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Bioactive glass/hydroxyapatite composites: Mechanical properties and biological evaluation

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ABSTRACT

Bioactive glass/hydroxyapatite composites for bone tissue repair and regeneration have been produced and discussed. The use of a recently developed glass, namely BG_Ca/Mix, with its low tendency to crystallize, allowed one to sinter the samples at a relatively low temperature thus avoiding several adverse effects usually reported in the literature, such as extensive crystallization of the glassy phase, hydroxyapatite (HA) decomposition and reaction between HA and glass. The mechanical properties of the composites with 80 wt.% BG_Ca/Mix and 20 wt.% HA are sensibly higher than those of Bioglass® 45S5 reference samples due to the presence of HA (mechanically stronger than the 45S5 glass) and to the thermal behaviour of the BG_Ca/Mix, which is able to favour the sintering process of the composites. Biocompatibility tests, performed with murine fibroblasts BALB/3T3 and osteocites MLO-Y4 throughout a multi-parametrical approach, allow one to look with optimism to the produced composites, since both the samples themselves and their extracts do not induce negative effects in cell viability and do not cause inhibition in cell growth.

1. Introduction

One of the most interesting research areas of materials science deals with the development of bioceramics for bone reconstructive surgery, aiming to restore functions of damaged calcified tissues of the body. Examples comprise periodontal treatments and dental fillings as well as spinal surgery, cranio-maxillofacial reconstruction, joint replacement, fracture treatment and, in particular, healing of deteriorated bone or bone defects with bone-graft substitutes [1–3]. An ideal bone-graft substitute must be able to integrate with the remaining bone, thus providing structural stability with neo-ossification through osteoconduction and osteoinduction [4]. Moreover, the artificial substitute is gradually replaced by a new tissue with no transient loss of mechanical support. Among bioceramics, hydroxyapatite (HA) is the most similar material to the mineral component of bones; it exhibits biocompatibility, osteoconductivity and bioactive behaviour, being able to bond to the bone directly. For these reasons, HA has been employed in powder form or granules in many oral, maxillofacial and orthopaedic applications [5,6], and to realize bioactive coatings on metal implants in order to provide them with an osteophilic surface, which promotes a stronger bone-to-implant bonding [7,8]. Unfortunately, although HA is bioactive, its reactivity with existing bone tissue is low. In fact, HA implants suffer minimal degradability in physiological environment and they cannot be resorbed or replaced by a newly formed bone [1]. For these reasons, the failure of HA-based implants may occur through the fracture of the HA-bone interface or the implant itself. One promising way to deal with these problems is to reinforce HA by adding a bioactive glassy phase in order to obtain a composite. Bioactive glasses, in fact, are characterized by a higher bioactivity index compared to HA, and specific compositions, such as the widely used 45S5 Bioglass®, are able to bond to soft tissues as well as to hard ones [6]. It is reported that glass-reinforced HA shows greater biological activities with respect to commercial HA [9,10]. This occurs because silicate-based glasses release critical concentrations of ions (such as Si, P or Ca) *in vivo*, which are able to stimulate bone formation and to play an important role both in angiogenesis and in neo-vascularization [11,12]. The improvement in the mechanical properties of HA associated with addition of bioglass is also documented by several authors [13,14]. Probably the main drawback of employing bioglasses in HA-based composites is the high temperature (1200 °C ÷ 1300 °C) required to sinter these samples, which may cause several adverse effects such as reactions between HA and glass, with the formation of new undesired phases, the decomposition of HA itself [15,16] and the crystallization of the glass, which is expected to decrease or even to inhibit the bioactivity of the final system [17–19]. In order to face these issues, new bioactive glasses with a high thermal stability are required. With this aim, a CaO-rich bioactive glass, named BG_Ca, was tested in previous works to produce HA-based composite materials with HA contents ranging from 50 wt.% to 80 wt.% [20,21]. The relatively high CaO-to-Na_2O ratio in the

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BG_Ca composition (4.6 mol% Na_2O, 45.6 mol% CaO, 2.6 mol% P_2O_5, 47.2 mol% SiO_2) was indeed responsible for a substantial reduction in the tendency to devitrify with respect to the "gold standard" 45S5 Bioglass® [22], thus facilitating the production of HA-based composites. Now, as a further improvement, the present contribution proposes to work with a potassium-modified formulation of the precedent BG_Ca, which is the new BG_Ca/Mix glass (2.3 mol% Na_2O, 2.3 mol% K_2O, 45.6 mol% CaO, 2.6 mol% P_2O_5, and 47.2 mol% SiO_2,) [23,24]. In fact, the presence of potassium oxide in the glass composition improves even more the thermal stability of the glass, thus increasing its crystallization onset temperature with respect to the starting BG_Ca and a fortiori with respect to 45S5 Bioglass®. As a matter of fact, the DTA performed on the BG_Ca/Mix powders revealed a glass transition temperature of about 720 °C and a crystallization temperature of 880 °C [23,24], while 45S5 Bioglass® starts its crystallization at temperatures as low as 610 °C [17–19]. The presence of potassium in the glass is expected to exert a positive effect also in terms of biological response of the implant. The inorganic phases of hard tissues are mainly composed of calcium and phosphorous, but they also contain considerable amounts of sodium, magnesium, CO_3^{2-}, fluorine and indeed potassium [25,26]. For this reason, in the past potassium has been extensively used both in glasses and glass-ceramics for biomedical applications [27–30]. The role of the aforementioned modifying elements and ions is even more relevant when bioactive glasses are mixed to synthetic HA in order to produce composites. In this case, several ionic substitutions may occur within the HA lattice, such as F^- for PO_4^{3-} or Na^+ for Ca^{2+}, thus giving rise to crystalline defects and microstresses which modify the original HA solubility, thus mimicking the behaviour of biological apatites, which are more reactive than synthetic ones. In the last decades, the use of phosphate and silicate glasses with the addition of MgO, CaF_2, Na_2O and K_2O has been widely exploited aiming to incorporate such ions within HA, in order to develop innovative composites with composition similar to that of the mineral part of bone [31–33].

In a recent introductory contribution, the potassium modified BG_Ca/Mix glass was tested for the first time to fabricate bioceramic composites [34]. Binary mixtures of BG_Ca/Mix and HA or β-tricalcium phosphate have been sintered at a relatively low temperature (818 °C or 830 °C depending on the sample) thanks to the peculiarities of the glass. In this way it was possible to almost preserve the glassy phase in the samples, thus obtaining binary composites which looked rather promising in terms of *in vitro* bioactivity [34]. However, the biocompatibility of such BG_composites with respect to cells was not tested.

In the present contribution, the attention has been focused on BG_Ca/Mix-HA composites and a step forward in the achievement of such materials has been taken. First of all, the formulation of the composites has been modified in order to account for the feasibility and properties of both glass-rich composites and HA-rich ones. In this sense, a first set of glass-based samples has been obtained by increasing the content of glass to 80 wt.% in order to exploit its bioactivity and its sintering capability at best. At the same time, a second set of HA-based composites containing as low as 30 wt.% BG_Ca/Mix has been discussed in order to investigate samples with a relatively low (but still sensible) amount of glass. Besides that, a substantial novelty of the present contribution is provided by *in vitro* tests performed on fibroblasts and osteocyte-like cells to determine the cytotoxicity of both types of composites.

45S5 Bioglass® powders have been also pressed and sintered in order to obtain reference samples as a term of comparison. The samples have been characterized from a mechanical point of view, using a depth-sensing micro-indentation technique to estimate the local elastic modulus and hardness. Moreover, the biocompatibility of new composites has been evaluated *in vitro* in cell culture, in comparison with 45S5 Bioglass®, throughout a multi-parametrical approach.

2. Materials and methods

2.1. Samples' preparation

BG_Ca/Mix was prepared by melting the raw materials (commercial SiO_2, $CaCO_3$, $Ca_3(PO_4)_2$, Na_2CO_3 by Carlo Erba Reagenti, Italy) in a crucible at 1450 °C for 1 h in air. Then the melted glass was cast into cold water to obtain a frit. After a 24 h drying step in an oven at 110 °C, the frit was crushed in a porcelain ball mill for 1 h and sieved to a grain size below 45 μm.

The BG_Ca/Mix glass powders were mixed with HA powders (CAPTAL® Hydroxylapatite, Plasma Biotal Ltd, UK) for 8 h in a plastic bottle using a roll shaker in order to prepare composites with the following compositions:

- 80 wt.% BG Ca/Mix and 20 wt.% HA powders ("BGCaM80");
- 30 wt.% BG_Ca/Mix and 70 wt.% HA powders ("BGCaM30").

The mixed powders were pressed at 45 MPa for 15 s using acetone as a liquid binder to obtain green bodies shaped in a disc form (12 mm of nominal diameter and 2 mm of thickness).

Several sintering tests were performed aiming to produce samples with the best compromise between adequate compactness and low crystallization of BG_Ca/Mix; in particular, the densification of the samples was monitored by measuring their volume shrinkage. The thermal treatment was set at a final temperature of 830 °C for BGCaM80 and 900 °C for BGCaM30, respectively. As previously mentioned, such temperatures led to a balance between densification and crystallization: lower temperatures were indeed associated to a partial compaction, with sensible residual porosity and poor mechanical properties (the samples could be broken by hand or easily scratched with a screwdriver), whereas higher temperatures caused a wide devitrification or even a sample deformation (overheating). It is interesting to note that the sintering temperature was lower for the BGCaM80 composite than for the BGCaM30 one, due to the higher content of glass which acts as a sintering aid. For both composite materials, the heating rate was set at 5 °C/min up to 500 °C and subsequently at 10 °C/min up to the sintering temperature. Both types of samples were heat-treated for 3 h at the maximum temperature and then extracted from the oven and left to cool to room temperature.

45S5 Bioglass® reference samples ("45S5") were also produced starting from commercial 45S5 Bioglass® powders (MO-SCI Health Care LLC, Rolla, MO, USA), which were pressed at 45 MPa for 15 s using acetone as a liquid binder. In this case, green bodies were treated at 1050 °C for 3 h, according to Ref. [35].

2.2. Microstructural and mechanical characterization

The polished cross-sections of the samples were observed with an environmental scanning electron microscope, ESEM (ESEM Quanta 200-FEI Company, Eindhoven, The Netherlands) coupled with an Energy-Dispersive X-ray (X-EDS) microanalysis system (INCA, Oxford Instruments, U.K.). The microscope was operated in a low-vacuum mode with a pressure of 0.5 Torr. The phase composition of the composites was determined by X-ray diffraction (XRD: X'Pert PRO; Panalytical, Almelo, the Netherlands). Data collection was performed by a 2θ scan method in the range of 10–70° using Cu-kα X-ray line with a step size of 0.017° 2θ and scan step time of 61.59 s.

In order to investigate the local mechanical properties of the composites, depth-sensing Vickers micro-indentations (OpenPlatform, CSM Instruments, Peseux, Switzerland) were performed on the polished cross sections by applying a maximum load of 2 N for 15 s, with a loading and unloading rate of 3 N/min. In particular, for each indentation, the penetration depth curve (i.e. depth vs applied load) was automatically recorded by the instrument and analysed according to the