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Research Article

Comparative Bioactivity Study of 45S5 and 58S Bioglasses in Organic and Inorganic Environment

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Abstract Melt derived bioactive glasses and sol-gel derived glasses have been extensively investigated and are reported to be bioactive. Investigation of the bioactivity was conducted in different media, like conventional Simulated Body Fluid (c-SBF), Dulbecco's Modified Eagle's Medium (DMEM) and Tris buffer solution. The aim of this work is to investigate and evaluate the *in vitro* bioactivity assessment of two commonly used bioactive glasses- 45S5 and 58S-soaked in DMEM and c-SBF, in order to compare the biological response of both glasses in organic and inorganic environments. For the samples immersed in c-SBF, the onset of apatite formation on the surface of 45S5 grains was slightly delayed in comparison to 58S. After 3 days in DMEM 58S powders revealed the formation of a crystalline HCAp phase on the surface of all grains in contrary to 45S5, where a sparsely developed amorphous apatite phase was developed.

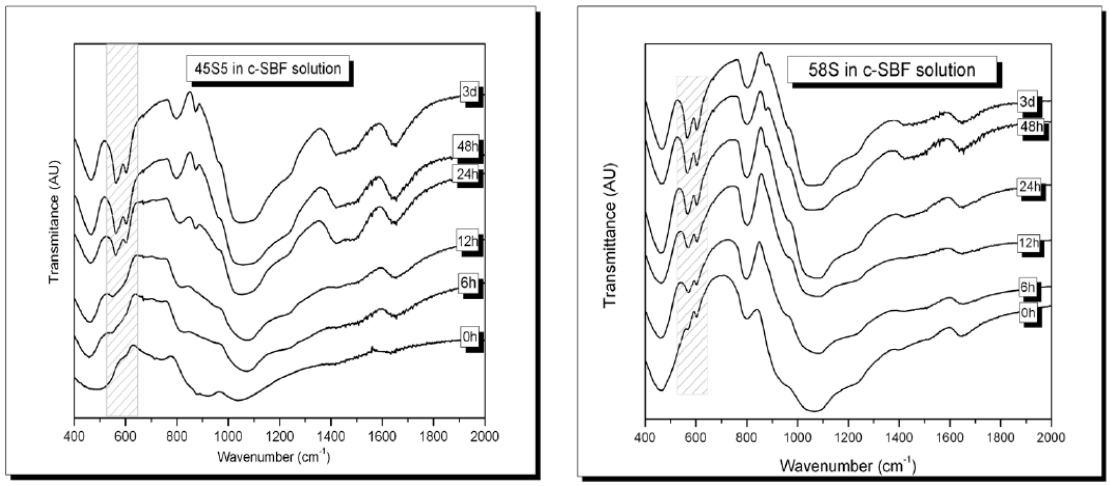
Keywords bioactive glass; renewal; FTIR; SEM; SBF; DMEM; bioactivity

1 Introduction

Bioactive glasses have the ability to bond to both soft and hard tissue and the promotion of bone growth has been well documented [7, 1]. Several studies in the past have evaluated the biological response of various bioactive glasses in different media, like conventional Simulated Body Fluid (c-SBF), Dulbecco's Modified Eagle's Medium (DMEM) and Tris buffer solution [3, 9]. Clupper et al. [3] suggested that the culturing media, which provide nourishment to cells used in tissue-engineering applications, are generally rich in inorganic ions and organic species such as basic carbohydrates and amino acids, indicating the necessity of testing candidate scaffolds materials in solutions of increasing complexity, i.e. SBF, which contains ions similar to blood plasma and DMEM, which in addition to containing similar ionic concentrations as blood plasma also contains proteins common to blood. Studies of the bioactivity in various solutions of melt-derived 45S5 and sol-gel 58S bioactive glasses have been reported, both *in vitro* [11] and *in vivo* [7]. Their behavior has been related to the formation of a biologically active hydroxycarbonate apatite layer (HCA) on the surface of the glasses [7]. Both the onset of apatite formation and the morphology of the developed apatite layer depend on the immersion media due to the different mechanism of apatite formation in each solution. To the best of the authors' knowledge, there is no published work on the comparison of the in vitro bioactive behavior a sol-gel-derived and a melt-derived bioactive glass. Consequently, the aim of this work was the evaluation of the bioactive behavior of melt-derived 45S5 and sol-gel-derived 58S bioactive glasses in two different culture media c-SBF and DMEM solutions, under renewal conditions.

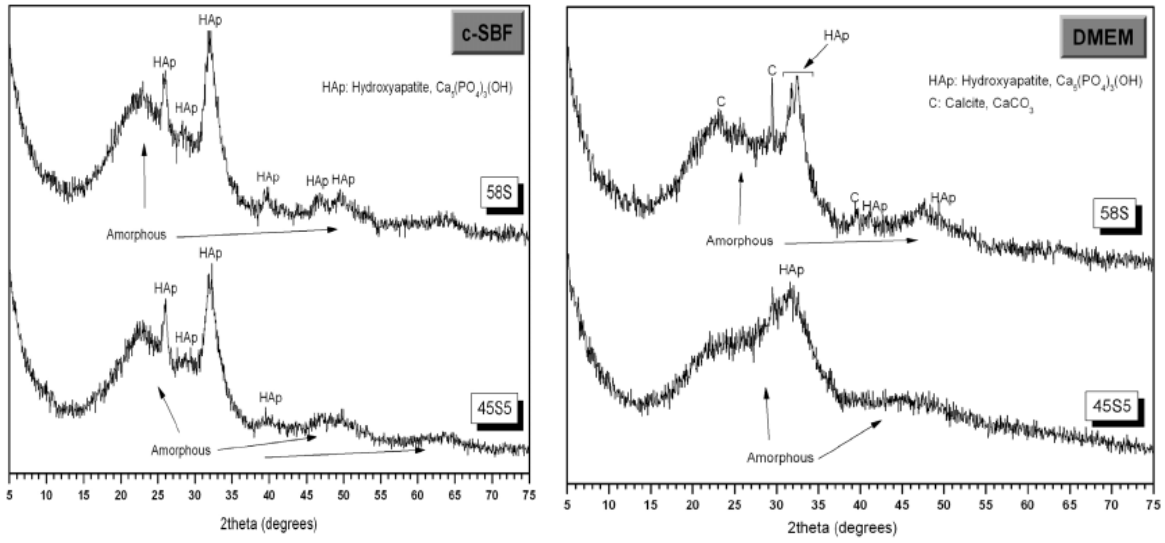
2 Materials and methods

Melt-derived (SiO_2 45, Na_2O 24.5, CaO 24.5, P_2O_5 6 in wt%) and sol-gel-derived (SiO_2 58, CaO 36, P_2O_5 6 in wt%) bioactive glasses were produced as described in literature [11]. The formation of both bioactive glasses—in the specific systems—was confirmed by Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and X-ray Diffractometry (XRD). Both materials were sieved in order to receive powder of 20–40 μm. Then, 75 mg of powder were immersed in 50 mL of both c-SBF solution—prepared as described by Kokubo et al. [8]—and DMEM® culture medium (Invitrogen, GIBCO, USA) supplemented with 10% FCS, for various immersion times. The immersion times for both bioactive glasses in c-SBF and DMEM® were 6, 12, 24, 48 h and 3 days. All experiments performed, were carried out under solution renewal conditions as it has been reported that



(a) (b)

Figure 1: FTIR spectra of (a) 45S5 and (b) 58S powder before and after immersion in c-SBF solution.



(a) (b)

Figure 2: XRD pattern of 45S5 and 58S powder, after immersion in (a) c-SBF and (b) DMEM solution, for 3 days.

this method simulate better the in vivo conditions by maintaining the ionic concentration and pH of the solution constant [10]. The renewal solution conditions were after 6 h, 24 h, and then 2 days. For the characterization of the reacted powders FTIR, XRD and SEM were used.

3 Results and discussion

FTIR spectra of 45S5 and 58S powders before and after immersion in c-SBF solution for 6, 12, 24, 48 h and 3 days are presented in Figures 1 and 2, respectively. Before immersion, both bioactive glasses presented the characteristic peaks of silicate glasses, as reported in literature [2]. Additionally, for the 58S bioactive glass, a double peak at 568 and 602 cm^{-1}, which is attributed to the bending of P-O vibrational mode, proves a preexistent Ca-P phase that is confirmed by XRD patterns (unshown data) [5].

In the case of c-SBF solution, even after 6 h of soaking, 45S5 powder formed a broad peak in the spectral area of 550–610 cm^{-1} in FTIR spectrum (Figure 1(a)), indicating the formation of an amorphous Ca-P phase [2]. However,

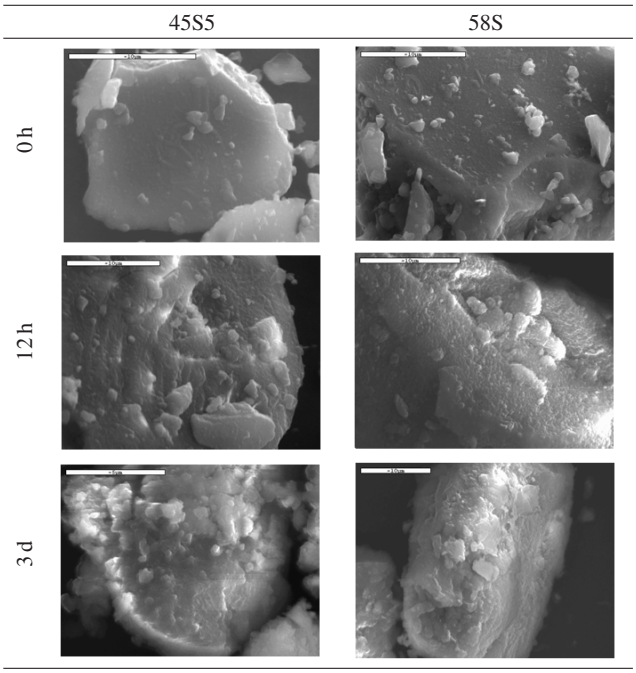
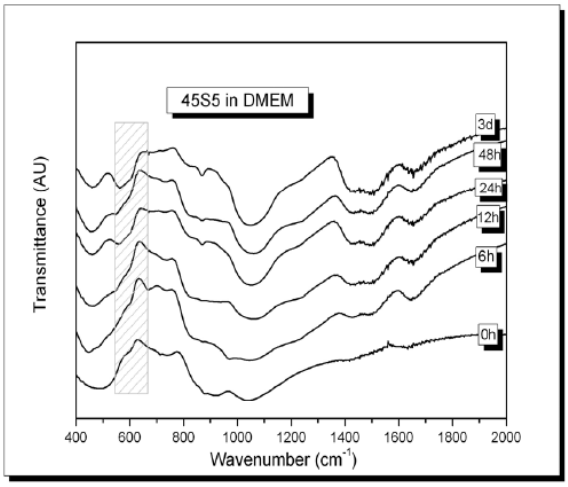


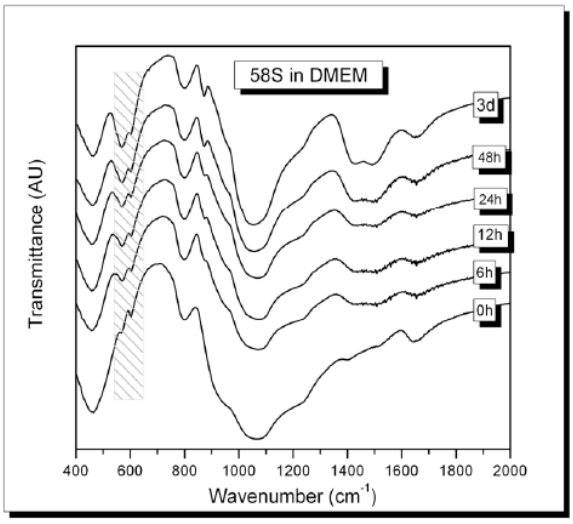
Table 1: SEM pictures of 45S5 and 58S samples in c-SBF.

only after 24 h a double peak at 562 and 602 cm^{-1} attributed to the bending of P-O vibrational mode was formed, and a broad peak at spectral area 1420–1508 cm^{-1} corresponding to the symmetric vibrational mode of CO_3 group, proving the crystallization of an HCAp phase on the grains' surface [2]. SEM microphotographs (Table 1) revealed that after 24 and 48 h of immersion in SBF a rather thin apatite layer has been developed on the surface of the grains of 45S5. These findings are confirmed by EDS analysis, which revealed a mean molar Ca/P ratio of about 1.81 for the samples immersed for 3 days in c-SBF, while SEM microphotographs and XRD patterns (Figure 2(a)) indicated that a quite thick and well formed apatite layer has covered the surface of the grains.

As shown in Figure 1(b), after 6 h no significant alteration took place at the 58S powder samples. However after 12 h the double peak at 562 and 602 cm^{-1} is sharper, indicating the onset of apatite formation on the grains surface [2,5]. Further change on the samples after 12 h is not shown on the FTIR spectra; however, SEM microphotographs (Table 1) reveal that after 12 h of immersion in c-SBF a rather thin apatite layer has been developed on the surface of the grains of 58S. Further confirmation of these findings resulted by EDS analysis, which revealed a mean molar Ca/P ratio of about 1.79 for the samples immersed for 12 h in c-SBF, while the participation of Si from the substrate is decreased. Moreover, after 3 days of immersion a well crystallized apatite layer is formed on the surface of the grains, as revealed by the XRD patterns (Figure 2(b)) and SEM microphotographs (Table 1).



(a)



(b)

Figure 3: FTIR spectra of (a) 45S5 and (b) 58S powder before and after immersion in DMEM solution.

The fast apatite formation of 45S5 bioactive glass in c-SBF solution, compared to previously reported data [2,4], is probably, attributed to the specific ratio of total surface area to volume of immersion liquid, since it is reported, that the decrease of the value of this specific ratio, induce a better bioactive response [4].

Powder samples of 45S5 and 58S were also soaked in DMEM solution and the FTIR spectra are shown in Figures 3(a) and 3(b) respectively. After 24 h, the broad peak in the spectral area of 550–610 cm^{-1} assigned to the bending of P-O vibrational mode, the shifting and the sharpening of the broad peak at 1050 cm^{-1} attributed to the asymmetric Si-O-Si stretching vibration mode

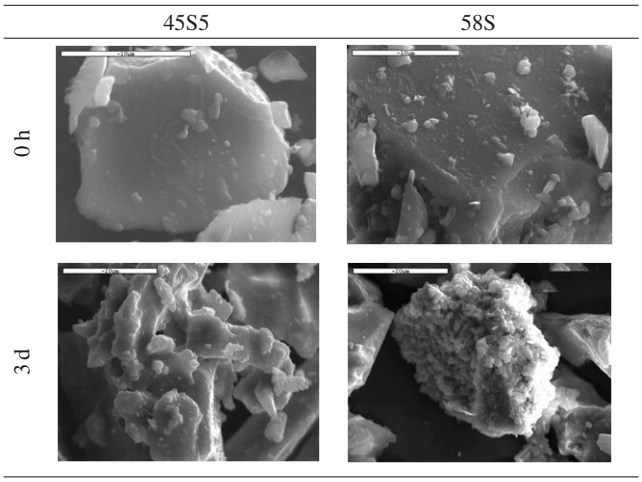


Table 2: SEM pictures of 45S5 and 58S samples in DMEM.

and the double peak at 1420 and 1500 cm^{-1} assigned to the symmetric vibrational mode of CO_3 group, indicate the formation of an amorphous Ca-P phase [2]. After 3 days SEM microphotographs (Table 2) and XRD patterns (Figure 2(b)) confirmed the formation on the surface of 45S5 samples of a sparsely developed amorphous apatite phase.

On the contrary, XRD patterns (Figure 2(b)) of 58S powders soaked in DMEM for 3 days revealed the formation of a crystalline HCAp phase on the surface of all grains. In addition EDS analysis present a mean molar Ca/P ratio of about 2. Even so, EDS analysis proved that, in contrary to the samples immersed in DMEM, those immersed in c-SBF for 3 days display a major elimination of Si and Na from the substrate, indicating a thicker and well-formed apatite layer. The delay of the onset of apatite formation on both materials immersed in DMEM solution, in comparison to those immersed in c-SBF solution, is assumed to be due to the organic constituents of DMEM, as reported by Suk-Woo Ha et al. [6]. The reason for this delay is, probably, the adsorption of the proteins on the amorphous Ca-P layer—that is initially formed on the grains upon immersion in DMEM—and the occupation of the sites where precipitation would occur. In the case of 58S, the high surface area and large pore sizes of the material [11] provide more unoccupied sites, where the crystallization of the amorphous Ca-P layer could be realized.

4 Conclusions

Ultimately, 45S5 bioactive glass presented enhanced bioactivity in c-SBF solution, as a result of the specific ratio of total surface area to solution's volume. In DMEM solution, the crystallization of a HCAp phase on the grains of 45S5 was prohibited by the organic constituents of DMEM, which occupy the sites where precipitation of apatite would occur. On the contrary, the bioactive behavior of 58S in DMEM solution was being better compared to 45S5 for the same soaking time, because of the porosity of the sol-gel-derived 58S glass that provides more sites for precipitation.

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