Preparation and Properties of Modified PHEMA Hydrogel with Sulfonated PEG Graft

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ABSTRACT: A novel poly(ethylene glycol) (PEG) macromer with a methacryloyl and sulfonic acid group at each end of the chain was prepared. Modified hydroxyethyl methacrylate (HEMA) based hydrogels were synthesized by crosslinking polymerization of HEMA in the presence of the above-mentioned PEG macromer. The effect of the sulfonated PEG graft was examined by comparing the swelling properties with those of a pure poly(hydroxyethyl methacrylate) (PHEMA) hydrogel. The modified PHEMA hydrogel exhibited increasing water absorbency with increasing sulfonated PEG content up to 15 wt %. These hydrogels with the sulfonated PEG graft exhibited a more hydrophilic character than the pure PHEMA gel. Also the swelling degree varied slightly with pH, showing increased swelling at higher pH probably due to the presence of the anionic sulfonate group on the PEG end chain. In addition, the protein adsorption test showed a lower level of fibrinogen adsorption from the sulfonated poly(ethylene glycol) (SPEG) modified gel than on the homo PHEMA hydrogel. Interestingly, scanning electron microscopy showed that the porous and rather uniform morphology of the gels changed with increasing sulfonated PEG content in PHEMA. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 2484–2489, 2007

Key words: PEG macromer; hydrogel; PHEMA; swelling; sulfonate group; protein adsorption

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. Hydrogels have been successfully used for a variety of biomedical applications including contact lenses, intraocular lenses, drug delivery devices, implants, and scaffolds for living cell encapsulations.[1] 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.[1,2] 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.[3]

Recently, there has been increasing interest in the use of scaffolds for tissue and organ reconstruction and substitution.[4] Hydrogel polymers are particularly appealing candidates for the design of highly functional tissue engineering scaffolds.[2,5] The scaffold functions as a substrate for the proliferation and differentiation of cells that are either seeded or infiltrated from the surrounding host tissue, thereby restoring the organ function. The cell-scaffold interaction is an important factor in organ regeneration, and is influenced by their structures particularly by the pore size.[6,7] The porosity can be controlled by a number of methods, including solvent casting/particulate-leaching, phase separation, freeze-drying, and gas-forming.[8]

Poly(ethylene glycol) (PEG) has a wide range of beneficial properties for biomedical applications, including low toxicity and nonthrombogenic.[2,9,10] PEG has been used to provide a nonfouling 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] In addition, it was reported that a material with a negatively charged surface is more compatible with the blood compatibility than a neutral one.[13,14] Han and Kim et al. showed that polyurethane grafted with sulfo-

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nated PEG exhibited a superior thrombo-resistance, as determined by the decreased platelet adhesion or protein adsorption, and less calcification than on the surface grafted with PEG only.[15–17] Accordingly, it is highly probable that PEG containing sulfonate end groups, i.e., sulfonated PEG (PEG-SO_3H), or a substrate with modified surface containing a PEG-SO_3H brush will enhance the biocompatibility of materials as a result of the synergistic effect of the dynamic mobility of PEG chains and the negatively charged sulfonate groups.[17,18]

The aim of this study was to prepare a PHEMA based hydrogel modified by a sulfonated PEG graft to identify a novel hydrogel with a biocompatible surface. An additional aim was to determine the effect of grafting on the swelling property, surface characteristics, and morphology of the hydrogel.

EXPERIMENTAL

Chemicals and measurements

A methacryloyl poly(ethylene glycol) macromer with a sulfonate end group, MA-PEG-SO_3H, was prepared using the method previously reported elsewhere.[19] Poly(ethylene glycol) methyl ether methacrylate (MA-PEG-OMe, $M_w = 1100$, Aldrich) was vacuum dried for 1 week before use. The polymeric hydrogel 2-hydroxyethyl methacrylate (HEMA) was purchased from Aldrich Chem and passed through an alumina column to remove the polymerization inhibitor before use. Ammonium peroxodisulfate (APS, Aldrich, 99%) and 1,3-propane sultone (PST, Aldrich, 98%) were used without further purification. Double distilled water was used as the reaction medium. Bicinchoninic acid (BCA) protein assay reagents (Micro BCA) were purchased from Pierce, Rockford, IL.

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. To investigate the pore structure and size, the morphology of the prepared gel scaffolds was observed by SEM (FESEM Model JSM6700F, JEOL, Japan). A porous gel sample were mounted on a metal stub with double-sided carbon tape and coated with Au-Pd for 60 s under an argon atmosphere using plasma sputtering (Sputter Coater 108auto, Cressington Scientific Instruments). Dynamic contact angle was measured by contact angle meter (Cahn, Model DCA-322, Germany) using disc-shaped dry gel specimen and the change was monitored as a function of wetting time.

Preparation of PEG macromer, MA-PEG-SO_3H

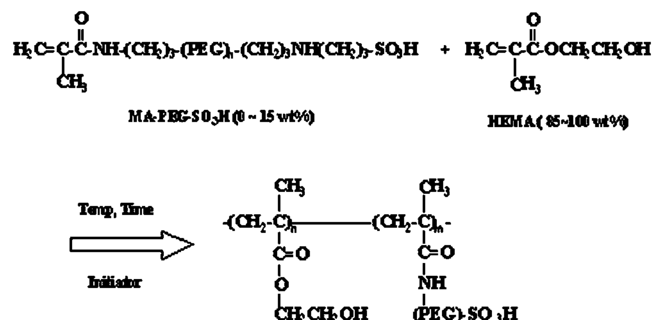
The MA-PEG-SO_3H macromer was prepared using the following procedure: 10% PST (2.45 g) in THF was added dropwise to a 10% PEG-diamine (M_w: 1100, 20 g) solution in THF, and allowed to react at 50°C for 5 h. After the reaction, the mixture was cooled to room temperature for some time to allow phase separation. The upper layer was then decanted. The remaining oily product was washed with cold THF, and dried in a vacuum at ambient temperature for several days to obtain the zwitterionic amino-PEG sulfonate (PEG-AS). Consecutively, 10% PEG-AS (3 g) in $CHCl_3$ was reacted with excess amounts of methacrylic anhydride (MAH, 0.55 g) at 25°C for 40 h in the presence of triethylamine (TEA, 0.09 g). The reaction mixture was precipitated in a large amount of diethyl ether. The resulting glue-like product was separated, dissolved in deionized water, and freeze-dried to yield a white powdery PEG macromer (MA-PEG-SO_3H) in an $\sim$ 70% yield. Elemental analysis showed the following values: C, 48.01; H, 9.14; N, 2.28; S, 2.61 wt %.

Gel preparation: Radical crosslinking polymerization of HEMA in the presence of the PEG macromer

HEMA and the copolymer hydrogels were prepared either on a silicone mold or in a small reaction ampoule. The reaction is shown in Scheme 1. Typically, a predetermined amount of MA-PEG-SO_3H (5, 10, 15 wt % of HEMA) and HEMA were added to the flame-dried vial and stirred to obtain a clear solution. No additional dimethacrylate 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 is transformed into a transparent solid gel. The prepared gel product was washed in distilled water for two days and freeze-dried to obtain the gel specimen.

Swelling measurement

The degree of swelling in different media was determined by conventional gravimetric analysis. A pre-



Scheme 1 Preparation of PHEMA hydrogel with tethered PEG sulfonate.

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:

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

Protein adsorption test

The level of fibrinogen (Fg) adsorption was evaluated using an ELISA assay described elsewhere.[19] Before the adsorption experiments, gel pieces, 1 cm^2 in size, were equilibrated in water at 37°C for 12 h. The adsorption was then carried out by gently shaking a fibrinogen (0.02 g/L) solution containing the fully swollen gel specimen at 37°C. After a definite time interval, the gel specimen was removed and washed three times with distilled water to remove any nonadsorbed proteins. The samples were then transferred to another tube, and 2 mL of a 1% w/v sodium dodecyl sulfate (SDS) in deionized water was added and incubated overnight in a shaking incubator at 37°C. The samples were then sonicated for 2 h and vortexed before pipetting 100 μL of the solution into a 96-well plate. The amount of adsorbed protein was measured using a BCA protein assay kit.

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 phosphate buffered saline (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.

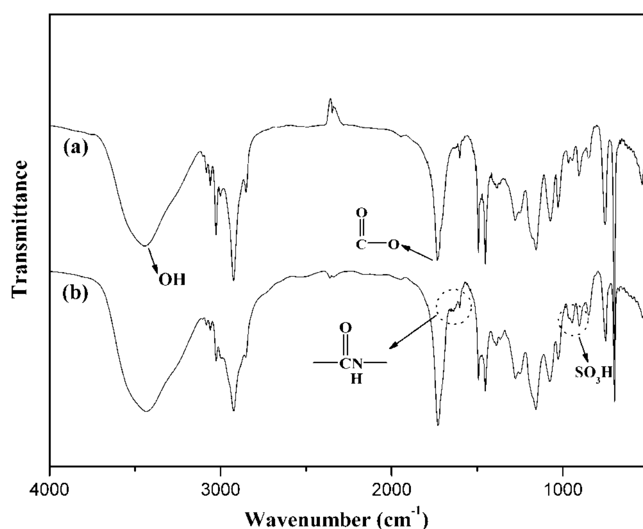


Figure 1 (a) FTIR spectra of the PHEMA and (b) PHEMA containing 10 wt % SPEG.

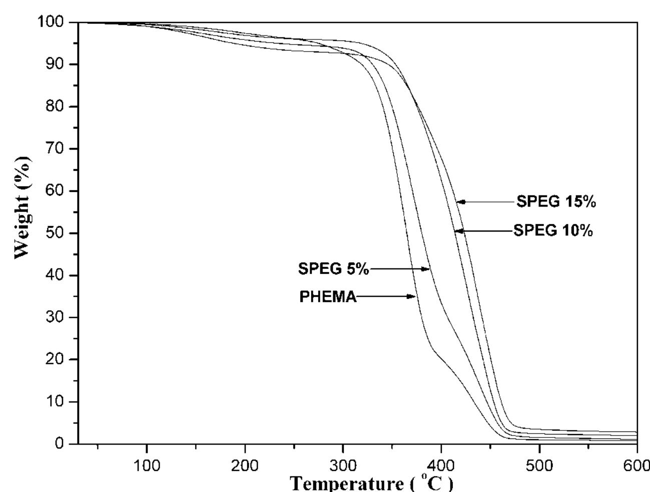


Figure 2 TGA thermograms of the SPEG-modified PHEMA gels.

RESULTS AND DISCUSSION

Preparation of hydrogel

A PEG macromer, MA-PEG-SO_3H, was introduced to the bulk HEMA gel preparation with different compositions to provide copolymeric gels containing PEG graft with sulfonate end group. MA-PEG-SO_3H was synthesized according to the procedure described previously,[16,17] and the structure was confirmed by ^{1}H-NMR analysis. All the gel samples were obtained as transparent solid sheets using an open Teflon mold. The prepared gel samples were washed by stirring the samples in distilled water for 48 h followed by freeze-drying under vacuum.

Figure 1 shows a typical FTIR spectrum of the HEMA copolymer gel (SPEG-10). The characteristic absorption band of the PEG backbone (C-O-C) occurs at 1115 cm^{-1}, along with a band at 993 cm^{-1} corresponding to -SO_3H. The band at 1643 cm^{-1} corresponds to the amide group. Figure 2 shows the TGA thermograms of different gels, which shows an improved thermal stability of the modified gels by the

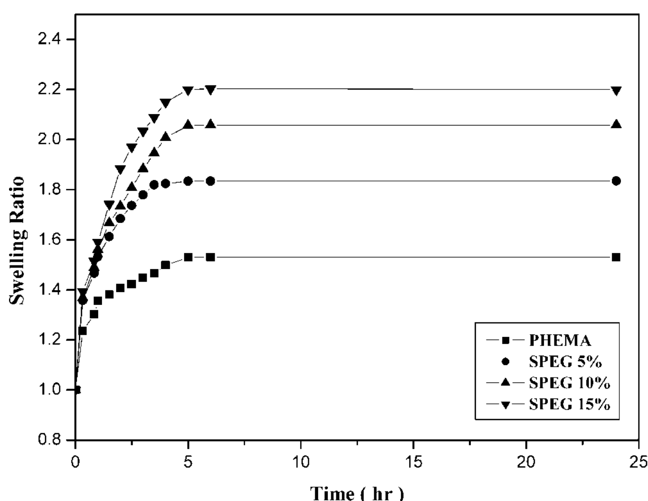


Figure 3 The swelling ratios of the PHEMA gels with a different SPEG content.

increased addition of the sulfonated PEG components into the PHEMA.

Swelling and wetting properties of SPEG-modified PHEMA hydrogel

Figure 3 shows the swelling ratios of the prepared gel in water at different SPEG contents. The swelling ratio in the gel increased with increasing SPEG content. The swelling ratio increased gradually from $\sim$ 1.5 to 2.2 as the SPEG content was increased from 0 to 15%. A more hydrophilic PEG graft was expected with additional acidic sulfonate terminal groups, which would result in a higher swelling capacity of the composite system. The swelling curve in Figure 3 shows that all the gel samples appeared to reach equilibrium in $\sim$ 5 h.

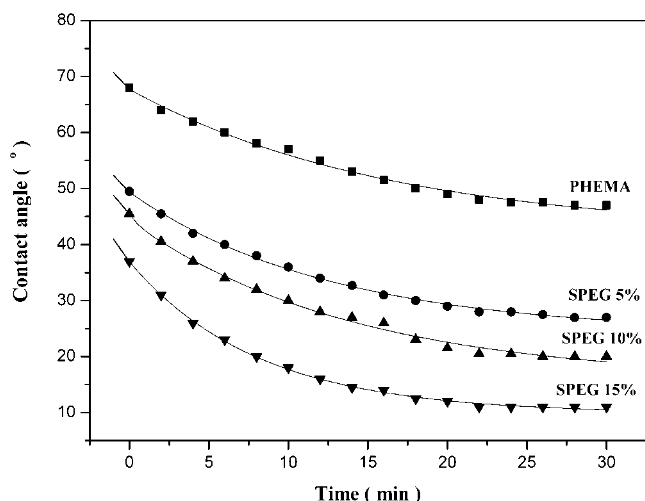


Figure 4 The dynamic contact angle measurement of the SPEG-modified PHEMA gel surface according to the elapsed wetting time.

The effect of pH on the swelling properties was checked by measuring the swelling ratio in a buffer solution at different pH. The degree of swelling was found to be higher in the alkaline solution (pH 10) than in the acidic or neutral pH, even though the differences were relatively small. As a typical example, the swelling ratio of SPEG 10% gel was 2.3 at pH 10 when compared with 2.1 at pH 7. As expected, the introduction of SPEG into the HEMA gel system altered the bulk properties of the resulting gels. On the other hand, the surface properties of the gel should also be modified to some extent by the introduction of SPEG grafts. The SPEG chains are expected to be spread over the gel surface because SPEG components are more hydrophilic than the HEMA matrix as evidenced by the swelling behavior. Thus the SPEG tethered chains will tend to locate preferentially on the surface of both bulk gel and internal pores in aqueous environment. Effects of sulfonated 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 4 shows a plot of the dynamic contact angles measured according to the elapsed wetting time. The starting contact angle was much lower in the SPEG-modified surfaces, and decreased with increasing SPEG content. This suggests that the gel surface becomes increasingly hydrophilic with increasing SPEG content. This means that the flexible PEG chains with a sulfonate end are located on the PHEMA matrix gel surface to modify its surface characteristics. This structural effect should alter the adsorption behavior of a certain protein.

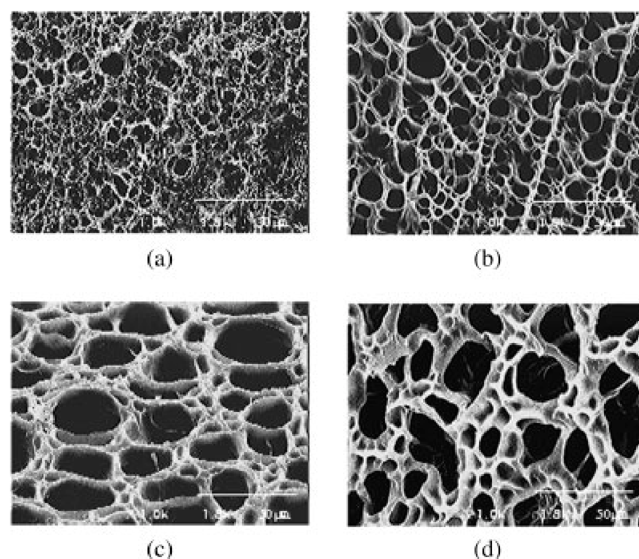


Figure 5 SEM images of freeze-dried PHEMA-SPEG hydrogel. (a) PHEMA; (b) P(HEMA-*co*-SPEG) (SPEG 5 wt %); (c) P(HEMA-*co*-SPEG) (SPEG 10 wt %); and (d) P(HEMA-*co*-SPEG) (SPEG 15 wt %).

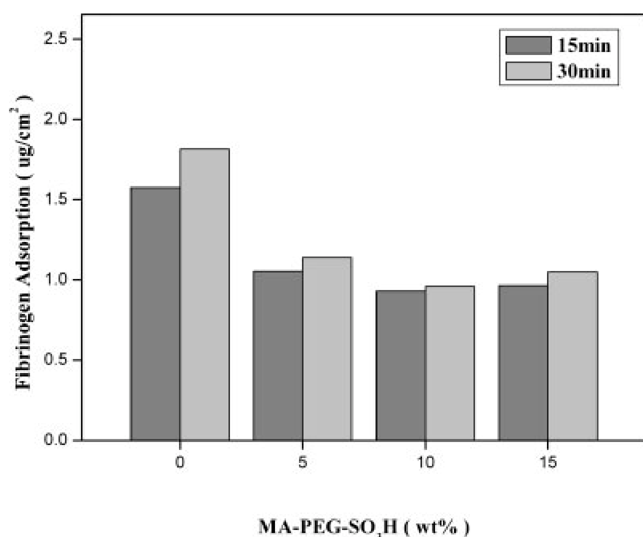


Figure 6 Fibrinogen adsorption to the SPEG-modified PHEMA gel surfaces.

Morphology of PHEMA hydrogel containing SPEG graft

A characteristic morphology, which changes with SPEG content, of the freeze-dried samples of the water-swollen hydrogel was observed by scanning electron microscopy. All the samples showed an open and well-connected cell structure, as shown in Figure 5. The homo PHEMA gel (a) has an irregular fibrous network structure with relatively small interstitial pores. The pores appear to be rather heterogeneous with several-micron sized pores along with larger pores ~ 10 μm diameter. By introducing the SPEG graft into PHEMA, the porosity of the network increased and the structure appeared to be regulated to some extent. At 5% SPEG, the overall pores grew in size to between 5 and 10 μm (the larger pores increased to ~ 15μm in size). When the SPEG content was increased to 10%, the main pores changed into a circular form and their size increased to 20–25 μm (the larger pores increased to ~ 30 μm in size). In addition, the cell wall thickened, and several small μm-sized pores began to form within the wall structures. This structural change might result from multiple interactions between the gel components including the PEG chains with an ionic sulfonate group. Even though the detailed mechanism is out of this communication, the increasing pore size with the increase of SPEG might be due to the microphase separation during the gel network formation including ionic repulsion of sulfonate groups of PEG pendant. The structure appeared to change at a SPEG concentration of 15%. Small pores originating from the wall were observed between the larger pores with concurrent thickening of the cell wall. The pores of the network did not increase in size, but appeared to shrink and change into distorted shapes with newly developed small pores (5–10 μm in diameter) in between. Therefore, the gel network structure appeared to have dual porosity. Additional studies correlating the gel morphology with the gel properties and their effect on the diffusion of different molecules within the gel matrix are currently under investigation.

Protein adsorption and cytotoxicity

Using the SPEG-modified PHEMA gel, fibrinogen (Fg) adsorption was evaluated *in vitro* by BCA protein assay.[20] The level of Fg adsorption by the SPEG-modified surfaces ranged from 45 to 60% was compared with the reference PHEMA gel (Fig. 6). However, the effect of the SPEG content on the level of adsorption

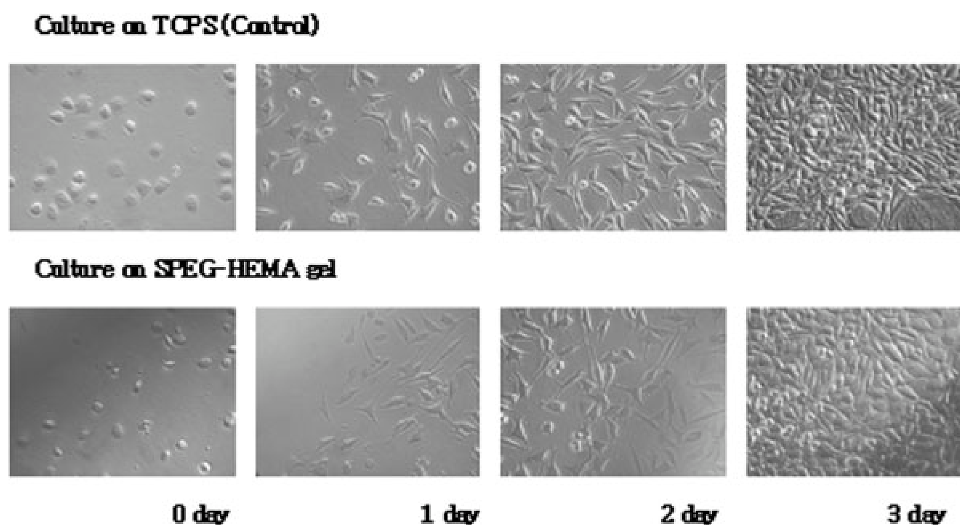


Figure 7 Micrographs of the L929 cells 12, 24, and 48 h after being plated on: (a) TCPS and (b) PHEMA-SPEG hydrogel (culture condition: 37.0°C, 5.0% CO_2, 3 mL DMEM with 10% FBS).

was relatively small. For the application as biomaterials, e.g., contact lens, the wetting property, and protein adsorption are very important for determining the biocompatibility of the related materials.[21] The preliminary results in this study showed the positive effect of the SPEG graft on the protein resistance of the material, even though this effect was not large.

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 (Fig. 7). The surfaces of the modified PHEMA hydrogel had a similar cell growth pattern to that of the TCPS control. Therefore, the SPEG-modified PHEMA hydrogels is a promising biocompatible material.

CONCLUSIONS

Novel HEMA based hydrogels modified by a sulfonated PEG graft were prepared by the crosslinking polymerization of HEMA in the presence of a methacryloyl PEG macromer with a sulfonate end group. The increasing degree of swelling and the decreasing surface contact angle highlight the increasing hydrophilic nature of the gels modified by incorporating SPEG. The fibrinogen adsorption test showed a lower level of adsorption from the SPEG-modified gel than the homo PHEMA. The SPEG-modified PHEMA gels had a regular porous network structure with pores with sizes ranging from several to tens of microns, which changed according to composition. This material might be applicable for controlled drug delivery and as a gel scaffold in tissue engineering.

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