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Hybrid alginate beads with thermal-responsive gates for smart drug delivery

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Polysaccharide-based thermo-responsive material was prepared by grafting PNIPAAm onto hybrid alginate beads, in which a biomineralized polyelectrolyte layer was constructed aiming to enhance the mechanical strength and ensure higher graft efficiency. XPS results demonstrated that the incorporation of PNIPAAm to the hybrid beads was successful, and the PNIPAAm-grafted beads were more hydrophilic than the ungrafted ones as indicated by their swelling behavior. The drug release behaviors revealed that the grafted beads were both thermo- and pH-sensitive, and the PNIPAAm existed in the pores of the alginate beads acted as the "on–off" gates: the pores of the beads were covered by the stretched PNIPAAm to delay the drug release at 25°C and opened to accelerate the drug release at 37°C because of the shrinking of PNIPAAm molecules. This paper would be a useful example of grafting thermo-responsive polymers onto biodegradable natural polymer substrate. The obtained beads provide a new mode of behavior for thermo-responsive "smart" polysaccharide materials, which is highly attractive for targeting drug delivery system and chemical separation. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: alginate; biomineralization; PNIPAAm; stimuli-sensitive polymers; drug delivery systems

INTRODUCTION

Preparation of stimuli-responsive substrates has attracted increasing attention due to their potential for applications in biological science and biomedical engineering.[1–3] Poly(*N*-isopropylacrylamide) (PNIPAAm) is one of the most important and intensively studied polymers, and exhibits a lower critical solution temperature (LCST) of $<32^{\circ}$C in aqueous solution that is attributed to alterations in the hydrogen-bonding interactions of the amide groups.[4] Attaching PNIPAAm or its copolymers to the surfaces of a given substrate is a promising strategy to create responsive interfaces, since the physical properties of the PNIPAAm-modified interfaces are readily controlled by changing temperature.[5] Furthermore, PNIPAAm might be a good material to bridge the gap between biological machines and multi-functional actuators for its water-solubility, thermo-sensitivity, and biocompatibility. Therefore, there have been many publications concerning the thermo-responsive properties of individual PNIPAAm linear chains or hydrogels on solid substrates.[6–11] All these thermo-responsive gating substrates are synthetic polymers[6,7] or mental substrates.[9–11] On the other hand, polysaccharides are natural polymers having broad applicability for tissue regeneration as a consequence of their bioactivity and ability to support many different human cell types.[12] Alginate, as one kind of natural polysaccharides, has also emerged as promising materials for cell encapsulation and drug delivery for its pH-sensitivity, biocompatibility, and biodegradability.[13–16]

Combining biopolymers with thermo-sensitive macromolecules (such as PNIPAAm) can prepare matrixes that present a dual and independent sensitivity to both pH and temperature. In former studies concerning PNIPAAm/alginate dual responsive system,[17–19] PNIPAAm exist in alginate semi-IPN network. Therefore, the squeezing property of PNIPAAm at 37°C can break the balance of the semi-IPN network and accelerate the disruption of alginate beads. So semi-IPN network is not a good system for natural polymers with dual-stimuli-responsive properties. As discussed formerly, many publications have reported the grafting of thermo-responsive polymers onto synthetic polymers or mental substrates. In the present work, thermo-responsive polymers PNIPAAm will be grafted onto the biodegradable alginate beads to prepare thermo-responsive "smart" polysaccharide materials. To overcome the rapid erosion and high release rate in a neutral pH condition, the alginate beads are introduced with a biomineralized polyelectrolyte layer[20] as illustrated in Scheme 1. The biomineralized layer formed between Ca^{2+} and HPO_4^{2-} and the polyelectrolyte layer formed from chitosan (positive charge), poly(sodium acrylate) (PAANa), and alginate (negative charge), both existed in the alginate beads aiming to enhance the mechanical strength.[21,22] PAANa with ultra high molecular weight ($M_\eta > 10^7$) has good flexibility and hydrophilicity. It is extensively used as an excellent food additive to enhance the flexibility of food in food industry and as

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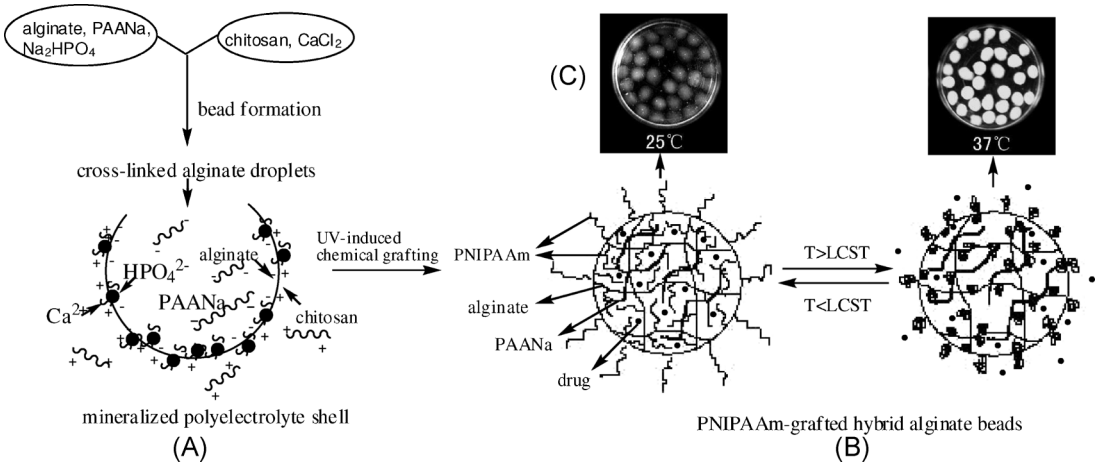

Scheme 1. Schematic illustration of the smart alginate beads. Biomineralized polyelectrolyte bead (A), PNIPAAm-grafted hybrid alginate bead (B), and digital photos (C) of PNIPAAm-grafted alginate beads (G1) at 25 and 37°C.

thickening and stabilizing agents in the formulation of pharmaceutical emulsions and suspensions.[23,24] With the assistance of biomineralized polyelectrolyte layer, the alginate beads could be kept intact and flexible when being grafted with PNIPAAm. Additionally, the alginate beads would be in a swollen state during the course of grafting because of the good swelling property of alginate in water. Therefore, it is easy for the NIPAAm molecules to permeate into the inner pores of alginate beads to finish grafting reaction. Instead of precipitating from the alginate network as discussed in semi-IPN PNIPAAm/alginate dual responsive system,[15,16] the shrinkage of PNIPAAm will not break the polymeric network in PNIPAAm-grafted alginate beads because PNIPAAm is mostly attached in the surface of the pores.

This study is aiming to prepare thermo-responsive polysaccharide materials with smart "on–off" gates by grafting PNIPAAm onto hybrid alginate beads, based on the opinion of mimicking natural process such as biomineralization. At the same time, inorganic minerals are combined with macromolecular chain to form organic–inorganic hybrid porous construction. To our knowledge, this is the first example of grafting thermo-responsive polymers onto the biodegradable natural polymer beads. The thermo-responsive swollen/shrunken property of PNIPAAm gates grafted in the pores of the alginate beads is presented in this paper: the pores of the beads are covered by stretched PNIPAAm to delay the drug release at temperatures below LCST, while opened to accelerate the drug release because of the shrinking of PNIPAAm at temperatures above LCST. These gating beads provide a new mode of behavior for thermo-responsive "smart" polysaccharide materials, which is highly attractive for targeting drug delivery systems and chemical separations.

EXPERIMENTAL

Materials

N-isopropylacrylamide (NIPAAm, Tokyo Chemical Industry Co. Ltd, Japan), acrylamide (AAm, Shanghai Chemical Regent Co. Ltd, China), ammonium persulfate (APS, Shanghai Chemical Regent Co. Ltd, China), sodium alginate (viscosity of 1% solution at 20°C = 20 cps, Shanghai Chemical Regent Co. Ltd, China), chitosan (viscosity = 55 cps, 92% degree of deacetylation, Shanghai Chemical Regent Co. Ltd, China), PAANa ($M_\eta = 1 \times 10^7$, Henan Kaite Chemical Industry General Co. Ltd, China), and indomethacin (Shanghai Houcheng Chemical, China) were used as received.

Preparation of biomineralized polysaccharide-coated alginate beads

Homogeneous aqueous solutions composed of sodium alginate (1.5%, w/v) containing NaCl (0.15 M), 5% (w/w) of PAANa (relatively to the weight of alginate) and 250 mM of Na_2HPO_4 were prepared. A homogeneous aqueous solution of chitosan in 1% (v/v) acetic acid containing 3% $CaCl_2$ was used as a coagulation fluid. The solution was mixed for 2 hr before use. The pH value of the coagulation fluid was adjusted to 6.4 ± 0.2 by adding NaOH solution (1 M). Thereafter, the mixture of alginate, PAANa, and Na_2HPO_4 was extruded in a form of droplets using a syringe in the coagulation fluid under mechanical stirring at 200 rpm. The resulting beads were kept for 30 min in $CaCl_2$/chitosan solution under stirring. The beads were washed with deionized water repeatedly. The resultant beads were dried in air overnight and then vacuum dried at 40°C for 24 hr.

Preparation of PNIPAAm-grafted alginate beads

Alginate beads were immersed in a H_2O_2/APS solution (50 ml/2 g) for 20 min under a UV lamp at 254 nm. After surface activation, the alginate beads were exposed to air for 15 min and then immersed in a 20% w/v NIPAAm aqueous solution, previously degassed with nitrogen at 50°C for 1.5 hr. After the reaction, the grafted beads were washed extensively with deionized water to remove the homopolymer and the unreacted monomer. The resultant beads were dried in air overnight and then vacuum dried at 40°C for 24 hr.

Drug loading of the beads

Homogeneous indomethacin solution (0.4 mg/ml) was obtained by adding 20 mg of indomethacin to 50 ml of phosphate buffer solution (PBS, pH 7.4, containing 10% (v/v) ethanol). Then alginate beads (248 mg) were immersed in the indomethacin solution at 50 rpm in a horizontal laboratory shaker and

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maintained at 25°C for 24 hr. After drug-loading, the beads were extensively washed with deionized water. The resultant beads were dried in air overnight and then vacuum dried at 40°C for 24 hr.

Characterization of biomineralized polysaccharide-coated beads

X-ray photoelectron spectroscopy (XPS) measurements were performed on a Kratos Axis Ultra spectrometer with monochomatic Al Kα radiation at 1486.92 eV. The FT-IR spectra of the samples were recorded with a Bruker Tensor 27 FT-IR spectrometer in the range of 4000–500 cm^{-1} using KBr pellets. The morphology and composition of the prepared beads was observed using scanning electron microscopy (SEM, FEI Quanta 200) at an accelerated voltage of 20 kV. Before being observed by SEM, the beads were stabilized on aluminum stubs using adhesive and sputter coated with an approximate 100 Å layer of gold. The formation of biomineralized polysaccharide membrane was confirmed by means of energy dispersive X-ray spectrometer (EDS, EDAX).

Swelling studies

The swelling behavior of the prepared beads was studied in PBS with pH 7.4 and in an HCl solution with pH 1.2 (similar to that of intestinal and gastric fluids, respectively) at temperatures of 25 and 37°C, respectively. At predetermined time intervals, the swollen beads were weighted after being wiped with soft paper tissue. The measurements were repeated three times typically for each condition. The degree of swelling for each sample was calculated by using the following expression: Swelling ratio $= (W_s - W_d)/W_d$, where W_s and W_d are the weights of the swollen beads and that of the dried beads, respectively.

Determination of the drug encapsulation efficiency of the beads

The prepared beads (10 mg) were dissolved in 100 ml of PBS (pH 7.4, containing 5% (v/v) ethanol) under stirring during 24 hr. The amount of free indomethacin was determined in the clear supernatant by UV spectrophotometry at 320 nm using a calibration curve constructed from a series of indomethacin solutions with standard concentrations. Such experiments allow the calculation of both the loading efficiency and the loading content. The loading efficiency is defined as the weight percentage of loaded drug based on feed amount.

In vitro release studies

The prepared beads (10 mg) were suspended in 50 ml of PBS with pH 7.4 or HCl solution with pH 1.2. This dissolution medium was stirred at 50 rpm in a horizontal laboratory shaker and maintained at 25 and 37°C. The sample (2 ml) was periodically removed and the withdrawn sample was replaced by the same volume of fresh medium. These experiments were performed at least three times. The amount of the released indomethacin was analyzed with a UV spectrophotometer as described previously.

Thermal-sensitivity of the PNIPAAm-grafted beads

Reversibility of drug release through the PNIPAAm-grafted beads in response to temperature change was measured at pH 7.4. The prepared beads (10 mg) were suspended in 50 ml of PBS with pH 7.4. This dissolution medium was stirred at 50 rpm in a horizontal laboratory shaker with temperature alternating between 25 and 37°C. The sample (2 ml) was removed and the temperature was switched every 1.5 hr. These experiments were performed at least three times. The amount of released indomethacin was analyzed with a UV spectrophotometer as described previously.

RESULTS AND DISCUSSION

Fabrication strategy

Biomineralized polyelectrolyte beads were produced by a one-step method as discussed in former literatures.[15,16] In the present work, PAANa with ultra high-molecular weight ($M_\eta > 10^7$) is introduced into the alginate beads together with the biomineralized polysaccharide layer. The excellent flexibility and hydrophilicity of PAANa with ultra high molecular weight could enhance the mechanical strength of alginate beads and control the permeability of the encapsulated drug in a neutral pH condition.[23,25] As illustrated in Scheme 1A and 1B, PAANa with ultra high-molecular weight are entangled in the cross-linked alginate beads. Polyelectrolyte could be formed between the opposite charges (the positive charges from chitosan, the negative charges from PAANa and alginate). At the same time, biomineralized layer could be formed between Ca^{2+} and HPO_4^{2-}. These two factors could enhance the mechanical strength and ensure higher graft efficiency of the hybrid alginate beads.

Characterization of PNIPAAm-grafted alginate beads

Scheme 1B presents the schematic illustration of PNIPAAm-grafted alginate beads. PNIPAAm existed in the pores of the alginate beads acts as the "on–off" gates according to the change of temperature. At 25°C, the pores of the beads are covered by the stretched PNIPAAm, the drug release rate is low; at 37°C, the pores of the beads are opened due to the shrinking of PNIPAAm resulting in an easier drug release from the beads interior. On the other hand, the alginate beads are in a swollen state during the grafting procedure because of the excellent swelling property of alginate.[26] It is very easy for NIPAAm to permeate into the inner pores of alginate beads. Therefore, it could be imagined that PNIPAAm has not only been grafted onto the surface but also onto the inner pores of the alginate beads in the present work.

Three samples have been prepared in our experiments as illustrated in Table 1, the ungrafted beads (U1), the PNIPAAm-grafted beads with PAANa (G1), and the PNIPAAm-grafted beads without PAANa (G2). The concentration of Na_2HPO_4 is 250 mM for all samples. It can be found that the drug content of U1 is only 0.368 (mg/10 mg beads). For G1 and G2, the value increases to 0.390 and 0.376, respectively. The difference in drug content is probably derived from the swelling property of the samples. It can be observed in drug loading experiment that the swelling ratio of the PNIPAAm-grafted beads is higher than that of the ungrafted ones (this point will be discussed carefully in the swelling study). Supposedly, the diffusion channels for indomethacin is wider in the swollen PNIPAAm-grafted beads, which allows more drug molecules to be permeated into; as a result, the drug loading content becomes higher than the ungrafted ones. Additionally, the biomineralized polyelectrolyte layer could prevent the permeation of the encapsulated drug in a

Table 1. Composition and drug loading efficiency of the studied beads

Sample	PAANa/alginate (wt%)	NIPAAm concentration (wt%)	Drug content (mg/10 mg beads)
Ungrafted alginate beads (U1)	5	0	0.368 ± 0.007
NIPAAm-grafted alginate beads with PAANa (G1)	5	20	0.390 ± 0.010
NIPAAm-grafted alginate beads without PAANa (G2)	0	20	0.376 ± 0.008

neutral pH condition, which also increases the drug content of the resulting beads.

Figure 1A shows the XPS N_{1s} core-level spectra of ungrafted and PNIPAAm-grafted alginate beads. A new peak emerged at binding energy (BE) of 398 eV can be found in the N_{1s} spectrum of PNIPAAm-grafted alginate beads G1, which cannot be found in case of the ungrafted sample U1 (the very small peak is attributed to the NH_2 group of chitosan). It can also be observed that the N_{1s} peak of G2 is smaller than that of G1, indicating that PAANa with ultra high-molecular weight could increase the hydrophilicity and ensure higher graft efficiency of the alginate beads as discussed formerly. Figure 1B and C shows the XPS O_{1s} core-level spectra of samples U1 and G1, respectively. Both of the two spectra can be curve-fitted with two peaks at BE of 529 eV for the C=O species and 531 eV for the C–O–C species, respectively. The peak of the C=O species in G1 is larger than that in U1, which means an increase in the content of –C=O–NH– group (derived from PNIPAAm) resulting in a relative decrease of the ether bonds. These results confirm that PNIPAAm has been successfully grafted onto the surface of the alginate beads.

From the digital photos of PNIPAAm-grafted beads as illustrated in Scheme 1C, it could be observed that the sample G1 in PBS (pH = 7.4) changes from almost colorless transparent to white, when the temperature increases from 25 to 37°C. This is in line with the previous observations in alginate/PNIPAAm semi-IPN beads.[27] The formation of the white region, generated above the LCST is due to the collapse and shrinkage of the PNIPAAM chain on the pores of alginate beads, which could lead to an increase in light diffusion.[27] This observation suggests that PNIPAAm has existed on the pores of the alginate bead, and also suggests that the temperature sensitivity of PNIPAAm chain is not affected by the presence of nearby physically entangled alginate chains, biomineralized polyelectrolyte layer, and PAANa.

The surface morphology of PNIPAAm-grafted and ungrafted beads was also observed with SEM as shown in Fig. 2. The

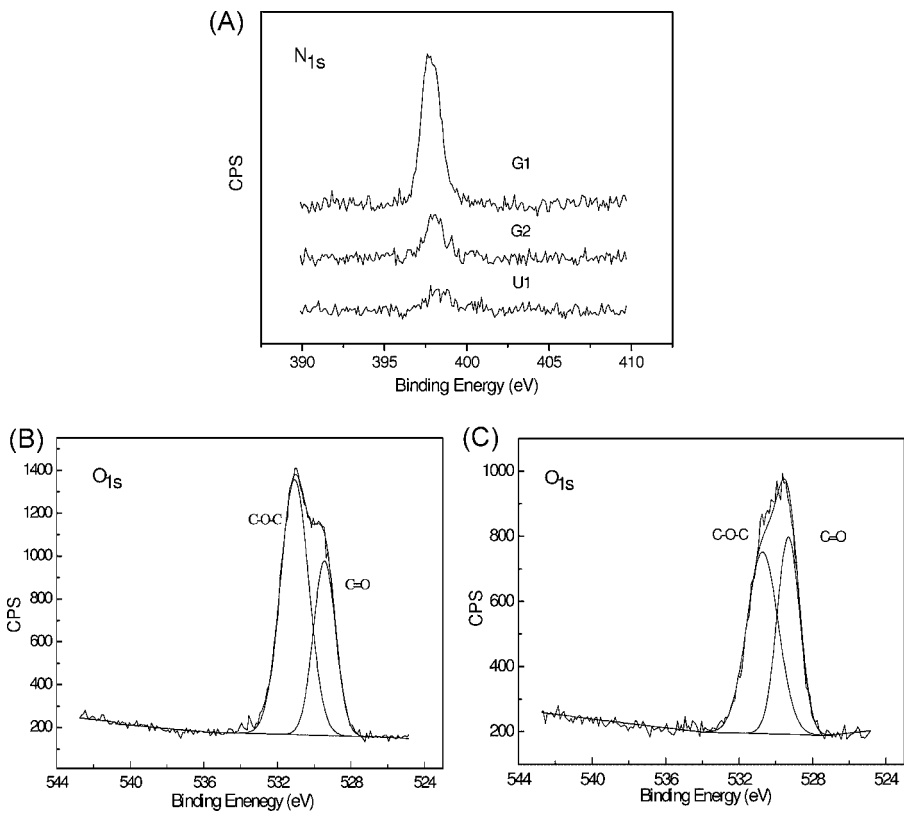

Figure 1. XPS N_{1s} core-level spectra of ungrafted beads and PNIPAAm-grafted beads (A), O_{1s} core-level spectra of ungrafted beads (B), and O_{1s} core-level spectra of PNIPAAm-grafted beads (C).

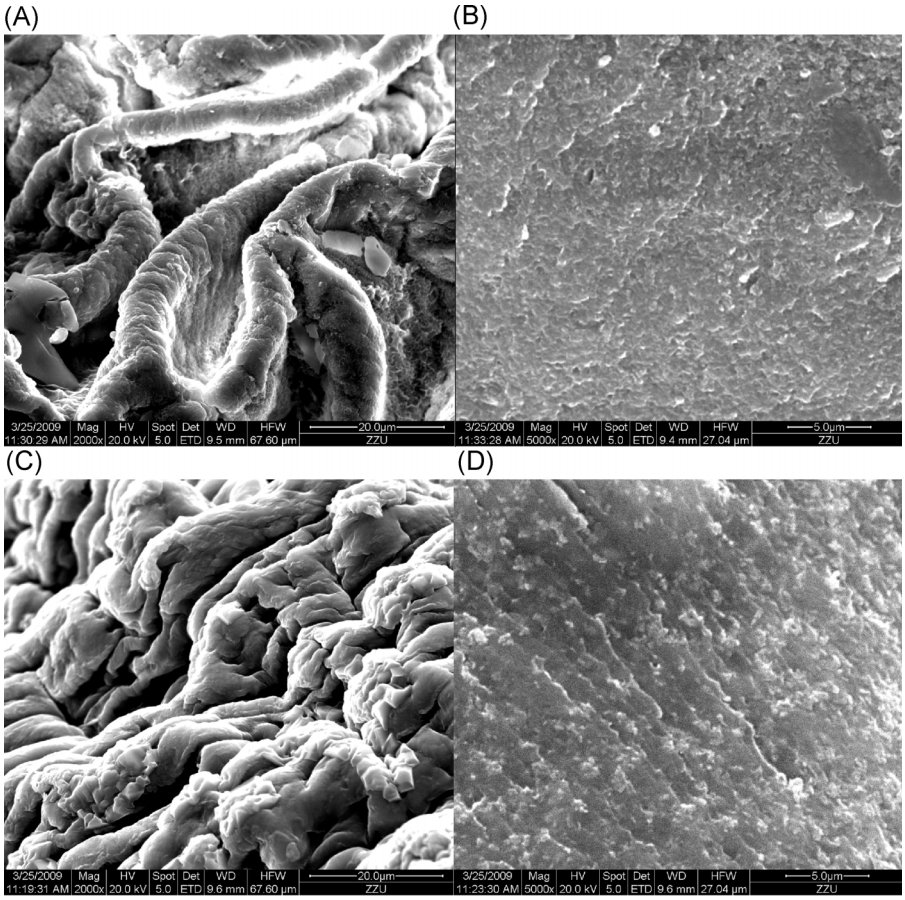

Figure 2. SEM images of ungrafted bead (surface) (A), ungrafted bead (cross-section) (B), PNIPAAm-grafted bead (surface) (C), and PNIPAAm-grafted bead (cross section) (D) (magnification: A, C × 2000, B, D × 5000).

surface of the PNIPAAm-grafted beads (Fig. 2C) become denser than that of the ungrafted ones (Fig. 2A),[28] indicating the incorporation of PNIPAAm layer in the pores of the alginate beads. These results confirm that, by using the polymerization method in the present study, PNIPAAm has been grafted onto both the outer surfaces and the inner pores of the hybrid alginate beads. However, there is no distinct difference between the cross-section of G1 and U1 as illustrated in Fig. 2D and B. Perhaps most of PNIPAAm are grafted on the surface of alginate beads, the content of PNIPAAm grafted on the inner pores of the alginate beads is relatively low. As a result, there is no distinct change in the cross-section after grafting. The presence of Ca and P elements within the outer biomineralized polyelectrolyte shell is confirmed by FT-IR and EDS analysis. For all the samples, three strong absorptions around 1035, 600, and 561 cm^{-1} assignable to the P–O bonds can be observed in their FT-IR spectra, suggesting the formation of biomineralized layer around the beads.[16] Additionally, as shown in the EDS spectrum of PNIPAAm-grafted beads (Fig. 3), a signal for the P element can be observed not only in the surface but also in the cross-section of the grafted beads, which is different from that in the ungrafted ones.[16,20] It should be noted that the contents of P, Ca, and O elements in the surface of the grafted beads are much higher than other elements, indicating that the outer shell is mainly composed of $CaHPO_4$. It also can be found that the contents of P and Ca elements in the cross-section are much lower than those in the surface of the grafted beads, indicating that it is relatively difficult for Ca^{2+} to diffuse into the inner pores of the alginate beads.

Swelling study

Figure 4 shows the swelling behavior of the prepared beads at different pH values and temperatures. It can be observed that at pH 7.4 the swelling ratio increases after grafting, from ~13.8% for sample U1 to ~25.6% for G1 and ~27.8% for G2 at 37°C. At 25°C, the value increases from ~10.7% for U1 to ~23.1% for G1 and ~24.9% for G2. Analysis by a Student's *t*-test showed that the differences between the swelling ratio of ungrafted beads and PNIPAAm-grafted beads are statistically significant (greater than 95% confidence) at pH 7.4. The *p*-value was in the range of 0.001–0.004. Therefore, it is apparent that the swelling ratio of the grafted beads is higher than that of the ungrafted ones at pH 7.4. This phenomenon can be explained as that the introduction of PNIPAAm increases the hydrophilicity of the alginate beads, which results in the increase of swelling ratio. Regarding G1 and G2, the small differences in the swelling behavior are not statistically significant (p-value > 0.1 analysis by a Student's *t*-test), both at 37 and 25°C.

It also can be observed from Fig. 4 that the small differences in the swelling behavior of grafted beads at different temperatures (37 and 25°C) are not statistically significant at pH 7.4 (p-value > 0.1 analysis by a Student's *t*-test). Reminding that

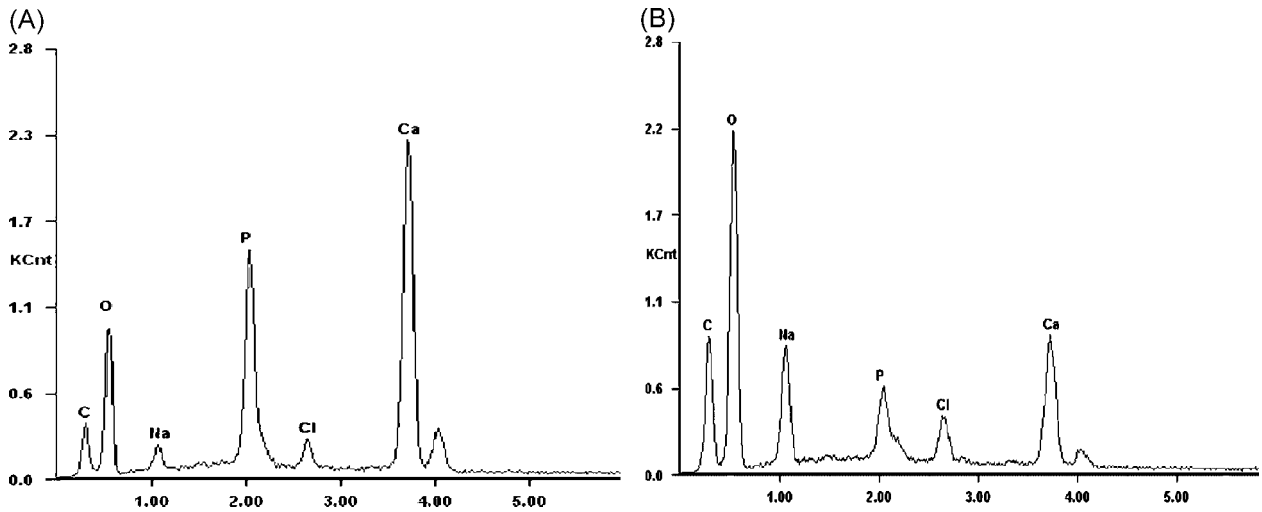

Figure 3. EDS spectrum of surface (A) and cross-section (B) of PNIPAAm-grafted beads.

the grafted PNIPAAm is hydrophilic at 25°C but hydrophobic at 37°C, it is extremely possible for the beads to take away more water at 25°C than at 37°C during the water wiping process because the hydrophobic grafted-PNIPAAm surface layer could prevent water inside the beads from escaping. This point was further supported by the optical photos of PNIPAAm-grafted beads in PBS (pH = 7.4) at 25 and 37°C, respectively, as shown in Fig. 5. The bead diameter decreased from 4.5 to 4 mm with increase in the temperature from 25 to 37°C, and the color of the bead changed from almost colorless to white, indicating the hydrophobic grafted PNIPAAm inside the alginate beads was in a shrunken state. It should be noted that such a size decrease was not as significant as that of the semi-IPN beads consisting of PNIPAAm and alginate as described in literature 15. PNIPAAm existed in the polymeric network for the semi-IPN beads, whereas it is mostly attached in the surface of the pores in PNIPAAm-grafted alginate beads. At the temperature higher than LCST, the polymeric network will not be broken by the shrinkage of PNIPAAm.[29,30]

In the low pH region (pH 1.2), most of the carboxylic acid groups in alginate are in the form of –COOH, as the pK_a of alginate is about 3.2. The hydrogen bonds between –COOH in alginate and –CONH– in PNIPAAm leads to polymer–polymer interactions predominating over the polymer–water interactions.[27] Therefore, the swelling ratio of the studied beads is very low as described in Fig. 4. These results demonstrate that the pH-sensitivity of the alginate beads are not affected by the grafted-PNIPAAm chains and the biomineralized polyelectrolyte layers. As the pH value changed to 7.4, the carboxylic acid groups become ionized and a small quantity of H^+ in water acts as a bridge within alginate molecules, resulting in the increase of the swelling ratio.

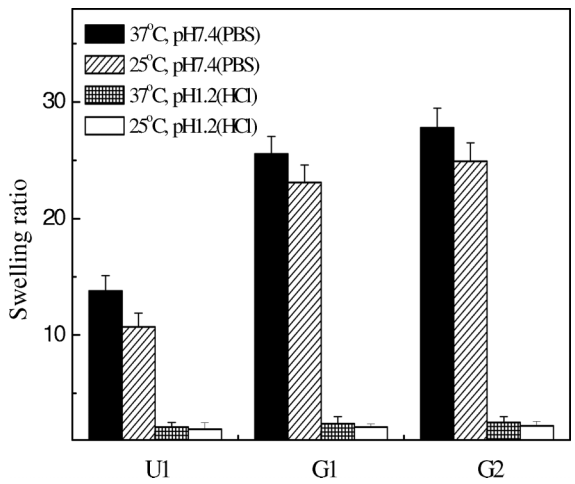

Figure 4. Temperature- and pH-dependent changes of equilibrium swelling ratio for the studied beads.

Drug release study

Figure 6 shows the indomethacin release profiles of ungrafted beads U1 and PNIPAAm-grafted beads G1 at different temperatures. Distinct difference can be observed that the release amount is larger than 60% within 12 hr at 37°C for G1, while a drug release amount of only 30% took the same period for the same sample at 25°C (Fig. 6A). It also can be found from Fig. 6A that the drug release between 37 and 25°C is slightly different in the first 5 hr. Then the drug release between 37 and 25°C is significantly different after 5 hr. The probable reason for this phenomenon is that the samples could reach the sufficient swelling condition. As a result, the beads are swollen completely

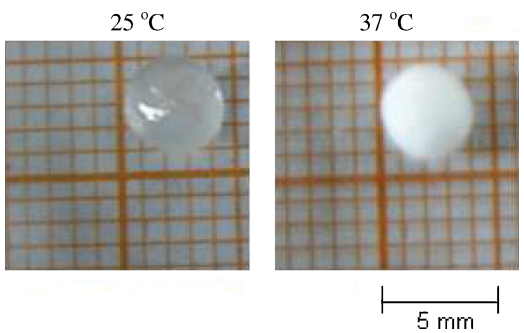

Figure 5. Digital photos of PNIPAAm-grafted alginate bead (G1) at 25 and 37°C. This figure is available in color online at wileyonlinelibrary.com/journal/pat

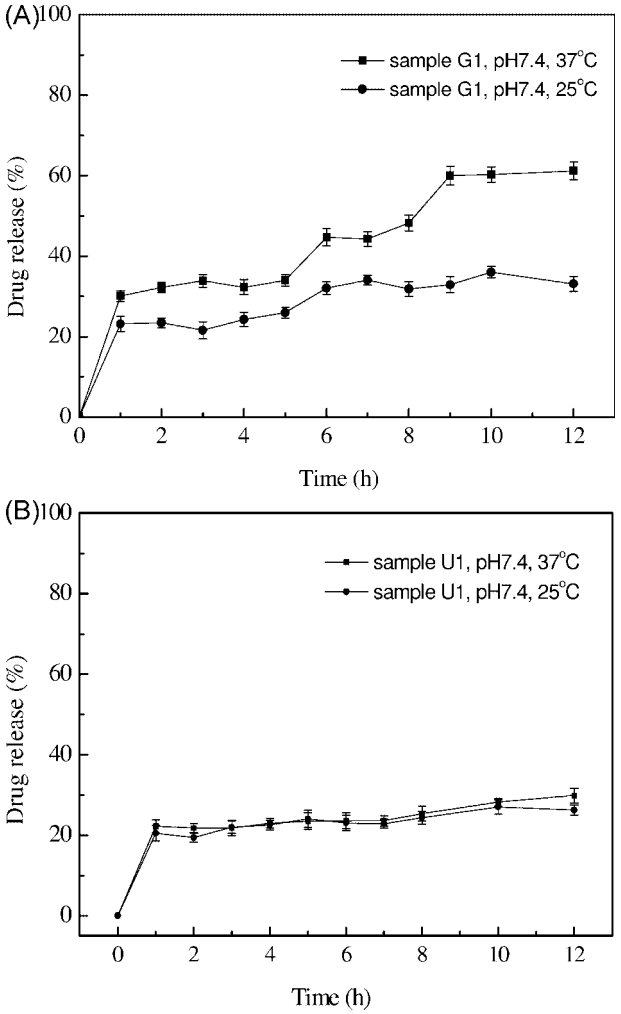

Figure 6. Release profiles of indomethacin from the PNIPAAm-grafted beads G1 (A) and ungrafted beads U1 (B) measured at 25 and 37°C.

and the pores of alginate beads are opened because of the shrinking of PNIPAAm, which result in the faster drug release. While, there is no distinct difference in the release profiles of sample U1 at different temperatures (Fig. 6B), the *p*-values obtained from Student's *t*-test analysis in comparison between the data of U1 at 37°C and 25°C were larger than 0.1. That is, the ungrafted beads show no temperature sensitivity for the absence of PNIPAAm in the pores of alginate beads. Grafted-PNIPAAm in the pores of alginate beads act successfully as temperature sensitive materials for indomethacin release.

The main reason for explaining the higher release rate for semi-IPN PNIPAAm/alginate beads at 37°C is the precipitation of PNIPAAm above LCST, which leads to the gating effect of the drug. Instead of precipitating from the alginate network as discussed in semi-IPN PNIPAAm/alginate dual responsive system,[20,21,28,30] the shrinkage of PNIPAAm will not break the polymeric network in PNIPAAm-grafted alginate beads because PNIPAAm is mostly attached in the surface of the pores. As discussed in the literature,[30] PNIPAAm-grafted alginate hydrogels would show suitable mechanical strength without collapsing during repeatable shrinkage and expansion changes, whereas bulk-grafted or semi-IPN PNIPAAm/alginate hydrogels collapsed easily. These results suggest that PNIPAAm-grafted alginate beads show a thermo-responsive release characteristic.[31] At temperatures below the LCST, the grafted PNIPAAm chains are swollen and the pores of the beads are covered by the stretched PNIPAAm; the release of indomethacin molecules across the beads is slow. In contrast, at temperatures above the LCST, the grafted PNIPAAm chains are shrunken and the pores of alginate beads are opened because of the shrinking of PNIPAAm, which result in the faster drug release.[32,33] Consequently, the release rate of the indomethacin molecules from the NIPAAm-grafted alginate beads is higher at 37°C than that at 25°C. The above results verified that the grafted PNIPAAm chains in the surface of the pores act successfully as a temperature sensitive valve for indomethacin release.[34] This temperature-dependent "on/off" characteristics of PNIPAAm-grafted alginate beads enable the release of the drug in a controlled way by simply adjusting the environmental temperature.

Reversibility of drug release through the PNIPAAm-grafted beads in response to temperature change was measured at pH 7.4 with temperature alternating between 25 and 37°C, as shown in Fig. 7. In the experiment, PNIPAAm-grafted beads (G1) were treated for the first 1.5 hr at 25°C. After 1.5 hr, sample G1 was moved from a horizontal laboratory shaker with 25°C to another shaker with 37°C. This drug release experiment with temperature alternating between 25 and 37°C was performed for 22.5 hr, and then a thermal-responsive release profile would be obtained as described in Fig. 7. It can be found that the drug release is relatively low when the sample was placed in 25°C, and the drug release will significantly increase when sample was placed in 37°C. This result can demonstrate that the temperature-dependent "on/off" characteristics of PNIPAAm-grafted alginate beads enable the release of the drug in a controlled way by simply adjusting the environmental temperature as discussed formerly. This "on/off" property can be kept for six cycles with significant response to temperature change as demonstrated in Fig. 7.

Two different pH values, pH = 1.2 and 7.4 (similar to that of gastric and intestinal fluids respectively), are selected as the pH conditions in our study to demonstrate the pH-responsive property of the studied membranes. Figure 8 presents the drug release behaviors at 37°C for sample G1 at pH 1.2 and pH 7.4. It is clear that at pH 7.4 the maximum value attained for drug release

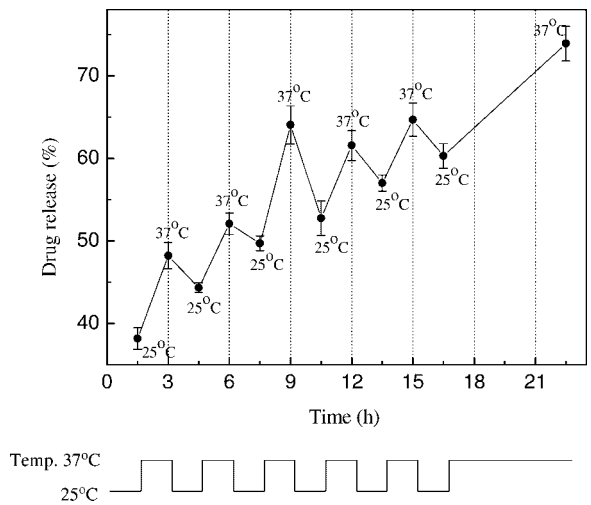

Figure 7. Reversibility of drug release through the PNIPAAm-grafted beads G1 in response to temperature change measured at pH 7.4.

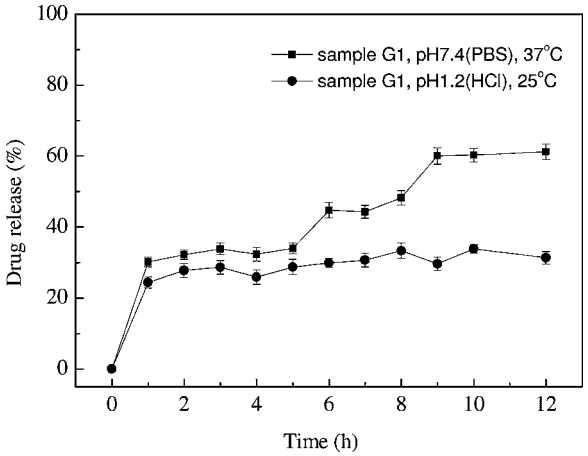

Figure 8. pH-dependent release profiles of indomethacin at 37°C from PNIPAAm-grafted beads G1 measured at pH 1.2 and 7.4.

is more than 60% after 12 hr, whereas at pH 1.2 the maximum drug release is only 30% after subjecting the beads to the same treatment. The relatively low release amount of the studied beads at pH 1.2 should be related to the low degree of the swelling ratio of the beads in acidic conditions as discussed in Fig. 4. Therefore, a pH-dependent response can be observed for PNIPAAm-grafted alginate beads. The pH- and thermo-responsive property of the hybrid alginate beads indicates that the grafted beads not only keep the temperature-dependent "on/off" characteristics, but also preserve the pH-sensitivity after the introduction of the biomineralized polyelectrolyte coating and the grafting process.

It should be noted that the solubility of indomethacin in acidic conditions is much lower than in neutral solutions. Therefore, besides the response of alginate to pH, as clearly detected in swelling, the release profile may also be dependent on pH due to the difference in indomethacin solubility. In fact, the drug content of samples in the present study is very low. As described in the text, the maximum indomethacin concentration is 0.004 mg/ml (indomethacin/PBS) and 0.008 mg/ml for the determination test of drug encapsulation efficiency and the drug release test, respectively. The solubility of indomethacin in the experimental medium is 0.32 and 0.36 mg/ml at pH 7.4 for 25 and 37°C, respectively; the value is 0.07 and 0.08 mg/ml at pH 1.2 for 25 and 37°C, respectively. The maximum indomethacin concentration is much lower than that of indomethacin's solubility in the present study. Therefore, indomethacin can be dissolved thoroughly at both pH 7.4 and 1.2.

CONCLUSIONS

This paper presented a useful example of grafting thermo-responsive polymer PNIPAAm onto the biodegradable alginate beads via UV-induced chemical method. The biomineralized polyelectrolyte layer was constructed aiming to enhance the mechanical strength and to ensure higher graft efficiency. The swelling behavior and the drug release behavior indicated that the PNIPAAm-grafted beads were pH- and thermo-sensitive. The temperature-dependent "on/off" characteristics of PNIPAAm- grafted alginate beads enabled the smart release for drug in a controlled way by simply adjusting the environmental temperature. These gating beads provide a new mode of behavior for thermo-responsive "smart" polysaccharide material, which is highly attractive for targeting drug delivery systems and chemical separations.

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