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Chemical characterization of hydroxyapatite obtained by wet chemistry in the presence of V, Co, and Cu ions

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ABSTRACT

A model system for the precipitation of hydroxyapatite (HA) from saturated solutions at basic pH was utilized to investigate the effects of V, Co, and Cu ions on crystallography and stoichiometry of the produced apatites. X-ray diffraction (XRD) was applied to analyze phase composition and crystallinity of powders obtained with different metal ion concentrations and annealed at different sintering temperatures. This procedure used the temperature-dependent phase transitions and decompositions of calcium phosphates to analyze the particular influences of the metal ions on apatite mineralization. Comparative XRD measurements showed that all metal ion species reduced crystallinity and crystallite size of the produced apatites. Furthermore the transformation of amorphous calcium phosphate (ACP) to HA was partially inhibited, as was deduced from the formation of α-tricalcium phosphate (α-TCP) peaks in XRD patterns of the heated powders as well as from the reduced intensity of the OH stretch vibration in FTIR spectra. The thermally induced formation of β-TCP indicated a significantly reduced Ca/P ratio as compared to stoichiometric HA. This effect was more pronounced with rising metal ion content. In addition, the appearance of metal oxides in the XRD patterns of samples heated to higher temperatures indicated the incorporation of metal ions in the precipitated apatites. Peak shifts showed that both the apatitic as well as the β-TCP phase apparently had incorporated metal ions.

1. Introduction

Recent approaches to equip materials for their application as orthopedic implants with bioactive and osteointegrative properties are based on the modification with low doses of inorganic bioactive ions, which provides the possibility of a controlled manipulation and support of implant-specific tissue reactions [1,2]. Many metal ions are present as low concentrated trace elements in the human body and play an essential role in biological processes, e.g. the regulation of cell functions, the activation or inhibition of enzymatic processes [3] or the triggered expression of specific proteins [4–6]. Vanadium, for example, supports osteogenesis by mitogenetic effects on osteoblasts and the inhibition of osteoclast formation [7,8] and has also been proven to show antitumoural activity [9]. However, the osteoclast inhibition must be regarded as a sensitive parameter, as osteoclasts also play a major role in the initialization of bone-remodeling. Copper promotes angiogenesis [10,11] and osteoblast activity [12] and has also antimicrobial properties in the μM range, whilst cobalt also shows angiogenetic effects [13–15] and stimulates osteoclast formation in concentrations of 0.01 to 1 μM [16,17]. An application of these ions in subtoxic concentrations excludes detrimental effects on cells such as mutagenic and carcinogenic effects [18]. In comparison to organic molecules metal ions have a lot of advantages: they can be stored for practically unlimited time, are easily sterilized, and last but not least they are inexpensive and easy to handle. Studies carried out so far merely concentrated on metal ion modified polymeric biomaterials, whilst ceramic materials based on calcium phosphates have been modified almost exclusively with silicon, magnesium, strontium or zinc ions [19–23]. However, when the release of metal ions from an orthopedic implant or a bone filling cement into adjacent tissue occurs, possible effects on the formation of new bone tissue have to be taken into account.

Bone mineralization is a complex process which is mainly initiated by the interaction of osteoblasts and collagen fibrils. The osteoblasts secrete matrix vesicles containing calcium complexes of phospholipids, basic proteins and alkaline phosphatase. After bursting of the vesicle membrane the calcium phosphate crystallites formed inside act as extracellular nucleation seeds, with the actual precipitation starting at the collagen fibrils serving as the organic template [24]. The mineralization process may be understood as a chemical precipitation from an aqueous solution locally oversaturated with Ca^{2+} and PO_4^{3-} ions, followed by transformation of calcium phosphate to HA crystallites, which finally align along the collagen fibrils to form a solid but elastic composite material of inorganic bone mineral and collagen.

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To understand these processes Blumenthal et al. studied several precipitation reactions for the synthesis of HA to examine how biomineralization is regulated by molecules occurring in blood plasma [25]. Pyrophosphate ions ($P_2O_7^{4-}$) and adenosine triphosphate (ATP) contain P–O–P bounds and can significantly reduce the formation rate of HA. Depending on its concentration ATP inhibits respectively delays the transformation of amorphous calcium phosphate (ACP) to HA. It is assumed that the molecules adsorb to the newly formed HA seeds by means of the P–O–P bounds and hence inhibit further crystal growth. Of particular interest for the study on hand were Blumenthal's experiments with the application of metal ions instead of inhibiting biomolecules [25,26], which showed inhibiting effects on HA formation in different mineralization model systems. Aluminium ions for example act in a similar way like ATP molecules and repress crystal growth by adsorption to active growth sites of the initially formed apatite seeds [25,27]. Experiments with titanium and vanadium ions which were carried out only for the direct crystallization of HA with and without the application of seed crystals showed similar results. However, in any case the formation of HA began immediately after mixing of the reactants. Obviously the metal ions only inhibited the progress of crystal growth, but not the formation of the first crystal seeds. The aim of the present study was to investigate in more detail the influences of vanadium, cobalt and copper ions on the mineralization of apatites from oversaturated solutions of Ca^{2+} and PO_4^{3-} ions and – by thermal treatment at different sintering temperatures – the crystallographic effects on the products. For this purpose a simplified model system for the formation of phase-pure HA was established, which then was contaminated with several different concentrations of the tested metal ions. The precipitated products were characterized by X-ray diffraction, FTIR spectroscopy and ICP-MS analysis with regard to their chemical and crystallographic composition. XRD was also applied to heat treated samples in order to study influences of the metal ions on thermally induced phase transformations. It should be stated that the metal ion concentrations applied in this study were much higher than the concentrations that would be expected from leaching processes or that would be suitable for biomaterial applications; they were chosen in order to obtain significant effects in the measurements, which could help to understand the interaction of HA mineralization with the metal ions at clinically relevant concentrations.

2. Materials and methods

2.1. Mineralization

Hydroxyapatite powders were obtained by precipitation according to the stoichiometric reaction of calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, Riedel-de Haën, Seelze, Germany) and diammonium hydrogen phosphate ($(NH_4)_2HPO_4$), Sigma-Aldrich, Munich, Germany):

$$5Ca(NO_3)_2 + 3(NH_4)_2HPO_4 + H_2O \rightarrow Ca_5(PO_4)_3OH + 6NH_4NO_3 + 4HNO_3 \quad (1)$$

Two mother solutions of the reactants were prepared by dissolution in deionized water in portions of 150 ml each. Solution 1 contained 0.025 mol $Ca(NO_3)_2$ and solution 2 0.015 mol $(NH_4)_2HPO_4$. Thus, the concentrations of the reacting ions in the resulting mixture of solutions 1 and 2 were $[Ca^{2+}] = 83.34$ mmol/l and $[PO_4^{3-}] = 50$ mmol/l.

The mixing of the solutions was carried out by slow addition of solution 2 to solution 1. This so-called "inverse" precipitation method [28] maintains a Ca/P ratio above 1.67, which promotes the formation of stoichiometric HA [29]. The addition of solution 2 occurred in three portions of 50 ml each. The first portion was added dropwise, the second one slowly flowing, and the last one was poured within a few seconds. Between the three steps the mixture was shaken very carefully to avoid the destruction of initial crystallization seeds. Immediately after the addition of the third portion of phosphate solution 20 ml of 25% ammonia solution (Grüssing GmbH Analytika, Filsum, Germany) were added to elevate the pH value to 12. This was necessary to counteract the pH reduction caused by the formation of nitric acid on the product side of Eq. (1). Furthermore the solubility of HA in aqueous solutions decreases with increasing temperature [30], therefore the mixture was heated for 10 min with a maximum temperature of about 80 °C and was then allowed to cool down and rest. After 20 h the precipitate was poured through a folded filter and washed several times to separate the insoluble product from the non-adsorbed ions remaining in the solution. Then the precipitates were stored on the filter paper in a furnace at 37 °C until they were completely dried. The solid products were crushed in a mortar and divided into portions. One of these portions was heated separately in unglazed porcelain crucibles in a sintering furnace to 1000 °C. The heating-up took 60 min to reach the maximum temperature, which was then maintained for 60 min. After thermal treatment the samples were allowed to cool down to about 100 °C with closed furnace door, before they were taken out. Additional heating experiments were carried out for selected powder samples at lower temperatures (600 or 900 °C).

For the introduction of V, Co and Cu ions into the model mineralization system metal salts with high solubility were used, namely vanadium(III) chloride 99% (VCl_3, metals basis, Alfa Aesar, Germany), cobalt(II) nitrate hexahydrate 98% ($Co(NO_3) \cdot 6H_2O$, Aldrich, Germany) and copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$, Merck, Germany). Prior to the mixing of solutions 1 and 2 a certain amount of one of the metal salts was weighed in and added to solution 1. The added concentrations were calculated in percentage related to the mass of calcium atoms in the expected reaction product according to the stoichiometric precipitation. This was done under the assumption that the incorporation of metal ions into the HA lattice occurred by substitution of Ca^{2+} ions. Therefore the amount m_s of metal salt to be added in order to substitute 1% of Ca^{2+} ions was calculated by the following equation:

$$m_s = \frac{M_s}{M_i} \cdot 0.01 \text{ g} \quad (2)$$

Here M_s and M_i are the molar masses of the metal salt and the concerning metal ion respectively. Hence the addition of 1% to the precipitation reaction corresponded to 30.9 mg for VCl_3, 49.4 mg for $Co(NO_3)_2 \cdot 6H_2O$, and 39.3 mg for $CuSO_4 \cdot 5H_2O$. Based on these values reactions with various additions of metal salts were carried out, whereby the percentages of ions were 1, 5, and 10%. The addition of the different amounts of metal salts occurred 10 min before mixing of the two mother solutions to ensure complete dissolution of all compounds. The mixing itself and the following treatment of the reaction mixtures were carried out according to the production of pure HA, as described above.

2.2. X-Ray diffraction

Phase composition of the obtained powders was determined by X-ray diffraction (XRD) in Bragg–Brentano geometry with a Siemens D5005 X-ray diffractometer (Bruker AXS, Karlsruhe, Germany) using Cu-Kα radiation with a voltage of 40 kV and a tube current of 40 mA. Prior to measurement, all samples were finely ground in a mortar and the resulting powders were filled into powder cuvettes. Diffraction patterns were recorded in a 2Θ range from 20 to 40° with a step size of 0.02° and scan speeds of 3 s/step. The Diffrac^{Plus} evaluation software (Bruker AXS) was utilized for both qualitative and semi-quantitative analyses, the latter one being carried out by evaluation of the net areas of the (0 0 2) diffraction peak, which was the only distinct peak in the patterns of unheated samples, as well as by estimation of crystallite size according to Scherrer's formula [31].

2.3. Fourier transform infrared spectroscopy

FTIR measurements were performed with a Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).