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Antibacterial properties of laser spinning glass nanofibers

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ABSTRACT

A laser-spinning technique has been used to produce amorphous, dense and flexible glass nanofibers of two different compositions with potential utility as reinforcement materials in composites, fillers in bone defects or scaffolds (3D structures) for tissue engineering. Morphological and microstructural analyses have been carried out using SEM–EDX, ATR-FTIR and TEM. Bioactivity studies allow the nanofibers with high proportion in SiO_2 (S18/12) to be classified as a bioinert glass and the nanofibers with high proportion of calcium (ICIE16) as a bioactive glass. The cell viability tests (MTT) show high biocompatibility of the laser spinning glass nanofibers. Results from the antibacterial activity study carried out using dynamic conditions revealed that the bioactive glass nanofibers show a dose-dependent bactericidal effect on *Sthaphylococcus aureus* (*S. aureus*) while the bioinert glass nanofibers show a bacteriostatic effect also dose-dependent. The antibacterial activity has been related to the release of alkaline ions, the increase of pH of the medium and also the formation of needle-like aggregates of calcium phosphate at the surface of the bioactive glass nanofibers which act as a physical mechanism against bacteria.

The antibacterial properties give an additional value to the laser-spinning glass nanofibers for different biomedical applications, such as treating or preventing surgery-associated infections.

1. Introduction

Bioactive glasses (BGs) are inorganic materials with interesting properties in biomedicine (Kokubo, 2008). Applications in the areas of orthopedics, dentistry or tissue regeneration have been developed on the basis of their ability to react in the presence of biological fluids leading bioactive intermediate stages and favoring the formation of new tissues (Hench and Wilson, 1993). An interesting property in the biomedical field, which has been described for some bioactive glasses, is the capacity of inhibiting growth or even killing different bacteria (Stoor et al., 1998; Munukka et al., 2008). There is a wide range of literature analysing the effect of the composition, particle size or morphology of the bioactive glasses on their antibacterial activity. However, the great variability of conditions used to perform the studies (e.g., bacteria studied, microbiological conditions, glass composition and morphology, etc.) hinders the specific factors influencing bactericidal capacity to be determined. In a previous paper, we have collected historical data and analysed them by a process named data mining using an artificial intelligence technique (neurofuzzy logic) (Echezarreta-López and Landin, 2013). Our results allowed us to conclude that the antibacterial activity is mainly determined by SiO_2 content, the release of alkaline ions to the medium and the increase of pH of the medium.

In the last few decades many researchers have established several methods to obtain improved bioactive glasses (Jones, 2013). The long term benefits of nanofeatures in those fields have been pointed out by different authors. Sato and Webster have shown the importance of nanostructures in orthopedic applications, suggesting that nanophase materials promote new bone formation due to the similarity with the nanometric dimensions of the bone tissue components (Sato and Webster, 2004). Biomaterial–biological interactions are related to modifications in the surface at nanometric level with important consequences in their applicability in tissue regeneration (Khang et al., 2010; Chiara et al., 2012) or dental integration (Bressan et al., 2013). Quintero et al. have developed a novel technique of producing glass fibers of nanometric diameters with specific and controllable chemical compositions using a laser spinning procedure. The developed technique allows the rapid

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production of dense glass nanofibers that can be used as bioactive reinforcement materials in composites, fillers in bone defects or scaffolds (3D structures) for tissue engineering (Quintero et al., 2009a).

On this basis, the aim of this study is to evaluate, for the first time, the potential antibacterial properties of the nanofibers of different compositions produced by the laser spinning technique. The two materials studied include, a high silicon content glass nanofibers (S18/12) and a high calcium content glass nanofibers (ICIE16). The significant variations in their composition make their dissolution process extremely different, which may have a potential effect on their antibacterial properties. Structural and surface modifications of nanofibers (SEM–EDX, ATR-FTIR and TEM) analysed before and after microbiological studies in dynamic conditions should allow the antibacterial activity to be explained.

2. Materials and methods

2.1. Glass nanofibers preparation

Glass nanofibers were obtained by a laser spinning technique as previously described by Quintero et al. (2009a). First, a blend of raw materials including soda, lime, silica and phosphorous oxide, were melted in a platinum crucible at 1500 °C and poured into a graphite mold to obtain flat plates with thickness of 5 mm and the desired compositions. The composition of these glass plates was analysed by X-ray fluorescence (XRF) obtaining the values included in Table 1. These glass plates were then employed as the precursor material for the laser spinning process. A high power CO_2 laser (Rofin Sinar DC 035) emitting, in continuous mode, a beam of infrared radiation with wavelength of 10.6 μm was focused over the glass plates to set irradiance to 1×10^5 W/cm^2 and advance speed of 10 mm/s. The assist gas employed was compressed air at 12 bar. The high speed gas jet is responsible for the extremely quick elongation and cooling of a small volume of the molten viscous material, thus high form factor fibers are formed.

Laser-spinning glasses nanofibers, as supplied, were immersed in Milli-Q water for 1 min without stirring, filtered through an acetate cellulose membrane (0.45 μm) and dried at 50 °C for 24 h.

2.2. Characterization studies

Some structural and morphological characteristics of glass nanofibers were analysed. Attenuated total reflectance-infrared spectroscopy (ATR-FTIR) analyses were performed on a 670IR Varian (USA) spectrometer equipped with a Gladi-ATR (Pike, USA). The spectra were recorded on an average of 256 scans at a resolution of 4 cm^{-1} in the 400–4000 cm^{-1} range. The glass nanofibers were analysed by FTIR before and after the antibacterial properties studies were carried out.

The surface morphology and composition modifications of glass nanofibers and bacteria were observed by scanning electron microscopy (SEM) (Leo 435VP, Cambridge, UK) with X-ray energy dispersive spectroscopy microanalysis (EDX, Oxford 300). The samples were coated with gold to eliminate charging effects. Transmission electron microscopy (TEM) (JEOL JEM-1011, Tokyo, Japan), according to Santhana Raj et al. (2007), were employed to evaluate physical action of the glass nanofibers on the bacteria.

Table 1
Laser spinning glass nanofibers composition (mol%).

Name	SiO_2	CaO	Na_2O	K_2O	P_2O_5	MgO
ICIE16	49.46	36.27	6.60	6.60	1.07	–
S18/12	71.1	9.3	12.3	0.3	–	6.4

2.3. Bioactivity studies

Bioactivity *in vitro* tests of individual samples of ICIE16 and S18/12 nanofibers were carried out by studying their dissolution process in simulated body fluid (SBF) for 5 days. The SBF solution was prepared using the standard procedure described by Kokubo et al. (1990). Six milligrams of each nanofiber was soaked in 25 mL of SBF (pH 7.35) at 37 °C. At pre-set times of 3, 6, 12, 24, 48 and 120 h, the nanofibers were washed and dried in air. Bioactivity was related to the variations of ionic concentrations in the solution with the set time. The Ca and Si concentrations in the solution were estimated by optical emission spectroscopy inductive coupled plasma spectroscopy (ICP–OES) using a PerkinElmer Optima 3300DV system (Norwalk, USA).

2.4. Biocompatibility assay

The *in vitro* cytotoxicity tests for glass nanofibers were performed on extracts prepared by elution of the samples (75 mg/mL) in Dulbecco's modified Eagle's medium (DMEM) (GIBCOTM), supplemented with 10% fetal bovine serum (FBS) and 1% gentamicin in duplicate at 37° for 24 h and 48 h. Cell viability tests were performed with BALB/3T3 cell line (CCL 163, ATCC, USA), according to the 10993–5 protocol of the International Standardization Organization (ISO). A cell suspension of 2×10^4 cells/well in 200 μl of DMEM was added into a 96-well plate and allowed to grow. After 24 h or 48 h, different concentrations of ICIE16 and S18/12 glass nanofibers extracts (0.5, 1 and 2%) were added to the cells and the plate was incubated at 37 °C in a humidified atmosphere with 5% CO_2. A control (cells without glass nanofibers extracts) was treated in the same way. The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay was used in this study to measure cell viability. MTT assay measures intracellular mitochondrial activity of the cells, which involves reduction of MTT by intracellular dehydrogenases of viable cells to blue formazan. Cells survival was evaluated through the measurement of absorbance at 550 nm using a microplate reader (BIORAD Model 680, Hercules, CA, USA). Each experiment was carried out in duplicate.

2.5. Antibacterial properties studies

The antibacterial properties of the laser-spinning glass nanofibers were evaluated in dynamic conditions using a Gram positive bacterial strain, *Sthaphylococcus aureus* CECT 240.

S. aureus was incubated in a tryptic soy broth (TSB) medium (Oxoid, Drongen, Belgium) pH 7.3 to reach a density of about 10^6 cells/mL. Then 100 μL of this culture were inoculated into an eppendorf tube containing 900 μL of fresh TSB medium and glass nanofibers. Bacteria cultured without glass nanofibers were used as a negative control. Different concentrations (5, 25 and 75 mg/mL) of pre-washed and sterilized nanofibers were incubated for 4 days at 37 °C in an Erlenmeyer flask sealed with Parafilm® M under agitation in an orbital shaker at 200 rpm. 50 μL were taken from this culture every 24 h, 10 μL were used to check bacterial presence using SEM and the rest to perform serial dilutions. Aliquots of 50 μL of dilutions 10^{-4}, 10^{-5} and 10^{-6} were plated in triplicate on tryptic soy agar (TSA) medium and incubated at 37 °C for 24 h. After counting plate colonies, a bacterial growth index (GI) was established according to the following parameters: Level 4: >>>300 colonies (countless); Level 3: >300 colonies (countable); Level 2: 30–300 colonies; Level 1: 0–30 colonies; Level 0: no colonies.

Additionally, pH medium was measured (pH Meter GLP22 Crison, Spain) at each set time.