Swelling Behavior and Cell Viability of Dehydrothermally Crosslinked Poly(vinyl alcohol) Hydrogel Grafted with *N*-Vinyl Pyrrolidone or Acrylic Acid Using γ-Radiation

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ABSTRACT: To improve equilibrium water content, dehydrothermally crosslinked poly(vinyl alcohol) (PVA) hydrogel was grafted with *N*-vinyl pyrrolidone (NVP) or acrylic acid (AA) monomer using γ-radiation. Swelling behavior of the grafted hydrogels was studied in phosphate-buffered saline, and cell viability was evaluated using fibroblast cells from mouse connective tissue. Equilibrium water content of AA- and NVP-grafted PVA hydrogel ranged between 40–60% and 60–80%, respectively, depending on radiation dose and monomer concentration. For maximum degree of swelling, the optimum monomer concentration and radiation dose were 20% by weight and 20 kGy, respectively. Fibroblast cells seeded on NVP-grafted hydrogel had an extended oval morphology while those seeded on AA-grafted PVA had a rounded spherical morphology. These results support the use of NVP for grafting PVA to increase swelling and improve cell viability. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 91: 2862–2868, 2004

Key words: hydrogels; poly(vinyl alcohol); networks; graft copolymers; radiation; swelling

INTRODUCTION

Hydrogels are three-dimensional crosslinked hydrophilic polymeric structures which are able to swell in the aqueous environment.[1] Due to their high water content, low water contact angle, high permeability, and low friction coefficient, hydrogels are studied extensively as a replacement for soft tissues.[2] Poly(vinyl alcohol) (PVA), due to its low friction coefficient and excellent mechanical properties in the swollen state,[3,4] is investigated as a replacement for degenerated articular cartilage. Results of previous studies indicate that the friction coefficient of PVA hydrogel significantly depends on the water content of the hydrogel;[5–9] as the water content increased, a significant decrease in friction coefficient was measured.

PVA hydrogels with high tensile strength and high tear resistance can be prepared by dehydrothermal treatment in which the fully hydrolyzed PVA chains are annealed above the glass transition temperature (T_g) to induce crystallization. The formation of crystallites act as permanent physical junctions to form a three-dimensional hydrogel network.[10] However, maximum equilibrium water content of these gels is near 40%, which is significantly lower than that of natural articular cartilage, which is approximately 65–80%. We hypothesized that the equilibrium water content of dehydrothermally crosslinked PVA could be improved by grafting a super-hydrophilic monomer such as *N*-vinyl pyrrolidone (NVP) or acrylic acid (AA) to PVA while retaining mechanical properties. PVA is miscible with NVP and AA over the entire composition range due to hydrogen bond formation.[11–13]

PVA hydrogel is grafted with a variety of monomers in order to improve physical, mechanical, or biological properties.[14] For example, PVA is grafted with acrylic acid,[15] methyl methacrylate,[16] or poly(organophosphazenes)[17] to improve or modify surface properties and to balance hydrophilicity. Similarly, it is grafted with *N*-isopropyl acrylamide[18] or 2-hydroxy ethyl methacrylate[19] to modify permeability, wettability, and water content. Alternatively, it is grafted with poly(ethylene glycol)[20] to reduce protein adsorption or cell deposition or grafted with poly(lactide-*co*-glycolide)[21] to adjust degradation rate.

The objective of this research was to graft NVP or AA to dehydrothermally crosslinked PVA hydrogel in order to increase equilibrium water content and retain mechanical properties and biocompatibility of PVA. High-energy γ-radiation was chosen for grafting because this technique does not require the use of chemical agents, which could adversely affect cell viability. The effect of grafting on equilibrium degree of swelling and cell viability was investigated.

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MECHANISM OF γ-RADIATION GRAFTING

The mechanism of grafting of polymers in aqueous solution by γ-radiation is reviewed by Peppas.[1] We have previously investigated γ-radiation crosslinking of poly(acrylic acid) in aqueous solution as a function of concentration and radiation dose.[22] The mechanism is discussed here briefly for γ-radiation grafting of PVA hydrogel. The aqueous solution contains semicrystalline PVA hydrogel (PVAH), a water solvent (HOH), and acrylic acid (AAH) or *N*-vinyl pyrrolidone (NVPH) monomer. The PVA chains, monomer, and solvent molecules absorb γ-radiation and go to a transient state in which a covalent bond is dissociated causing the formation of free radicals $PVA^{\bullet}$, $AA^{\bullet}$ or $NVP^{\bullet}$, $H^{\bullet}$, and $OH^{\bullet}$. The most important reactions in the absence of oxygen are as follows:

$$PVA^{\bullet} + PVA^{\bullet} \rightarrow PVA\text{-}PVA \quad (1)$$

$$PVA^{\bullet} + HOH \rightarrow PVAH + HO^{\bullet} \quad (2)$$

$$PVA^{\bullet} + {}^{n}AAH \text{ or } {}^{n}NVPH \rightarrow PVA\text{-}g\text{-}AA_n^{\bullet} \text{ or } PVA\text{-}g\text{-}NVP_n \quad (3)$$

$$PVAH + (H^{\bullet} \text{ or } HO^{\bullet} \text{ or } AA^{\bullet} \text{ or } NVP^{\bullet}) \rightarrow PVA^{\bullet} + (HOH \text{ or } AAH \text{ or } NVPH) \quad (4)$$

$$({}^{n}AA \text{ or } {}^{n}NVP) + (H^{\bullet} \text{ or } HO^{\bullet} \text{ or } AA^{\bullet} \text{ or } NVP^{\bullet}) \rightarrow PAA^{\bullet}{}_n \text{ or } PNVP^{\bullet}{}_n \quad (5)$$

$$(PAA^{\bullet}{}_n + PAA^{\bullet}{}_m) \text{ or } (PNVP^{\bullet}{}_n + PNVP^{\bullet}{}_m) \rightarrow (PAA_n + PAA_m) \text{ or } (PNVP_n + PNVP_m) \quad (6)$$

$$(PAA^{\bullet}{}_n \text{ or } PNVP^{\bullet}{}_n) + (HOH \text{ or } AA \text{ or } NVP) \rightarrow (PAA_n \text{ or } PNVP_n) + (H^{\bullet} \text{ or } HO^{\bullet} \text{ or } AA^{\bullet} \text{ or } NVP) \quad (7)$$

Reaction (1) causes additional crosslinks in the form of covalent bonds to form in the PVA hydrogel. In reaction (2), the PVA radical abstracts a hydrogen from the water molecule and transfers a radical from the PVA chain to the hydroxyl group, which reduces the rate of grafting. In reaction (3), a radical on a PVA chain reacts with a polymer radical with *n* AA or NVP monomer units, which forms an AA or NVP graft with *n* units (PVA-*g*-AA_n• or PVA-*g*-NVP_n•). In reaction (4), a radical is transferred from hydrogen, hydroxyl, AA, or NVP radical to a PVA chain, which increases the rate of grafting. In reaction (5), *n* AA or NVP monomer units react with hydrogen, hydroxyl, AA, or NVP radicals to form PAA or PNVP homopolymer radical with *n* repeat units. In reaction (6), two PAA or PNVP homopolymer radicals with *n* and *m* repeat units, respectively, react to form two terminated PAA and PNVP homopolymer chains with *n* and *m* repeat units, respectively. In reaction (7), the PAA or PNVP radicals terminate by chain transfer reaction with water or monomer molecules. The hompolymerization reaction (5) in the aqueous phase limits the extent of grafting. As the monomer concentration is increased, the rate of grafting as well as the rate of homopolymerization is increased. Also, as the radiation dose is increased, more radicals are formed which favor the grafting as well as homopolymerization reactions.

EXPERIMENTAL

Materials

AA and NVP monomers were obtained from Sigma-Aldrich (Munich, Germany). Acrylic acid monomer was distilled at 50°C under a reduced pressure of 5 mmHg to remove hydroquinone mono-methyl ether inhibitor and stored at −20°C. NVP monomer with purity of greater than 99% was used as received. Fully hydrolyzed PVA with degree of hydrolysis greater than 99.4% (Mowiol 36-99) was obtained from Hoechst (Frankfurt, Germany). The viscosity of the 4% by weight PVA in distilled deionized (DD) water at 20°C was 36 mPa s.

Preparation of PVA hydrogel

The following procedure was used to prepare the hydrogel. A 10% w/w aqueous solution of fully hydrolyzed PVA was prepared by dissolving PVA in DD boiling water. Varying amounts of glycerol, ranging from 10% to 40% by weight of PVA, were added to the aqueous solution to act as a plasticizer in the annealing stage. The solution was placed in an oven, and the temperature was increased gradually from 25 to 100°C to slowly evaporate the water and obtain homogeneous film. The dried film was annealed at 120°C for 1 h at a reduced pressure of 30 mmHg to induce crystallization. The annealed film was allowed to swell in DD water for at least 48 h at ambient conditions.

γ-radiation grafting

Different amounts of AA or NVP monomer, ranging from 5% to 30% by weight based on PVA, was added to the equilibrium swollen PVA in a Petri dish, purged with nitrogen, sealed with plastic wrap, covered, and exposed to γ-radiation. Each solution was exposed to four different doses of radiation including 6.5, 10, 20, and 25 kGy. The source of γ-radiation was ^{60}Co, and the dose rate was 1.39 Gy/s such that the irradiation time ranged from 1 to 5 h. Gamma cell calibration was performed with the exposure time of the system based

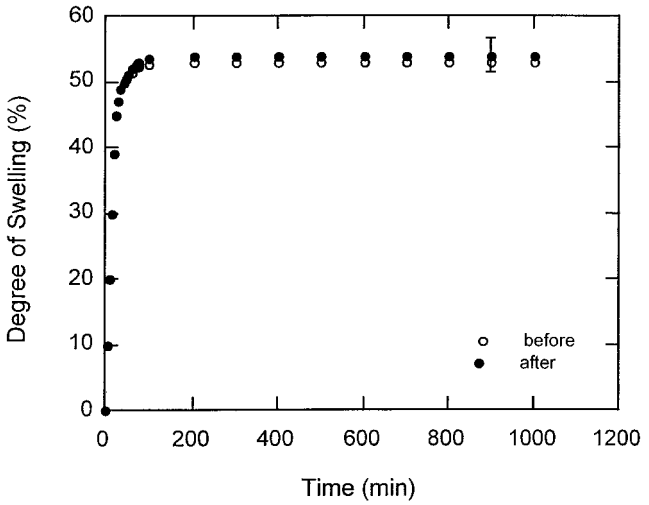

Figure 1 Degree of swelling of dehydrothermally crosslinked PVA hydrogel as a function of time in PBS at 25°C before and after irradiation with 25 kGy of γ-radiation.

on dosimetry using a Frick chemical dosimeter. It should be noted that the radiation doses used were similar to the range employed in typical γ-sterilization. After irradiation, the sample was washed twice with DD water to remove the unreacted monomer. The degree of grafting, α, was determined by:

$$\alpha = (W_{ag,d} - W_{bg,d})/W_{bg,d} \times 100 \quad (8)$$

where $W_{ag,d}$ and $W_{bg,d}$ were the weight of dry sample after and before grafting with γ-radiation, respectively. The degree of swelling, S, was determined by:

$$S = (W_s - W_d)/W_s \times 100 \quad (9)$$

where W_s and W_d were the weight of swollen and dry sample, respectively.

Cell viability studies

Cell viability was determined using the ASTM-F813 standard procedure.[23] The AA- or NVP-grafted PVA hydrogel sample was sterilized by UV radiation and washing with 70% ethanol solution. Serum-free primary culture media was prepared by dissolving the following into 1 liter of DD water: 13.4 g of Dulbecco's Modified Eagle Medium (DMEM), 3.7 g of sodium bicarbonate, and 10 mL of antibiotic and antimycotic solution containing penicillin G, streptomycin, and Fungizone in PBS. The pH of the media was adjusted to 7.2 by adding 1*N* HCl. The primary media was sterilized by filtration using a sterile 0.2-μm cellulose acetate membrane filter unit and stored at 4°C before use. Fibroblast cells from mouse connective tissue were obtained from the cell banks of Iran Pasteur Institute (Tehran, Iran). The cells were expanded in sterile tissue culture plates by seeding and culturing the initial cell suspension in primary media for 7 days. The fibroblast cells, at a concentration of 1.3×10^5 cells/mL, were added to each Petri dish containing the PVA hydrogel and placed in a 5% CO_2 incubator for 24 h. After cell culture, the sample was washed with PBS and the remaining cells on the hydrogel surface were fixed with gluteraldehyde and stained with trepan blue for microscopic observation.

Statistical analysis

Results are reported as means ± standard deviations. Statistical significance was determined using a paired *t*-test. Statistical significance was assumed for $P < 0.05$.

RESULTS AND DISCUSSION

γ-Irradiation with a dose of 25 kGy did not have an appreciable effect on the swelling behavior of dehydrothermally crosslinked PVA hydrogel (Figure 1). Therefore, in the absence of AA or NVP monomer, radiation did not have a significant effect on crosslinking or degradation of the PVA gel, implying that the rate of reaction (1) was lower than the other reactions. Figure 2 shows the effect of radiation dose on the degree of grafting of AA to PVA hydrogel for AA concentrations ranging from 10% to 30%. In general, the degree of grafting increased with increasing radiation dose. When the radiation dose was increased from 20 to 25 kGy, there was a statistically significant increase in the degree of grafting for all concentrations of AA. The same trend was also observed for the

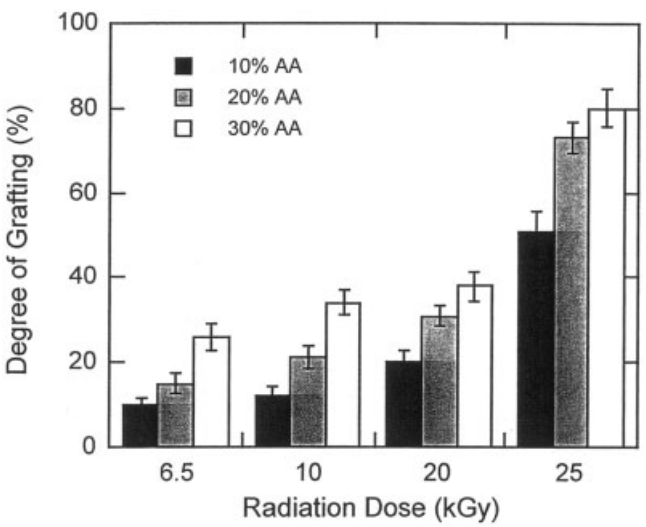

Figure 2 Effect of radiation dose on the degree of grafting of AA-grafted PVA hydrogel for AA concentrations of 10%, 20%, and 30% by weight based on PVA.

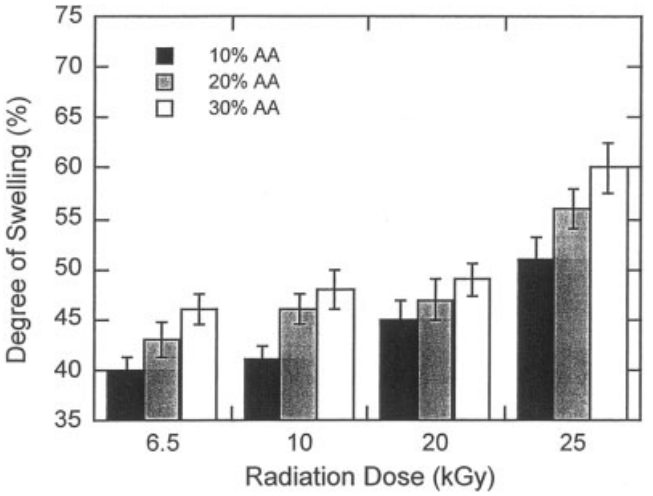

Figure 3 Effect of radiation dose on the degree of swelling in PBS of AA-grafted PVA hydrogel for AA concentrations of 10%, 20%, and 30% by weight based on PVA.

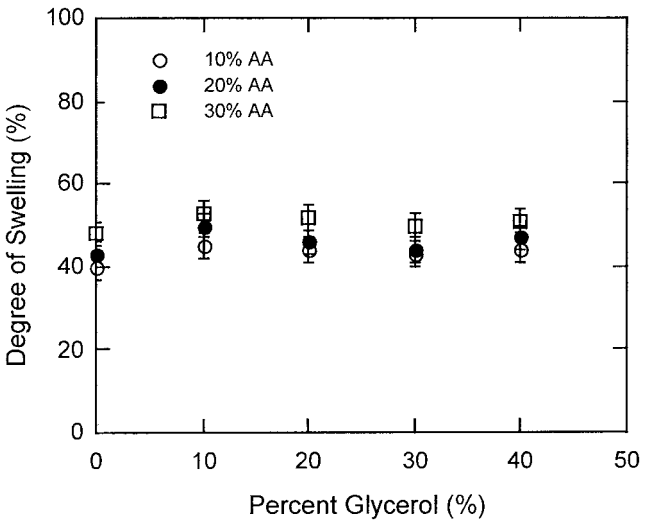

Figure 4 Effect of glycerol concentration on the degree of swelling in PBS of AA-grafted PVA hydrogel with irradiation dose of 20 kGy. AA concentrations include 10%, 20%, and 30% by weight based on PVA.

degree of swelling in PBS as a function of radiation dose and AA concentration (Figure 3). For example, for 10% AA concentration, as the radiation dose was increased from 20 to 25 kGy, the degree of swelling increased significantly from 45% ± 2% to 51% ± 2%; for 20% AA concentration, it increased from 47% ± 3% to 56% ± 2%; and for 30% AA concentration, it increased from 49% ± 2% to 60% ± 3%. For a given dose of radiation, the degree of swelling did not change significantly as the AA concentration was increased, as shown in Figure 3. For example, for 10%, 20%, and 30% AA concentrations at a radiation dose of 20 kGy, the degree of swelling was 45% ± 3%, 47% ± 3%, and 49% ± 3%, respectively. For samples with 40% or higher AA concentrations, the aqueous solution gelled after γ-radiation. Therefore, in order to minimize the extent of homopolymerization and have maximum degree of swelling, radiation dose of 20 kGy was used for later experiments.

Previous studies indicate that the addition of low molecular weight polyols such as glycerol to PVA, as a plasticizer, affects the degree of crystallinity and size of crystallites when the sample is annealed at elevated temperatures.[24,25] Figure 4 shows the effect of addition of glycerol to PVA in the annealing stage on the degree of swelling for different concentrations of AA grafted at 20 kGy of γ-radiation. According to this figure, the concentration of glycerol did not have a statistically significant effect on the degree of swelling of grafted PVA for all concentrations of AA. For example, for 30% AA concentration, as the glycerol content was increased from zero to 10%, 20%, 30%, and 40%, the degree of swelling changed from 48% ± 2% to 53% ± 2%, 53% ± 2%, 50% ± 2%, and 51% ± 2%, respectively. Also, at a given concentration of glycerol, a modest increase in swelling was observed when the AA concentration was increased. For example, for 20% glycerol concentration, the degree of swelling changed from 43% ± 3% to 46% ± 3% and 51% ± 3% as the AA concentration was increased from 10% to 20% and 30%, respectively.

Figure 5 shows the effect of glycerol concentration on the degree of swelling of PVA for different concentrations of NVP ranging from 10% to 30% grafted with 20 kGy of γ-radiation. In general, glycerol concentration had a greater effect on swelling of NVP-grafted PVA than that of AA. Moreover, there was a statistically significant decrease in the degree of swelling

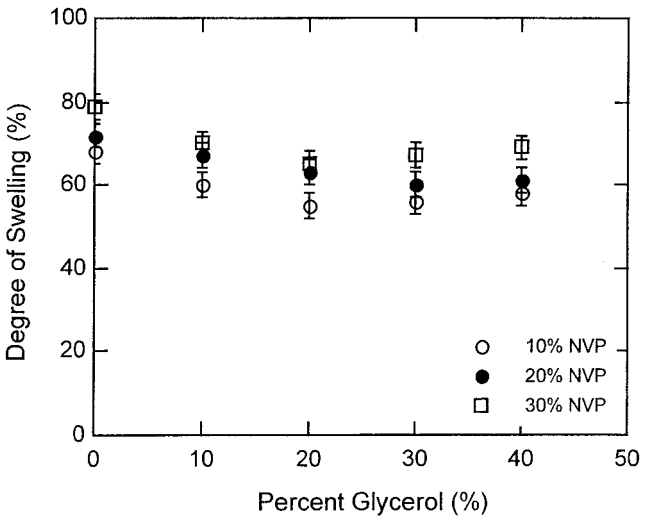

Figure 5 Effect of glycerol concentration on the degree of swelling in PBS of NVP-grafted PVA hydrogel with irradiation dose of 20 kGy. NVP concentrations include 10%, 20%, and 30% by weight based on PVA.

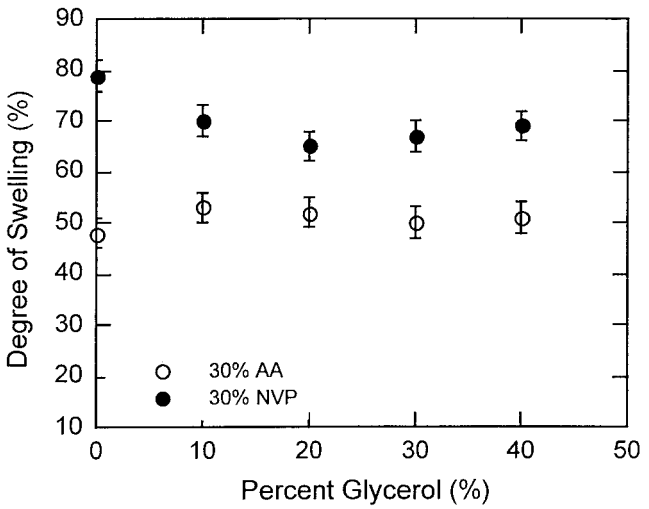

Figure 6 Degree of swelling in PBS of 30% AA and 30% NVP-grafted PVA hydrogel with irradiation dose of 20 kGy as a function of glycerol concentration.

between no glycerol and 10–40% glycerol content. For example, for 30% NVP concentration, as the glycerol concentration was increased from 0 to 10%, 20%, 30%, and 40%, the degree of swelling varied from 79% ± 3.4% to 70% ± 3.0%, 65% ± 2.6%, 67% ± 3.1%, and 69% ± 3.0%, respectively. This can be explained by an increase in the crystallinity of PVA with glycerol addition, which reduced the degree of swelling. At a given concentration of glycerol, a modest increase in swelling was observed when NVP concentration was increased. For example, for 10% glycerol concentration, the degree of swelling changed from 60% ± 3% to 67% ± 4% and 70% ± 4% as the NVP concentration was increased from 10% to 20% and 30%, respectively. Scanning electron microscopy (SEM) of the freeze-dried NVP-grafted PVA with 40% glycerol showed a homogeneous film with no porosity. This can be attributed to complete miscibility of PVA and glycerol for all compositions.

Figure 6 compares the degree of swelling of 30% NVP- and 30% AA-grafted PVA as a function of glycerol concentration. Statistically, there was a significant difference between the degree of swelling of NVP grafted PVA hydrogel which varied between 79% ± 4% and 60% ± 3% compared to that of AA which varied between 53% ± 2% and 43% ± 2%. The lower degree of swelling of AA-grafted PVA hydrogel can be explained by shielding of carboxylate ions of AA by the mobile ions of PBS.[26] Also, dissociation of carboxylic acid groups of AA, with pK_a of 4.5, to carboxylate anions and hydrogen cations in PBS significantly reduces hydrogen bonding ability of AA with PVA.[27] On the other hand, NVP is a neutral monomer with strong interaction with PVA by hydrogen bonding and its swelling behavior is independent of ionic strength of the medium. Based on the above results, for maximum degree of swelling, the optimum concentration of monomer, dose of radiation, and glycerol concentration for NVP-

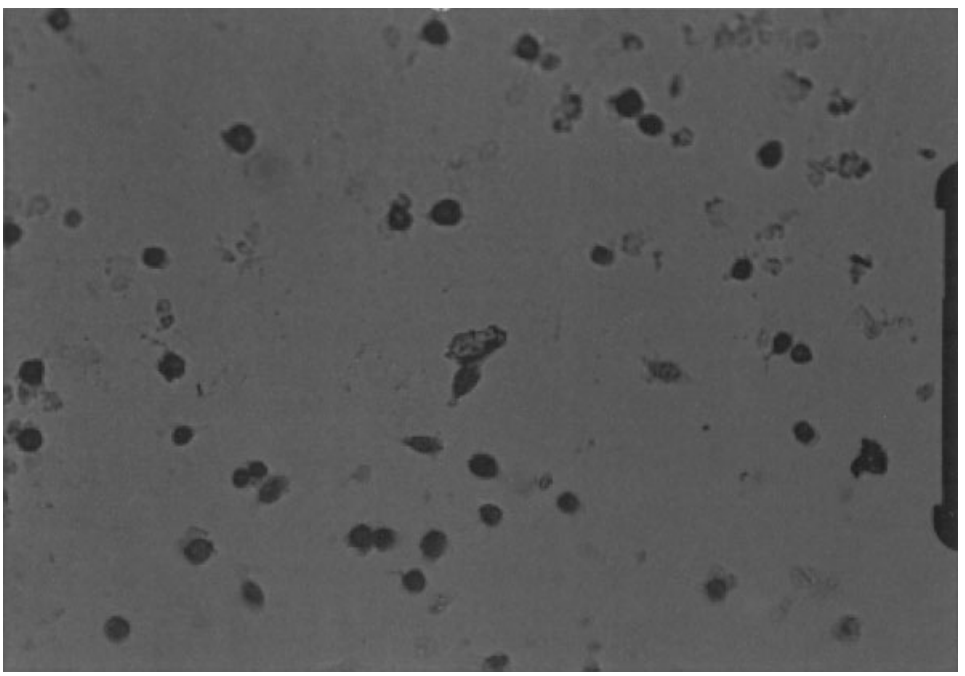

Figure 7 Microscopic image of fibroblast cells on the surface of NVP-grafted PVA hydrogel at 100× magnification after incubation for 24 h, washing with PBS, fixing with gluteraldehyde, and staining with trepan blue. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

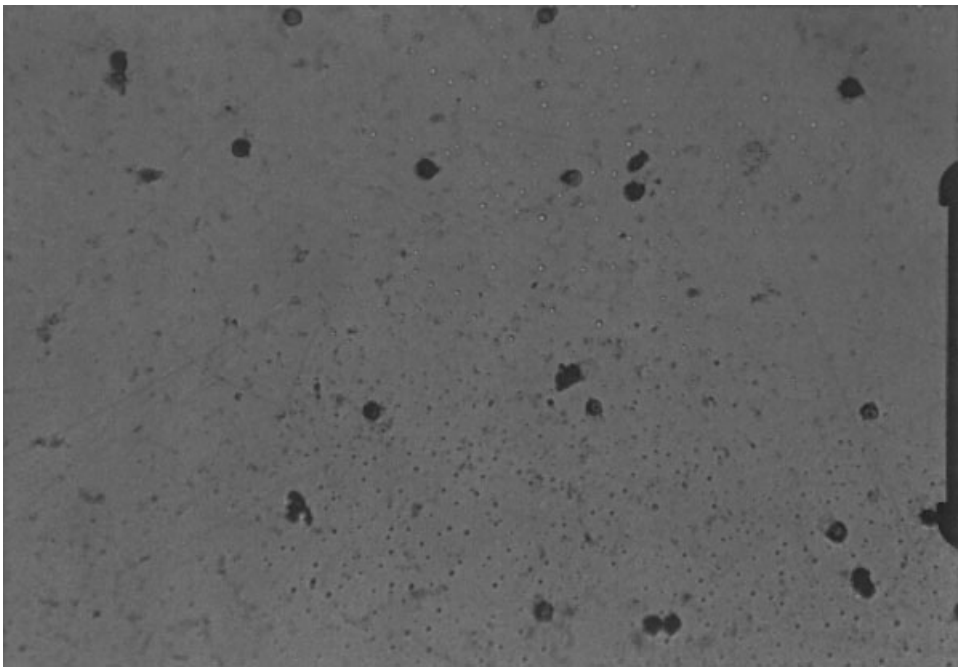

Figure 8 Microscope image of fibroblast cells on the surface of AA-grafted PVA hydrogel at 100× magnification after incubation for 24 h, washing with PBS, fixing with gluteraldehyde, and staining with trepan blue. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

grafted PVA hydrogel were 20% by weight, 20 kGy, and zero, respectively.

Figures 7 and 8 show micrographs of the 20% by weight NVP- and AA-grafted PVA hydrogel surfaces, respectively, with irradiation dose of 20 kGy without glycerol, after fixation with gluteraldehyde and staining with trepan blue. Qualitatively, cell density on the NVP-grafted PVA hydrogel was higher than that of AA-grafted PVA, while the degree of swelling of the NVP-grafted PVA was higher than that of AA (i.e.,

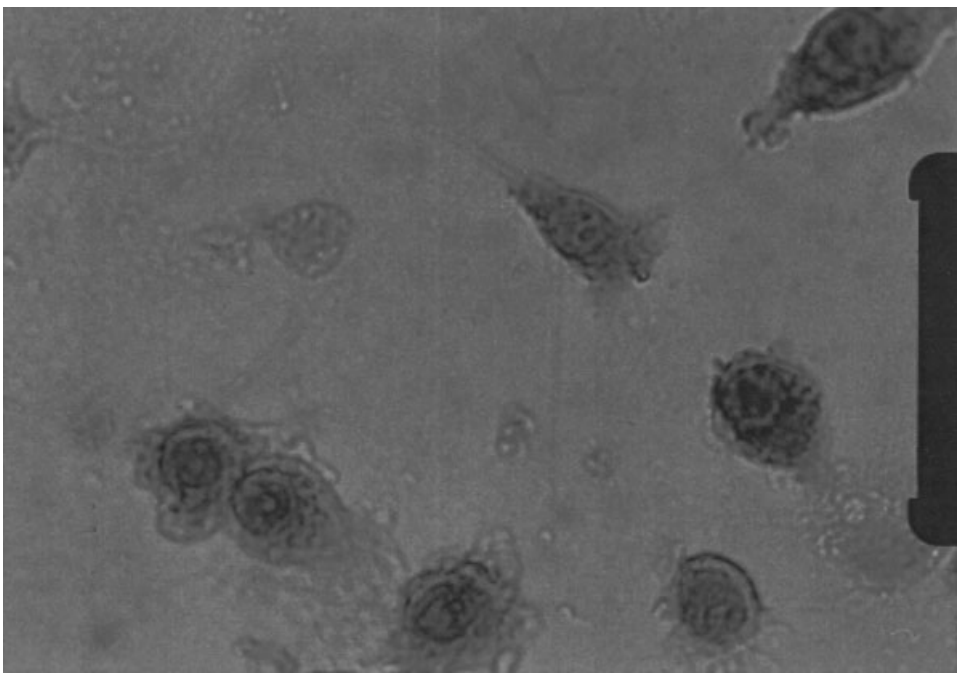

Figure 9 Microscope image of fibroblast cells on the surface of NVP-grafted PVA hydrogel at 400× magnification. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

72% ± 4% vs. 50% ± 3%). Figure 8 shows that cells on the AA-grafted surface have a rounded spherical morphology compared to that of NVP, which had an extended oval-shaped morphology (Fig. 9), indicating that fibroblast cells had a stronger attachment to the NVP-grafted surface. This can be explained by repulsion between the negatively charged carboxylate anions of AA and the anionic groups on the cell surface which reduced cell attachment on the AA grafted PVA. Based on these results, NVP-grafted PVA has a higher equilibrium water content and higher cell viability compared to that of AA-grafted PVA.

CONCLUSION

Acrylic acid (AA) and *N*-vinyl pyrrolidone (NVP) monomers were grafted to dehydrothermally crosslinked poly(vinyl alcohol) (PVA) hydrogel using γ-irradiation in order to improve equilibrium water content while retaining the desirable mechanical properties of PVA. In this work, equilibrium water content of the grafted PVA hydrogels was measured and cell viability was evaluated. Addition of glycerol added in the annealing stage to PVA, as a plasticizer, had no effect on equilibrium water content of AA-grafted PVA hydrogel while it reduced the degree of swelling of NVP-grafted PVA. For a given radiation dose and glycerol content, monomer concentration did not have a significant effect on the degree of swelling. There was a significant difference between the degree of swelling of NVP-grafted PVA hydrogel, which was between 79% ± 4% and 60% ± 3%, depending on the radiation dose and the monomer concentration, compared to that of AA-grafted PVA, which was between 53% ± 2% and 43% ± 2%. Cell density of the NVP-grafted PVA hydrogel was higher than that of AA-grafted PVA, while the degree of swelling of the NVP-grafted PVA was higher than that of AA. Moreover, cells on the AA-grafted surface had a rounded spherical morphology compared to that of NVP, which had an extended oval-shaped morphology, indicating that the fibroblast cells had a stronger attachment to the NVP-grafted surface. Based on these results, NVP-grafted PVA has higher equilibrium water content and higher cell viability compared to that of AA-grafted PVA.

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