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Ethanol-based sol-gel synthesis of nano-crystalline hydroxyapatite with different calcium phosphorus ratios (Ca/P)

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One of the easy ways to prepare nanocrystalline hydroxyapatite (nano-HAp) is the sol-gel method that due to its unique advantages compared to conventional methods, has raised research interest among scientists. The purpose of this study was to investigate the properties of nano-HAp powders which were synthesized by ethanol-based sol-gel method with stoichiometric ratio of Ca/P = 1.67 and comparing it with closer ratios of 1.5 and 1.6. To do so, we used the aqueous solution of phosphorus pentoxide (p_20_5, MERCK) and four-watered calcium nitrate ($Ca(NO_3)_2.4H_2O$, MERCK) in ethanol. The resulted nano-powders and sintered pellets made of it were analysed and compared with X-ray diffraction analysis (XRD). Also, the sintered pellets' bioactivity in simulated body fluid (SBF) solution was examined by SEM. In powders which were calcined at the temperature of 600 °C, the most amorphous phase (49%) in comparison to Ca/P = 1.5 was observed. After sintering, the main phase of 1.67 ratio was hydroxyapatite (HAp) and the main phase of 1.5 ration was β-TCP, although in 1.6 ration a composite of HAp and β-TCP was observed. According to the results, sol-gel method for producing nano-crystalline HAp was an appropriate method; in addition, compared with the different ratios of Ca/P, the stoichiometric nano-crystalline may have better bioactivity.

Key words: Hydroxyapatite, Sol-gel, tricalcium phosphate, Bioactivity.

Introduction

Calcium phosphate (CP) bioceramics' family are because of their apatite structure important biomaterials for bone regeneration and many studies have been conducted on various CP´s [1-4]. Hydroxyapatite (HAp; $Ca_{10}(PO_4)_6(OH)_2$) is the major CP in natural bone; also, synthetic HAp is a bioactive ceramic which is widely used in the form of particulates, not only for the replacement for lost bone tissue (or repairing them) [5], but also as coatings for scaffolds to improve their biological properties [6]. In addition, HAp has been used recently as a matrix for controlled drug delivery in biomedical applications [7], or even direct pulp capping in dentistry [8]. In addition to HAp, particular attention has been placed to tri-calcium phosphates (TCPs, Ca_3PO_4) because of their wide uses in the field of medicine [9]. The phases of β-TCP and α-TCP showed various applications in the form of single-phase or biphasic such as in bone regeneration.

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Nano-sized CP-based ceramic powders has some benefits over conventional ones (in micron size) due to their high surface area and similarity with nano-sized mineral crystals in natural bone [10]. Furthermore, since nano-sized HAp is a huge reservoir of calcium and phosphate ions which are necessary for a wide variety of metabolic functions, it has a crucial function for organisms [11]. Moreover, HAp nanocrystalline powders, due to their high surface area, have shown improved sinterability and densification leading to e.g. improved fracture toughness and mechanical strength [12].

So far, several methods have been used in the literature to produce HA nano-powder like co-precipitation [13], mechanical alloying [14], ultrasound precipitation [15], sol-gel [16], etc. In recent years, researchers have shown interests in the use of sol-gel method [12, 17-27]. The sol-gel method is a chemical process in a moist environment, which is advantageous to the dry process compared to which the products can be obtained with high purity. To illustrate, this method allows calcium and phosphorus precursors be mixed together at a molecular level which can improve the homogeneity and product purity [24].

Synthesis of stoichiometric nano-HAp powders using sol-gel synthesis is relatively easy and their well-known inherent advantages have already been highlighted in

the literature [28], such as the ability to create nano-crystalline powders, bulk amorphous monolithic solids and thin films. According to an study conducted by Fathi *et al.*, they synthesized HAp nano-powders consisting of crystallites of 20-30 nm by ethanol based sol-gel method and compared its characteristics after sintering at temperatures ranging from 600 °C to 900 °C. They demonstrated that crystallinity and morphology of stoichiometric HAp were dependent on sintering temperature [29].

Different Ca/P molar ratios can, also, affect the final proportions of HAp and TCP in sintered CP ceramics [30]. With regard to the fact that CPs with different Ca/P molar ratios synthesized by different methods have been compared in the literature [30-35], the aim of this study was to investigate nano-HAp synthesized by ethanol-based sol-gel synthesis with stoichiometric molar ratio of Ca/P = 1.67 and compare it with ratios of 1.6 and 1.5 with respect to structure and *in vitro* bioactivity.

Materials and Methods

Sample preparation

CP powders with stoichiometric Ca/P molar ration of 1.67 and its relative rations of 1.5 and 1.6 were synthesized by ethanol-based sol-gel method, phosphorus pentoxide (P_2O_5, MERCK) and calcium nitrate ($Ca(NO_3)_2.4H_2O$, MERCK) were employed as a chemical precursor for phosphorus and calcium, respectively. The aqueous solution of 0.5 molar phosphorus pentoxide and 1.5, 1.6, and 1.67 molar of calcium nitrate in pure ethanol was obtained separately. Then phosphorus precursor solution was slowly poured into the solution containing calcium precursor which was stirring vigorously with mechanical stirrer (1000 rpm) and then the resulting sol was stirred for additional 30 minutes. For obtaining gel, through the process of aging, the resulted transparent sol was kept in a closed container for 48 hours at room temperature. Then it was dried in oven at 60 °C for 1 day, and finally, to be calcined, the powders were kept at 120 °C for 3 hrs, whereafter the temperature was increased with heating rate of 1 °C min^{-1} up to 600 °C and held at this temperature for 1 hr. In the next stage, utilizing a press machine and with the pressure of 0.15 MPa, the powders were turned into 10 mm diameter pellets and stored at 1100 °C temperature for 3 hrs [36].

Sample characterization

The resulting samples surfaces were analyzed by X-ray diffraction analysis (XRD, voltage of 40 kV, current settings of 40 mA, Cu Kα radiation, Philips, X'PERT). XRD diagrams were recorded in the interval of $20° \leq 2\theta \leq 80°$ at scan speed of 2 ° min^{-1}. The synthesized samples morphology and microstructure were investigated using scanning electron microscope (SEM, Vega, Tescan, Philadelphia, PA) after coating them with a thin layer of gold by sputtering (EMITECH K450X, UK).

With respect to the XRD diagrams, to calculate the degree of crystallinity the Landi method was employed [37]:

$$X_c = 1 - \frac{v_{112/300}}{I_{300}} \quad (1)$$

where $V_{112/300}$ is the intensity of the hollow between HAp (112) and HAp (300) reflections, and $I_{300,}$ the peak intensity of the HAp (300) reflection.

Crystallite size of the HAp in the calcined powders and the pressed and sintered pellets were estimated according to the Scherrer equation [33]:

$$d = \frac{0.9\lambda}{w\ cos\theta} \quad (2)$$

where d is the crystallite diameter (nm), λ is the wavelength of X-rays (nm), w is the main peak width at half height in radians, and θ is the angle of the main peak.

Biological evaluation

We prepared simulated body fluid (SBF), in which the ion concentrations were nearly equal to its concentration in human blood plasma, according to the literature [38]. Briefly, reagent grade NaCl, KCl, $NaHCO_3$, $MgCl_2.6H_2O$, $CaCl_2$ and KH_2PO_4 were dissolved into distilled water and buffered at pH 7.25 with Tris buffer ($(CH_2OH)_3CNH_2$) and 1 N HCl at 37 °C. All chemicals were purchased from Merck, Inc. The pellets had been immersed in SBF at a solid/liquid ratio of 100 mg/ml at 37 °C for 21 days and then were evaluated by SEM analysis.

Results and Discussion

Synthesizing stoichiometric HAp according to ethanol-based sol-gel synthesis was demonstrated in various sintering temperatures from 600 °C to 900 °C [21, 29]; however, in the current study, we synthesized and characterized stoichiometric HAp by this method at sintering temperature 1100 °C, and compared it with two closer Ca/P molar ratios of 1.5 and 1.6.

The term of Ca-deficient hydroxyapatite (CDHA, Ca_{10-x} $(HPO_4)_x$ $(PO_4)_{6-x}$ $(OH)_{2-x}$,$(0 < x < 1)$) is applied on CPs with a range of Ca/P molar ratio between 1.5 and 1.67 without any heat treatment [9]. Since it was demonstrated that differentiation between CDHA nanocrystals with different molar ratios of Ca/P is difficult using XRD diagrams [35], in the current study, we just characterized both calcined and sintered HAp nanocrystals with different molar ratios.

Fig. 1 shows XRD patterns of powders which had been calcined at 600 °C temperature from gels with Ca/P ratio of 1.5, 1.6 and 1.67, respectively, and the phases of the powders have been identified and

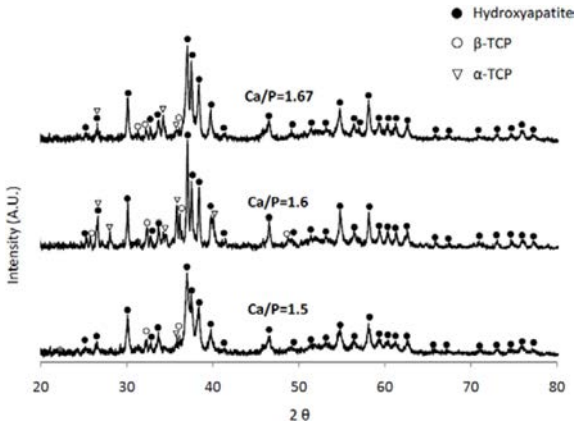

Fig. 1. XRD pattern of synthesized powder by sol-gel method and calcined at 600 ºC temperature for 3 hrs at different Ca/P ratios.

Table 1. Crystallization degree of calcined powders at temperatures 600 ºC for 3 hours with different ratios of Ca/P.

Ca/P ratio	1.5	1.6	1.67
Crystallization degree (%)	51	64	59

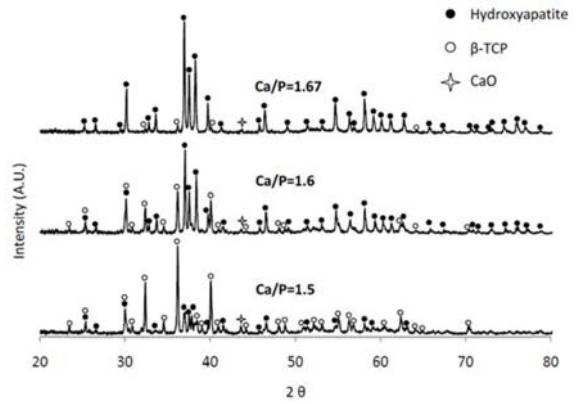

Fig. 2. XRD pattern of sintered pellets at 1100 ºC temperature for 3 hrs from calcined powders with different Ca/P ratios.

marked. It is clear that the three powders, especially the powder with Ca/P molar ratio of 1.5, contain large amorphous phases. The degree of crystallinity of the calcined powders was assessed using the Landi method (Table 1). This confirms that a large amount of the powders are amorphous, with up to 49% amorphous phase for the powder which was calcined from gel with Ca/P molar ratio of 1.5. In this powder, the main crystalline phase was HAp and only a little amount of TCP phases could be observed. For the powders synthesized with 1.6 and 1.67 Ca/P molar ratios more crystalline phase was formed and the amorphous phase was reduced to 36% and 41%, respectively. Our results are consistent with literature [21, 29]; for instance, Fathi *et al.* demonstrated 39% amorphous phase in stoichiometric HAp after sintering at 600 ºC [29]. Kannan *et al.* studied the XRD patterns of powders calcined at 700 ºC temperature, and found that the amorphous phase increased from Ca/P molar ratio 1.67 to 1.41 [30].

Table 2. The main peak width at half height and HA crystallite size in the calcined powder and sintered pellet samples with different Ca/P ratio.

Ca/P ratio	Samples	Peak width (rad)	Particle size (nm)
1.5	Powder	0041/0	41
	Pellet	0034/0	49
1.6	Powder	0027/0	62
	Pellet	0020/0	82
1.67	Powder	0034/0	49
	Pellet	0027/0	62

Fig. 2 shows XRD patterns of 10 mm diameter pellets derived from the understudy calcined powders. At the Ca/P molar ratio of 1.6, after sintering at temperature 1100 ºC, we obtain a composite made of HAp and β-TCP. Absence of α-TCP can be due to the intensity of â-TCP and HAp peaks compared with the small amount of this phase or conversion of this semi-stable phase to the stable phase of β-TCP during the sintering process. In the ratio of Ca/P = 1.67 too, as expected [34], we have seen the main phase of HAp and a slight amount of β-TCP phase. However, the main phase of pellets with Ca/P molar ratio of 1.5 was transformed into β-TCP. In fact, although no β-TCP phase was found for nano-powders with Ca/P molar ratio of 1.5 calcined at 600 ºC (Fig. 1), it was formed after sintering at 1100 ºC (Fig. 2). Consistent with the literature, β-TCP with Ca/P molar ratio of 1.5 can be obtained at temperatures higher than 800 ºC [39], and after sintering at temperature 1100 ºC the main phase is β-TCP [32]. However, in another study, pure β-TCP was obtained from sintering at 1100 ºC of the powders produced from Ca/P ratio of 1.41. [30]. This difference may be due to different experimental conditions, temperature and pH.

CaO phase was observed in all understudy pellets XRD diagrams after sintering temperature of 1100 ºC. It is demonstrated in the literature that as the temperature is increased to 700 ºC or above it, HAp phase could be decomposed into CaO and β-TCP phases [29].

The Scherrer equation was used to access both the calcined powders and the pressed and sintered pellets (Table 2). We used cobalt anode that emits a wavelength equal to 0.179 nm, and the diffraction angle of the main peak of HAp at all times is equal to 18.5 º. Both powders and pellets demonstrated nanostructures in range between 41 to 62 nm and 49 to 82 nm, respectively. This is consistent with the SEM analysis of the powders for all three Ca/P ratios (Fig. 3). Also, for understudy Ca/P molar ratios, by pressing the powder into pellets and sintering them at temperature 1100 ºC for 3 hrs, the diameter of the crystallite increased, probably owing to the process of grain growth and reduction of its borders because of the density of the particles and rising of

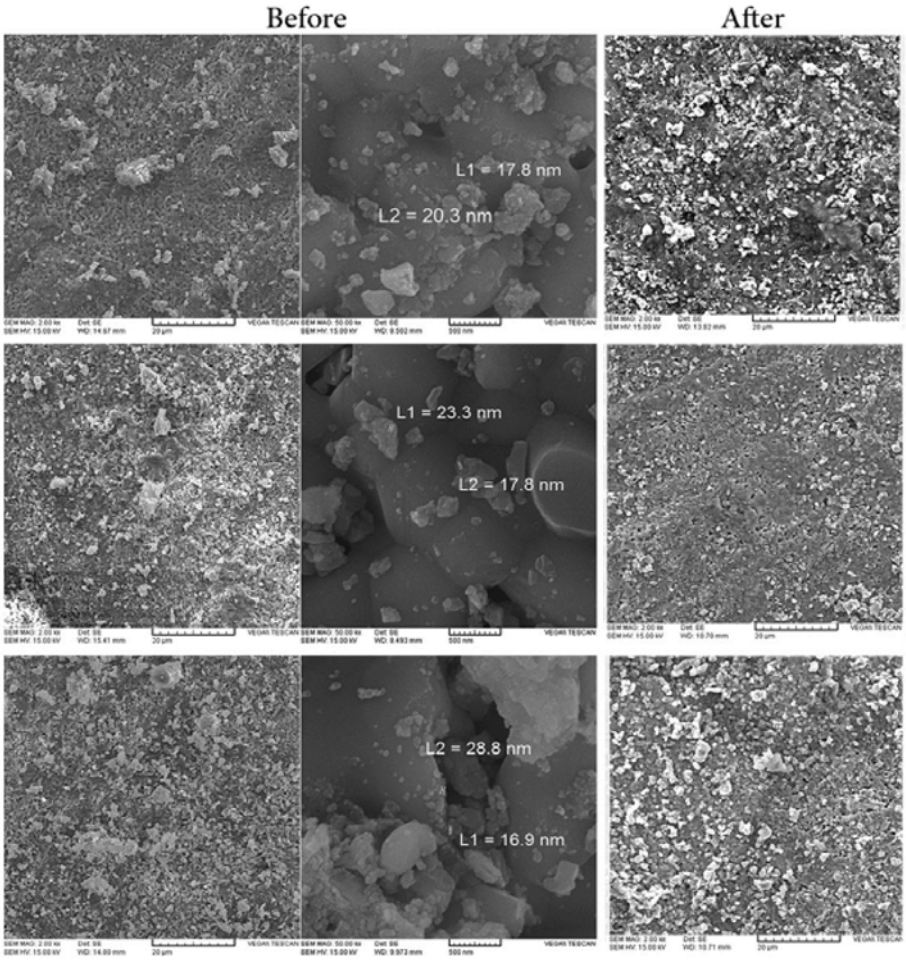

Fig. 3. SEM images of studied groups before and after placing in SBF. From up to down, Ca/P ratio is 1.5, 1.6, 1.67.

Table 3. Comparison of the peak intensity of pages (211) in the calcined powder and sintered pellets.

Ca/P ratio	Samples	Peak intensity
1.5	Powder	588
	Pellet	313
1.6	Powder	755
	Pellet	1123
1.67	Powder	673
	Pellet	1471

temperature [30]. Recently, it was established that both calcination temperature and duration have direct effect on both crystallite size and crystallinity of HAp nanopowders [18]. However, the lowest increase in crystallite size was seen for the Ca/P molar ratio of 1.5.

Table 3 shows the peak intensity of (211) reflection in the HAp phase. By the sintering process, the intensity of the main peaks of HAp phase in two samples with the Ca/P molar ratios of 1.6 and 1.67 have been raised, and in a sample with stoichiometric ratio of Ca/P = 1.67, after sintering this intensity is more than doubled, indicating the crystallization of the amorphous phase and also growth of HAp grains [31]. In a samples with a ratio of Ca/P = 1.5, the process is quite different and with sintering, HAp peak intensity decreases and in contrast, the β-TCP phase has showed growth (Fig. 2).

Fig. 3 shows the SEM images of understudy groups. It has been shown previously that bioactive materials which form apatite on their surfaces in SBF have demonstrated bonding to living bone *in vivo* through the apatite layer [38, 40]; therefore, observations of surface apatite formation in SBF can be taken as a measure of the potential *in vivo* bioactivity of the material. Understudy pellets placed in SBF for 21 days at 37 ºC revealed surface apatite formation (Fig. 4). However, it seems that by reducing the Ca/P molar ratio the amount of apatite formed on the surface was diminished and its dispersion was also decreased, suggesting that a reduced Ca/P molar ratio may also lead to reduced bioactivity (Fig. 4). In the literature, apatite formation on sintered HAp surface in SBF and its bonding to living bone through an apatite layer *in vivo* was observed [41]. Also, it is confirmed that â-TCP can band to alive bone though apatite formation have not

been shown on its surface in SBF or *in vivo* [42].

Conclusions

Ethanol-based sol-gel synthesis is an appropriate synthesis route for the production of HAp nano-crystalline powders. All synthetized powders demonstrated crystallization at sintering temperature of 1100 °C. Both crystallinity and morphology of CPs synthesized by this method were dependent on Ca/P molar ratios. Moreover, each pellets with different Ca/P molar ratios showed different phases formed after staying at sintering temperature of 1100 °C for three hours.

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