The first pamidronate containing polymer and copolymer†

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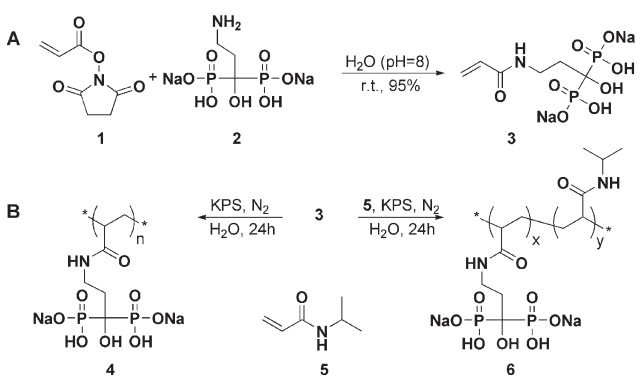
Here we report the synthesis, characterization and hydrogelation of polymers consisting of pamidronate, a useful therapeutic agent.

This paper describes the synthesis, characterization and application of the polymers consisting of pamidronate as the pendant group. Pamidronate (**2**) belongs to a group of drugs that contain a geminal bisphosphonate bond and inhibits bone resorption because its P–C–P motif (like pyrophosphate) chelates with calcium ions to result in high affinity for bone mineral. Bisphosphonates have found clinical applications as drugs for treating bone diseases (*e.g.* Paget's disease, hypercalcemia of malignancy and osteoporosis).[1] In recent years, the biomedical applications of bisphosphonates have expanded rapidly. For instance, bisphosphonates, administered to women with breast cancers, provide a supportive treatment to patients with bone metastases.[2] As inhibitors for farnesyl pyrophosphate synthases, bisphosphonates promise a new class of antiparasitic agents.[3] Additionally, hydrogels containing bisphosphonate are effective for treating uranium wounds because of the high affinity of bisphosphonate group towards uranyl ion (UO_2^{2+}).[4] The specificity of bisphosphonates towards bones has led to the research on conjugates of bisphosphonates with near-infrared dyes or macrocyclic chelating ligands for bone imaging and with steroids or proteins for bond therapy.[5,6] Particularly, a dendritic tetra(bisphosphonic acid) improves targeting of proteins to bone *via* multivalent interactions originated from the tetramer,[6] which implies that the polymers of pamidronate should confer high affinity to bone and serve as a new type of polymers for potential applications targeted to bones. Therefore, it is necessary to develop a feasible strategy for presenting pamidronate in polymeric form.

Incorporation of drugs or inhibitors to a polymer is a powerful strategy for exploring and expanding the applications of existing therapeutic agents in polymeric forms.[7,8] In addition to affording a platform for the controlled release of drugs,[7] the attachment of multiple copies of inhibitors to a polymer creates polyvalent or multivalent drugs.[9] Despite excellent therapeutic properties of bisphosphonates, only a few works have explored the incorporation of bisphosphonates onto the reactive side chains of polymers *via* grafting,[10] while the amount of bisphosphonates incorporated is quite limited. To avoid such a limitation, it is worthwhile developing a versatile bisphosphonate-based monomer, as recently demonstrated by Schrader and co-workers,[11] which polymerizes or copolymerizes easily for further exploration of polymeric bisphosphonates. Therefore, we have designed and synthesized a novel monomer—*N*-acryl pamidronate (**3**), which polymerizes to form poly(*N*-acryl pamidronate) (**4**) and poly(*N*-acryl pamidronate-*co*-*N*-isopropylacrylamide) (**6**). To the best of our knowledge, this is the first time that polymeric pamidronates have been synthesized. Inspired by the work on the mineralization of hydroxyapatite (HA) using the hydrogels as a scaffold,[12,13] we also synthesized a new class of hydrogel containing polymeric pamidronate (*via* the crosslinking of **6**) and evaluated the mineralization of HA on the hydrogel made of **6**. Since the incorporation of pamidronate moiety into the hydrogel augments its affinity towards calcium ion, the mineralized hydrogel (*i.e.* composite of the hydrogels and HA) may serve as a suitable biomaterial for mimicking natural bone.

Monomer **3** was synthesized by reacting *N*-acryloxysuccinimide (**1**) with a molar equivalent of pamidronate disodium salt (**2**) in water (Scheme 1(A)). After 24 h reaction at room temperature, the addition of absolute ethanol to the reaction mixture precipitated **3** in 95% yield. The polymerization of **3** was allowed to take place in aqueous solution since it dissolves well in water. We used potassium persulfate (KPS) as the initiator and found that KPS was able to initiate the homopolymerization of **3** in water at 70 °C (Scheme 1(B)). We also found that oligomers formed only when the amount of KPS was less than 5 mol% of **3**. The bisphosphate groups probably terminated the free radicals during the reaction so that more KPS was required to prepare the homopolymer (**4**). The determined molecular weight of **4** produced at 5 mol% of KPS is low (5068 g mol^{-1}) with a polydispersity of 1.15. In order to improve the molecular weight of **4**, we first carried out the polymerization reaction using 2 mol% of KPS at 70 °C. After 12 h, we added additional 2 mol% of KPS under N_2 protection and



Scheme 1 Synthesis of pamidronate-containing monomer (**3**), homopolymer (**4**) and copolymer (**6**).

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Table 1 Reaction conditions for preparing the hydrogels and their corresponding compositions

Hydrogel	**3** (mg)	**5** (mg)	BIS[a] (mg)	DHEBA[a] (mg)	KPS[b] (mg)	H_2O[c] (mL)
Gel I	66.6	429.4	6.0	0.0	9.2	5.7
Gel II	140.6	429.4	6.5	0.0	9.7	6.0
Gel III	66.6	429.4	0.0	8.0	9.2	5.7

[a] BIS and DHEBA as a crosslinker (1.0 mol% of total monomers). [b] KPS as an initiator (0.85 mol% of total monomers). [c] H_2O as a solvent (total monomers concentration is 0.7 mM).

allowed the reaction to continue for another 12 h. After purification, we successfully obtained **4** with a molecular weight of 25 273 g mol^{-1} and polydispersity of 3.10.

We choose *N*-isopropylacrylamide (**5**) as the comonomer because it has good solubility in water and is the most often used monomer for the synthesis of thermosensitive hydrogels.[14] Scheme 1(B) shows the synthetic route to poly(*N*-acryl pamidronate-*co*-*N*-isopropylacrylamide) (**6**). After the two monomers were mixed in water under an N_2 atmosphere, the solution in a flask was immersed in a water-bath at 70 °C and stirred with a continuous bubbling of nitrogen. After the addition of the initiator, KPS, the reaction was carried out for 24 h to ensure the completion of the copolymerization. The structure of **6** was determined by ^{1}H NMR spectroscopy (see ESI†). The molecular weight of **6** was 46 058 g mol^{-1} with a polydispersity of 3.07 (see ESI†).

Compound **5** is also a useful monomer for making crosslinked poly(*N*-isopropylacrylamide) (PNIPAAm), one of the most studied polymeric hydrogels for biomedical application.[14] Therefore, we used **5** as the comonomer of **3** to produce a new type of bifunctional hydrogel (*i.e.* bioactivity of pamidronate and thermosensitivity of PNIPAAm) by copolymerizing **3** and **5** in the presence of a crosslinker, *N*,*N*′-methylene bisacrylamide (BIS) or *N*,*N*′-(1,2-dihydroxyethylene) bisacrylamide (DHEBA). Three crosslinked hydrogels were prepared. Table 1 gives the amount of each component. In a typical procedure,[15] after the removal of oxygen by degassing, KPS was added to the mixture, which was transferred to a vial, sealed and placed in a 70 °C water-bath for 24 h. The resulting hydrogels (Fig. 1(A)) were soaked in deionized water for 4 days (the medium was changed daily) to remove remaining monomers and non-crosslinked polymers. Then, the hydrogels were dried until reaching constant weight. The corresponding SEM image (Fig. 1(B)) reveals that it consists of a porous network. This porous pamidronate hydrogel can be useful for controlled drug release, enzyme immobilization, cancer treatment, or as tissue engineering scaffolds such as directing mineralization of hydroxyapatite to mimic bone structure.

The use of polymer films to direct the mineralization of HA for mimicking bones has attracted considerable efforts. The hydrogel consisting of pamidronate is an excellent candidate because the pamidronate moiety has high affinity for calcium ion. Thus, we examined the growth of HA on the surface of the hydrogel (gel I) according to literature method.[13] In the first procedure (urea-mediated process), gel I was soaked in an acidic solution (pH = 2.5–3) of HA containing urea (2 M). Upon gradually heating the mixture from room temperature to 90–95 °C (within 2 h and without stirring), urea started to decompose, and the pH slowly increased to around 8. After reaching 95 °C, the mineralization took place for 24 h. The strong affinity between calcium ion and the pamidronate moieties on the surface of gel I led to the growth of HA crystals forming spherical aggregates (Fig. 1(C)), a typical morphology observed with crystalline apatite grown on a polymeric scaffold by using the same mineralization method.[13] The calibrated energy dispersive spectroscopy (EDS) area analysis on the mineral surface of the composite reveals a Ca/P ratio (1.6 ± 0.1), similar to that of synthetic HA (see ESI†). Furthermore, X-ray powder diffraction (XRD) of the composite material gives peaks that match typical reflections ([002] and [112]) of crystalline HA (Fig. 2(A)), which differs from the XRD of gel I (Fig. 2(C)). In the second procedure (ammonia-mediated process), the hydrogel was soaked in an acidic solution (pH = 2.5–3) of HA. Then the whole mixture was put under an atmosphere of ammonia. Upon the diffusion of the ammonia, the pH of the mixture slowly increased, and HA grew onto the hydrogel after 24 h. Though the second procedure produced HA with a slightly different morphology (Fig. 1(D)), XRD confirmed these flowerlike aggregates were crystalline HA (Fig. 2(B)). Moreover, EDS analysis

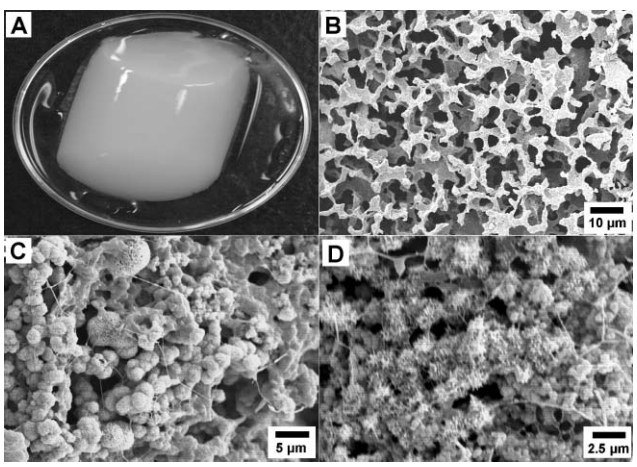

Fig. 1 (A) Optical and (B) SEM images of the as-prepared gel I. SEM images of surface of gel I after mineralization (C) using a urea-mediated process and (D) using an ammonia-mediated process.

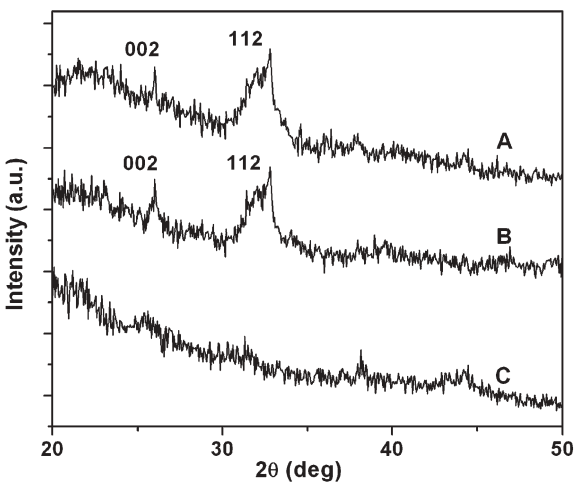

Fig. 2 X-Ray diffraction pattern of gel I–HA composites obtained by urea-mediated process (A), ammonia-mediated process (B) and gel I itself (C).

performed on the mineral bundles again reveals a Ca/P ratio (1.66 ± 0.1), which matches that of HA (see ESI†). Based on the SEM, EDS and XRD results, HA layers form successfully onto the hydrogel of the crosslinked copolymer of pamidronate.

In summary, we have prepared a new monomer, *N*-acryl pamidronate (**3**), which copolymerizes with *N*-isopropylacrylamide to provide a crosslinked hydrogel that serves as the scaffold for mineralization of HA. This rather simple monomer provides a new and useful building block to synthesize derivatives of pamidronate because it combines the polymerization capability of the acryl group and the bioactivity of pamidronate to produce a series of homopolymers, copolymers and block polymers as candidates of new biomaterials.

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