Applied Surface Science 423 (2017) 557–565

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Full Length Article

Osteoconductive properties of two different bioactive glass forms (powder and fiber) combined with collagen

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ARTICLE INFO

Article history:
Received 25 April 2017
Accepted 14 June 2017
Available online 19 June 2017

Keywords:
Bioactive glasses
Collagen
Composite
Fibers
Powder
Bone repair

ABSTRACT

Bioactive Glasses (BG) is a group of synthetic silica-based materials with the unique ability to bond to living bone and can be used in bone repair. Although the osteogenic potential of BG, this material may have not present sufficient osteoconductive and osteoinductive properties to allow bone regeneration, especially in compromised situations. In order to overcome this limitation, it was proposed the combination the BG in two forms (powder and fiber) combined with collagen type I (COL-1). The aim of this study was to evaluate the BG/COL-based materials in terms of morphological characteristics, physicochemical features and mineralization. Additionally, the second objective was to investigate and compare the osteoconductive properties of two different bioactive glass forms (powder and fiber) enriched or not with collagen using a tibial bone defect model in rats. For this, four different formulations (BG powder – BGp, BG powder enriched with collagen – BGp/Col, BG fibers – BGf and BGp fibers enriched with collagen – BGf/Col) were developed. The physicochemical and morphological modifications were analyzed by SEM, FTIR, calcium assay and pH measurement. For *in vivo* evaluations, histopathology, morphometrical and immunohistochemistry were performed in a tibial defect in rats. The FTIR analysis indicated that BGp and BGf maintained the characteristic peaks for this class of material. Furthermore, the calcium assay showed an increased Ca uptake in the BG fibers. The pH measurements revealed that BGp (with or without collagen) presented higher pH values compared to BGf. In addition, the histological analysis demonstrated no inflammation for all groups at the site of the injury, besides a faster material degradation and higher bone ingrowth for groups with collagen. The immunohistochemistry analysis demonstrated Runx-2 and Rank-L expression for all the groups. Those findings support that BGp with collagen can be a promising alternative for treating fracture of difficult consolidation.

1. Introduction

A series of different artificial materials have been developed to be used as bone graft substitutes [1]. Among those, Bioactive Glasses (BG) comprise a group of synthetic silica-based materials with the unique ability to bond to living bone by forming a biologically active bone-like apatite layer on their surface [2–5]. The original BG, named Bioglass® 45S5, is a melt-derived glass with four components (46.1% SiO_2, 24.4% Na_2O, 26.9% CaO and 2.6% P_2O_5, in mol) and it is known as one of the most bioactive bone-bonding glasses. It has been used in many clinical procedures, including the repair of periodontal bone defects, maxillofacial defects reconstruction, spinal surgery and bone replacement [6,7]. Although the osteogenic potential of BG, this material may have not present sufficient osteoconductive and osteoinductive properties to allow bone regeneration, especially in compromised situations such as osteoporosis or fractures of great extension [8].

In order to overcome these limitations, many composite grafts have been developed trying to associate different characteristics from distinguished materials toward approximating the bone-forming components, providing a better environment for bone formation [9,10]. In this context, some researchers have been combining collagen to bioceramics and bioactive glasses with the intention of mimicking the organic and inorganic part of the bone tissue [11,12]. It is well known that collagen, mainly type I (COL-1),

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http://dx.doi.org/10.1016/j.apsusc.2017.06.152
0169-4332/

is the major organic component in the extracellular matrix (ECM) and this protein level is critical for mechanical and biological roles [13].

Besides the composition, another essential point for the graft success is the material structure [13]. In bone tissue engineering, BG have been used mainly in powder, blocks and scaffolds [14,15]. Although, in most cases these biomaterials serve as support of tissue formation, being able of stimulating cell adhesion and angiogenesis, they do not have the ability of acting as fillers for bone defects with irregular shapes [16]. In this context, the moldability found in fibrous materials is a desired characteristic necessary for grafts, allowing to fit irregularly shaped bone defects [17,18]. Additionally, fibrous materials are capable of supporting cell attachment, mineralization and new bone formation in the site of the defect [19,20].

Therefore, the obtainment of malleable fibers from bioactive glasses enriched with collagen seems to be a promising therapeutic approach targeting bone repair. Toward this goal, a fibrous glassy material, with four components (46.1% SiO_2, 24.4% Na_2O, 26.9% CaO and 2.6% P_2O_5, in mol) [7], have been recently developed [21]. In addition, the bioactive material was enriched with type-1 collagen in order to introduce an organic part to the ceramic.

Since there is a growing interest in the development of biomaterials with improved osteogenic properties, it was hypothesized that the introduction of collagen to BG would improve the *in vivo* bioactive properties of the material, providing a bone graft with additional advantages for clinical use. Furthermore, the hypothesis that the fibrous glassy material would present a more adequate morphology, facilitating cell migration and vascularization, was raised. Consequently, the aims of the present study were systematized into 2 points: first, to evaluate the BG/COL-based materials in terms of morphological characteristics, physicochemical features and mineralization. Second, to investigate and compare the osteoconductive properties of two different bioactive glass forms (powder and fiber) enriched or not with collagen using a tibial bone defect model in rats. To this end, samples in 4 different compositions (BG powder: BGp; BG powder enriched with collagen: BGp/COL; BG fibers: BGf; and BG fibers enriched with collagen: BGf/COL) were analyzed by scanning electron microscopy (SEM), x-ray diffraction (XRD), calcium deposition and pH. Also, the biomaterials were implanted into non-critical tibial defects in rats. Histopathological, histomorphometry and immunohistochemistry analyzes were evaluated after 15 days of implantation.

2. Materials and methods

2.1. Materials

2.1.1. BG powder

For the obtainment of BG powder, mineral silica 98.0 wt% powder was purified by attack with hot hydrochloric acid (Merck, P.A.) followed by filtration (fast paper filtration Whatmman 40) and held 30 washings with boiling distilled water for elimination of impurities (R_2O_3) and analyzed by XRF to ensure 99.5 wt% purity. Additionally, the following reagent analytical-grade as sodium hydroxide (NaOH 97.0 wt%, heavy metals ≤ 0.003 wt%, Cl- ≤ 0.005 wt%, Fe ≤ 0.001 wt%, Hg ≤ 0.1 ppm, K ≤ 0.02%, Na2CO3 ≤ 1.0 wt%, NH4OH ≤ 0.02 wt%, Ni ≤ 0.001 wt%, PO43- ≤ 0.001 wt%, SO42- ≤ 0.003 wt%, absorbed water ≤ 2.0 wt%; Nuclear, São Paulo, Brazil), Calcium oxide (CaO 97.0 wt%, heavy metals ≤ 0.005 wt%, Cl- ≤ 0.05 wt%, SO42- ≤ 0.5 wt%, Fe ≤ 0.5 wt%, insolubles ≤ 0.01 wt%, absorbed water ≤ 2.0 wt%; Química Moderna, São Paulo, Brazil), Sodium Phosphate (Na_3PO_4 99.0 wt%, heavy metals ≤ 5 ppm, insolubles ≤ 0.01 wt%, SiO_4 0.005 wt%, PO_4^{3-} ≤ 0.001 wt%, Fe ≤ 5 ppm, Na_2CO_3 ≤ 0.02 wt%, NH_4OH ≤ 0.01 wt%, Ca and Mg ≤ 0.01 wt%, SO_4^{2-} ≤ 0.004 wt%, Cl- ≤ 0.1 ppm; Química Moderna, São Paulo, Brazil) were also used. The chemicals were weighed and mixed for 30 min in a polyethylene bottle. Premixed batches were melted in an alumina crucible at a temperature of 1500 °C (Lindberg Blue vertical super kanthal furnace – USA). The melting time was fixed as 2 h. Samples were quenched in deionized water and milled to powder grain (with sizes ranging from 260 to 600 μm). No annealing was performated.

2.1.2. BG fibers

BG fibers were obtained using the same process described above. However, in spite of quenching in water, the melt was quenched in a Hager-Rosengarth apparatus [21,22]. The Hager-Rosengarth process leads the melt to a centrifugal acceleration, leaving the centrifuging disc and cooled in air which is flowing together with the fiber due to this angle speed [23].

2.1.3. Collagen

Collagen type I used in this study was provided by Consulmat (São Carlos, Brazil). Type I collagen from bovine bone was obtained in three main steps: (i) demineralization of bovine cortical bone in chloridric acid (HCl); (ii) dissolution of the cortical bone collagen in 0.5 M acetic acid ($C_2H_4O_2$) at 40 °C and (iii) pH adjustment with ammonium hydroxide (NH_4OH). Granules <270 μm were obtained.

2.2. Composite preparation

2.2.1. Enrichment of BG powder with COL

Layers of COL and BG powder were alternately deposited on a substrate (Petri dish) insomuch that the resulting particles were completely coated by COL. Samples were dried during 48 h in an oven at 40 °C and the composite was milled to obtain powder.

2.2.2. Enrichment of BG fibers with COL

For the fibrous composition enriched with COL, the collagen solution was homogeneously sprayed on the fibers tappet until it had acquired the wet appearance. Subsequently, the material was dried for 48 h at 40 °C.

2.2.3. Material sterilization

Before use, the materials were sterilized using a 25 kGy dose of gamma irradiation (IPEN, São Paulo, Brazil).

2.3. Characterization of the materials

2.3.1. Morphological characteristics

The morphology of the different materials was first examined by SEM observation (Phenom™, FEI Co.). The composites were mounted on aluminum stubs using carbon tape and sputter-coated with gold/palladium prior to examination.

2.3.2. Fourier transform infrared spectroscopy (FTIR)

FTIR (Thermo Nicolet Nexus 4000, USA) was performed to evaluate the chemical bonds present in the materials. The examinations were done in the range of 400–1800 cm^{-1} with a resolution of 2 cm^{-1}. The samples were scanned 128 times for each measurement and the spectrum acquired was the average of all these scans.

2.3.3. Calcium assay

The Ca release/uptake capacity of the bioactive materials, BGp and BGf, was evaluated according to Kokubo and Takadama [24]. Samples (0.05 g) were placed in glass vials containing 3 mL of Simulated Body Fluid (SBF) at 37 °C on a shaker table (70 Hz) for up to 5 days, with refreshment on days 1, 3 and 5. Subsequently to each refreshment, the solution of the previous period was saved for analysis of the calcium content in SBF by the orthocresolphtalein