Materials Science and Engineering C 77 (2017) 121–128

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Reline-assisted green and facile synthesis of fluorapatite nanoparticles

Mohammad Karimi [a,*], Majid Rastegar Ramsheh [a], Seyed Mohammad Ahmadi [b], Mohammad Reza Madani [c], Mehdi Shamsi [a], Reyhaneh Reshadi [d], Farahnaz Lotfi [d]

[a] Tracheal Diseases Research Center (TDRC), National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Department of Biomedical Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran
[c] Chronic Respiratory Diseases Research Center (CRDRC), National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] Department of Biomedical Engineering, Yazd Branch, Islamic Azad University, Yazd, Iran

ARTICLE INFO

Article history:
Received 21 November 2016
Received in revised form 4 February 2017
Accepted 23 March 2017
Available online 24 March 2017

Keywords:
Fluorapatite
Nanoparticles
Deep eutectic solvent
Reline
Green synthesis
Biocompatibility

ABSTRACT

A fast, simple and sustainable method based on choline chloride-urea deep eutectic solvent (known as Reline) was employed to synthesize nanosized fluorapatite (FA) particles. Using XRD, FESEM, TEM, EDS, and FTIR, the formation of FA nanoparticles with average crystal size of ~34 nm, percent crystallinity of 93%, particle size of ~45 nm, and high crystal, elemental, and structural purity was confirmed. The MTT cytotoxicity assay endorsed the non-toxicity of as-synthesized FA nanoparticles. The good biocompatibility, osteogenity and mineralization ability of as-synthesized FA nanoparticles were confirmed by Alizarin red staining, Acridine orange staining and ALP activity tests. After synthesis of the nanoparticles, the Reline solvent was recovered successfully using freeze-drying method with 71% yield of recovery revealing the green, sustainable and economical nature of the developed synthesis method. According to the results, owing to its alkalinity, high ionic strength and 3D bulky configuration, the Reline solvent provides the optimum conditions required for formation of FA with maximum crystallinity and the particle size controlled in the nanometer range. Providing a simple, cost-effective, and green method for synthesis of FA nanoparticles with potential biological applications is the most innovative aspect of this study.

1. Introduction

The development of new biomaterials for hard tissue repair and regeneration has become a pioneer research field in biomedical engineering. The main origin of the field is to engineer, design and fabricate novel structures and nanostructures toward managing the process of tissue regeneration in the body [1,2]. In this sense, calcium phosphate (CP) nanostructures as the main foundation of human hard tissues are of the utmost importance [1,3,4]. Accordingly, in recent years, a vast number of scientific researches have been conducted on CP-based biomaterials which some of them have led to the creation of commercial products and technologies [5–7]. Nevertheless, design-fabrication of novel and third generation bioceramics supporting the tissue reconstruction and accelerating the rehabilitation processes remains serious challenge [2,8]. In this arena, the rapid growth of nanotechnology in recent years has sparked the remarkable progresses in development of nanoscale and nanostructured bioceramics with bolstered biological and biomechanical properties for a variety of orthopedic and dental applications [9].

Owing to its interesting bioactivity and biocompatibility, nanostructured calcium hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), the major mineral component of bone and dental hard tissues in mammals, is widely used in biomedical applications like bone grafts, dental implants, drug delivery systems, etc. [10,11]. HA found in the tissue is mostly in the form of impure apatite with complicated structures of HA nanoparticles and organic components which constitutes about 50% of dentin and bone as well as 98% of enamel by weight [12]. The impure nature of biological HA originates from the intrinsic tendency of its constituents calcium-Ca^{2+}, phosphate-PO_4^{3-}, and hydroxyl-OH^- ions to be substituted with essential biological ions such as magnesium-Mg^{2+}, sodium-Na^+, or strontium-Sr^{2+} (rather than Ca^{2+}), carbonate-CO_3^{2-} (rather than PO_4^{3-} or OH^-) and fluoride-F^- or chloride-Cl^- (rather than OH^-). In addition, biological HA is a calcium deficient HA with Ca/P ratio in the range of 1.63 to 1.61 less than the stoichiometric ratio of 1.67 [13,14].

Fluoride substitution in the calcium apatite structure leads to an interesting compound known as fluorapatite (FA, $Ca_{10}(PO_4)_6F_2$) which is considered as an replacement for HA due to its desirable physical and biological properties [12,15,16]. In fact, smaller and more symmetrical fluoride ions can fit into the calcium phosphate crystal structure more easily and better than hydroxyl ions; this makes FA more stable and lower soluble/degradable than HA [17–19]. The results show that

* Corresponding author.
E-mail addresses: m.karimi@merc.ac.ir, karimi.merc@gmail.com (M. Karimi).

http://dx.doi.org/10.1016/j.msec.2017.03.217
0928-4931/

fluoride substitution causes increased cell activity and reduced erodibility of FA which in turn leads to a range of orthopedic and dental applications [9,17]. In addition, as compared to HA, FA shows higher mechanical strength and thermal stability so that it remains unchanged at high temperatures (melting point ~1650 °C) [20]. Biological tests have demonstrated that fluorine can stimulate the extracellular matrix formation both in vitro and in vivo as well as strengthen the bone cortex [16,17,19]. Compared to other forms of calcium phosphate, fluoride ion in fluorapatite causes a positive stimulating effect on cell proliferation, alkaline phosphatase activities and, osteocalcin levels to meet the requirements set for the reparation of hard tissues like bones and teeth [15,18,19].

Up to now, various methods have been developed for the synthesis of FA with nanoscale structure. Among them the precipitation method [21], hydrothermal synthesis [8], sol-gel method [22], mechanochemical synthesis [23], and flame spray pyrolysis [24] can be pointed out. Using poisonous and unsafe precursors, solvents and surfactants as well as the inclusion of additional ions, such as ammonia and other ions resulted from precursors dissolution in FA structure (that strongly affect the chemical, physical and biological properties of FA) are the major challenges faced by the foregoing methods. In addition, most of these methods need to use expensive raw materials along with harsh and time consuming processing conditions that are in contrary to the principles of green chemistry [8,14].

In this research, FA nanoparticles with high crystal purity have been synthesized via a simple, green and biocompatible pathway based on ionothermal precipitation of phosphate, calcium and fluoride precursors in the choline chloride-urea deep eutectic solvent (DES) known as Reline. As a new generation of ionic liquids (ILs), DESs are mainly formed by complexation between a quaternary ammonium salt (QAS, choline chloride) and a hydrogen bond donor (HBD) such as a variety of alcohols, amines, multifactorial carboxylic acids and amides [25,26]. The interesting properties of DESs such as low vapor pressure, biocompatibility, high heat resistance, high solubilizing power, high ionic strength and safety, making them attractive candidates to be used in the synthesis of inorganic nanomaterials such as bioceramics [26,27]. The as- synthesized FA nanoparticles have been analyzed in terms of crystallinity, microstructure, particle size, elemental purity, functional groups and cytotoxicity by techniques of X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDS), Fourier transform infra-red spectroscopy (FTIR) and MTT assay.

2. Experimental

2.1. DES preparation

To obtain choline chloride-urea DES (commercialized under the trade name of Reline®), choline chloride ($C_5H_{14}ClNO$, Merck) and urea (CH_4N_2O, Merck) were mixed in 1:2 M ratio and heated to 90 °C until a homogeneous liquid was formed.

2.2. Synthesis of FA nanoparticles

In a typical synthesis of FA nanoparticles according to the Fig. 1, 2.5 g of anhydrous calcium chloride ($CaCl_2$, Merck) was dissolved in 200 ml of the as-prepared Reline maintained under vigorous stirring at 150 °C. Two concentrated aqueous solutions containing 1.78 g of di-ammonium hydrogen phosphate ($(NH_4)_2HPO_4$, Merck) in 5 ml of double distilled water and 0.17 g of ammonium fluoride (NH_4F, Merck) in 5 ml of double distilled water were prepared separately and then were mixed together. The resulted aqueous solution was added to the Reline solution containing calcium ions at 150 °C. The as-formed precipitates after 1 h were filtered, washed with deionized water and dried in vacuum oven at 60 °C for 10 h. To recover the Reline solvent, the filtrate was dewatered using two different methods: (i) oven-drying at 100 °C for 48 h; and (ii) freeze-drying at −51 °C and 0.5 Torr for 48 h.

2.3. Characterization of nanoparticles

The X-ray diffraction (XRD) analysis was performed on a X-ray diffractometer D-500 (Siemens, Germany) equipped with a CuKα (λ = 1.542 Å) rotating anode. The diffraction patterns were collected at room temperature over an angular range of 20° to 60°, with 0.02° step-size and scan speed of 2°/min.

The average size (D) of FA nanocrystals was calculated from XRD data using the Debye-Scherrer equation [28]:

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

where K is shape factor (0.9), λ is the X-ray wavelength (0.154 nm), θ is the Bragg diffraction angle of the XRD peak with 100% relative intensity (211), and β is the line broadening in radian measured from the full

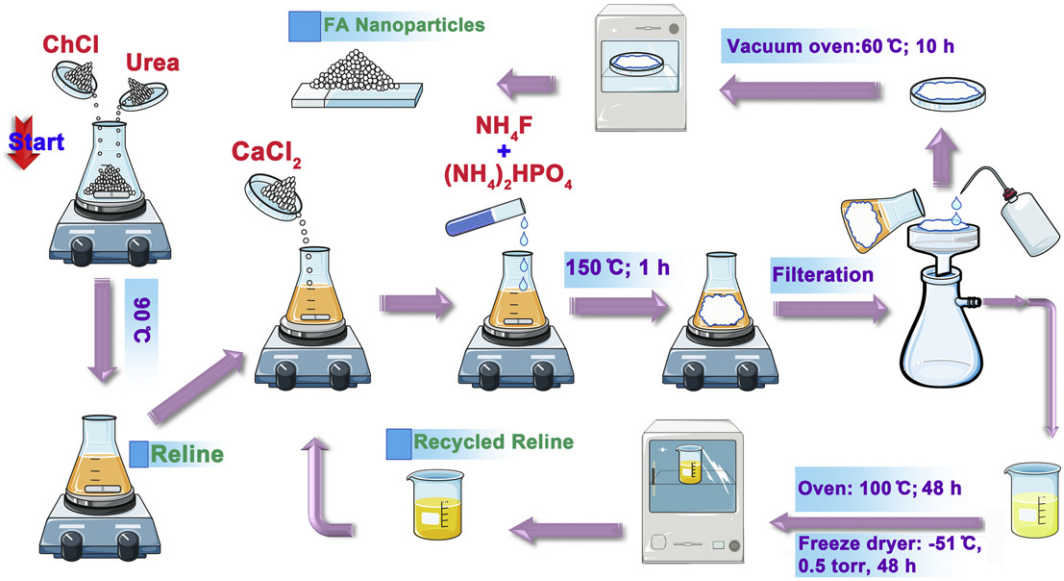

Fig. 1. The Scheme of FA synthesis process.