Research Article In Vitro Bioactivity Studies of Sol-Gel Derived Dental Ceramics/Bioactive Glass Composites in Periodically Renewed Biomimetic Solution

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Abstract The aim of this work is the investigation of the in vitro bioactivity of sol-gel derived dental ceramics/bioactive glass (DC/BG) composites in periodically renewed SBF, as they are reported to promote the development of an apatite layer on their surfaces even under static conditions. Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) were used to characterize the reacted samples as powders and as thermally treated disk shaped specimens. DC/BG composites-both as powders and as thermally treated specimens-presented bioactivity in periodically renewed c-SBF. The onset of apatite formation on the surface of all powdered samples was observed even after three days of immersion, while the apatite formation on the surface of the heated composite specimens was slightly delayed. Moreover, it was observed an almost linear delay of apatite development to the dental ceramic concentration, attributed to the, accordingly, increased non-bioactive leucite content.

Keywords sol-gel; experimental dental ceramics; 58S bioactive glass; SBF; composites, FTIR, XRD, SEM

1 Introduction

In vitro studies are applied to predict the behavior of bioactive glasses inside the human body, taking into account the relationship between the carbonate hydroxyapatite (HCAp) layer formation *in vitro* and the bone bonding ability to induce bone formation *in vivo* [7]. Dental ceramics/bioactive glass composites fabricated by sol gel methods have been reported to develop hydroxycarbonate apatite after immersion in biomimetic solutions, in particular in Simulated Body Fluids (SBF) under static conditions [2]. However, remarkable variations of the calcium phosphate layers grown on bioactive glasses have been reported under different in vitro tests (static vs dynamic or renewal solution conditions), suggesting the possibility of different interactions on the glass/tissue interface [8]. Regarding the ionic concentration and that SBF is supersaturated with respect to apatite [4], the apatite formation in static environment is favored compared to in vivo conditions, questioning the validity of the extrapolated data of the in vitro results. In vitro tests, which include the renewal of the SBF solution, simulate better the in vivo conditions, compared to the static in vitro tests keeping a constant pH. Thus, dynamic conditions allow predictions that better approach the behavior of these glasses at the implantation site. The aim of this work was the investigation of the in vitro bioactivity of sol-gel derived DC/BG composites as powders and as disk shaped heated specimens in periodically renewed SBF.

2 Materials and methods

The composites were prepared by the sol-gel method as reported in literature [9], while during the gelation various wt% ratios of the dental ceramic till 70% were added in the mixture. The resulting mixtures were sieved to a powder of $< 40 \,\mu$ m. The powders were mixed with distilled water and cylindrical specimens were fabricated as reported in literature [5]. In brief, ceramic powder was mixed with modelling liquid using the same liquid to powder ratio (0.620) for the different ceramics, transferred into polysiloxane moulds and condensed using a vibrator, to accomplish water removal. The disks were removed by gentle hand pressure and sintered at 930 °C with a rate of 60 °C/min in vacuum, according to the firing cycle suggested by the dental ceramic manufacturer [5]. The in vitro bioactivity of the composites (powders and specimens) was tested for various immersion times in c-SBF solution that was renewed after 6 h, 24 h and then every 2 days, as proposed by Zhong and Greenspan [9]. The FTIR



Figure 1: FTIR spectra of all composite powders before and after 3, 6, 9 days in c-SBF.

transmittance spectra by the KBr pellet technique were obtained using a Perkin-Elmer Spectrometer Spectrum 1000 in MIR region with a resolution 4 cm^{-1} , the FTIR reflectance spectra were obtained using a Bruker IFS 113v spectrometer in MIR region with a resolution 2 cm^{-1} . Topographical evaluation and surface elemental compositions of the samples after being carbon coated were performed with a scanning electron microscope with associated EDS (JEOL J.S.M. 840A, Tokyo, Japan).

3 Results and discussion

The transmittance FTIR spectra of the DC/BG composite powders before and after immersion in c-SBF are presented in Figure 1.

The spectra of all powder samples before immersion prove the coexistence of the two constituents in the mixture, by revealing the characteristic bands of both dental ceramic and bioactive glass [3]. It is important to mention that the intensity of the band at 714 cm^{-1} that is assigned to leucite (KAlSi₂O₆, Lt), which is a dominant phase in dental porcelains, is linearly related to the amount of the dental ceramic in the composite. Additionally, the presence of a weak double peak in the spectra of all powdered sample at 568 and 604 cm⁻¹ that is attributed to the bending of P-O mode on the spectra of all powders before immersion, reveal the crystallization of an apatite phase [6], which was defined as hydroxyapatite (HAp) by the XRD analysis [3].

The enhancement of the double peak at 568 and 604 cm⁻¹ on the spectra of all powdered samples, even after 3 days of immersion, is indicative of the onset of apatite formation on the surface of all powdered samples. The shifting and sharpening of the broad peak at $1100 \,\mathrm{cm}^{-1}$ attributed to the asymmetric Si-O-Si stretching vibration mode indicates the crystallization of apatite and is only observed after 3, 6 and 9 days of immersion for the 50:50, 60:40 and 70:30 DC/BG composites respectively. This is further confirmed by the gradual appearance of a weak peak at 1420 cm⁻¹ that is attributed to the symmetric vibrational mode of CO₃ group suggesting the formation of carbonated hydroxyapatite [6]. SEM microphotographs that are presented in Table 1 reveal the development of apatite aggregates on the surface of the grains of the 50:50 DC/BG composite even after 3 days of immersion, while EDS analysis revealed a mean molar Ca/P ratio of about 1.6-1.8

However, after 9 days the surface of all grains was covered by apatite aggregates that presented a mean molar Ca/P ratio of about 1.6, while the participation of Al and Si from the substrate is decreased, suggesting that all grains are fully covered by apatite aggregates.

In the case of 60:40 and 70:30 DC/BG composites, apatite aggregates were first observed after 6 and 9 days, respectively. For the respective days of immersion, EDS analysis presented a mean molar Ca/P ratio of about 1.68 and 1.5, while Al and Si from the substrate were only slightly decreased, suggesting a sporadic development of apatite that is, also, observed in SEM microphotographs (Table 1). The bioactive behaviour of disk shaped specimens of 50:50 DC/BG composite were not investigated as the specific system presented poor mechanical integrity [3] and is, probably, not suitable for use in restorative dentistry. The in vitro bioactivity of the specimens was tested up to 18 days (Table 2).

SEM microphotographs revealed no significant alteration of the surface of the specimens for both DC/BG composites after 9 days of immersion. On the contrary after 18 days the surfaces of all specimens are fully covered by a well structured apatite layer consisting of spherulitic aggregates. EDS analysis revealed a molar Ca/P ratio of



Table 1: SEM microphotographs of all powders.



Table 2: SEM microphotographs of all specimens.

about 1.62 and 1.71 for the 60:40 and 70:30 specimens respectively, while the participation of Si and Al from the substrate have disappeared, suggesting a thick and well-formed apatite layer. SEM observations and EDS analysis are confirmed by the FTIR reflectance spectra of the DC/BG specimens after immersion in c-SBF for 18 days that are presented in Figure 2. In both spectra can be followed the double peak at 580 and 600 cm⁻¹ assigned to the bending of P-O mode and the sharp peak at 1050 cm⁻¹ attributed to P–O stretching vibration [1].

4 Conclusions

Dental ceramics/bioactive glass composites—both as powders and as thermally treated specimens—presented bioactivity in periodically renewed c-SBF. As it was expected, HCAp layer was developed faster on the surfaces of the powdered samples compared to the respective heated specimens, due to their higher surface energy. Even at high dental ceramic concentrations (70:30 DC/BG) the composites were bioactive, probably due to the excess of the CaO content from the sol gel glass in the amorphous glassy network of the composites. Moreover, it was observed an almost linear



Figure 2: FTIR spectra of both specimens before and after 18 days in c-SBF.

delay of apatite development to the dental ceramic concentration, attributed to the accordingly increased non-bioactive leucite content.

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