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Notes

Preparation and Properties of PEG-Modified PHEMA Hydrogel and the Morphological Effect

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Introduction

Hydrogels are highly biocompatible on account of their low surface tension, their similar hydrodynamic properties to those of natural biological gels and tissues, and their minimal mechanical irritation in the soft and rubbery state. Currently, a wide variety of clinically important hydrogels are being employed as short and long term materials in kidney dialyzers, blood oxygenators, heart valves, vascular grafts, contact lenses, etc. Increasing interest has been devoted to the preparation and novel application of polymeric hydrogels based on poly(hydroxyethyl methacrylate) (PHEMA) in a variety of medical and biological applications.[1-4] PHEMA is one of the most well-studied synthetic hydrogel polymers. It is nontoxic, biocompatible, swells but does not dissolve in aqueous media, and meets the nutritional and biological requirements of cells. By the bulk polymerization of 2-hydroxyethyl methacrylate (HEMA), a glassy and transparent polymer is produced, which is hard like poly(methyl methacrylate). When immersed in water, PHEMA swells and becomes soft and flexible. Although it allows the transfer of swelling agents and some low molecular weight solutes, this kind of PHEMA is considered non-porous.

Recently, there has been increasing interest in the use of scaffolds for tissue and organ reconstruction and substitution.[5] Hydrogel polymers are particularly appealing candidates for the design of highly functional tissue engineering scaffolds and also as supports for delivery of bioactive agents (drugs) either locally or systemically.[2,6] In both broad application areas, the rate of transport of both small and large molecules, and indeed cells, through the polymer network, critically determines their efficacy. The cell-scaffold interaction is an important factor in organ regeneration, and is influenced by their structures particularly by the pore size.[7,8] Porosity can be controlled by a number of methods, including solvent casting/particulate-leaching, phase separation, freeze-drying, and gas-forming.

PEG has a wide range of beneficial properties for biomedical applications, including low toxicity and non-thrombogenic.[2,9,10] PEG has been used to provide a non-fouling surface in different molecular forms for various biomedical applications in contact with the blood or tissue. A variety of strategies for tailoring the surfaces of materials with PEG-grafts have been developed.[11,12]

The aim of this study was to prepare a PHEMA based hydrogel modified by a PEG graft in order to identify a novel hydrogel with a biocompatible surface and controlled porosity. An additional aim was to determine the effect of grafting on the swelling property and morphology of the hydrogel which can affect the rate of transport of bioactive molecules.

Experimental

Chemicals and Measurements. Poly(ethylene glycol) methyl ether methacrylate (MA-PEG-OMe, Aldrich) of three different molecular weight were vacuum dried for 1 week prior to use. 2-Hydroxyethyl methacrylate (HEMA) was purchased from Aldrich Chem. Co. and passed through an alumina column to remove the polymerization inhibitor prior to use. Ammonium peroxodisulfate (APS, Aldrich, 99%) was used without further purification. Biphenyl acetic acid (BPAA), as a model drug was purchased from Aldrich Chem. Co. Double distilled water was used as the reaction medium.

The IR spectra were obtained using a Perkin Elmer FTIR spectrometer (Model SPECTRUM 2000). Thermal analysis was carried out using a Perkin Elmer DSC/TGA7 Series thermal analysis system. In order to investigate the pore structure and size, the morphology of the prepared gel scaffolds was observed by scanning electron microscopy (SEM, ESEM Model XL30 ESEM-FEG, Phillips). A porous gel sample was mounted on a metal stub with double-sided carbon tape and coated with Pt for 20 min under vacuum (10^{-3} Torr) using plasma sputtering (Ion sputter coater HC-21). Dynamic contact angle was measured by contact angle meter (Erma Optical Co., Model G-1 type) using disc-shaped dry gel specimen and the change was monitored as a function of wetting time.

Gel Preparation: Radical Crosslinking Polymeriza-

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and the copolymer hydrogels were prepared either on a silicone mold or in a small reaction sample. Typically, a predetermined amount of MA-PEG-OMe (5, 10, 15 wt% of HEMA) and HEMA were added to the flame-dried vial and stirred to obtain a clear solution. No additional dimeth-acrylate compound was added to this gel preparation. The vial was capped with a rubber septum, and the atmosphere was replaced by repeated vacuum and nitrogen purging through a three-way stopcock. The initiator (APS) solution was added to the above using a microsyringe, and the mixture was allowed to react at 45 °C for 20 h, where it transformed into a transparent solid gel. The prepared methoxy-PEG (MPEG) grafted PHEMA gel product was washed in distilled water for two days to leach-out any remaining soluble fraction, and then freeze-dried to obtain the gel specimen.

Swelling Measurement. The degree of swelling and the rate in different media were determined by conventional gravimetric analysis. A pre-weighed piece of dry-gel (W_{dry}) was immersed into a swelling medium and allowed to swell. The swollen piece was then removed, pressed gently in between two filter papers to remove any excess water and weighed. The procedure was continued until equilibrium swelling was obtained. The weight of the swollen gel (W_{swell}) was then measured. The swelling ratio (or water absorbency) was expressed as follows:

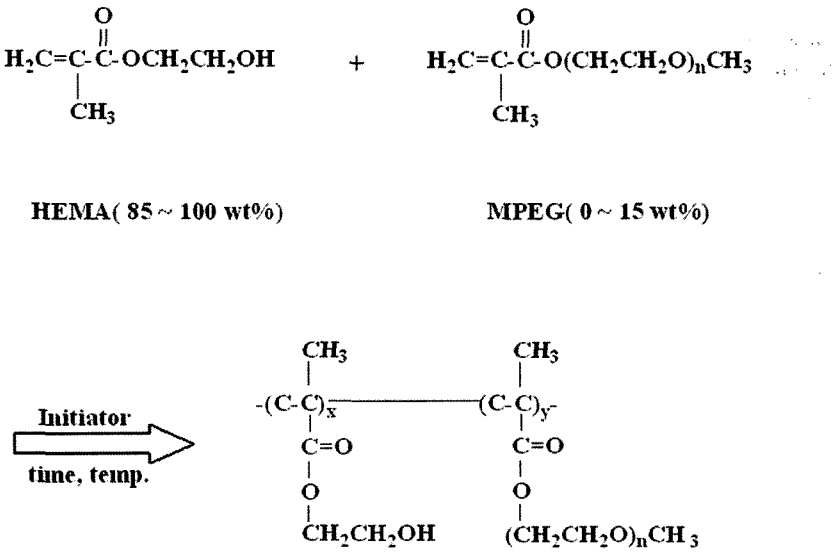
$$\text{Swelling Ratio} = W_{swell} / W_{dry}$$

Drug Loading and Release Behavior. Hydrogel samples containing BPAA(4 mg), a model drug, were prepared and their release behavior was investigated; First, a solution of HEMA and PEG-macromer containing 2 μg of BPAA was prepared and degassed several times before initiator (APS) solution was injected by microsyringe. The solution was poured onto a silicon mold and reacted for 20 h at 45 °C under nitrogen atmosphere. The final drug-loaded gel block was placed in a steel mesh, and the release experiment was carried out in 200 mL of phosphate buffer saline (PBS) solution at 37 °C in water bath. At periodic intervals, 3 mL aliquots were withdrawn and the absorbance spectrum was obtained on a PerkinElmer Lambda 5 ultraviolet-visible spectrometer (λ_{max} at 252 nm for BPAA). The release media were replaced periodically with an equal volume of fresh medium to create an off-sink condition.

Cytotoxicity Test. The cytotoxicity and biocompatibility of the prepared hydrogels were determined using L929 fibroblasts cell lines. The hydrogels were cut into $\pi \times 1.3 \times 1.3$ cm^2 pieces and placed in 6-well plates with tissue culture polystyrene (TCPS) as a control substrate. All the hydrogels were sterilized with 70% ethyl alcohol and UV irradiation, and then washed with PBS. The L929 fibroblasts cells were cultured in the growth medium, which was made up of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) at 37 °C in a 5% CO_2 atmosphere for 3 days. The L929 fibroblasts were purchased from the Korea cell line bank. The cell response was observed everyday using a microscope.

Results and Discussion

Preparation of PEG-Modified PHEMA Gel. PEG macromer, MA-PEG-OMe, of three different chain length (Mw ca. 500, 1,000, and 2,000) was introduced to the HEMA gel preparation with different compositions. All the gel samples were obtained as transparent, 2 mm thick solid blocks using a silicone mold. The prepared gel samples were washed by



Scheme I. Preparation of PHEMA hydrogel with tethered methoxy-PEG.

stirring the samples in distilled water for 48 h followed by freeze-drying under vacuum.

Figure 1 shows typical FTIR spectra of the HEMA and copolymer gel. The characteristic absorption band of the PEG backbone (C-O-C) occurs at 1115 cm^{-1} additionally to those of homo PHEMA. Figure 2 shows the TGA thermograms of different gels. The materials were thermally stable up to about 300 °C, and showed a shift of weight loss pattern to higher temperature with the addition of the PEG components into the PHEMA.

Swelling and Wetting Properties of PEG Modified PHEMA Hydrogel. Swelling curves in Figure 3 show that the gel samples reach equilibrium swelling in approximately 5-6 h and remain constant thereafter. Figure 4 shows the plot of equilibrium swelling ratios of the prepared gels in water as a function of content for the different chain length of PEG graft. The swelling ratio increased gradually as the PEG content was increased from 0 to 15%, and also as the PEG chain length increased, the difference in swelling degree became more prominent. A more hydrophilic PEG graft would result in a higher swelling capacity of the composite system. The swollen gel became more and more soft and flexible in proportional to the swelling capacity, i.e. the amount of PEG components. As expected, the introduction of PEG into the HEMA gel system altered the bulk properties of the resulting gels including physical properties. On the other hand, the surface properties of the gel are also expected to be modified to some extent by the PEG grafts, which will spread out from the surface of both bulk and internal pores. The effects of tethered PEG on the surface are interesting because the surface nature and interfacial properties of the biomaterials are extremely important in determining the biocompatibility of the given materials. Figure 5 shows a plot of the dynamic contact angles measured according to the elapsed wetting time. The starting contact angle was much lower in the PEG modified surfaces, and decreased with increasing PEG content. This suggests that the gel surface becomes increasingly hydrophilic with increasing PEG content. This means that the flexible

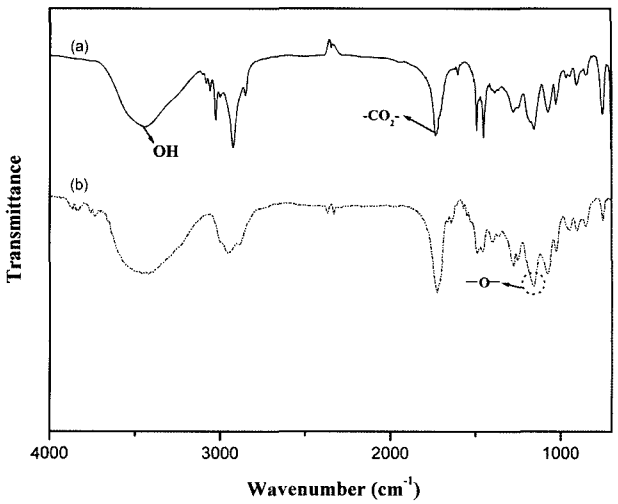


Figure 1. FTIR spectra of the PHEMA and PHEMA with PEG graft.

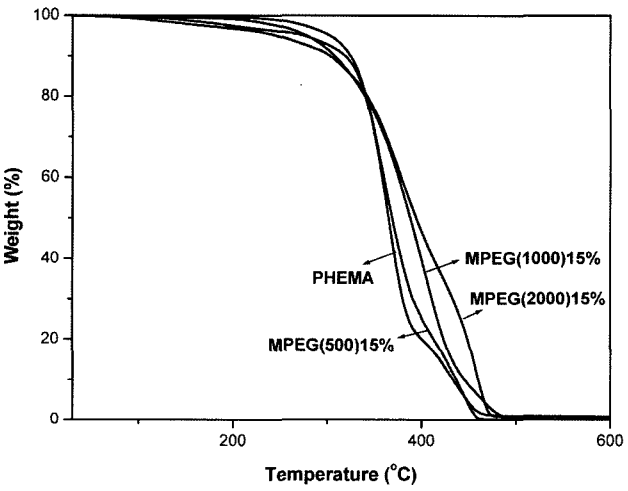


Figure 2. TGA thermograms of the PEG modified PHEMA gels.

PEG chains are located on the PHEMA matrix gel surface to modify its surface characteristics. The PEG chains

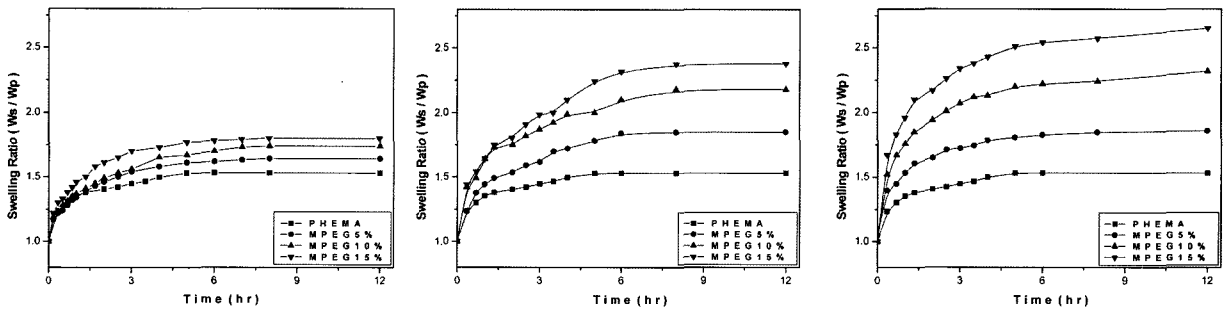


Figure 3. The swelling curves of the PEG modified PHEMA gels. (a) MPEG 500, (b) MPEG 1000, and (c) MPEG 2000.

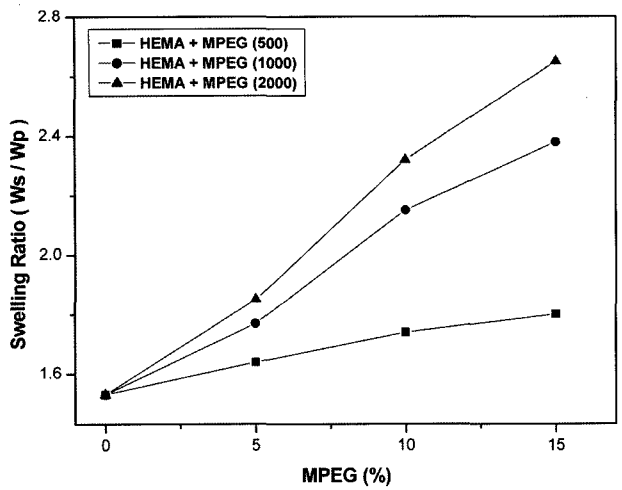


Figure 4. Plot of swelling ratios as a function of MPEG content and the chain length.

anchored on the surface of the pores can also work very importantly to affect the interaction between salute molecules and materials. In addition, this structural effect should alter the adsorption behavior of a certain protein on the material, however, the related study is out of the scope of this communication.

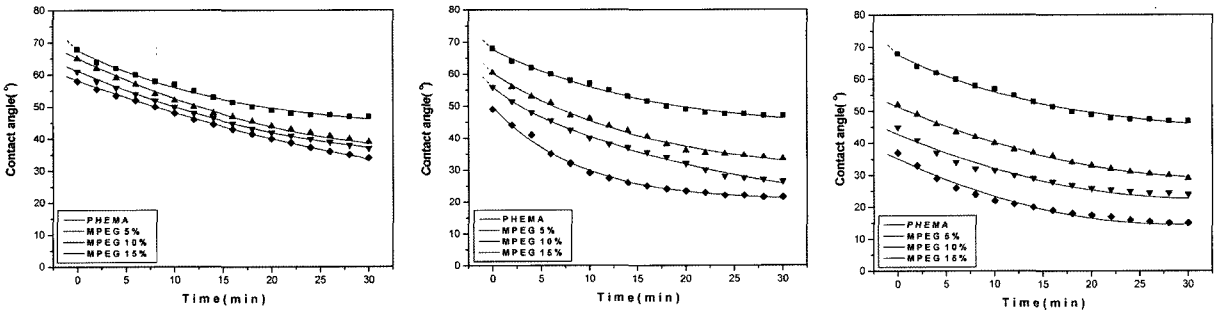
Morphology of PHEMA Hydrogel Containing PEG Graft. A characteristic morphology, which changes by both content and chain length of PEG graft, of the freeze-dried samples of water-swollen hydrogel was observed by SEM as shown in Figure 6. Bulk PHEMA gel is transparent and possesses a very dense structure without pores discernible. By introducing the PEG graft into PHEMA, the porosity of the network began to be developed and the pore size is gradually increased. Basically nonporous homo PHEMA turned to microporous structure, where the pore size seemed to be regulated to a certain range from submicron to tens of micron size by changing the content and chain length of PEG chain. Especially the change in pore size by PEG chain length was observed quite dramatic. This structural change must result from the complex interactions among the gel components including water molecules, but both the controllable porosity and the chemical nature of pore surface may be exploited beneficially for the current drug delivery systems. The related studies on the gel morphology with its effect on the diffusion of bioactive molecules within the gel matrix are interesting and currently under investigation.

Cytotoxicity and Drug Release Behavior. The L929 fibroblasts cell line was used to evaluate the cytotoxicity and biocompatibility of prepared hydrogels. The cellular behavior on a biomaterial is an important factor determining its biocompatibility. The L929 fibroblast cell line was cultured for 3 days and the surface morphology was observed by optical microscopy (Figure 7). The surfaces of the modified PHEMA hydrogel had a similar cell growth pattern to that of the TCPS control. Thereby, the PEG modified PHEMA hydrogel is considered to be biocompatible material.

To study the morphological effects on the release behavior, we prepared model drug incorporated PEG-*g*-PHEMA gels and the simple release behavior was observed. As a model drug, BPAA, an anti-inflammatory and analgesic drug, was used. The release patterns are shown in Figure 8. Overall the release seemed to be governed by simple diffusion and the release rate of drug tended to increase in proportion to both the PEG content and its chain length, even though the differences among samples were not very large. Compared to homo PHEMA, the initial release rate and the level of released drug in the final stage were higher in PEG-*g*-PHEMA hydrogels.

Conclusions

Novel HEMA based hydrogels modified by a PEG graft were prepared by the crosslinking polymerization of HEMA in the presence of a methacryloyl PEG macromer with different chain length. The increasing degree of swell-



(a) (b) (c)

Figure 5. The dynamic contact angle measurement of the PEG modified PHEMA gel surface according to the elapsed wetting time. (a) MPEG 500, (b) MPEG 1000, and (c) MPEG 2000.

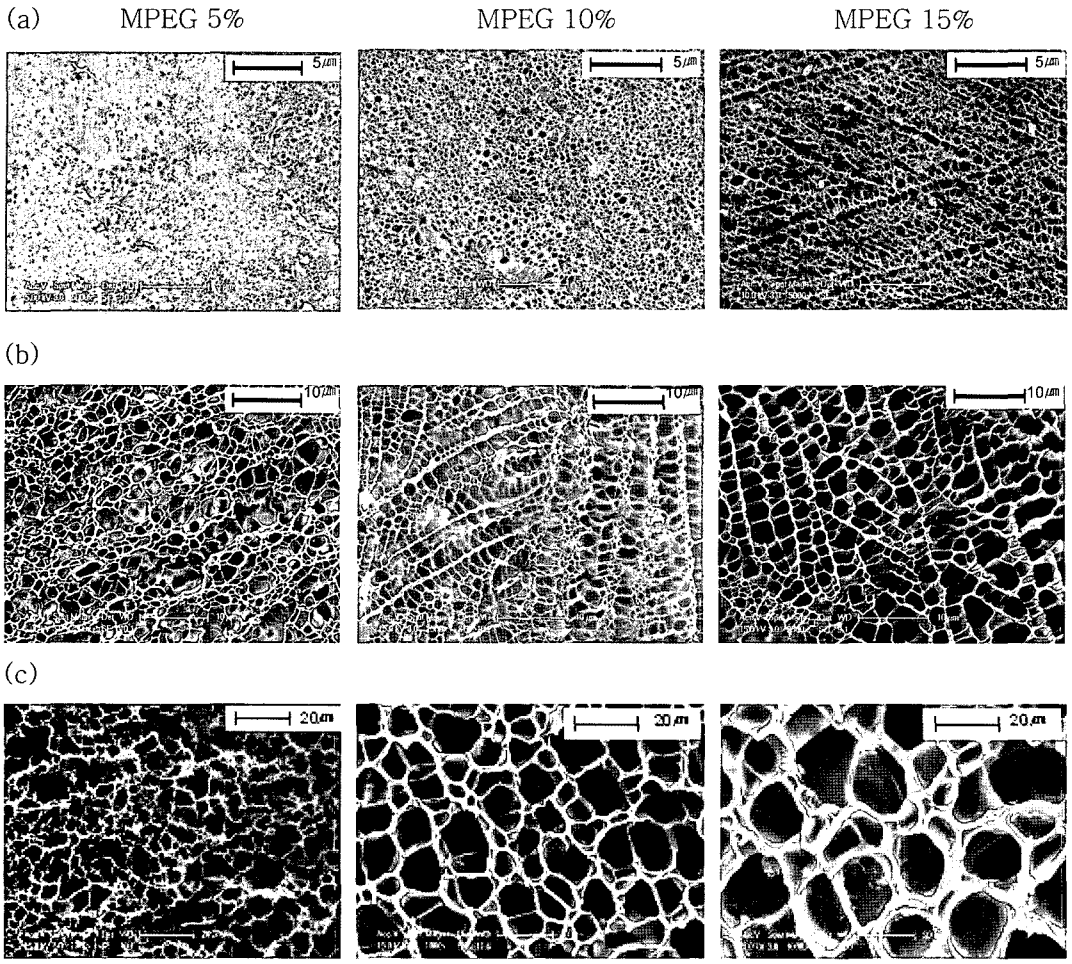


Figure 6. SEM images of freeze-dried PHEMA gel modified by PEG with different chain length. (a) MPEG 500, (b) MPEG 1000, and (c) MPEG 2000.

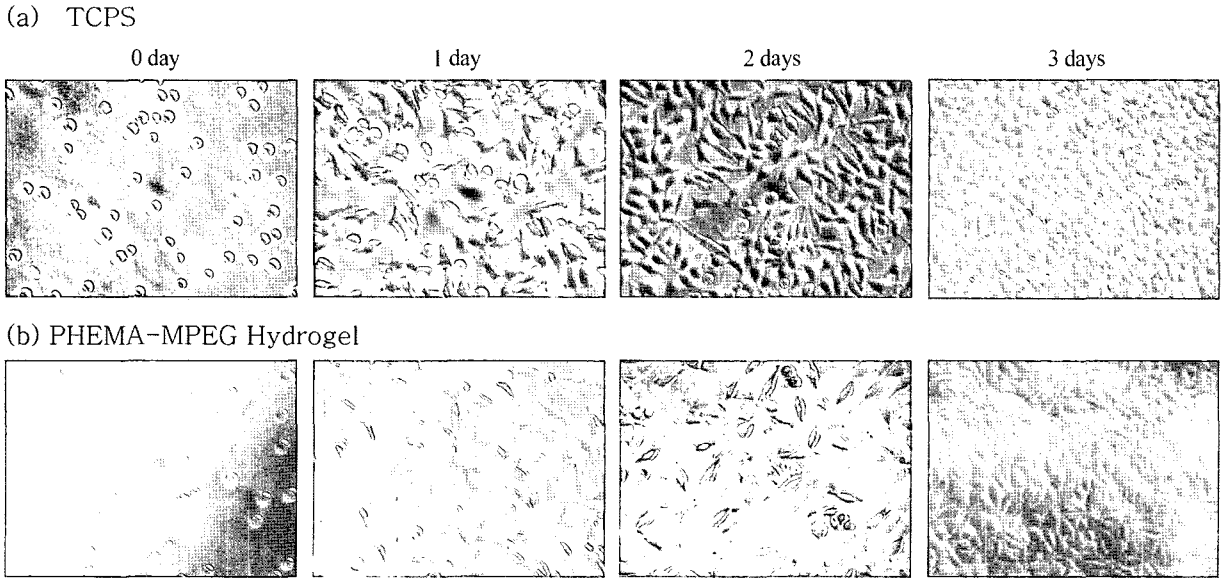


Figure 7. Micrographs of the L929 cells 1 day, 2 days, and 3 days after being plated on (a) TCPS and (b) PHEMA-MPEG Hydrogel (×500).

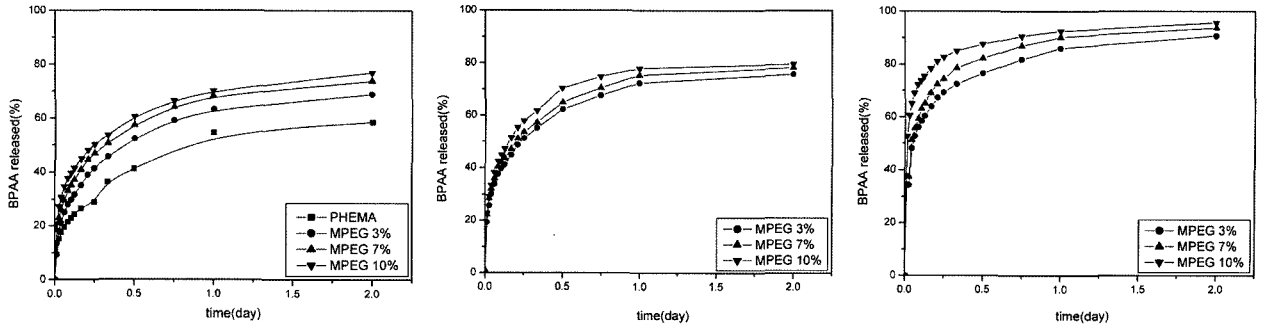


Figure 8. Release behavior of BPAA from the MPEG modified PHEMA hydrogels. (a) MPEG 500, (b) MPEG 1000, and (c) MPEG 2000.

ing and the decreasing surface contact angle highlight the increasing hydrophilic nature of the gels modified by incorporating PEG. The PEG modified PHEMA gels had a porous network structure with pore sizes ranging from submicron to tens of microns, which changed according to composition. From the cytotoxicity test, the modified gel was found to be non-toxic and biocompatible. This material might applicable as a material for controlled drug delivery and as a gel scaffold in tissue engineering.

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