Mechanosynthesis of carbonate doped chlorapatite–ZnO nanocomposite with negative zeta potential

Abbas Fahami ^{a,*}, Gary W. Beall ^{b,c},

^a *Ingram School of Engineering and Materials Science, Engineering and Commercialization Program, Texas
State University, San Marcos 78666, TX, USA*

^b *Texas State University-San Marcos, Department of Chemistry and Biochemistry, 601 University Drive,
San Marcos 78666, TX, USA*

^c *Physics Department, Faculty of Science, King Abdulaziz University, Jeddah, 21589, Saudi Arabia*

Abstract

Carbonate doped chlorapatite –ZnO composite nanopowders with negative zeta potential were synthesized by one step mechanochemical process as a novel bioceramic. Results indicated that the formation of carbonate doped chlorapatite–ZnO composite was varied by incorporation of different ZnO contents. In the presence of 3, 6, 9, 12, and 15 wt% ZnO, carbonate doped chlorapatite–ZnO composite nanopowders were produced successfully after 1 h of milling. There was no trace of carbonate doped chlorapatite based composite as ZnO content increased to 20 wt%. According to the X-ray diffraction data, the average crystallite size, and the degree of crystallinity of the product decreased slightly as ZnO contents increased from 0 to 15 wt%. Conversely, lattice strain increased gradually. Subsequent annealing at 800 °C for 1 h of carbonate doped chlorapatite–12 wt% ZnO composite indicated larger crystallite size and the degree of crystallinity. The zeta potential of nanoparticles (12 wt% ZnO sample) suspended in physiological saline (0.154 M NaCl) was determined. The zeta potential was almost zero for pH=3 and negative values for pH= 5–11 and -22.52 ± 1.96 mV at pH 7.4. Negative zeta potential is reported to favor apatite nucleation, bone regeneration, as well as osseointegration. Microscopic observations illustrated that the final product had a cluster-like structure containing ZnO platelets as well as polygonal and spherical particles with an average particle size of approximately ranged from 20 ± 10 to 50 ± 10 nm. To our knowledge, this is the first report of the synthesis of carbonate doped chlorapatite–ZnO composite nanopowders; the nanocomposite reported here can be a promising candidate for use in biomedical applications.

Keywords: Carbonate doped chlorapatite; Zinc oxide; Mechanochemistry; Zeta potential; Microscopic observations.

*Corresponding author. Tel.: +15129600009; fax: +15122453675
E-mail address: fahami@txstate.edu (A. Fahami).

1. Introduction

Hydroxyapatite (HAp) as a bioactive ceramics has been widely investigated, owing to its similarity to the inorganic components of bone and teeth. Excellent biocompatibility, the ability to promote cellular functions, and osteoconductivity are outstanding properties of HAp [1]. However, synthetic HAp has poor corrosion resistance in an acidic environment, weak mechanical properties, high dissolution rate in a biological system and poor chemical stability at high temperatures [2]. Therefore, the modification of HAp has been done by various processes such as substitution of hydroxide (OH^-) or phosphate (PO_4^{3-}) groups in HAp structure by other species such as F^- , Cl^- and CO_3^{2-} which formed fluorapatite (FAp), chlorapatite (CAp) and carbonated hydroxyapatite (CHAp), respectively [3-5]. According to the literature, the fluorine ion in FAp structure boosts tooth mineralization and bone density; however it decreases the solubility of FAp and fluorhydroxyapatite (FHAp) in chemical and biological media. This deficiency can be compensated by the presence of carbonate ion concentrations simultaneously in apatite structure. The significance of chloride ions is correlated to their ability to develop an acidic environment on the surface of bone which resulted in the stimulation of the osteoclasts in the bone resorption process [6].

Generally, 4–8 wt% of calcified tissues in human body is formed by CHAp which has better chemical and physical properties as well as its bone bonding capability in comparison with HAp [7]. Moreover, the presence of carbonate into the apatite structure leads to a reduction in crystallinity, a change in crystal morphology, and an improvement of chemical reactivity due to the weak bonding owing to carbonates geometry [8–10]. Indeed, the CHAp increases the local concentration of calcium and phosphate ions that are essential for new bone generation [11]. Recently, the incorporation of carbonate ion into fluorapatite and chlorapatite structure has been investigated by our research group [12–13].

In terms of mechanical properties, HAp is brittle and it is not appropriate for load bearing applications. Therefore, various methods such as bioinert ceramics incorporation into matrix [14], and adding dopants have been done by many research groups in order to promote the characteristic [15]. However, an ideal reinforcing material for the calcium phosphate-based composites is a big challenge. According to literature, zinc is an active participation in a variety of cellular processes including DNA synthesis, bone formation, and growth as well as wound healing. The addition of zinc to the deficient diets leads to a stimulation of both bone growth and mineralization, decreases the inflammatory response and has an antibacterial effect [16]. Recently, Cheng et al. [17] reported on HAp/ZnO-nanorod composite coatings as a great potential in osseointegration applications. Huang et al. [18] investigated corrosion resistance and biocompatibility of SrHAp/ZnO composite implant coating on titanium. Their results revealed that the mentioned composite coating had a lower corrosion rate with better the *in vitro* biocompatibility than the pure HAp-coated sample. This leads to a growing interest in the development of such composite materials.

Literature reports have given ample evidence that negative values of zeta potential have a significant favorable effect on apatite nucleation, the attachment and proliferation of bone cells as well as osseointegration of dental implants [19]. Zeta potential has also been used to explain ions effects on coagulation in blood including the thrombosis and the aggregation of cholesterol particles [20]. Some attempts were reported in literature as to negative zeta potential, Doostmohammadi et al. [21] investigated zeta potential of bone-derived hydroxyapatite which was -9.25 ± 0.9 mV at pH 7.4. Teng et al. [22] tried to prepared synthetic HA particles with more negative zeta potential through electrical polarization treatment.

The main incentive for current research is to synthesis and characterization of carbonate doped chlorapatite–zinc oxide (CCAp–ZnO) composite nanopowders with negative zeta potential via a novel facile mechanochemical process. To our knowledge, this is a first study on characterizing of CCAp–ZnO composite nanopowders and evaluating zeta potential. To determine the optimal amount of zinc oxide, the structural features of the samples in the presence of different zinc oxide contents were examined by using XRD, FT-IR, FE-SEM, TEM, and EDS. In addition, the zeta potential of the prepared composite particles was determined.

2. Experimental procedures

2.1. Materials and composite preparation

Zinc oxide (≥ 99.0 wt% ZnO), calcite (≥ 99.0 wt% CaCO_3), phosphorus pentoxide (≥ 98.0 wt% P_2O_5), and calcium chloride (≥ 97.0 wt% CaCl_2), powders were purchased from Sigma Aldrich. All the reagents were of analytical grade and used without other purifications. The mole ratio of calcium to phosphorus was in accordance with the stoichiometric Ca/P content in the composition of CCAp which was equal to 1.9. The substitution degree of PO_4^{3-} by CO_3^{2-} was chosen equal to 1.0. Hence, the general form of the mechanochemical reaction (R1) is as follows:

To prepare the CCAp–ZnO Nanocomposite, different values of zinc oxide (0, 3, 6, 9, 12, 15 and 20 wt%) were mixed with the desired amounts of CaCO_3 , P_2O_5 , and CaCl_2 and the blended powders were milled for 60 min in a high–energy ball mill (8000M Mixer/Mill) using hardened chromium steel vials (vol. 65 ml) and steels balls under air atmosphere. In all cases, the weight ratio of ball-to-powder (BPR) were 20:1. To crystalized mechanosynthesized CCAp– 12 wt% ZnO composite, the thermal treatment was done at 800 °C for 1h at air atmosphere. The heating rate from room temperature to the desired temperature was fixed 10 °C min⁻¹.

2.2. Characterization techniques

The phase analysis of the specimens was examined by X–ray diffraction (Bruker D8 Advance ECO (XRD)) equipped with Cu-K_α radiation ($\lambda \approx 1.54\text{\AA}$), and $10^\circ \leq 2\theta \leq 80^\circ$). The XRD profiles were compared to standards compiled by the Joint Committee on Powder Diffraction and Standards (JCPDS), which involved card #27-0074 for CAP, #036-1451 for

ZnO, #005-0586 for CaCO₃. The Williamson-Hall equation was used to determine the crystallite size and lattice strain of the products [23]:

$$B\cos\theta = 0.9\lambda/D + \eta\sin\theta \quad (I)$$

where λ , D , η and θ are the wavelength of the X-ray used (0.154056 nm), crystallite size, internal micro-strain and the Bragg angle (°), respectively. Note that B in the above equation is the peak width (in radians) after subtracting the peak width due to instrumental broadening from the experimentally recorded profile. Therefore, when $B\cos\theta$ was plotted against $\sin\theta$, straight lines were obtained for samples with the slope as η and the intercept as $0.9\lambda/D$.

In addition, the crystallinity degree (X_c) was estimated by taking the sum total of relative intensities of individual characteristic peaks according to the following equation [12]:

$$X_c = \frac{SUM(I_1: I_n)_{CCAP}}{SUM(I_1: I_n)_{Standard}} \times 100 \quad (III)$$

The lattice parameters (a , b , and c) can be evaluated from the following relation

$$\frac{1}{d^2} = \frac{4}{3} \frac{h^2 + hk + k^2}{a^2} + \frac{l^2}{c^2} \quad (IV)$$

where h , k , and l are the Miller indices [12]. The unit cell volume (V) of CCAP was determined using the following equation [23]:

$$V = (a^2c)\sin60^\circ \quad (V)$$

Fourier transform infrared spectroscopy (FT-IR, Bruker Tensor 27, USA) was performed to evaluate the functional groups and structural changes of CCAP–ZnO during milling. All spectra were recorded at ambient temperature in the range 4000–400 cm⁻¹. The morphological features of CCAP–ZnO were evaluated using FE–SEM (FEI Helios Nanolab 400 SEM). A more detailed morphological analysis was executed using TEM (JEOL JEM 1200 EXII) that operated at the acceleration voltage of 120 kV. X-ray energy dispersion spectroscopy (EDS) and Elemental mapping analysis system attached to the FESEM were utilized to determine the elemental composition of samples and distribution of materials. The zeta potential of the particles was performed with a Malvern Zetasizer (Nano-ZS with Autotitrator). To roughly simulate *in vivo* ionic environments, CCAP–12 wt% ZnO composite and heat treated samples were suspended in 0.154 M NaCl solution (physiological saline) at different pH ranged from 3 to 11. The appropriateness of such *in vitro* analyses was reported by Bagambisa et al. [24] and Doostmohammadi et al. [21], who found that an aqueous *in vitro* model yielded complementary outcomes when compared to the *in vivo* results owing to the ubiquitous presence of water [24]. The potential was measured six times (each time being the average of 100 runs) and the mean values and standard deviations were calculated. The instrument calculates electrophoretic mobility (U), and zeta potential automatically based to Smoluchowski's equation [25]:

$$\zeta = \frac{U\eta}{\epsilon} \quad (VI)$$

where ζ is the zeta potential, η the medium viscosity, U the electrophoretic mobility and ε is the dielectric constant.

3. Results and discussions

3.1. XRD analysis

Fig. 1 displays the XRD profiles of 60 min milled samples with the different amounts of ZnO (0, 3, 6, 9, 12, 15, 20 wt%). In the absence of ZnO, all the peaks corresponding to reagents had disappeared completely and only those of CCAP were noticeable after 60 min of mechanical activation. With the addition of 3 wt% ZnO, a CCAP-ZnO composite nanopowders was formed during 60 min of milling. A similar trend was observed as ZnO values increased from 6 to 15 wt%. However, some discrepancies were noticeable with the increase of ZnO contents. First, the intensity of characteristics peaks decreased gradually which could be due to the amorphization of the product. Secondly, an increase in the peak broadening with ZnO addition was seen which could be explain on the basis of crystallite refinement and micro-lattice enhancement. This indicates that the final product are nanocrystalline in nature. With increasing the ZnO content to 20 wt%, the weight percent of (wt%) of calcium and phosphorous reagents were not enough to form carbonate doped chlorapatite. Consequently, no mechanochemical reaction was done during 60 min of milling. In this condition, only the peaks related to the calcite and ZnO were detected. It is worthy to note that P_2O_5 peaks were not observed due to very high deliquescent nature of P_2O_5 and further phosphoric acid formation. The XRD profile of the milled CCAP-12 wt% ZnO nanocomposite after subsequent annealing at 800 °C for 1h is shown in Fig. 2. It is clear that CCAP and ZnO were the main components of the heat treated specimen, and a considerable increase in the peak intensity and a significant decline in breath of the fundamental diffraction peaks had occurred. From this figure a CCAP-12 wt% ZnO composite nanopowders with high crystalline structure was obtained.

3.2. Structural features

The crystallite size, lattice strain, crystallinity degree, lattice constants and unit cell volume of samples as a function of various ZnO values (wt %) are shown in Fig. 3. According to Fig. 3a, the crystallite size of specimens declined gently from 39 ± 1 to 18 ± 2 nm as ZnO contents increased from 0 to 15 wt%. After annealing CCAP-12 wt% ZnO sample at 800 °C, this value increased to 198 ± 2 nm. In contrast, the lattice strain rose remarkably from 0.0073 ± 0.0001 to 0.0105 ± 0.0002 when ZnO contents (wt%) increased and reached a maximum of 0.0122 for the annealed CCAP-12 wt% ZnO sample.

Based on Fig 3b, crystallinity degree was expressed in accordance with the line broadening at the (300) and (211) diffraction peaks for CCAP. The results showed that the crystallinity degree decreased slightly from 18 ± 1 to 21 ± 2 as ZnO increased in the samples from 0 to 15 wt%. This value obtained almost doubled, 37 ± 2 , for the heat treated sample

(CCAP-12 wt% ZnO) at 800 °C in comparison with milled sample. The lattice constants and unit cell volume of CCAP as a function of ZnO contents is shown in Fig. 3c. Based on the data of standard chlorapatite (JCPDF#0027-0074), *a*-axis, and *c*-axis were 9.642 and 6.776 Å. As compared with the data obtained from equation IV, the *a*-axis and *c*-axis values were different; however it shows an overall downward trend as ZnO contents increased in samples. This is also consistent with lattice contraction that has been observed. It can also be observed that carbonate substitution into the phosphate sites in the Ca-analog carbonated chlorapatite led to a small decrease in the *a*-axis and a slight increase in *c*-axis. This phenomenon demonstrated B-type substitution of carbonated chlorapatite, which is good agreement with our recent study [26]. Fig. 3d illustrates that the unit cell volumes of CCAP declined moderately as ZnO contents escalated from 0 to 15 wt% with a rapid reduction in 3 wt%, which were 531.72 in CCAP and 512.93 in CCAP- 15 wt% ZnO. This value grew slightly for the annealed sample (CCAP- 12 wt% ZnO), 519.66 Å³, in comparison with the milled one (CCAP- 12 wt% ZnO), which was 517.22 Å³. Likewise, the obtained result can be attributed mainly from decreases in the *a* constant, rather than from the *c* parameter and could possibly be related to the structural changes of CCAP during the milling process. These findings showed that the structural features of the mechanosynthesized CCAP-ZnO nanocomposite was influenced by the various weight percent of ZnO.

3.3. FT-IR spectra

The FT-IR spectra of CCAP based composite nanopowders with different amount of ZnO (0–15 wt%) displayed in Fig. 4. There are two bands belonging to the vibration of the adsorbed water in apatites appeared at 3600–2600 and 1800–1660 cm⁻¹ [27]. The absence of the bands at 630 and 3568 cm⁻¹ corresponding to OH⁻ libration mode and the appearance of bands at 669-706 cm⁻¹ are attributed to a predominantly Cl⁻ chain [28]. The FT-IR spectra presented that all the samples had the certain bands of phosphate groups of the apatite structure at about 478 cm⁻¹ (*v*₂), 570 and 605 cm⁻¹ (*v*₄), 954-968 cm⁻¹ (*v*₁), and 1032–1058 cm⁻¹ (*v*₃) [29]. It can be seen that PO₄³⁻ bands became broaden as ZnO values boosted in the samples which are attributed to the decline of crystallinity [30]. The presence of three bands at about 1455, 1410 and 871 cm⁻¹ were related to carbonate vibrations in type B carbonated chlorapatite which demonstrated some substitution of PO₄³⁻ sites by CO₃²⁻ ions [31]. This suggests that the synthesized nanopowders can present greater bioactivity and osseointegration with higher osteoconductivity properties and earlier bioresorption [32]. The stretching vibration of the Zn–O appeared as a broad band in the range of 700– 500 cm⁻¹ [27], which has also intensified with increasing of ZnO values in specimens. Based on FT-IR spectra, the mechanosynthesized CAP–ZnO composite nanopowders had high chemical purity which is good agreement with XRD results. This purity is essential in biomedical applications especially for Implants coatings.

3.4. FE-SEM and TEM observations

Fig. 5 shows FE-SEM and TEM images of CCAp-12 wt% ZnO composite nanopowders. Based on this figure, the milled sample had high tendency to agglomeration due to its relatively large specific surface area of powders and van der Waals interactions [33]. In terms of mechanochemistry, two particles share the same crystallographic orientation when two neighbor primary particles collide. As a result, two primary particles combine into a secondary one so that the agglomeration can be extended [34]. From FE-SEM images of Fig. 5a and b, CCAp-12 wt% ZnO nanopowders had a cluster-like structure which was composed of polygonal and spherical nanoparticles as well as ZnO micro-platelets. Based on these observations, the addition of ZnO nanopowders as a second phase had little effect on the morphology of matrix (CCAP) which was found from previous study [26].

Typical TEM images of CCAp-12 wt% ZnO nanocomposite produced through one step mechanochemical process are shown in Fig. 5c and d. It can be seen that, the particles exhibit high tendency to agglomerate which include of tiny polygonal crystallites with spherical morphology. This is in good agreement with FE-SEM images. The average particle size of CCAp-12 wt% ZnO powders was approximately ranged from 20 ± 10 to 50 ± 10 nm. The particle sizes obtained from TEM observation were almost identical with the crystallite size calculated from XRD pattern. It seems that this range of particle size is similar to that of natural bone (HAP) which have been reported in 20–40 nm range [35].

3.5. EDS analysis and elemental mapping

In this study, the EDS analysis and elemental mapping were employed to obtain a better understanding of the XRD results. The measurements of elemental composition of CCAp-12 wt% ZnO are presented in Fig. 6. According to the EDS spectra (Fig. 6a), the main components of the nanocomposite powders were oxygen, calcium, phosphor, carbon, zinc and chlorine. Since carbon dioxide was the only by-product of milled sample, no other chemically stable contaminants were observed. Moreover, Fe contamination resulting from the severe wearing of the steel vials and balls was not detected owing to short milling time (60 min). In accordance with elemental mapping analysis (Fig. 6b), the nanocomposite powders showed an appropriate distribution of elements which confirmed the formation of homogenous microstructures during mechanochemical procedure. Due to the high volume fraction of ZnO micro-platelet (12 wt%) in this specimen, the distribution of Zn was dominant in some part of sample. However, Ca and P as representative of CCAP had comparatively better distribution patterns than the other components. These findings were in good agreement with XRD results.

3.6. Zeta potential analysis

The zeta potential of milled and annealed CCAp-12 wt% ZnO particles as a function of pH was successfully measured in physiological saline as shown in Fig. 7. Based on Fig. 7a, the zeta potential of milled particles ranged from 0.001 ± 0.0004 mV at pH=3 to -21.20 ± 1.32 mV at pH 11. The maximum negative zeta potential value (-33.08) was observed at pH=9, while at a typical physiologic pH of 7.4 the zeta potential was -22.52 ± 0.9 mV. Fig. 7b shows the measured zeta

potential of annealed particles ranged from -1.47 ± 0.06 mV at pH=3 to -16.26 ± 1.25 mV at pH 11. This value reached a maximum negative at pH=7.4, which was -20.85 ± 1.30 mV. In brief, the zeta potential varied with pH and was almost zero at acidic pH and negative at neutral and basic pH. To the best of the authors' knowledge, there are no previously published data giving the zeta potential of CCAP–ZnO composite nanopowders. However, some reports indicating a positive zeta potential value for HAp and other bioceramics have been reported [21,36]. The reported zeta potential for hydroxyapatite and beta tricalcium phosphate (β -TCP) were a positive value, while for osteoblast cells and bone were 20.85 ± 1.30 and -70 , respectively [21]. The results reported for milled and annealed samples here show that at pH 7.4 (found in many situations *in vivo*), the zeta potential of CCAP–12 wt% ZnO in physiological saline were -22.52 ± 0.9 and -20.85 ± 1.30 mV, respectively. This negative zeta potential for CCAP based nanocomposite could be a beneficial property when if the material is implanted in bone containing viable cells [36]. The difference between the reported zeta potential values for HAp and CCAP–12 wt% ZnO particles produced in this study could be related to ions (e.g. Cl^- and CO_3^{2-}) or reinforcements (e.g. ZnO) that exist in chlorapatite based nanocomposite that are not present in HAp. Further investigation on recognizing the source of the negative zeta potential for CCAP–ZnO composite nanopowders should be the subject of a future detailed research.

4. Conclusions

Pure carbonate doped chlorapatite based composite nanopowders with different amount of ZnO were derived from a facile mechanochemical process. Results demonstrated that the formation and structural features of CCAP–ZnO were influenced significantly by incorporation of different weight percent of ZnO. Based on the XRD results, the crystallite size, crystallinity degree, and unit cell volume of specimens declined gently as ZnO contents increased from 0 to 15 wt%, conversely the lattice strain of composites increased gradually. The annealing process affected the crystal structure of CCAP–12 wt% ZnO sample and increased the crystallite size and crystallinity. FTIR spectra demonstrated some substitution of phosphate ions by carbonate ions (B type) in chlorapatite structure, which led to the shrinkage of lattice constants as obtained in XRD results. Microscopic observations showed a cluster-like structure of product containing ZnO micro-platelets which were accompanied by polygonal and spherical particles with an average particle size of around 50 ± 10 nm.

The most remarkable finding was a negative zeta potential of product in normal physiological saline at a pH of 7.4. A negative zeta potential of milled and annealed product is a promising way to facilitate bone cell activity and might prove to be useful as implant coatings and other biological applications.

Acknowledgement

This research was supported by the National Science Foundation (PREM center for interfaces, DMR-1205670) and the Robert A. Welch Foundation (AI-0045).

References

- [1] A.C. Tas, Synthesis of biomimetic Ca-hydroxyapatite powders at 37 °C in synthetic body fluids, *Biomaterials*, 21 (2000) 1429–1438.
- [2] F. Mohandes, M. Salavati-Niasari, M.H. Fathi, Z. Fereshteh, Hydroxyapatite nanocrystals: Simple preparation, characterization and formation mechanism, *Mater. Sci. and Eng. C* 45 (2014) 29–36.
- [3] B. Nasiri-Tabrizi, A. Fahami, R. Ebrahimi-Kahrizsangi, A. Khazraei, M.R. Yazdani, M.J. Kajibafzadeh, A study on mechanochemical behavior of $\text{CaO-P}_2\text{O}_5\text{-CaF}_2\text{-ZrO}_2$ system to produce fluorapatite–zirconia composite nanopowders, *Powder Tech.* 243 (2013) 59–70.
- [4] S.J. Kalita, A. Bhardwaj, H.A. Bhatt, Nanocrystalline calcium phosphate ceramics in biomedical engineering, *Mater. Sci. Eng. C* 27 (2007) 441–449.
- [5] D.P. Minh, N. Dung Tran, A. Nzihou, P. Sharrock, Novel one-step synthesis and characterization of bone-like carbonated apatite from calcium carbonate, calcium hydroxide and ortho phosphoric acid as economical starting materials, *Mater. Res. Bull.* 51 (2014) 236–243.
- [6] B. Nasiri-Tabrizi, A. Fahami, R. Ebrahimi-Kahrizsangi, Phase transitions and structural changes of nanostructured chlorapatite under thermal treatment, *Ceram. Int.* 40 (2013) 901–910.
- [7] S. Kannan, J.H.G. Rocha, J.M.F. Ferreira, Synthesis of hydroxy- chlorapatites solid solutions, *Mater. Lett.* 60 (2006) 864–868.
- [8] A.A. Baig, J.L. Fox, J. Su, Z. Wang, M. Otsuka, W.I. Higuchi and R.Z. Legeros, Effect of carbonate content and crystallinity on the metastable equilibrium solubility behavior of carbonate apatite, *J. Colloid Interf. Sci.* 179 (1996) 608–617.
- [9] W.L. Suchanek, P. Shuk, K. Byrappa, R.E. Riman, K.S. Tenhuisen, V.F. Janas, Mechanochemical-hydrothermal synthesis of carbonated apatite powders at room temperature, *Biomaterials* 23 (2002) 699–710.
- [10] S.A. Redey, M. Nardin, D.B. Assolant, C. Rey, P. De-lannoy, L. Sedel, P.J. Marie, Behavior of human osteoplastic cells on stoichiometric hydroxyapatite and type-A carbonated apatite, *J. Biomed. Mater. Res.* 50 (2000) 353–364.
- [11] I.D. Ana, S. Matsuya, K. Ishikawa, Engineering of carbonate apatite bone substitute based on composition-transformation of gypsum and calcium hydroxide, *Eng.* 2 (2010) 344–352.
- [12] A. Fahami, B. Nasiri-Tabrizi, Mechanochemical behavior of $\text{CaCO}_3\text{-P}_2\text{O}_5\text{-CaF}_2$ system to produce carbonated fluorapatite nanopowders, *Ceram. Int.* 40 (2014) 14939–14946.

- [13] A. Fahami, B. Nasiri-Tabrizi, G. W. Beall, B. Pingguan-Murphy, Effect of ion concentration on mechanosynthesis of carbonated chlorapatite nanopowders, *Mater. Lett.* 146 (2015) 16-19.
- [14] A. Fahami, R. Ebrahimi-Kahrizsangi, B. Nasiri-Tabrizi, Mechanochemical synthesis of hydroxyapatite/titanium nanocomposite, *Solid State Sci.* 13 (2011) 135–141.
- [15] S. Aryal, K. Matsunaga, W.Y. Ching, Ab initio simulation of elastic and mechanical Properties of Zn- and Mg-doped hydroxyapatite (HAP), *J. Mech. Behave. Biomed.* In Press, doi:10.1016/j.jmbbm.2015.03.018.
- [16] F. Velard, D. Laurent-Maquin, J. Braux, C. Guillaume, S. Bouthors, E. Jallot, J.M. Nedelec, A. Belaaouaj, P. Laquerriere, The effect of zinc on hydroxyapatite mediated activation of human polymorphonuclear neutrophils and bone implant-associated acute inflammation, *Biomaterials*, 31 (2010) 2001-2009.
- [17] K. Cheng, Z. Guan, W. Weng, H. Wang, J. Lin, P. Du, G. Han, Hydroxyapatite/ZnO-nanorod composite coatings with adjustable hydrophilicity and Zn release ability, *Thin Solid Films*, 544 (2013) 260–264.
- [18] Y. Huang, H. Zeng, X. Wang, D. Wang, Corrosion resistance and biocompatibility of SrHAp/ZnO composite implant coating on titanium, *Appl. Surf. Sci.* 290 (2014) 353– 358.
- [19] A. Doostmohammadi, A. Monshi, M.H. Fathi, O. Braissant, A comparative physico-chemical study of bioactive glass and bone-derived hydroxyapatite, *Ceram. Int.* 37 (2011) 1601-1607.
- [20] V. Uskoković, R. Odsinada, S. Djordjevi, S. Habelitz, Dynamic light scattering and zeta potential of colloidal mixtures of amelogenin and hydroxyapatite in calcium and phosphate rich ionic milieus, *Arch. Oral. Biol.* 56 (2011) 521-532.
- [21] A. Doostmohammadi, A. Monshi, R. Salehi, M.H. Fathi, S. Karbasi, U. Piesles, Preparation, chemistry and physical properties of bone-derived hydroxyapatite particles having a negative zeta potential. *Mater. Chem. Phy.* 132 (2012) 446-452.
- [22] N.C. Teng, S. Nakamura, Y. Takagi, Y. Yamashita, M. Ohgaki, K. Yamashita, A new approach to enhancement of bone formation by electrically polarized hydroxyapatite, *J. Dent. Res.* 80 (2001) 1925-1929.
- [23] G.K. Williamson, W.H. Hall, *Acta Metall.* X-ray line broadening from fided aluminium and wolfram, 1 (1953) 22–31.
- [24] F.B. Bagambisa, U. Joos, W. Schilli, Mechanisms and structure of the bond between bone and hydroxyapatite, *J. Biomed. Mater. Res.* 27 (1993) 1047–1055.
- [25] D.A. Oppermann, M.J. Crimp, D.M. Bement, In vitro stability predictions for the bone/hydroxyapatite composite system, *J. Biomed. Mater. Res.* 42 (1998) 412–416.
- [26] A. Fahami, G. W. Beall, B. Nasiri-Tabrizi, B. Pingguan-Murphy, Effect of high-energy ball milling on the formation and microstructural features of carbonated chlorapatite nanopowders, *Ceram. Int.* 41 (2015) 4750–4758.

- [27] I. Nikcevic , V. Jokanovic , M. Mitric, Z. Nedic, D. Makovec, and D. Uskokovic, Mechanochemical synthesis of nanostructured fluorapatite/fluorhydroxyapatite and carbonated fluorapatite/fluorhydroxyapatite, *J. Solid State Chem.* 177 (2004) 2565–2574.
- [28] C. C. Maiti, F. Freund, Incorporation of chlorine into hydroxy-apatite, *J. Inorg. Nucl. Chem.* 43 (1981) 2633-2637.
- [29] C. H. Yodera, J. D. Pasteris, K. A. Krola, V. L. Weidner, R. W. Schaeffer, Synthesis, structure, and solubility of carbonated barium chlor- and hydroxylapatites, *Polyhedron* 44 (2012) 143-149.
- [30] B. Nasiri-Tabrizi a,b, E. Zalnezhad, B. Pingguan-Murphy, W.J. Basirun, A.M.S. Hamouda d, S. Baradaran, Structural and morphological study of mechanochemically synthesized crystalline nanoneedles of Zr-doped carbonated chlorapatite, *Mater. Lett.* 149 (2015) 100-104.
- [31] M. Fleet, Carbonated hydroxyapatite: materials, synthesis, and applications, Pan Stanford Publishing (2015) ISBN: 978-981-4463-67-6.
- [32] E. Landi, G. Celotti, G. Logroscino, A. Tampieri, Carbonated hydroxyapatite as bone substitute, *J. Euro. Ceram. Soc.* 23 (2003) 2931-2937.
- [33] P. Balaz, Mechanochemistry in nanoscience and minerals engineering, 1st ed. Germany: Springer Berlin Heidelberg; 2008.
- [34] C. Suryanarayana, Mechanical alloying and milling, *Prog. Mater. Sci.* 46 (2001) 1–184.
- [35] A. Ruksudjarit, K. Pengpat, G. Rujijanagul, T. Tunkasiri, Synthesis and characterization of nanocrystalline hydroxyapatite from natural bovine bone, *Curr. Appl. Phys.* 8 (2007) 270–272.
- [36] K. Cheng, W. Weng, H. Wang, S. Zhang, In vitro behavior of osteoblast-like cells on fluoridated hydroxyapatite coatings, *Biomaterials* 26 (2005) 6288–6295.

List of Figures Caption

Fig. 1 XRD patterns of the powder mixture in the absence and presence of different amounts of ZnO nanopowders (0–20 wt%) after mechanical activation for 60 min.

Fig. 2 XRD pattern of CCAP–12 wt% ZnO sample heat treated at 800 °C for 1h.

Fig. 3 (a) crystallite size, and lattice strain, (b) the crystallinity degree, (c) the lattice constants, and (d) unit cell volume of CCAP as a function of ZnO contents (Heat: CCAP–12 wt% ZnO sample annealed at 800 °C for 1h)

Fig. 4 FT-IR spectra of CCAP–ZnO composite nanopowders as a function of ZnO contents (0–15 wt%)

Fig. 5 FE–SEM (a,b) and TEM images (c,d) of CCAP–12 wt% ZnO composite nanopowders

Fig. 6 (a) EDS analysis and (b) elemental mapping of CCAP–12 wt% ZnO composite nanopowders

Fig. 7 The zeta potential of milled (a) and annealed (b) CCAP–12 wt% ZnO composite nanopowders as a function of pH

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7