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Facile synthesis of biocompatible polyglycerol hydrogel based on epichlorohydrin

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ABSTRACT: A series of hydrophilic polyglycerol (PG) hydrogel was designed and synthesized via one pot with epichlorohydrin (ECH), H_2O, and NaOH as the starting materials. The equilibrium swelling ratios of PG hydrogels could be tuned by simply changing the feed amount of NaOH. The gels were characterized by carbon nuclear magnetic resonance (^{13}C NMR) spectroscopy, X-ray photoelectron spectroscopy, and Fourier transform infrared spectroscopy. The As-synthesized PG hydrogels showed temperature-sensitive swelling behaviors. The results of MTT assay suggested that the PG hydrogels prepared by this novel synthesis method showed comparable cytocompatibility with the recognized poly(ethylene glycol) hydrogel. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43451.

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INTRODUCTION

Hydrogels are crosslinked hydrophilic three-dimensional networks that can absorb and retain large amounts of water.[1–3] Due to their high water content and elastic nature, polymer hydrogels have been used extensively in biomedical field, such as bioadhesives,[4] drug delivery vehicles,[5,6] and tissue engineering scaffolds.[7,8] With the rapid development of biomaterials, there is also an increasing demand for hydrogels with excellent mechanical performances and multifunctional properties, such as adjustable swelling behaviors and thermosensitive properties.

Polyglycerol (PG) is usually synthesized from glycidol or glycerol, and has received particular attention due to its good biocompatibility[9,10] and structure of aliphatic polyether–polyol.[11] PG is also a environmentally friendly material in natural conditions owing to the presence of the ether group, which is very sensitive to photochemical oxidation and then causes a chain scission of the polymer.[12] PG has many intriguing characteristics, such as dendritic structures, high chemical stability, and dense surface functionality, which endow it with great ability to fabricate hydrogels. Hennink *et al* reported a strategy for the fabrication of PG hydrogel for biomedical and tissue engineering applications with good biocompatibility.[13] Oligo-PG ($M_n = 2000$ g/mol) was first prepared via controlled anionic polymerization of glycidol using a partially deprotonated triol as alkoxide initiator under anhydrous conditions. Subsequently, methacrylate groups were incorporated into oligo-PG by a transesterification reaction, and then radical copolymerization or UV induced photopolymerization was employed to generate PG hydrogel. Haag *et al.* utilized glycerol and tris-epoxide as starting materials to synthesize defined-size PG microgels *via* miniemulsion polymerization under acid conditions.[14] Dubé *et al.* synthesized PG hydrogel with etherification of glycerol at high temperature using sulfuric acid as catalyst under inert gas, and first reported that PG hydrogels are pH- and thermoresponsive.[15] However, multistep procedures were usually needed for the preparation of PG hydrogels.

In this study, we report a facile route to synthesize PG hydrogel with epichlorohydrin (ECH), H_2O and NaOH as the starting materials. Owing to the higher electrophilic activity of oxirane ring of ECH under alkaline conditions, the reactions to form PG hydrogel can occur in mild conditions in aqueous medium and room temperature. The chemical structures of PG hydrogels were characterized by ^{13}C NMR, XPS, and FT-IR. The hydrogels with different equilibrium swelling ratios (ESR) were prepared by changing the feed amounts of NaOH. The thermosensitive property and cytotoxicity of the PG hydrogels were evaluated in details.

EXPERIMENTAL

Materials and Methods

Materials. ECH was purchased from Shanghai Lingfeng Chemical Reagent (China), NaOH, and cetyltrimethylammonium bromide

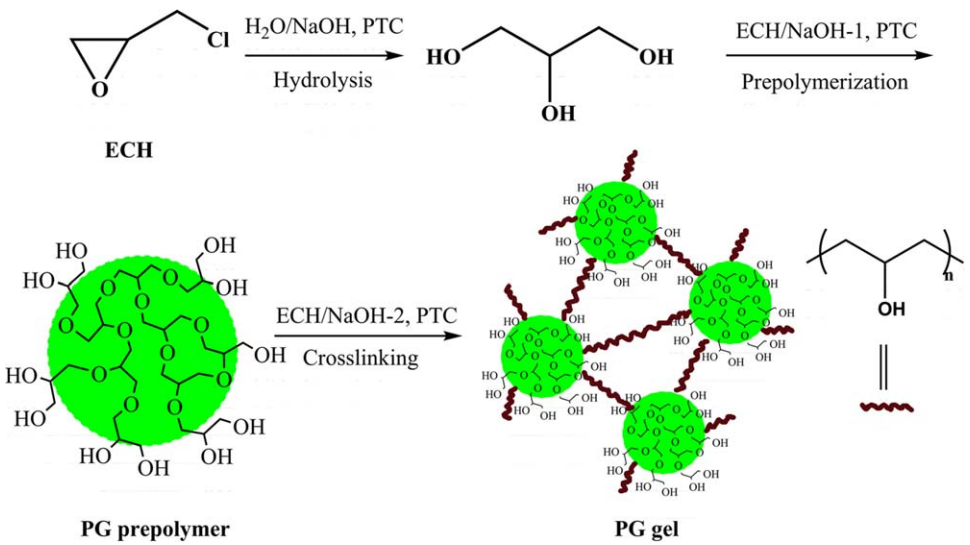

Figure 1. Schematic route for synthesis of PG gel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

(CTAB) were purchased from Sinopharm Chemical Reagent Co., (China). All of the chemicals and other reagents were of analytical grade and used as received.

Synthesis of PG Hydrogels. As illustrated in Figure 1, a facile one-pot method was developed for the synthesis of PG hydrogels. The feed ratios for the synthesis of PG gels are summarized in Table I.

The preparation procedure was as follows: 40 mL of ECH, 18 g of NaOH (30%), and 0.3 g of CTAB were mixed in a 100 mL round bottom flask with magnetically stirring at room temperature (~20 °C) for 48 h. Then 2 g of NaOH (NaOH-1) was added and stirred for 24 h. Finally, after addition of different amounts of NaOH (NaOH-2) and stirred for another 48 h, polymer gels with different equilibrium swelling ratios (ESR) were formed. Thereafter, the obtained polymer gels were cut into discs (10 mm in diameter, 3 mm in thickness). The discs were immersed in distilled water for 3 days at room temperature and the water was replaced several times to remove the residual monomers and other impurities. Some of the swollen hydrogels were freeze-dried before further characterization.

Characterization of PG Hydrogels. ^{13}C NMR measurement was carried out on a Unity Inova 600 (Varian) NMR spectrometer at 600 MHz. The PG prepolymers were dissolved by methanol and followed by reprecipitation using acetone, then the white precipitates were dried and dissolved in D_2O for ^{13}C NMR analysis. The freeze-dried PG gels were swollen in DMSO-δ_6 for six days prior to detection. XPS characterization was performed on a XSAM800 (Kratos) electron spectrometer employing Mg-K X-rays. The electron takeoff angle to the spectrometer was 90° from the specimen surface. FT-IR spectrum was obtained on a NEXUS 670 FT-IR spectrometer. The freeze-dried gels were fractured carefully in liquid nitrogen, sputter-coated with gold and subjected to observation under scanning electron microscopy (SEM, Hitachi-X650, Japan).

Table I. Synthesis of PG Gels

Sample	ECH (mL)	30% NaOH (g)	NaOH-1 (g)	NaOH-2 (g)
PG-1	40	18	2	8
PG-2	40	18	2	6
PG-3	40	18	2	4
PG-4	40	18	2	2
PG-5	40	18	2	1

Swelling Behavior of PG Hydrogels. The swelling behavior was measured gravimetrically by incubating the specimen in distilled water up to a constant weight at the predetermined temperature. After immersion in distilled water, the hydrogels were removed from water bath and blotted with a wet filter paper to remove excess water on the hydrogel surface and then weighed. The dry weight of each sample was determined after dried to constant weight under vacuum at 70 °C overnight. The average values among three measurements were taken for each sample. The ESR and the water uptake (WU) were calculated as following equations, respectively:

$$\mathrm{ESR} = \frac{m_{\mathrm{wet}}}{m_{\mathrm{dry}}} \quad (1)$$

$$\mathrm{WU} = \frac{m_{\mathrm{wet}} - m_{\mathrm{dry}}}{m_{\mathrm{wet}}} \times 100 \quad (2)$$

where m_{wet} is the weight of the wet hydrogel after reaching equilibrium at a predetermined temperature, and m_{dry} is the dry weight of each hydrogel.

Cell Cytotoxicity of PG Hydrogels. Bovine cartilage chondrocytes were isolated from the articular cartilage of a 2-month old calf and cultured in Dulbecco's Modified Eagles Medium (DMEM high glucose, Hyclone) supplemented with 10 m*M* HEPES, 0.1 m*M* nonessential amino acids, 0.4 m*M* proline, 50 mg/L vitamin C, 10% fetal bovine serum, and 1% penicillin–streptomycin (Invitrogen). Passage 2 cells were used in this study. Cytotoxicity of the hydrogels was investigated via MTT (MTT = 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide) assay according to our previous work.[16] Hydrogel discs were placed on the bottom of a 24-well plate.

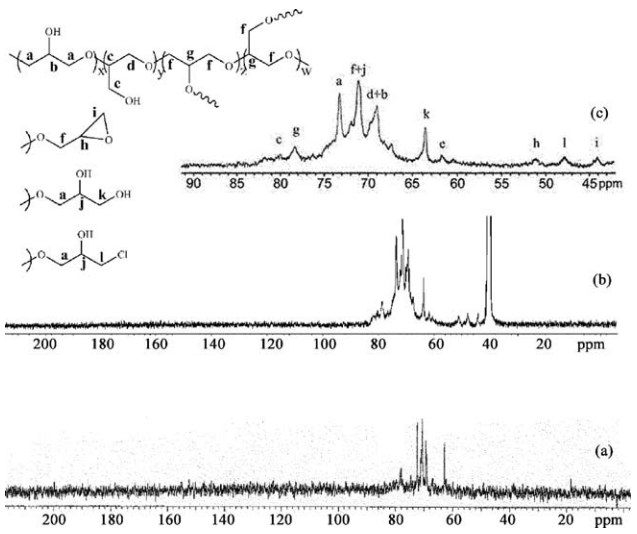

Figure 2. ^{13}C-NMR spectrum of PG prepolymer (a) (in D_2O), PG-1 gel (b) (swollen in DMSO-δ_6, 30 °C) and the (42–90 ppm) expanded region (c) of PG-1 gel, along with the proposed structural units of PG-1 gel.

The hydrogels were washed with sterile PBS, 70% ethanol, and sterile PBS for 24, 12, and 48 h, respectively. 7.5×10^3 cells were seeded on the hydrogel in each well and incubated for 24 h at 37 °C in the presence of 5% CO_2. The culture medium was removed and 200 μL of DMEM and 20 μL of MTT (0.5 g/L) solution in PBS was added into each well and incubated for 4 h. Then 500 μL of DMSO was added after DMEM and MTT solutions were removed, and the 24-well plate was shaken at 100 rpm for 30 min in an incubator. The absorbance was measured at 570 nm on a plate reader (Bio-tek Synergy MX). The cell viability was calculated as follows:

$$\text{Cell viability} = \frac{\text{OD}_{\text{treated}}}{\text{OD}_{\text{control}}} \times 100\% \quad (3)$$

where $\text{OD}_{\text{treated}}$ was obtained from the cells treated by the hydrogel, and $\text{OD}_{\text{control}}$ was obtained from the cells treated by the PEG hydrogel. The data are given as mean ± standard deviation (SD) based on three measurements.

RESULTS AND DISCUSSION

Synthesis of PG Hydrogels. The PG gels were fabricated *via* the partial hydrolysis of ECH to produce glycerol, copolymerization of ECH initiated by glycerol or hydroxyl groups (NaOH-1), and cross-linking (NaOH-2) of the oligo-PG, in the presence of NaOH (Figure 1). Chemically, two reactions were involved in the formation of PG gel, i.e., the nucleophilic ring-opening addition of oxirane ring, and next the elimination of hydrochloride from the reaction intermediate, which caused the reactions of ECH hydrolysis, polymerization, and copolymer crosslinking. The reactions to generate PG gels can be well controlled by manipulating the addition and consumption of NaOH during the preparation process. According to the preparation procedure, a series of PG hydrogels were successfully synthesized using ECH, H_2O, NaOH, as starting materials and CTAB as a phase transfer catalyst (PTC) in an alkaline/water medium at room temperature.

Characterization of PG Hydrogels. The chemical structures of PG prepolymers and PG gels were characterized with ^{13}C-NMR spectra and illustrated in Figure 2. And the PG prepolymer was a soluble product before the addition of NaOH-2. PG gels were characterized with ^{13}C-NMR spectra of DMSO-d_6 swelled specimen as PG gel did not dissolve in any organic solvent or water. Theoretically, there are two possible reaction pathways for the elimination of hydrochloride from the reaction intermediate in the alkaline medium. One of the pathways is that base attacks a hydrogen of hydroxyl to form epoxy compound. Other may give as the first intermediate a vinyl alcohol by base attacking β hydrogen, which undergoes rearrangements to a stable methyl ketone. From Figure 2(a,b), no signals related to methyl carboxide (~206.4 ppm) groups were observed, indicating that the elimination of hydrochloride from the reaction intermediate occurs only in accordance with the former pathway to give oxirane ring during the preparation process. The carbon signals of the gels appeared mainly range from 60 to 80 ppm, indicating that almost all of the carbons of PG prepolymers and gels were linked with oxygen atoms as expected. The expanded region (42–90 ppm) of the ^{13}C-NMR spectra is presented in Figure 2(c). Three possible subunits are suggested from ^{13}C-NMR spectrum in ppm to be as following: (1) —CH_2CH (OH)CH_2O—: 73.4 (a, $2CH_2$), 69.2 (b, CH); (2) —CH(CH_2OH)CH_2O—: 80.3 (c, CH), 69.8 (d, CH_2O), 61.9 (e, CH2OH); (3) —$CH_2CH(O)CH_2O$—:78.6 (g, CH), 72.2 (f, $2CH_2$) and —CH(CH_2—)CH_2O—: 78.6 (g, CH), 72.2 (f, $2CH_2$). The carbon signals at 71.3 ppm and 63.7 ppm were assigned to terminal groups HC—OH (signal j) and H_2C—OH (signal k), respectively. These results are in good agreement with the ^{13}C NMR spectroscopy of polyglycerol.[17,18] In addition, the carbon signals of glycidyl group [Figure 2(c), signal h and i: 51.1 and 44.1 ppm[19]] as well as chloromethyl group (H_2C—Cl) at 47.8 ppm[20] [Figure 2(c), signal l] were also observed.

The detected H_2C—OH and HC—OH group indicated the occurrence of the addition reaction of water molecules on the oxirane ring of the reactants. Moreover, a low intensity signal of the carbon of chloromethyl group (H_2C—Cl) revealed that most of the chlorine atoms of ECH disappeared in the obtained copolymer, which was further confirmed by only 0.24% of chlorine determined by XPS measurement (Figure 3 and Table II). Obviously, the chlorine atom in the material ECH could be removed under the employed reaction conditions *via* an intramolecular or intermolecular elimination of hydrochloride after ECH was ring-opened by hydroxyl anion or alkoxyl group of resultant oligo-PG derivatives. These resulted in the reactions of ECH hydrolysis, copolymerization, and copolymer crosslinking.

The structure of PG gel was also supported by the results from FT-IR spectrum. As shown in Figure 4, hydroxyl group band (3433.1 cm^{-1}), alkyl group band (2923.4, 2875.8, and 1460.4 cm^{-1}), ether band (1115.0 cm^{-1}), and epoxy band (851.6 cm^{-1}, weak) are recorded for PG gel specimens. And the peak at 1644.3 cm^{-1} is identified as the bending vibration of residual water in the sample.[3,21–24]

XPS technique was used to measure the composition and chemical combination state of elements in the cross section of the gel. The curve fitting of the XPS spectrum was carried out by using XPS peak 4.1 program. The measured and processed

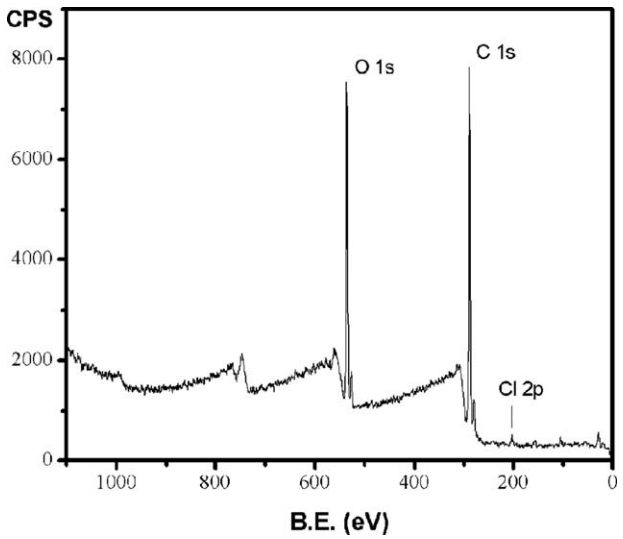

Figure 3. XPS wide scan of PG-1 gel.

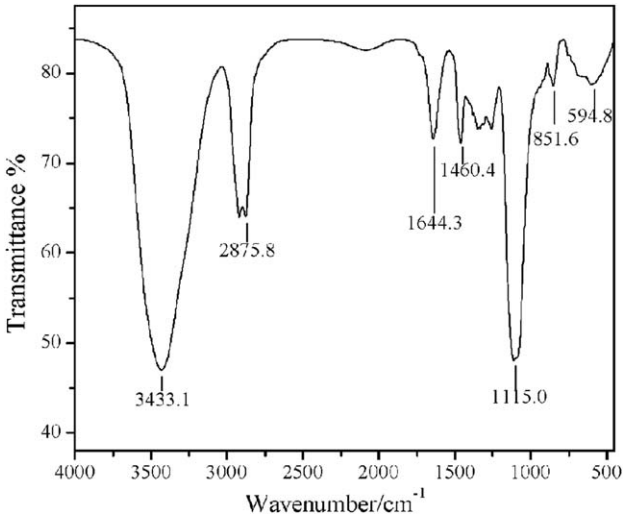

Figure 4. Typical FT-IR spectrum of PG gel.

results of elements in the PG-1 gel as an example are summarized in Table II. The result showed that the gel is composed of elements of O, C, and small amount of Cl. The atom percentage content of O increased from 20% in ECH to about 38.9% for the PG-1 gel, while the atom percentage content of Cl decreased significantly from 20% to around 0.24%. The increase in the O content implicated that H_2O molecules participated in the formation of PG gel and hydrolysis took place. Only 0.24% of Cl was detected from the gel-1 by XPS, indicating that the Cl in ECH is eliminated in the presence of NaOH during the preparation process. The processed result of curve fitting of O showed that atomic percent of O in the C—O—C and C—O—H combined state were 28.4 and 10.5%, respectively. This indicated the percentage of ether chain and hydroxyl groups were around 73.0 and 27%, respectively. Compared with the 60% or more of hydroxyl groups content in hyperbranched polyglycerol ($M_n = 6000$ g/mol),[17] the result suggested that PG-1 gel should be crosslinked PG and had higher crosslinked density.

The morphologies of the produced gel are showed in Figure 5. Three-dimensional macroporous structure was observed for all the hydrogels. It is also observed that the compactness of hydrogels is related to the feed amount of NaOH-2. When more amount of NaOH-2 was used, the hydrogel could be tighter.

Based on the results of characterization, in combination with the swelling property, the structure of resulting PG gel was assigned as cross-linked PG, which is a nonionic hydrogel.

Table II. XPS Analysis (atomic percent %) of PG Gels

				O[b]		Cl[b]	
Sample	C[a]	O[a]	Cl[a]	C—O—C	C—O—H	—Cl	Cl^-
PG-1	60.5	38.9	0.6	28.4	10.5	0.24	0.36

[a] Measured results.
[b] Processed results. Parameters of binding energy: C—O—C: 532.400 eV; C—O—H: 532.800 eV; —Cl: 199.990, 201.590 eV; Cl^-: 198.100, 199.700 eV.

Swelling Behavior of PG Hydrogels. The swelling behavior of the resulting gels prepared *via* this synthetic route was evaluated in terms of ESR and WU by incubating them in distilled water at 25 °C. The effect of amount of NaOH-2 on ESR of the PG gels is shown in Figure 6(a). When 1, 2, 4, 6, and 8 g NaOH-2 was used in the crosslinking reaction, the average ESR of PG hydrogels were 78.3, 35.9, 21.0, 10.9, and 4.2, respectively. The WU of PG gels is in the range of 76.1–98.8% in distilled water at 25 °C. The appearance of the hydrogels changed from opaque (PG-1) to transparent (PG-5) as the amount of NaOH-2 decreased (Figure 7), exhibiting a very obvious difference between the hydrogels. The transparent appearance of hydrogel is because it absorbs large amounts of water, leading to increase in its network diameter that closes to lightwave.[25] With lager amount of NaOH-2 for the crosslinking reaction, the resulting hydrogels are more tightly crosslinked and swell less in water. Obviously, the ESR of the hydrogels is in inverse proportion to the feed amount of NaOH-2, which indicates that the swelling of the PG hydrogels *via* this synthetic route can be favorably controlled by varying the feed amount of NaOH-2 during the preparation process.

The equilibrium swelling behavior of the PG hydrogels in distilled water at different temperatures was also investigated (Figure 6). As shown in Figure 6(b), the ESR of the PG hydrogels decreases with the increase of the temperature. The PG hydrogels showed the heat-induced shrinkage phenomenon in response to the variations in temperature. This swelling behavior is similar to that of PEG-based hydrogels,[26] and is in agreement with the previous researches.[15]

This temperature dependence should be attributed to the interaction of hydrogen bonding between large amount of hydroxyl groups, ether chains in the PG hydrogel and water molecules. As temperature increases, hydrogen bonding between the hydrophilic polymer network and water molecules has been gradually weakened and destroyed, and part of water molecules bound by the hydrophilic polymer network escapes. As a result, the hydrogel is in lower ESR at higher temperature. It is also observed, from Figure 6, that the crosslinking density has a great impact

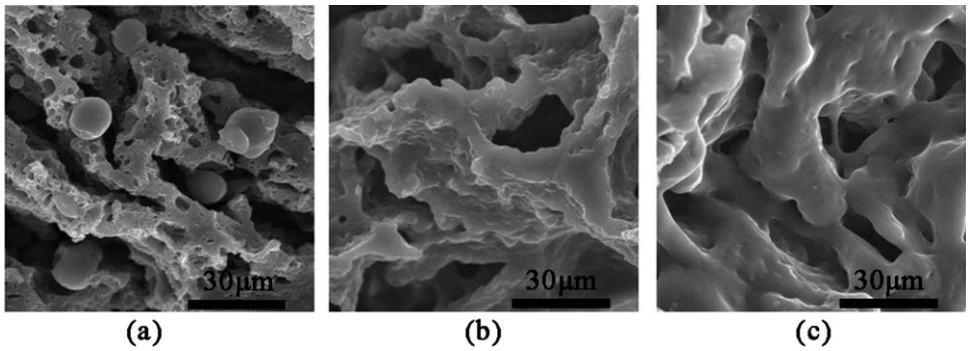

Figure 5. SEM images of the liquid N_2 fractured surface of PG gels at dried state, (magnification ×1000) (a) PG-1, (b) PG-3, and (c) PG-4.

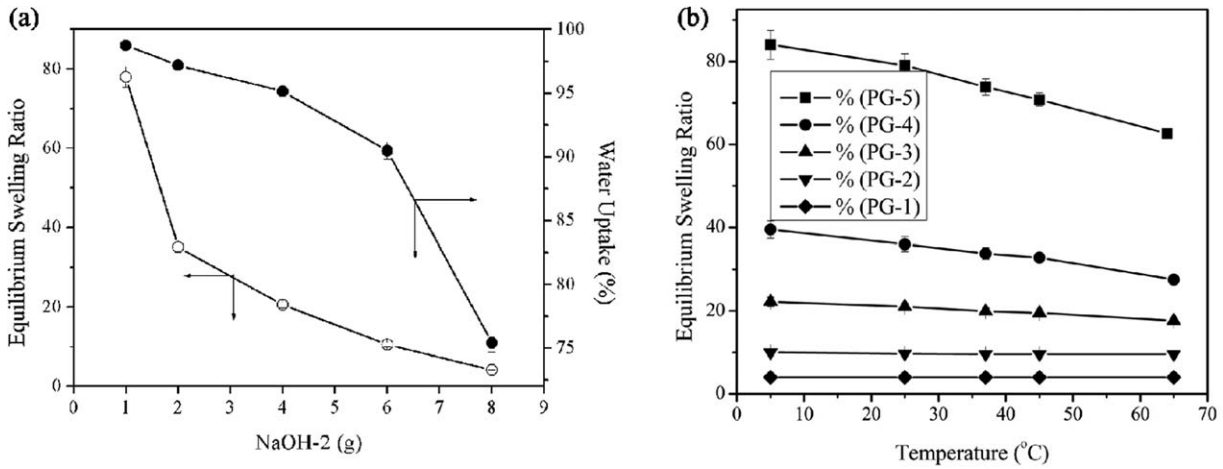

Figure 6. (a) Effect of amount of NaOH-2 and (b) temperature on ESR of PG gels in distilled water.

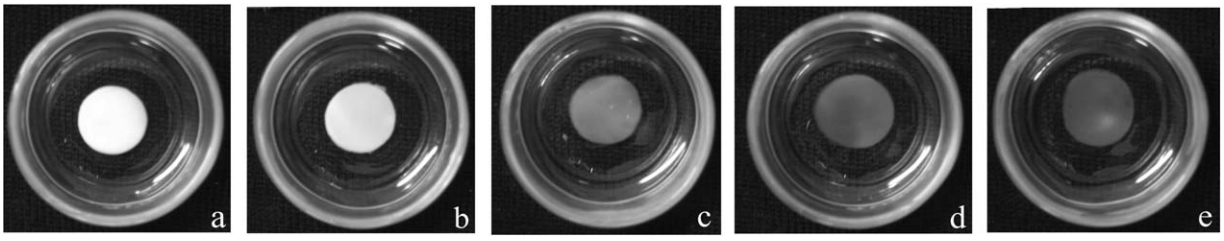

Figure 7. Optical photos of the PG hydrogels prepared by various ECH/NaOH feed ratio: (a) PG-1, (b) PG-2, (c) PG-3, (d) PG-4, (e) PG-5.

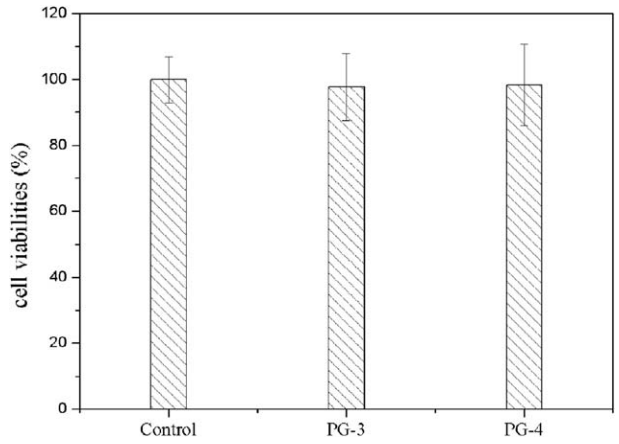

Figure 8. Viability of passage 2 cells of bovine cartilage chondrocytes cultured for 24 h on the pure PEG hydrogels, PG-3, and PG-4.

on the heat shrinkage swelling behavior of the PG hydrogels. The hydrogels with lower ESR (PG-1 and PG-2 gel) almost do not appear obvious heat-shrinkage swelling behavior, whereas the hydrogels with higher ESR (PG-4 and PG-5 gel) show obvious shrinkage change as temperature increases, especially for PG-5 gel which contracts more than 20 of ESR units when temperature decreases from 60 to 5 °C.

Cell Cytotoxicity of PG Hydrogels. MTT assay was performed to evaluate the cytocompatibility of PG hydrogels prepared by this novel synthetic route. Since hydrogels made from PEG3400 has been widely used as drug delivery carrier and tissue engineering scaffolds due to their excellent cytocompatibility,[16] we utilized PEG hydrogel as a control to study the cytotoxicity of the as-prepared PG hydrogel. As shown in Figure 8, no statistically significant difference was observed between the control and PG hydrogels (PG-3 and PG-4) after 1 day of cell culture.

This result suggests that PG hydrogels prepared by this novel synthetic route showed comparable cytotoxicity with PEG hydrogels and could be used safely in biomedical applications.

CONCLUSIONS

A series of PG hydrogels were designed and synthesized using ECH, H_2O, and NaOH as starting materials, and CTAB as a PTC in an alkaline/water medium at room temperature. Three steps are involved in the preparation of PG hydrogels: the hydrolysis of part of ECH, copolymerization, and thus cross-linking. The ESR of the hydrogels is inversely proportional to the feed amount of NaOH-2, which indicates the swelling of the hydrogels could be favorably tailored by varying the amount of NaOH-2 during the preparation process. The PG hydrogel (i.e., PG-5 with minimum degree of cross-linking) showed obvious heat-shrinkable swelling behavior. These results indicate the potential force in the development of more functional PG hydrogels. The result of MTT assay suggests that PG hydrogels prepared by this novel synthesis route showed comparable cyto-compatibility with PEG hydrogels. A new method with advantages of simple procedures, plentiful raw materials, and mild conditions was developed for the preparation of PG hydrogels.

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