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Original Research

Co-doping of hydroxyapatite with zinc and fluoride improves mechanical and biological properties of hydroxyapatite

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Abstract

Hydroxyapatite (HA) co-doped with Zn^{2+} and F^{-} ions was synthesized by precipitation method for the first time in this study. FTIR spectroscopy revealed Zn^{2+} and F^{-} ions incorporation into HA structure. Co-doping of Zn^{2+} and F^{-} ions decreased unit cell volume of HA and decreased grain sizes. Zn^{2+} or 5 mol% F^{-} addition into HA significantly improved its density. Microhardness was increased with Zn^{2+} addition and further increase was detected with F^{-} co-doping. Zn^{2+} and F^{-} co-doped samples had higher fracture toughness than pure HA. Zn^{2+} incorporation to the structure resulted in an increase in cell proliferation and ALP activity of cells, and further increase was observed with 1 mol% F^{-} addition. With superior mechanical properties and biological response 2Zn1F is a good candidate for biomedical applications.
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Keywords: Hydroxyapatite; Co-doping; Microstructure; Microhardness; Biocompatibility

1. Introduction

HA is a ceramic compound which is the main constituent of the inorganic part of the bone structure. It has the chemical formula of $Ca_{10}(PO_4)_6(OH)_2$. There are two different crystal structures of synthetic HA (monoclinic and hexagonal). Both of these structures are closer to the mineral phase of the bone. However, monoclinic HA crystal structure, the most ordered and thermodynamically stable form, can be obtained only at very high temperatures [1]. It also shows more stoichiometric structure compared to hexagonal HA. HA has different mechanical properties in different ranges depending on the sintering temperature and duration used [2]. Compressive strength between 133 and 193 MPa, bending strength between 38 and 250 MPa and tensile strength between 49 and 174 MPa have been reported for HA in literature [2,3]. These results were also affected by porosity, impurity and test directions according to the bone axis etc. Modulus of elasticity had a range from 11.5 to 27 GPa showing that HA is a brittle material [3,4]. Low fracture toughness values are also the indicator of the brittleness of HA [5]. The fracture toughness for HA was reported between 0.6 and 1 MPa $m^{1/2}$, whereas these values are between 2 and 12 MPa $m^{1/2}$ for bone [6,7].

HA is a biocompatible and bioactive material that can make chemical bonds with bone [8]. Improvement of biological and physicochemical properties of HA can be achieved by doping with ions that are usually present in natural apatites of bone. Most natural apatites are non-stoichiometric because of the presence of minor constituents such as cations (Mg^{2+}, Mn^{2+}, Zn^{2+}, Na^{+}, Sr^{2+}) or anions (HPO_4^{2-} or CO_3). Hexagonal HA structure enables some ionic substitutions according to charge type, charge size and ionic radii. These substitutions change structural, mechanical and biological properties of HA [9]. Zn ion has +2 charge and can be incorporated to the HA

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structure by replacement with Ca^{2+} ion. Zn^{2+} incorporation decreased the grain sizes and increased the solubility of pure or carbonated HA [10,11].

Zn doping of HA is of major interest because biological tissues like bone and enamel of human teeth are composed of HA containing zinc. This crucial element has an important role in the activity of many enzymes and its uptake and release are strongly mediated by the bone reservoir in the body [12]. Additionally, antibacterial and antifungal effects of Zn doped HA against *Escherichia coli, Staphylococcus aureus* and pathogen yeast *Candida albicans* were also documented in solid and liquid media [13]. Moreover, Zn–HA nano-rods presented improved performance to decrease oral cavity bacteria [14].

Its stimulatory effect on bone formation and mineralization has been reported with in vivo and in vitro studies [15,16]. Zn^{2+} doped HAs were synthesized to investigate their antimicrobial characteristics, microstructure, sintering behavior, and in-vivo responses [17–21]. It was shown that Zn incorporation into implants promotes bone formation around the implant and decreases the inflammatory response [16,22]. It was also reported that Zn^{2+} ion results in osteoclastic inhibition [23]. Low amount of Zn^{2+} doping is favored due to cytotoxic effects of high amounts of Zn^{+2} doping [24]. Moreover, high amounts of Zn^{2+} doping (up to 15–20 mol%) inhibits HA phase formation [25]. Surface characteristics of HA were also investigated in the presence of Zn^{2+} ions [26–28]. Zn^{2+} doped HAs were coated on metals to investigate the surface microstructure, porosity, and cell responses etc. [29–31]. Zn^{2+} including HAs with other systems were also synthesized as composite, microsphere, and scaffold [32–34].

F^- ion can also be incorporated to HA structure and it competes with OH^- ion. F^- addition resulted in a decrease in solubility and an increase in density of HA after sintering. Improvement in strength and hardness by two or four fold was detected with F^- addition as well as improvement in crystallinity [35]. F^- effects on cell proliferation and attachment were reported in several studies [29,52]. The investigations showed that F^- doping of HA could improve the biological properties (i.e. cell attachment, proliferation, functionality) of HA if the level of doping was done at optimum levels (0.4 mol F^-/1 mol HA) [36,37]. In study of Wang et al., it was reported that osteoblast-like MG63 cells spreaded on surface of fluoridated samples with their filopodium and lamellipodium which was the indicator of good cell viability and good cell-biomaterial interactions [38].

HA co-doped with Zn^{2+} and F^- ions was synthesized by precipitation method for the first time in this study. The synthesized samples were sintered at 1100 °C for 1 h. The changes in structural, mechanical and biological properties of HA were investigated after co-doping of F^- and Zn^{2+} ions. Moreover, the effect of F^- amount on Zn^{2+} doped HA was investigated. There are several studies that investigated the effect of Zn^{2+} and F^- on HA structure separately. However, this study is the one which combines the effects of these two ions on structural, mechanical and biological properties of HA.

2. Experimental

2.1. Preparation of pure and doped HA

HA was synthesized by solution precipitation method in this study [39]. To synthesize pure HA, calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, Merck, Germany) and diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, from Merck, Germany) powders were dissolved in distilled water separately by adjusting the amounts to obtain theoretical Ca/P ratio of 1.67. After 1 h stirring, ammonia solution (NH_4OH, from Merck, Germany) was added to the $(NH_4)_2HPO_4$ solution to bring the pH to a value between 11 and 12. After 10 min of stirring, $Ca(NO_3)_2 \cdot 4H_2O$ solution was added to the ammonia and $(NH_4)_2HPO_4$ solution mixture. At the same time with addition of $Ca(NO_3)_2 \cdot 4H_2O$ solution, ammonia solution was also added drop wise to adjust the pH level between 11 and 12. The reaction begins as $Ca(NO_3)_2 \cdot 4H_2O$ solution is added to the ammonia and $(NH_4)_2HPO_4$ solution mixture. A milky solution appears with the first drop of addition of $Ca(NO_3)_2 \cdot 4H_2O$ solution. After stirring for 2 or 3 h, the mixture was heated until it boiled. The boiling step is required for decreasing pH, which triggers re-precipitation reaction. The re-precipitation reaction removes the remaining particles of TCP which is a more stable phase than HA. This is true only when the temperature is higher than 1150 °C, otherwise HA is a more stable phase than TCP.

After 10 min of boiling the heater was turned off and the mixture was left for stirring overnight at room temperature. After 1 day of aging, the mixture was filtered using a vacuum filter. The obtained wet cake was left for drying overnight at 200 °C for removal of excess water and ammonia. Dried powder was then sintered at 1100 °C for 1 h. For Zn^{2+} and F^- doped samples, zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$, from Riedel-deHaën, Germany) was added to $Ca(NO_3)_2 \cdot 4H_2O$ precursor for Zn^{2+} doping and ammonium fluoride (NH_4F, from Aldrich, Germany) was added to $(NH_4)_2HPO_4$ precursor for F^- doping. The rest of the process was the same as the process followed for pure HA synthesis. Table 1 represents the synthesized samples with their ion addition amounts.

2.2. Characterizations

For density measurements, Archimedes method was used. Dry weight and the weight in distilled water were measured, respectively. By using the formula below, the density of the

Table 1
Naming, percentages of doping by mole and Ca/P ratio for prepared samples.

Sample name	Mole % Zn^{2+}	Mole % F^-	Ca/P
Pure	0	0	1.67
2Zn	2	0	1.63
2Zn1F	2	1	1.63
2Zn2.5F	2	2.5	1.63
2Zn5F	2	5	1.63

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