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Synthesis of hydroxyapatite nanorods assisted by Pluronics

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Abstract Pluronics F127, P123, and F87 were employed to synthesize hydroxyapatite nanorods for biomedical applications. The calcium phosphate precipitates were characterized by XRD, TEM/EDS, FTIR, and TGA. Pluronics affected the phase evolution of the calcium phosphate precursors in the mother solution at room temperature. The hydroxyapatite nanorods with a diameter of 20 nm, a length of 100 nm, and a Ca/P ratio of 1.70 were obtained after the precursors were heated at 140 °C for 3 h in a Teflon-lined autoclave. There is about 2 wt% Pluronic on the surface of hydroxyapatite. The hydroxyapatite with a small amount of organics on the surface can be potentially used as fillers in biomedical composites with excellent biological and mechanical properties.

Introduction

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) is widely used as implants, drug carriers, and bone tissue engineering scaffolds, because its phase structure and chemical composition are similar to those of the mineral phase of hard tissue in the body [1, 2]. Furthermore, HA has an ability to form chemical bond with bone tissue in vivo (i.e., bioactivity) and to conduct bone tissue growth on the implants (i.e., osteoconductivity) [2]. Many methods have been developed to synthesize HA, including wet chemistry methods (co-precipitation, sol-gel method, hydrothermal route, non-aqueous solution, self-assembly technique, and electrochemical deposition) [2–4], solid state reaction [5, 6], microwave sintering [7], mechanochemistry [8], sonochemistry [9], and physical methods (magnetron sputtering and chemical vapor deposition) [10].

The solution method can simulate the biomineralization condition in organism, which can easily control the phase, structure, and chemical composition of the final product. Moreover, the bone-like apatite can be obtained using the organic or selected macromolecules as modifiers under mild condition. Pluronics (i.e., poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) triblock copolymers) as non-ionic surfactant are widely used in medical and pharmaceutical fields and have been used to assist synthesis of rod-like nanostructures, such as nanostructured Au [11], TiO_2 [12], and PbS [13]. Furthermore, Pluronic F127, incorporated into HA based calcium phosphate particles, can increase the properties of handling and moldability of the implant without compromising osteoconductivity [14]. Hence, Pluronics (F127, P123, and F87) were utilized to synthesize HA nanorods and the effects of different type Pluronics on the phase evolution of the calcium phosphate precipitates were also investigated by XRD, FTIR, and TEM in this study.

Experimental sections

Synthesis of calcium phosphates

Calcium chloride and Pluronics (F127, P123, and F87, their chemical formulations are presented in Table 1) with a

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Table 1 The PPO content in the three Pluronics

Pluronic	General formula	Molecular weight	PPO content (mol%)
F87	(EO)62(PO)40(EO)62	7700	24
P123	(EO)40(PO)70(EO)40	5820	47
F127	(EO)106(PO)70(EO)106	13388	35

Note: EO stands for ethylene oxide structure unit in Pluronic block copolymer, $–CH_2–CH_2–O–$; PO stands for propylene oxide structure unit in Pluronic block copolymer, $–CH(CH_3)–CH_2–O–$

The number beside the parentheses is repeated number of structure unit

molar ratio of 1:1 (the structure unit of Pluronics) were dissolved in 50 mL deionized water to form 0.4 M $CaCl_2$ solution. Dipotassium hydrogen phosphate was dissolved to obtain a clear phosphate solution, which was added dropwise into the Pluronic-containing $CaCl_2$ solution. The reaction was conducted at room temperature (about 23 °C) and the pH value was kept at 10 by adding 0.1 M sodium hydroxide solution during the reaction. The precipitates were collected by centrifuging after aging for 0.5 h as reported in our previous paper [15]. The precipitates were washed repeatedly by deionized water and anhydrous ethanol, respectively, to remove the contaminated ions such as K^+, Na^+, and Cl^-, and dried at room temperature for more than 24 h. Alternatively, the mother solution containing the fresh precipitates aging for 0.5 h before centrifuging was transferred into a Teflon-lined stainless autoclave. The autoclave was placed into an oven and heated at 140 °C for 3 h and the heated precipitates were washed repeatedly by deionized water and anhydrous ethanol, respectively, and dried at 120 °C for more than 24 h. The control samples were synthesized without adding any Pluronics using the same process as described above.

Characterization of calcium phosphates

The Fourier Transform Infrared (FTIR) spectroscopy was conducted in a Nicolet magna-IR FTIR system 550 spectrophotometer with 2 cm^{-1} resolution from 4000 to 400 cm^{-1} using KBr pellet method. Powder X-ray diffraction (XRD) patterns were recorded on a Rigaku Rotaflex diffractometer using CuKα radiation over the range $10° \leq 2\theta \leq 70°$ with a step of 0.02° using a scanning rate of 2°/min. The morphologies of the precipitates were observed on a JEOL 2010 transmission electron microscope (TEM) operating at 200 kV with an Oxford Link EDX system. The TEM specimens were prepared by placing a drop of the calcium phosphate precipitates on a 300-mesh copper grid with a supporting polymer film, which were dispersed in ethanol solution. The solvent was evaporated completely at room temperature. Thermal gravimetry analysis (TGA) was examined on Netzsch STA449C thermal analyzer with a heating rate of 10 K/min under N_2 atmosphere in a range of 30–900 °C.

Results and discussion

Figure 1 presents XRD patterns of the fresh precipitate precursors collected from the mother solution before hydrothermal treatment. The XRD results show that the precursors synthesized in the presence of P123 are amorphous, which have a characteristic broad diffraction peak at $2\theta = 30°$ [16]. The other three precursors synthesized in the presence and absence of Pluronics (F87 and F127, respectively) are crystalline, whose diffraction peaks agree with those of the standard HA phase (ICDD Card No 09–432). Figure 2 gives FTIR spectra of the fresh precipitate precursors collected from the mother solution before hydrothermal treatment. The unsplit IR bands at about 600 and 1000 cm^{-1} in the precursors synthesized in the presence of P123 are the characteristic of amorphous calcium phosphate [16]. The split IR bands at about 600 and 1034 cm^{-1} in the precursors synthesized in the presence and absence of Pluronics (F123 and F87, respectively) are assigned to the crystalline phosphate group in apatite [17]. These FTIR results are consistent with the XRD results in Fig. 1. The bands at 1455 and 875 cm^{-1}, and 1421 cm^{-1} are ascribed to bending mode (v_4) and stretching mode (v_3) of carbonate, respectively [17]. It is worthy to note that an unexpected shoulder at 1490 cm^{-1} appears and its intensity decreases with increase in the crystallinity of the precipitates (Fig. 2) which may be due to carbonate group in amorphous structure [18]. These results imply that all the

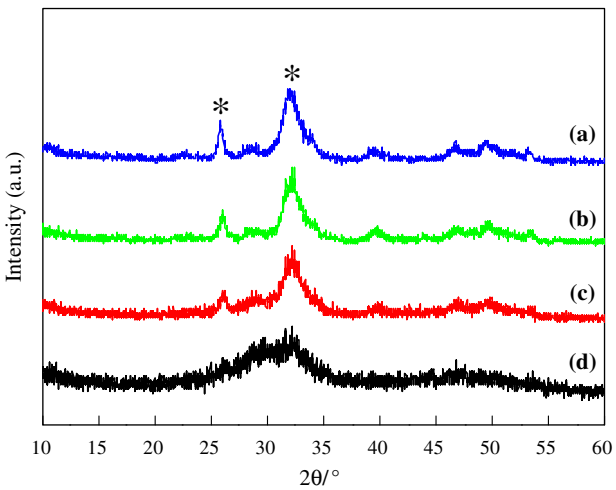

Fig. 1 XRD patterns of the calcium phosphate precursors synthesized in the absence and presence of Pluronics (Ca/Pluronic=1:1) at room temperature after aging for 0.5 h. * Hydroxyapatite, (a) control, (b) F127, (c) F87, and (d) P123

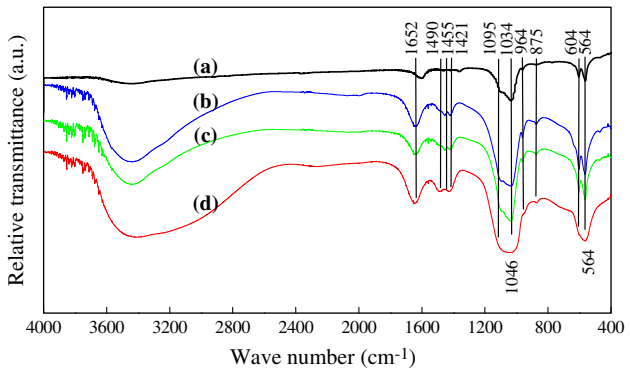

Fig. 2 FTIR spectra of the calcium phosphate precursors synthesized in the absence and presence of Pluronics (Ca/Pluronic=1:1) at room temperature after aging for 0.5 h. (a) Control, (b) F127, (c) F87, and (d) P123

four precursors are carbonated. Carbonate came from the air during the reaction.

C–O–C group in polymer can interact with Ca^{2+} in the aqueous solution to form a complex. This complex affects the transformation and/or conversion of ACP into apatite as reported by Li et al. [15]. That is, the C–O–C group stabilizes amorphous phase in the mother solution through the complex of Ca^{2+} and C–O–C group [15]. C–O–C group from both PEO and PPO blocks in Pluronics can form the above complex. Moreover, PPO block has stronger ability to stabilize ACP in the solution than PEO block because the -CH_3 side group in PPO block has strong steric hindrance. An amorphous phase was obtained due to highest PPO content of P123 in these three Pluronics (The PPO content in the three Pluronics is listed in detail in Table 1). The PPO block can retard the transformation and/or conversion of ACP into apatite in the aqueous mother solution, effectively.

Figure 3 presents the TEM images of the four precursors before hydrothermal treatment. The morphologies of the precursors are different. The control sample has normal needle-like morphology. The abnormally spindle-like structures are observed in the precursors synthesized in the presence of F87 and F127. A typical sphere-like structure of amorphous matter is obtained in the precursor synthesized in the presence of P123. The spindles are composed of the smaller calcium phosphate nanofibers observed from TEM images. The abnormal spindle-like structure may be as a result of self-assembly behaviors of initial particles directed by Pluronics as reported [19, 20], which needs to investigate further in the next work.

Figure 4 shows the effects of different type Pluronics on the phase of the calcium phosphate precipitates after hydrothermal treatment at 140 °C for 3 h. The calcium phosphate precipitates are all crystalline apatite (ICDD Card No 09-432). However, the calcium phosphates synthesized in the presence of Pluronics have more clear diffraction peaks than the one in the absence of Pluronics. There are two bands at 1458 and 1418 cm^{-1} in the FTIR spectra (Fig. 5), which belong to the bending and stretching mode of carbonate group in B-type carbonated apatite, respectively [6, 21]. A band at 875 cm^{-1} is due to bending

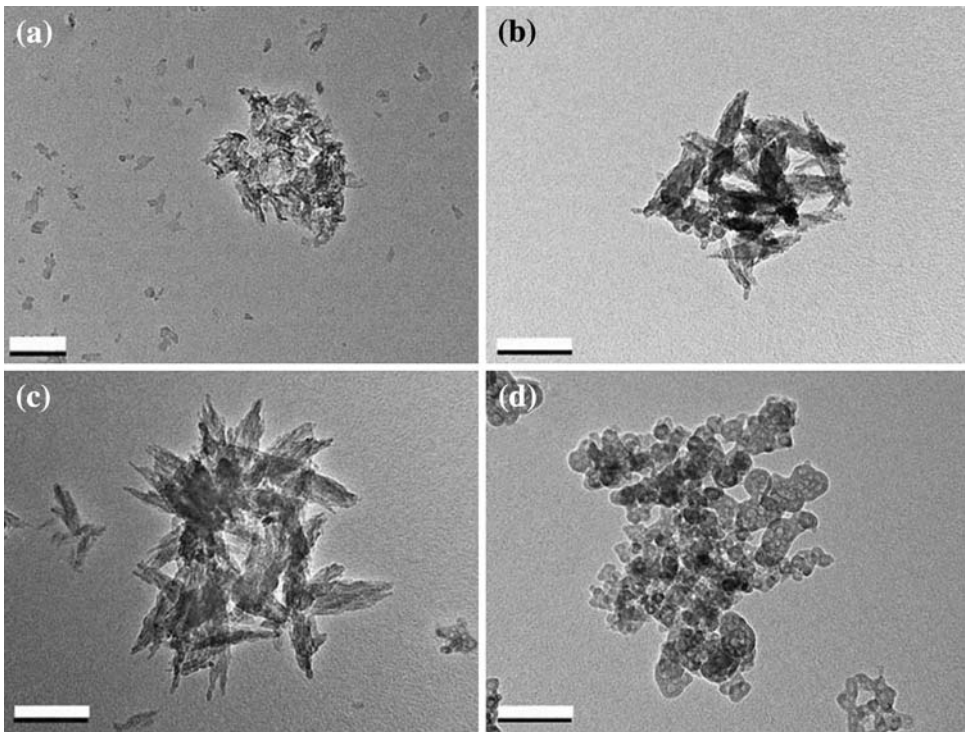

Fig. 3 TEM images of the calcium phosphate precursors synthesized in the absence and presence of Pluronics (Ca/Pluronic=1:1) at room temperature after aging for 0.5 h. **a** Control, **b** F127, **c** F87, and **d** P123. The bars are 100 nm

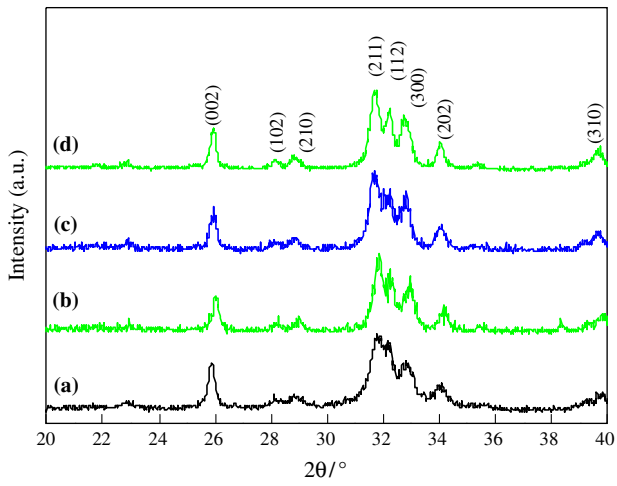

Fig. 4 XRD patterns of the calcium phosphate precipitates synthesized in absence and the presence of Pluronics after hydrothermal treatment at 140 °C for 3 h. (a) Control, (b) F127, (c) P123, and (d) F87

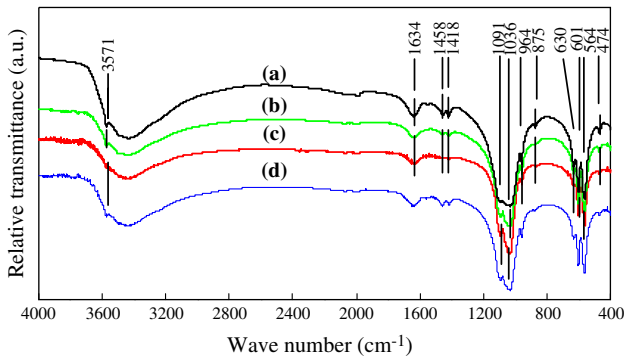

Fig. 5 FTIR spectra of the calcium phosphate precipitates synthesized in the absence and presence of Pluronics after hydrothermal treatment at 140 °C for 3 h. (a) Control, (b) F127, (c) F87, and (d) P123

mode of carbonate group in carbonated apatite [17]. This means our precipitates are B-type carbonated apatite. A shoulder at 1490 cm^{-1} in the poor crystalline precipitates disappeared due to crystallization during hydrothermal treatment. Bands at 1095 and 1034 cm^{-1} are assigned to triple degenerated asymmetric stretching mode (v_3) of the P–O bond of the phosphate group [17]. A band at 964 cm^{-1} is due to non-degenerated symmetric stretching mode (v_1) of the P–O bond of the phosphate group [17]. Bands at 604, 564, and 474 cm^{-1} are ascribed to triple degenerated bending mode (v_1) of the O–P–O bond of the phosphate group [17]. Bands at 3571 and 630 cm^{-1} are indicators of stretching mode (v_S) and libration mode (v_L) of the hydroxyl group, respectively [17]. It is needed to point out that the bend at 875 cm^{-1} also belongs to characteristic vibration of hydrogen phosphate group [17].

Figure 6 shows that the carbonated apatite has a rod-like structure in all four samples. The control sample has smaller size than the other three samples. The morphologies of the carbonated apatite synthesized in the presence of different type Pluronics are identical with a diameter of about 20 nm and a length of about 100 nm. It means the three types of Pluronics can assist in the synthesis of HA nanorods under hydrothermal condition.

The TGA results in Fig. 7a show that there are three regions of weight loss in the range of 30 to 800 °C in the F127 assisting sample while there is only one region in the control sample. Weight loss in region I is ascribed to water loss below 200 °C. There is about 2 wt% weight loss in the range of 200 to 550 °C in region II in the F127 assisting sample, whereas there is almost no weight loss in the control sample. This weight loss should be assigned to the decomposition of Pluronic. Weight loss at about 800 °C is due to release of CO_2 in region IV. The EDS spectrum in Fig. 7b shows the HA nanorods have a Ca/P molar ratio of 1.70. It implies that incorporation of carbonate into the apatite structure increases the Ca/P molar ratio of the carbonated apatite. There is not any contaminated ion in the EDS spectrum except Ca, P, O, and C elements. Cu element comes from the copper grid.

HA is limited to be applied in non-loading or low-loading fields due to its poor and/or brittle strength. Recently, HA is used as fillers to prepare HA/polymer composites to improve its mechanical properties [22]. The interface between the fillers and matrix determines the mechanical properties of the composite, which will be enhanced if the fillers are modified by organics [22, 23]. It is very interesting to find that there is ~2 wt% Pluronic in the HA nanorods even after washing repeatedly by deionized water and anhydrous ethanol, respectively. The Pluronics in the HA nanorods could improve the interfacial strength of HA based inorganic–organic composites. This is similar to the surface modification by organic chemicals [23, 24]. Our HA nanorods with a small amount of Pluronics on the surface can be potentially used as fillers in biocomposites with excellent bioactivity and high mechanical properties. Moreover, Pluronics existing in our HA precipitates will improve their handling and moldability during the implantation as reported by Zhou et al. [14].

Summary

HA nanorods were synthesized with assistance of Pluronics under hydrothermal condition. Pluronics affect the formation of the precursors at room temperature, but do not change the phase composition and morphology of HA after hydrothermal treatment. The HA nanorods are carbonated apatite with a diameter of about 20 nm and a length of about 100 nm, which is similar to mineral phase in the

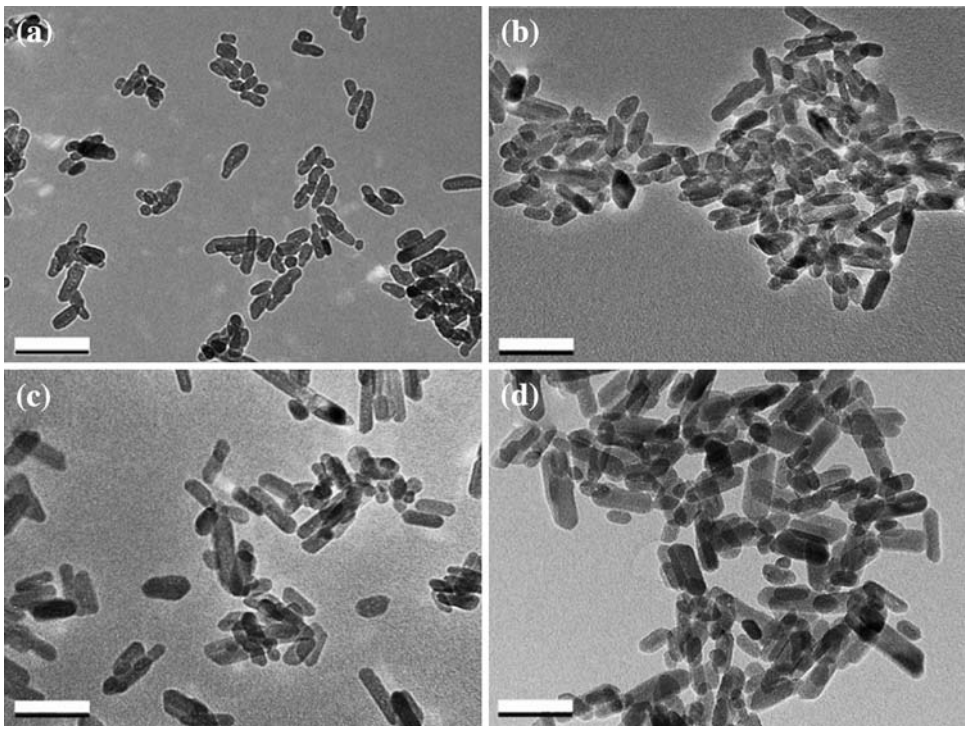

Fig. 6 TEM images of the calcium phosphate precipitates synthesized in the absence and presence of Pluronics after hydrothermal treatment at 140 °C for 3 h. **a** Control, **b** F127, **c** F87, and **d** P123. The bars are 100 nm

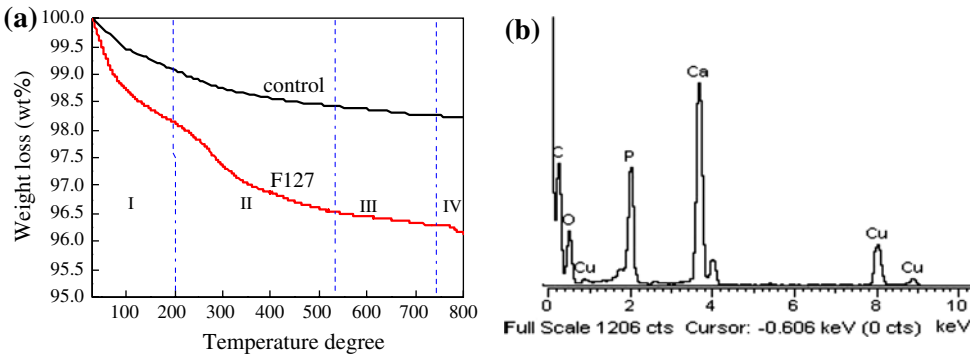

Fig. 7 TGA curves (**a**) and EDS spectrum (**b**) of typical calcium phosphate precipitates synthesized in the absence and presence of F127 after hydrothermal treatment at 140 °C for 3 h. Cu (**b**) is from the copper grid

bone in the phase structure, chemical composition, and morphology.

HA nanorods can be synthesized by wet chemistry method with assistance of different surfactants such as cetyltrimethylammonium bromide (CTAB) and sodium bis(2-ethylexyl) sulfosuccinate (AOT). But few works have investigated the organic reserved in the precipitates. Moreover, organics almost are burnt at about 800 °C to obtain pure calcium phosphates. Pluronic on the HA nanorods are kept in present study. In one word, Pluronic not only act as a template to synthesize HA nanorods, but also improve the interface between HA fillers and polymer matrix in the biocomposites.

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