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Original research paper

RADIO-OPAQUE MATERIALS BASED ON HYDROXYAPATITE AND BISMUTH*

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Abstract. *This study relates to a new biocement prepared from mixtures of Bi-substituted hydroxyapatite and calcium sulphate dihydrate using deionized water as liquid phase. The Bi-substituted hydroxyapatite nanopowder was produced by the co-precipitation method using $Ca(OH)_2$, H_3PO_4 and $Bi(NO_3)_3{\cdot}5H_2O$ raw materials as calcium, phosphorous and bismuth sources. The XRD diffractograms of the cement powders indicate only hydroxyapatite and $CaSO_4$ phases. This biocement exhibits radio-opacity, thus enhancing its utility in the dental and medical fields.*

Key words: *Biocement, hydroxyapatite, bismuth, radio-opaque*

1. INTRODUCTION

The hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA), well known for its excellent bioactivity, biocompatibility and osteoconductivity, is one of the most important calcium phosphate based bioceramic materials with important applications in medicine and chemistry[1].

The literature reports demonstrate the flexibility of the hydroxyapatite structure as regards the substitution with other different cations in the "Ca" sites [2-5]. The properties of hydroxyapatite biomaterials including morphology, lattice parameters, surface characteristic, solubility, mechanical and biological properties can be affected by the incorporation of foreign cations.

The biomaterials with excellent optical properties were intensively studied in the last decade due to the increasing demand for efficient light-sensitive materials not only for the optoelectronic devices, but also for a broad range of applications in the biomedical field. Radiopaque compounds are widely used in medical formulations and selected for the manufacture of components used in a wide variety of minimally invasive devices. Current research involves the design of biomaterials that are ideal for applications such as radiopaque implants as well as computed tomography nanoparticle contrast agents [6].

For more than a century the bismuth salts have been used as a remedy for some maladies such as gastrointestinal disorders, syphilis and hypertension [7]. Today, the two major medicinal use of bismuth compound is focused on antimicrobial agent and anticancer agent [8,9]. Bismuth compounds, due to their radio-opacity, are also added to various bone and dental implants, catheters and surgical instruments in order to make them detectable by X-rays and computed tomography [10,11].

The bismuth is regarded as "the wonder metal" because of its diverse oxidation states. Its behavior as smart optically active centers in diverse materials has been noticed in the last decades [12]. Due to these properties the bismuth compounds have important applications in areas of biomedicine, white light illumination and lasers.

In dental and orthopedic applications, radio-opaque materials may be used as filler in the composition of the biocement paste in order to enhance absorption of X-rays, and therefore for improving the visibility of the cement under X-ray examination.

Calcium phosphate biocement that based on hydroxyapatite shows the ability to conform perfectly to the defects in hard tissues such as tooth, bone, etc. This is due to its ability to set at body temperature leading to the formation of a solid phase material [13].

In this work, a new biocement was produced based on a mixture of bismuth-substituted hydroxyapatite and calcium sulphate dihydrate, with deionized water as liquid phase. This biocement can be used as a bone substitute material for filling in parts of defect bone, being capable of setting itself in the body. The Bi-doped hydroxyapatite was prepared by means of wet chemical method based on co-precipitation reactions. The radio-opacity of the biocement samples was analyzed.

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2. EXPERIMENTAL

2.1. Materials and synthesizing methods

Calcium hydroxide $Ca(OH)_2$, orthophosphoric acid H_3PO_4 (85 %), bismuth nitrate pentahydrate $Bi(NO_3)_3{\cdot}5H_2O$, calcium sulphate dihydrate ($CaSO_4{\cdot}2H_2O$), ethanol and sodium hydroxide were purchased from Sigma-Aldrich (Germany). All chemicals were of analytical grade.

The hydroxyapatite and bismuth-substituted hydroxyapatite nanoparticles were synthesized by wet chemical precipitation method from $Ca(OH)_2$, H_3PO_4 and $Bi(NO_3)_3{\cdot}5H_2O$ as calcium, phosphorous and bismuth sources, respectively. The method was described in our previous works [14,15].

The pure hydroxyapatite powder was prepared by mixing appropriate amounts of $Ca(OH)_2$ (0.1 M) and H_3PO_4 (0.1 M) aqueous solutions to achieve predetermined Ca/P atomic ratio of 1.67. The suspension obtained was aged for 3 h and then filtered and washed with ethanol and triply distilled water. The obtained powder was calcined 1 h at 800 °C in an electrically heated furnace in order to increase its crystallinity.

The bismuth-substituted hydroxyapatite powder was prepared similarly to the pure hydroxyapatite powder, as described above. The $Ca(OH)_2$ (0.1 M) aqueous solution was dispersed into an mixed aqueous solution of $Bi(NO_3)_3{\cdot}5H_2O$ and H_3PO_4 (0.1 M). The (Bi+Ca)/P atomic ratio was kept at 1.67 value, while the Bi/(Bi+Ca) atomic ratio (denoted as X_{Bi}) in the solution was 0.1. The following procedure stages were the same as described above for the preparation of pure hydroxyapatite powder.

Cement powder was prepared from a mixture of hydroxyapatite (45 %) and $CaSO_4{\cdot}2H_2O$ (55 %), milled to produce fine particles. Deionized water was used as liquid phase for the preparation of the biocement. The powder was incorporated into liquid phase by successive fractions and kneaded with the aid of a spatula between each addition to produce a paste of workable consistency. After a mixing time of 5 min, carried out on a glass plate at 20±2 °C, the paste was loaded into the molds, clamped and stored at 30 °C.

2.2. Samples characterization

The phase composition, degree of crystallinity and size of crystallites of the samples were estimated by X-ray diffraction analysis (XRD) with X'PERT PRO MRD diffractometer using CuKα radiation (λ = 0.15418 nm).

The morphology of the samples was studied by scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDX) with QUANTA 200 3D microscope.

The radiographs of the samples were obtained in a dental X-ray system (X-Mind™ AC, SATELEC, France). The samples were placed onto occlusal radiographic film and exposed, along with a graduated aluminum (99.5 % pure) step wedge with thickness varying from 1 to 10 mm in 1 mm increments. Radiographs were digitized using a desktop scanner (VistaNet/VistaScan PERIO PLUS). Then, the digitized images were imported into the Gendex Dental Systems VixWin 2000 software where a tool was applied to identify equal-density areas in the radiographic images. This procedure allowed comparison between the densities of different samples and the radiopacity of different degrees of thickness of the aluminum step wedge. The area corresponding to the specimen was selected (by using the computer mouse) from each radiographic image, in order to verify which thickness of the aluminum step wedge was detected by the software as equivalent to the specimen's radiographic density. This assessment determined the equivalence of radiopacity of the sample compared to a particular thickness of aluminum, measured in millimeters.

3. RESULTS AND DISCUSSION

3.1. Hydroxyapatite powders characterization

The incorporation of foreign ions in the hydroxyapatite structure is favorable when the difference between the ionic radii is small, closeness to the ionic radius of Ca^{2+} ion. Having comparable dimensions, the Bi^{3+} ions can substitute Ca^{2+} ions during the synthesizing process.

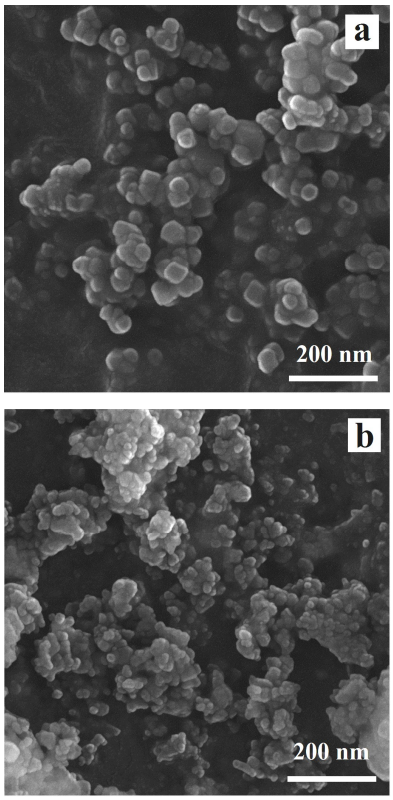

Figure 1. SEM images of the undoped hydroxyapatite (a) and Bi-substituted hydroxyapatite (b) calcined samples

The phase composition, lattice parameters, degree of crystallinity and size of crystallites of the hydroxyapatite and Bi-doped hydroxyapatite powders were determined by XRD analysis [14,15]. The XRD

patterns (figure not shown) indicate that undoped hydroxyapatite and Bi-substituted hydroxyapatite calcined samples have the characteristic peaks in the 2θ regions of 21°-29°, 32°-34°, 39°-41°, 46°-54°, in good agreement with the hexagonal (space group P63/m) hydroxyapatite phase (JCPDS Data Card 09–0432).

The undoped hydroxyapatite and Bi-substituted hydroxyapatite calcined samples exhibit nanosized spherical shapes and agglomeration with intergranular micropores, as shown SEM micrographs in Fig. 1.

The radio-opacity of the undoped hydroxyapatite and Bi-substituted hydroxyapatite samples was analyzed. The Bi-substituted hydroxyapatite sample shows a much stronger radio-opacity compared with undoped hydroxyapatite, as can see in Fig. 2.

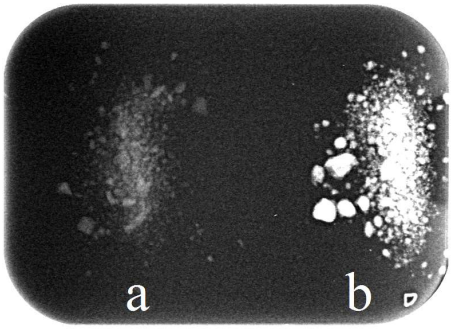

Figure 2. Radiograph images of the undoped hydroxyapatite (a) and Bi-substituted hydroxyapatite (b) calcined samples

3.2. Biocement samples characterization

Figure 3 shows the XRD diffractograms of the undoped hydroxyapatite and Bi-doped hydroxyapatite biocement samples. In the both samples the major phases, as expected, are hydroxyapatite (which is confirmed by comparing data obtained with the JCPDS Data Card 09–0432) and $CaSO_4$. Another phase is calcium oxide (CaO), identified at 37.46° (2θ) of much lower intensity.

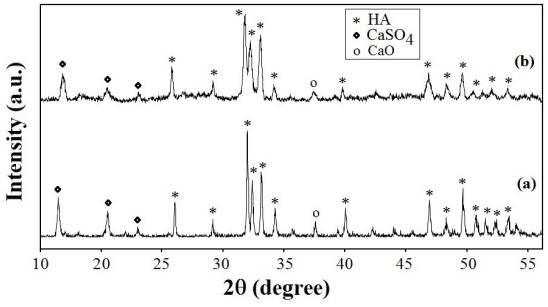

Figure 3. XRD diffractograms of the undoped hydroxyapatite (a) and Bi-doped hydroxyapatite biocement (b) samples

It is important to mention that the deionized water added to the formulation provided good workability and the mixture was easily poured into the mold. On the other hand, when more water was added, the cement mixture was too wet to work on which also affected the curing time.

The SEM results (presented in Fig. 4) show that the biocements with deionized water has no individual structure of calcium sulphate indicating that calcium sulphate has completely mixed together with hydroxyapatite.

Figure 4 also shows that the biocements contain interconnected pores. Porosity is also visible in radiograph images (Fig. 5) by black dots visible on the surface of the samples. The porosity and the pores interconnectivity would be desirable for uses such as the biocements for bone tissue engineering, which involves placing bone forming cells into the porous ceramic bodies (scaffolds) and implanting the cell-loaded scaffolds into a bone defect, followed by bone healing. At the same time, high porosity and interconnected porous structure can also accelerate the circulation of nutrients as well as the exhaust of the metabolic waste [16].

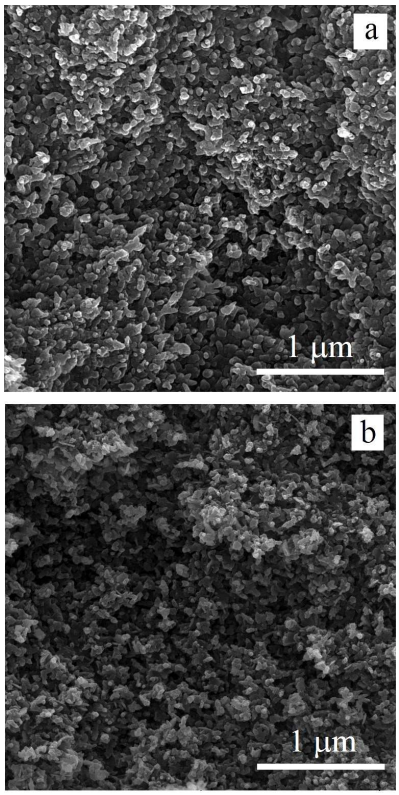

Figure 4. SEM images of the undoped hydroxyapatite (a) and Bi-doped hydroxyapatite (b) biocement samples

Radio-opacity is a fundamental requirement for restorative dental materials. ISO standard 4049/2009 (Dentistry – Polymer-based restorative materials) stipulate that the minimum radiopacity for a restorative material should be equal to or higher than its equivalent thickness in aluminum [17]. Metallic dental materials like amalgam, gold, cobalt chromium, and stainless steel have high radio-opacity. None of the composite material has radio-opacity like that of metallic materials because of absence of metallic elements in it.

This study has proven the radio-opacity of Bi-substituted hydroxyapatite based cements. The radio-opacity of the undoped hydroxyapatite and Bi-substituted hydroxyapatite cement samples was analyzed. The cement based on Bi-substituted hydroxyapatite shows a much stronger radio-opacity compared with cement based on undoped hydroxyapatite, as can see in Fig. 5.

In the present study, we constructed a 10-step aluminum step wedge ranging in thickness from 1 mm to 10 mm. The results demonstrated that Bi-substituted hydroxyapatite biocement sample presented the greatest radiopacity and was equivalent to 8.92 of aluminum. The undoped hydroxyapatite biocement sample exhibited the lowest radiopacity and was equivalent to 2.86 mm of aluminum. This implies that Bi-substituted hydroxyapatite cements can be candidates for filling materials in terms of their radiopacity.

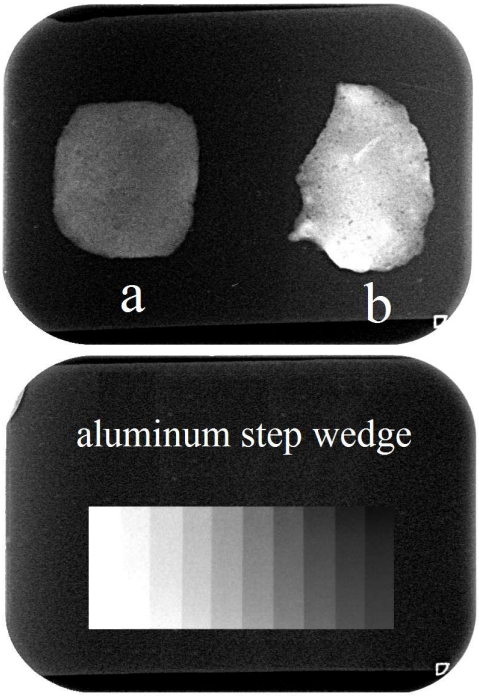

Figure 5. Radiograph images showing the radio-opacity of the undoped hydroxyapatite (a) and Bi-doped hydroxyapatite (b) biocement samples and equivalence to that of the aluminum step wedge

As revealed in the current study, due to their radio-opacity, the Bi-substituted hydroxyapatite biocements can be added to various bones and dental implants, catheters and surgical instruments in order to make them detectable by X-rays and computed tomography.

However, further studies are required to ascertain their potential clinical benefits and limitations, due to differences in the radio-opacity of the composites.

4. Conclusions

A new biocement was successfully prepared from mixtures of Bi-substituted hydroxyapatite and calcium sulphate dihydrate using deionized water as liquid phase. The Bi-substituted hydroxyapatite nanopowder was produced by the co-precipitation method using $Ca(OH)_2$, H_3PO_4 and $Bi(NO_3)_3{\cdot}5H_2O$ raw materials, as calcium, phosphorous and bismuth sources. The XRD diffractograms of the cement powders indicate only hydroxyapatite and $CaSO_4$ phases. The deionized water added to the formulation provided good workability and the mixture was easily poured into the mold. The Bi-substituted hydroxyapatite cement is radio-opaque, being detectable by X-rays and computed tomography.

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