Temperature-responsive Hydrogels Synthesized from Photo-Polymerizable Poloxamer Macromers for Topical Skin Moisturizing

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Temperature-responsive hydrogels were fabricated by the photo-polymerization of bis-acryloyl poloxamer tri-block copolymers, and their temperature-responsive behavior and physiological effect on skin were investigated. For this, poloxamer macromers were prepared by end-capping both terminal hydroxyl groups of poloxamer tri-block copolymers with acrylates. They were then photo-polymerized to generate polymeric hydrogels with structural integrity that allowed excellent stability against dilution in an aqueous environment. The resulting hydrogels exhibited dramatic reversible swelling behavior upon changes in temperature. A demonstration experiment, in which the hydrogels were applied topically to the skin, showed that they shrank rapidly as a result of the increase in temperature, owing to the contact with the skin surface, highlighting their effectiveness for instantaneously moisturizing the skin.

Keywords: Thermo-sensitive hydrogels, Bis-acryloyl poloxamer, Reversible swelling behaviors, Skin moisturizing

Introduction

Hydrogels are hydrophilic polymer networks that can absorb large amounts of water and aqueous ingredients.[1–3] Polymeric hydrogels have been widely used in the fields of medicine, pharmaceuticals, environmental protection, and other advanced technologies.[4,5] Traditionally, most hydrogels in practical industrial applications have been fabricated by the phase mixing and cooling of an aqueous solution of the formulated polymers. Upon the application of an external stress, such as heat, pH, light, etc., these polymer-based hydrogels, which exhibit a gel structure at room temperature or in the absence of other external stresses, show a phase transition to a sol state. However, the transition kinetics are markedly slow. Hence, only a small portion of the active ingredients loaded in the hydrogel phase can be delivered to the target area. In dermatology and cosmetics, hydrogels have been used widely as vehicles for delivering active ingredients.[6–8] Although most hydrogels are designed to supply aqueous ingredients to the skin, the efficiency of delivery is limited mainly by the passive diffusion-based release process.[9,10]

Polymeric hydrogels fabricated from smart macromers have been used in many areas for the sustained and/or controlled delivery of bioactive molecules.[11–14] A good example can be found in the case of using poloxamers, tri-block copolymers, poly(ethylene oxide)(PEO)-*b*-poly(propylene oxide)(PPO)-*b*-poly(ethylene oxide)(PEO). They can self-associate to form micelles in an aqueous solution in dilute conditions. At large concentrations, typically above ~20% (w/w) at room temperature, they exhibit temperature-dependent sol–gel transition behavior. In principle, this thermo-sensitivity originates from the lower critical solution temperature (LCST) phase behavior. If a hydrogel is made of this type of poloxamer, it would swell and shrink reversibly at the LCST, which is basically due to the entropy-induced hydrophobic interaction of the polymer chains.[7] Taking advantage of this semisolidified physical gel structure, a smart hydrogel system with structural integrity upon dilution with other aqueous fluids can be fabricated.

The purpose of this study was to fabricate temperature-responsive hydrogels from bis-acryloyl poloxamer macromers. These macromers were synthesized by end-capping the poloxamers with polymerizable acrylates. The poloxamer macromers were photo-polymerized to form a hydrogel phase.[15–17] The essence of this approach is to use a poloxamer macromer that is useful for the synthesis of smart hydrogels, with controlled phase transition behaviors according to temperature. This temperature-mediated phase transition of hydrogels can have practical applications to topical skin treatment. To demonstrate this, we performed a feasibility experiment in which the poloxamer-based hydrogel was used to regulate the skin moisturizing effect.

Experimental

Materials. Poloxamer 407 ($(PEO)_{99}(PPO)_{69}(PEO)_{99}$) was purchased from (BASF, Germany). Acryloyl chloride and triethylamine were obtained from (Merck, New Jersey, USA). *n*-Hexane, methylene chloride, and other reagents were of analytical quality and used without further purification. Deionized double-distilled water was used for all experiments. Agar-based hydrogels and cellulose-based hydrogels were commercially obtained from the local market.

Synthesis of Bis-Acryloyl Poloxamer. The poloxamer 407 copolymers were dried at 110°C under vacuum for 4 h. The dried poloxamer 407 (40 g, 3.18 mmol) and triethylamine (1.00 mL, 7.24 mmol) were dissolved in 100 mL methylene chloride and placed in a 500-mL round-bottomed flask. Acryloyl chloride (0.50 mL, 12.72 mmol) was added drop-wise. The mixture was stirred at 4°C for 6 h and then at room temperature for a further 6 h. The reaction mixture was filtered to remove the salt and triethanolamine hydrochloride and evaporated completely. The remnant salts and unreacted material were washed continuously in excess *n*-hexane and dried overnight under vacuum. The extent of acrylation was determined by ^{1}H-nuclear magnetic resonance (NMR, Brucker DRX 400 spectrometer operating at 400 MHz) spectroscopy. The chemical shift (δ) was measured in ppm, using $CDCl_3$ as an internal reference. The incorporation of functional groups was investigated by Fourier transform infrared (FT-IR, BOMEM MB-154) spectroscopy. After most of the solvent has been removed at room temperature, the samples were placed in a vacuum oven and dried for 2 days. Poloxamer 407 and the bis-acryloyl poloxamer macromers were mixed separately with KBr to prepare two thin pellets. Sixteen scans were signal-averaged at a resolution of 2 cm^{-1}.

Preparation of Hydrogels by UV-induced Polymerization. The poloxamer macromers were dissolved in degassed deionized water. The concentration was controlled with three different levels, 12, 15, and 18% (w/w). In general, a photoinitiator is incorporated to facilitate radical generation.[18,19] However, in this study, the hydrogels were prepared without a photoinitiator. The solution was stored overnight at 4°C and then poured into a Teflon tray at room temperature. The tray had a cuboid shape (3 cm in length, 3 cm in width, and 0.5 cm in depth). The solution was exposed to long-wavelength UV radiation from a 250-W UV source (λ_{max} 365 nm) (Forcelamp, Incheon, Korea) for 15 min, which was sufficient to induce photo-polymerization. The UV source apparatus positioned at the top of the cabinet interior provided a wide area of illumination. After preparing the hydrogels, they were washed in deionized water to completely remove unreacted macromers.

Characterization of the Swelling Behavior. To investigate the swelling behavior, the hydrogels were immersed overnight in 10 mL of aqueous solution at room temperature. The solution was composed of glycerin (5 wt%), 1,3-butylene glycol (5 wt%), sodium hyaluronate (0.1 wt%), and water (up to 100 wt%). The swelling ratios were determined gravimetrically in triplicate by dividing the final wet hydrogel weight by the initial hydrogel weight prior to immersion in the aqueous solution. The deswelling ratios were also determined using the same method. The deswelling ratios were calculated at predetermined times over a 30-min period. The temperature was set to 4 and 25°C to observe the swelling behaviors. When examining the deswelling behaviors, the temperature was set to 37°C, considering the body temperature.

Evaluation of Skin Moisturizing. The moisturizing ability of three different hydrogels (poloxamer photo-cross-linked, agar-based, and cellulose-based hydrogel) on the skin was studied using a corneometer (Skino-Mat, Cosmomed, Germany). First, all hydrogels were prepared with the same specifications, such as shape, length, width, and height, and they were fully immersed in the same aqueous environment to entrap water molecules. The forearm skin of healthy volunteers (n = 5) was treated with the hydrogel produced with the following dimensions for 30 min: 3 cm in length, 3 cm in width, 0.5 cm in height. After treatment, moisturizing was measured at 0.5, 2, 4, and 8 h in a temperature- and humidity-controlled room (20 ± 2°C and 50 ± 10% humidity). Hydrogel-untreated sites were used as negative control.

Degradation Study of Photo-cross-linked Hydrogels. The degradation profiles of photo-cross-linked hydrogels were investigated over 6 months. Photo-cross-linked hydrogels were immersed in 20 mL of aqueous solution at room temperature. The solution was composed of glycerin (5 wt%), 1,3-butylene glycol (5 wt%), sodium hyaluronate (0.1 wt%), preservative(q.s.), and water (up to 100 wt%). The extent of degradation at each interval (1, 2, 3, and 6 months) was determined gravimetrically in triplicate by dividing the hydrogel weight by the initial hydrogel weight prior to immersion in the aqueous solution.

Results and Discussion

The molecular structure of bis-acryloyl poloxamer 407 macromer synthesized via the molecular design is shown in Figure 1. In detail, this macromer was prepared by di-acryloyl chloride reacting to two hydroxyl terminal groups of poloxamer 407 in methylene chloride and triethylamine. Figure 2 shows that poloxamer 407 has no characteristic ^{1}H NMR peaks, while the bis-acryloyl poloxamer 407 macromer has characteristic peaks of acryloyl protons at each terminal (5.8–6.5 ppm). From the ^{1}H NMR result, the degree of substitution of the acryloyl groups was approximately 81.4%. This was determined by measuring the relative peak ratio of the acryloyl protons at each terminal of the poloxamer macromer (CH_2, 5.8–6.5 ppm) and of the three protons of the methyl group of the propylene oxide unit (CH_3, 1.1 ppm). FT-IR spectroscopy was conducted to confirm that the acrylated group had been conjugated with the hydroxyl group of poloxamer 407. Figure 3 shows the

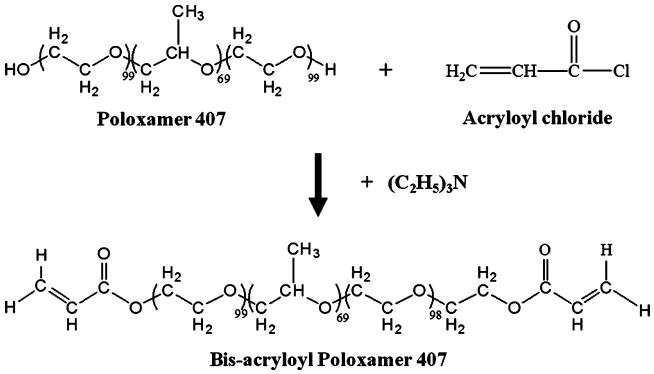

Figure 1. Synthetic scheme of bis-acryloyl poloxamer 407 macromers.

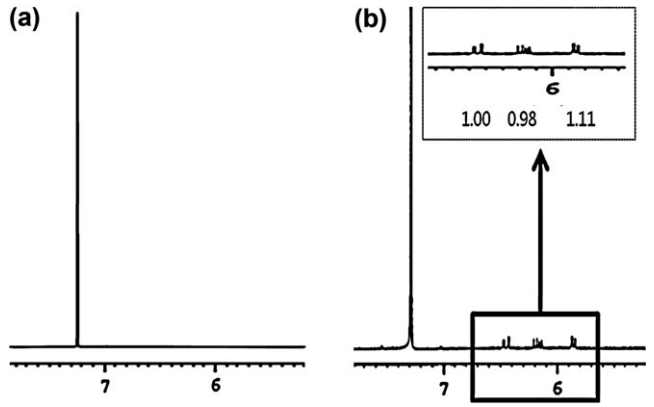

Figure 2. ^{1}H NMR data of bis-acryloyl poloxamer 407 macromers.

characteristic bands at 1640 and 1720 cm^{-1}, corresponding to the stretching vibration of a terminal double bond and to the asymmetric stretching vibration of an ester group, respectively. The data confirmed the synthesis of a polymerizable double-bond end-capped macromer.

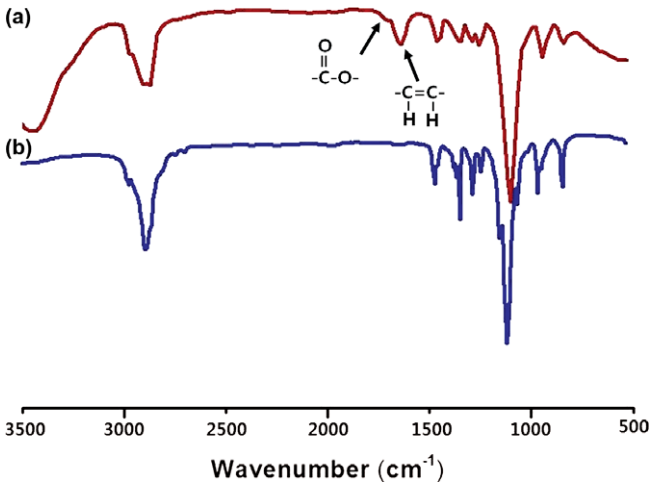

Figure 3. FT-IR spectra of bis-acryloyl poloxamer 407 (a) and poloxamer 407 (b).

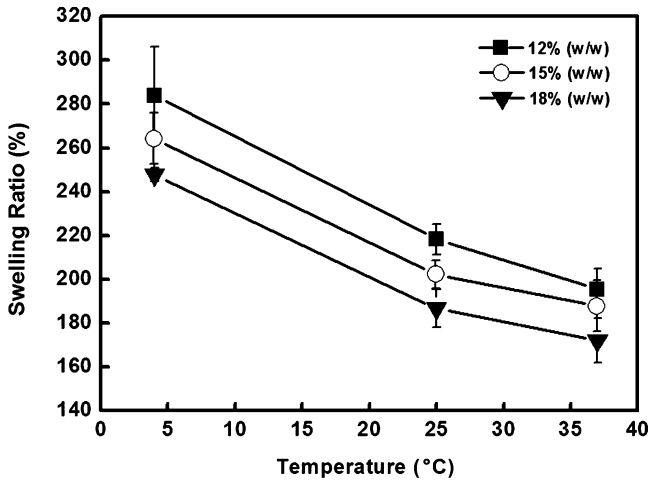

Figure 4. Swelling ratios of hydrogels as a function of temperature and concentration of macromers.

Photo-polymerization was carried out to transform the macromers to a hydrogel phase. The radical initiation of the two double bonds at both ends of the macromer led to the formation of a three-dimensionally cross-linked hydrogel network. A methodological feature of this approach is that photo-polymerization was performed in the absence of a photoinitiator. This was to avoid the presence of any unreacted residues in the hydrogel, which might have adverse effects on biocompatibility in the final applications. Successful photo-polymerization was achieved when the bis-acryoyl poloxamer concentration in the solution was above 12 wt%. The macromers self-assemble to form micelles in solution.[20,21] To effectively interconnect the micelles, they should be closely packed so that a sufficient number of polymerization sites can be provided through the hydrogel phase during the chain propagation stage. This facilitates the entrapment of micelles within the fully cross-linked gel network.

When the temperature is raised above a certain critical micelle temperature, PPO blocks of the micelle are dehydrated and associated with each other to form hydrophobic domains, whereas hydrophilic PEO blocks are still hydrated and sterically stabilize PPG domains. Thus, a hydrogel made of the poloxamer micelles is temperature responsive. As shown in Figure 4, the swelling ratios decreased continuously with increasing temperature. This suggests that the entrapped poloxamer micelles are indeed important and play a role in controlling the swelling ratio of the resulting hydrogel.

At low temperatures, a disintegrated poloxamer micelle structure, which is induced by the weak hydrophobic interactions between PPO blocks in the micelle core, allows the expansion of the surrounding polymer network, resulting in an increase in the swelling ratio. As the temperature increased, however, the swelling ratios decreased gradually over a wide range of temperatures, from 4 to 37°C. This is because the hydrophobic interaction between PPO blocks

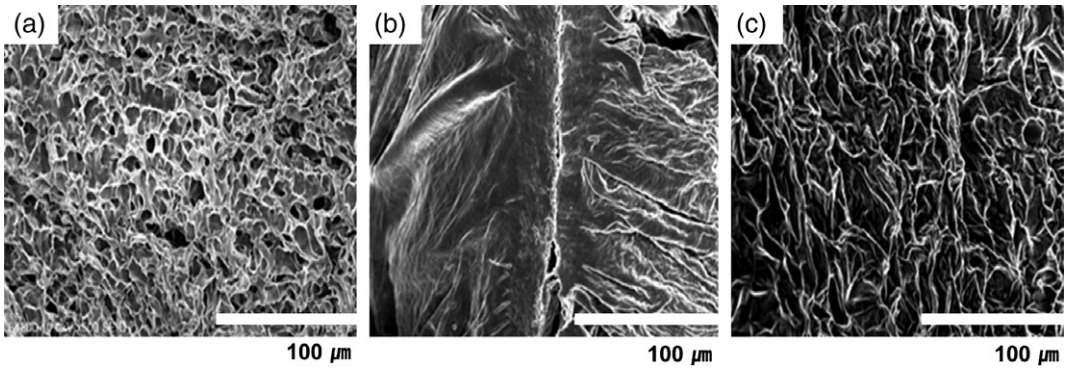

Figure 5. Inner structure of the hydrogels observed by SEM. (a) poloxamer photo-cross-linked hydrogel, (b) agar-based hydrogel, and (c) cellulose-based hydrogel.

becomes stronger at elevated temperatures. As a result, poloxamer micelles would pack more densely in the hydrogel network, which consequently collapses the polymerized chain network around the micelles, leading to a decrease in swelling ratio. The concentration of the macromers incorporated also affects the swelling ratio; a higher concentration decreased the swelling ratio over a given temperature range. The closer packing of the micelles appears to have allowed the hydrogel to have a higher cross-linking density.

To confirm the morphological difference in the hydrogel prepared from the poloxamer macromers, the inner structures of the hydrogels synthesized using the different precursor polymers were observed by scanning electron microscopy (SEM). Interestingly, the hydrogel made from the poloxamer macromers exhibited a porous structure, with pore sizes of 10–20 μm (Figure 5(a)). On the other hand, the other two hydrogels made from agar and cellulose had no characteristic pores in their inner structures (Figure 5 (b) and (c)). Regarding this morphological uniqueness, it was assumed that the pores in the hydrogels might be generated or enlarged because of the solid–liquid phase separation during the drying process for the SEM preparation.

The hydrogels showed typical biphasic deswelling profiles: faster linear deswelling to the 10 min mark, followed by much slower deswelling over a 30-min period (Figure 6). The hydrogel containing a higher concentration of macromers showed a much slower deswelling profile, which is also associated with the dense packing of the poloxamer micelles at the higher concentration. Moreover, the test temperature condition was found to be critical for the deswelling trends because the poloxamer micelles are temperature-responsive. These results suggest that the swelling and deswelling kinetics of these hydrogels can be regulated by tuning the test temperature and the concentration of macromers. Figure 7 shows a schematic illustration for temperature-sensitive hydrogel behavior. The shrinking of the hydrogel was attributed to the micellization of poloxamer 407 upon increasing temperature. When the temperature was raised, hydrophobic PPO blocks were dehydrated and self-associated, while hydrophilic PEO blocks were hydrated, resulting in the formation of a core–shell-type polymeric micellar structure.[22]

As they are biocompatible and intelligently hold and release water, their applicability as a smart skin moisturizer was evaluated. The moisturizing effect was quantitatively determined by measuring the relative capacitance on the skin surface between before and after applying hydrogel, as shown in Figure 8. The hydrogel fabricated in this study showed a remarkably rapid moisturizing profile in the early stages, which is comparable to the hydrogels made from typical water-soluble polymers such as agar and cellulose. This might be due to the structure of cross-linked hydrogels

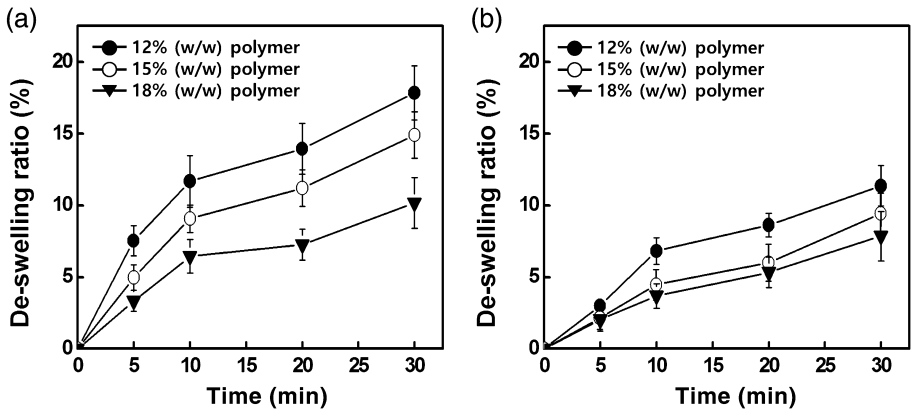

Figure 6. Deswelling ratios of bis-acryloyl 407 poloxamer hydrogels under two different incubation conditions. The initial temperature was (a) 4°C and (b) 25°C, respectively.

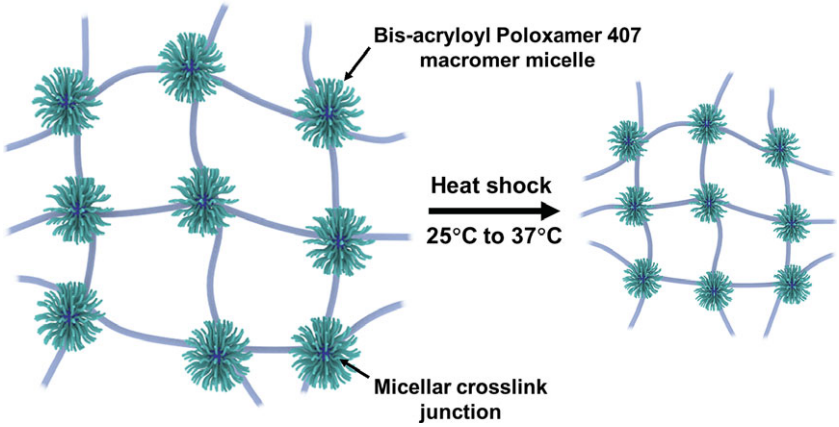

Figure 7. Schematic illustration for the temperature-sensitive phase transition of bis-acryloyl 407 poloxamer hydrogels.

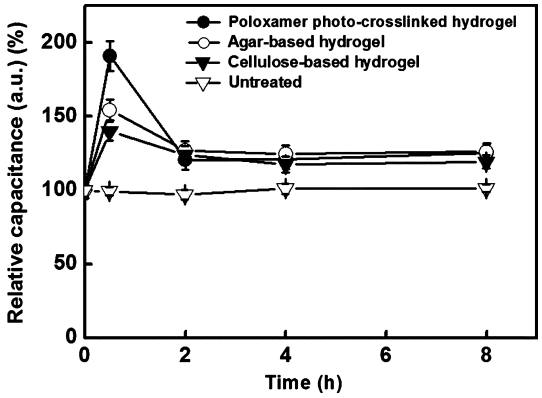

Figure 8. Skin surface capacitance corresponding to the moisturizing ability of the stratum corneum layer after treatment with various hydrogels.

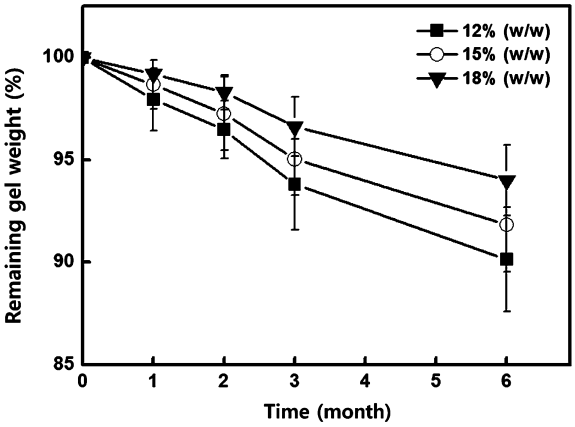

Figure 9. Degradation profiles of bis-acryloyl 407 poloxamer hydrogels as a function of storage time at room temperature.

and the extent of entrapped water. Because photo-cross-linked hydrogels have a porous structure, they could entrap a large amount of water compared to agar- and cellulose-based hydrogels (data not shown). In general, the water molecules trapped in the hydrogels diffuse out via passive transport induced by the concentration gradient. These results can be interpreted considering that, after being applied to the skin, the poloxamer hydrogel physically squeezes out the water molecules trapped in the gel phase in response to the increase in temperature, which appears to provide a stronger driving force for achieving such prompt moisturizing performance.

To ensure the structural stability of the hydrogel phase in complex formulations, we observed the gel stability as a function of the storage time in room temperature (Figure 9). The three hydrogel samples prepared in this study were degraded by less than 10% of mass over the long storage time of 6 months. The structural stability could be also improved by regulating the cross-linking density. From a commercial point of view, it is an acceptable level as a normal product. These results imply that although the hydrogels could be degraded in the complex aqueous environment, the extent of degradation was quite low and would not cause any problem in practical applications.

Conclusion

Bis-acryloyl poloxamer macromers were synthesized to prepare temperature-sensitive hydrogels by photo-polymerization. The hydrogels produced in this study displayed unique swelling characteristics because the segregated micelles in the gel matrix exhibited temperature responsiveness owing to the delicate balance of the hydrophilic and hydrophobic moieties of the poloxamer chains. The topical application of the hydrogel to the skin could enhance the moisturizing effect, which is comparable to the typical hydrogels made using aqueous polymers. The temperature-responsive poloxamer hydrogels are expected to find practical applications in the dermatological and cosmetic fields.

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