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Preparation and Properties of PEG Modified PNVP Hydrogel

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Abstract: Polymer hydrogel has attracted considerable interest as a soft material which is finding expanding applications in pharmaceutics and various biomedical fields. In this work, modified PNVP hydrogels were synthesized by crosslinking polymerization of NVP monomer in the presence of PEG macromer with a methoxy end. The effect of the tethered PEG chain on the properties of the hydrogel was investigated in terms of its swelling capacity, compression gel strength, and the morphology of the resulting hydrogels. These PEG-modified PNVP hydrogels possessed good biocompatibility and a decreased protein (fibrinogen) adsorption, thereby indicating their potential as novel drug delivery matrices and scaffold for tissue engineering.

Keywords: polymeric hydrogel, PNVP, PEG, biocompatibility.

Introduction

Hydrogels have many desirable properties with applications in many different fields, such as biomedicine, agriculture, electrophoresis, water treatment, etc. Recently there has been increasing interest in the use of hydrogels for novel drug and cell delivery and also as tissue engineering scaffold.[1-7] Poly(*N*-vinyl pyrollidone), PNVP, are well known hydrophilic polymer, which generally possess excellent biocompatibility with living tissues and very low cytotoxicity. PNVP has been considered as a plasma substitute, a soluble drug carrier, a modifier for enzymes and a copolymer in UV-curable bioadhesives.[8] PNVP is also a very good choice for generating hydrogels, which has been used successfully as a basic material for the manufacturing of hydrogel wound dressing.[9-12] They show usually good biocompatibility and widely applied, not only as wound dressings but also as drug delivery systems. Poly(ethylene glycol), PEG, has a wide range of beneficial properties for biomedical applications, including low toxicity and non-thrombogenic.[13-16] PEG has been used to provide a non-fouling surface and stealthy characteristics 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.[17,18] Therefore it is promising to combine PNVP and PEG in graft form to produce a novel polymeric hybrid with modified bulk and surface properties.

In this study, PNVP based hydrogels modified by PEG graft were prepared by the crosslinking polymerization of vinyl pyrollidone in the presence of PEG macromer with varying amount of different chain length. We investigated the effects of tethered PEG onto gel matrix, looking at the swelling properties, gel strength, morphology, and protein adsorption.

Experimental

Chemicals and Measurements. Poly(ethylene glycol) methyl ether methacrylate (MPEG, Aldrich) of three different molecular weight (M_n = 475, 1,100, and 2,080) were vacuum dried for 1 week prior to use. 1-Vinyl-2-pyrollidone (NVP, Aldrich, 99%) was passed through an alumina column to remove the polymerization inhibitor prior to use. Ethylene glycol dimethacrylate (EGDMA, 98%, Aldrich) was used as crosslinking reagent. Ammonium peroxodisulfate (APS, Aldrich, 99%) was used without further purification. *N,N,N',N'*-tetramethyl ethylene diamine (TEMED, 99%, Aldrich) was used as an activator of APS. Double distilled water was

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used as the reaction medium.

The IR spectra were obtained using a Perkin Elmer FT-IR spectrometer (Model SPECTRUM 2000). Morphology of the dried gel scaffolds was observed by scanning electron microscopy (ESEM Model XL30 ESEM-FEG, Phillips Co.). 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). Gel strength was measured using Universal Testing Machine (Lloyd, England) at 0.5 mm/min crosshead speed. For each test, five times of measurements were obtained on different hydrogel specimens and the average taken.

Gel Preparation. Radical Copolymerization of NVP and MPEG in the Presence of EGDMA: PNVP and the copolymer hydrogels were prepared either on a silicone mold or in a small reaction sample. Typically, a predetermined amount of MPEG (0, 5, 10, 15 wt% of NVP) and NVP were added to the flame-dried vial and stirred to obtain a clear solution. EGDMA as the crosslinking reagent 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 in deionized water and TEMED were 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 resulting gel blocks were washed in distilled water for two days, 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:

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

Cytotoxicity. 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 (bovine) 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.

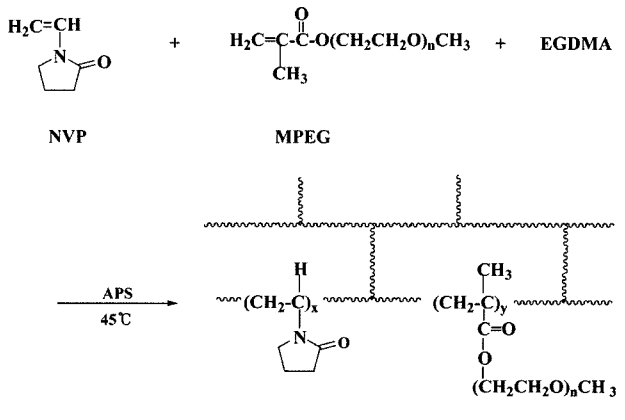
Protein Adsorption. The level of fibrinogen (Fg) adsorption was evaluated using an ELISA assay described elsewhere.[16] Prior to the adsorption experiments, gel pieces, 1 cm^2 in size, were equilibrated in water at 37 °C for 12 h. The adsorption was then carried out by gently shaking a fibrinogen (0.02 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 non-adsorbed 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.

Results and Discussion

Preparation and Swelling Property of PEG Modified PNVP Hydrogels. Methacryloyl PEG macromer of different molecular weight was introduced to the bulk PNVP gel preparation with different compositions to provide copolymeric gels containing tethered PEG chain as the reaction was shown in Scheme I. All the gel samples were obtained as transparent solid blocks using an open Teflon mold. Figure 1 shows FT-IR spectra of the PNVP and the copolymer gels with MPEG (MW 1,000). The characteristic absorption band of the PEG backbone (C-O-C) occurs at 1105 cm^{-1}, with increasing intensity as the PEG content. The bands at 1750 and 1652 cm^{-1} corresponds to both the amide of pyrollidone and ester group of EGDMA moiety.

Swelling curves in Figure 2 show that the gel samples absorb water very quickly and reach equilibrium swelling in a few minutes. Figure 3 shows the plot of equilibrium swelling ratios of the prepared gels in water as a function of content for PEG of MW 1,000. The swelling ratio was observed in the range of ca. 5-6, which decreased gradually as the PEG content increased from 0 to 15%. The PEG is also a hydrophilic component, but it seems to act adversely to the overall swelling in this particular system. Nevertheless, the

Scheme I



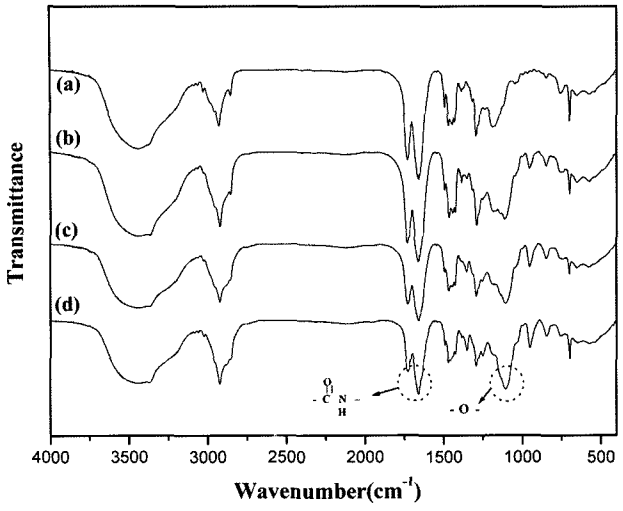


Figure 1. FT-IR spectra of (a) PNVP, (b) P(NVP-MPEG 5 wt%), (c) P(NVP-MPEG 10 wt%), and (d) P(NVP-MPEG 15 wt%) hydrogels.

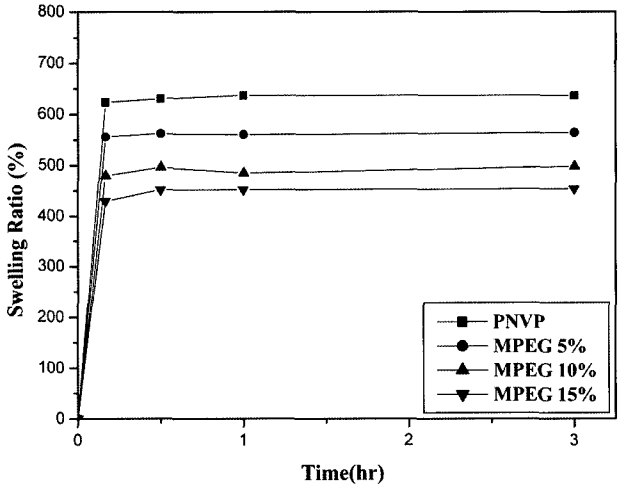


Figure 2. Swelling curves of P(NVP-MPEG) gel (EGDMA 5 wt%).

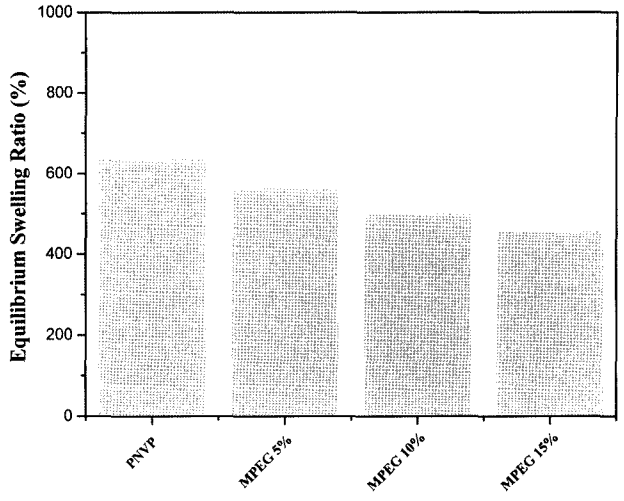


Figure 3. Swelling dependence on PEG content in PEG modified PNVP gel (EGDMA 5 wt%).

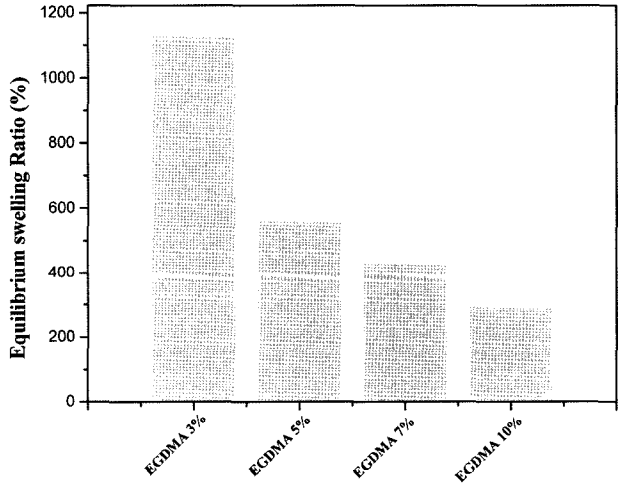


Figure 4. Swelling degrees as a function of EGDMA content in PEG modified PNVP gel (MPEG 5 wt%).

effect to the extent of swelling was found to be dependent on the chain length of PEG. The amount of crosslinking reagent, of course, altered the swelling capacity very much as the results are shown below. Figure 4 shows the swelling change as a function of EGDMA content for 5 wt% MPEG (MW 1,000) copolymer gel. The swelling degree decreased significantly by increasing EGDMA content from 3 to 10 wt%, which is simply expected from the increasing crosslinking density of the gel network. Again, the effect of PEG content on the swelling were different depending on the length (or molecular weight) of PEG chain used. Figure 5 shows the results from PNVP-PEG copolymer gel system containing 5 wt% EGDMA content. Each of 5 and 10 wt% PEG of different chain length are introduced and the equilibrium swelling degrees are represented. As the PEG MW increased, the swelling increased linearly. In case of the PEG MW of 500 and 1,000, the swelling degrees were lower than homo PNVP gel, and the higher the PEG content the lower the swelling ratio, as the same trend in result was discussed previously. Interestingly, however, the result came out oppositely for PEG of MW 2,000. The swelling degree increased to the level of homo PNVP gel at 5 wt% PEG, and increased more when the content was raised to 10 wt% in this system which is in contrast to other PEGs of lower MW. Probably PEG chain of MW 2,000 provides large enough free volume to raise the overall hydrophilicity of composite gel system. Morphology change of these composite hydrogels by introducing PEG tether seems to be responsible for these changes in this swelling behavior,

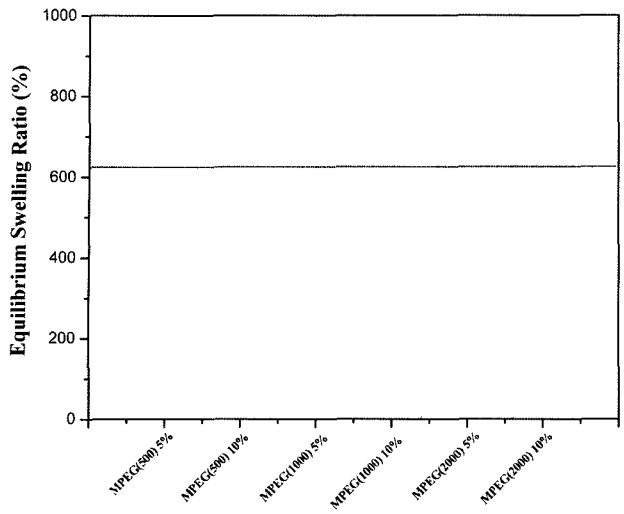


Figure 5. Swelling of PEG modified PNVP gel with different PEG chain length (EGDMA 5 wt%).

however, the detailed discussion is out of the scope of this report.

Morphology of PNVP Hydrogel Containing PEG Graft. A characteristic morphology, which changes by content of PEG (MW 1000) graft, of the freeze-dried samples of water-swollen hydrogel was observed by scanning electron microscopy as shown in Figure 6. Bulk PNVP gel (a) was transparent and possessed no particular texture without pores discernible. By introducing the PEG graft into PNVP, a phase-separated two-phase structure was developed and the cell size seemed to be regulated as the PEG content gradually increased up to 10 wt%. Basically nonporous homo PNVP turned to microphase-separated structure, where the cell size appeared rather regular in the range from tens of micron to a few micron size by changing the content of PEG graft. The mechanism to explain the formation of this morphology and the related studies on their effect to the physicochemical properties of the resulting gels are interesting and currently under investigation.

Mechanical Strength of PNVP Hydrogel Containing PEG Graft. Figure 7 shows the gel strength data obtained for PEG modified PNVP hydrogels. As shown in the Figure 7(a), the compression strength at yield of swollen gel increased significantly when PEG (MW 1,000) graft has been

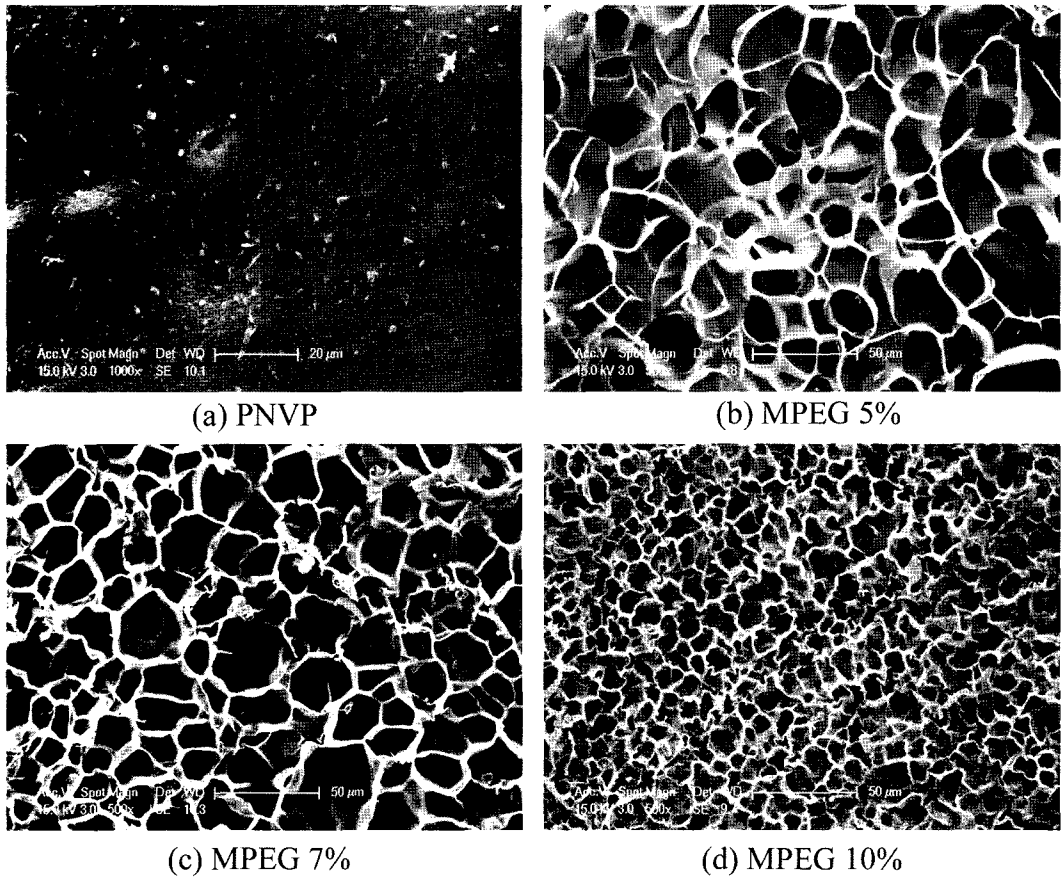


Figure 6. SEM images of freeze-dried gel. (a) PNVP, (b) P(NVP-MPEG(1000) 5 wt%), (c) P(NVP-MPEG(1000) 7 wt%), and (d) P(NVP- MPEG(1000)10 wt%).

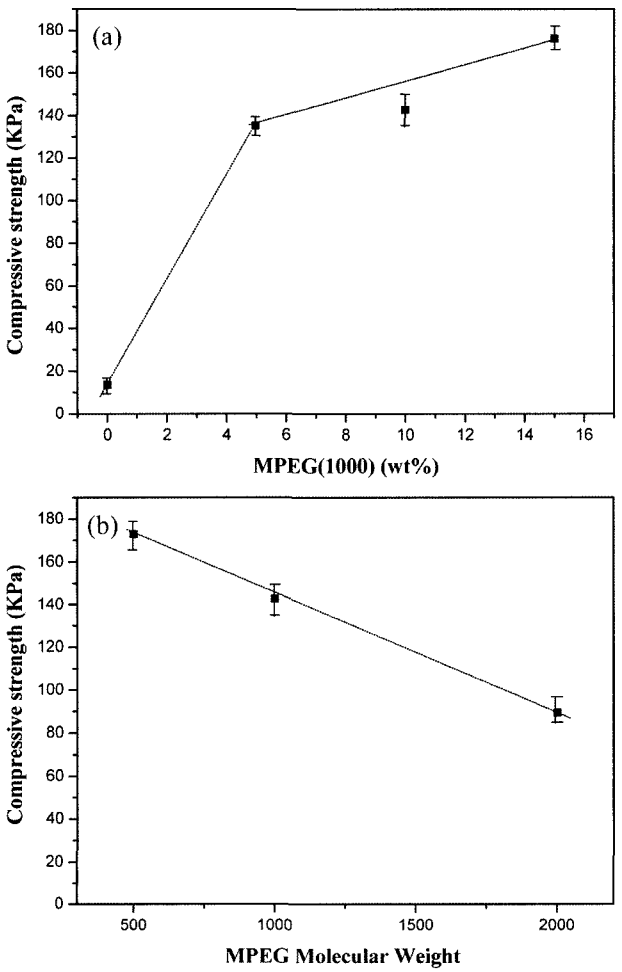


Figure 7. Mechanical strength of PEG modified PNVP hydrogels.

introduced. By introducing 5 wt% MPEG, the compression strength increased over 10 times compared to homo PNVP gel. At higher contents of 10 or 15 wt%, the increase was marginal. Flexible and mobile PEG chains imbedded in the

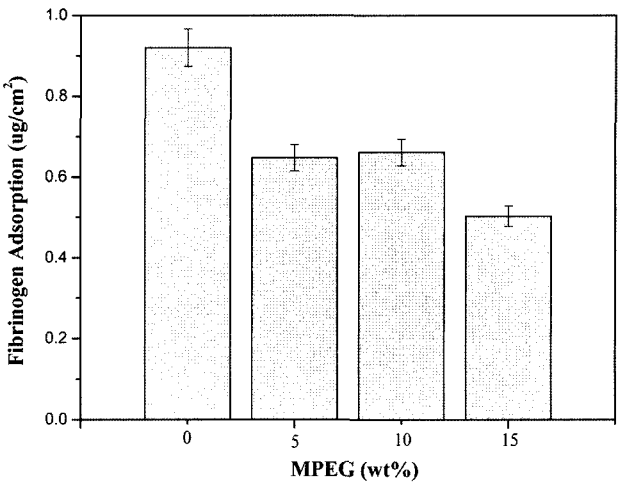


Figure 9. Fibrinogen adsorption on the PEG modified PNVP hydrogel surfaces.

polymer network and regularly phase-segregated morphology might be responsible for this enhanced gel strength. Figure 7(b) shows the same data for three different chain length of MPEG (10 wt% content). The compression strength value were higher at lower molecular weight MPEG, decreasing linearly with molecular weight. The shorter but more dense PEG chains appear to be more efficient to support compression load.

Biocompatibility of PEG-Modified PNVP Hydrogels. As above discussed, the introduction of PEG into the PNVP gel system altered the bulk hydrogel properties including physical properties. On the other hand, the tethered PEG may play a very important role in determining the biocompatibility of the given materials because the surface and interfacial properties of the biomaterials are another factor for their performance. The L929 fibroblasts were 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

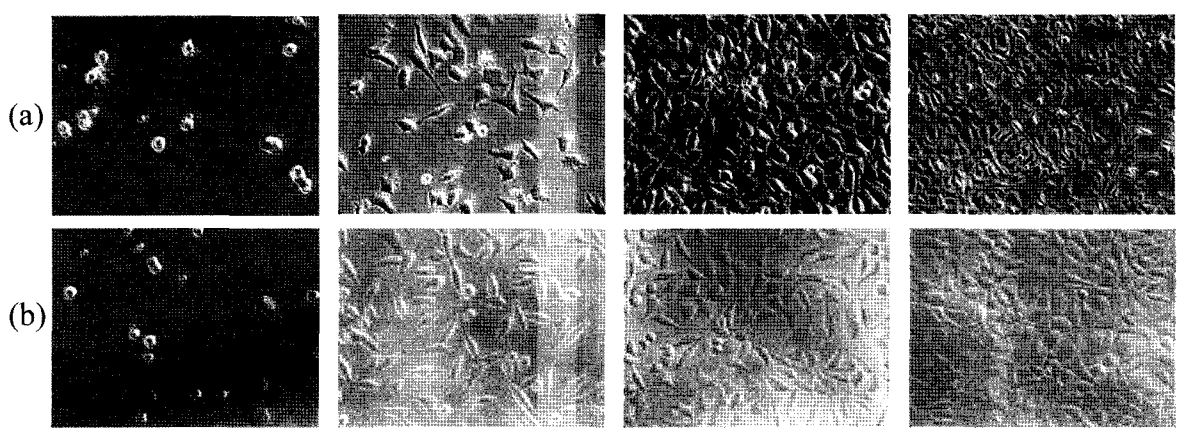


Figure 8. Microscopy photographs of the L929 cells after they were plated on (a) TCPS and (b) PNVP-MPEG(1000) (MPEG 10 wt%).

fibroblast were cultured for 3 days and the surface morphology was observed by optical microscopy (Figure 8). The surfaces of the modified PNVP hydrogel had a similar cell growth pattern to that of the TCPS control. Thereby, the PEG modified PNVP hydrogel is considered to be biocompatible material.

Using the PEG (MW 1,100) modified PNVP gel, fibrinogen (Fg) adsorption was evaluated *in vitro* by BCA protein assay.[19] The level of Fg adsorption by the PEG modified surfaces ranged from ca. 55 to 68% compared with the reference PNVP gel (Figure 9). However, the effect of the PEG content on the level of adsorption was rather 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.[20] Preliminary results in this study showed the positive effect of the PEG graft on the protein resistance of the material.

Conclusions

Novel PNVP based hydrogels modified by a PEG graft were prepared by the crosslinking polymerization of NVP in the presence of a methacryloyl PEG macromer with different chain length. The degree of swelling changed depending on the PEG content and the molecular size as well as the amount of crosslinking reagent, EGDMA. The PEG modified PNVP gels exhibited a phase-separated, regular cell structure with the cell sizes ranging from submicron to tens of microns. The compression strength of hydrogel has increased significantly by the introduction of PEG, depending on both content and chain length of PEG. From the cytotoxicity test, the modified gel was found to be non-toxic and biocompatible.

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