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Synthesis and Characterization of Glucosamine Modified Poly(ethylene glycol) Hydrogels via Photopolymerization

Qunfang Wang,[1,2,3] Li Ren,[1,2] Caixia Xu,[1,2] Zhichen Zhai,[1,2] Jia-an Zhou,[1,2] Yongchang Yao,[1,2] Huan Xia,[1,2] Yingjun Wang[1,2]

[1]Biomaterial Research Institute, School of Materials Science and Engineering, South China University of Technology, Guangzhou 510641, People's Republic of China

[2]National Engineering Research Center for Human Tissue Restoration and Function Reconstruction, Guangzhou 510641, People's Republic of China

[3]Experimental Center of Instruments, South China Normal University, Guangzhou 510631, People's Republic of China

Correspondence to: L. Ren (E-mail: psliren@scut.edu.cn) or Y. Wang (E-mail: imwangyj@163.com)

ABSTRACT: This study presented the synthesis and characterization of glucosamine (GlcN) modified poly (ethylene glycol) (PEG) hydrogels. The chemical structure was characterized by Fourier transform infrared (FTIR) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. The morphology of hydrogels was observed by scanning electron microscopy (SEM). The results indicated that GlcN was successfully incorporated into PEG hydrogel network. Moreover, the data of the swelling ratio showed that the ratio of GlcN-modified PEG hydrogels was lower than that of pure poly(ethylene glycol) diacrylated (PEGDA). Biocompatibility of unreacted GlcN monomer and GlcN-modified hydrogels was also evaluated *in vitro*. Compared with glucosamine hydrochloride, 2 and 5 m*M* *N*-acroloyl-glucosamine monomer exhibited no toxicity against bone marrow stromal cells (BMSCs), while with the concentration increased to 10 m*M*, cell viability appeared to decrease. However, when BMSCs were encapsulated in GlcN-modified hydrogels via photopolymerization method, cells remained vigorous viability. Metabolic activity of the encapsulated cells demonstrated GlcN-modified hydrogels was favorable for cell proliferation. Compared with free GlcN, covalent binding GlcN showed lower cytotoxicity and higher cell proliferation properties. As a result, GlcN-modified PEGDA hydrogels could be used as safe and injectable cell carriers for *in situ* tissue engineering applications. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: hydrogel; glucosamine; poly(ethylene glycol); photopolymerization; biocompatibility; bone marrow stromal cells

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INTRODUCTION

Hydrogels are highly swollen and insoluble network, which can provide a three-dimensional environment for tissue regeneration.[1] Poly(ethylene glycol) (PEG) was chosen for this study because of its hydrophilicity and biocompatibility. Moreover, PEG diacrylate or dimethacrylate can be photopolymerized to form hydrogels in direct contact with cells without deleterious effects.[2,3] Nevertheless, there are some limits for the application of PEG, such as no interaction sites for proteins and cells in polyethylene diacrylate (PEGDA) hydrogels. Studies have been reported that PEGDA hydrogels which were modified with the bioactive molecules, such as cell adhesive ligands[4–6] and transform growth factors,[7–9] could establish interaction domains for cells. The way of binding bioactive molecules to hydrogels structure is well acknowledged as an effective approach to improve interactions between materials and cells.[10]

Monosaccharide or carbohydrate conjugate plays an important role in cell signaling and cell–cell communication, such as recognition, inflammation, and immune response.[11] Lopina et al. reported that carbohydrate-modified star polyethylene oxide hydrogels supported long-term culture and differentiated function of primary rat hepatocyte.[12] It can be seen that carbohydrate conjugated to synthetic polymer is another approach to construct a microenvironment for cells. Glucosamine (GlcN) is an amine monosaccharide, which is a principal constituent of glycosaminoglycans (GAGs) and synovial fluid.[13] In clinic, GlcN is widely used for osteoarthritis patients to relieve pain and slow down the rate of joint space narrowing.[14–16] Elisseeff et al. reported that GlcN as a supplement added into media, enhanced chondrogenic differentiation of murine embryonic stem cells in hydrogels,[17] promoted aggrecan and collagen type II production,[18] and upregulated the mRNA levels of transforming growth factor-β1 (TGF-β1) in chondrocytes.[19] On account

of the biological importance of GlcN, the design and applications of covalent binding GlcN to construct hydrogels have been emerged. The GlcN-modified polymer hydrogels increased adhesion with the host neural tissue, improved vascularization, and encouraged infiltration of non-neuronal cells of the host.[20] D-glucosamine-based supermolecular hydrogel facilitated the wound healing as well.[21] These studies suggested that GlcN residues played a substantial role in promoting tissue organization and regeneration. Therefore, it is reasonable to hypothesize that hydrogels covalently bonding GlcN may exert similar bioactivity and will establish a communication bridge between materials and cells.

Glycopolymer has been widely accepted as one of the most important materials for biomedical application.[20,22,23] Glucosamine is a reactive functional group; most of methods reported previously were based on introduction of an acryloyl group to form a monomer and subsequent polymerization, such as preparation of *N*-acryloyl-glucosamine and *N*-isopropyl-acrylamide copolymer using reversible addition fragmentation chain transfer technique[24] and synthesis of *N*-acryloyl-glucosamine homopolymer in presence of ammonium persulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine initiator system.[22] In addition, GlcN-modified hydrogels have been obtained using following approaches: (1) GlcN-based hydrogelator molecular self-assembly technology[21]; (2) macromolecule reacted with GlcN.[12,25] Unfortunately, due to the use of noxious reagents and deleterious reaction conditions which led to serious cellular damage, these hydrogels could not be applied for *in situ* formation and three-dimensional cells culture. Furthermore, the rates of network gelation, reported by previous literatures, are considerably slower than those demonstrated by photoinitiated chain polymerization. And it is impossible to spatially and temporally control the formation of hydrogels by these ways. In this study, *N*-acryloyl-glucosamine was copolymerized with PEGDA using the photopolymerization method. Compared with the aforementioned approaches, the photopolymerization has several advantages. It offers a fast and efficient method to encapsulate cells with minimal cell death due to spatial and temporal control. In addition, photopolymerization reaction can be carried out in a biocompatible manner, such as in water solution, at room temperature, and even *in situ* formation in a minimally invasive device. By this means, the novel GlcN-modified PEGDA hydrogel could be injectable and convenient for *in situ* forming constructs and cell delivery, as well as introduced GlcN group into PEGDA crosslinking network. Herein GlcN-modified hydrogels was synthesized and characterized with nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infrared spectroscopy (FTIR) spectra. Specifically, to evaluate the effect of covalent-binding GlcN on the fate of cells, rat bone marrow stromal cells (BMSCs) were photoencapsulated in gels prepared from *N*-acryloyl-glucosamine and PEGDA copolymer and cultured *in vitro*. The cells viability in these gels was investigated. At the same time, the cytotoxicity of *N*-acryloyl-glucosamine monomer was estimated using MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) method.

MATERIALS AND METHODS

Materials

Acryloyl chloride was purchased from Aladdin reagent factory (Shanghai, P. R. China). D-glucosamine hydrochloride was obtained from Sigma–Aldrich. Other chemical reagents were purchased from reagent factory (Guangzhou, P.R. China). All cell-culture-related reagents, except which was mentioned especially, were purchased from Gibco.

Sample Preparation

Preparation of N-Acryloyl-Glucosamine. *N*-acryloyl-glucosamine (AGA) was prepared from D-glucosamine hydrochloride and acryloyl chloride according to literature method.[26] Briefly, 0.04 mol glucosamine hydrochloride was dissolved in 20 mL of 2 mol L^{-1} K_2CO_3 solution. The reaction vessel was subsequently cooled in an ice bath, and then 0.048 mol acryloyl chloride was added dropwise under vigorous stirring. The mixture was maintained at 0°C for 2 h and then slowly warmed to room temperature for 1 day. The crude glycomonomer was further dried under vacuum and then purified by column chromatography on silica gel with a methanol/ethyl acetate mixture as eluent (20/80 v/v). AGA was finally recrystallized twice in a methanol/ethyl acetate mixture (20/80 v/v) at 4°C and characterized using NMR (Bruker Avance Digital 400 Hz) and ESI-MS (Finnigan, LCQ Deca XP MAX).

Synthesis of Poly(ethylene glycol) Diacrylate. PEG diacrylate (PEGDA) was synthesized as previously reported[27] from poly(ethylene glycol) and acryloyl chloride. Briefly, 0.01 mol anhydrous PEG with a molecular weight of 4000 was dissolved in 250 mL of anhydrous methylene chloride, and then 0.08 mol triethylamine (TEA) was added to the PEG solution. The mixture was purged with nitrogen for 10 min, and then 0.08 mol acryloyl chloride was added dropwise to the PEG solution in an ice bath under stirring. After 2 h, the mixture was transferred from the ice bath and stirred at 35°C over night under nitrogen. The insoluble triethylamine salts that were formed during the reaction were removed by filtration, and the PEG diacrylate product was precipitated by the addition of cool diethyl ether. The PEGDA precipitate was dried for 24 h in a vacuum oven at 35°C. The dry solid was further purified by dialyzing against deionized water for 1 day (molecular weight cutoff 500 Da) and then lyophilized. The product was characterized using nuclear magnetic resonance (NMR) (Bruker Avance Digital 400 Hz) and gel permeation chromatography (GPC) (Waters 515).

Photo-Crosslinked PEGDA Hydrogels Containing Glucosamine Group. Hydrogels were fabricated by dissolving PEGDA macromer and AGA monomer in deionized water containing 0.05%(W/V) I 2959 (Irgacure 2959, 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone, Ciba). The formulation of hydrogels was composed of 20% (W/V) PEGDA and varied AGA concentration: 0, 2, 5, and 10 m*M*, respectively. The precursor solution was pipetted into 96-well tissue-culture polystyrene plate with diameter 3 mm and the hydrogels were polymerized under a UV light (365 nm, Japan, MUV-165) at the intensity of 7 mW cm^{-2} for 5 min.

Characterization and Property of Hydrogels

Chemical Structure of Hydrogels

^{1}H-NMR. Proton nuclear magnetic resonance spectra of the precursor solution and hydrogels were carried out with Bruker drx400 instrument. Deuterium oxide (Cambridge Isotope Laboratories) was used as the solvent.

ATR-FTIR. FTIR spectra of lyophilized hydrogels were recorded on Nicolet Nexus Infrared Analysis equipped with an attenuated total reflectance (ATR) accessory.

Morphology of Lyophilized Hydrogels. The lyophilized hydrogel sections were cut quickly in liquid nitrogen. The specimens were coated with gold to improve the electrical conductivity. Surface and cross section morphologies of freeze-dried hydrogels were observed using a scanning electron microscope (SEM, XL 30, Philips, the Netherlands).

Swelling Measurements. Hydrogels were lyophilized after gelation and weighed as W_i. Then the samples were swollen in deionized water (deionized water was refreshed every 8 h) at 37°C for 48 h, and blotted dry and weighed as W_s. After that, the samples were lyophilized and weighed as W_d. The swelling ratio of the hydrogels (Q) was calculated as:

$$Q = (W_s - W_d)/W_d$$

Sol faction was calculated for different hydrogels as:

$$\text{Sol faction} = (W_i - W_d)/W_i$$

Experiments were conducted three times for the swelling ratio and sol faction measurements.

***In Vitro* Cell Culture Studies**

Isolation of Sprague–Dawley (S-D) Rat BMSCs. BMSCs were isolated from femurs of 4- to 5-week old **S-D** rats.[28] Briefly, the femora harvested from S-D rat were washed in low glucose Dulbecco's modified Eagle medium (L-DMEM), supplemented with 10% fetal bovine serum (FBS) (v/v). Both ends of the femurs were cut away from the epiphysis, and the bone marrow was flushed out of the diaphysis using 10 mL of media in a syringe. The marrow was collected into a sterile 25-mL centrifuge tube, and centrifuged at 2000 rmp for 20 min. The cell pellet was resuspended in 10 mL of L-DMEM supplemented with 10% FBS and cultured in two T-25 polystyrene flasks at 37°C in a humidified 5% CO_2 atmosphere. After 3 days, the media was changed and the nonadherent cells were washed away. Once reaching 90% of confluence, the cells were subcultured with 1 : 3 rations. Passage 4 BMSCs were used in this study.

Cytotoxicity of N-Acryloyl-Glucosamine. BMSCs were seeded into the 96-well plate at a density of ~3000 cells mL^{-1} and then incubated in a humid atmosphere under 37°C and 5% CO_2. Afterward, D-glucosamine hydrochloride and *N*-acryloyl-glucosamine were dissolved in culture media to make a final concentration of 2, 5, and 10 m*M*, respectively. Then the solution was filter-sterilized (0.22 um pore diameter). Two hundred microliters of each solution was added in the wells. In parallel, 200 μL of normal cell culture media was also added to the well as a positive control. Cell viability was evaluated by using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, Sigma) assay following a standard protocol at different time points.[29]

Cell Encapsulation and Viability. BMSCs were trypsinized and harvested by centrifuge (250 G, 5 min) followed by resuspension with sterile phosphate buffered saline (PBS, PH 7.4) (cell density 20 million mL^{-1}). PEGDA-AGA precursor solution (20% PEGDA in sterile PBS) containing I2959 was sterilized by means of filtration and added to cells to make suspension at density of 1×10^7/mL. The final concentration of I2959 was 0.05% (w/v). The solution was pipetted into 96-well culture plate and polymerized under a UV light (365 nm) at the intensity of 7 mW cm^{-2} for 5 min. Each construct was placed into 24-well plates containing 600 μL of culture medium [low-glucose DMEM supplemented with 10% FBS] and incubated at a humid atmosphere under 37°C and 5% CO_2. For evaluation of cell viability, Calcein AM/EthD-III assay kit (Biotium Incorporation) was used for Live/Dead staining. Briefly, after incubation up to 14 days, a disk of construct was washed with PBS three times and incubated in 200 μL of the "Live/Dead" dye solution (2 μM calcein AM and 4 μM EthD-III PBS solution) for 30 min at room temperature. The construct was then observed under the fluorescence microscope (ZEISS Axioskop 40). Green and red staining indicated viable and dead cells, respectively.

Metabolic Activity of the Encapsulated Cells in Different Hydrogels. Culture media was removed, and water-soluble tetrazolium salt (Cell count kit-8, CCK-8) cell proliferation assay agent (10 μL CCK-8 per 100 μL medium) was added to each well, according to the manufacture's protocol. Hydrogel-cells constructs ($n = 4$) were cultured for 4 h and CCK-8 was reduced by dehydrogenases in cells to give a yellow-colored product (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye was measured by spectrophotometer at $\lambda = 450$ nm.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS 11.0) software package was used to perform the statistical analysis. All the experiments were repeated with $n = 3$ biological replicates and the results were represented as the mean ± standard deviation. Repetitive one way analysis variance (ANOVA) and Tukey's multiple comparison tests were used to determine statistical significance ($P < 0.05$ or $P < 0.005$).

RESULTS AND DISCUSSION

Chemical Structure of PEGDA and AGA

The chemical structure of PEGDA and AGA was listed in Figure 1, and the ^{1}H NMR spectrum of them was shown in Figure 2. ^{1}H NMR spectrum of AGA indicated that the vinyl protons of acryl group exhibited in 6.06–6.31 ppm (H8, H9, anti) and 5.68–5.73 ppm (H9, syn) [Figure 2(a)]. Calculated results of $C_9H_{15}NO_6$ (AGA) + Na^+ is 256.08, it was found 256.14 in ESI-MS of AGA.

^{1}H NMR spectrum of PEGDA was illustrated in Figure 2(b). The δ (5.90–5.92 ppm, CH_2=; 6.09–6.16 ppm and 6.34–6.38 ppm, CH_2=CH—) represented the terminal vinyl protons of acryl group. The ethylene protons of PEG repeating units were present in 3.41–3.78 ppm (—CH_2—CH_2—). The degree of substitution of the PEG terminal hydroxyl group for acrylate was calculated using the method of Dust et al.[30] Acrylation substitution was 81%. GPC analysis suggested that the acrylation and purification procedure did not affect the molecular weight of PEG chain.

Characterization and Property of Hydrogels

In this study, photopolymerization was employed to obtain *N*-acryloyl-glucosamine and PEGDA copolymer hydrogels. The chemical structure of GlcN-modified PEGDA hydrogel was

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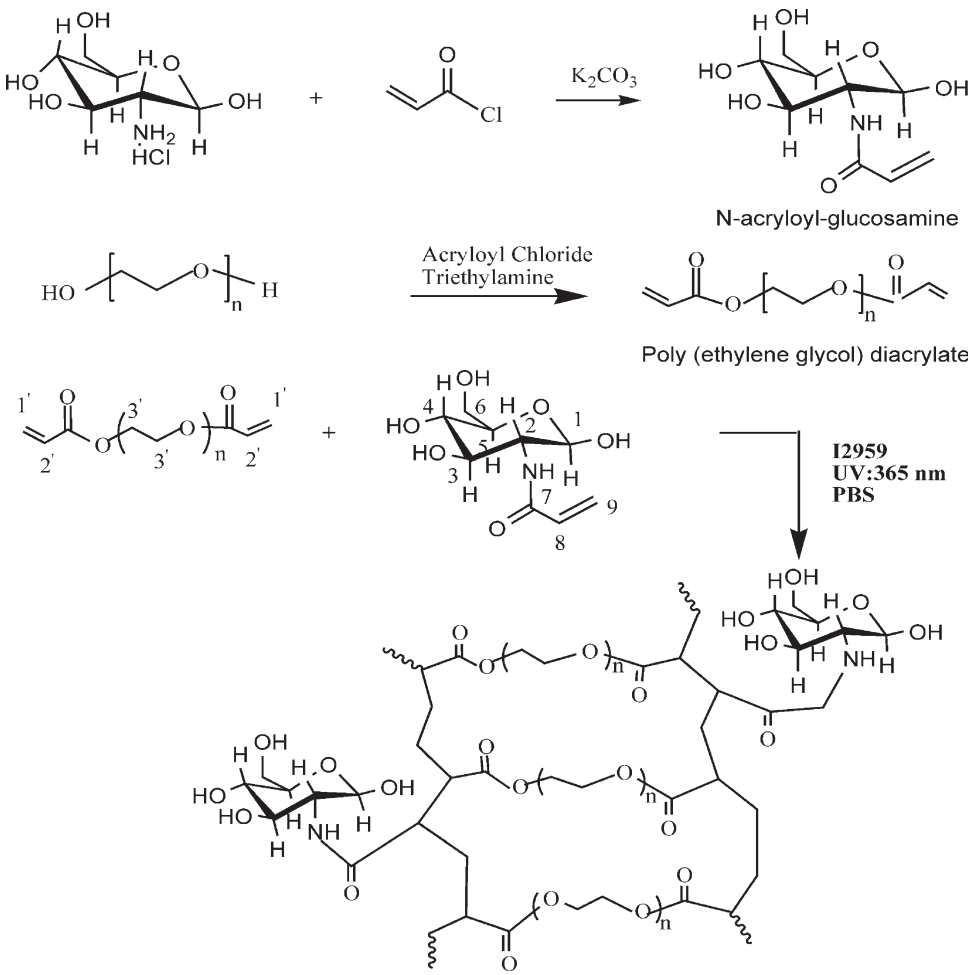

Figure 1. Photo-crosslinking reaction for fabrication of glucosamine modified PEGDA hydrogel.

illustrated in Figure 1. Poly(ethylene glycol) with two terminal acrylate groups was a divinyl macromer, while amino group in GlcN was acrylated to form a vinyl monomer. ^{1}H NMR spectra of the precursor solution showed typical resonance signals of terminal vinyl protons of acryl group [Figure 2(c): chemical shift of CH_2=CH— in PEGDA: 5.90–6.38 ppm; chemical shift of CH_2=CH— in AGA: 6.09–6.24, 5.67–5.68 ppm]. After UV light irradiation, the absorbance signals of vinyl protons in ^{1}H NMR spectra disappeared [Figure 2(d)]. ^{1}H NMR spectra results indicated that carbon–carbon double bonds of PEGDA and AGA monomers were photo-polymerized in presence of initiator I2959.

FTIR-ATR results (Figure 3) exhibited that the PEGDA/AGA copolymer possessed three characteristic absorbance bands of PEGDA backbone at 2910, 1728, and 1100 cm^{-1}, which were assigned to stretching vibration of CH_2, C=O, and C—O, respectively. In addition, the PEGDA/AGA copolymer lyophilized hydrogels exhibited three new bands at 3390, 1646, and 1548 cm^{-1}. The first was assigned to OH and NH, and the latter two were attributed to the stretching vibrations I and II of amide carbonyl groups, respectively. FTIR-ATR results confirmed that AGA was successfully copolymerized with PEGDA.

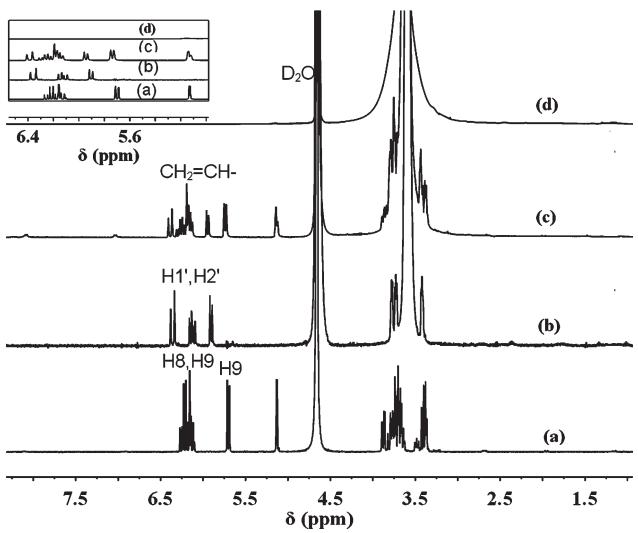

Figure 2. ^{1}H NMR spectra of PEGDA/AGA in D_2O: (a) AGA; (b) PEGDA; (c) PEGDA+5 mM AGA precursor solution; (d) PEGDA/5 m*M* AGA gel.

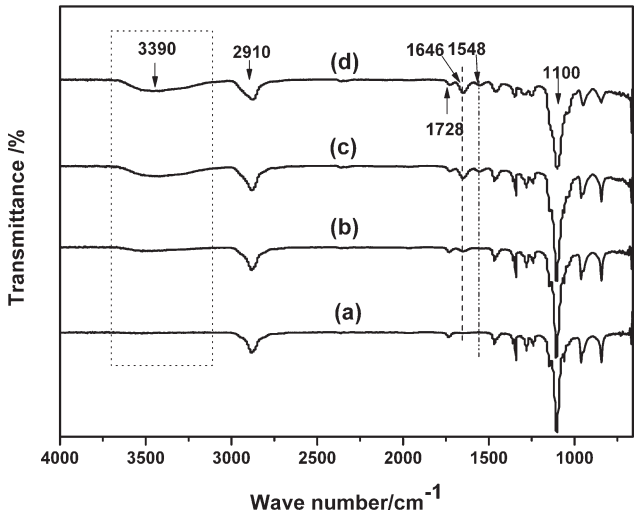

Figure 3. ATR-FTIR spectra of PEGDA/AGA dry gels:(a) PEGDA; (b)PEGDA/2 m*M* AGA; (c) PEGDA/5 m*M* AGA; (d)PEGDA/10 m*M* AGA.

Upon photo-polymerization of PEGDA and AGA, a final network structure with polyacrylate kinetic chains connected with PEG and GlcN was obtained.[31]

The equilibrium swelling ratios and sol fraction of PEGDA, PEGDA/2 m*M* AGA, PEGDA/5 m*M* AGA, and PEGDA/10 m*M* AGA were showed in Table I. With the increase of AGA concentration in the initial precursor solution, the swelling ratios of hydrogels were decreased. Property of hydrogels was strongly affected by various kinds of inter- or intramolecular interaction, such as hydrogen-bonding interaction, hydrophobic interaction, electrostatic interaction, etc.[32] In PEGDA/AGA hydrogel net, the

Table I. Effect of AGA Concentration in PEGDA/AGA on Sol Fraction and Swelling Ratio

PEGDA [%(w/w)]	AGA (m*M*)	Swelling ratio	Sol fraction (%)
10	0	12.5 ± 0.9	12.8 ± 0.3
10	2	11.6 ± 0.3	11.1 ± 0.4
10	5	10.4 ± 0.1	13.4 ± 0.4
10	10	9.4 ± 0.8	13.6 ± 0.4

Data reported as mean ± standard deviation.

oxygen atoms of PEG chain is an acceptor, the hydroxyl of GlcN groups is a donor. And the hydrogen-bond is formed between PEG chain and GlcN group. To a certain extent, the formation of intramolecular hydrogen-bond in PEGDA/AGA gel net led to decreasing the swelling capability of gels.[33] Thereby, the swelling ratios of hydrogels were decreased. There was no significance in sol fraction of various samples. The components of leaching solution were measured by high performance liquid chromatography (HPLC) (the data were not listed here.). And HPLC results verified that sol fraction could be removed completely just after changing solvent once.

The SEM examination of the lyophilized hydrogels cross-section illustrated that these materials were porous and contained some interconnected pores network (Figure 4). The pore size of GlcN-modified PEGDA gel was relatively uniform and ranged from 30 to 200 μm. Nevertheless, most of the pores in pure PEGDA gel were dispersed and large. Several investigations confirmed that the properties of hydrogels depended on the monomer concentration of the initial mixtures.[34,35] With total higher initial monomer concentration, swelling ratio would decrease, and the hydrogels would have a tighter network structure. The properties of GlcN-modified PEGDA gels showed analogous to

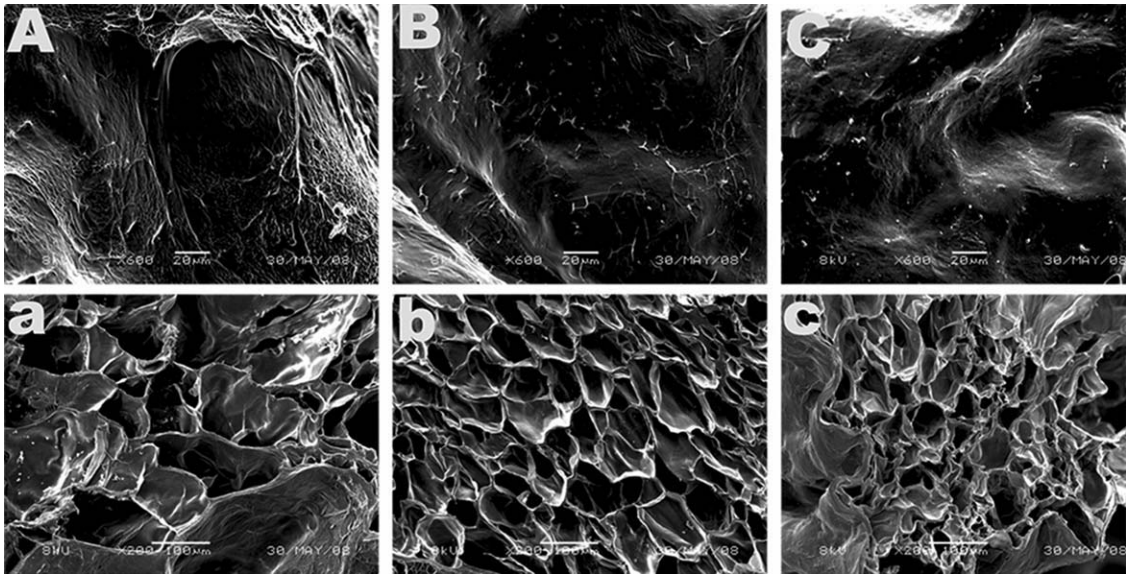

Figure 4. SEM micrographs of dried hydrogels: (A) the surface of PEGDA hydrogel; (B) the surface of PEGDA/5 m*M* AGA hydrogel; (C) the surface of PEGDA/10 m*M* AGA hydrogel. Images of all hydrogels surface were acquired at ×600 magnification, the scale bar was 20 μm. (a) the cross section of PEGDA hydrogel; (b) the cross section of PEGDA/5 m*M* AGA hydrogel; and (c) the cross section of PEGDA/10 m*M* AGA hydrogel. Images of all hydrogels cross section were acquired at ×200 magnification, Scale bar = 100 μm.

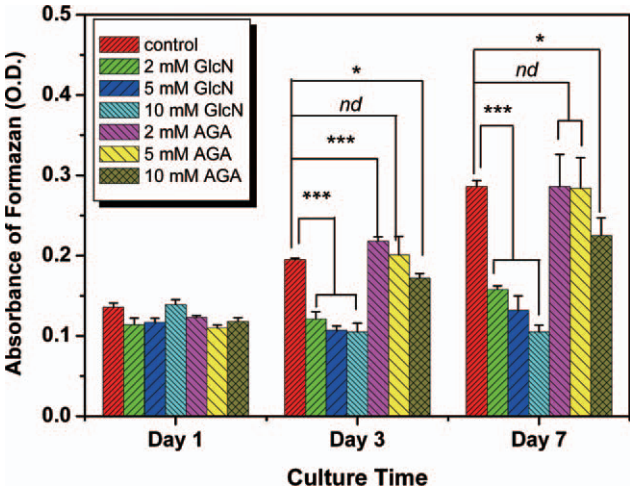

Figure 5. Rat BMScs proliferation in presence of various concentration of glucosamine hydrochloride and *N*-acrylol-glucosamine after 1, 3, and 7 day incubation at 37°C, BMSCs standard media as a control. $***P < 0.0005$; $*P < 0.05$; and nd means no significant difference. Cell toxicity evaluation of monomer was performed by MTT assay. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

previous studies. SEM photos of the lyophilized hydrogel surface showed that there was no obvious difference between PEGDA and PEGDA/AGA hydrogels. PEGDA was the major component of PEGDA/AGA hydrogels, so the surface morphology of lyophilized hydrogels was maintained as PEGDA gels.

Cells Culture *In Vitro*

Cytotoxicity of N-Acryloyl-Glucosamine. Studies have reported that the exogenous GlcN inhibited cell proliferation. The inhibitory effect of GlcN on proliferation exhibited a dose-dependent manner.[17,18,36] The cytotoxicity of AGA monomer with different concentrations (2, 5, and 10 m*M*) was tested via MTT method. Also the results were compared with those of adscititious GlcN under the same experimental condition. As shown in Figure 5, presence of GlcN (2, 5, and 10 m*M*) resulted in significant decrease of cell metabolic activity with increasing of GlcN concentration during the whole incubation period. This decrease in cell proliferation was statistically significant between GlcN group and control ($P < 0.005$). Interestingly, the MTT results of AGA monomer showed entirely different metabolic activity of BMSCs. The presence of 2 m*M* AGA promoted proliferation of cells significantly at Day 3. When the concentration of AGA was increased to 5 m*M*, there was no significant difference between control and 5 m*M* AGA groups. After 7 days incubation, there was no significance in cell metabolic activity between control and 2 or 5 m*M* AGA groups. However, the increase of the concentration to 10 m*M* AGA monomer showed a remarkable reduction ($P < 0.05$). The results of MTT indicated that exposure to GlcN inhibited cells proliferation, which was consistent with those obtained by other research groups.[17,37] The inhibitory effect of the GlcN molecule was attributed to the unsubstituted amino group. Therefore, the addition of AGA (2 and 5 m*M*) did not result in inhibitory effect of GlcN. Stolzing et al. reported high glucose in medium increased apoptosis and a decreased rate of rat MSCs proliferation.[38] AGA is a derivant of glucose, and as a result, increasing concentration of AGA will inhibit proliferation of rat MSCs. However, as a cells carrier, monomer or unreacted residual should be biocompatible. MTT results demonstrated that AGA showed no cytotoxicity but facilitated proliferation of BMSCs when AGA concentration was 2 and 5 m*M*.

Cell Encapsulation Studies. The MTT assay is unsuitable to evaluate metabolic activity of encapsulated cells because of producing a water-insoluble purple formazan upon in the presence of living cells. Therefore, CCK-8 agent, which is reduced to a water-soluble formazan dye in viable cells, was adopted to assess the proliferation of encapsulated cells. The data of metabolic activity of encapsulated BMSCs were shown in Figure 6. The number of viable cells in PEGDA hydrogel was remarkably reduced during the period of culture. Nevertheless, metabolic activity in PEGDA/AGA (10 m*M*) group was significantly higher than those in control group ($P < 0.005$). Nuttelman et al. confirmed that the viability of human MSCs encapsulated in PEG gel dropped to 60% after 1-week culture in BMSCs basal media.[39] Our results agreed with their study in PEGDA hydrogel. However, in our study, we found that the number of cells increased in PEGDA/AGA (10 m*M*) hydrogels after 1 week, and remained ~95% in PEGDA/AGA (10 m*M*) hydrogel during culture period. Sol fraction results indicated that not every AGA molecule was incorporated into the hydrogel network during photo-polymerization. When the hydrogel immersed in media, unreacted AGA would be released from the hydrogel. However, the unreacted components were removed via changing medium. The HPLC results of leaching solution confirmed it. Thus, the effect of sol fractions on cells should not be considered.

Images of live/dead staining in two experimental groups were shown in Figure 7. BMSCs were encapsulated within PEGDA

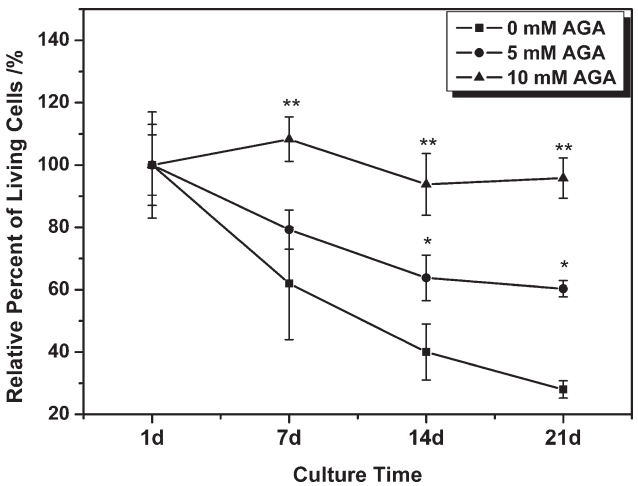

Figure 6. Metabolic activity of the encapsulated cells after 1, 7, 14, and 21 days' incubation. There were three groups in total—(1) BMSCs encapsulated into PEGDA hydrogel (0 m*M* AGA group); (2) BMSCs encapsulated into PEGDA/5 m*M* AGA hydrogel (5 m*M* AGA group); (3) BMSCs encapsulated into PEGDA/10 m*M* AGA hydrogel (10 m*M* AGA group) $**P < 0.005$; $*P < 0.05$. Metabolic activity of the encapsulated cells was assessed by CCK-8 agent.

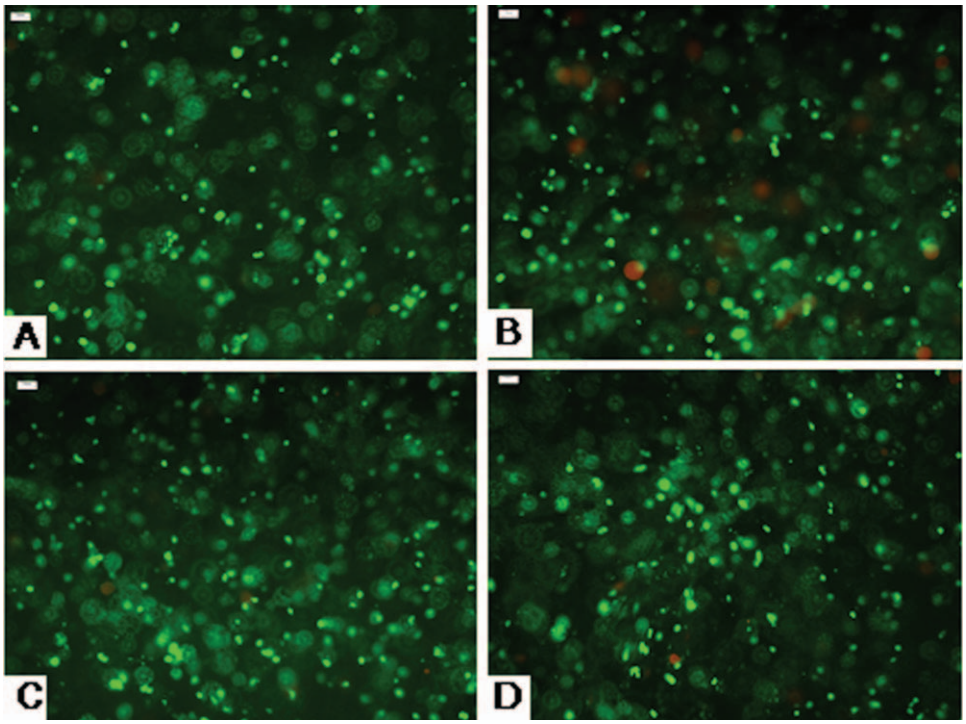

Figure 7. Images of live/dead fluorescent staining after 14-day incubation. Green and red staining indicated viable and dead cells, respectively. Scale bar = 50 μm. (A, B) BMSCs encapsulated in PEGDA hydrogels, (C, D) BMSCs encapsulated in PEGDA/5 m*M* AGA and PEGDA/10 m*M* AGA gels, respectively. A, C, D incubated with standard BMSCs medium. B incubated with standard medium containing 2 m*M* glucosamine hydrochloride. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and PEGDA/AGA hydrogels and examined after 14 days culture. There were >95% of viable cells in PEGDA hydrogel without GlcN treatment [Figure 7(A)], while only 60% of cells were observed viable in PEGDA hydrogel treated with 2 m*M* GLcN [Figure 7(B)]. However, the amount of live cells in PEGDA/AGA hydrogels [Figure 7(C,D)] was comparable to that in pure PEGDA hydrogel. And the effect of AGA concentration (5 and 10 m*M*) on cells viability showed insignificant.

Live-dead staining images directly reflected the cells viability in hydrogels. The viable cells were <60% in GlcN-treated group, demonstrating that 2 m*M* GlcN was harmful to cells. Interestingly, BMSCs retained vigorous vitality and high cells density in GlcN-modified hydrogels.

Carbohydrates are a major component of the extracellular matrix, such as glycosaminoglycans. They form a microenvironment (or "niche") for stem cells and play many active roles in determining cell normal function.[40] Accordingly, GlcN group crosslinked with PEGDA improved the interaction between rat stem cells and PEGDA hydrogels, moreover, facilitated their survival in these hydrogels.

Based on the results obtained from MTT analysis and live-dead staining study, it could draw a conclusion that GlcN-modified hydrogels by our means were safe and promising to apply *in situ*. Hydrogels could make BMSCs keep in spherical morphology, which mimic the native condition of chondrocytes.[41] Moreover, GlcN was found to be effective for chondrogenesis of embryonic stem cells.[17] Therefore, GlcN-modified hydrogels would be a potential scaffold for cartilage regeneration. In the future, we expect to perform more detailed cell studies using this novel hydrogels.

CONCLUSIONS

GlcN-modified hydrogel was synthesized by photopolymerizing *N*-acrolyl-glucosamine monomer with PEGDA macromer. Both FTIR-ATR and ^{1}H NMR data demonstrated that GlcN was successfully incorporated into PEGDA hydrogel network. The equilibrium swelling ratios decreased with the increase of GlcN ratio. MTT results showed that 2 and 5 m*M* *N*-acroloyl-glucosamine monomer exhibited no toxicity against rat bone marrow stromal cells (BMSCs), while with the concentration increased to 10 m*M*, cell viability appeared to decrease. BMSCs was successfully encapsulated in GlcN-modified PEG hydrogels and showed favorable viability. Metabolic activity of the encapsulated cells and live-dead staining revealed that BMSCs proliferated better in 10 m*M* GlcN-modified hydrogels than those in PEGDA. We believe that GlcN-modified PEG hydrogels will be a potential cell carrier for cartilage regeneration.

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