Approach for fabricating bioglass coatings on reticulated vitreous carbon foams for tissue engineering applications

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Approach for fabricating bioglass coatings on reticulated vitreous carbon foams for tissue engineering applications

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Abstract. Reticulated vitreous carbon foams have recently been used in the fabrication of scaffolds for biomedical applications, and have been shown to support cell adhesion. In this research, a methodology for coating reticulated vitreous carbon foams with two different bioglasses synthesized using the sol-gel technique is presented, towards enhancing the bioactivity of the reticulated vitreous carbon scaffolds. These coatings were morphologically characterized by confocal optical microscopy and scanning electron microscopy. Moreover, reactivity of the coated foams was also evaluated through in vitro tests by immersion in physiological solution. The results show successful deposition of the bioglass coating on the surface of the foams without compromising foam porosity. Coating morphological studies revealed a relationship between the composition of the bioglass and the final morphology of the coating. Additionally, preliminary in vitro tests showed that coating reactivity was also related to initial coating composition. Although the coatings dissolved after 21 days in PBS, the precipitation of a calcium-phosphate layer indicated the potential presence of the initial phases of the apatite formation process.

1. Introduction

Tissue engineering is an area of biomedicine that focuses mainly on the regeneration of tissues and organs in order to repair defects caused by disease or injury. Towards creating environments that promote cell regeneration, one of the fundamental fields in tissue engineering research is the development of scaffolds, which are materials that are intended to serve as support for the growth of new tissue at the defect site. In this context, reticulated vitreous carbon (RVC) foams have emerged as an interesting alternative, since recent studies have shown that RVC foams provide a porous and suitable support for cell growth [1]. Nonetheless, one aspect that could be improved is the bioactivity of RVC foams. Therefore, the incorporation of bioactive materials such as bioglass could aid in enhancing this feature, and thus, favor tissue regeneration within RVC scaffolds. In this sense, one of the challenges associated with the preparation of coatings on foams is the development of a coating fabrication process that does not compromise the morphology of the base material. In the present work, a methodology for the deposition of bioglass coatings over commercially available RVC foams is proposed, based on the use of the sol-gel and dip-coating techniques. Successful deposition of bioglass coatings was achieved without compromising the morphology of the base foam, and thus, the porosity required for cell infiltration [2]. Furthermore, preliminary in vitro testing indicated that coating dissolution in phosphate buffered saline solution was related to the composition of the bioglass used.
2. Materials and methods

2.1. Bioglass sol-gel synthesis and characterization
Two different bioglass compositions were evaluated: i) 65% SiO₂, 5% P₂O₅ and 30% CaO, and ii) 45% SiO₂, 5% P₂O₅, and 50% CaO. Tetraethyl orthosilicate (Sigma-Aldrich), triethyl phosphate (Alfa Aesar), and calcium nitrate tetrahydrate (Panreac) were used as the silica, phosphate and calcium sol-gel precursors, respectively. Solutions were prepared based on previously reported procedures [3]. In order to define favorable viscosity conditions for the preparation of bioglass coatings on the RVC foams, a gelation curve was obtained for each sol, which was carried out at room temperature on a DV III Brookfield viscometer at 240rpm. Xerogels were then produced by heat treating the sols at 60°C for 6h, followed by heating at 700°C, using a ramp of 3°C/min and 1h dwell time. Finally, the composition of the resulting bioglass powders was characterized by X-Ray Fluorescence (XRF), using a Bruker XRF S8 TIGER spectrometer with 4kW dispersive-wavelength.

2.2. Fabrication of bioglass-coated RVC foams
The methodology used to coat the RVC foams was developed based on the dip-coating technique and the procedure reported by Boccaccini [4], who found that immersion in ethanol prior to coating enhanced the hydrophilicity of the substrate and therefore, favored coating adhesion. The RVC foams were obtained from ERG Aerospace (Duocel®, USA), and were disks of 10mm in diameter, 5mm in height and a linear porosity of 20PPI. Upon pretreatment in ethanol, the foams were dried at room temperature, followed by immersion in the bioglass sol for 15min. To remove the excess sol within the RVC foam, samples were placed in a propylene tube fitted with a mesh on one end so as to ensure that the foam was kept in place while air was gently blown through, in order to push the excess sol out. After this, the coated foams were dried for 24h at room temperature prior to heat treatment at 700°C (3°C/min, 1h dwell time) under a nitrogen atmosphere (0.15mL/min) to achieve densification of the coating [5]. Finally, the morphology of the resulting coated RVC foams was characterized using confocal microscopy. Further evaluation of the bioglass coatings was performed via scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX), using a FEI Quanta 650 FEG microscope.

2.3. In vitro reactivity test
In order to test the ability of the fabricated coatings to form apatites, in vitro experiments were conducted. In these, bioglass-coated RVC samples were placed in 12-well plates and immersed in 3mL of phosphate buffered saline (PBS) for 7, 14 and 21 days at 37°C, performing PBS changes every 2 days in order to simulate the fluid exchange and circulation processes that occur naturally in the body. Preliminary characterization to evaluate potential apatite formation was conducted using SEM and EDX.

3. Results and Discussion

3.1. Sol-gel rheological characterization
As it can be seen in Figure 1, the 65% SiO₂ sol reached the gel point (the end of the polycondensation process) after 5h and 30min of aging at 60°C. On the other hand, for the 45% SiO₂ sol, gelation occurred approximately after 6h and 15min of aging, showing a lower gelation rate. This indicated that the hydrolysis processes occurred at a lower rate in the 45% SiO₂ sol, which was possibly caused by a lower concentration of the silica precursor that serves as a crosslinking agent [6]. Moreover, Table 1 shows the XRF results for the synthesized glasses. As it can be noted, the actual concentration of SiO₂, CaO, and P₂O₅ in the bioglasses was very close to its theoretical value, with some expected error that most likely stemmed from the preparation of the precursor solutions.
Table 1. FRX analysis of the bioglass powders.

| Bioglass type | Theoretical composition | Actual composition |
|---------------|-------------------------|--------------------|
| 45% SiO₂      | 45% SiO₂                | 42.75% SiO₂        |
| SiO₂          | 50% CaO                 | 50.12% CaO         |
|               | 5% P₂O₅                 | 6.97% P₂O₅         |
| 65% SiO₂      | 65% SiO₂                | 62.44% SiO₂        |
| SiO₂          | 30% CaO                 | 29.71% CaO         |
|               | 5% P₂O₅                 | 7.67% P₂O₅         |

Figure 1. Sol viscosity versus aging time at 60°C: 65% SiO₂ sol, and 45% SiO₂ sol.

3.2. RVC foam coating
Two different sol viscosities were selected for the preparation of bioglass coatings: 5 and 10cP. Figure 2 shows representative confocal images of foams coated with sols of varying viscosity. Qualitative comparison of the images appeared to indicate that a sol viscosity of 5cP resulted in more homogeneous coatings, relative to the foams coated using sols at 10cP.

Figure 2. Micrographs (50X) of RVC foams: (a) 45% SiO₂ (5cP), (b) 45% SiO₂ (10cP), (c) 65% SiO₂ (5cP), (d) 65% SiO₂ (10cP).

3.3. In vitro tests
Figure 3 shows SEM images of coated RVC foams prior to immersion in PBS. It can be seen that the 45% SiO₂ bioglass layer (Figure 3(a)) has a more porous structure than that of the 65% SiO₂ bioglass layer (Figure 3(b)). Also, it can be seen that the thickness of the 65% SiO₂ bioglass layer (Figure 3(d)) was around 5μm, while the 45% SiO₂ layer (Figure 3(e)) was about 14μm thick. The relationship between the composition of the sintered bioglass and the final morphology of the coating is evident. These results are consistent with a previous study [7], which indicated that an increase in the concentration of silicon in bioactive glasses causes an increase in network connectivity and the reduction of non-bonding oxygens, thus obtaining a denser bioglass. Moreover, a preliminary assessment of the potential reactivity of bioglass coatings prepared from 5cP sols was conducted. This was achieved by immersion of the coated RVC foams in PBS at 37°C for 7, 14 and 21 days. The SEM images in Figure
show that after 7 days of immersion, the 45% SiO$_2$ coating (Figure 4(a)) was partially dissolved in PBS. On the other hand, the 65% SiO$_2$ coating (Figure 4(b)) showed faster dissolution, as well as the eventual formation of large salt crystal deposits (Figures 4(d)-(e), indicated by a red arrow).

Furthermore, Figure 5 shows the EDX results obtained for the bioglass-coated RVC foams before (day 0) and after 14 days of immersion in PBS. Upon 7 days of immersion in PBS, SiO$_2$ dissolution in the 45% SiO$_2$ coating was due to the low connectivity of the silicate network and the high presence of network modifiers, compared to the 65% SiO$_2$ counterpart (data not shown). On the other hand, the formation of a layer rich in phosphorus and calcium in the 45% SiO$_2$ sample indicates the precipitation of phosphorus ions from the solution (Figure 5c). This ion adsorption process occurs prior to the formation of an apatite layer, which is considered an indirect indicator of the bioactivity of a material. After 14 days in PBS, it appears that the 65% SiO$_2$ coating completely dissolved (Figure 5d), and the formation of sodium chloride crystals on the surface was observed. The precipitation of a layer of calcium and phosphate for the 45% SiO$_2$ coating evidenced an interaction with the media solution, which could be a potential indicator of the initial phase of apatite formation, based on the literature [8].

Apatite formation on bioglass coatings has been previously reported [9-10]. A great number of studies have used simulated body fluid (SBF) as the in vitro testing media, instead of PBS. The ionic content of SBF is similar to that of blood plasma [11]; however, its composition does not consider that, biologically, a lot of the calcium ions in plasma are bound to proteins and therefore, cannot contribute to apatite precipitation [12]. In this sense, SBF is a solution that is supersaturated with respect to calcium, which inherently favors apatite precipitation, regardless of the material that is being tested. This could mask the actual reactive behavior of a material towards apatite formation [12]. On the other hand, PBS is a highly stable simulated physiological solution, which is widely used in in vitro cell culture given its isonotic nature, and the fact that it provides efficient pH regulation in biological assays. Relative to SBF, PBS is more stable and can be prepared more reproducibly [12]. Also, bioglass-containing porous systems have shown apatite formation when immersed in PBS [12]. Because of these reasons, PBS was chosen over SBF for the present in vitro studies. Fagerlund's research compares the behavior of SBF and PBS in in vitro tests with bioglass, concluding that each type of solution has a different effect on
experimental variables such as pH and concentration of ions in the solution, which in turn affect reaction kinetics [13]. Therefore, it is possible that a different outcome could have been observed in terms of apatite formation, if SBF had been used as the incubation media, instead of PBS.

Figure 4. SEM micrographs of bioglass coatings after immersion in PBS: (a) 45% SiO$_2$, 7 days. (b) 65% SiO$_2$, 7 days. (c) 45% SiO$_2$, 14 days. (d) 65% SiO$_2$, 14 days. (e) 65% SiO$_2$, after 21 days.

Figure 5. EDX spectra of coatings before (Day 0) and after 14 days of immersion in PBS at 37°C: (a) 45% SiO$_2$, (b) 65% SiO$_2$, (c) 45% SiO$_2$, (d) 65% SiO$_2$. 
4. Conclusions
A methodology for the deposition of bioglass coatings over commercially available RVC foams was successfully developed, based on the use of the sol-gel and dip-coating techniques. Microscopy analysis confirmed the deposition of the ceramic coatings, without compromising the morphology of the base foam. Moreover, a viscosity of 5cP appeared favorable for the precursor sol, as it resulted in more homogeneous bioglass coatings, relative to foams coated with the 10cP sols. SEM analysis indicated that the 65% SiO2 coating was a more compact network, although its degradation rate in vitro was higher than that observed for the 45% SiO2 samples. Based on the results from preliminary in vitro tests in PBS, the 45% SiO2 coatings could potentially induce apatite formation. Future studies should include different simulated physiological solutions, as well as a wider range of bioglass compositions.

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