DENTAL MATERIALS XXX (2018) XXX–XXX

Available online at www.sciencedirect.com

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Niobium addition to sol-gel derived bioactive glass powders and scaffolds: In vitro characterization and effect on pre-osteoblastic cell behavior

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ARTICLE INFO

Article history:
Received 28 March 2018
Received in revised form
14 May 2018
Accepted 7 June 2018
Available online xxx

1. Introduction

Bioactive glasses are synthetic biocompatible materials that are able to interact with host tissue due to ion release when in contact with living tissues. Both composition and microstructure of glasses are important for its ability to promote bone deposition [1]. Sol–gel route has been used as an easier and successful route to produce both bioactive glass powders [2] and porous tridimensional structures [3]. Bioactive glass powder synthetized by sol–gel route results in particles with superficial porosity and high surface area, while gel cast foaming has been shown to produce scaffolds with controlled porosity and pore size to mimic bone structure allowing material to act as a framework for deposition of new tissue [4].

The release of ions in the moment of implantation is the most important feature to ensure materials bioactivity as it influences cell activity regulating cell proliferation, differentiation and gene expression [5–7]. The role of dissolution products has been investigated for bioactive glasses and is shown to be a key mechanism to the ability of osteoinduction in this class of materials [8,9]. The release of different ions may stimulate distinct reactions [7,10–12]. Niobium (Nb) is a

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https://doi.org/10.1016/j.dental.2018.06.014

metal that has showed high biocompatibility in several studies in medical materials showing potential to increase bioactive glass properties [13–17]. Niobium addition to biomaterials has shown successfully results being associated to reduction in cytotoxicity [18] and increase in alkaline phosphatase activity [19], increasing calcification in bone defects and improving biological properties in metallic surfaces [20,21]. The addition of Niobium to sol–gel derived bioactive glasses could represent an alternative to increasing in bone deposition. However, no study attempt to use Niobium in a sol–gel derived bioactive glasses. For this reason, the aim of this study was to synthetize bioactive glass powders and scaffolds through sol–gel route with Niobium addition.

2. Materials and methods

2.1. Material's synthesis

Niobium was added to bioactive glasses during mixture of precursors Niobium Chloride ($NbCl_2$ — 0.4 g) was mixed to 8.3 ml ethanol and 0.17 ml distilled water and kept under stirring for 20 min. Sol was prepared by mixing Tethaethilorthosilicate (TEOS, $Si(OCH_2H_5)_4$ — 33.5 ml), Thriethilphosphate (TEP, $(C_2H_5)_3PO_4$ — 2.9 ml), Calcium Nitrate ($Ca(NO_3)_2$ — 20,13 g), Sodium Nitrate ($NaNO_3$ — 13.52 g) were added to 250 ml of Nitric Acid (HNO_3) 1 M solution under stirring during 45 min each. For powder synthesis the obtained sol was stored during 5 days in room temperature until complete gelification. Gel was submitted to ageing in 70 °C for 24 h, drying in 120 °C for 24 h and kept for 24 h at 700 °C to stabilization and nitrates vaporization.

To produce glass scaffolds, sol was mixed with sodium lauryl sulphate (SLS — 1.50 ml) and Fluoridric Acid (HF5% — 3.00 ml) under vigorous agitation during 10 min until the gel point. Porous gels were stored during 5 days at 18 °C for further gelification. Gels were divided in cylindrical samples (6 mm diameter × 8 mm height) and were submitted to a heat treatment at 70 °C, 120 °C and 700 °C for 24 h each. After heating the final size of the samples was 3 mm diameter × 4 mm height.

2.2. X-ray diffraction

X-Ray diffraction was performed for powders and scaffolds. The diffractometer (X'PertPRO,PANalytical MPD, Netherlands) was operated using CuKα radiation at 40 kV–40 mA at angular range between 5° e 100°, with step size 0.02° during 2 s.

2.3. Raman analysis

Powders and scaffolds were analysed with Raman Spectroscopy (SENTERRA model, Bruker Optics, Germany) operated with a diode laser of 785 nm wavelengths, 100 mW of intensity, for 5 s and 2 co-additions. The range of analysis was 400–1800 cm^{-1}.

2.4. Morphology

Scanning electron microscopy was used to analyse powders and scaffolds morphology. Samples were gold coated, placed in an aluminium sample holder and analysed in an electronic microscope (Jeol JSM-6060 Germany) with 10 kV acceleration voltage.

2.5. pH

Glass were immersed in 200 ml of deionized water or 1 M tris(hidroximetil)aminometano (TRIS) solution. Glasses were immersed in both solutions and kept under stirring and at room temperature. A digital pHmetre (D-22 Digimed-São Paulo, São Paulo Brazil) was used to measure the initial pH measurement of solution as well as the measurements in the following moments: 0.5, 1, 2, 5, 15, 30, 60, 120, 240, 360 min and 1, 2, 7 and 14 days. For each measurement 10 ml of the solution was taken and immediately filtered in a 22 μm filter.

2.6. Specific surface area and particle size

Nitrogen adsorption measurements were carried out using an Autosorb Quantachrome Nova 1200 (Quantachrome Instruments Corporate Headquarters, USA) instrument. Powder specific superficial area was calculated by Brunauer–Emmett–Teller (BET) method based on the Nitrogen adsorption isotherm data. Particle size distribution was assesses using a laser diffraction particle size analyzer (CILAS 1180, France).

2.7. X-ray computed microtomography

Specimens were scanned using a high-resolution micro-CT system (SkyScan 1272; Bruker microCT, USA). The scanner operated at 70 kV and 142 mA (0.5 mm Al filter) with 1224 resolution. For each specimen, evaluation was performed in 200 slices. CTAn v.1.14.4.1 software (Bruker-Massachussets, USA) was used for the 3-dimensional (3D) quantitative analysis of porosity (%), pore size (μm), connectivity density ($1/mm^3$), scaffold surface/volume ratio (1/mm), trabecular thickness (μm) and trabecular separation (μm) of each specimen.

2.8. In vitro cell behaviour

MC3T3-E1 cells (Banco de Células do Rio de Janeiro, Rio de Janeiro, Brazil) were cultured in alpha minimum essential medium (α-Men-Thermo Fisher Scientific, USA) supplemented with 10% foetal bovine serum (FBS — Thermo Fisher Scientific, USA) and 1% Penicillin (Thermo Fisher Scientific, USA) and stored in a 5% CO_2 at 37 °C. Medium was changed every 3 days. To test cell behaviour, conditioned medium was produced upon immersion of two concentration (2,5 mg and 5 mg) of powders and one size of scaffold (3 mm diameter × 4 mm height) in 1 ml of supplemented culture medium during 24 h at 37 °C. For all cell studies, three specimens were used for each group and all tests were performed in triplicate. Both powders and scaffolds were sterilized in autoclave prior to immersion.

2.8.1. Cell proliferation

Sulphoronamide-B (SRB) assay was used. Cells (5×10^3) were seeded in 96-well plates and after 24 h each well was treated with 100 μl of conditioned medium. After 72 h in culture,

Please cite this article in press as: Balbinot GdS, et al. Niobium addition to sol-gel derived bioactive glass powders and scaffolds: In vitro characterization and effect on pre-osteoblastic cell behavior. Dent Mater (2018), https://doi.org/10.1016/j.dental.2018.06.014