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Gallium-containing hydroxyapatite for potential use in orthopedics

P. Melnikov[a,*], A.R. Teixeira[a], A. Malzac[a], M. de B. Coelho[b]

[a] Department of Clinical Surgery, Faculty of Medicine, Federal University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil
[b] Brazilian Agricultural Research Corporation - EMBRAPA, Brazil

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ABSTRACT

A novel material that may be recommended for grafts and implants stimulating bone growth has been obtained by introducing gallium ions (up to 11.0 mass%) into crystalline lattice of hydroxyapatite. The doping was carried out using gallium nitrate and sodium gallate solutions. In both cases, lattice parameters of gallium-doped hydroxyapatite are identical to those of pure synthetic hydroxyapatite. Gallium does not replace calcium as a result of heterovalent substitution and consequently produces no distortions in the framework of hydroxyapatite matrix. It remains strongly fixed in the form of solid solution of intercalation. According to scanning electron microscopy images gallium insertion does not cause any morphological alterations in hydroxyapatite structure and the product developed meets physico-chemical criteria for biomaterial to be employed in orthopedic practice and local handling of traumatic injuries. Its future usage opens the opportunity to enhance osteosynthesis and calcium retention *in loco*.

1. Introduction

Sufficient evidence has accumulated that lends credit to the possibility of direct and relevant participation of gallium in skeleton metabolism. Because of its strong affinity to bone tissue this trace element at present is widely used in the form of its radioactive isotope Ga-67 for diagnostic purposes. Gallium ions also are clinically effective against bone resorbtion and for the treatment of osteoporosis and cancer-related hypercalcemias [1,2]. They increase calcium and phosphorus content of the bone and have direct nontoxic effects on osteoclasts. Such a method of administration for local purposes has two disadvantages: on one hand, the low compliance by the patient and, on the other, the risk of reaching toxic doses. But if the drug is introduced in a molecular level, it can be slowly released locally [3]. Nevertheless, no orthopedic biomaterials containing gallium for local applications are accessible so far. On the other hand, hydroxyapatite, calcium oxophosphate $Ca_5(PO_4)_3(OH)$, preferably in its porous form is used as a bone-graft substitute in metaphyseal, vertebral and maxillofacial [4] defects as a routine procedure. It may also be applied along with autogenuous bone or demineralized bone matrix [5], which would conceivably create a composite with both osteoconductive and osteoinductive properties. The idea of the present investigation is that these properties can be further enhanced by direct intercalation of gallium ions into hydroxyapatite crystalline lattice. Other drugs, such as biphosphonates, calcitonin and fluoride tested until now do not seem to provide overwhelming protective and osteoinducing effects. It is logical to suggest that gallium goes to the specific structural sites that in natural hydroxyapatite are normally occupied by yttrium, cerium and other trivalent and rare earth elements [6,7]. The scope of the present work is the preparation of gallium-containing hydroxyapatite for potential use in orthopedic surgery. First results on this subject have been recently presented at the 8th World Biomaterials Congress [8].

2. Materials and methods

Starting reagents were dibasic ammonium phosphate $(NH_4)_2HPO_4$ (Synth), ammonium hydroxide (Synth), sodium hydroxide (Synth), calcium nitrate $Ca(NO_3)_2{\cdot}4H_2O$ (Merck), gallium nitrate $Ga(NO_3)_3{\cdot}xH_2O$ (Sigma–Aldrich), commercial hydroxyapatite (Sigma–Aldrich), and alcoholic solution of alizarin yellow GG (Merck). The amount of water present in gallium nitrate hydrate (6 moles) was previously established by thermogravimetric analysis. Sodium gallate solution was specially prepared from Ga_2O_3 (Gosreagent, spectrally pure). All reagents were of analytical grade purity with the exception of $Ga(NO_3)_3{\cdot}xH_2O$, which was spectral pure, 99.999%.

Crystalline phases were investigated using X-ray diffraction method. Powder patterns were registered with a Siemens Kristalloflex diffractometer (Ni-filtered Cu Kα radiation) provided with a graphite monochromator, 2θ range 10–60°. Energy dispersive analysis (EDAX) was performed using a Princeton Gamma Tech PGT instrument provided with SiLi detector. Scanning electron microscopy (SEM) was carried out using a SM-300-TOPCON instrument. Thermogravimetric analysis (TGA) was carried out using a thermal analyzer (50H Shimadzu Instrumentation) under nitrogen flux, ramping 5 °C min^{-1}. Thermomechanical analysis was carried out using a Shimadzu TMA-50 analyzer with a load of 1 g, ramping 10 °C min^{-1}.

* Corresponding author at: Department of Clinical Surgery, Faculty of Medicine, Federal University of Mato Grosso do Sul, Rua Gabinete 560, Vila Ipiranga, 79080-680 Campo Grande, Mato Grosso do Sul, Brazil. Tel.: +55 6733457031.
E-mail address: petrmelnikov@yahoo.com (P. Melnikov).

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2.1. Preparation of sodium gallate solution

2.69 g of gallium oxide were mixed with 15 g of granulated sodium hydroxide. The mixture was placed in a platinum crucible and heated until the flux melted. After the sample dissolved and gave clear liquid, the melt was swirled around the side of the crucible to aid its subsequent removal. The crucible was cooled and solid product lixiviated with hot water leading to precipitation of $Ga(OH)_3$. The hydroxide was washed out with water and immediately mixed with a 20% aqueous NaOH solution, added dropwise under stirring. The procedure should be performed on fresh warm $Ga(OH)_3$ because of its prompt ageing and polymerization blocking gallate formation. The solution obtained was used for subsequent syntheses.

2.2. Preparation of gallium-doped hydroxyapatite using gallium nitrate

50 ml of aqueous solution containing 0.26 g of $Ga(NO_3)_3{\cdot}xH_2O$ were mixed with 100 ml of solution containing 41.37 g of $Ca(NO_3)_2{\cdot}4H_2O$. Then 9.90 g of $(NH_4)_2HPO_4$ were dissolved in 75 ml of water and an alcoholic solution containing 5–10 drops of alizarin yellow were added dropwise to the above mixture, leading to precipitation of a dense colloidal mass. This mass was diluted with water in proportion 1:1 and pH brought to 11.0 with concentrated NH_4OH. During the treatment with NH_4OH colloidal solution is destroyed and a fine suspension is formed. As a rule, about 4 ml of ammonium hydroxide were necessary to achieve pH 11.0, producing color change of alizarin to clear yellow. The usage of pH strips is not recommended for two reasons: colloidal solution blocks ions diffusion and ammonia vapors in the beaker make the indicator show false higher pH values. The suspension was heated at 40 °C for 2 h and put for 24 h into a closed vessel under nitrogen atmosphere, at room temperature. After that, the clear liquid and the precipitate were heated at 95 °C for more 3 h, the precipitate was filtered out and washed four times with water. NH_4OH solution was added to filtrate as assurance of complete precipitation. No calcium phosphate was detected. The product was dried over P_2O_5, then at 60 °C until constant mass weight, and finally sintered in air at 1100 °C.

2.3. Preparation of gallium-doped hydroxyapatite using sodium gallate

50 ml of aqueous solution containing sodium gallate prepared from 2.69 g Ga_2O_3 (equivalent to 2.0 g metallic Ga) were mixed with 100 ml of a solution containing 41.37 g of $Ca(NO_3)_2{\cdot}4H_2O$. Then 9.90 g of $(NH_4)_2HPO_4$ dissolved in 75 ml of water and an alcoholic solution containing 5–10 drops of alizarin yellow were added dropwise to the above mixture, leading to precipitation of a dense colloidal mass. Then the procedure was the same as described above.

3. Results and discussion

The energy dispersive X-ray spectra (EDAX) of the samples prepared by both methods (Figs. 1 and 2) show calcium (Kα, $K_{\beta1}$, Lλ) and phosphorus (Kα) lines, dominating the pattern, and a net presence of gallium incorporated. Sodium sometimes appears in trace quantities as a shoulder on gallium peak. Phosphorus and gallium mappings of the samples doped with gallium nitrate and sodium gallate (see SEM images in figures) assert that the elements are distributed statistically. Gallium content calculated from EDAX data for the sample obtained with the usage of $Ga(NO_3)_3$ was 3.74%, while for the sample obtained with sodium gallate, it was 11.0%. Since washing of doped hydroxyapatite after its preparation was carried through to completion, this gallium can be considered as firmly fixed in the crystal lattice. It is in conformity with the content of trivalent metals steadily attached to natural apatite from

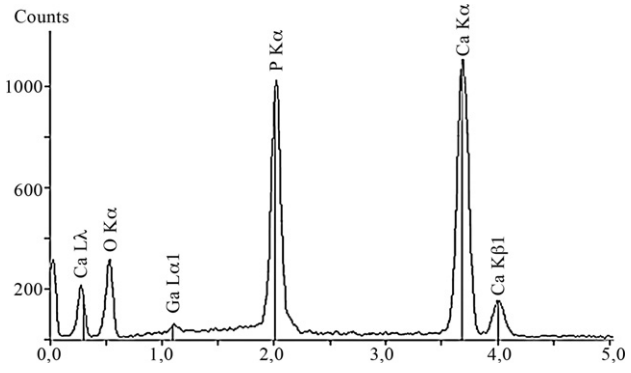

Fig. 1. Energy dispersive X-ray spectrum of sample obtained using gallium nitrate.

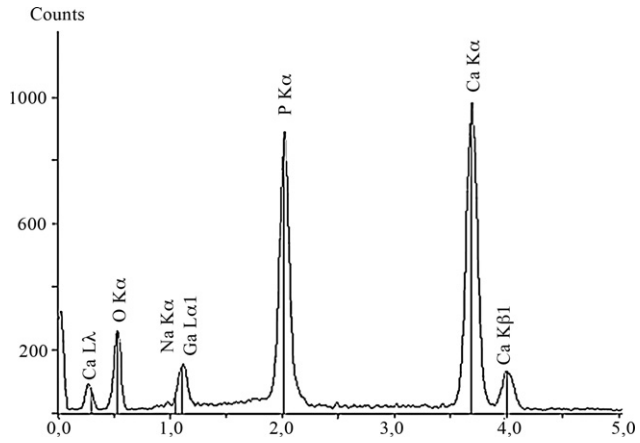

Fig. 2. Energy dispersive X-ray spectrum of sample obtained using sodium gallate.

Kola Peninsula, about 2.0 mass% [9]. Apparent discrepancy in concentrations may be due either to a limited availability of rare earth metals in the Kola environment or to a higher affinity of gallium to the apatite matrix.

It is expected that *in vivo* conditions, at slightly acidic pH gallium will slowly come out facilitating the osteointegration of the implant.

Fig. 3 shows that X-ray diffractograms of both samples of hydroxyapatite are identical. Lattice parameters $a = 9.421$ Å and $c = 6.884$ Å calculated from these data are the same as given in the above mentioned ICSD file 09-0432 for synthetic hydroxyapatite. However, X-ray pattern of the sample obtained with sodium gallate is more complex, but all reflections could be satisfactorily accounted for by computing the whole set of feasible Miller indexes and comparing them with those present in the diffractogram. No additional phases were detected, so no decomposition of hydroxyapatite/Ga took place during thermal treatment.

There are two possible ways of trivalent metal incorporation into crystalline lattice of hydroxyapatite: through heterovalent substitution and through intercalation, e.g. by forming solid solutions of substitution/intercalation [10]. As the sum of ionic radii

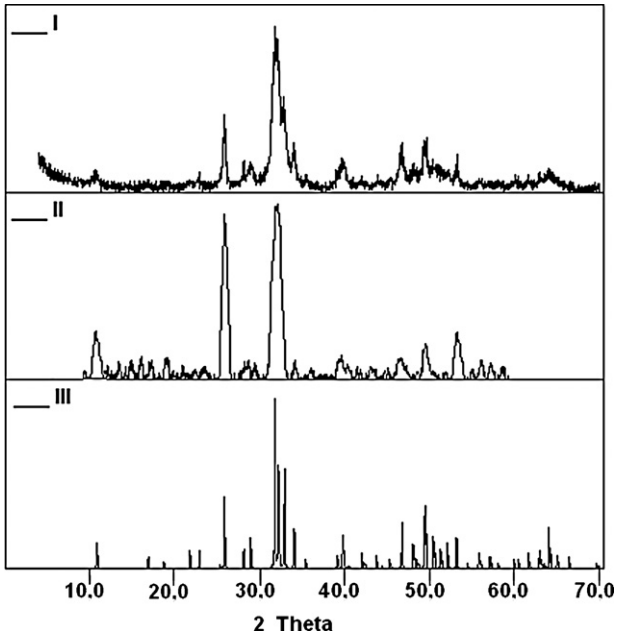

Fig. 3. X-ray diffractograms of gallium-doped hydroxyapatite. (I) Sample obtained using gallium nitrate; (II) sample obtained using sodium gallate; (III) commercial hydroxyapatite.