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Anti-bacterial glass formulations for bone tissue engineering applications

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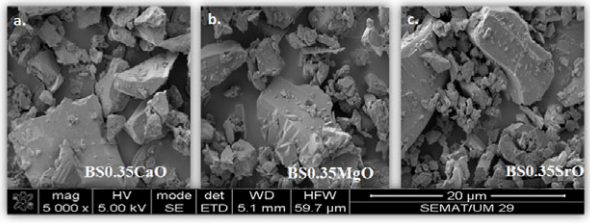
Introduction: One of the major problems in Bone Tissue Engineering (BTE) is the high probability of occurring bacterial contamination during preparation and surgical procedures. Bacterial strains such as *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* proved to be hard to eliminate from bone grafts [1]. Silica-based bioglasses, namely the silica-borate system, can take advantage of the well-known boron antibacterial properties [2, 3]. In fact, bioglasses have been used in BTE for several decades due to their biological activity and mechanical performance. The aim of this study is to evaluate the anti-bacterial properties of silica-borate glass formulations to use them within a BTE perspective.
Materials and methods: Silica-borate glass compositions of general formula $0.20B_2O_3$: $0.40SiO_2$: xMgO: yCaO: $(0.35\text{-}x\text{-}y)$SrO: $0.05Na_2O$ (molar ratio, where x, y = 0.35 or 0.00, and $x \neq y$) were synthesized by melt quenching. The glass particles were ground and sieved to collect the particles with a size <63 μm. Glass particles' cytotoxicity was assessed by direct contact with human osteosarcoma cell line during 3 days of incubation (37°C and 5% CO_2). At different time points cell proliferation (DNA) and metabolic activity (MTS) was determined. Microbial susceptibility was measured using standardized inocula of *P. aeruginosa* ATCC27853, *E. coli* CECT 434, *S. aureus* ATCC 29213 in contact with glass samples. *Preliminary studies were performed using a*dapted EUCAST Diffusion Method for 24 hours exposure. Broth dilution method was altered and performed by adding known amounts of glass particles directly in bacterial cultures for 24 hours period. After it colony forming units (CFUs) was assessed. All experiments were performed at least in triplicate.
Results: Silica-borate glass compositions were synthesized by melt quenching (≈ 1300°C) and successfully ground to a particle size <63 μm (Fig. 1). Cytotoxicity assessment results allow us to state that cells remain viable during the 3 days of culture, while preliminary diffusion tests (executed to evaluate the antibacterial capacity of the glass compositions against *P. aeruginosa* and *E. coli*) showed that the BS0.35SrO glass inhibits bacterial growth at 9 and 18 mg/mL. Broth dilution tests strongly supported that glass BS0.35SrO 18 has bactericidal effect on *P. aeruginosa* (Fig. 2), with more than 3 logarithms reduction in the number of microorganisms, presenting also bacteriostatic activity against *E. coli*.



Glass samples	Molar ratio	Melt Temp. (°C)	≈ size (μm)
BS0.35CaO	0.2B2O3:0.4SiO2:0.35CaO:0.05Na2O	1300	63
BS0.35SrO	0.2B2O3:0.4SiO2:0.35SrO:0.05Na2O	1300	63
BS0.35MgO	0.2B2O3:0.4SiO2:0.35MgO:0.05Na2O	1300	63

0.20B2O3:0.40SiO2:xMgO:yCaO:(0.35-x-y)SrO:0.05Na2O (molar ratio, where x, y = 0.35 or 0.00, and x ≠ y)

Figure 1. SEM micrographs of the synthesized glass particles, BS0.35CaO (a.), BS0.35MgO (b.), BS0.35SrO (c.), and their composition.

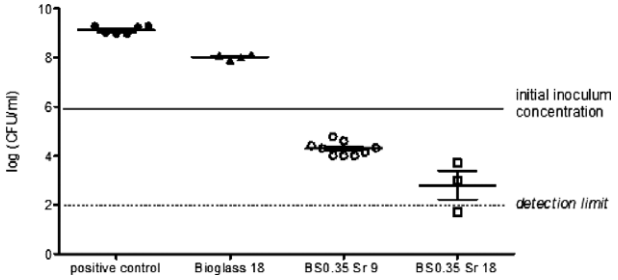


Figure 2. Example of *P. aeruginosa* growth after 24 hours of contact with glass particles. Bioglass was used as control. Error bars correspond to standard deviations.

Discussion and conclusions: Silica-borate glass compositions were easily melted and ground in a reproducible way. The results indicate that all the glass compositions were not cytotoxicity during 3 days of culture. Bacterial tests indicate that BS0.35SrO has bacteriostatic activity for more than one species and demonstrated specific bactericidal activity against *P. aeruginosa* for 18 mg/mL. Results also revealed a concentration dependence on the anti-bacterial properties.

Acknowledgments: Portuguese Foundation for Science and Technology (Ph.D. grant BD/73162/2010). The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007–2013) under Grant Agreement No. REG-POT-CT2012-31633-POLARIS. M. Martins id financed by the project "Tissue engineering of connective tissues" (Ref. RL3 – TECT - NORTE-01-0124-FEDER-000020) cofinanced by North Portugal Regional Operational Programme (ON.2 – O Novo Norte), under the National Strategic Reference Framework (NSRF), through the European Regional Development Fund (ERDF).

Disclosure: Authors have nothing to disclose.

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