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A comparative in vivo evaluation of bioactive glasses and bioactive glass-based composites for bone tissue repair

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ABSTRACT

In this work a set of novel materials for bone tissue regeneration have been tested in vivo in an animal model. In fact, despite many studies have been devoted to amorphous 45S5 Bioglass®, there is lack in the literature of works aimed to study the in vivo performance of heat-treated – and thus partially crystallized – 45S5. As widely reported, crystallization limits the bioactivity of 45S5 and is the main reason that prevents a broader use of this material. Thus, in the present work, a recently developed bioactive glass (BG_Ca/Mix) is tested, since previous investigations demonstrated that BG_Ca/Mix is particularly promising by virtue of both its high bioactivity and lower tendency to crystallize with respect to 45S5. BG_Ca/Mix sintered powders and two composites, which contain BG_Ca/Mix and an increasing percentage (20 wt% or 70 wt%) of hydroxyapatite (HA), were considered. As a term of comparison, 45S5 sintered powders were also studied. The samples were implanted in rabbits' femurs and harvested after 8 weeks. The histological analysis demonstrated that BG_Ca/Mix has an osteoconductive ability slightly higher than that of 45S5 glass-ceramics, followed by that of the composites, which may represent the starting point for obtaining systems with degradation rate tailored for a given clinical application. Moreover, the 45S5 samples were locally cracked, probably because of a non-uniform dissolution in the physiological environment. On the contrary such cracks, which could lead to implant instability and unsuitable mechanical performance, were not observed in BG_Ca/Mix.

1. Introduction

The biomaterials field has grown significantly over the last 50 years, evolving through three different generations: biointert materials (first generation), bioactive and biodegradable materials (second generation) and, finally, materials designed to induce appropriate responses at the molecular level (third generation) [1]. Recent developments include hybrid and inorganic materials for delivery, therapy, sensors, etc. [2–5]; nanoparticles for drug and gene delivery [6–8], metal nanostructures for photothermal therapy [9,10], systems with antibacterial and anti-inflammatory properties [8,11,12], functionally graded materials [13], advanced bio-coatings [14–15], tissue and bone substitutes [16,17]. In this context, the pathologies associated with the musculoskeletal system involve hundreds of millions of people all over the world. The need for new surgical materials to treat musculoskeletal infirmities and, in particular, bone loss due to cancer, trauma, osteoporosis and jaw atrophies, has focused the interest of materials' scientists on calcium phosphate ceramics (CPs), which currently play a fundamental role in orthopaedics, hand surgery, maxillofacial and oral surgery [18,19]. In fact, thanks to their high biocompatibility, CPs are ideal candidates for the realization of prostheses, orthopaedic devices and synthetic bone grafts. Among CPs, hydroxyapatite (HA) is probably the most widely used material, by virtue of its close crystal and chemical resemblance to the inorganic component of biological hard tissues (i.e., bone and teeth). In fact, HA is highly osteoconductive and it is able to form a strong bond with the surrounding living bone [20].

Bioactive glasses are an attractive alternative to HA, as they are typically able to bond to bone more rapidly than other bioceramics. 45S5 Bioglass® [21], a degradable glass in the Na_2O–CaO–SiO_2–P_2O_5 system, was the first bioactive glass discovered. Bioactive glasses have unique properties, if compared to CPs and HA. In addition to their high reactivity in vivo, in vitro studies reported that the ions released during the 45S5 Bioglass® dissolution (e.g. Si, P, Ca) seem to induce angiogenesis, neo vascularisation and stimulate osteoblasts proliferation and new bone growth [22]. Moreover, certain compositions of bioactive glasses are able to bond to both bone and soft connective tissues [23].

Probably, the main disadvantage of Bioglass® and, in general, of bioactive glasses, is their tendency to crystallize during the heat treatments which are necessary for the production of special products, such as scaffolds, coatings and composites with CPs as second phase. In fact,

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crystallization may reduce the bioactivity of the final system [24]. For these reasons, the investigation of new bioactive glasses with low tendency to devitrification, to be used whenever a heat treatment is necessary, is particularly important. Among such novel compositions, the so-called BG_Ca/Mix (47.3 mol% SiO_2, 45.6 mol% CaO, 2.3 mol% K_2O, 2.3 mol% Na_2O, and 2.6 mol% P_2O_5) [25,26], which has been developed in recent years, looks particularly promising: while a crystallization temperature of about 650 °C is reported for Bioglass® [27], BG_Ca/Mix starts to crystallize at temperatures as high as 880 °C. Moreover, BG_Ca/Mix is characterized by a slower ion leaching in physiological solution with respect to Bioglass®, thus determining pH values which can be considered optimal for cell adhesion, proliferation and differentiation [25].

In previous works, BG_Ca/Mix has been successfully used to deposit coatings and, in particular, for the realization of HA-based composites containing up to 80 wt% of glass [28–30]. The production of HA/bioactive glass composites is especially interesting, as it is possible to overcome the intrinsic limits of the glassy and the ceramic phases when considered singularly. The main objective is to tailor the biodegradation rate and the bioactivity of the resulting system by varying the volume fractions of the two constituents. Moreover, the use of bioactive glasses can be exploited to incorporate ions of biological interest within HA lattice, such as silicon, strontium, magnesium, etc., in order to simulate the behaviour of the so-called biological apatite, i.e. the mineral part of bone, which is typically nonstoichiometric and characterized by several ionic substitutions [18,31]. Thanks to the peculiarities of BG_Ca/Mix, it was possible to sinter the HA-based composites at lower temperatures with respect to samples with the same HA/Bioglass® ratio, thus reducing the crystallization of the glassy phase and avoiding the decomposition of HA and/or reactions between HA and glass. The in vitro biocompatibility of both BG_Ca-Mix and of HA/BG_Ca-Mix composites has been successfully proved in recent investigations [28,32].

In the present work, for the first time, the novel BG_Ca/Mix (sintered powders) and the HA/BG_Ca-Mix composites have been tested in vivo in an animal model. The samples were implanted bilaterally in the midshafts rabbit femurs and harvested after 8 weeks. Two composites with an increasing content of HA were considered. It should be noted that, although the literature reports several studies dealing with HA-based composites with phosphate bioglasses as second phase, only in the last years silicate glasses, such as Bioglass®, have been employed in combination with HA to realize biocomposites, by virtue of their osteoinductivity. Therefore, in the literature, there is lack of investigations dealing with these systems and based on animal models. Furthermore, in this work also 45S5 sintered powders were implanted and considered as a term of comparison. In fact, the in vivo behaviour of 45S5-derived glass ceramics is fundamental in order to evaluate the feasibility of specific implants, such as 45S5 scaffolds, whose production may require the consolidation of glass powders. Although in the last 40 years innumerable studies have been devoted to amorphous 45S5 [24,33], only in recent years the efforts of several researches have been addressed to understand the connections between sintering, crystallization and in vivo bioactivity of heat-treated 45S5.

2. Materials and methods

2.1. Bioactive glasses and composites preparation

Bioactive glass powders were produced by melting the commercial raw materials (SiO_2, $Ca_3(PO_4)_2$, $CaCO_3$, Na_2CO_3, all reagent grade - Carlo Erba Reagenti, Italy; to prepare BG_Ca/Mix, part of Na_2CO_3 has been replaced by K_2CO_3) in Pt crucibles at 1450 °C. The following thermal cycle was employed: from room temperature to 1100 °C at 10 °C/min; an isothermal step at 1100 °C for 1 h to allow decarbonation; from 1100 °C to 1450 °C at 10 °C/min. The molten glasses were quenched into water to obtain two frits, which have been left to dry in an oven at 110 °C for 24 h. The 45S5 and BG_Ca/Mix frits have been subsequently crushed in dry conditions in a porcelain jar and finally sieved to obtain a powder (grain size < 67 μm). Subsequently, 45S5 and BG_Ca/Mix powders were wetted with acetone and pressed to produce green bodies. As previously reported in refs [25,34], 45S5 and BG_Ca/Mix green bodies were sintered for 3 h at 1050 °C and 800 °C, respectively. A heating rate of 10 °C/min was used for both glasses. At the end of the thermal treatments the samples have been extracted from the oven and left to cool down at room temperature.

The BG_Ca/Mix glass powders were mixed with commercial HA powders (CAPTAL® Hydroxylapatite, Plasma Biotal Ltd., UK) for 6 h in a polyethylene bottle using a roll shaker to obtain the following set of composites:

- 80 wt% BG_Ca/Mix and 20 wt% HA powders ("80BG20HA");
- 30 wt% BG_Ca/Mix and 70 wt% HA powders ("30BG70HA").

80BG20HA and 30BG70HA powders were then wetted with acetone and pressed to produce the green bodies, which have been heat-treated for 3 h at 830 °C and 900 °C, respectively, as reported in previous works [28]. Finally, the fully dense bodies (45S5, BG_Ca/Mix and composites) were abraded to obtain samples in form of prismatic rods (~6 × 2 × 2 mm), which were subsequently sterilized in ethylene oxide before implantation.

2.2. Animals and surgery

Eight healthy six-months-old white New Zealand rabbits (Harlan Laboratories S.r.l., Correzzana, Monza e Brianza, Italy) with an average body weight of 5 kg were used. The animals were maintained for acclimation to housing conditions with food and water ad libitum at least for one week before surgery. The night before surgery, each animal was fasted to be ready for anesthesia. The experiments were carried out according to the Bioethical Committee of the Italian National Institute of Health and authorized with Decrees of the Italian Ministry of Health (Protocol Number: 210/2013-B). Animal care, maintenance, and surgery were conducted in accordance with Italian law (D.L. no. 26/2014) and European legislation (EEC no. 63/2010).

The animals were weighed and submitted to the same surgical procedure under general anesthesia with a mixture of xylazine (4 mg/kg body weight) (Sedaxylan®, Dechra Veterinary Products S.r.l., Turin, Italy) and ketamine (30 mg/kg body weight) (Imalgene 1000®, Merial Italia S.p.A., Milan, Italy). If necessary, further sedation was obtained by means of propofol (7 mg/kg) (Propovet®, Ecuphar S.r.l., Piacenza, Italy) administrated in the marginal ear vein.

After induction of anesthesia, shaving and antisepsis were carried out on the legs to be operated. A 3 cm long skin incision was made on the antero-lateral surface of the tight; after blunt dissection of muscles, the proximal diaphysis of the femur was reached. An incision was made on the periosteum by a scalpel to expose the femur cortex. Two cortical holes of 3.5 mm in diameter and 7 mm in depth in the midshafts rabbit femur were bilaterally drilled under continuous saline irrigation, using a bone trepan bur (227B.204.040; Komet Italia S.r.l., Milan, Italy; motor system and drilling procedure: run at a speed of 1700 rpm, Physiodispenser 7000, Nouvag AG; Switzerland).

Three rods of different bioactive glass/composite and one sham (without implant) were placed in the left and right femur of each rabbit, as shown Fig. 1. The shape of the samples allowed them to remain stuck in the drilled cylindrical hole, applying the stabilization criterion needed in the bone grafting procedure [35–37]. Sham holes remained empty as controls, with the aim of evaluating the spontaneous bone healing (Fig. 1(c)). The fascia-periosteal flaps were sutured by 4.0 glycolide/L-lactide copolymer (Vicryl®, Ethicon, Johnson & Johnson, Livingston, UK) and the skin with 3.0 silk (Perma-hand® Silk Suture, Ethicon, Johnson & Johnson, Livingston, UK).

Post-operatively, single intramuscular injections of antibiotics (enrofloxacin, 10 mg/kg body weight) (Baytril 5%®, 50 mg/ml, Bayer S.p.A., Milan, Italy) and analgesic (buprenorphine, 0.05 mg/kg body