Hollow, pH-Sensitive Calcium–Alginate/Poly(acrylic acid) Hydrogel Beads as Drug Carriers for Vancomycin Release

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ABSTRACT: In this study, hollow calcium–alginate/poly(acrylic acid) (PAA) hydrogel beads were prepared by UV polymerization for use as drug carriers. The hollow structure of the beads was fortified by the incorporation of PAA. The beads exhibited different swelling ratios when immersed in media at different pH values; this demonstrated that the prepared hydrogel beads were pH sensitive. A small amount (<9%) of vancomycin that had been incorporated into the beads was released in simulated gastric fluid, whereas a large amount (≤67%) was released in a sustained manner in simulated intestinal fluid. The observed drug-release profiles demonstrated that the prepared hydrogel beads are ideal candidate carriers for vancomycin delivery into the gastrointestinal tract. Furthermore, the biological response of cells to these hydrogel beads indicated that they exhibited good biological safety and may have additional applications in tissue engineering. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 1878–1886, 2010

Key words: drug delivery systems; hydrogels; stimuli-sensitive polymers

INTRODUCTION

Vancomycin is a glycopeptide antibiotic used in the prophylaxis and treatment of infections caused by Gram-positive bacteria. However, it is seldom used for the first-line treatment of *Staphylococcus aureus* infections.[1,2] One of the reasons for this is that vancomycin must be administered intravenously because it cannot cross the intestinal lining.[3] Vancomycin is a large hydrophilic molecule that exhibits poor diffusion across the gastrointestinal mucosa. Oral vancomycin therapy is only indicated for the treatment of pseudomembranous colitis, in which case it can reach the site of infection in the colon only if administered orally.[4] However, its absorption efficiency in this form is considerably low (<5%). To increase the efficiency of vancomycin application, research efforts have been directed toward the development of formulations for its sustained and controlled release. Attempts to use antibiotic-loaded beads for oral drug delivery have proven fruitful for the treatment of gastric diseases such as peptic ulcers[5–7] and ulcerative colitis, carcinomas, and intestinal infections.[8,9]

In this study, we developed a system involving the use of pH-sensitive hydrogel beads composed of alginate (Alg) and poly(acrylic acid) (PAA) for the oral delivery of vancomycin. Hydrogels that have a water content of more than 20% are three-dimensional hydrophilic polymer networks that swell instead of dissolve when placed in biological fluids [e.g., simulated gastric fluid (SGF) and simulated intestinal fluid (SIF)] or water because of their chemical or physical crosslinks.[10] Because of the characteristic swelling/deswelling behavior exhibited by hydrogels with changes in their environment (e.g., temperature and pH changes), these remarkable systems have been extensively applied in drug-release techniques.[11,12]

Alg is a natural polyacid that possesses unique gel-formation properties, in that it forms crosslinks with multivalent cations such as Ca^{2+} to produce an egg-box structure.[13,14] If an Alg hydrogel is soaked in an environment containing monovalent cations such as Na^{+}, these monovalent cations substitute for the multivalent cations in the gel and thus bring about its decomposition.[15] Such Alg degradation cannot be controlled and may limit the application of Alg in controlled drug release. The combination of Alg and PAA to form hydrogels has been studied extensively in recent years. Hua and Wang[16] found that the introduction of sodium humate into the Alg-*g*-PAA system could enhance the water absorbency and the superabsorbent containing 10 wt % sodium humate acquired the highest water absorbency. Yin et al.[17] synthesized hydrogels by the graft crosslink copolymerization of Alg and acrylic acid

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(AAc) with *N*,*N*′-methylenebisacrylamide (NMBA) as a crosslinker. They found that an overshooting effect was observed in acidic media; that is, the gels first swelled to a maximum value followed by a gradual deswelling until equilibrium. Two different chemical strategies for the introduction of acrylic moieties (acidic and basic) onto sodium–alginate (Na–Alg) were investigated by Laurienzo et al.[18] An interpolymer complex based on electrostatic interactions was created, and these interactions were able to modulate the release rate of low-molecular-weight drugs. Insulin-loaded Alg/cysteine/PAA/cysteine microparticles were prepared and evaluated *in vitro* by Greimel et al.[19] These microparticles were obtained by ionic gelation and stabilized via disulfide bonds. They showed the highest drug loading, and they could achieve the controlled release of insulin over 3 h in SIF.

In this study, hollow, pH-sensitive calcium–alginate (Ca–Alg)/PAA hydrogel beads were prepared by UV polymerization for use as gastrointestinal tract drug carriers. The hollow structure of the beads was fortified by the incorporation of PAA. The ability of PAA to stabilize Alg renders it an attractive candidate as a carrier in controlled drug-release systems. We investigated the swelling ratio, water content, and mechanical properties of the Alg hydrogel and the *in vitro* release profile of vancomycin from these hydrogel beads in SGF and SIF. In addition, the biological response of cells to these hydrogel beads was examined to investigate their biocompatibility for use in novel tissue-engineering applications.

EXPERIMENTAL

Materials

Anhydrous AAc in the monomeric form and Na–Alg (low-viscosity grade, 250 cp in a 2% solution at 25°C) were obtained from Sigma-Aldrich (St. Louis, MO). $CaCl_2$ pellets and NMBA were purchased from J. T. Baker (Phillipsburg, Europe) and Fluka (St. Louis, MO), respectively. Vancomycin (Acros, Geel, Belgium), which was used as the model drug, and 98% 2-ketoglutaric acid (Acros, Geel, Belgium), which was used as a photoinitiator, were supplied by Acros. 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt (MTS) was obtained from Promega (Madison, WI). All of the chemicals used were analytical grade.

Preparation of the Ca–Alg/PAA hydrogel beads

The desired amount of Na–Alg powder was dissolved in double-distilled water and stirred continuously with a homogenizer (Polytron; Kinematica, AG, Lucerne, Switzerland) at 15,000 rpm for the desired amount of time. During homogenization, 50 mL of vancomycin (100 ppm) was added to the solution to prepare hydrogel beads for the drug-release experiments. The resulting solution was released dropwise into 40 mL of $CaCl_2$ solution (0.55*M*) containing 5.49 mL of AAc solution (2*M*), 0.12 g of NMBA (used as a crosslinking agent), and 0.12 g of 2-ketoglutaric acid (used as a photoinitiator). The solution containing the gel beads in 250-mL flask was stirred with a magnetic stirrer, and it was then polymerized by UV irradiation (2200 W) for 15 min. The distance between the top of the flask and the lamp was 15 cm. We then separated the hydrogel beads from the PAA coating by breaking the PAA gel. The beads were then washed with ethanol and distilled water and subsequently freeze-dried for further analysis.

Morphological analysis

The surface and cross-sectional morphologies of the beads were examined under scanning electron microscopy (SEM; S-3000N, Hitachi, Tokyo, Japan). The beads were mounted on metal grids with double-sided tape and were coated with gold *in vacuo*.

Mechanical testing

Because it was impossible to compress the hydrogel beads directly under the platens, an alternative method was used to conduct the compression testing. The hydrogel beads that were not freeze-dried were fully contained inside a 2×2 cm^2 poly(ethylene terephthalate) bag. This bag was then subjected to compression testing (AG-IS, Shimadzu, Tokyo, Japan) at a crosshead speed of 1 mm/min. The test was stopped when the beads inside the poly(ethylene terephthalate) bag were broken. The average compression modulus and stress at breaking point of the hydrogel beads were calculated for 10 bags. Because the thickness of the bag was about 0.2 mm, we assumed that the compressive mechanical properties contributed from the bag were negligible.

Swelling characteristics and water content of the Ca–Alg/PAA gel beads

The swelling characteristics of the Ca–Alg/PAA gel beads were determined by immersion of the dried specimens in 20 mL of phosphate buffer solution (PBS; 0.2 g of KCl, 8.0 g of NaCl, 2.16 g of Na_2HPO_4, and 0.2 g of KH_2PO4 in double-distilled water added to attain a volume of 1 L; pH 7.4) at 37°C or in 20 mL of SGF (0.08*M* HCl containing 0.2% NaCl; pH 1.2)[20] at 37°C. The swollen gel beads were periodically removed, blotted with filter paper to remove the surface moisture, and weighed. All experiments were performed in triplicate. The

swelling ratios (Q_s's) of the specimens were calculated by the following expression:

$$Q_s = (W_s - W_d)/W_d \quad (1)$$

where W_s is the weight of the swollen specimen and W_d is the weight of the dried specimen. Furthermore, the water content (Q_w) of the specimens was calculated by the following expression:

$$Q_w = (W_s - W_d)/W_s \quad (2)$$

Loading of vancomycin

In a typical loading study, 10 mg of vancomycin was dissolved in 100 mL of double-distilled water and stirred continuously with a homogenizer. Then, we added 50 mL of vancomycin solution to the Alg solution and followed the rest of the procedure as mentioned previously to prepare hydrogel beads for the drug-release experiments.

The hydrogel beads containing vancomycin were then broken by a stainless steel spatula to release the vancomycin from the gel beads. This solution was set for 2 days to ensure the complete release of vancomycin. The amount of free vancomycin was analyzed in the supernatant by the Bradford method with a UV–visible spectrophotometer (UV 2401 PC, Shimadzu) at 280 nm. The entrapment efficiency (E%) of vancomycin was calculated as follows:

$$E\% = \frac{\text{Total vancomycin} - \text{Free vancomycin}}{\text{Total vancomycin}} \times 100\% \quad (3)$$

All measurements were performed in triplicate and averaged.

In vitro drug-release experiments

The *in vitro* release profile of vancomycin from the hydrogel beads was investigated with two methods. In the first method, the hydrogel beads containing vancomycin were exposed to SIF (pH 7.4) or SGF (pH 1.2) at 37°C. In the second method, the hydrogel beads containing vancomycin were first released in SGF at 37°C, which simulated the conditions in the gastric tract, and were subsequently immersed in SIF (0.05*M* KH_2PO_4, pH 7.4),[21–23] which mimicked the conditions of the intestinal tract. All of the drug-release experiments were performed in triplicate; approximately 0.003 g of hydrogel beads composed of 3 wt % Ca–Alg and 2*M* PAA were put into a 1000-mL beaker filled with the appropriate medium. The beaker was placed in a circulating water bath equipped with a stirrer for mixing the medium. The temperature and stirring rate were maintained at 37°C and 100 rpm, respectively. Aliquots (10 mL) were withdrawn from the release medium at each sampling time and were analyzed on a UV–visible spectrometer (Lambda 25, PerkinElmer, Walthams, MA) at a wavelength of 280 nm to determine the amount of vancomycin that was released.

Cell culture

To examine the cytotoxicity of the materials used in the drug-delivery system, we cultured adipose stem cells (ADSCs) on the prepared hydrogels. Human adipose tissue samples obtained from donors by liposuction under local anesthesia were provided by the Department of Plastic Surgery, National Cheng Kung University Hospital, Taiwan. To isolate the ADSCs, lipoaspirates were washed with large volumes of PBS until the subfractions cleared. The lipoaspirates were digested with an equal volume of 0.075% type I collagenase at 37°C for 30–60 min and subsequently neutralized with 10% fetal bovine serum. The suspension was filtered through 100-mm nylon mesh to remove the tissue fraction, and the filtrate was centrifuged at 1200 g for 10 min. The pellet was resuspended in the control medium (Dulbecco's modified Eagle's medium, which contained 10% fetal bovine serum and 1% antibiotic/antimycotic solution) and incubated overnight at 37°C and at 5% CO_2. For the subculture, 0.05% trypsin solution was added to the medium, and the medium was replaced three times per week. To prevent spontaneous differentiation, the cells were maintained at 90% confluence. Cells arrested at the fourth passage were used for all of the experiments. The prepared hydrogel beads were then washed three times with double-distilled water and freeze-dried before sterilization. These beads were first sterilized under UV light (2200 W) for 30 min and were then moved to a laminar flow hood and sterilized continuously under UV light for 1 day. Furthermore, they were placed in 96-well plates, one bead for each well, and incubated with PBS containing penicillin, streptomycin, and fungizone for 24 h at 37°C in a 5% CO_2 humidified atmosphere for two cycles. The ADSCs were seeded in the wells containing the hydrogel beads at a density of 1×10^5 cells/mL. The plates were incubated at 37°C for 30 min in a 5% CO_2 humidified atmosphere and were subsequently equilibrated in 0.1 mL of Dulbecco's modified Eagle's medium. The medium was replaced, and samples were withdrawn for further analysis after seeding for the desired time.

MTS test

The MTS assay was performed to evaluate the cell vitality and proliferation in all of the experiments. This method is based on the reduction of the tetrazolium ring of MTS by mitochondrial dehydrogenase

produced by living cells. This reaction yields a formazan product that is soluble in the cell culture medium. The amount of formazan produced is proportional to the number of viable cells. After seeding for the desired amount of time, 20 μL of MTS was added to each well. After 3 h of incubation under standard conditions of 5% CO_2 and 37°C, the purple formazan product (indicative of reduction of MTS) was visible. The medium was withdrawn and was analyzed on an enzyme-linked immunosorbent assay microplate reader (Anthos 2010; Tecan Sunrise, Männedorf, Switzerland) at 490 nm.

Determination of the cell concentration on the beads

The beads were removed from the wells and washed with PBS to remove the unwanted material and nonadherent cells. The adherent cells were digested with trypsin, retrieved, and stained with trypan blue. The mean number of cells determined for at least three replicates was used to calculate the cell proliferation inhibition index (CPII), which was expressed as a percentage of the mean number of cells in the control cultures with the following equation:[24,25]

$$\text{CPII} = 100 - (\text{Mean number of cells in the test culture}/\text{Mean number of cells in the control culture} \times 100) \quad (4)$$

Cell morphology as observed under an SEM

To prepare samples for observation under an SEM, the specimens were separated from the medium and washed twice in distilled water. Specimens of the freeze-dried hydrogel beads were then carefully fractured and coated with gold. The internal morphology of the cell-seeded hydrogel beads was observed under SEM.

RESULTS AND DISCUSSION

Preparation of the Ca–Alg/PAA hollow hydrogel beads

Alg is a natural, biocompatible, and biodegradable compound; hydrogel beads composed of Alg crosslinked with Ca^{2+} exhibit low mechanical strength and are, therefore, rapidly degraded after oral administration. To overcome this limitation, we used AAc to reinforce the structure of the Alg hydrogel and to increase its strength. A series of hydrogels were prepared by the mixture of Alg with various weight percentage concentrations (1–8 wt %) with a fixed concentration of AAc (2*M*). We found that when the Alg concentration was higher than 5 wt %, the solution became extremely viscous. In fact, we were unable to stir the solution when a high Alg concentration of 8 wt % was used. Furthermore, we found that the use of a high Alg concentration resulted in the production of deformed beads (data not shown). The optimal Alg concentration was identified as 3 wt %; a series of hydrogels could be made with this specific Alg concentration and various AAc concentrations (2–8*M*) without any problem.

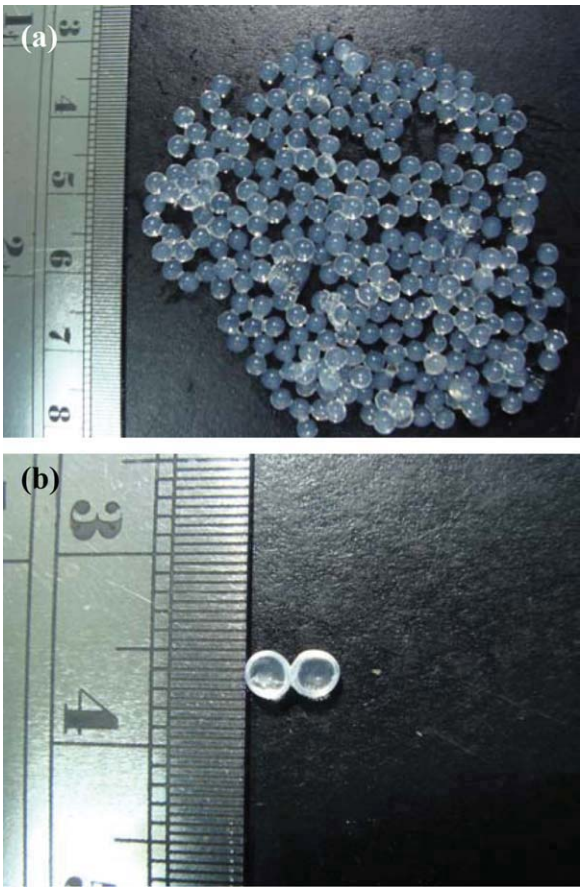

Figure 1 Photographs of the (a) morphology and (b) cross-sectional appearance of Ca–3 wt % Alg/2*M* PAA hydrogel beads. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Alg solution was released dropwise into the solution containing AAc and $CaCl_2$. The COO^- ion of Alg was bound to Ca^{2+} and formed the egg-box structure; this resulted in the production of Ca–Alg hydrogel beads. The solution containing the Ca–Alg hydrogel beads was then polymerized by UV irradiation; during this process, we strengthened the structure of the beads by coating them with PAA. Subsequently, we obtained the Ca–Alg/PAA hydrogel beads [Fig. 1(a)] by breaking apart the PAA gel surrounding them using a stainless steel spatula. The average diameter of the hydrogel beads was 0.226 ± 0.011 cm, and their average water content was 85.34%.

When the Alg solution was released dropwise into the $CaCl_2$ solution containing AAc, NMBA, and 2-ketoglutaric acid, the calcium ions diffused into the Alg droplets. Alg chain backbones in an egg-box crosslinked fashion were formed through rapid

crosslinking by calcium ions. The Ca–Alg beads then acted as a template for polymerization. The AAc monomers were polymerized and crosslinked inside the generated Ca–Alg beads after the solution containing the gel beads was subjected to UV irradiation. In this study, we found that the structure of the obtained Ca–Alg/PAA hydrogel beads might be either solid or hollow, which was dependent on the reaction time before the solution containing the gel beads was subjected to UV irradiation. For the short reaction time (e.g., <9 min for the specific compositions in our study), the egg-box crosslinked structure only formed on the surface of the Ca–Alg beads; furthermore, the AAc, NMBA, and 2-ketoglutaric acid molecules might not have had sufficient time to diffuse into the Ca–Alg beads. The polymerization and crosslinking of AAc in the central region of the beads was, therefore, prevented and occurred only in the outer layer of the beads after the solution containing the gel beads was subjected to UV irradiation. The Ca–Alg/PAA hydrogel beads formed in this way were found to have a hollow structure after they were freeze-dried. Figure 1(b) shows the photograph of the cross-sectional appearance of Ca–3 wt % Alg/2*M* PAA hydrogel beads. The cross-sectional SEM morphology of this hollow hydrogel bead is shown in Figure 2(a). On the other hand, when the reaction time before the solution containing the gel beads being subjected to UV irradiation was long enough (e.g., >9 min for the specific compositions in our study), the AAc, NMBA, and 2-ketoglutaric acid molecules could diffuse toward the center of the beads. In this case, the AAc monomers were polymerized and crosslinked inside the preferentially generated Ca–Alg beads after the solution containing the gel beads was subjected to UV irradiation. Solid beads of the Ca–Alg/PAA hydrogel were then formed after freeze drying. Figure 2(b) shows the cross-sectional SEM morphology of this solid hydrogel bead. Khorram et al.[26] adopted the method from Park and Hoffman[27] to prepare poly(isopropylacrylamide) hollow heads as reservoir drug-delivery systems. They found that the formation of hollow beads was achievable when the Na–Alg and *N*-isopropylacrylamide concentrations in the monomer solution were high enough to induce phase separation (SA) between Na-Alg and *N*-isopropylacrylamide.[28] In this study, we found that the reaction time might have also played a significant role in determining the structure of the hydrogel beads.

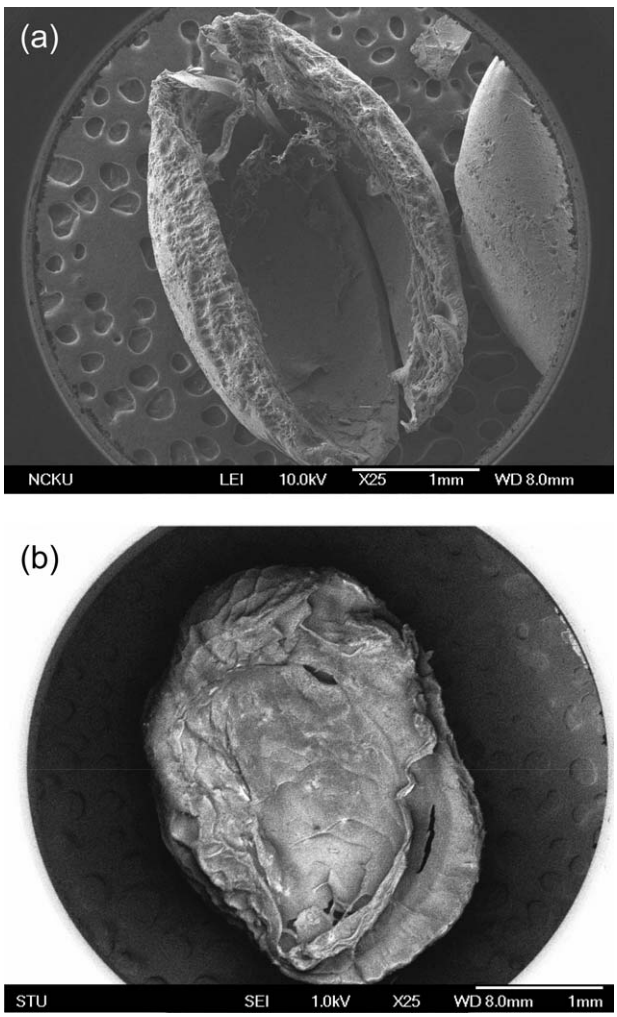

Figure 2 Cross-sectional SEM morphology of the Ca–Alg/PAA hydrogel: (a) hollow bead and (b) solid bead.

Morphology of the hollow Ca–Alg/PAA hydrogel beads

Photographs of the hollow Ca–Alg/PAA hydrogel beads as observed under an SEM are shown in Figure 3. Figure 3(a,b) shows the typical morphology of the beads and demonstrates the presence of a skin layer on the bead surface. This skin layer may have played a role in sustained drug release from the hydrogel beads. The cross-sectional morphology of the beads is illustrated in Figure 3(c,d). Our observations confirmed that the hydrogel beads prepared in this study possessed a hollow structure, which could have improved the loading efficiency of the incorporated drug. The shell of the hydrogel bead was basically composed of two layers. The outer layer comprised PAA containing macropores, which provided a path for drug release from the bead cavity. The inner layer, which was the wall of the bead cavity, comprised a Ca–Alg segment lacking pores; this segment could have served as a reservoir for the incorporated drug. Thus, the architecture of the hydrogel beads prepared in this study rendered them suitable for use as a drug carrier.

Swelling ratio and water content of the hollow Ca–Alg/PAA hydrogel beads

The swelling profiles of the hollow hydrogel beads when immersed in PBS (pH 7.4) and SGF (pH 1.2) are shown in Figure 4. The Ca–Alg (Alg

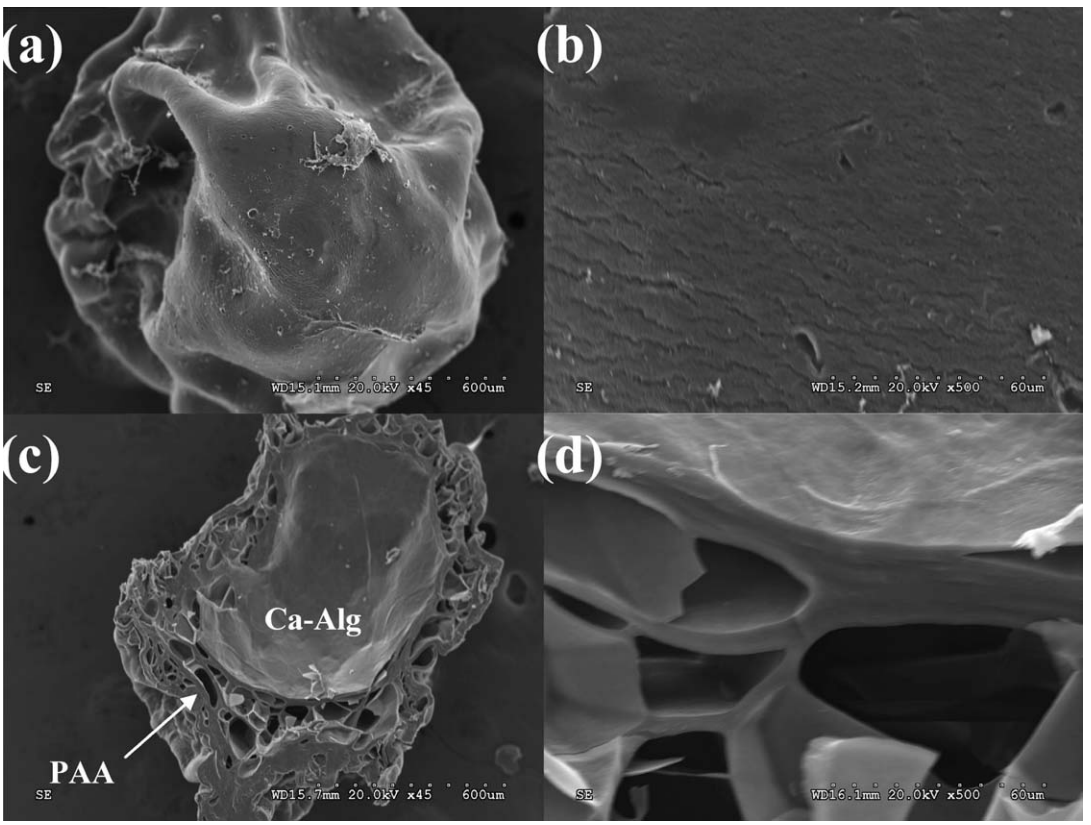

Figure 3 SEM images of Ca–3 wt % Alg/2*M* PAA hydrogel beads: surface morphology at (a) 45 and (b) 500× magnification and cross-sectional morphology at (c) 45 and (d) 500× magnification.

concentration = 3 wt %) hydrogel beads were observed to disintegrate approximately 3 h after their immersion in PBS because of the replacement of Ca^{2+} with univalent ions or the capture of Ca^{2+} by phosphate. The water content of these beads was approximately 92.5%. On the other hand, the swelling ratio of the Ca–Alg/PAA (Alg concentration = 3 wt %, PAA concentration = 2*M*) hydrogel beads monolithically attained an approximate value of 46.6 (w/w) after their immersion in PBS for 6 h. After this, the swelling ratio gradually increased over the next 18 h and attained a value of 63.5 (w/w) after 24 h. The hydrogel bead structure was evidently reinforced by the surrounding PAA chain segments, which prevented the release of Ca^{2+} and tremendously extended the degradation time of the beads. The water content of these hydrogel beads was as high as 98.6%, but their swelling ratio was extremely low (ca. 3.9% w/w) when they were immersed in SGF for 24 h. These results suggest that the hydrogel beads prepared in this study were pH sensitive because of the nature of the carboxyl groups present in PAA and Alg.[21,29,30]

The carboxylic acid in PAA ($pK_a = 4.75$) and Alg ($pK_a = 3.5$)[31] did not undergo ionization at pH 1.2; thus, the hydrogel bead structure was considerably stable at this pH. However, as the pH was increased to 7.4, ionization occurred, the intramolecular and intermolecular electrostatic repulsive forces of PAA and Alg increased, and the hydrogel beads swelled under these conditions. The use of such pH-sensitive hydrogel beads as drug carriers provides an advantage over other systems, because the pH conditions differ in the gastric and intestinal tracts.

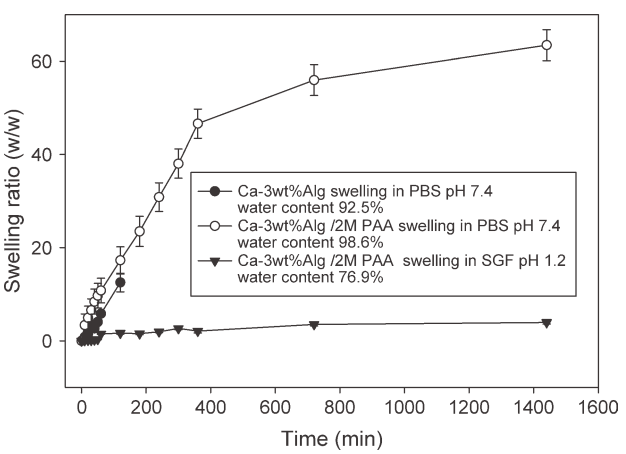

Figure 4 Swelling profiles of the hydrogel beads immersed in PBS (pH 7.4) and SGF (pH 1.2).

TABLE I
Compressive Modulus and Stress at Break Point Values of the Hydrogel Beads Prepared with Different Concentrations of Alg and PAA

Sample	Modulus (MPa)	Stress at break point (MPa)
Ca–1 wt % Alg/2*M* PAA	1.80 ± 0.03	0.84 ± 0.07
Ca–2 wt % Alg/2*M* PAA	2.24 ± 0.15	1.02 ± 0.05
Ca–3 wt % Alg/2*M* PAA	2.31 ± 0.14	1.49 ± 0.63
Ca–4 wt % Alg/2*M* PAA	2.35 ± 0.18	2.42 ± 0.22
Ca–5 wt % Alg/2*M* PAA	2.08 ± 0.10	3.05 ± 0.15
Ca–3 wt % Alg/3*M* PAA	2.39 ± 0.11	3.15 ± 0.15
Ca–3 wt % Alg/4*M* PAA	2.10 ± 0.01	3.89 ± 0.19
Ca–3 wt % Alg/5*M* PAA	1.98 ± 0.05	2.22 ± 0.02
Ca–3 wt % Alg/8*M* PAA	2.03 ± 0.12	2.28 ± 0.50
Ca–3 wt % Alg	1.57 ± 0.15	0.25 ± 0.01
Ca–3 wt % Alg/2*M* PAA exposed to PBS	2.41 ± 0.18	0.73 ± 0.00

Mechanical properties of the hollow Ca–Alg/PAA hydrogel beads

We examined the mechanical properties of the hydrogel beads by varying the concentration of either Alg or PAA, and the results of this analysis are listed in Table I. The stress at break point and compressive modulus of the Ca–3 wt % Alg/2*M* PAA hydrogel beads were both higher than those obtained from the unreinforced hydrogel beads (Ca–3 wt % Alg). High mechanical properties were achieved by the formation of an interpenetrating network between PAA and Alg. The breaking-point stress of the hydrogel beads increased with the Alg content of the beads, whereas the PAA concentration was maintained at 2*M*. The modulus values of these beads were comparable. Furthermore, the modulus values of the hydrogel beads were also comparable when the PAA concentration was varied with the Alg concentration maintained at 3 wt %. The breaking-point stress of these beads was maximal at a PAA concentration of 3 or 4*M*. However, the administration of drug carriers containing such high PAA concentrations (3 or 4*M*) may be detrimental to internal tissues. Therefore, we considered Ca–Alg/PAA (Alg concentration = 3 wt %, PAA concentration = 2*M*) hydrogel beads that exhibited comparable modulus (2.31 MPa) and breaking-point stress values (1.49 MPa) as the optimal system for the *in vitro* drug-release experiments.

To investigate the effects of body fluids on the mechanical properties of these specific hydrogel beads, the beads were immersed in PBS for 24 h and subsequently subjected to mechanical testing. The results indicated that the modulus values of the beads remained relatively constant (2.41 MPa) after their immersion in PBS; however, the breaking-point stress values were reduced to 0.73 MPa. Under these conditions, it was possible that the water molecules from PBS would attack the amorphous phase of the hydrogel beads and thus lead to the progress of degradation. Nevertheless, its mechanical properties were higher than those of the hydrogel beads (Ca–3 wt % Alg) that were not reinforced with PAA, as indicated in Table I.

In vitro release of vancomycin from the hollow Ca–Alg/PAA hydrogel beads

We used two methods to investigate the release profile of vancomycin from the hydrogel beads after its loading at an efficiency of up to 88.15 ± 8.64%. Because the hydrogel beads prepared in this study were pH sensitive, the release profiles of vancomycin from them differed, depending on the pH of the medium. The vancomycin release rate from the hydrogel beads (Fig. 5) was found to be higher in SIF (pH 7.38) than in SGF (pH 1.2). Within 24 h, up to 71.15% of the incorporated vancomycin was released in SIF, whereas only approximately 15.64% was released in SGF. At neutral pH, the affinity of phosphate for calcium was higher than that of Alg, and this led to the formation of a calcium–phosphate complex. Thus, it appeared that that the Ca–Alg gel matrix disintegrated in SIF; this resulted in a release of large amounts of the incorporated drug.[32] The fact that the drug-release rate of our system differed in the two media used increases its practical applicability for the topical administration of drugs.

Following oral administration, drugs are normally retained in the gastric tract for 6–10 h, after which they move into the intestinal tract and remain there for the next 8 h. To simulate the normal route of drug transport, the hydrogel beads containing vancomycin were first maintained in SGF for 6 or 10 h and were subsequently maintained in SIF for the next 8 h. Because hydrogel beads are highly stable in an acidic environment, only 6.51% of the incorporated vancomycin was

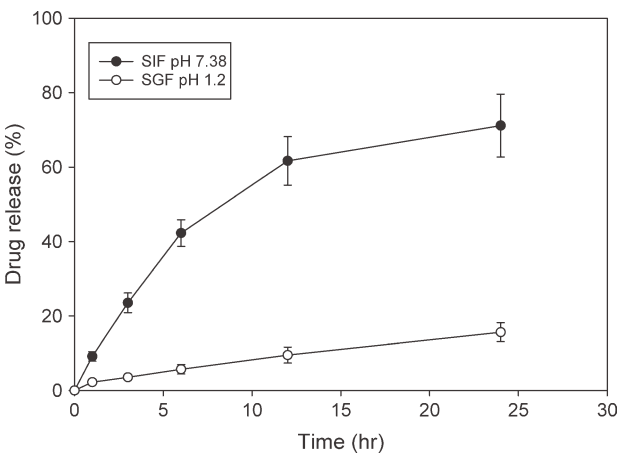

Figure 5 Vancomycin release profiles of the Ca–3 wt % Alg/2*M* PAA hydrogel beads in SIF (pH 7.38) and SGF (pH 1.2).

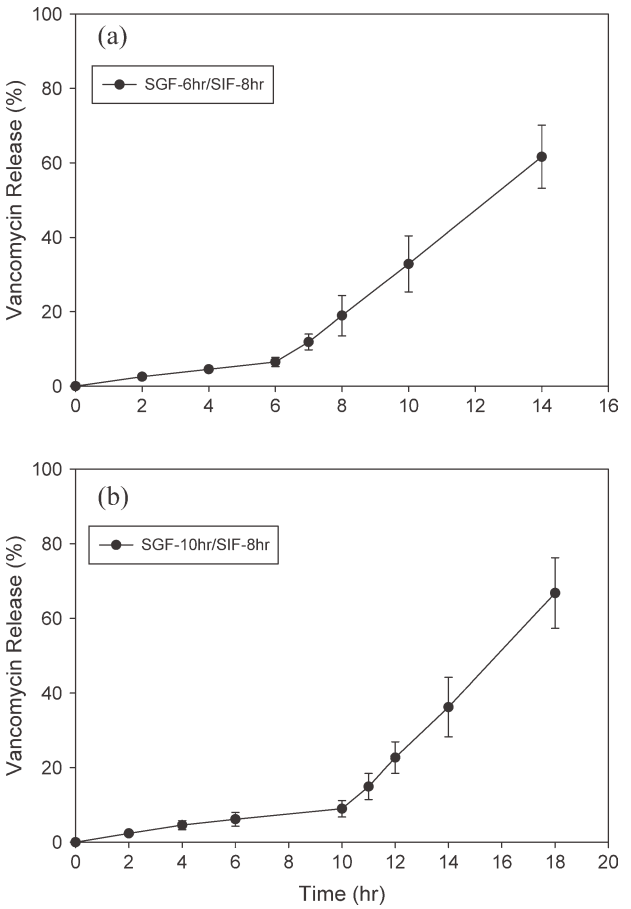

Figure 6 Vancomycin release profiles of the Ca–3 wt % Alg/2*M* PAA hydrogel beads (a) when maintained in SGF (pH 1.2) for 6 h and subsequently in SIF (pH 7.38) for 8 h and (b) when maintained in SGF (pH 1.2) for 10 h and subsequently in SIF (pH 7.38) for 8 h.

released within 6 h, and 8.98% was released within 10 h [Fig. 6(a,b)]. In contrast, the hydrogel beads rapidly swelled in the neutral environment, and the incorporated drug was released at a very fast rate. Up to 61.56% of the incorporated vancomycin was released from the hydrogel beads within 14 h in SIF following the prior immersion of the beads in SGF for 6 h. On the other hand, up to 66.78% of the drug was released within 18 h after the prior immersion of the beads in SGF for 10 h. Sustained drug release was observed in SIF, regardless of whether the beads had been maintained in SGF for 6 or 10 h. This indicated that fortification of the hydrogel bead structure with PAA delayed the degradation of the beads.[21] On the basis of the observed drug-release profiles, we consider our system applicable for drug delivery into the gastrointestinal tract, wherein it is necessary that a drug is released in small amounts in the gastric tract but in large amounts and in a sustained manner in the intestinal tract.[33] The results of the vancomycin release profile were consistent with those obtained from the swelling behavior of the hydrogel beads.

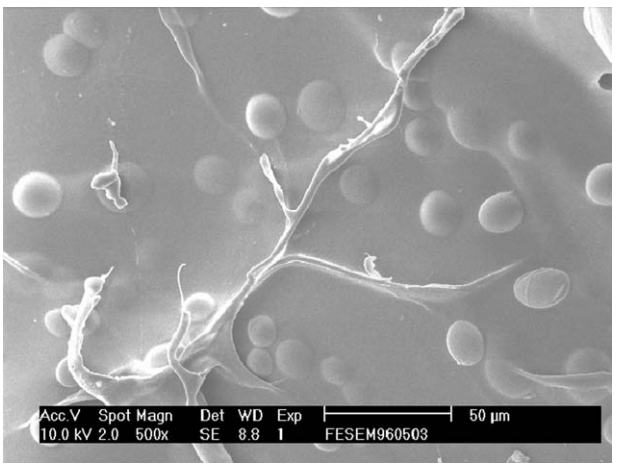

Figure 7 Morphology of the stem-cell-seeded Ca–3 wt % Alg/2*M* PAA hydrogel beads after culturing for 3 days.

Biological response

We examined the cytotoxicity of the prepared hydrogel beads by culturing ADSCs on them and comparing these cultures with control cultures (stem cells grown on polystyrene). After 3 days of culturing, the ADSCs seeded on Ca–3 wt % Alg/2*M* PAA hydrogel beads (Fig. 7) appeared spherical rather than flat. The cell morphology suggested mild cytotoxicity for the matrix due to the acidic nature of PAA. Furthermore, the stem cells uniformly adhered to the surface of the hydrogel beads. The number of cells increased with the culture time in all cases, as shown in Figure 8. On day 3, the absorbance of cells seeded on the Ca–3 wt % Alg/2*M* PAA hydrogel beads was lower than that of the cells seeded on the Ca–3 wt % Alg hydrogel beads because of the acidic nature of the former. Nevertheless, the viabilities of the cells seeded on the two types of beads were comparable.

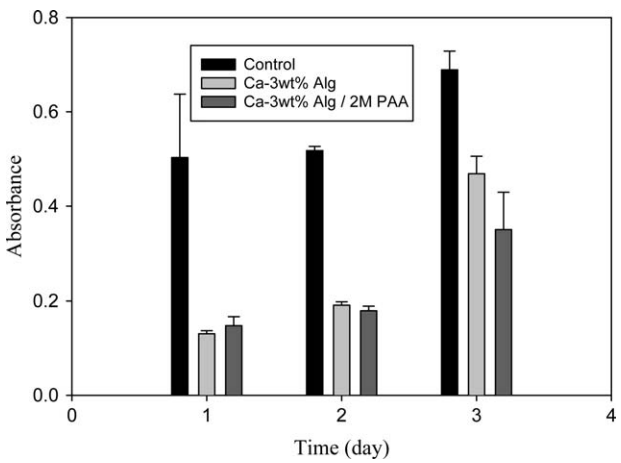

Figure 8 Absorbance of the control cells, cells on the Ca–3 wt % Alg hydrogel beads, and cells on the Ca–3 wt % Alg/2*M* PAA hydrogel beads as determined by MTS tests.

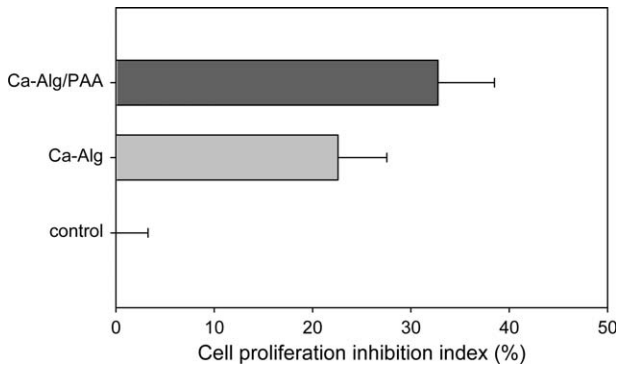

Figure 9 CPIIs of the controls, cell-seeded Ca–3 wt % Alg hydrogel beads, and cell-seeded Ca–3 wt % Alg/2*M* PAA hydrogel beads after 3 days of culture.

The CPII values determined for all of the gel materials investigated are shown in Figure 9; this parameter provides a direct measurement of cytotoxicity. As shown in the histogram, on day 3, CPII of the Ca–3 wt % Alg hydrogel beads was approximately 22.6%, whereas that of the Ca–3 wt % Alg/2*M* PAA hydrogel beads was 32.8%. The high value in the latter case was attributed to the acidic nature of these beads. De Groot et al.[25] used dextran to modify hydroxyethyl methacrylate and reported its CPII to range from 25 to 40%. Thus, they reported that these biopolymers were not cytotoxic. The CPII values of the hydrogel beads prepared in this study ranged from 32.8 to 22.6%; this demonstrated the biological safety of these materials.

CONCLUSIONS

Hollow Ca–Alg/PAA hydrogel beads were successfully prepared via UV polymerization. PAA played a significant role in fortifying the structure of these beads. Their swelling behavior indicated that the prepared hydrogel beads were pH sensitive; this is an advantage for practical drug delivery into the gastrointestinal tract. In addition, the biological response of cells to these hydrogel beads indicated that these hydrogel beads exhibited good biological safety and could have additional applications in tissue engineering.

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References

1. Deresinski, S. Int J Antimicrob Ag 2009, 34S, S43.
2. Iyer, S.; Jones, D. H. J Am Acad Dermatol 2004, 50, 854.
3. Small, P. M.; Chambers, H. F. Antimicrob Agents Chemother 1990, 34, 1227.
4. Levine, D. P. Clin Infect Dis 2006, 42, S5.
5. Shu, X. Z.; Zhu, K. J. Int J Pharm 2000, 201, 51.
6. Remuñañ-López, C.; Portero, A.; Lemos, M.; Vila-Jato, J. L.; Nuñez, M. J.; Riveiro, P.; López, J. M.; Piso, M.; Alonso, M. J. J Pharm Sci 2000, 10, 69.
7. Orienti, I.; Cerchiara, T.; Luppi, B.; Bigucci, F.; Zuccari, G.; Zecchi, V. Int J Pharm 2002, 238, 51.
8. Shah, S.; Qaquish, R.; Patel, V.; Amiji, M. J Pharm Pharmacol 1999, 51, 667.
9. Tozaki, H.; Odoriba, T.; Okada, N.; Fujita, T.; Terabe, A.; Suzuki, T.; Okabe, S.; Muranishi, S.; Yamamoto, A. J Controlled Release 2002, 82, 51.
10. Peppas, N. A.; Khare, A. R. Adv Drug Deliv Rev 1992, 11, 1.
11. Zhang, X. Z.; Wu, D. Q.; Chu, C. C. Biomaterials 2004, 25, 3793.
12. Liu, W.; Zhang, B.; Lu, W. W.; Li, X.; Zhu, D.; Yao, K. D.; Wang, Q.; Zhao, C.; Wang, C. Biomaterials 2004, 25, 3005.
13. Lin, H. R.; Yeh, Y. J. J Biomed Mater Res (Appl Biomater) 2004, 71B, 52.
14. Lin, H. R.; Sung, K. C.; Vong, W. J. Biomacromolecules 2004, 5, 2358.
15. Bouhadir, K. H.; Mooney, D. J. In Methods of Tissue Engineering; Atala, A.; Lanza, R. P., Eds.; Academic: New York, 2002; p 653.
16. Hua, S.; Wang, A. Carbohydr Polym 2009, 75, 79.
17. Yin, Y.; Ji, X.; Dong, H.; Ying, Y.; Zheng, H. Carbohydr Polym 2008, 71, 682.
18. Laurienzo, P.; Malinconico, M.; Mattia, G.; Russo, R.; La Rotonda, M. I.; Quaglia, F.; Capitani, D.; Mannina, L. J Biomed Mater Res 2006, 78A, 523.
19. Greimel, A.; Werle, M.; Bernkop-Schnürch, A. J Pharm Pharmacol 2007, 59, 1191.
20. Goh, L. M. L.; Barlow, P. J. Food Chem 2004, 86, 195.
21. Anal, A. K.; Stevens, W. F. Int J Pharm 2005, 290, 45.
22. Krasaekoopt, W.; Bhandari, B. Int Dairy J 2004, 14, 737.
23. Mandal, S.; Puniya, A. K.; Singh, K. Int Dairy J 2006, 16, 1190.
24. Schiraldi, C.; D'Agostino, A.; Oliva, A. Biomaterials 2004, 25, 3645.
25. De Groot, C. J.; Van Luyn, M. J. A. Biomaterials 2001, 22, 1197.
26. Khorram, M.; Vasheghani-Farahani, E.; Dinarvand, R. J Controlled Release 2006, 116, e31.
27. Park, T. G.; Hoffman, A. S. J Polym Sci Part A: Polym Chem 1992, 30, 505.
28. Park, T. G.; Choi, H. K. Macromol Rapid Commun 1998, 19, 167.
29. Pasparakis, G.; Bouropoulos, N. Int J Pharm 2006, 323, 34.
30. Liua, Y.-Y.; Shao, Y.-H.; Lu, J. Biomaterials 2006, 27, 4016.
31. Tapia, C.; Montezuma, V.; Yazdani-Pedram, M. AAPS PharmSciTech 2008, 9, 1198.
32. Dainty, A. L.; Goulding, K. H.; Robinson, P. K.; Sinpkins, I.; Trevan, M. D. Biotechnol Bioeng 1986, 28, 210.
33. George, M.; Abraham, T. E. Int J Pharm 2007, 335, 123.