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Sustained drug release on temperature-responsive poly(*N*-isopropylacrylamide)-integrated hydroxyapatite

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A hybrid temperature-responsive hydroxyapatite–poly(*N*-isopropylacrylamide) (HAP–PNIPAAm) gel has been synthesized by the interpenetration of PNIPAAm into a sintered HAP disk through a radical-initiated polymerization of NIPAAm monomers under N_2 atmosphere, and shows sustained positive thermo-responsive drug release profile over a month at PBS buffer.

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HAP] ceramics have been extensively studied as bone substitutes because of their biocompatibility and osteoconductivity.[1] Their cation exchange property[2] and the ability of adsorbing organic molecules[3] have widely extended their applications in industrial and medical fields. A sufficient pore dimension, an interconnected pore structure, and nonbiodegrability are required for bone ingrowth.[4] High strength HAP-based bioceramics have been also synthesized through nanostructure processing.[5] Covalent immobilization as well as physical adsorption of functional polymers on metal oxides or metal phosphates has been also studied for selective separation of benzene derivatives on HPLC.[6]

Synthetic polymers that undergo discontinuous volume phase transitions in response to external stimuli such as temperature,[7] pH,[8] ionic strength,[9] and electric field[10] have received increased attention in the last few decades owing to their possible versatile applications such as controlled drug delivery and selective catalysis. For example, PNIPAAm hydrogel changes its volume dramatically at its lower critical solution temperature (LCST, 34 °C) due to the hydrophobic interaction of isopropyl groups of the polymer in water.

We here report that HAP–PNIPAAm prepared by interpenetration of NIPAAm monomers into a sintered HAP disk followed by a polymerization shows thermosensitive properties and a positive thermo-responsive drug release profile over a month. The highly crystalline sintered HAP network showed no change in its crystallinity after the release experiment.

HAP was molded into a disk (13 mm diameter, 2 mm thickness) and sintered at 1000 °C for 10 h. A HAP–PNIPAAm composite material was prepared by the radical-initiated polymerization of NIPAAm (monomer) and *N*,*N*′-methylene bisacrylamide (NMBA) with ammonium persulfate on a HAP disk in water.[11] After the polymerization PNIPAAm outside of the composite was cut off. The dried composite was immersed in a saturated solution of indomethacin in EtOH–water (8 : 2, v/v) overnight and dried over a period of 3 days at room temperature. The drug-loaded composite was immersed in 10 mL of phosphate buffer (pH 7.4, 10 mM). Solutions containing the drug-loaded composite was shaken in an Environ Shaker for stepwise temperature changes between 25 and 40 °C. The indomethacin concentration of the solution was measured using a UV–Vis spectrophotometer at different time intervals. After each measurement, 10 mL of PBS buffer was replaced.

HAP used in this study has a porous structure with > 1.0 μm pore diameter and a specific surface area of 67 $m^2\ g^{-1}$, which is close to those of natural bone materials. After sintering at 1000 °C for 10 h, its surface area was decreased to 11 $m^2\ g^{-1}$ without changing the pore diameter. The swelling for the sintered HAP and HAP–PNIPAAm composite was studied with respect to temperature as shown in Fig. 1. The hydrophilic HAP shows constant water swelling with a very slight decrease with increase of temperature. However, the HAP–PNIPAAm composite shows a pronounced deswelling with increased temperatures at 34 °C (LCST). The swelling of PNIPAAm incorporated in thermostable HAP can be severely hindered, showing dramatically decreased swelling at low temperature relative to that of a pure PNIPAAm hydrogel.[11] The BET results obtained by an N_2 sorption experiment indicate that the small specific surface area of the hybrid material is caused by sintering with concomitant disappearance of the original micropores after sintering at high temperature. The crystallinity of the materials and their chemical composition were investigated by powder X-ray diffraction (XRD). This shows that HAP is highly crystalline (no amorphous phases) after sintering and even after release test, and reveal no other significant components, with the presence of minimal amounts ($\leq$0.5%) of MgO (2θ = 42.88) and CaO (2θ = 37.42). The FT-IR spectra prove the presence of an infiltrated PNIPAAm network with no other significant components. Bands are observed at 1645.0 for carbonyl (amide), 1386.6 and 1367.8 cm^{-1} for the isopropyl group of PNIPAAm, and 1044.5, 961.0 cm^{-1} for P–O bands of the HAP network, respectively. Thermogravimetric analysis on the HAP–PNIPAAm material was conducted up to 500 °C (5 K min^{-1}). A significant weight loss (15.0 wt%) in the HAP–PNIPAAm sample was detected by TGA and a large endothermic transition was observed at 325 °C in the DTG, caused by the endothermic dissociation of organic PNIPAAm infiltrated into the HAP network. By contrast, no weight loss and no peak in the DTG spectrum of the HAP sample were observed. Scanning electron microscopy (SEM) showed incorporation of organic PNIPAAm in the HAP network and a dense PNIPAAm layer at the outer surface of the pallet sample (Fig. 2). The grain size of the HAP used in this study was 30–40 nm diameter, and energy dispersive X-ray spectroscopy (EDX) analysis showed the calculated ratio Ca/P was 1.38, *i.e.* phosphorus-rich, lower than that (1.67) of natural HAP ceramics.[1]

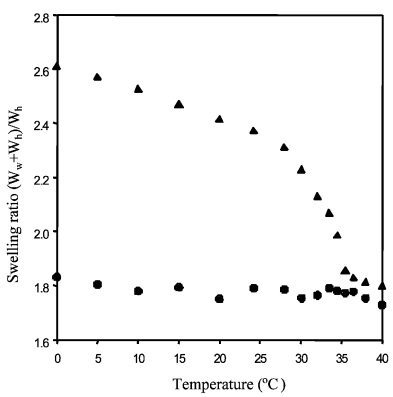

Fig. 1 The equilibrium weight swelling ratio, $(W_w + W_h)/W_h$, where W_w is the weight of adsorbed water and W_h is the hybrid composite weight, of sintered HAP and HAP–PNIPAAm composite in PBS as a function of temperature: (●) sintered HAP; (▲) HAP–PNIPAAm.

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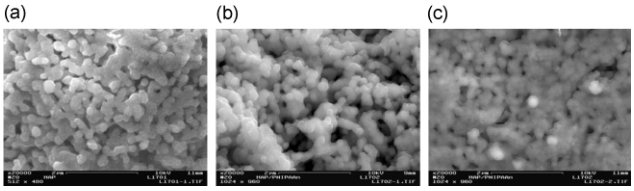

Fig. 2 SEM images of (a) HAP and (b, c) HAP–PNIPAAMm (b: cross-section, c: flat surface).

Indomethacin, known as an antiinflammatory drug for treatment of chronic rheumatism, was used to study thermosensitive drug release on HAP–PNIPAAm. The total loading content of IMC was determined by the total amount of released IMC until no IMC was detected by UV, and was determined to be about 6 mg of IMC per g of composite. Drug release experiment was investigated by stepwise temperature changes between 25 and 40 °C. An extremely sustained release pattern was observed (Fig. 3). At 40 °C slow but constant drug release rate was observed while no release rate was observed during the low temperature period (25 °C). In principle, pores in HAP are too large and thermostable to control IMC release rate in this condition. An 'on–off' pattern of drug release is caused by the swelling and deswelling of thermosensitive PNIPAAm inside the pores of HAP. At low temperature (off process) swollen PNIPAAm traps the drug inside the HAP pores. During the initial high temperature period the drug is squeezed in to pore channels by the PNIPAAm shrinking and diffuses out of the channels at the continuous high temperature. A positive release profile of IMC on HAP–PNIPAAm was sustained over a month,[12] while drug release on other composite systems such as polymer–metal oxides and polymer–polymer composites last for less than two weeks.[13] This observation suggests that the interaction between IMC and Ca^{2+} plays an important role in sustained drug release. IMC with a carboxylate group can interact with Ca^{2+} ion easily either by hydrogen bonding or by chemical coordination through a water molecule, which would be very stable in PBS buffer at room temperature. However, at the elevated temperature (40 °C) IMC molecules are released slowly because heat aids the disruption of the hydrogen bond-type interaction. Fig. 4† shows the interaction between IMC and Ca^{2+} in aqueous solution, and for the solid composite matrix. The complex between Ca^{2+} and IMC in aqueous solution (Fig. 4(b)) shows a different electronic transition pattern from free Ca^{2+} ions in water (Fig. 4(a)). UV spectra of the complex of IMC in calcium phosphate solution prepared *in situ* (Fig. 4(c)) and of IMC in the HAP–PNIPAAm composite (Fig. 4(d)) are

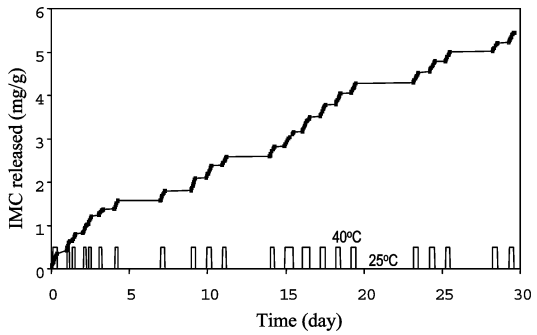

Fig. 3 Sustained release of IMC from HAP–PNIPAAm in response to stepwise temperature changes in PBS (pH 7.4).

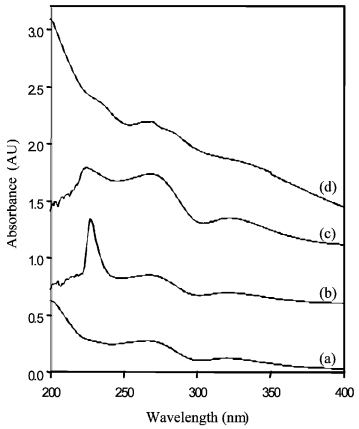

Fig. 4 UV spectra for IMC in different solutions and in a matrix: (a) 0.02 mM IMC in H_2O; (b) 0.02 mM IMC in $Ca(NO_3)_2$ solution; (c) 0.02 mM IMC in $Ca_3(PO_4)_3$ solution prepared *in situ*; (d) IMC in HAP–PNIPAAm.

very similar with little difference in peak intensity, and are also similar to the UV spectrum of the complex between Ca^{2+} ion and IMC in aqueous solution (Fig. 4(b)) with only minor differences, suggesting that IMC interacts with Ca^{2+} ions in the HAP–PNIPAAm composite.

In conclusion, hybrid HAP gel interpenetrated with thermosensitive PNIPAAm hydrogel has been developed for controlled drug release, and shows a sustained positive thermosensitive release profile when the temperature was maintained above the LCST. The sustained release is caused by the attractive interaction between Ca^{2+} in HAP and carboxylate of IMC. This concept can be applied for biodegradable polymers in osteophoresis.

Notes and references

† Baselines for each UV spectra were subtracted using the original blank solution.

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