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Development of Sr and CO_3 co-substituted hydroxyapatites for biomedical applications

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Abstract

Sr and CO_3 co-substituted hydroxyapatite (SrCHA) nanopowder was synthesized by neutralization. The powder was characterized. The improved solubility in Hanks' balanced solution of SrCHA granules (400–600 μm of dimensional range), potentially usable as bone filler, was assessed and compared with that of an analogous carbonate free granulate. SrCHA porous bodies with interconnected micro- and macro-porosity, which mimic the morphology of spongy bone, were prepared by the impregnation of cellulose sponges with suspensions of the SrCHA powder and controlled sintering. SrCHA porous scaffolds sintered at 850 °C, in flowing CO_2 atmosphere, showed satisfying compressive strength (4.58 ± 0.75 MPa) for a porosity value of 45 vol.% and retained the desired ionic substitutions (Sr/Ca = 0.11 and CO_3 = 6.8 wt.%). The possibility of widely modulating, by acting on the chemical–physical–geometrical features of the material, the prolonged in situ release of therapeutic Sr, together with the fundamental (Ca, PO_4) and main substituting (CO_3) ions that constitute the bone mineral phase, makes the use of SrCHA as resorbable bone filler or bone substitute scaffolds promising, especially when pathologies related with Sr deficiency are present. In vitro and in vivo tests are in progress.

Keywords: Strontium carbonate apatite; Porous scaffold; Solubility test; Mechanical properties; Biomedical applications

1. Introduction

Synthetic apatites represent, in general, elective bone substitutes since they resemble the inorganic phase constituting bone, i.e. nanocrystalline non-stoichiometric hydroxyapatite (HA). Among the hetero-ions substituting in biological HA, CO_3^{2-} is the most abundant (2–8 wt.% [1]), and partially substitutes both in the PO_4^{3-} site (B-type CHA) and the OH^- site (A-type CHA) of the HA structure, with a preference for the former. The high reactivity of young bone could be related to the greater presence of B-CO_3 compared with in old bone. B-type carbonated hydroxyapatites showed improved solubility, collagen deposition in vitro and reabsorption in vivo, compared with stoichiometric HA and/or to A-type CHA [2–4].

The development of innovative biomaterials for bone substitution and regeneration must consider that the final users are mainly elderly patients, who either have lower osteogenesis or are affected by osteoporosis [5–7]. The presence of bone rarefaction and biological "drawback" due to osteoporosis negatively influence the physiological healing processes of a fracture and the osteointegration of bone-implanted orthopaedic prostheses [8].

In vitro and in vivo studies have indicated that orally administered strontium (as strontium chloride, ranelate, lactate) increases bone formation, the number of bone-forming sites and bone mineral density, and reduces bone resorption, leading to a gain in bone mass and improved bone mechanical properties in animals and humans [9,10]. Accordingly, it could be hypothesized that one feature of osteoporosis is a certain degree of Sr deficiency, but data on bone Sr in normal humans are scarce [11]. While low doses of Sr have been shown to stimulate bone formation, high doses have deleterious effects on bone

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mineralization, through reduction in calcium absorption and possibly alterations of the mineral properties [12].

Sr is chemically and physically closely related to calcium, thus it is a natural bone-seeking trace element that accumulates in the skeleton. The incorporation of strontium into bone after oral strontium treatment depends on the skeletal site (diaphysis of the femur < lumbar vertebra < iliac crest) and on bone turnover (thus it is higher in cancellous than in cortical bone, and in young than in old bone) [9,10].

Stimulatory effects on bone collagen synthesis in cell cultures have been specifically correlated to Sr administration, since neither calcium nor sodium salts were effective [10].

Unfortunately, even at high doses of oral strontium (3 mmol Sr day^{-1}, 13 weeks) less than 1 Ca^{2+} ion in 10 can be substituted by Sr^{2+} in the newly formed bone mineral [10]; thus it is difficult to improve bone characteristics following only an oral administration of Sr.

A positive influence of Sr in preventing dental caries has also been proposed, considering the significant differences in strontium content of human enamel: 104.1 and 184.0 μg g^{-1}, respectively, for high and low caries populations [13].

Sr-substituted apatites powders have been prepared by ion exchange of Sr^{2+} for Ca^{2+} using a biomimetic approach [14], as well as by chemical synthesis [15,16]. Differences in interfacial bondings of Sr-containing HA with cancellous and cortical bone have been recently reported for a rabbit hip replacement model, pointing out the good affinity of an apatite cement with both types of bone, and remarkable osteointegration with the cancellous bone [17,18].

The final aim of this work is the preparation of porous bone substitutes, for cancellous bone replacement, or as fillers in orthopaedic or dental fields, that release Sr^{2+} ions in situ over long periods of time, to enable the Sr to influence osteointegration, the regeneration of new bone and its properties. The release in situ of Sr^{2+} ions directly from the synthetic bone substitute during its resorption could overcome the problems and limitations related to the incorporation of Sr in bone after oral administration (dose, duration of treatment and skeletal site, limited substitution of calcium in the apatite, etc.). The direct synthesis of Sr-substituted CHA having Sr amounts close to the values present in young animals (12 mol.% of the Ca^{2+} [14]), and thus higher than those present in old animals (2 mol.% of Ca^{2+} [14]) or achievable in biological bone through the Sr oral administration (0.5–3 mol.% [9]), has been successfully attempted. Co-substituting B-carbonate should improve the biological response of the SrHA, exploiting the enhanced bioactivity of carbonated apatite.

2. Materials and methods

The classical neutralization synthesis of HA based on $Ca(OH)_2$ and H_3PO_4 was performed, including the additions of strontium nitrate (as in Ref. [19]) and sodium hydrogen carbonate as sources of substituting ions. A water suspension containing 100 g of $Ca(OH)_2$ (Aldrich 95% pure) in 600 ml of deionized water was prepared, stirred and heated at 37 °C. Then 54.25 g of $Sr(NO_3)_2$ (Riedel de Haen, 99.9% pure) was dissolved in 400 ml of deionized water and the resulting solution was added to the suspension. A solution containing 88.8 g of H_3PO_4 (Aldrich 85 wt.% pure) in 600 ml of deionized water was prepared and dropped (1–2 drops s^{-1}) together with 400 ml of $NaHCO_3$ (Merck, A.C.S., ISO) solution into the basic Sr^{2+}-added $Ca(OH)_2$ suspension over a period of 3–4 h, with constant temperature and with continuous stirring. The starting Sr/Ca and CO_3/PO_4 molar ratios were 1/5 and 1/6, respectively. The synthesis process is ''self-controlled at high pH'', thus no additions of other chemicals (e.g. ammonia) are needed to maintain the conditions needed for the precipitation of the apatite phase (pH > 11) instead of other calcium phosphates. The suspension of the reaction product was maintained under stirring and at a constant temperature of 37 °C for 2 h, then left to cool to room temperature for 24 h without stirring. The precipitate was separated from the mother liquor by centrifugation, then washed and centrifuged three times. After that, the precipitate was freeze-dried and finally sieved at 150 μm.

The specific surface area of the powders was evaluated by the Brunauer–Emmett–Teller method (Sorpty 1750, Carlo Erba, Milano, Italy). Sample (3 g) outgassing was performed at 100 °C for 24 h before the analysis.

For the agglomerate size distribution measurement, the powders were analysed by sedimentography (Sedigraph 5100, Micromeritics, Norcross, GA) after ultrasonic dispersion of 4 g of material in deionized water for 10 min using Calgon as the dispersant.

Chemical analysis was performed by inductively coupled plasma-optical emission spectrometry (ICP-OES, Liberty 200, Varian, Clayton South, Australia): 20 mg of powder was dissolved in 2 ml of HNO_3 and the solution volume was increased up to 100 ml with deionized water.

Fourier transformed infrared (FT-IR) spectroscopy (Thermo Nicolet-Avatar 320 FT-IR) was also performed to determine the HA stoichiometry deviations, in particular of carbonate ions partially substituting respectively for PO_4^{3-} and/or OH^- groups. A pelleted specimen was prepared by cold pressing a mixture of the powder with KBr using a weight ratio of 1:100; the air spectrum, detected immediately before the specimen analysis, was automatically subtracted from the specimen spectrum.

Simultaneous (thermogravimetric and thermodifferential) thermal analysis (Netzsch Geratebau STA 409) was used to explore the thermal transformation process and the thermal stability of the powder, and to estimate the carbonate content of the apatite. This analysis was performed on specimen of about 20 mg and using a heating rate of 10 °C min^{-1} up to 1400 °C in air or flowing CO_2.

The carbonate amount of the powders was also evaluated with a C elemental analyser (LECO C/S, Leco Corporation, St. Joseph, Michigan, USA).

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