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Synthesis and characterisation of nanophase hydroxyapatite co-substituted with strontium and zinc

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ABSTRACT

In order to develop new bioactive calcium phosphate (CaP) materials to repair bone defects, it is important to ensure these materials more closely mimic the non-stoichiometric nature of biological hydroxyapatite (HA). Typically, biological HA combines various CaP phases with different impurity ions, which substitute within the HA lattice, including strontium (Sr^{2+}), zinc (Zn^{2+}), magnesium (Mg^{2+}), carbonate (CO_3^{2-}) and fluoride (F^-), but to name a few. In addition to this biological HA have dimensions in the nanometre (nm) range, usually 60 nm in length by 5–20 nm wide. Both the effects of ion substitution and the nano-size crystals are seen as important factors for enhancing their potential biofunctionality. The driving hypothesis was to successfully synthesise nanoscale hydroxyapatite (nHA), co-substituted with strontium (Sr^{2+}) and zinc (Zn^{2+}) ions in varying concentrations using an aqueous precipitation method and to understand their chemical and physical properties. The materials were characterised using Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), X-Ray Photoelectron Spectroscopy (XPS) and Transmission Electron Microscopy (TEM) techniques. The FTIR, XRD and XPS results confirmed that the nHA was successfully co-substituted with Sr^{2+} and Zn^{2+}, replacing Ca^{2+} within the nHA lattice at varying concentrations. The FTIR results confirmed that all of the samples were carbonated, with a significant loss of hydroxylation as a consequence of the incorporation of Sr^{2+} and Zn^{2+} into the nHA lattice. The TEM results showed that each sample produced was nano-sized, with the Sr/Zn-10% nHA having the smallest sized crystals approximately 17.6 ± 3.3 nm long and 10.2 ± 1.4 nm wide. None of the materials synthesised here in this study contained any other impurity CaP phases. Therefore, this study has shown that co-substituted nHA can be prepared, and that the degree of substitution (and the substituting ion) can have a profound effect on the attendant materials' properties.

1. Introduction

Synthetic hydroxyapatite (HA) [$Ca_{10}(PO_4)_6(OH)_2$] has been used extensively as a bone augmentation biomaterial, producing scaffolds for bone tissue engineering and for coating implants to promote bone healing and more effectively enhancing osseointegration [1,2]. It is well understood that nanoscale hydroxyapatite (nHA) with an average particle size of less than 20 nm can optimise the biofunctionality and bioactivity of HA, by increasing osteoblast cell proliferation [2].

Typically, biological apatite is non-stoichiometric and incorporates various impurity ions within the HA crystal lattice, Table 1 summarises the numerous substitutions which can occur, of those listed strontium and zinc have been shown to add significant enhancements to the properties of the HA individually. However, it has been suggested that co-substitutes of strontium and zinc could deliver a range of significant benefits over single ion substitutes [3,4]. Strontium and zinc co-substituted within the nHA lattice can optimise osteoconductivity by assisting new bone growth and improve osteoinductivity, by encouraging the differentiation of mesenchymal stem cells to osteoblasts, which could work effectively to prevent osteoporosis [5–7]. Both ions have also been found to produce an antimicrobial response and zinc in particular may inhibit the growth of *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* [8,9]. Therefore, both ions co-substituted together may also provide an effective antimicrobial treatment to combat conditions such as osteomyelitis [8].

The driving hypothesis of this work was to successfully synthesise nHA in a stoichiometric form, along with nHA co-substituted with Sr^{2+} and Zn^{2+} ions at a range of different weight percentage(wt%) concentrations, Sr/Zn-2.5%nHA, Sr/Zn-5%nHA and Sr/Zn-10%nHA. From previous research, one would expect the crystallinity, particle morphology and phase purity to vary as the level of ionic co-substitution was increased from 2.5% to 5–10%Sr/Zn [10,11]. Each sample was

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Table 1
Examples of various ions which can substitute within the HA lattice.

Substituted ion	Position of substitution	Ionic radius (Å) Ca (0.99Å)	Biological function and effect	Ref.
Strontium (Sr^{2+})	Ca^{2+} Ca(I) Low Concentrations Ca(II) High Concentrations	1.12	High concentration where new bone is forming. Boosts osteoblast (OB) cell proliferation. Reduces osteoclast (OC) activity and therefore rate of bone resorption. Encourages an antimicrobial effect	[5,6,8,9,32–34].
Zinc (Zn^{2+})	Ca^{2+} Interstitial Insertion at Low Concentrations Ca(II) High Concentrations	0.74	Inhibits OC resorption of bone. Encourages OB activity stimulating new bone formation. Strong antimicrobial effect. Anti-inflammatory effects	[6–8,30,31,35–39].
Magnesium (Mg^{2+})	Ca^{2+} Ca(II)	0.72	Assists calcium ions to promote bone strength and health. Stimulates OB adhesion, proliferation and differentiation. Promotes osseointegration and bone ingrowth. Reduced β-TCP crystallinity, enhancing bone resorption	[8,40–42].
Silver (Ag^{+})	Ca^{2+} Ca(I)	1.26	Exhibits an antibacterial effect on *Staphylococcus aureus*, *E-coli*, *Pseudomonas aeruginosa*, *Candida albicans* and gram negative bacilli, with relatively low cytotoxicity. A minimum inhibitory concentration (MIC) of silver must be reached and maintained to inhibit later stage bacterial colonisation	[8,43].
Lithium (Li^{+})	Ca^{2+}	0.76	Causes a reduction in bone remodelling, reducing bone turnover rates with lower amounts of bone loss. Stimulates the canonical Wnt signalling pathway, activating a major osteogenic signalling cascade, allowing MSC's to differentiate to OB's. Suppresses OC formation	[34,44–47].
Copper (Cu^{2+})	Ca^{2+}	0.73	Antimicrobial effect against *E. coli* and *Candida albicans*.	[48,49]
Silicon (SiO_4^{4-})	PO_4^{3-} Substitutes for phosphate group at the 6 h position	5.86	Increases solubility and dissolution. Optimises cellular proliferation, neovascularisation and formation of mineral nodules and bone apposition. Encourages OB differentiation and collagen type-1 production in MG63 cells. Osteoconductive silicon calcium phosphate scaffolds produce proliferation and differentiation of HBMSCs to matrix producing OBs, without any external osteogenic factors	[8,30,50–52].
Carbonate (CO_3^{2-})	CO_3^{2-} A type substitutes for hydroxyl ions B type substitutes for phosphate ions (A/B ratio = 0.7–0.9)	4.35	Crystal morphology evolves as level of carbonate substitution increases from plate-like to needle-like to spherical. Reduced grain size and hardness value 200% larger than HA values, but not suitable for major load bearing applications. Fast dissolution times, in vivo and in vitro due to carbonate weakening the bonds within the HA lattice. Bioactive and osseointegrative, forming a bone-like surface apatite layer in 7 days compared to 24–28 days for stoichiometric HA	[8,16,53].
Fluoride (F^{-})	OH^{-} Substitutes for hydroxyl groups	1.33	Reduces dental caries, dissolving in acidic environment of mouth after consumption of sugars and starches. Exhibits an antimicrobial effect. Boosts bone growth and formation, successfully treating osteoporosis.	[8,54]