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Wet chemical synthesis of strontium-substituted hydroxyapatite and its influence on the mechanical and biological properties

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Abstract

Strontium is used orally in the medical treatment of osteoporosis because of its stimulative effect on bone formation and, simultaneously, its inhibiting effect on bone resorbing cells. Due to these effects, it might also be used for calcium phosphate-based bone substitutes. We hypothesise that strontium-substituted hydroxyapatite can be synthesised using a wet chemical reaction and a subsequent thermal treatment. Up to 15 wt% of strontium was successfully incorporated into the lattice of hydroxyapatite without affecting the apatite structure. Higher concentrations led to a destabilisation of the structure and the formation of β-tricalcium phosphate as a secondary phase. The bending strength of the composite materials was up to 30 MPa, independent of the strontium content. Initial cell culture experiments proved the cytocompatible behaviour of the material. We therefore conclude that strontium-substituted hydroxyapatite can be synthesised with suitable biological and mechanical properties as potential bone substitute material for osteoporotic patients.

Keywords: A: Powders: chemical preparation; D: Apatite; E: Biomedical applications; Strontium

1. Introduction

As a result of physical strain, natural bone tissue is continually reforming. An imbalance between bone building and resorption results in dysfunctions in the bone structure. One of the most prevalent diseases related to these bone remodelling activities is osteoporosis. In the case of osteoporotic bone tissue, bone resorption predominates and bone density decreases over time. Osteoporosis is treated with strontium ranelate in the form of oral drugs in order to oppose this unbalanced process.

At the beginning of the 20th century Lehnerdt identified strontium as having a positive impact on bone building [1]. His investigations revealed that the addition of strontium to nutrition resulted in an increased activation of bone-forming cells (osteoblasts) and thereby a strong formation of new bone tissue [1]. Further studies demonstrated that an inhibition of bone resorbing cells (osteoclasts) occurred by means of strontium-inoculated culture of bone cells with increasing concentrations of strontium [2]. This effect is the result of strontium's ability to activate the Wnt/β-catenin signalling pathway [3], which stimulates osteogenesis, the self-regeneration of stem cells and their differentiation into osteoblasts [4]. This effect is used for the treatment of patients with osteoporosis [3].

Synthetically derived hydroxyapatite is an important bone substitute material because this calcium phosphate is the main component of the mineral phase of sound natural bone. Its bioactive properties stimulate the ongrowth of the surrounding hard tissue onto the surface of an implant. It is possible in principle to substitute calcium with strontium within the hydroxyapatite lattice. The synthesis can be carried out by precipitation titration and a subsequent heat treatment whereby a very pure hydroxyapatite phase can be synthesised [5]. One method for the synthesis is the addition of a solution of diammonium hydrogen phosphate to a suspension of calcium nitrate, in which strontium nitrate can be supplemented to synthesise strontium-containing

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hydroxyapatite [6]. Another method to synthesise hydroxyapatite is the titration of calcium oxide and phosphoric acid [7]. However, using a precipitation titration method results in very small amounts of synthesised material and requires a lot of time. Therefore, thermal reaction offers an alternative method for the synthesis of strontium-containing hydroxyapatite, for example by sintering tertiary strontium phosphate and strontium carbonate in water vapour [8].

Earlier studies exhibited that the incorporation of 15 mol% strontium for calcium ions into the lattice of hydroxyapatite caused deformation of the elementary cells and therefore a decrease in crystallinity [9]. Kim et al. achieved comparable results and found an increased formation of β-tricalcium phosphate phase with an increasing content of added strontium [6]. The latest research into the dissolving behaviour revealed that the release of strontium-containing calcium phosphate is higher with the higher strontium content, if the composition is created by a reaction between strontium carbonate and a calcium phosphate cement (precursor powder) [10]. The release is constant if strontium is incorporated into the structure by exchanging strontium carbonate for calcium carbonate during the synthesis of the precursor preparation [10].

We proposed to synthesise pure and strontium-substituted hydroxyapatite by means of a wet chemical reaction between calcium carbonate, strontium carbonate and phosphoric acid, and a subsequent heating process. The advantage of this method is less effort and a greater producible quantity in comparison to conventional methods using precipitation titration. The latter has to be carried out quite slowly to achieve pure phases. As a result, it requires a lot of time to synthesise an adequate amount of material. In contrast to other methods, the pH value receives no consideration in the process we developed. We hypothesise that strontium-substituted hydroxyapatite can be synthesised using a two-step manufacturing process. Due to its biological and mechanical properties it can be suitable as a bone substitute material for the biomedical application.

2. Materials and methods

2.1. Synthesis of pure hydroxyapatite

Hydroxyapatite was synthesised using a wet chemical reaction between calcium carbonate and phosphoric acid solution (Eq. 1).

$$5CaCO_3 + 3H_3PO_4 \rightarrow Ca_5OH(PO_4)_3 + 5\ CO_2 + 4H_2O \quad (1)$$

After mixing 500 g distilled water and calcium carbonate (calcium carbonate p.a., AppliChem, Darmstadt, Germany) in a ratio of 5 is to 3, the suspension was mixed with 700 g 20 mm-ZrO_2 grinding spheres (20 mm, Tosoh, Japan) and kept on a rolling bench for 30 min. Afterwards, a 40 wt% phosphoric solution (phosphoric acid 85% p.a., AppliChem, Darmstadt, Germany) was added by pivoting the bottle so that the ratio between calcium and phosphor was 1.67, necessary to create a pure hydroxyapatite structure. The composition was given time to react for 24 h. After this period, each batch of about 100 g of the mixture was ground together with 900 g 1 mm-ZrO_2 grinding spheres (1 mm, Tosoh, Japan) in an agitator ball mill for 20 min (900 rpm). Subsequently, the grinding spheres were removed and the mixture was given time to react again for 24 h. After further grinding and holding time of another 24 h, part of the mixture was dried at 80 °C and pestled to 200 µm rechecked by a microstainer. The powder was tempered in a furnace (L9/13, Nabertherm, Lilienthal, Germany) at 1000 °C for 2 h to induce the apatite structure. The rate of heating, as well as the rate of cooling, was 5 °C/min. After pestling the powder again to 200 µm and rechecking it with a microstainer the powders were analysed using an X-ray diffraction. In addition, the particle size of the powder was analysed by laser granulometry (Mastersizer 2000, Malvern, Herrenberg, Germany). The laser analysis was carried out using two procedures in order to investigate both single particles and agglomerates formed by several particles. To this end, the powder was treated in an ultrasound bath filled with water to break down agglomerates (wet analysis). Additionally, the dry powder was examined (dry analysis). The residual slip which was not dried was set aside for further experiments.

2.2. Syntheses of strontium-containing hydroxyapatite by a thermal reaction

The synthesised slip of calcium phosphates (cf. 2.1) was split and added to different amounts of strontium carbonate (strontium carbonate p. A., AppliChem, Darmstadt, Germany) to adjust Sr/Ca-ratios of 0, 5, 10, 15, 18, 26, and 34 wt% (designated Sr0HA, Sr5HA, etc.). To bring the Ca/P-ratio to 1.67, specific amounts of a 40 wt% H_3PO_4 were added to the suspensions. After the syntheses were kept on a rolling bench for 24 h, 20 g of each slip was dried, pestled to 200 µm rechecked by a microstainer and tempered as described earlier. Subsequently, the powders were pestled again to 200 µm rechecked by the microstainer and analysed by the X-ray diffraction and laser granulometry in the same way as mentioned earlier in Section 2.1. X-ray fluorescence analyses were conducted to investigate the elementary composition.

2.3. Mechanical strength evaluation

The *ball on three balls* test was conducted to determine the strength of the different hydroxyapatite variation specimens [11]. The specimens for this test were manufactured as follows. The individual powders were mixed with 6 wt% of an aqueous solution of organic ingredients consisting of 15 wt% Dolapix CE 64 (Zschirmer and Schwarz, Lahnstein, Germany), 35 wt% Optapix PAF 35 (Zschirmer and Schwarz, Lahnstein, Germany), and 50 wt% distilled water as a compressing aid. To ensure homogenous dispersion, the powders were processed using a speed mixer (SpeedMixer DAC 150.1 FVZ, Hausschild, Hamm, Germany) at 3000 rpm for 1 min. At least 30 samples of each synthesised powder were manufactured by pressing 240 mg with 1500 N to a thin disc, respectively, (initial load 50 N, testing speed 5 mm/min, holding time 60 s) using a universal testing

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