Preparation and Swelling Behavior of Biodegradable Hydrogels Based on α,β-Poly(*N*-2-hydroxyethyl-DL-aspartamide)

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ABSTRACT: Biodegradable polymers and the hydrogels have been increasingly applied in a variety of biomedical fields and pharmaceutics. α,β-Poly(*N*-2-hydroxyethyl-DL-aspartamide), PHEA, one of poly(amino acid)s with hydroxyethyl pendants, are known to be biodegradable and biocompatible, and has been studied as an useful biomaterial, especially for drug delivery, via appropriate structural modification. In this work, hydrogels based on PHEA were prepared by two-step reaction, that is, the crosslinking of polysuccinimide, the precursor polymer, with oligomeric PEG or PEI-diamines and the following nucleophilic ring-opening reaction by ethanolamine. Soft hydrogels possessing varying degrees of gel strength could be prepared easily, depending on the amount of different crosslinking reagents. The swelling degrees, which were in the range of 10–40 g–water/dry gel, increased somewhat at higher temperature, and also at alkaline pH of aqueous solution. A typical hydrogel remained almost unchanged for 1 week, at 37°C in phosphate buffer of pH 7.4, and then seemed to degrade slowly as time. A porous scaffold could be fabricated by the freeze drying of water-swollen gel. The PHEA-based hydrogels have potential for useful biomaterial applications including current drug delivery system. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 3741–3746, 2003

Key words: hydrogels; biodegradable; swelling; polyamides; biomaterials

INTRODUCTION

The importance of biodegradable polymers and the hydrogel materials is being increasingly recognized, and extensive studies has been conducted on their uses in various biomedical applications.[1,2] Poly(amino acid)s, which have protein-like linkages, are known to be biodegradable, and have been investigated in various biomedical applications including drug delivery systems. Poly(aspartic acid), PASP, is one of promising water-soluble and biodegradable polymer, which can be produced from the hydrolysis of polysuccinimide (PSI).[3–5] PSI, the precursor polymer, is prepared by the thermal bulk polycondensation of aspartic acid or the ammonium salts of maleic acid and malic acid.[5,6] PASP is currently in commercial use as a dispersing agent. When neutralized and crosslinked, PASP has a high water absorbency, which is pH and electrolyte sensitive in water and body fluids.[7–10] α,β-Poly(*N*-2-hydroxyethyl-DL-aspartamide), PHEA, is another important polymer, derived by coupling polysuccinimide with ethanolamine, and has been proposed as a potential plasma extender and carriers for macromolecular prodrugs.[5,11] The presence of hydroxyl groups in the side chain provides the possibility of forming a covalent linkage between the polymer and drug molecules with proper spacer groups. This polymer has been demonstrated to possess suitable physicochemical characteristics for development of macromolecular prodrugs, such as biodegradability, high water solubility, multipoint drug attachment, excellent biocompatibility, and low cost. Extensive studies on this material have been done by Giammona group recently.[6,11,13–15]

Hydrogels based on both natural and synthetic polymers have continued to be interest for encapsulation of drugs, and most recently, such hydrogels have become especially attractive to the new field of "tissue engineering" as matrices for repairing and regenerating a wide variety of tissues and organs.[16–18] Hydrogels are hydrophilic polymer networks that may absorb from 10–20% (an arbitrary lower limit) up to hundreds of times their dry weight in water. Crosslinking of PHEA to prepare the corresponding gel can be accomplished by means of both chemical and physical processes. Recently, Giammona et al. has

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reported on the hydrogels by gamma-irradiation of PHEA or modified-PHEA with methacryloyl groups.[11,15] In this work, chemical hydrogels based on PHEA were prepared by the crosslinking reaction of polysuccinimide, the precursor polymer, with oligomeric PEG and PEI-diamines, and the subsequent nucleophilic ring-opening reaction by ethanolamine under controlled reaction condition. The preparation of gels and their swelling behavior, depending on the different medium, temperature, and pH, are investigated. Fabrication of porous scaffold was attempted by freeze-drying method. Also, some results on the hydrolytic degradation are presented.

EXPERIMENTAL

Chemicals and measurements

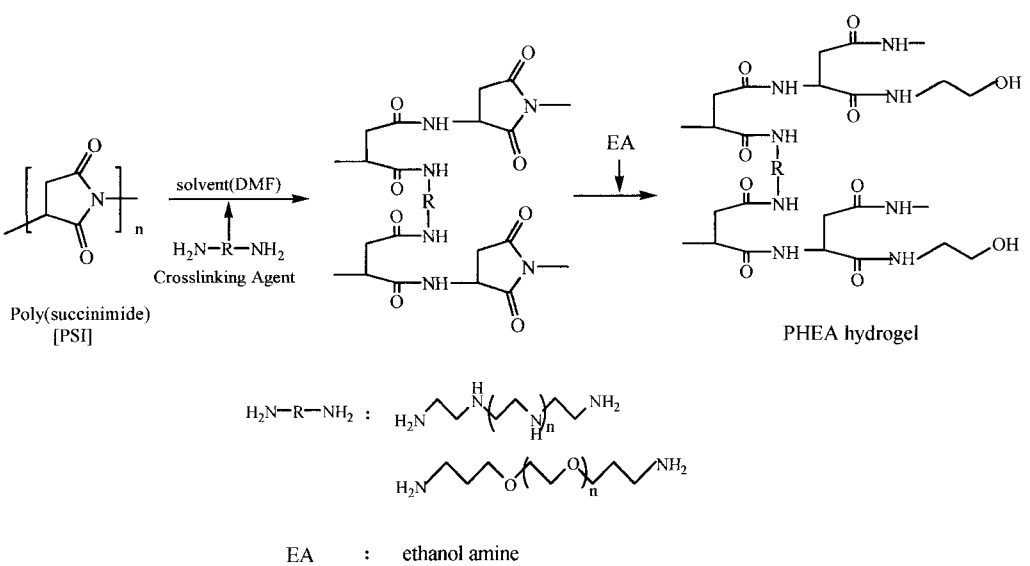
The L-aspartic acid and *o*-phosphoric acid were purchased from Aldrich Chemical Co. Ethanolamine (99%) and linear *a*,ω-diamino polyethyleneimine (L-PEI diamine, MW 423) were purchased from Aldrich Chemical Co. and used as received. PEG-diamine (MW 1000) was a gift from NOF, Japan. *N,N*-dimethylformamide(DMF) was dried over CaH_2 and fractionally distilled under reduced pressure. All the other chemicals purchased were of high quality and used without purification.

The IR spectra were obtained on a Unicam 1000 FTIR spectrometer. The thermal analysis was carried out on a Perkin-Elmer DSC/TGA7 Series thermal analysis system. The solution viscosity was measured in an Ubbelohde capillary viscometer using DMF as the solvent.

Surface morphology of the prepared scaffolds were observed by SEM (Model XL30 ESEM-FEC, FEI Co.) to investigate the pore structure and pore size. Porous powder sample were mounted on the metal stub with double-sided tape and coated with platinum for 30 s under argon atmosphere using plasma sputter.

Preparation of crosslinked gels

The preparation and characterization of polysuccinimide, the precursor polymer, has already been described in previous literature.[5,6] The molecular weight of PSI was estimated from the solution viscosity using empirical equation shown in the literature. Crosslinked gels based on PHEA structure were prepared by the two successive processes; that is, the crosslinking reaction of PSI with diamine compound and the subsequent reaction with ethanolamine in anhydrous DMF. Typically, into the 100-mL three-necked flask equipped with nitrogen inlet, outlet, and condenser was added 1 g of PSI and dissolved completely with 20 mL of DMF. PEG-diamine (10 mol %), the crosslinking agent, in 5 mL of DMF was added and the mixture was stirred for 4 h at room temperature (25–30°C) under nitrogen atmosphere, and then 2.4 mol times of 2-ethanolamine in 5 mL of DMF was added dropwise using a funnel, and the reaction mixture was stirred for another 2 h to complete the reaction. The resulting viscous solution (sometimes in soft gel-form) was precipitated into a large amount of cold 2-propanol, and the fine particulate products were centrifuged, washed, and collected to dry in vacuum. For the preparation of porous scaffold, the swelled gel, dialyzed in distilled water, was frozen in liquid nitrogen and liphophilized by freeze drying. The overall yields were about 50–60%.



Scheme 1 Preparation of hydrogels based on PHEA.

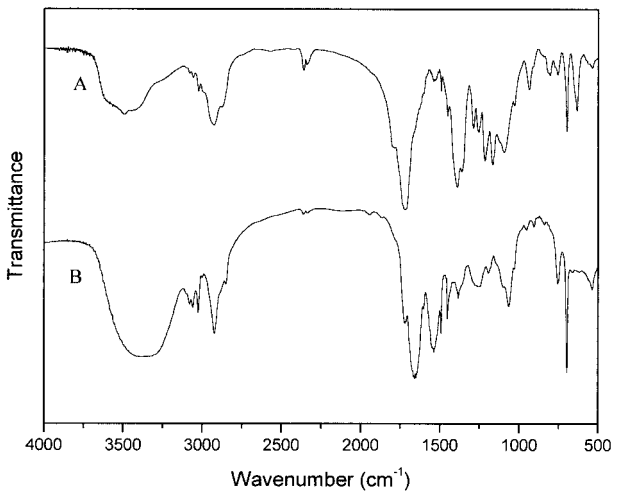


Figure 1 IR spectra of crosslinked PSI by PEG-diamine (A) and the PHEA gel (B).

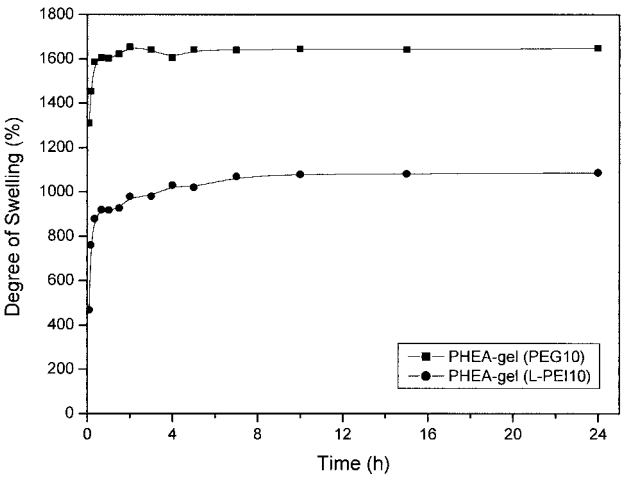


Figure 2 Typical swelling curves of the prepared gels in water.

Measurement of swelling degree

The water absorbency and swelling rate were measured by using a simple tea-bag method in different media. A certain amount of dry gel powder (W_d) was weighed into an empty bag, and then placed in distilled water until near equilibrium swelling was obtained. The weight of the swollen gel including the bag was measured, and the weight of the wet tea-bag without the sample was subtracted to obtain the pure weight of the swollen gel (W_s) resulting from the dry polymer. The swelling percent was simply calculated using the equation below:

$$\text{Swelling } \% = (W_s - W_d)/W_d \times 100 \qquad (1)$$

RESULTS AND DISCUSSION

Preparation and characterization of PHEA hydrogels

Relatively high molecular weight PSI was prepared by the thermal condensation of L-aspartic acid in the presence of *o*-phosphoric acid under reduced pressure. The polymer possessed a reduced viscosity of 38 mL/g in DMF. The molecular weight was estimated to be about 100,000 Da, as calculated from an empirical equation relating the solution viscosity to the molecular weight.[5] Hydrogels based on the PHEA structure were prepared by reacting PSI with diamine compound (PEG or L-PEI diamine) and subsequent reaction with excess amount of ethanolamine as the reaction scheme is shown below (Scheme 1). A viscous mixture, in some cases as a clear gel form, was formed as the reaction proceeded. In this study, it was interesting to introduce water-soluble oligomeric diamine compounds with oxyethylene or iminoethylene structural unit, which might give more hydrophilic and biocompatible nature into the material.

Figure 1 shows the IR spectra of the crosslinked PSI with PEG-diamine (A) and its gel product after EA reaction (B). The spectrum (A) shows characteristic bands of succinimide at 1727, 1393, 1217, and 1163 cm^{-1}, plus additional weak absorption bands due to amide (1666, 1539 cm^{-1}) and alkylene ether groups (1029, 757 cm^{-1}). The gel form (B) shows broad characteristic hydroxyl groups (3200–3600 cm^{-1}) and amide (1610, 1530 cm^{-1}) linkage of PHEA backbone. From the TGA thermograms of the prepared gels, the materials were found to be stable up to about 220°C in nitrogen. DSC of hydrogel samples showed weak and broad glass transitions around 110–120°C.

Swelling behavior and morphology of PHEA hydrogels

The prepared gels were tested with regard to their swelling in aqueous solutions by the tea-bag method. Figure 2 shows a typical water-swelling curve for the gel samples from the two different diamines of L-PEI and PEG. The initial fast swelling seemed to reach equilibrium in several hours, and the swelling degree remained almost constant thereafter. The swollen gels exhibited relatively good gel strength. Table I shows a

TABLE I
Swelling Degrees of PHEA Hydrogels

Crosslinking agent (Diamine/mol %)		Swelling %	
		In water	In 0.9 wt % NaCl
L-PEI	5	1570	1720
	10	1000	950
PEG	5	4200	1700
	10	1610	1280

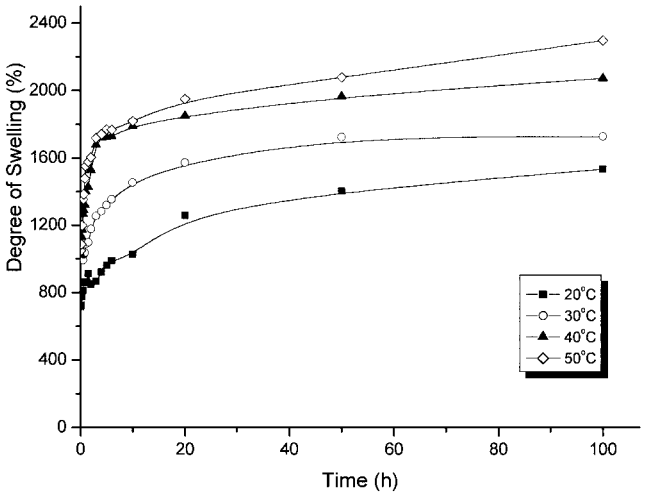


Figure 3 Degree of swelling at several different temperatures (sample: PHEA gel from PEG 10).

comparison of the typical swelling degrees of the gel samples from two different diamines, with each 5 and 10 mol % content, in both distilled water and 0.9 wt % NaCl solution. The swelling degrees in the salt solution (0.9 wt % NaCl solution) were not much different compared to those in pure water (except PEG 5% sample), suggesting a nonionic nature of the PHEA gel. At the same content of the crosslinking agent, the hydrogels from PEG-diamine exhibited higher swelling degrees, which may be caused by a higher molecular size of the PEG chain and its favorable interaction with water molecules.

Figure 3 shows the swelling degree measured at several different temperatures as a function of soaking time (sample, PEG10). The degree of swelling tended to increase somewhat at a higher temperature, probably due to the thermal expansion effect. Also, a gradual increase in the swelling was observed as the soaking period lengthened. Figure 4 shows the swelling at different pHs for the two different gels (PEG10 and PEI10), where the swelling experiment was conducted in a standard buffered solution at room temperature (20°C). The swelling was observed to be higher at an alkaline pH, yet those at neutral and acidic pHs were relatively low with little differences. In the case of gel from L-PEI, the swelling degree at pH 2 was higher than those at pH 4–7, probably caused by the slightly basic ethyleneimine structures introduced, which could be protonized to some extent in acidic medium.

The aspect of gel degradation in an aqueous environment was investigated by monitoring the swelling degree and weight loss of the material as a function of immersion time. A typical result is plotted in Figure 5. A series of swollen gels within tea-bags was placed in a buffered solution of pH 7.4, controlled at 37°C and sampled out after a given period of time to check the water absorbency and weight loss. The weight was measured by freeze drying of the remaining gel. As shown by the curves, no appreciable change in weight was observed during the initial 1 week, but a gradual decrease was observed thereafter. In contrast, the swelling degree of the samples continued to increase up to about two times of the initial swelling through 6 weeks. In seventh week, the apparent swelling then decreased abruptly to a negligible degree, suggesting all the material are drained out of bag as water-soluble liquid. It seems that the gel begin to degrade partially after a certain period of time caused by the hydrolysis of the amide linking groups in excess water, which can be autocatalyzed by the generated carboxylic group within the matrices.

Recently, hydrogels have become increasingly studied as matrices for tissue engineering.[16–18] PHEA-based hydrogel also has a potential for this purpose

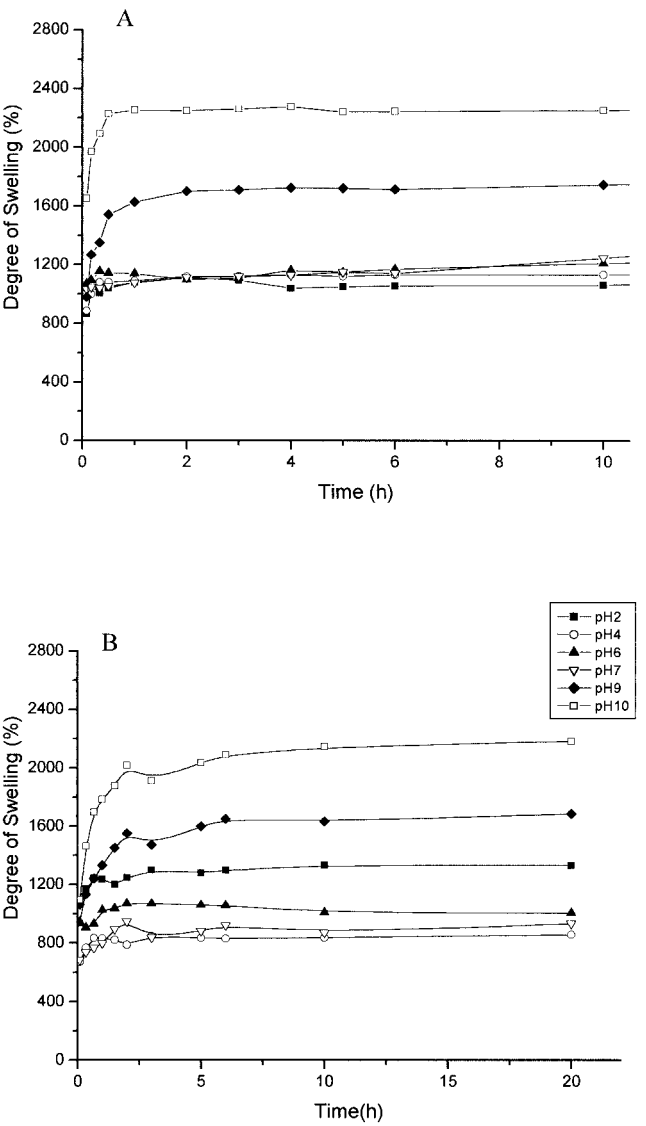


Figure 4 The pH dependence of swelling behavior. (A): PEG 10, B: L-PEI 10.

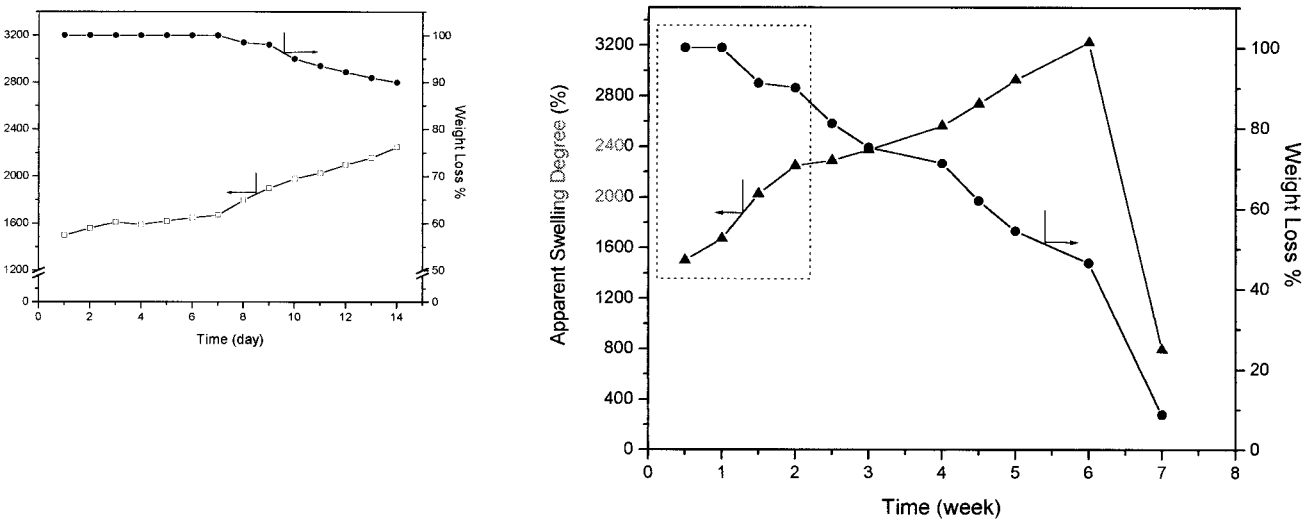


Figure 5 Stability of hydrogel as a function of immersion time at 37°C in PBS solution. (sample: PHEA gel from PEG 10).

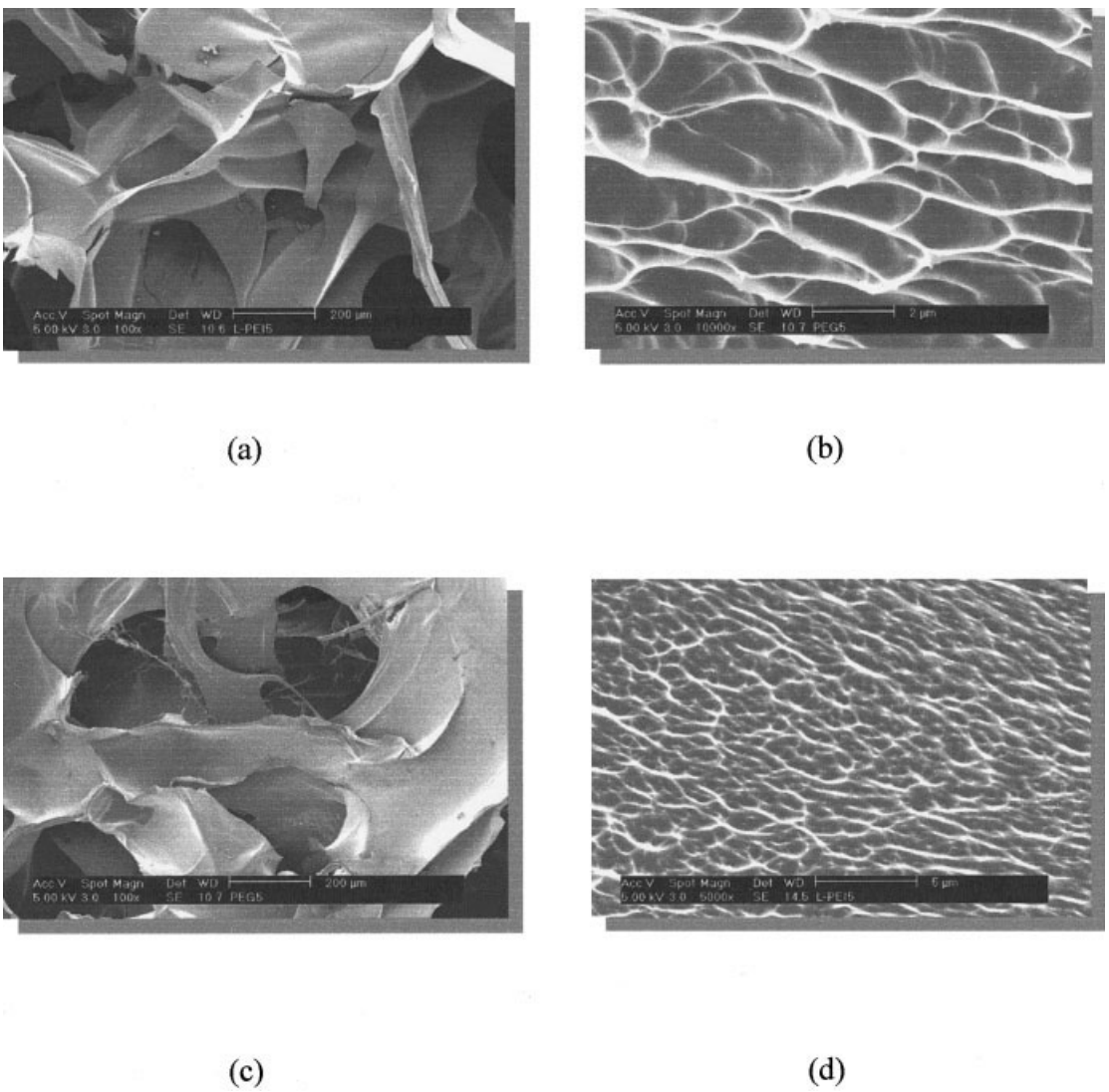


Figure 6 SEM micrographs of freeze-dried PHEA hydrogels [(a),(b): PEG 5; (c),(d): PEI 5].

because of the several advantageous properties. This hydrogel possesses biocompatible and biodegradable property, chemical modification is viable using hydroxyl pendant, and relatively strong gel with different pore size might be possible by changing crosslinking density using proper crosslinking agent. PHEA-based hydrogels prepared in this work could be shaped into porous sponge-like scaffold by simple freeze-drying methods of water-swollen gel. Figure 6 showed typical SEM micrographs of the above gel samples indicating microporous structure of the scaffold. Observation of sponge-like PHEA-gel [Fig. 6(a)] demonstrates a network structure composed of a ribbon-type wall with relatively large pores. Comparatively, Figure 6(b) shows higher magnification of the surface of thin plate, with fine and irregular shells interconnected each other. The similar morphology was observed from PHEA-gel (PEI 5) as shown in Figure 6(c) and (d). A detailed view on the morphology of the scaffold, changing by the different composition of sample and fabrication conditions are now under investigation, and the results will appear in the next communication.

CONCLUSIONS

Polymeric hydrogels based on PHEA were prepared and the swelling behavior was investigated. These materials exhibited a low to medium swelling degree (10–40 g water/g-dry gel) depending on the content of the different crosslinking agent. The prepared gels were found to decompose within a several weeks at 37°C in an aqueous buffer solution. A porous scaffolds are resulted from freeze drying of the swollen gel, as the pore structures were observed by SEM. Applications of this PHEA hydrogel in the field of current drug delivery and tissue engineering are potentially promising.

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