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Photopolymerized Hydrogel Composites from Poly(ethylene glycol) and Hydroxyapatite for Controlled Protein Delivery *In Vitro*

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ABSTRACT: The incorporation of hard particles into soft hydrogels can improve the mechanical properties and provide necessary bioactivity to the hydrogels for desired biomedical applications. Hydrogel composites containing hydroxyapatite (HA) are promising materials for orthopedic applications. In this study, injectable poly(ethylene glycol) (PEG) hydrogel precursor solutions containing HA particles and model protein bovine serum albumin (BSA) were synthesized *in situ* by photopolymerization. *In vitro* BSA release properties from the hydrogel composites containing various amounts of HA were investigated and discussed. Fourier transform infrared spectroscopy and scanning electron microscopy were employed to investigate the interaction between HA and the hydrogel network and the morphology of the hydrogel composites. It is found that PEG hydrogel composites containing HA sustained the release of BSA for at least 5 days and the presence of HA slowed down BSA release. Photopolymerized hydrogel composites containing HA may find potential use as a drug delivery matrix for orthopedic tissue engineering. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

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INTRODUCTION

Hydrogels are three dimensional polymeric networks that can absorb large amount of water but remain insoluble in water due to the formation of chemical and/or physical crosslinks between polymer chains. Hydrogels have found tremendous application in the biomedical field for tissue engineering, drug delivery, and biosensors.[1–5] Injectable hydrogels are of special interest due to their convenience and benefits associated with minimally invasive surgery.[6–9] Injectable hydrogels have low viscosity and/or shear-thinning properties as sols for easy injection via a needle. Forming hydrogels by the photopolymerization of sols is advantageous because the reaction is fast and there is on demand control over the initiation and termination of the reaction by controlling the light source.[10]

Versatile applications of photopolymerized hydrogels have been demonstrated as reviewed previously.[11,12] For example, Anseth and coworkers extensively investigated the photopolymerization of hydrogels for cell encapsulation, drug delivery, and tissue engineering.[13–17] Hennink and coworkers evaluated the effect of photopolymerization on stem cells embedded in hydrogels and the authors demonstrated that the viability of the encapsulated cells in the hydrogel is not adversely affected by photopolymerization.[18] We have previously shown that photopolymerized pH and glucose sensitive hydrogels can be synthesized *in situ* to construct microfabricated continuous glucose monitoring system.[19] Photopolymerization of poly(ethylene) glycol (PEG)-based hydrogels are perhaps the most intensively studied materials. Adhesion peptide functional groups such as Arg-Gly-Asp (RGD) have been frequently introduced into PEG hydrogels to increase its affinity to cells for tissue engineering applications.[15,20,21]

Hydrogels synthesized from photopolymerization of PEG, however, do not have sufficient mechanical properties for certain biomedical applications. Recently, efforts have been made to address the drawbacks of the PEG hydrogel by incorporating particles to make hydrogel composites with enhanced mechanical properties.[22–25] Hydrogel composites have been increasingly studied due to their synergistic benefits offered by each component. Hydroxyapatite (HA) has been frequently used in bone and dental applications as HA is a major component of bone and tooth enamel. Recent studies have confirmed the feasibility of photopolymerized composite biomaterials for soft tissue restoration in both rodents and a pilot clinical testing in human patients.[26] Previously it was shown that the incorporation of HA into PEG hydrogels via photopolymerization not only enhanced the hydrogels' mechanical properties but also provided osteoconductivity to the hydrogel as demonstrated by preosteoblast attachment and spreading onto the hydrogel

composites.[23] Drug release from photopolymerized PEG hydrogels has been studied previously by others.[13,27,28] However, drug release from photopolymerized PEG hydrogel composites containing particles has been rarely investigated and no protein release study has been performed on photopolymerized PEG/HA hydrogel composites.

In this work, we developed photopolymerized hydrogel composites composed of PEG and HA. These composites have the potential to act as bone tissue scaffolds as well as delivery matrices for growth factors such as bone morphogenetic protein 2 (BMP-2). We used the bovine serum albumin (BSA) as a model protein to study protein release from the PEG hydrogels and their composites. Fourier transform infrared spectroscopy (FTIR) was employed to investigate the interaction between HA and PEG. Scanning electron microscopy (SEM) was used to investigate the structures of the hydrogel composite and HA. The effect of HA on the protein release was presented and discussed in the article.

MATERIALS AND METHODS

Materials

PEG with a molecular weight M_w of 10,000, acryloyl chloride, triethylamine, and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich St. Louis, MO. BSA (Alexa Fluor 488 conjugate) was purchased from Invitrogen Grand Island, NY. HA was also procured from Sigma-Aldrich. Irgacure 2959 photoinitiator was obtained from Ciba Chemicals Tarrytown, NY.

PEG-diacrylates (PEGDA) were synthesized by methods developed by Cruise et al.[29] Fifty grams of PEG (M_w 10,000) were dissolved in 750 mL of benzene in a 1000-mL round-bottom flask and 250 mL of benzene were azeotropically distilled to remove water. Triethylamine, in four-fold molar excess over the hydroxyl groups of PEG, was added in one portion to the flask and four-fold molar excess of acryloyl chloride over hydroxyl groups of PEG was added drop-wisely to the reaction mixture. The reaction was allowed to proceed under stirring and nitrogen for overnight at 35°C. Thereafter, the mixtures were filtered to remove the insoluble triethylamine salts and the PEGDA were precipitated out by adding 1.4 L cold diethyl ether. The white PEGDA were collected by filtration. The products were redissolved in 100 mL methylene chloride and then reprecipitated by adding 1.4 L cold diethyl ether. The polymers were dried for several days in a vacuum oven at room temperature.

Preparation of PEG/Hydroxyapatite Nanocomposite Hydrogels

PEG/HA hydrogel nanocomposites containing HA were photopolymerized between two glass slides separated by a 0.8 mm thick Teflon spacer. First, stock solutions of PEGDA (40 wt % in PBS), BSA (2.5 mg/mL in water), and Irgacure 2959 (0.4 wt % in PBS) were prepared. Appropriate amount of PEGDA, HA, and Irgacure 2959 were added to a vial and the mixture was vortexed for 10 min and then sonicated for 20 min. For example, to make a PEG/HA composite containing 15 wt% HA, 0.75 mL PEGDA, 0.325 mL Irgacure 2959, and 0.225 g HA were combined. BSA (0.2 mL) was then added and the mixtures were briefly vortexed before they were injected into a cavity between two glass slides separated by the Teflon spacer. Alexa Fluor 488 conjugated BSA was chosen due to its superior photostability and, therefore, ease of handling and detection. The cavities containing the compositions were exposed to a UV lamp with a maximum intensity of 21.7 mW/cm^2 (UVP, B-100AP, Ultra-Violet Products, 365 nm) for 20 min. The hydrogel composites were removed from the cavity and cut using a biopsy punch (1 cm diameter) to obtain circular-shaped hydrogel composites.

Fourier Transform Infrared Spectroscopy Study of Hydrogel Composites

Photopolymerized PEG hydrogel and PEG/HA composites samples were dried for FTIR analysis. PEG hydrogel, PEG/HA composites, and HA were placed on the top of a diamond surface for attenuated total reflectance FTIR (Thermo Scientific Nicolet iS10 FTIR Spectrometer) measurements.

Protein Release from Hydrogel Nanocomposites

PEG/HA hydrogel composites loaded with BSA were placed in a 20 mL vial and 5 mL PBS (50 m*M*, pH 7.4) was used as the eluting solution. The experiments were performed in triplicate. The vials were placed in a water bath at 37°C with gentle agitation. At certain time points, 0.2 mL of supernatant PBS buffer was collected and replaced with an equal amount of fresh PBS. The cumulative protein release was quantified by fluorescent assay. Briefly, 0.1 mL of the sample solutions were placed in a 96-well plate for fluorescence quantification using a plate reader (CytoFluor Series 4000) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. The protein concentrations in the samples were determined using a standard BSA calibration curve constructed from known BSA concentrations.

Hydrogel Composites Water Content

PEG/HA hydrogel composites were allowed to swell for several days in PBS buffer and the weight of the hydrogels (W_{wet}) were measured after their surface water was lightly blotted with Kimwipes. The hydrogel composites were then dried (W_{dry}) in a vacuum oven at about 50°C for overnight. The water content of the hydrogel composites was determined based on the ratio of $(W_{wet} - W_{dry})/W_{wet}$.

Scanning Electron Microscopy of Hydroxyapatite and Hydrogel Composite

A HA suspension was prepared in water at 1 mg/mL and one drop of the HA suspension was placed on the surface of a clean glass slide and dried overnight prior to SEM imaging (FEI Helios Nanolab 600i Dual Beam). A PEG hydrogel composite containing 5 wt % HA was first allowed to swell in deionized water for several days with daily water change to reach equilibrium. The hydrogel composite was then quenched in liquid nitrogen and freeze-dried for two days before SEM imaging (FEI Quanta 3D FEG).

RESULTS AND DISCUSSION

Table I lists the compositions of the hydrogel composites used in this study. The amount of BSA loaded in each composite was constant (0.5 mg). The size of the HA used in this study was 1–3 μm with a small population of ~20 μm particles as seen in Figure 1. The particles consisted of aggregates of HA needles as seen from the right image of Figure 1. Figure 2 shows SEM

Table I. Hydrogel Compositions Used in This Study

Samples	HA (wt %)	PEGDA 10,000 (wt %)	Water (wt %)
0% HA	0	20	80
1% HA	1	20	79
5% HA	5	20	75
15% HA	15	20	65

images of the PEG/HA hydrogel composite containing 5 wt % HA at various magnifications. Figure 2 shows that the hydrogel composite has a broad range of pore sizes ranging from less than 10 nm [Figure 2(d)] to several μm.

Figure 3 shows FTIR data for HA, PEG hydrogel, and their composites. The PEG/HA hydrogel composite preserved the characteristic peaks of both HA and PEG hydrogel. The peaks shown around 1096 and 1060 cm^{-1} are attributed to the stretching of ether groups from PEG.[30] The peak at 1021 cm^{-1} corresponds to the vibrations of phosphate groups from HA.[31,32] One can note that the characteristic peaks from PEG shifted slightly in the PEG/HA composite, suggesting the presence of hydrogen bonding between HA and PEG hydrogels.

Figure 4 presents the hydrogel composite water content prior to swelling and after equilibrium in PBS as a function of HA. The data show that the PEG hydrogel and PEG/HA composites exhibited significant swelling in PBS. Linear regression analysis indicates that the equilibrium water content of the swollen PEG/HA composites has a linear and inverse relationship with the amount of HA. Higher HA amounts result in lower equilibrium water contents.

Hydrogel swelling equilibrium is reached when the total free energy reaches a minimum value or when the chemical potential of each mobile species becomes the same in the coexisting phases.[33] It is well-known that PEG can form hydrogen bonds with water. Decreased hydrogel water content with increasing amount of HA suggests that fewer sites from PEG polymer chains are available to form hydrogen bonds with water. Since some of the PEG sites have already formed hydrogen bonds with HA, as suggested by FTIR shown in Figure 3, there are fewer sites from PEG that can interact with water to form hydrogen bonds than pure PEG hydrogels. We speculate that the most likely hydrogen bonds are formed between hydroxyl groups from HA and hydroxyl groups from PEG. Meanwhile, HA may act as fillers to occupy the void spaces within the hydrogel and therefore lead to a more compact hydrogel that has less swelling capabilities compared to pure PEG hydrogels.[23] Another factor that can influence the degree of hydrogel swelling is the osmotic swelling pressure of the hydrogel composites. We have recently shown that the HA surfaces were negatively charged as measured by zeta potential.[32] The presence of negative charges on the surface of HA makes the hydrogel composite comparable to polyelectrolyte hydrogels. This should increase the degree of swelling for the hydrogels due to the osmotic swelling pressure primarily originating from the mobile counter-ions within the hydrogels. However, the overall degree of swelling of the hydrogel decreased with increasing HA amount, suggesting that the degree of swelling is dominated by hydrogen bonding and HA's filler effect.

Figure 5 shows the BSA release kinetics from PEG hydrogels and PEG/HA composites. The fastest release came from the PEG hydrogels with 20 wt % released by 120 h. The release rates decreased with increasing HA amount from 1 to 5 wt % and then increased with 15 wt % HA.

Diffusion rates of proteins within hydrogels can be affected by the size of the protein, molecular weight between crosslinks, degree of hydrogel swelling, hydrogel crosslinking density, and hydrogel network defects.[34–36] There is no significant initial burst release for all of the samples suggesting that there is little BSA on the hydrogel surface. The cumulative release of BSA (20 wt %) from our PEG hydrogel is comparable to other studies of

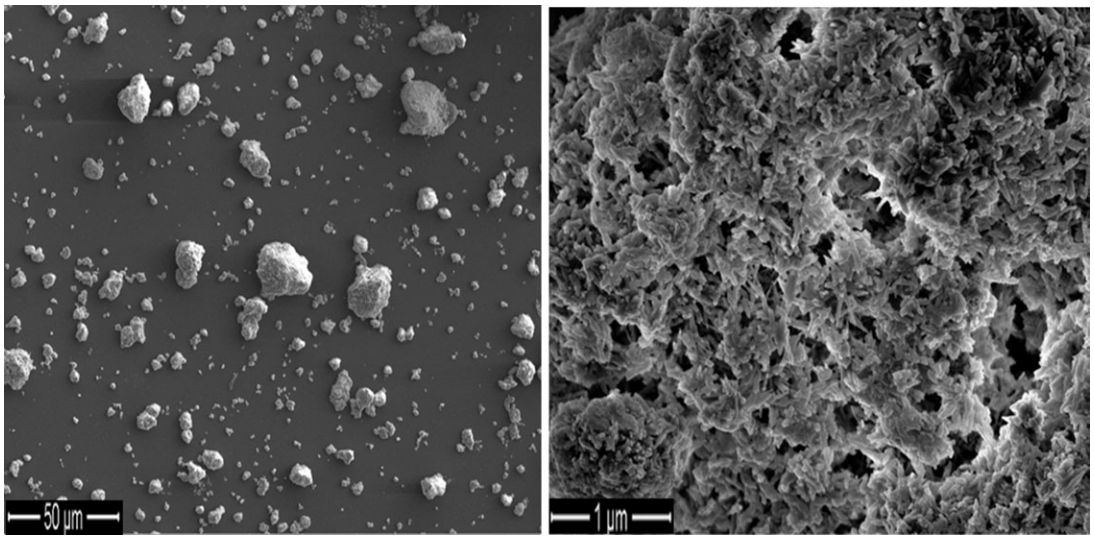

Figure 1. SEM images of HA showing HA particles (left) and one particle (right).

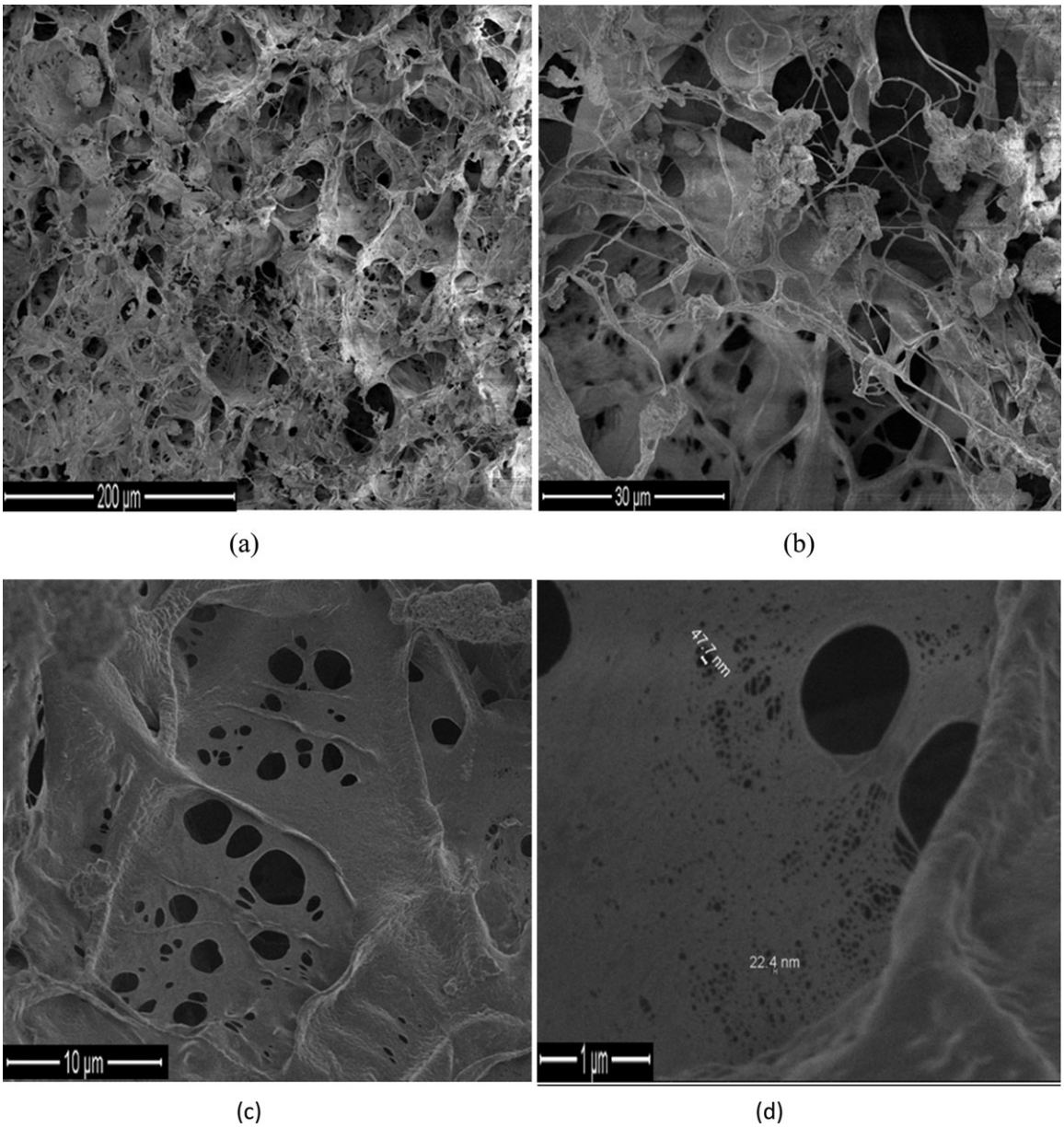

Figure 2. SEM of PEG/HA hydrogel composites at increasing magnification from (a) to (d).

protein release from photopolymerized hydrogels.[27,28] The decreased cumulative release of protein can be attributed to protein aggregation, denaturation, or protein conjugation to the polymer network and the encapsulated particles. We attribute the sustained and slow release of BSA to its large size (4.2 nm diameter by 14.4 nm length[37]) and to the comparable or even smaller pore sizes of hydrogels seen in Figure 2(d). We expect that the cumulative release will increase with increasing concentration of protein in the hydrogel. Earlier work has shown that higher amount of protein loaded into PEG hydrogel resulted in higher cumulative release.[27] It is likely that there is some degree of reaction of BSA with the PEG polymer during the photopolymerization process. Lin and Metters[28] have shown that the free radicals generated from photoinitiator during photopolymerization may react with the highly reactive N-terminal residues of BSA to form protein–polymer conjugates. Protection of proteins during photopolymerization may be achieved by adding an appropriate amount of radical scavenger such as vitamin C, transfection agents, and metal chelating ligand.[28,38]

As seen in Figure 5, the presence of HA reduced the release kinetics of BSA from the PEG hydrogels. Hydrogel water content is an important factor that affects the release behavior of the encapsulated drugs. Figure 4 shows that the hydrogel equilibrium water content decreased with increasing HA content. This contributes at least partially to the reduced BSA release for hydrogel composites containing HA as compared to pure hydrogel. Another reason for the reduced BSA release from hydrogel composite is that the potential adsorption of BSA onto the HA

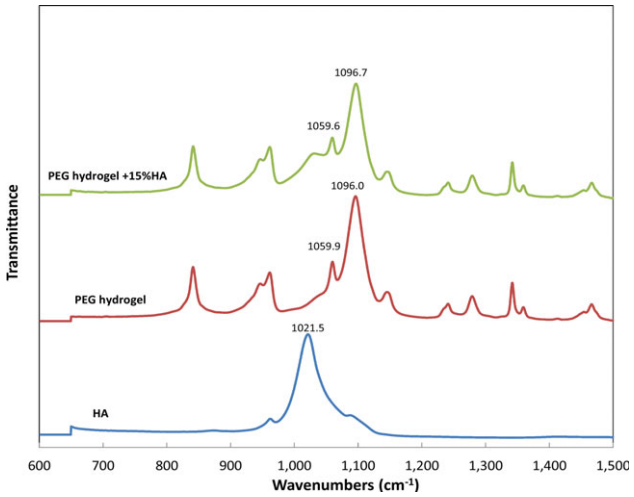

Figure 3. FTIR of HA, PEG hydrogel, and PEG hydrogel/HA composite. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

surfaces as demonstrated by Boonsongrit et al.[39] It is interesting to note, however, that the slowest BSA release was observed at 5 wt % instead of at 15 wt %. There are several possible reasons for the enhanced release of BSA from the 15 wt % HA PEG/HA composites. As the turbidity of the precursor solutions comprising of PEGDA and HA increased with increasing amount of HA, more UV light might be blocked for the hydrogel composites containing 15 wt % and therefore more network defects were generated during synthesis. Network defects can promote the diffusion of the solute in the hydrogel and further defects can be intentionally induced by encapsulating particles.[40–42] Another contribution might be the initial degree of swelling of the hydrogel upon immersion in PBS eluting buffer solution. As seen in Figure 4, hydrogel water content difference between prior to swelling and after equilibrium increased with increasing amount of HA. The hydrogel composite containing 15% HA

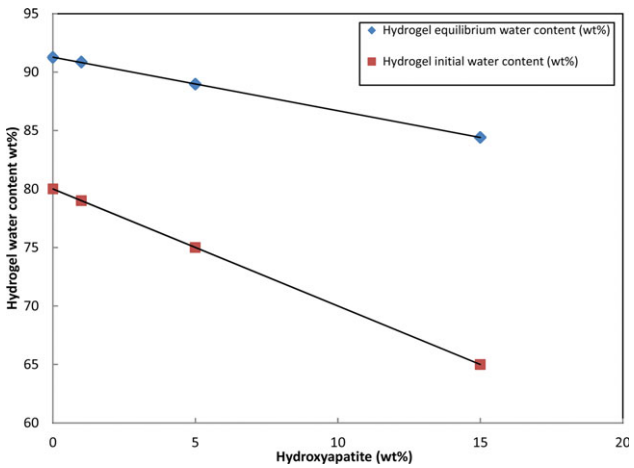

Figure 4. Water content of PEG hydrogel and PEG//HA hydrogel composites prior to swelling and after equilibrium in PBS buffer (50 m*M*). Lines were fit to the data by linear regression analysis. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

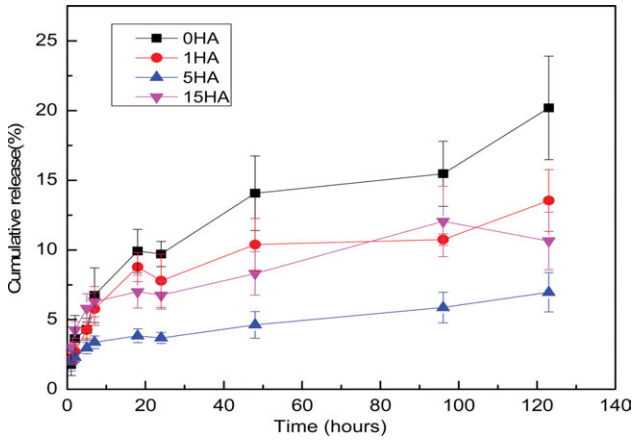

Figure 5. BSA release from 0.333 mg/mL BSA in photopolymerized hydrogel composites containing various amounts of HA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

has the largest water content change of 19.4%, whereas the water content changes for 0% HA, 1% HA, and 5% HA are 11.2%, 11.9%, and 14%, respectively. It is also possible that the larger amount of HA created larger pores for the hydrogels so the BSA diffusion from 15 wt % is faster than that from 5 wt%.

CONCLUSIONS

This work demonstrates the formation of PEG/HA photopolymerizable hydrogels that allow the controlled release of proteins. The hydrogel composites are suitable for sustained BSA release for at least 5 days. Protein release can be tailored by controlling the HA amounts. The presence of 1 and 5 wt % HA reduced the BSA release but at higher HA concentration (15 wt %) the release is increased due to increased network defects and increased swelling at higher HA concentrations. These materials have promise as injectable, osteogenic scaffolds, and drug delivery matrices for bone tissue engineering.

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