Tissue Engineering Scaffolds from La$_2$O$_3$ – Hydroxyapatite\Boron Glass Composites

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Abstract
Bioactive composites of bovine derived hydroxyapatite and boron containing bioactive glass were doped with 5, 10 and 15 wt.% lanthanum oxide (La$_2$O$_3$). Results showed that at low sintering temperatures (800 °C) the doping oxide of La$_2$O$_3$ promoted transformation to crystalline phases. Formation of secondary phases, such as rhenanite, NaCaPO$_4$, and calcium silicate, Ca$_4$SiO$_4$, were also found. La was found to act as modifier and influenced the viscosity. The increase of La$_2$O$_3$ content led to decrease of pore size. Porosity amount 48.1 %, at 15-240 µm size range were reduced, to 34.4 %, with 5-80 µm size range and particle sizes of 0.3-6 µm to 0.09-2 µm, respectively. In addition to that compression strength (MPa) and microhardness (HV) of the performed composite bioceramics were in the range of 25 – 110 MPa, and 121 – 364 HV, respectively. Our investigations suggested that the porosity type has a strong influence on the compressive strength and microhardness properties of the final bioceramic products.

Keywords: Scaffolds; biocomposite; borate bioglass; biological hydroxyapatite; sintering

1. INTRODUCTION
Over the last years, numerous research studies have been dedicated to the design and testing of artificial bone scaffolds. Bone is a complex and hierarchical tissue, whose major mineral component (~70%) is constituted by a non-stoichiometric hydroxyapatite (HA) type compound. Nowadays, bone defects, resulting either by traumatic events (accidents) or induced by congenital, chronic or age-related diseases, is a major concern in medicine. Over 2.2 million bone graft implants are presented yearly worldwide [1-2]. Numerous biomaterials or treatment possibilities are available to repair bone defects, for example autograft, allograft and synthetic graft [1-2]. Recently, extensive attention has been directed to the development of synthetic tissue engineering scaffolds for the bone reconstruction. Such synthetic bone grafts claim certain perforce features: biocompatibility, controlled biodegradability, osteoconductivity/osteoinductivity, structural similarity to bone, mechanical compatibility to the surrounding tissue (to avoid the creation of residual stress in bone), and attractive costs [3]. In view of developing production technologies for a new generation of bone tissue scaffolds, the physical and chemical properties of the synthetic candidate materials should come into focus. In this study the role of porosity and pore structure will be assessed. Biocomposites, based on hydroxyapatite (HA) and bioactive glass (a material possessing the highest index of bioactivity have been successfully used in medicine) [1-11]. Nevertheless, various complications are known to occur in post-surgical period. Several studies have attempted to remedy these problems by intentional doping of composites with small amounts of cations such as Ag, Cu or La [1-7]. Particularly, during time, La doping of HA has attracted significant interest for various biomedical applications [9-15]. By substituting the Ca$^{2+}$ cation (ionic radius=99 pm)
with the bulkier La\textsuperscript{3+} cation (115 nm) an expansion of the HA lattice is produced along with a higher crystal lattice energy, which imprint a better thermo-stability of material, and lowers the risk of HA decomposition during thermal processing [12,19]. Moreover, many other remarkable benefits of La doped HA materials have been identified: accelerated osteointegration [12,16-18], high antimicrobial resistance and anticoagulant properties [21], higher flexural strength, lower dissolution rate [19,21], better mechanical properties of both bulk [20,21] and coatings [12], increase in crystallinity and crystal coherence length with doping concentration [21,22]. In our previous studies [23-25], we have succeeded to produce promising biocomposites based on biologically derived hydroxyapatite (using bovine femoral bones as a cheap sustainable resource) and Na\textsubscript{2}O-B\textsubscript{2}O\textsubscript{3}-SiO\textsubscript{2} bioactive glass. Boron containing bioglasses are well-known for their excellent ability to facilitate and preserve the good health of bones [9, 26]. Dietary exposure to varying levels of boric acid affects the increase of the strength of vertebrae in compression forces [9]. Moreover, boron seems to be an essential mineral in the conversion of vitamin D to its active form [27]. Additionally, it has been shown that increased osteoblast proliferation activity was observed by using boron-containing bioglasses in cell culture studies [26]. However, the introduction of boron into the classical Bioglass\textsuperscript{®} [3] composition lowers the melting point from 1450°C [26] to around 950-1050°C [26, 28].

Up to now, only the effect of Fe\textsubscript{3}O\textsubscript{4}, Fe and Cu doping on the magnetic properties and biological behavior of these biological hydroxyapatite/glass ceramics has been studied [6]. The influence of La\textsubscript{2}O\textsubscript{3} doping on sintering, stability, structure and mechanical properties of such HA/bioglass composites has not been systematically studied.

Therefore the aim of this study is to investigate the effect of various lanthanum oxide (La\textsubscript{2}O\textsubscript{3}) doping on the structure and properties of biological HA/boron-containing bioglass (BHA-BBG) composites.

2. MATERIALS and METHODS

2.1 Materials and sample preparation procedure

The raw materials consisted of powder of: BHA (prepared as presented in Ref.) [23, 29], boron bioglass powder with the composition (wt.%): SiO\textsubscript{2}-45.7, Na\textsubscript{2}O-26.1, and B\textsubscript{2}O\textsubscript{3}-28.2, and La\textsubscript{2}O\textsubscript{3}.

The La\textsubscript{2}O\textsubscript{3}-doped BHA-BBG composite bioceramics were prepared by a two-step sintering process, according to the method described in previous studies [23-25]. Briefly, the BHA-BBG composites were prepared by the mechanical mixing of 50 wt.% BHA powder and 50 wt.% boron glass powders. The powder mixture was first sintered at 1100°C and then crashed into a fine powder. Three different amounts of La\textsubscript{2}O\textsubscript{3} powder (5, 10 and 15 wt.%) were added to the BHA-BBG powder mixtures, and then the batches were mechanically mixed by ball milling. The resulted powders were pressed by uniaxial cold pressing in a stainless steel mold at 150 MPa according to the British Standards (No. 7253), and subsequently sintered at 800°C in air for 2 h (using heating and cooling rates of 10 °C/min and 1 °C/min, respectively).

2.2 Characterization techniques

a) The microstructure, porosity, pore size and pore interconnectivity were investigated by scanning electron microscopy (SEM, JEOL JSM 6490LV), and quantified with dedicated image analysis software (SIAMs PhotoLab).

b) The identification of the crystalline status and phases of composites was performed by X-ray diffraction (XRD, DRON-3, with Cu\textsubscript{K\alpha} radiation).

c) The effect of La\textsubscript{2}O\textsubscript{3} doping on volume changes and mass loss of composite samples was studied by traditional ceramic processing.

d) The samples’ density was estimated by (Archimedes) method by immersing them in toluene.

e) The compression strength of the sintered samples was evaluated using a universal tensile testing machine (PSU-50). For the Vickers microhardness measurements, a Shimadzu machine (HV) was employed by applying a 200 g load for 20 s.

f) The evaluation of the in vitro solubility of composite samples (at 36-37 °C) was carried out by a technique reported in our previous work [24]. In order to resemble the physiological fluids for in vitro investigations, a solution of 0.9% NaCl was used as a model medium.

3. RESULTS and DISCUSSIONS

Figure 1 shows the typical XRD pattern of La\textsubscript{2}O\textsubscript{3}-doped composite. All the doped samples display similar diffractograms. The La\textsubscript{2}O\textsubscript{3}-doped bioceramic composite is composed of mainly non-stoichiometric HA phase (Ca\textsubscript{9,74}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2,06}; JCPDS No. 86-1199). Related to the other phases, rhenanite (NaCaPO\textsubscript{4}; JCPDS No. 29-1193), stoichiometric HA (Ca\textsubscript{5}(PO\textsubscript{4})\textsubscript{6}(OH); JCPDS No. 866740), and calcium silicate (Ca\textsubscript{2}SiO\textsubscript{4}; JCPDS No. 06-0511) are also detected in the La\textsubscript{2}O\textsubscript{3}-doped composite. In other words, two points indicate that La dopant both promotes interaction of crystalline (HA) and amorphous (glass) phases, and favors formation of secondary phases (name which ones).
Fig. 1. X-ray diffractogram of BHA-BBG composite doped with 10 wt.% La$_2$O$_3$.

Fig. 2. Effect of La$_2$O$_3$ content on volume change of BHA-BBG composites after sintering.

Fig. 3. Effect of La$_2$O$_3$ content on mass loss of BHA-BBG composites after sintering.
According to earlier studies, the XRD analysis of the La-free (non-doped) composites show only HA phase [25]. Thus, the phase of La2O3 was not detected [30-33]. Consequently, it can be postulated the possibility of La2O3 to act as a network modifier, with La captions entering into the glass structure.

Figure 2 depicts the volume change of La2O3-doped composites after the final sintering performed at 800°C. It is noted that the incorporation is not typical for undoped composites. A significant volume growth of the sample doped with 5 wt.% La2O3 was recorded. The increasing level of La2O3 doping leads to a progressive volume shrinkage. It is worth emphasizing that the shrinkage of the 15 wt.% La2O3 doped composite is two times lower than the shrinkage of undoped samples (10.2 %) [24]. It is well known that La2O3 in amount 5–40 wt.% is used for preparation optical glass, production lenses and prisms for cameras and astronomical purposes. Additionally, the properties of La2O3 are similar to that of B2O3, which is also used for preparation of optical glass. At high temperatures, B2O3 reduces the viscosity of the glass. On the other hand, at low temperatures, when B2O3 content is up to 15 wt.%, it increases the viscosity. Also, a high B2O3 content leads to the diminution of viscosity [34]. Therefore, one can assume in the case of La2O3-doped BHA-BBG composites, lanthanum oxide could lead to an increases of the glass viscosity.

Furthermore, the mass loss recorded after sintering increased 4 times when doping the composite with 5 wt.% La2O3 (Figure 3). The increase of the La2O3 doping level is accompanied by a continuous increase of mass loss. (Figure 3).

It can be attributed to the property of lanthanum oxide in an easy way of forms such as hydroxide in air that decomposes in temperature range 300-1100 °C and forms La2O3 [35].

Typical SEM micrographs of La2O3-doped BHA-BBG composites (polished surfaces) are presented in Figure 4. It can be seen that the composite materials contain crystalline particles, amorphous glass and numerous macro- and micropores. The quantitative analysis of the pore and particle size distribution indicated that the an increasing La2O3 content leads to the decrease of pore sizes from 15-240 μm to 5-80 μm (Figure 5). This can be attributed to the effect of La2O3 on the viscosity of glass during sintering. Interestingly, the BHA-BBG composite doped with 5 wt.% La2O3 presents two maximum pore sizes, whilst the other two composite materials only one. Thus, an increasing content of lanthanum oxide leads to transformation of the porous structure of bioceramics from poly porous to monoporous.

A similar effect is observed for particle size distribution of La2O3-doped BHA-BBG composites (Figure 6). The particle size decreases from 0.3-6 μm to 0.09-2 μm with increasing La2O3 content, but all doped composites have a single particle size distribution. The total porosity decreases with the increasing La2O3 content (Table 1), but still remains 1.5 times higher than that of the undoped BHA-BBG composite. Moreover, the value of open porosity increases with increasing of La2O3 content, but is lower than that of with the undoped BHA-BBG composite. The open porosity for La2O3-doped BHA-BBG composites ranges between 0.1 and 0.7.

<table>
<thead>
<tr>
<th>Content of La2O3, wt. %</th>
<th>Porosity, (±0.5 %)</th>
<th>Microhardness (HV)</th>
<th>Compressive Strength, (MPa)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>total</td>
<td>open</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>48.1</td>
<td>5.6</td>
<td>121±5</td>
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<tr>
<td>10</td>
<td>37.8</td>
<td>7.3</td>
<td>242±4</td>
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<tr>
<td>15</td>
<td>34.4</td>
<td>24.9</td>
<td>364±3</td>
</tr>
</tbody>
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Fig. 4. Effect of $\text{La}_2\text{O}_3$ content on the microstructure of BHA-BBG composites.

Fig. 5. Effect of $\text{La}_2\text{O}_3$ content on pore size distribution for BHA-BBG composites.

Fig. 6. Effect of $\text{La}_2\text{O}_3$ content on particle size distribution for BHA-BBG composites.
The compression strength and microhardness values (Table 1) of the composites increase with the \( \text{La}_2\text{O}_3 \) doping content, and correlate well with the total porosity trend. The values of compression strength and microhardness recorded for prepared \( \text{La}_2\text{O}_3 \)-doped bioceramics are closed to that of human bone [36].

It was established that the solubility of \( \text{BHA-BBG} \) samples in physiological solution (0.9 % NaCl) after 2 days does not depend on \( \text{La}_2\text{O}_3 \) content (0.4 wt.%). However, that solubility is around 2 times higher than the solubility of undoped composites (0.18 wt.%) [24, 37].

The solubility tests in seawater revealed that dissolution of composite samples occurs only after 14-days of soaking. One can notice that the solubility of the doped composites depends on the \( \text{La}_2\text{O}_3 \) content (Figure 7). An increasing \( \text{La}_2\text{O}_3 \) doping content leads to the decrease of \( \text{BHA-BBG} \) solubility. This behavior can be connected to the total porosity of the samples, which decreases with the increasing \( \text{La}_2\text{O}_3 \) doping levels.

**4. CONCLUSIONS**

The results indicated that the \( \text{La}_2\text{O}_3 \) addition promotes the interaction between the crystalline (BHA) and amorphous (BBG) phases at the sintering temperature of 800 °C. The formation of mixed BHA-BBG secondary phases, such as rhenanite NaCaPO₄ and calcium silicate Ca₂SiO₄ has been observed. Moreover, \( \text{La}_2\text{O}_3 \) acts as a network modifier into the glass structure of increases the viscosity during sintering. According to the SEM analysis, it was established that an increasing \( \text{La}_2\text{O}_3 \) content leads to the decrease of pore and particle sizes from 15-240 \( \mu \text{m} \) and 0.3-6 \( \mu \text{m} \) to 5-80 \( \mu \text{m} \) and 0.09-2 \( \mu \text{m} \), respectively. The in vitro studies showed that the solubility of \( \text{La}_2\text{O}_3 \)-doped \( \text{BHA-BBG} \) composites depends on the \( \text{La}_2\text{O}_3 \) content. The compression strength (25-110 MPa) and microhardness values (121-364 HV) of composites correlates with the total porosity evolution, which decreased from 48.1 % to 34.4 % with the increasing of \( \text{La}_2\text{O}_3 \) content.

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