Controllable Properties and Microstructure of Hydrogels Based on Crosslinked Poly(ethylene glycol) Diacrylates with Different Molecular Weights

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ABSTRACT: This work describes a comprehensive study of hydrogels based on polyethylene glycol diacrylates (PEGDAs) with the molecular weight (MW) range of 400–2000. The blends of low- and high-molecular weight PEGDA macromers with different ratios were photopolymerized under visible light irradiation, using a blue light sensitive photoinitiator Irgacure819, at the total polymer concentration of 60 wt %. Swelling ratios, wetting property, elastic moduli, transparency, and the microstructure of the resulting hydrogels were investigated. Among them, equilibrium water contents, hydrophilicity, and mesh size of the hydrogels increased while the elastic moduli decreased when increased the PEGDA MW or the content of higher MW PEGDA in the blends. Most of the hydrogels possessed excellent transparency in visible region. The viability of L929 cells on the surface of hydrogel was also estimated. All the selected hydrogels exhibited a relatively high proliferation rate, which demonstrated this hydrogel system with photoinitiator Irgacure819 had good biocompatibility. These results show the properties of PEGDA hydrogel could be easily adjusted by varying PEGDA MW or the ratios of low- and high-MW macromers in the composites. It could be helpful for the design of proper PEGDA hydrogels in the applications as tissue engineering or drug delivery system. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 531–540, 2011

Key words: hydrogels; biomaterials; polymer blends

INTRODUCTION

Hydrogels, consisting of crosslinked hydrophilic polymers, are a large group of materials widely used for biomedical purpose,[1] such as cell encapsulation,[2,3] contact lens materials,[4,5] wound dressings,[6] drug delivery vehicles[7,8] and artificial organs,[9] for their good biocompatibility and tissue-like elasticity. In addition, hydrogels have high permeability for oxygen, nutrients, and other water-soluble metabolites.[10–12] These characteristics make hydrogel a great potential candidate for tissue engineering scaffolds.[13,14] With the increase of demands for various biomedical applications, the synthesis and characterization of different sorts of hydrogels has been the focus of extensive research.

Photopolymerization is a very useful and common polymerizing approach which has been used in biomedical applications for more than a decade. Compared with thermal polymerization usually requiring elevated temperatures, photopolymerization can be carried out at or below room temperature, which is very important in some biochemical applications, such as immobilization of enzymes with hydrogel. By the means of photopolymerization, liquid monomers or macromers can be converted to crosslinked hydrogels in a fast and controllable manner under ambient or physiological conditions.[12] Meanwhile, hydrogels can be created *in situ* to form complex shapes with a minimally invasive manner like injection, which has been used in the development of injectable intraocular lens (IOL).[15,16]

Polyethylene glycol (PEG) possesses a simple molecular structure, yet exhibits many unique properties such as high hydrophilicity, flexibility, nontoxicity, and nonimmunogenecity, which make it an important

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type of polymer in biomedical applications. So far it has been widely used, separately or/and in combination with other materials, in drug delivery systems[17] and tissue engineering.[18] For good properties, PEG is usually used in the modification of material surfaces to improve the biocompatibility.[19,20] For example, by grafting PEG chains on the IOL surface, posterior capsular opacification of intraocular lenses was effectively avoided in cataract surgery.[21] PEG diacrylates (PEGDAs) are derivatives of PEGs containing terminated acrylate groups in their chains. So PEGDAs can form chemical gels by themselves or with other polymeric monomers through crosslinking reactions, such as radical polymerization. Patel et al.[22] compared the viscosity, elastic, and viscous moduli of PEGDA hydrogel with human abdominal adipose tissue, finding that the PEGDA hydrogel was an appropriate candidate for initial implantation into the soft tissue defect. In the research of Weiner et al.,[23] PEGDA (MW575 and MW700) as a reactive additive in the photocrosslinked polyanhydride (PA) networks improved the system properties for a bone replacement. Additionally, PEGDA-based products have also been successfully used for injectable drug delivery systems[24–26] and cell culture mediums.[27–29] Although the composite systems of PEGDA and other materials have been extensively developed and used, few studies directly addressed the effects of PEGDA molecular weights on the properties of hygrogels with same polymer concentration.

In this work, we prepared a series of photopolymerized hydrogels from PEGDA macromers of various MWs and their blends with various ratios. Our goal is to conduct a comprehensive investigation in the effects of PEGDA MWs as well as the mixed ratios on the properties and the microstructure of hydrogels with same polymer concentration. For this purpose, properties of these resulting hydrogels, such as swelling behaviors, water-wettability, mechanical performance, and transparency were characterized and discussed. Besides, microstructures of the hydrogels composed of PEGDA400 and 2000 were observed by scanning electron microscope. Furthermore, the proliferation of L929 cells on the surface of hydrogels was estimated *in vitro*. The experimental results provide the basis for future work involving design of proper hydrogels according to their special biomedical applications.

EXPERIMENTAL PROCEDURES

Materials

PEGDA400, PEGDA600 were purchased from Sartomer Company (Guangzhou, China). The photoinitiator Irgacure819 and PEGDA1000 were offered by Ciba Specialty Chemical Corp. (Beijing, China) and Shin-Nakamura Chemical CO., LTO (Japan), respectively. PEG2000, Tetrahydrofuran (THF), petroleum ether (60–90°C), triethyl amide and acryloyl chloride were purchased from Yili Chemical Company, (Beijing, China). THF was dried by sodium and then distilled at 85°C.

PEGDA 2000 was synthesized through the esterification between the terminal hydroxyl groups of PEG2000 and acryloyl chloride in our laboratory. [yield: 95%, ^{1}H NMR ($CDCl_3$): δ(ppm) 3.4653743.7 (-CH_2-O-*CH_2*-*CH_2*-O-CH_2-, -*CH_2*-CH_2-OOC-, 175.7H), 4.2653744.4 (-CH_2-*CH_2*-OOC-, 3.7H), 5.8653746.5 (-*CH65309CH_2*, 5.6H)].

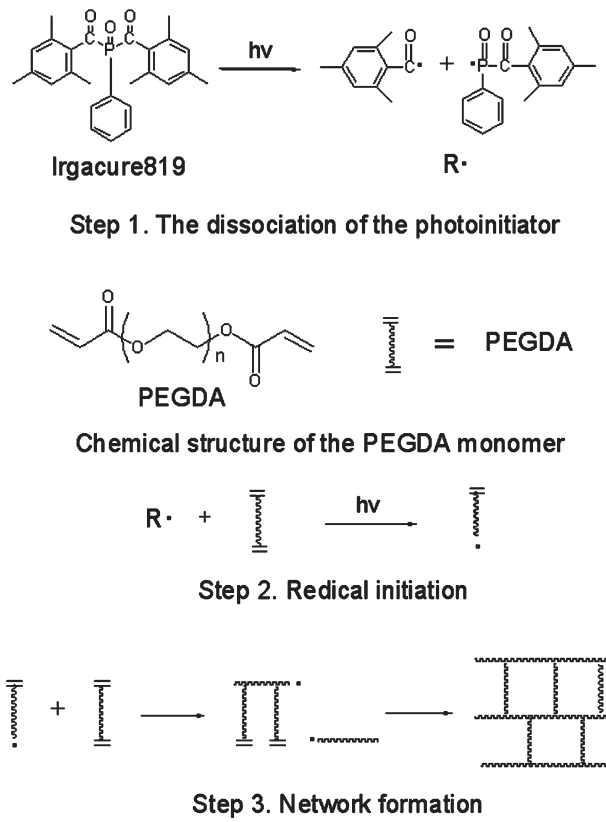
Preparation of PEGDA hydrogels

The water-soluble PEG diacrylates are reactive macromolecules whose acrylate groups can be easily polymerized with initiator under ultraviolet radiation or heat initiation. As most photoinitiators are sensitive to UV light (below 400 nm), which may be damaging to the human body, especially to the eyes,[16] so Irgacure 819 which can generate free radicals under visible light irradiation (under blue light) and initiate the radical polymerization was chosen as initiator in our experiment. To analyze the influence of MW on the properties of hydrogels, the solutions of PEGDA macromers with different MWs and their blends were used to prepare hydrogels, respectively. The chemical structure of Irgacure819 and the polymerization process is presented in Scheme 1. Briefly, two kinds of PEGDAs with predetermined feed ratios were dissolved in deionized water to make aqueous solutions (60 wt %). The photoinitiators (Irgacure819, 12.5 mg/mL in ethanol) were added into the solutions and mixed throughly (Irgacure819/PEGDA = 0.02 wt %). Then the solutions were poured into the cylindrical glass molds with 12 mm diameter and 2 or 4 mm height and photocrosslinked under 420 nm blue light of 5–8 mW/cm^2 for 5 min to form the hydrogel. The resulting hydrogels were taken out from the molds and immersed in deionized water for 72 h to remove the unreacted precursor and residual chemicals, during which the deionized water was refreshed periodically until the hydrogels reached an equilibrium state. The swollen hydrogels were taken out and freeze-dried to constant weight.

Characterization

Nuclear magnetic resonance (^{1}H NMR) spectrum of PEGDA2000 was recorded on a DMX-400 spectrometer (Bruker, Rheinstten, Germany) operating at 400 MHz in CDCl3 solution.

Fourier transform infrared spectrometer (FTIR, PerkinElmer, USA) was used to obtain evidence of



Scheme 1 The photopolymerization process of PEGDA hydrogels.

the polymerization of the PEGDA macromers. The macromers were coated directly on the potassium bromide flake and the dried hydrogel powder was mixed with potassium bromide and laminated. Then the flakes were scanned with FTIR spectrometer between 500 and 4000 cm^{-1}.

The changes of temperature during the polymerization were investigated by inserting a probe of digital thermoscope into the mold filled with the precursor solution of PEGDAs. Then polymerization was carried out on the function of light for 6 min, during which the temperature was recorded once every 20 s.

The swelling properties of the PEGDA hydrogels were determined by equilibrium water contents (EWCs). The crosslinked hydrogels were immersed into deionized water until the swelling equilibrium was achieved, and then the swollen hydrogels were weighed and freeze-dried to constant weight. The equilibrium water contents of PEGDA hydrogels were calculated according to the equation

$$\mathrm{EWC} = (W_s - W_d)/W_d \times 100\% \quad (1)$$

W_s is the weight of the swollen hydrogel and W_d is the weight of dried hydrogel. All experiments were performed in triplicate.

Crosslinking denstities (CD, mol/mL) of Pegda hydrogels were calculated by the Flory-Rehner equation,[30–32]

$$\mathrm{CD} = -[\ln(1 - \nu_2) + \nu_2 + \chi_{12}\nu_2^2][V_1(\nu_2^{1/3} - 2\nu_2/f)]^{-1} \quad (2)$$

where χ_{12} is the polymer-solvent interaction parameter, f is the crosslinking functionality (for PEGDA, $f = 4$), V_1 is the molar volume of water (18.062 mL/mol), and ν_2 is the volume fraction of polymer in the hydrogel when it reaches the equilibrium swelling state. The polymer-solvent interaction parameter χ_{12} for PEG-water system has been reported to be 0.45.[33]

Contact angles (CAs) of PEGDA hydrogels were measured by using an OCA15 contact angle analyzer (Dataphysics, Germany) at ambient humidity and temperature. Drops of deionized water about 1.0 μL in volume were applied to the sample surface.

The elastic moduli of swollen PEGDA hydrogels were measured at ambient temperature using SES-1000 materials testing machine (SHIMADZU Corporation, Japan). A hydrogel sample was placed on the down plate of the instrument and was compressed with the up plate at a constant speed of 0.50 mm/min, during which the plot of stress versus compressing distance was recorded by computer. The elastic modulus of hydrogel was determined from the slope of linear part of the plot above. All experiments were performed three times.

The microstructure of hydrogel was observed using scanning electron microscope (SEM, Cambridge S360, U.K.) The swollen PEGDA hydrogels were freeze-dried, then coated with gold in vacuum to test.

The transmittance of PEGDA hydrogel was measured from 200 to 800 nm with V-570 spectrophotometer (JASCO Corp., Japan). The samples of PEGDA hydrogels were prepared using cylindrical-shaped mold with the diameter of 12.0 mm and the height of 2.0 mm.

Cell culture

L929, a mouse connective tissue fibroblast cell line, was used to evaluate cytocompatibility by a direct contact test. L929 was maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum (FBS, BIOCHROM AG), together with 100 U/mL penicillin (GIBCO) and 100 μg/mL streptomycin (GIBCO) at 37°C in a humidified atmosphere containing 5% CO_2. When the cells reached 80% confluence, they were trypsinized with 0.25% trypsin containing 1 m*M* EDTA (GIBCO) and counted by a hemacytometer prior to further use.

Cells in an initial concentration of 5×10^4 /well were seeded in 24-well plate (Costar) in DMEM supplemented with 10% FBS for 24 h. The prepared hydrogels (10 mm diameter and 2 mm height) were soaked in adisinfectant solution (75% ethanol in sterile water) followed by a sufficient rinse with sterile water. Then they were placed into the wells and the cells were allowed to attach to the surfaces of hydrogels for 7 days. On Days 1, 2, 4, and 7, the viability of cells was quantitatively measured by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay,[34] the morphology of the cells, which were stained with crystal violet, was observed using an inverted phase contrast microscope (Leica). All experiments were performed in triplicate at least.

RESULTS AND DISCUSSION

FTIR spectra of the PEGDA macromer and hydrogel

The FTIR spectra of the PEGDA macromers and the freeze-dried hydrogel are shown in Figure 1(a,b), respectively. As shown, the peaks centered at 1640, 990, and 810 cm^{-1} are attributed to the C=C bonds of the acrylates (Fig. 1-1) in the PEGDA macromers, whereas these peaks reduce greatly in the polymerized gel (Fig. 1-2), suggesting that most of the C=C bonds in the macromers have reacted under the experimental conditions. The peaks at 1725 cm^{-1} and 1110 cm^{-1} are respectively, assigned to the C=O stretching vibration and the C—O asymmetric stretching vibration of C—O—C groups. Compared with the spectra of PEGDA macromers, the heights of these peaks change little in polymerized gel, which indicates the light intensity in our experiment was proper but weak, so that the inherent structure of PEGDA except C=C bonds was not damaged.

Temperature changes during polymerization

The heat released during the hydrogel polymerization is one of important factors for its practical use, especially in the use as cell encapsulation or injectable materials. If too much heat is released, the encapsulated cells or the tissue nearby the injecting place may be affected even damaged. To investigate the heat releasing during hydrogel polymerization, the temperature changes during polymerization were recorded and presented as temperature-time curves, shown in Figure 2. A similar tendency was observed for PEGDA400, PEGDA600, PEGDA1000, and PEGDA2000: the temperatures changed a little within the first 20 s followed by a sudden increase to the maximum at 60 s, then reduced gradually and became constant in the end. The sudden increase of temperature during the polymerization might be caused by the auto-accelerating of radical polymerization.[35] Owing to the increased viscosity and chain entanglements during the hydrogel polymerization, the atuo-acceleration arose when the rate of diffusion-controlled termination was reduced, which means the energy resulted from polymerization could not be released easily and then the temperature increased quickly. Besides, it is noted that the increasing rates of temperature were different in the samples polymerized with the different MWs macromers. Concretely, the temperature increased by 13°C for PEGDA400, while increased by 11°C for PEGDA600, 6°C for PEGDA1000, and 1°C for PEGDA2000. The possible reason for the phenomenon is that at the same mass concentration, PEGDA macromers with lower MW have more reactive acrylate groups which contribute to the heat released during the reaction. Therefore, in the polymerization of hydrogel with low MW PEGDA macromers, more heat may be released in unit time and consequently the high temperature is observed. Conversely, the change of temperature became calm after 5 min polymerization, which indirectly demonstrated the polymerization time (5 min) we chose was proper for the formation of hydrogels in the experiment.

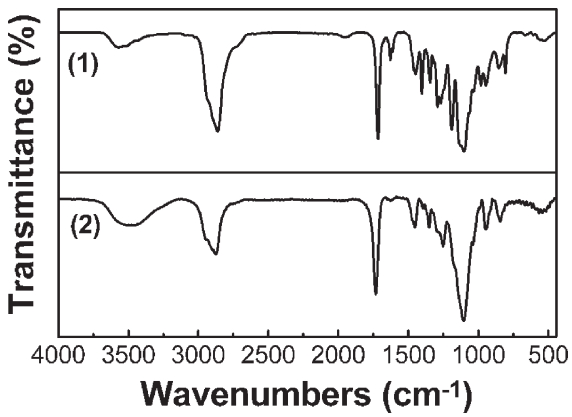


Figure 1 FI-IR spectra: (1) blend of PEGDA400 and PEGDA2000 macromers, (2) PEGDA400/2000 copolymer.

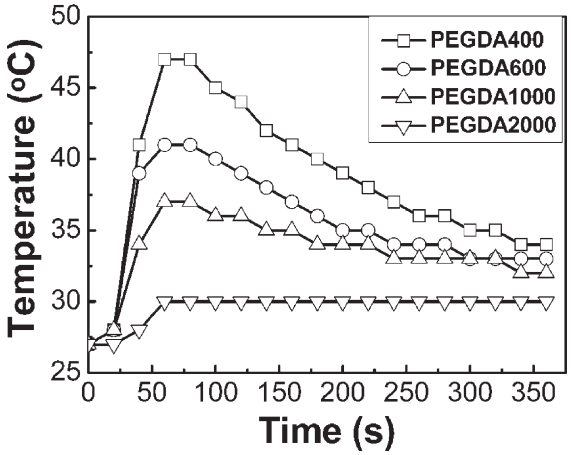


Figure 2 The temperature-reaction time curves of the hydrogels with different MW PEGDAs during photopolymerization.

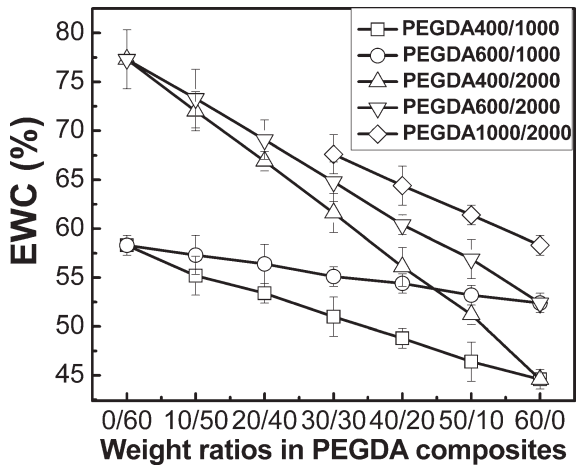


Figure 3 Influence of blend ratios on the EWC of PEGDA hydrogels.

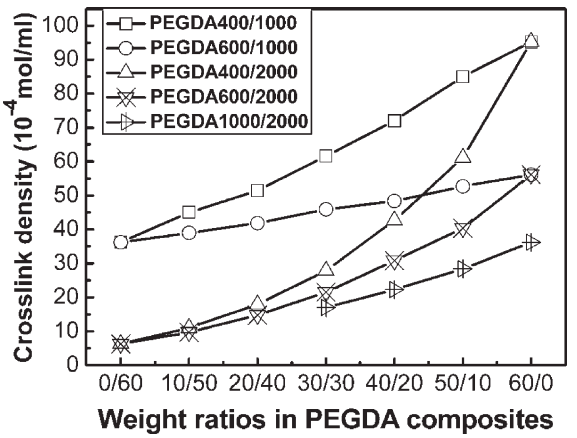


Figure 4 Crosslink density of PEGDA hydrogels with different blend ratios.

EWCs and CDs of PEGDA hydrogels

Figure 3 shows the EWCs of hydrogels prepared with the blends of different MW PEGDA macromers at various weight ratios. Obviously, the EWC of hydrogel decreased with increasing content of lower MW PEGDA macromers in blend. For instance, the EWC was about 72% as the weight ratio of PEGDA400/2000 was 10/50, while it decreased to 52% when the ratio increased to 50/10. We consider the variety of EWC is relative to the crosslinking density of different PEGDA hydrogels. For the hydrogels with a constant concentration of the macromers (60 wt %), the PEGDA hydrogel made from the lower MW macromers possessed higher crosslinking density because of the higher content of acrylate groups, and consequently the less water was absorbed in swelling process (crosslinking density shown in Fig. 4). The maximum value of EWC could reach 77.3% in the PEGDA2000 hydrogel and the minimum value was only 44.6% in PEGDA400 hydrogels, which indicates that hygrogels with different swelling ratios could be obtained for various biomedical applications through adjusting the MW of PEGDA macromers or the weight ratios of PEGDA mixtures.

CD, an important parameter correlating with the properties of hydrogel, such as swelling behaviors and mechanical characteristics has been calculated according to the Flory-Rehner equation and presented in Figure 4. The CDs of PEGDA400, 600, 1000, 2000 were about 95.27, 56.10, 36.20 and 6.29 $\times$ 10^{-4} mol/mL, respectively. In line with expectations, for the hydrogels of PEGDA blends with different weight ratios, the CDs increased with the increase of lower MW PEGDA content i.e., the increase of crosslinkable acrylate groups. These calculation results revealed that the CDs of PEGDA hydrogels could be easily adjusted by altering the molecular weight of PEGDA macromers or/and their blend ratios in hydrogels.

Wetting property of PEGDA hydrogels

The contact angle (CA) on surface of swollen PEGDA hydrogel, which reflects the wetting property of hydrogel, was measured at ambient humidity and temperature. Generally speaking, materials with good hydrophilicity are more proper for biomedical application but not the higher the better in some cases. For instance, in the application of hydrogel as intraocular lenses, too high hydrophilicity of hydrogel facilitates the adsorption and deposition of protein and Ca^{2+} to the lens materials, which is the major cause for the turbidness even opacity of artificial lenses.[36] Thus, adjusting the degree of hydrophilicity for materials is very useful in practical applications. As seen from the curves of CA versus the wetting time (Fig. 5), the initial CAs of all hydrogel samples are below 60° and get to constant values quickly within 50–100 s, which indicates the good hydrophilicity of PEGDA hydrogels. However, it should be noted that the CAs at the steady state

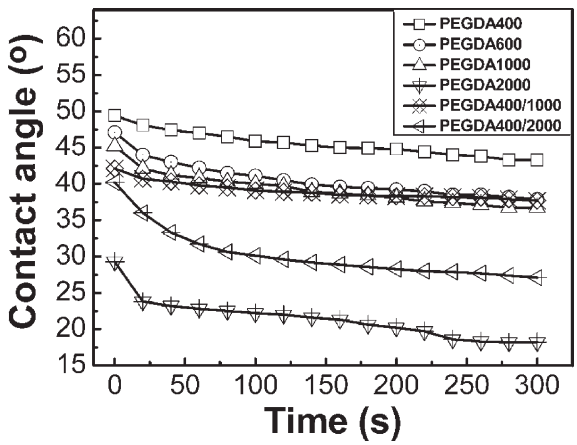


Figure 5 The contact angle curves of PEGDA hydrogels of PEGDA400, 600, 1000, 2000, PEGDA400/1000 (30/30 wt %) and PEGDA400/2000 (30/30 wt %) at equilibrium swelling state.

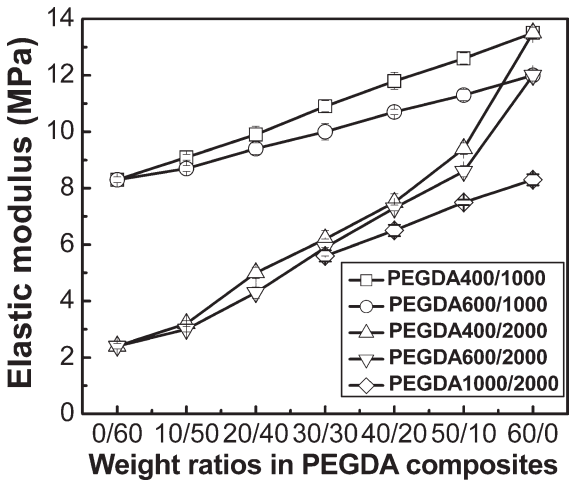


Figure 6 Elastic moduli of PEGDA hydrogels with different blend ratios.

decreased with the increase of PEGDA MW. For PEGDA400, 600, 1000, and 2000, the CAs at steady states were about 46 ± 2°, 41 ± 1°, 40 ± 1°, and 23 ± 2° respectively, while about 39 ± 1° and 30 ± 1° for PEGDA400/1000 (30/30 wt %) and PEGDA400/2000 (30/30 wt %). Although all these hydrogels were composed of PEGDA macromers, the different wetting-abilities were observed. This fact might be attributed to the different EWCs of these hydrogels. The higher EWC the hydrogel has, the better wetting property the hydrogel presents, and consequently higher CA value is obtained.

Mechanical properties of PEGDA hydrogels

Mechanical property is an important parameter for biomedical engineering materials, such as artificial bones and soft tissues. The requirement for the mechanical property of hydrogel is different according to the various intended uses. For example, when hydrogel used as a substitute of cartilage, the high elastic modulus is needed, while in ophthalmic application, the low one may be more suitable. Crosslinking density of the polymer is a significant factor that affects hydrogel mechanical property. In this experiment, the elastic moduli of columniform hydrogel samples were determined from the plots of stress versus compressing distance. Compared with the crosslinking density of hydrogel, we found that increasing the content of low MW macromers in the blend increased the crosslinking density of the resulted hydrogel, thereupon increased the elastic modulus of the experimental samples. As shown in Figure 6, the elastic moduli of swollen PEGDA hydrogels at equilibrium swelling states were about 2.4 ± 0.1, 8.3 ± 0.1, 12.0 ± 0.2 and 13.5 ± 0.2 MPa, respectively, for hydrogel PEGDA2000, 1000, 600, and 400. For the composite hydrogels, the elastic moduli of PEGDA400/1000, PEGDA600/1000, PEGDA400/2000, and PEGDA 600/2000 increased from 9.1 ± 0.1 to 12.6 ± 0.2, 8.7 ± 0.1 to 11.3 ± 0.2, 3.2 ± 0.1 to 9.4 ± 0.2, and 3.0 ± 0.1 to 8.6 ± 0.2 MPa, respectively, when increasing the ratios of lower macromers from 10/50 to 50/10. Because with increase of the hydrogel crosslinking density, the rigider network of intermolecular crosslinking can be formed to withstand the higher compressive loads. On the basis of the above results, PEGDA hydrogels with controlled elastic modulus can be obtained by adjusting the PEGDA MW or weight ratios in the composite.

Microstructures of PEGDA hydrogels

Scanning electron microscope (SEM) was employed to observe microstructure of the freeze-dried PEGDA hydrogels under swollen state. According to the SEM results (Fig. 7), obvious porous microstructures had been observed in PEGDA400/2000 hydrogels, but no obvious porous structure was observed in pure PEGDA400 hydrogel [Fig. 7(a)]. For PEGDA400/2000 composites and pure PEGDA2000 hydrogel, the porous microstructures with average pore size from 0.5 to 5.5 μm could be simply controlled by tuning the weight ratios of PEGDA400 and PEGDA2000, as presented in Figure 7(b–e). The pore size enlarged gradually with the content of PEGDA2000 increasing in PEGDA400/2000 composites and became the biggest in pure PEGDA2000 with the pore size about 5.0–7.5 μm. While the pore sizes were about 0.3–0.9 μm, 0.6–1.8 μm, and 0.9–2.2 μm, respectively, in PEGDA400/2000 composites with weight ratios of 40/20, 30/30, and 20/40. Figure 7(f) shows the change of average pore size with the content of PEGDA2000 in the hydrogel PEGDA400/2000. It is obvious that there is a nonlinear increase of average pore size with the content of PEGDA2000 increasing in the hydrogel PEGDA400/2000. Generally, the pore size of hydrogel network is dependent on the crosslinking density and the amount of water absorbed by the hydrogel: the higher the crosslinking density, the less the water absorbed, then the smaller the pore size left in the dried hydrogel. The concentration of acrylate group in the hydrogel PEGDA400 was the highest so it possessed the highest crosslinking density and the lowest EWC, as a result PEGDA400 hydrogel hardly appeared porous structure, while for PEGDA2000 hydrogel the porous structures with the largest size were obtained. The variable porous microstructure which facilitates the permeability of oxygen, nutrients, and other water-soluble metabolites may avail the cells or tissue culture in tissue engineering.

Transparency of PEGDA hydrogels

A UV-Vis spectrophotometer was employed to investigate the transmittances of PEGDA hydrogel samples with thickness of 2 mm. Most of PEGDA hydrogels in

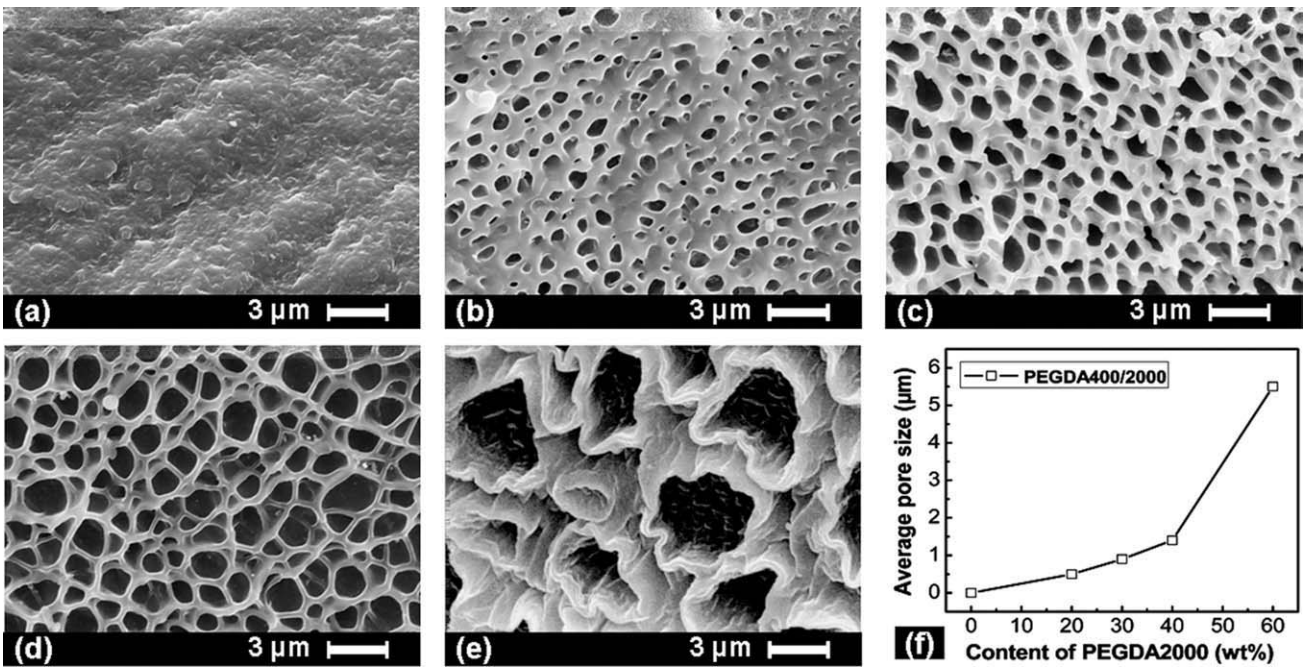


Figure 7 SEM images and average pore size of dried PEGDA hydrogels: (a) PEGDA400; (b) PEGDA400/2000 (40/20 wt %); (c) PEGDA400/2000 (30/30 wt %); (d) PEGDA400/2000 (20/40 wt %); (e) PEGDA2000; (f) Average pore size of PEGDA400/2000 hydrogels with different composite ratios.

the experiment had excellent transparency in visible region, which is proper for a material used for eyes, such as IOL or contact lens. As shown in Figure 8, the transmittances of these hydrogels with the mixed ratio (30/30 wt %) were over 80% within 400–800 nm which are higher than that of human lenses at age of 25.[16] However, for PEGDA400/2000 hydrogels, the transparency depends on the mixed ratios of PEGDA macromers. Figure 9(b) shows the transmittances of PEGDA400/2000 hydrogels before/after swelling at 555 nm (the photopic sensitivity peak for eyes). For the hydrogels before swelling, shown in Figure 9(b-1), the transmittance could be kept over 80% and then decreased slightly to 75% when increasing the blend ratio from 0/60 to 50/10, and then the transmittance dropped to 15% when increasing the ratio to 55/5. When the ratio increased to 60/0 (pure PEGDA400), the transmittance recovered to over 80%. For the swollen hydrogels, shown in Figure 9(b-2), the change tendency of transmittance with the blend ratios was more obvious. The pictures of these hydrogel samples before and after swelling are shown in Figure 9(a). It is clear that before swelling, hydrogel samples of G1, H1, and I1 with blend ratios of 53/7, 55/5, and 57/3 respectively, were opaque while sample E1 and F1 are slightly opaque. However, after swelling, hydrogel samples E2, F2, G2, H2, and I2 with blend ratios in the range from 40/20 to 57/3 were opaque while D2 was translucent. The depressed transmittance in hydrogel PEGDA400/2000 can be attributed to by the phase separation of PEGDA macromers with different MWs. As known, phase separation usually appears in the multicomponent hydrogels especially when these components are not compatible.[37,38] Although PEGDA400 and PEGDA2000 have the same structure and both are water-soluble, their hydrophilic extents are different due to the different mass ratios of hydrophobic acrylate group in one PEGDA chain. PEGDA400 with short chain length possesses high mass ratio of acrylate group and exhibits relatively low hydrophilicity while PEGDA2000 with a longer chain is more hydrophilic. This crucial difference between PEGDA400 and 2000 caused a microphase separation

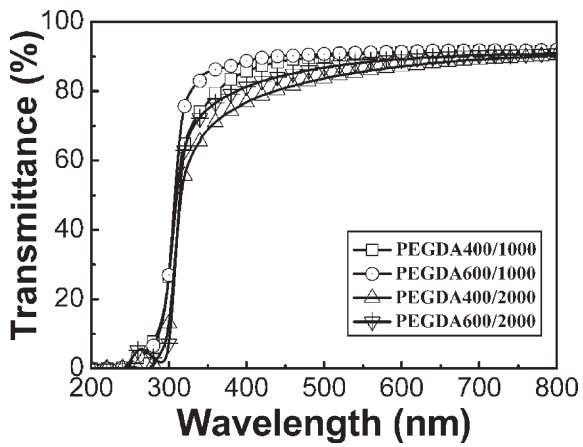


Figure 8 Transmittance spectra of hydrogels of PEGDA400/1000, PEGDA600/1000, PEGDA400/2000, and PEGDA600/2000 with a blend ratio of 30/30 wt % at equilibrium swelling state.

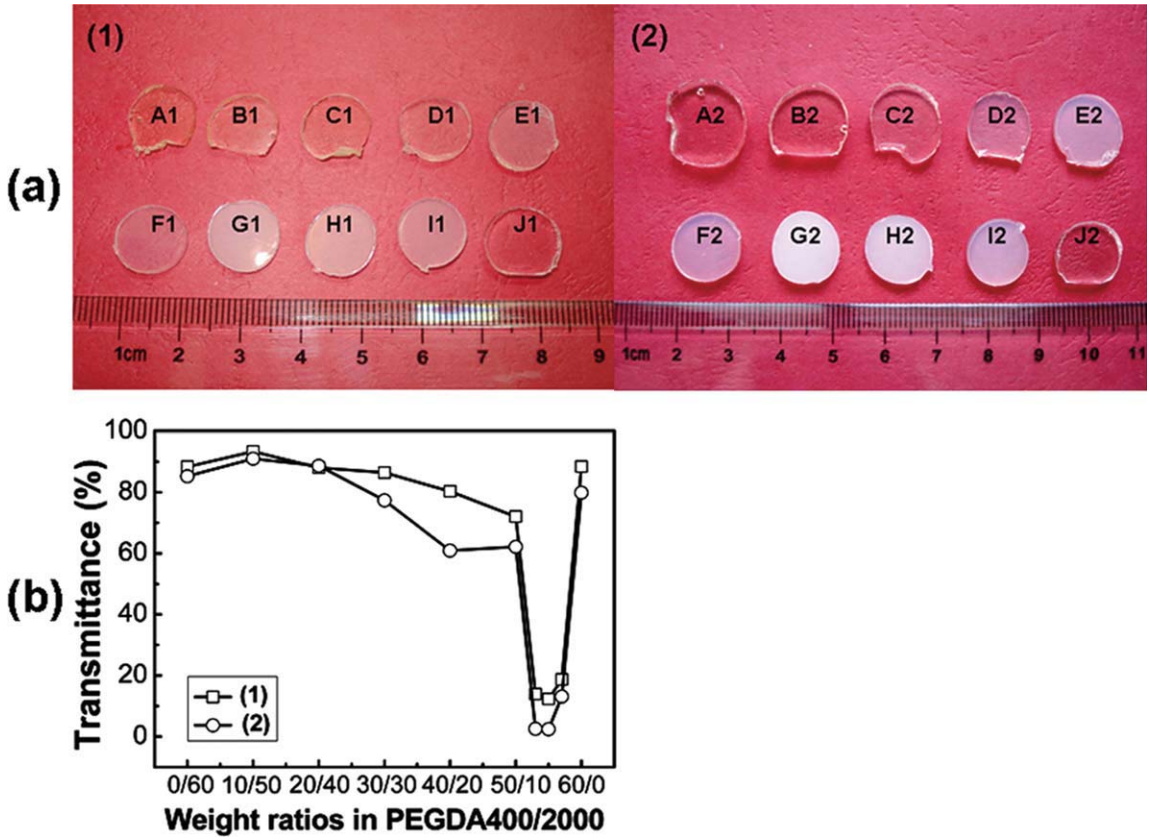


Figure 9 The pictures and transmittance curves (at 555 nm) of PEGDA400/2000 hydrogels: (a-1)&(b-1) hydrogels before swelling in deionized water; (a-2)&(b-2) hydrogels after swelling in deionized water. The weight ratios of PEGDA400/2000 from A to J: 0/60, 10/50, 20/40, 30/30,40/20, 50/10, 53/7, 55/5, 57/3 and 60/0, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

during the polymerization when their blend ratios were from 40/20 to 57/3. Besides, the opacity of hydrogels enriched after swelling compared with those before swelling. The possible reason may be that the presence of free water in swollen hydrogels formed another separate phase as well,[39] which make them more opaque.

Cell viability and proliferation on the surface of PEGDA hydrogels

Biocompatibility is significant for materials in biomedical applications. For photopolymerized materials, the initiator usually has potential toxicity to human body, especially at a high dosage, which could influence the materials biocompatibility. To evaluate the biocompatibility of the PEGDA hydrogels polymerized using initiator Irgacure819 at the dosage in our experiment, L929 mouse fibroblasts were cultured for 7 days in direct contacts to the hydrogels (PEGDA400, PEGDA400/2000, PEGDA2000). Cell viability on the hydrogel surface was morphologically investigated by crystal violet staining. Figure 10(a) shows phase contrast microscopy images obtained respectively, after one, two, four, and seven days of incubation with hydrogels. It was clear that fibroblast L929 cells proliferated well and maintained a polygonal shape with stretched filapodia which was typical morphology for L929, on all the hydrogel surfaces. In the MTT assay, L929 cells presented a relatively high proliferation rate during the seven days [Fig. 10(b)]. Besides, the rate of L929 cells proliferation was higher on the surface of hydrogel PEGDA2000 than those of PEGDA400 and PEGDA400/2000. This might relate to the hydrophilicity and surface roughness of different PEGDA hydrogels. Anyway, from the initial cytotoxicity experiment, we know that the PEGDA hydrogels with Irgacure819 have relatively low cytotoxicity to cells, which is one of indispensable characteristics for biomaterials.

CONCLUSIONS

In this study, a series of crosslinked hydrogels from the PEGDA macromers with different MWs and their composites were prepared through photopolymerization with initiator Irgacure819 by 420 nm blue light. The microstructure and properties such as water-absorbent ability, wetting property, mechanical property and transparency of the hydrogel were investigated, and were found to be dependent on

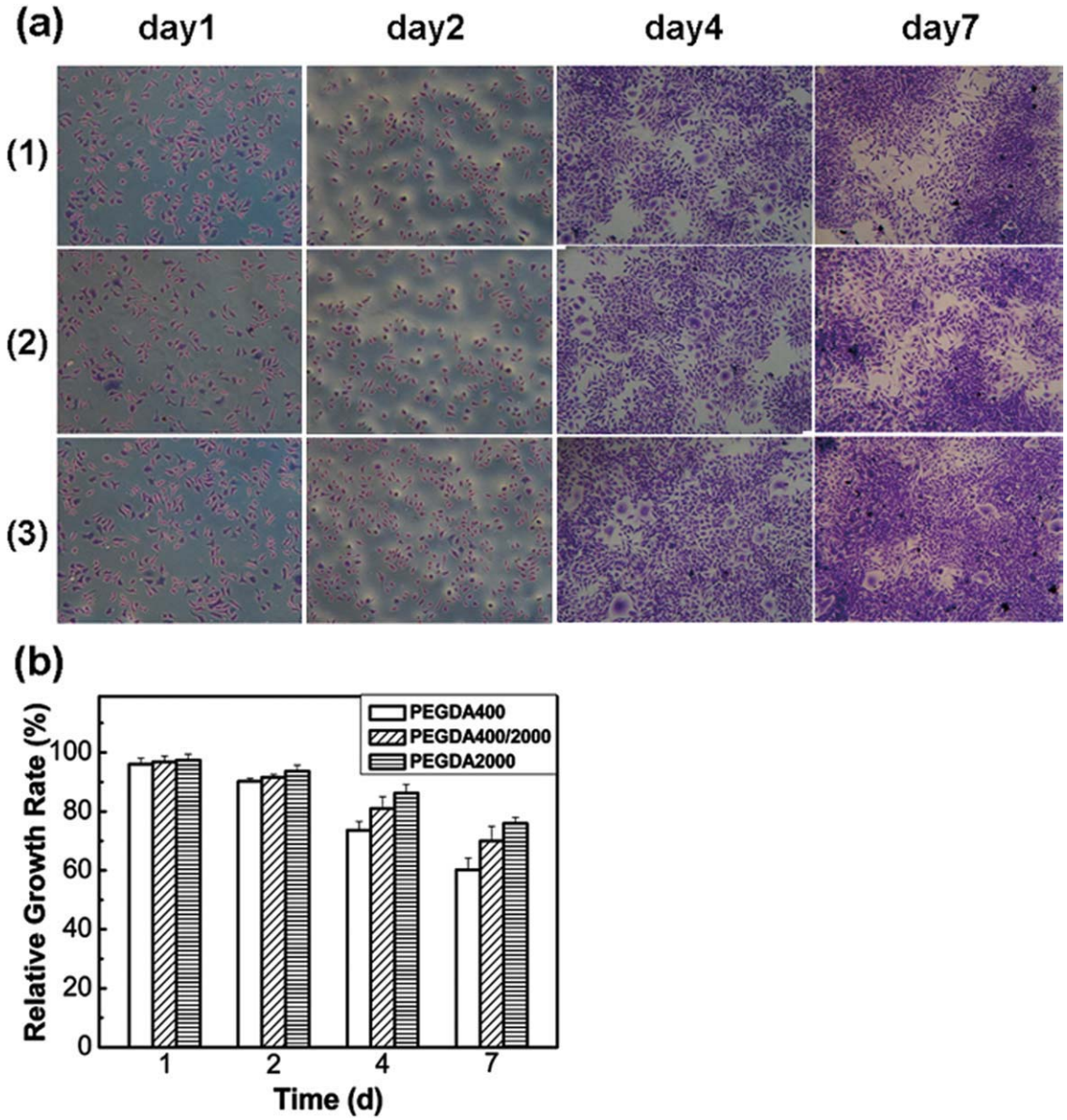


Figure 10 (a) Inverted phase contrast microscope images of PEGDA hydrogels: (1) PEGDA400, (2) PEGDA 2000, (3) PEGDA400/2000 (30/30 wt %); (b) Relative growth rate of mouse connective tissue fibroblasts on surfaces of PEGDA400, PEGDA 2000, PEGDA400/2000 (30/30 wt %) hydrogels ($n = 3$, mean $\pm$ SD). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the molecular weight and the weight ratio of the macromers in the composite. In this work, the higher the molecular weight or/and the higher ratio of the bigger macromer the hydrogel has, the lower the crosslinking density it gets, and then the lower the elastic modulus, the higher equilibrium water content and hydrophilicty are presented. Most hydrogels had excellent transparency except the hydrogel PEGDA400/2000 in the range from weight ratio 40/20 to 57/3 because of the possible phase separation. The experimental data indicate that the PEGDA hydrogels show well-regulated properties and microstructures with the changes of PEGDA molecular weight or/and the blend ratios in the composites. Furthermore, all hydrogels exhibit good biocompatibility which was evaluated in L929 cell lines. The obtained results could be helpful to design proper PEGDA hydrogels for their applications in the fields of tissue engineering, drug delivery system and three-dimensional cell culture.

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