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A method to fabricate hydroxyapatite/poly(lactic acid) microspheres intended for biomedical application

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Abstract

The interaction between organic and inorganic materials enables one to fabricate inorganic/organic microsphere composites. In a novel microemulsion method, we have prepared surfactant-free poly(lactic acid) (PLA)/hydroxyapatite (HAp) microspheres by using the interfacial interaction between inorganic and organic materials, because surfactants are undesireble materials for biomedical applications due to their non-biodegradability. The studies that varied the ion concentrations in oil/water microemulsions revealed that HAp was nucleated at the end group PLA, COOH, and the array of the PLA end groups determined the crystal phase of the calcium phosphate as HAp under the low ion concentration condition. PLA induced the nucleation and precipitation of HAp at the oil/water interface, simultaneously, the precipitated HAp stabilized the microemulsion without surfactants. This novel process made surfactants unnecessary for organic/inorganic microsphere fabrications.

Keywords: Apatite; Composite; Biomedical applications; Powders-chemical preparation; Microsphere

1. Introduction

A number of novel syntheses inspired by biomineralization have recently been reported to form inorganic–organic hybrid composites with controlled hierarchical structures.[1–4] The bioinspired syntheses has prompted many studies on the specific precipitation of inorganic materials on self-assembled organic materials, such as microemulsions.[5–15] The microemulsion methods have been extensively used to fabricate various microspheres which have potentials for catalysis and the controlled release of drugs, dyes, inks, etc. The microspheres fabricated by previous methods, however, were not sufficient for biomedical applications, because they were usually prepared with the aid of surfactants which were suspected of allergy-like reactions due to their non-biodegradability. Previously, we reported a novel method to form surfactant-free microspheres from microemulsions intended for biomedical applications.[16] The microspheres consisted of only biodegradable materials, poly(lactic acid) (PLA) and hydroxyapatite (HAp), being expected for use in biomedical applications. In this report, we have examined the influence of different ion concentrations in O/W emulsions on the resulting microspheres to clarify the interfacial interaction between inorganic–organic materials in microemulsions used to prepare surfactant-free microspheres.

2. Experimental procedure

The synthesis of PLA/HAp microspheres was carried out using oil-in-water microemulsions prepared as follows. Various concentrations (6.7–66.7 mol/m^3) of 200 ml $Ca(CH_3COO)_2$ solutions were mixed with 0.36 g PLA dissolved in dichloromethane (30 ml) to produce emulsions. 200 ml $(NH_4)_2HPO_4$ solutions adjusted to the Ca/P molar ratio of 1.67 (4.0–40.0 mol/m^3) were added dropwise to the emulsions with mechanical stirring. The mixtures were kept at room temperature for 24 h to remove dichloromethane. The pH values of the suspension after dichloromethane removal were 5.6 ± 0.2. Samples were collected by filtration, washed with distilled water and dried at 50 °C for 24 h. The mor-

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phology of the samples were observed by scanning electron microscopy (SEM) using a HITACHI 3000N operating at 5–20 kV. The X-ray diffraction analysis was carried out using a MAC science MXP^3 powder diffractmeter equipped with Cu Kα radiation (40 mA, 20 kV) and optical microscopy using a Nikon SZX-1500. An energy-dispersive X-ray micro-analyzer (EDX, HORIBA, EMAX-7000) was used for the elemental analysis. FT-IR spectroscopy was performed on KBr pellets in a JASCO FT/IR-8000S.

3. Results and discussion

Surfactant-free microspheres were prepared by the precipitation of calcium phosphate. The concentrations of the $Ca(CH_3COO)_2$ and $(NH_4)_2HPO_4$ solutions affected some properties of the samples as the threshold concentrations were 16.7 and 10.0 mol/m^3, respectively. An SEM analysis of the samples revealed that when the concentrations of the $Ca(CH_3COO)_2$ and $(NH_4)_2HPO_4$ solutions were the threshold values or less, only microspheres were observed (Fig. 1a). In contrast, at high concentrations more than the threshold values plate-like precipitations other than the micorospheres occurred (Fig. 1b). An EDX analysis confirmed the presence of the Ca and P elements on the microspheres and the plate-like precipitations. XRD patterns of the samples indicated that the crystal phases of the calcium phosphate were changed by the concentration change (Fig. 2). A broad peak around 20° was ascribed to the amorphous structure of PLA. Broad peaks around 26° and 32° represented the existence of incompletely crystallized hydroxyapatite. The peaks other than those of PLA and HAp were observed in the samples prepared with highly concentrated solutions greater than the thresholds (Fig. 2a,b), indicating the precipitation of dicalcium phosphate dihydrate (DCPD). The IR spectra of the microspheres is shown in Fig. 3. The band around 3580 cm^{-1} corresponding to the stretching vibration of O–H in a PLA end-group, COOH, was absent in the samples prepared with low concentrations (Fig. 3c–e left), suggesting that the carboxyl end groups of PLA would be ionized and bind Ca^{2+} ions resulting in the nucleation sites of the HAp precipitation. The appearance of the band in the highly concentrated samples would be explained by the O–H vibration in H_2O of DCPD (Fig. 3a,b, left). A C=O band at 1755 cm^{-1} assigned to a PLA side group was shifted to a lower wavenumber in the samples prepared with the highly concentrated solutions greater than the thresholds (Fig. 3a,b, right), although the band in the low concentration samples was same as the band of the as received PLA (Fig. 3c–e, right). These results allowed us to assume the following. When the concentrations were higher than the thresholds, DCPD which has a characteristic plate-like morphology was precipitated as homogeneous nucleation in the solutions because DCPD was the most stable phase around pH 5.6. The precipitated DCPD would be adsorbed on the C=O site of the PLA, which would make the C=O band shift to a lower wavenumber in the IR spectra. In

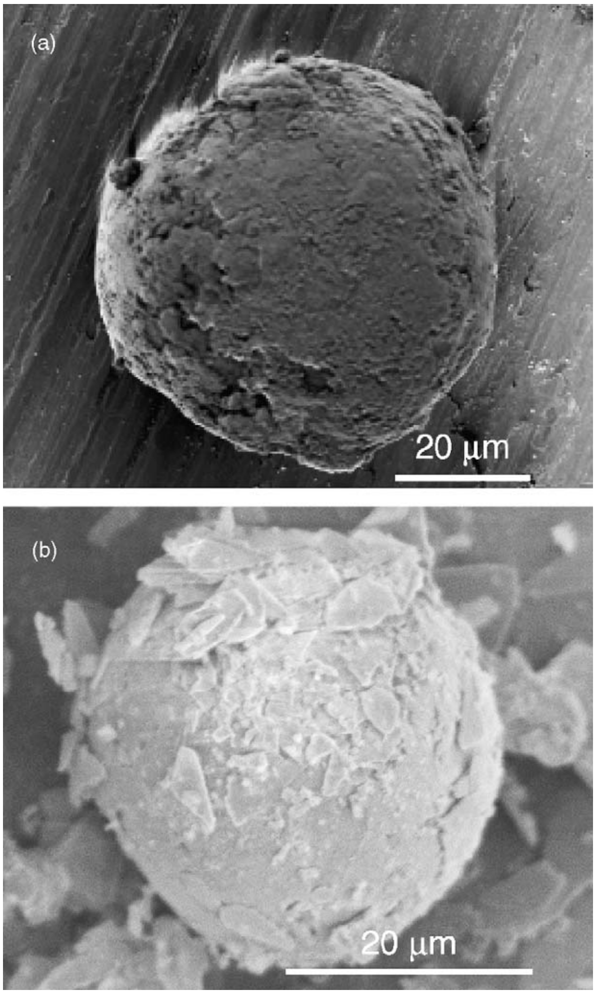

Fig. 1. SEM images of the samples prepared with different concentrations of $Ca(CH_3COO)_2$ and $(NH_4)_2HPO_4$ solutions: (a) 17.0 10.0 and (b) 41.7, 25.0 mol/m^3.

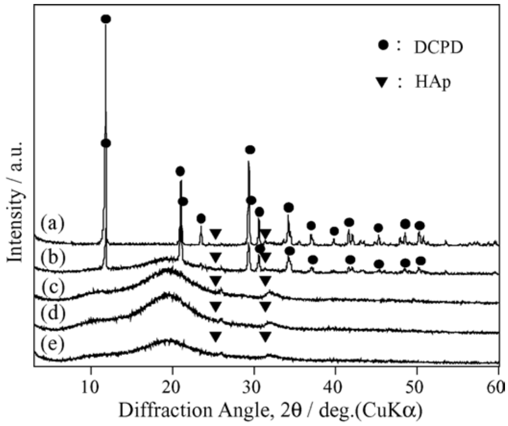

Fig. 2. XRD patterns of the samples prepared with various concentration of $Ca(CH_3COO)_2$ and $(NH_4)_2HPO_4$ solutions: (a) 66.7, 40.0, (b) 41.7, 25.0, (c) 16.7, 10.0, (d) 12.5, 7.5 and (e) 6.7, 4.0 mol/m^3.

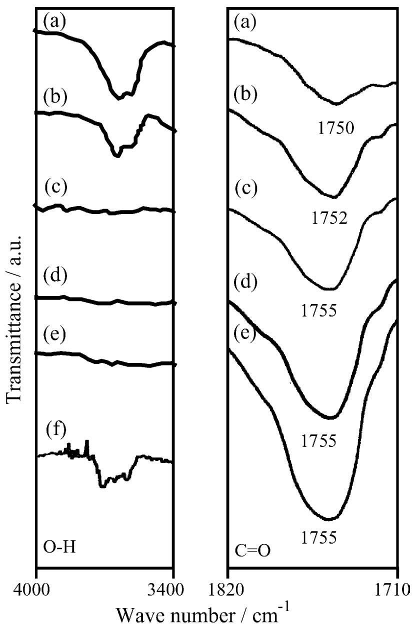

Fig. 3. IR spectra corresponding to the O—H and C=O vibration of the samples prepared with various concentration of $Ca(CH_3COO)_2$ and $(NH_4)_2HPO_4$ solutions: (a) 66.7, 40.0, (b) 41.7, 25.0, (c) 16.7, 10.0, (d) 12.5, 7.5, (e) 6.7, 4.0 mol/m^3 and (f) PLA as received.

contrast, HAp would be precipitated on the PLA end groups at the oil/water interface in microemulsions from low concentration solutions. The array of the PLA end groups at the interface might induce the precipitated crystal phase to HAp which was nevertheless an unstable phase under the conditions around pH 5.6. The precipitated HAp would also play a role as a stabilizer for the microemulsions. The PLA/HAp microsphere fabrication can occur by an interfacial interaction between the PLA end groups and the precipitated HAp.

4. Conclusion

In this paper, we have presented that the concentrations of $Ca(CH_3COO)_2$ and $(NH_4)_2HPO_4$ solutions affected the crystal phases and the binding sites of the PLA/HAp microspheres. In low ion concentrations, the PLA end-group, COOH, directly induces the nucleation of the HAp precipitate, and no precipitate other than the microspheres was observed. The precipitated HAp stabilized the microemulsions to form microspheres, simultaneously, the array of the PLA end groups at the interface would induce the precipitated crystal phase to HAp. The interaction between PLA and HAp at the oil/water interface would play an important role in fabricating the surfactant-free microspheres. The surfactant-free PLA/HAp microspheres would be expected to be used for biomedical applications.

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