Chinese Chemical Letters 26 (2015) 1121–1128

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Original article

Water dispersible hydroxyapatite nanoparticles functionalized by a family of aminoalkyl phosphates

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ARTICLE INFO

Article history:
Received 25 February 2015
Received in revised form 26 March 2015
Accepted 23 April 2015
Available online 7 May 2015

Keywords:
Hydroxyapatite
Hydrocolloid
Aminoalkyl phosphate
Cytotoxicity

ABSTRACT

A series of aminoalkyl phosphates (AAP-n, with carbon number n ranging from 2 to 6) are used as surface modifiers to prepare hydroxyapatite hydrocolloids. The resulting nanoparticles (Cn-HA) possess a core-shell structure where an ionized layer of calcium-(AAP-n) complex [$^{+}H_3N$-$(CH_2)_n$-OPO_3Ca] encapsulates each hydroxyapatite core. Long-term colloidal stability is achieved due to the electrostatic repulsion among the suspending particles. The incorporation of AAP-n results in a preferential crystal growth along *c*-axis, showing an increasing aspect ratio of particles from C2-HA to C6-HA. Preliminary cell culture using osteoblast-like MG63 cells shows no cytotoxicity associated with the as-prepared Cn-HA particles. The functional amino groups around the nanoparticles could be used to graft various organic chains to prepare homogeneous HA/polymer composites as bone grafting materials.

1. Introduction

Hydroxyapatite (HA), as the main inorganic component of bones and teeth, has been widely used in hard tissue reconstructions, like coatings for artificial hip joints [1], bone cements [2] as well as scaffolds in craniofacial orthopedics [3], thanks to its excellent biocompatibility and bioactivity [4–6]. However, the intrinsic brittleness of pure HA still hinders its load-bearing applications like in cortical bone substitutions. This has stimulated the development of various HA/polymer nanocomposites whose mechanical properties, in most cases, are still largely inferior to those of natural cortical bones [7–9]. The main reasons are thought to be the agglomeration of nanoparticles and the lack of strong interactions between the two phases [10].

Surface modification is the key to improve the dispersibility of nanoparticles in polymer matrices. Fore instance, alkyl chains or biodegradable polyester oligmers have been grafted onto HA nanoparticles [11–14]. These modifications are carried out on previously aggregated HA particles and some aggregation still remains after that. The resultant nanocomposites just show minor improvement in mechanical properties [13,14]. Recent advances in HA hydrocolloids [15–21] provide another possibility to prepare highly homogeneous nanocomposites through colloidal chemistry. The HA colloidal particles, stabilized by some amino acids [15], aminoethyl phosphate [16,17], citrate ions [18], as well as silk fibroin [19], can be directly blended with water soluble polymers like polyvinyl alcohol to make a homogeneous suspension which is then cast and dried to obtain the corresponding nanocomposite. However, this method is not suitable for hydrophobic polymers like polylactide and polycaprolactone which are more widely used in bone grafts. To solve this problem, a new type of amphiphilic stabilizer, dihydrogen phosphate-terminated poly(ethylene glycol)s, has been explored to stabilize HA colloid in both water and organic solvents [20,21]. Based on these findings, a homogeneous HA/polyurethane nanocomposite with 10 wt% of HA in it was generated through a simple solution casting method, with a gain of 75% in tensile strength relative to that of pure polyurethane [9].

Most of the previous surface modifiers can tightly bond onto HA surface, but still lack reactivity with polymer matrices, which may hinder further improvement of mechanical properties of the resultant nanocomposites. We recently synthesized a family of aminoalkyl phosphates [22] that can stabilize HA colloids while generating reactivity for further modifications. Their effects on the structure and properties of HA nanoparticles are reported in this study, as the first step toward new nanocomposites for bone reconstructions.

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http://dx.doi.org/10.1016/j.cclet.2015.04.035

2. Experimental

2.1. Materials

A series of ω-aminoalkyl ammonium hydrogen phosphates, H_2N-$(CH_2)_n$-OPO_3HNH_4, (AAP-n, with carbon number n ranging from 3 to 6) were synthesized in our lab according to previously described method [22]. Aminoethyl dihydrogen phosphate (AAP-2) was purchased from Sigma–Aldrich (St. Louis, Missouri, USA). Sodium dihydrogen phosphate and anhydrous calcium chloride were from Kelong Factory of Chemical Reagents (Chengdu, Sichuan, China) and served as P and Ca precursor of HA, respectively.

2.2. Synthesis of aminoalkyl phosphates functionalized hydroxyapatites (Cn-HA)

As shown in Scheme 1, each functionalized HA was synthesized by quickly adding a calcium solution into a phosphate solution. The phosphate solution consisted of NaH_2PO_4 (0.0021 mol) and one type of the surface modifiers AAP-n (n = 2–6, 0.0036 mol) in 15 mL of distilled water. The calcium solution contained 0.006 mol of $CaCl_2$ in the same volume of water. The pH values of both the phosphate solution and the final mixture were adjusted to 9–10, using a solution of 1 mol/L NaOH. The resulting precipitate in the mother liquid was aged at 80 °C for 16 h to obtain a light blue hydrocolloid. A control synthesis without any surface modifier only led to HA precipitate. Note that the molar ratios of the organic phosphate from AAP-n and mineral phosphate from NaH_2PO_4 to Ca^{2+} ions were kept at 0.6:1 and 0.35:1, respectively, in all the syntheses. The functionalized HAs were designated as Cn-HA, where n represents the carbon number in the structure of the used surface modifier.

Five milliliters of each Cn-HA hydrocolloid was kept for colloidal stability observation. The rest was centrifuged at 50,000 rpm for about 10 min on a Beckman L8-80 M ultracentrifuge (Beckman Coulter, Brea, CA, USA) to obtain a translucent gel-like precipitate which was washed three times with water to remove the soluble impurities. All the samples were freeze dried at −50 °C and 10 Pa for 24 h and air dried at 60 °C for 10 h to obtain powders for various characterizations.

2.3. Characterization

2.3.1. Structures and compositions

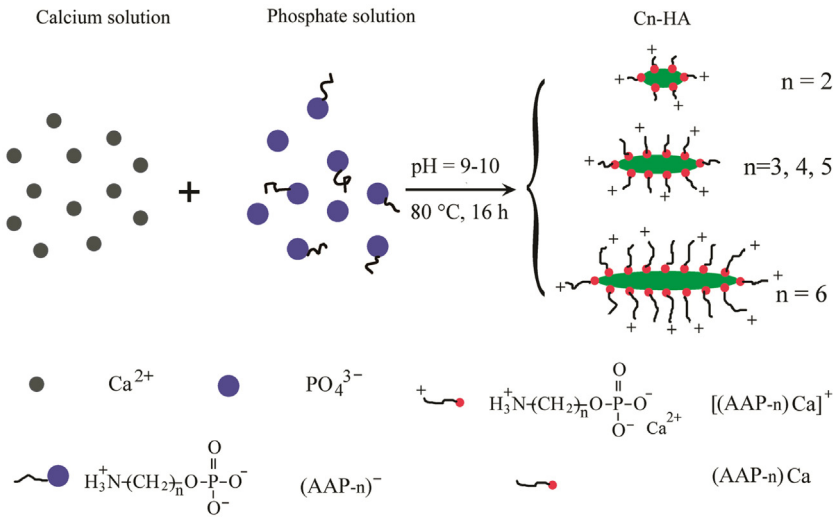
The Fourier transformed infrared (FTIR) spectra of Cn-HA powders were recorded on a Nicolet IR 2000 spectrophotometer (Thermo Nicolet, USA) through KBr disks, with 32 scans between 400 and 4000 cm^{-1} at a resolution of 4 cm^{-1}. The X-ray diffraction (XRD) patterns were obtained on an X'Pert Pro MPD diffractometer (PANalytical BV, Netherlands) equipped with Cu Kα radiation source operated at 40 kV and 45 mA. The HA (002) reflection was selected to measure the crystallite size (D) according to the Scherrer formula $D = 0.89\lambda/(\beta\cos\theta)$ [23], where λ is the X-ray wavelength (1.54056 Å), β the width in radian at half-maximum height of the diffraction peak, and θ the Bragg angle (12.92°).

The presence of the organic modifiers on Cn-HA particles was tested by thermal gravity analysis (TGA) on a TG 209 F1 thermogravimetric analyzer (Netzsch Group, Germany) from room temperature to 800 °C, at 10 K/min in a nitrogen flow of 30 mL/min.

Total Ca and P contents were measured on VG PQ ExCell inductive coupled plasma (ICP) emission spectrometer (TJA Corp., USA). The amount of mineral P from PO_4^{3-} groups was determined by spectrophotometry of yellow phosphovanadomolybdate complex (organic phosphate cannot form this complex) according to ISO 11400. In parallel, each sample was digested with concentrated nitric acid to transform organic phosphate into inorganic one [24] prior to another determination of total P content using the same spectrophotometry. The difference between the total and mineral P content was the organic content. The C and N contents in each Cn-HA sample were analyzed using EA3000 elemental analyzer (Euro Vector, Italy).

2.3.2. Colloidal property and stability

The particle size and size distribution of each Cn-HA hydrocolloid were measured by photo correlation spectroscopy using Zetasizer 3000 HSa particle analyzer (Malvern Instruments Ltd., Malvern, UK). Zeta potential was also measured on the same instrument. All samples were filtered through 0.45 μm membrane prior to each measurement (n = 3). The dried powders were re-dispersed into various organic solvents by ultrasonication for 4 h (KQ-300DE ultrasonic cleaner, Kunshan Ultrasonic Instruments Co., Ltd, Kunshan, Jiangsu, China). The colloidal stability in water and dimethylformamide (DMF) was recorded by photography using a digital camera.



Scheme 1. Synthesis of hydroxyapatite nanoparticles functionalized by aminoalkyl phosphates (AAP-n), which serve as both surface modifiers and colloidal stabilizers. Preferential crystal growth along c-axis is observed in C2-HA to C6-HA samples.