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In situ grafting polyethylene glycol chains onto amorphous calcium phosphate nanoparticles to improve the storage stability and organic solvent redispersibility

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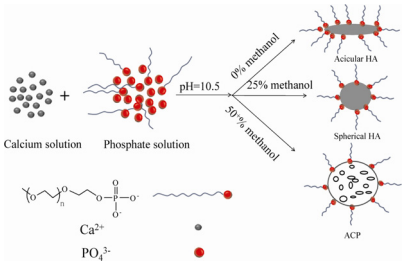
HIGHLIGHTS

- Amorphous calcium phosphate (ACP) nanoparticles were synthesized in methanol/water mixtures.
- The ACP colloidal particles were stabilized by polyethylene glycol chains around each particle.
- The ACP powders remained amorphous for over 1 year.

GRAPHICAL ABSTRACT

Amorphous calcium phosphate colloidal particles were prepared in methanol/water mixtures, stabilized by polyethylene glycol chains around individual core particles.



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ABSTRACT

Amorphous calcium phosphates (ACPs) usually exhibit superior bioactivity and bioresorbability over crystalline calcium phosphates. However, due to the tendency of transforming into more stable hydroxyapatites (HAs) during storage, the commercialization of ACPs is still restricted. In this work, a monophosphate-terminated methoxy-poly(ethylene glycol) (mPEG-OPO_3H_2) was used as an in-situ chemical stabilizer and surface modifier to ACPs' nanoparticles which were synthesized in methanol/water mixtures. The results indicate that the high content of methanol (50% and 75%, v/v) favors the formation of ACPs and the low content (0% and 25%) of methanol results in HA crystals. All the calcium phosphates (CaPs) displayed a colloidal appearance in the corresponding mother solution. In addition, the centrifuged precipitates can be redispersed in some organic solvents. For example, the methanol colloids still showed narrow particle size distributions (20 and 120 nm) without any sedimentation after 3 months. The ACP powders can maintain their amorphous structure up to 1 year. Such long-term storage stability and unique organic redispersibility are attributed to the formation of polyethylene glycol (PEG) brushes onto the individual ACP nanoparticles.

1. Introduction

Amorphous calcium phosphates (ACPs) usually exist in the early stage of skeletal calcification and most of them convert to hydroxyapatite (HA) crystals [1–4] in mature bones. With the Ca/P molar

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Table 1
Solvent composition for synthesis of CaP samples.

Sample	Phosphate solution				Calcium solution				
	mPEG-OPO_3H_2 (g)	$(NH_4)_2HPO_4$ (g)	H_2O (ml)	MeOH (ml)	$Ca(NO_3)_2$•$4H_2O$ (g)	H_2O (ml)	MeOH (ml)	MeOH (vol.%)	Structure
CaP0	1.18	0.236	250	0	2.36	50	0	0	HA
CaP25	1.18	0.236	225	25	2.36	0	50	25	HA
CaP50	1.18	0.236	150	100	2.36	0	50	50	ACP
CaP75	1.18	0.236	75	175	2.36	0	50	75	ACP
Controls									
CaP-PEG0–75: using mPEG instead of mPEG-OPO_3H_2 in the phosphate solution									HA
CaP-BLK0–75: without any surface modifier in the phosphate solution									HA

ratio ranging from 1.0 to 2.2 [5], ACPs have demonstrated better osteoconductivity [6,7] and crack resistance than stable HAs [8]. In addition, they can release Ca^{2+} and PO_4^{3-} ions more rapidly than traditionally biodegradable tricalcium phosphates [9], thereby facilitating local bone healing. Due to such superior bioactivity and bioresorbability, ACPs are increasingly used in hard tissue reconstructions, such as orthopedic cements [10,11], bone grafts [12–14], and dental composites [15–17].

ACPs can be synthesized at low temperatures [5,9], in organic solvents [18,19], or through a flame spraying-fast quenching method [13,20]. However, the produced ACPs were not stable during storage, e.g., the vacuum-dried ACP powder was transformed into apatite crystallites through absorbing water in air [21]. To improve the storage stability, various stabilizers have been attempted during syntheses including inorganic ions (Mg^{2+}, $P_2O_7^{4-}$, CO_3^{2-}, Zn^{2+}, $P_3O_{10}^{5-}$) [15,22], organic compounds (adenosine triphosphate [23], β-cyclodextrin [24]), and polymers (polyethylene glycols [25,26], polyelectrolytes [27], hydrophilic–hydrophobic block copolymers [14,28,29]). Unfortunately, few of them are sufficient to guarantee long-term stability. According to the previous reports, we found that only two agents, stearic acid [30] and a poly(acrylic acid-*b*-isoprene) (PAA_{78}-*b*-PI_{97}) [28], could stabilize ACP for over eight months in the form of dry powder and hydrocolloid, respectively.

The main biomedical application of ACPs is biocomposites, in which ACPs are involved as one phase [10–17,20,26]. The mechanical and biological properties of the biocomposites are closely related to the homogeneity of ACP particles in the matrices, which however largely remains a Holy Grail to achieve, particularly in polymers at a nano-scale. Previous researchers have dispersed ACP nanoparticles in hydrophobic polymers, such as polyacrylate resins [15–17] and biodegradable polyesters [12–14,20,26], which serve as dental fillers and bone substitutions. Nevertheless, the ACPs used were either non-modified [12,13,16,17,20] or slightly modified by weak chelating agents like polyethylene glycols [14,26], therefore, it is hard to assure their homogeneity in polymers. In this case, new strategies are desired to be explored.

Interestingly, various phosphoproteins containing phospho-*L*-serine residues are able to stabilize ACP nanoclusters in milk and blood [31]. This highlights the importance of organic phosphates to stabilize ACPs. Inspired by this clue, we synthesized a novel dihydrogen phosphate-terminated PEG, which can be employed as an *in-situ* surface modifier to HA nanoparticles during the synthesis [32]. In this study, the functions of this newly developed modifier which renders ACP nanoparticles long-term storage stability and organic solvent redispersibility are addressed.

2. Materials and methods

2.1. Materials

Analytical grade aqueous NH_3 (25 wt%), $Ca(NO_3)_2$•$4H_2O$ and $(NH_4)_2HPO_4$ were purchased from Chengdu Kelong Chemical Reagent Co. (Chengdu, Sichuan, China). Methoxy-poly(ethylene glycol) (mPEG, M_n = 200 Da) and $POCl_3$ were provided by Shanghai (Shanghai, China) and Haihong (Chengdu, Sichuan, China) Chemical Reagents Plant, respectively.

2.2. Synthesis of α-methoxyl, ω-dihydrogen phosphate poly(ethylene glycol)(mPEG-OPO_3H_2)

This substance is employed as a surface modifier to ACP particles in this work, and its synthesis route was described elsewhere [32]. Briefly, 3 g (15 mmol) of mPEG in 10 ml of chloroform was slowly dropped into a solution of $POCl_3$ (13.8 ml, 150 mmol) in chloroform (10 ml) at about 0 °C. After 7-h reaction at a reduced pressure (0.07 MPa), the resulting mixture was poured into 150 ml of petroleum ether, and rested for 2 h in an ice bath. The bottom layer with the phosphorylated intermediate was collected and hydrolyzed for 2 h by adding 5 ml of water. The hydrolyzed product was dehydrated by rotation evaporation and azeotropic distillation with ethanol, followed by washing with 20 ml × 3 of ethyl ether. The residual solvent was removed again by rotation evaporation, to obtain the target product α-methoxyl, ω-dihydrogen phosphate poly(ethylene glycol) (mPEG-OPO_3H_2, M_n = 280 Da) as a viscous liquid. Yield: 23.8%. FTIR, ^{1}H NMR and mass spectra confirmed its chemical structure [32].

2.3. Synthesis of ACP nanoparticles

With the purpose of finding the synthesis art for long-term stable ACPs, four calcium phosphate (CaP) samples were prepared in methanol/water mixtures using the same raw materials and surface modifier, and just changing the solvent compositions, as listed in Table 1. The samples were denoted as CaP0, CaP25, CaP50, and CaP75, respectively, where the number represents the volume percentage of methanol used for each synthesis. The surface modifier mPEG-OPO_3H_2 (1.18 g, 4.2 mmol) and the phosphate precursor $(NH_4)_2HPO_4$ (0.236 g, 1.8 mmol) were dissolved in 75–250 ml of water followed by adding 0–175 ml of methanol, making a nominal 250-ml phosphate solution (Table 1). The calcium solution consisted of 2.36 g of $Ca(NO_3)_2$•$4H_2O$ (10 mmol) and 50 ml of water or methanol. The pH values of both solutions were adjusted to 10.5 using aqueous ammonia. To prepare the four samples, each calcium solution was dropped into the corresponding phosphate solution at room temperature in 15 min followed by 1-h stirring, during which the pH value was kept at 10.5. Thereafter, the mixture was aged at 55 °C for 5 h under magnetic stirring. The resultant light blue colloid was subjected to 30-min ultrasonication (KQ-300DE ultrasonic cleaner, Kunshan Ultrasonic Instrument Co., Ltd., Kunshan, Jiangsu, China) and then conditioned overnight before purification.

Each colloid was centrifuged (TGL-20M centrifuger, Shanghai Lu Xiangyi Centrifuge Instrument Co., Ltd., Shanghai, China) at 15,000 rpm, followed by solvent rinsing and re-dispersing (here the solvent was the same as that used in each synthesis, e.g., 25 vol.% methanol in water was used for CaP25). Such process was