consequently, the contamination of the surface is reduced. In this study, when the surface was observed through SEM after adding 2-methacryloyloxyethyl phosphorylcholine thereto, a smoother surface appeared compared to that of the Ref. combination, showing the same tendency as with the study results obtained by Kim *et al.*[13] The protein deposition is thought to be reduced because of the said effect. The absorbance values of Ref. and MPC-10 are compared in Table 2 and Fig. 5.

Table 2. Protein absorption of samples

Sample	Absorbance
HEMA	0.2598
MPC-10	0.2250

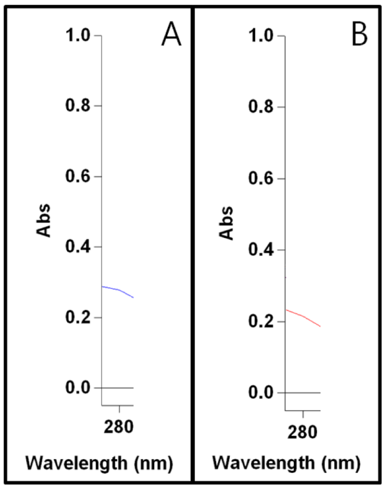

Fig. 5. Absorbance of samples [(a) Ref. (b) MPC-10].

5. Conclusion

After copolymerization by increasing the additive, 2-methacryloyloxyethyl phosphorylcholine, at 1-10% ratios, with HEMA, MMA, and AA, which are mainly used as a material of hydrophilic ocular lenses, and a cross-linking agent, EGDMA, as a basic combination, the physical properties of the contact lenses, and the degree of protein deposition, were measured and analyzed.

As the results of this experiment, when 2-methacryloyloxyethyl phosphorylcholine was used as an additive, the general physical properties of the hydrogel lens were satisfied. In addition, as the amount of 2-methacryloyloxyethyl phosphorylcholine increased, the refractive index decreased, and due to such effect, the water content increased and the wettability also tended to increase as an effect of the water content increase. Moreover, a smooth layer was created on the surface as an effect of the addition of 2-methacryloyloxyethyl phosphorylcholine, which interfered with the protein deposition. As a consequent antibacterial effect can also be expected, 2-methacryloyloxyethyl phosphorylcholine will be actively used for clinical studies.

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