Co-Sr doped carbonated hydroxyapatite: A biomaterial with enhanced mechanical and bioactivity properties

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Abstract: The present study is aimed at investigating the role of two biologically important cations, cobalt (Co²⁺) and strontium (Sr²⁺) doped into carbonated hydroxyapatite (CHA) crystal structure. Four different compositions of Co-Sr CHA powders were synthesized via nanoemulsion method at ambient temperature. Among the as-synthesized powders, Co-Sr CHA 1 (0.0082wt% Co, 0.0547wt% Sr) and Co-Sr CHA 2 (0.0085wt% Co, 0.0261wt% Sr) were chosen as the optimum compositions and used for fabrication of dense products. Sintering was then performed on the Co-Sr CHA compacted samples at 900°C in air, followed by cooled down in dry CO₂ atmosphere at temperature of 200°C. Regardless of their compositions, the sintered samples remained as single phase B-type CHA. Co-Sr CHA 1 has the optimum mechanical properties where the relative density and diametral tensile strength (DTS) values obtained were 89.15% and 7.67 MPa, respectively.

In vitro bioactivity test revealed that the formation of apatite layer were detected on the surface of all sintered samples after soaking in SBF solution for 7 days, with Co-Sr CHA 1 showing the fastest apatite formation as compared to CHA and Co-Sr CHA 2. Results obtained indicate that simultaneous doping of Co²⁺ and Sr²⁺ into CHA structure gives rise to improved mechanical strength as well as enhanced the bioactivity of the apatite.

1. Introduction

Calcium phosphate-based materials are widely used for biomedical applications as bone implants to substitute damaged or ailing bones1,2. Hydroxyapatite (HA) with a chemical formula of Ca₁₀(PO₄)₆(OH)₂ is one of illustration that has received wide attention among the researchers in the previous decades. This is due to its affinity to bone minerals because of their ability as biocompatibility, bioactivity and osteoconductivity properties1,3. However, HA has few drawbacks that contrasted to the biogenic apatite, particularly, its resorption rate in vivo which has been reported to be too sluggish in inducing a massive new bone formation1. In term of mechanical properties, HA ceramic is known to be less suitable to be used in a load-bearing application. Some studies found that dense HA ceramic has potential to fail by fatigue after a period of clinical utility4,5.

Biological and mechanical properties of HA can be enhanced by introducing new elements in their structure that are generally present in natural apatites of bone. Carbonated hydroxyapatite (CHA) has been reported to have a much closer compositional similarity to the mineral in natural bone compared to stoichiometric HA6,7. The amount of carbonate ions present in human bone is in the range of 4-8 wt% depending on the age of the individual1,7. The introduction of carbonate ions into HA structure was found
to increase the dissolution rate in solution and enhance its osteointegration rate\textsuperscript{5,9}. Hence, CHA is seem as better choice of material relative to HA due to its enhanced properties biocompatibility, bioactivity, and resorbability\textsuperscript{9,10}. Over the past couple of decades, many researchers have demonstrated that variety of ionic substitutions can be incorporated into synthetic HA. This approach is seem as one of the effective ways to improve the properties of HA in order to produce a mineral composition closely mimic to the natural bone tissue\textsuperscript{11}. Interestingly, the high flexibility, and stability of the apatite crystal structure allow the wide selection for replacement of cations (mainly Ca\textsuperscript{2+}) and anions (OH\textsuperscript{-} and (PO\textsubscript{4})\textsuperscript{3-}) according to charge type, charge size and ionic radii\textsuperscript{12,13}. The introduction of trace metal ions such as Ag\textsuperscript{+}, Na\textsuperscript{+}, Cu\textsuperscript{2+}, Mg\textsuperscript{2+}, Mn\textsuperscript{2+}, Zn\textsuperscript{2+}, Sr\textsuperscript{2+} and Co\textsuperscript{2+} into HA crystal structure can affect the surface charge, lattice parameters, crystallinity and morphology, which resulting in changes in thermal stability, mechanical properties, solubility and bioactivity of HA\textsuperscript{2,14}. For example, the substitution of Co\textsuperscript{2+} was reported to improve the angiogenesis properties of HA\textsuperscript{15,16}. The same goes for Sr\textsuperscript{2+} into HA structure which has been reported enhance the solubility and stimulated faster bone formation. Besides, the present of Sr ions also encouraged better cell attachment and proliferation\textsuperscript{12,17}.

The main aim of this work is to investigate the influences of the ionic substitutions by the incorporation of cobalt (Co\textsuperscript{2+}) and strontium (Sr\textsuperscript{2+}) ions simultaneously into the carbonated hydroxyapatite (CHA) structure. Several characterization techniques were used to analyze the as-synthesized powders and sintered multi-doped CHA such as X-ray Diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Field Emission Scanning Electron Microscopy (FESEM) and Energy dispersive X-ray analysis (EDX). Besides that, Diametral Tensile Strength (DTS) was used to determine the compressive strength of the sintered multi-doped CHA. For bioactivity testing, the sintered multi-doped CHA pellets were immersed in Simulated Body Fluid (SBF) for 7 days followed by surface analysis using FESEM to detect the formation of apatite layer.

2. Experimental
2.1 Preparation of Powders

Co-Sr doped Carbonated Hydroxyapatite (Co-Sr CHA) powders were chemically synthesized via nanoemulsion method according to the previous studies reported in the literature\textsuperscript{1,18}. The chemicals used were ammonium bicarbonate ((NH\textsubscript{4})HCO\textsubscript{3}), di-ammonium hydrogen phosphate ((NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4}), calcium nitrate tetrahydrate (Ca(NO\textsubscript{3})\textsubscript{2}.4H\textsubscript{2}O), sodium hydroxide (NaOH), strontium nitrate (Sr(NO\textsubscript{3})\textsubscript{2}) and cobalt nitrate hexahydrate (Co(NO\textsubscript{3})\textsubscript{2}.6H\textsubscript{2}O. Initially, aqueous solutions were prepared by dissolving 7.942g of ((NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4}) and 3.162g of ((NH\textsubscript{4})HCO\textsubscript{3}) in 60 ml of deionized water separately. For the doping precursor, 0.14g of Co(NO\textsubscript{3})\textsubscript{2}.6H\textsubscript{2}O was dissolved in deionized water until achieve the desired concentration (7.41×10\textsuperscript{-7}g) and directly poured into carbonate-phosphate mixture (solution 1). 0.012g Sr(NO\textsubscript{3})\textsubscript{2} was weighed and directly added to solution 1 and continuously stirred until well dissolved (solution 2). Meanwhile, a solution 3 was prepared by dissolving 23.409g of Ca(NO\textsubscript{3})\textsubscript{2}.4H\textsubscript{2}O in 100 ml of acetone, which was then poured into solution 2 to create emulsion. In this work, CHA powders was used as a standard reference for Co-Sr doped CHA powders. The amount of raw materials used in the synthesis is shown in Table 1.

| Samples            | Ca\textsuperscript{2+} | PO\textsubscript{4}\textsuperscript{3-} | CO\textsubscript{3}\textsuperscript{2-} | Co\textsuperscript{2+} | Sr\textsuperscript{2+} |
|--------------------|-------------------------|--------------------------------------|-------------------------------------|-------------------------|------------------------|
| CHA                | 23.409                  | 7.942                                | 3.162                               | -                       | -                      |
| Co-Sr CHA 1        | 23.409                  | 7.942                                | 3.162                               | 7.41×10\textsuperscript{-7} | 0.012                  |
| Co-Sr CHA 2        | 23.409                  | 7.942                                | 3.162                               | 7.41×10\textsuperscript{-7} | 0.006                  |
2.2 Fabrication of Dense Co-Sr CHA

0.5 g of Co-Sr doped CHA powders were pelletized using uniaxial hydraulic hand press with 50 MPa to produce compact green body. Sintering was then performed on the compacted powders at 900°C followed by cooled down under a wet CO2 atmosphere at temperature of 200°C.

2.3 Characterizations Techniques

2.3.1. X-ray diffraction. X-ray diffraction (XRD) was carried out using a Bruker D8 XRD. The diffractometer was operated at 40 kV and 110 mA at 2θ range from 0’ to 90’ with step size of 0.02° using the Cu Kα radiation (λ = 1.542 Å), operating with PANalytical X’pert PRO diffractometer. Stoichiometric HA with ICDD file number of 09-0432 was used as the reference pattern.

2.3.2. Fourier Transform Infrared Spectroscopy. Fourier transform infrared spectroscopy (FTIR) was performed to determine the chemical constituent presence in the as-synthesized powders and dense multi-doped CHA produced. Samples were ground and mixed with potassium bromide (KBr) and pelletized using hand press at a pressure of 5 MPa and hold for 2 minutes. The wave range was set up to be in the range of 4000 to 400 cm⁻¹. The samples was scanned for four times in transmittance mode.

2.3.3. Field Emission Scanning Electron Microscopy. The morphology of the as-synthesized powders and sintered samples before and after bioactivity study were observed using Zeiss Supra 55VP Field Emission Scanning Electron Microscope (FESEM). The as-synthesized Co-Sr CHA powders and sintered samples were initially coated with gold before observed under FESEM. Besides that, Energy Dispersive X-Ray (EDX) was used to analyze the elemental of each sample. This technique detects x-rays emitted from the sample during the bombardment by an electron beam to characterize the elemental composition of the analyzed sample.

2.3.4. Density and Porosity Measurement. Bulk density and porosity of the sintered multi-doped CHA pellets were determined using Standard Archimedes method in ASTM C830-0010. The dry weight of the samples (D) were measured using microbalance followed by submerged in deionized water by placing them in the desiccator in order to vacuum the samples for 120 minutes to allow water filled the open pores of the samples. Then, the suspended mass (S) and saturated mass (W) of the samples were weighed using Sartorius Balance and Bulk Density Apparatus.

2.3.5. Diametral Tensile Strength. Diametral Tensile Strength (DTS) was performed using INSTRON 3367 universal tensile machine in order to determine the tensile strength of the sintered samples. Prior to testing, the diameter and thickness of each sintered sample was measured. The sintered sample was then placed vertically between the two stainless steel plates. Compression load was then applied at the rate of 0.5 mm/min until the sample was broken.

2.3.6. Evaluation of Bioactivity. The bioactivity characteristic of dense multi-doped CHA samples was evaluated by observing the ability of the apatite to form on its surface when soaked in a simulated body fluid (SBF) for a certain period of time. Prior to bioactivity test, SBF solution was prepared according to the recipe proposed by Kokubo & Takadama (2006)¹⁹,²⁰. After soaking for 7 days, the samples were gently taken out from the SBF solution, washed carefully using deionized water and finally observed under FESEM. The formation of apatite layer on the sample was confirmed by performing EDX to detect the element present on the surface.

3. Results and discussion

3.1 Physico-chemical Properties

XRD results of the sintered samples of CHA, Co-Sr CHA 1 and Co-Sr CHA 2 is shown in Figure 1. Regardless of the compositions, all sintered samples produced corresponds to typical HA pattern (ICSD 09-0432). The main peaks were detected at 31°, 32°, 33° and 34° in 2θ which represents (211), (112), (300) and (202) respectively. Other planes such as (002), (130), (222), (213) and (004) matched with HA pattern were also found. It is widely accepted that substitution of doping elements into apatite structure causes a change of lattice parameters and induces the contraction in a-axis and expansion in c-axis. This
is due to the large radii atom of Sr\textsuperscript{2+} substitution for the small radii atom of Ca\textsuperscript{2+} while replacement of large radii atom Ca\textsuperscript{2+} by small radii atom of Co\textsuperscript{2+} where the atomic radius of: Sr\textsuperscript{2+} = 1.13 Å, Co\textsuperscript{2+} = 0.7 Å and Ca\textsuperscript{2+} = 1.00 Å\textsuperscript{17,21}. However, due to small amount of Co\textsuperscript{2+} and Sr\textsuperscript{2+} ions substituted in this study, no significant change in the crystallographic apatite structure was observed\textsuperscript{21}.

The obtained FTIR spectra (Figure 2) showed that all sintered samples exhibited the characteristic bands of carbonate groups (CO\textsubscript{3}) of the apatite structure at 1461.30 and 1419.22 cm\textsuperscript{-1} which corresponds to B-type CHA\textsuperscript{22}. The main characteristic bands of PO\textsubscript{4}\textsuperscript{3-} groups (ν\textsubscript{4}) vibrations of sintered Co-Sr CHA detected at 568.31 cm\textsuperscript{-1} and 601.04 cm\textsuperscript{-1}. While ν\textsubscript{1}, ν\textsubscript{2} and ν\textsubscript{3} detected at 474.81, 961.04 and 1040.52 cm\textsuperscript{-1}, respectively of of PO\textsubscript{4}\textsuperscript{3-} groups. The bands about 3443.55 cm\textsuperscript{-1} and 1638.71 cm\textsuperscript{-1} correspond to absorbed water. The band at 3571.77 cm\textsuperscript{-1} were assigned to stretching vibration of OH\textsuperscript{-} groups of the apatite structure\textsuperscript{3,9}.

### 3.2 Mechanical Study of the Sintered Samples

The mechanical properties of sintered multi-doped CHA were evaluated in terms of relative density and diametral tensile strength (DTS). It is observed that the relative densities of Co-Sr CHA 1 and Co-Sr CHA 2 achieved higher if compared to the sintered CHA which is 89.15% and 72.14%, respectively. For DTS, Co-Sr CHA 1 was showed the highest value between other two sintered samples which are 7.67 MPa while Co-Sr CHA 2 and CHA about 5.13 MPa and 1.95 MPa. These results reveal a fact that incorporating Sr\textsuperscript{2+} ions into the apatite structure helps to improve the mechanical properties especially in terms of density relative and compressive strength relative to pure CHA\textsuperscript{23,24}. The present of Co\textsuperscript{2+} ions in the HA structure was reported to have potential effect on enhancing the biological performances to a great extent particularly in inducing angiogenesis properties of the apatite structure\textsuperscript{25}. However, these ions does not showing significant impact on the mechanical properties of Co-Sr CHA as in agreement with the literature. Besides that, the presence of CO\textsubscript{2} atmosphere during cooling stage not only had assisted in compensating the carbonate loss from the sintered samples during sintering but also aids in densification (Table 2)\textsuperscript{10}.

Table 2: Comparison for relative density and diametral tensile strength of the sintered Co-Sr CHA

| Samples      | Relative density (%) | Diametral tensile strength (MPa) |
|--------------|----------------------|----------------------------------|
| CHA          | 56.80                | 1.95                             |
| Co-Sr CHA 1  | 89.15                | 7.67                             |
| Co-Sr CHA 2  | 72.14                | 5.13                             |
3.3 Biological Evaluation of the Sintered Samples

Bioactivity of the sintered samples evaluated in vitro by immersed in SBF solution for 7 days. The formation of apatite layer on the surface of Co-Sr CHA 1 sample was fastest as compared to CHA and Co-Sr CHA 2 sintered samples (Figure 3). The present of layer apatite on the surface of the sample was confirmed by the detection of calcium and phosphate ions. The EDX analysis was performed to show the presence of Ca and P by carried out the Ca/P ratio. The values of Ca/P ratio for CHA, Co-Sr CHA 1 and Co-Sr CHA 2 are 1.93, 1.87 and 1.89, respectively. This finding prove that besides improved the mechanical properties, the presence of Sr and Co into CHA structure also enhanced the biological activity by encouraging the apatite layer formation.

![Figure 3: Surface morphologies of the sintered samples after immersed in SBF solution. (a) CHA, (b) Co-Sr CHA 1 and (c) Co-Sr CHA 2 (dotted line: formation of thin apatite layer in granular-like form on the surface of the sample)](image)

4. Conclusion

The new elements such as of strontium (Sr) and cobalt (Co) as ion substitutions into the apatite structure play an important role to enhance the physical, chemical, mechanical, and biological properties of HA. In terms of mechanical properties, the amount of ion substitutions that incorporated into HA structure were influenced the mechanical properties of final product. As indicated by biological study results, the amount of ion substitutions also influenced biological properties such as more soluble and bioactive material if compared to stoichiometric HA due to the ability to encourage the apatite formation. At this stage, it can be concluded that Co-Sr CHA 1 has the best properties as compared Co-Sr CHA 2 and CHA alone for both bioactivity and mechanical properties in order to create a product which is closer to the cancellous bone.

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