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Green and safe in situ templating of bioactive glass scaffolds for bone tissue engineering

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This communication reports a new process for the synthesis of bioactive glass foams. This process is based on the use of gelatin as a template during the foaming of a sol, the gelled gelatin template formed in situ maintains the foam structure during further condensation of the glass network.

In the field of bone regeneration, one of the most promising solutions is the use of bone tissue engineering. This emerging technique for the development of grafts needs a scaffold, that is to say a support were the bone cells would be able to attach and grow to develop new bone. The exact description of the scaffold has difficulties to reach a consensus but a general approval is met around a 3D macroporous and interconnected network made with a bioactive and resorbable material. The macropore diameters should be of a few hundreds micrometers to allow cell invasion[1,2].

Among all the possible materials, sol-gel derived bioactive glasses are of great potential because of their high biocompatibility, bioactivity and resorbability. To realize a scaffold from a sol, several techniques already exist like the endo or exo-templating[3] or a sol-gel foaming process. The endo-templating refers to a material mimicking the shape of the template, for example a polyurethane or other type of polymeric foam.[4,5] The exo-templating refers to a sol filling the pores of the template to create a reverse print after the removal of the template. It can be done by the use of a pileup of polymeric beads.[6] These templating techniques generally need a synthetic polymer, and correspond to a two-step process where the first step consists in the manufacture of the template.

A one-step technique exists, the sol-gel foaming process that consists in the vigorous stirring of sol to incorporate air bubbles that are stabilized with a surfactant. The gelation of the sol is quickly provoked with the use of a strong catalyst, the hydrofluoric acid (HF).[7-9] Such a rapid gelation makes possible to retain the foam structure after stopping agitation. However, the use of HF means a highly hazardous product has to be handled; in this case it is not a minor concern since the foaming process is a turbulent mixing process which generates projections and thus important risks of exposure for the operator.

This communication reports the synthesis of a bioactive glass foam with macroporous structure into a one-step templating process. This process consists on the use of natural gelatin in the foaming of the sol to avoid the use of HF. Some authors already reported the mixing of silica sol and gelatin for the synthesis of hybrid materials,[10,11] or the synthesis of hybrids through the sol-gel foaming process with the addition of a polymer like polyvinyl alcohol[12] or gelatin[13]. But in those syntheses, hydrofluoric acid is also necessary. To the best of our knowledge it is the first time that gelled gelatin is used as an in situ template to form a 3D glass macroporous network. Indeed, contrary to processes where HF is used to polymerize the TEOS and to solidify the porous structure, here, the TEOS is not polymerized at the end of the foaming, it is the hardening of gelatin that maintain the porous structure. This process has several advantages over the techniques presented before: it involves the use of a natural and biocompatible polymer instead of a synthetic one; it does not need repeated operations of impregnation that expand the synthesis time;[4] finally this process overcomes the security problems associated with handling HF in a foaming process.

Here a sol was made by mixing 6.97 mL of TEOS (tetraethyl orthosilicate) with 1.12 mL of HCl (Hydrochloric Acid at 2M in water before mixing) and 6.74 mL of deionized water under strong stirring in order to realize the hydrolysis of TEOS without the use of a co-solvent. After 30 minutes, 2.63 g of calcium nitrate ($Ca(NO_3).4H_2O$) are added and the sol is left for aging for 48 hours under continuous stirring. The quantities of reagents were calculated to obtain a final composition of 75 wt. % of SiO_2 and 25 wt. % of CaO for the glass, a composition which has been demonstrated to be bioactive.[14]Gelatinpowder was then added with a gelatin to sol ratio of 0.1g/mL. Hence, for 15 mL of sol, 1.5 g of gelatin (Porcine type A) and 2 mL of deionized water were added. The solution is mixed during 10 minutes and then vigorously stirred in order to generate air bubbles into a foaming process inspired from processes that allow to obtain pure gelatin foams[15] or gelatin foam containing a ceramic charge.[16,17]Montufar et al.[18] showed that gelatin could serve as a foaming agent to realize injectable hydroxyapatite foam. They also showed that a high concentration of gelatin improved the stability of the foam but decreased the interconnection of the foam. Thus, we decided to add 0.25 mL of a surfactant (teepol) inspired from the sol-gel foaming process developed by Sepulveda and Jones[7,8] to stabilize the foam, avoiding the addition of too much gelatin which could be detrimental for the interconnectivity of the foam. Aging of the sol during 48 hours increases its viscosity and helps stabilizing the final foam. After 30 minutes of foaming, the obtained foams were left at ambient air during 5 days for the solvent to evaporate and the silica network to polymerize.

Figure 1: optical pictures of agelatin-bioactive glass foam before calcination (left) and a bioactive glass foam obtained after calcination (right)

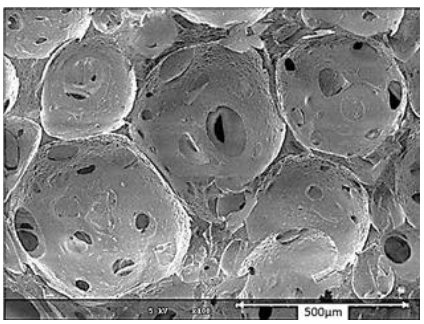

Figure 2: SEM pictures of the bioactive glass foams after calcination.

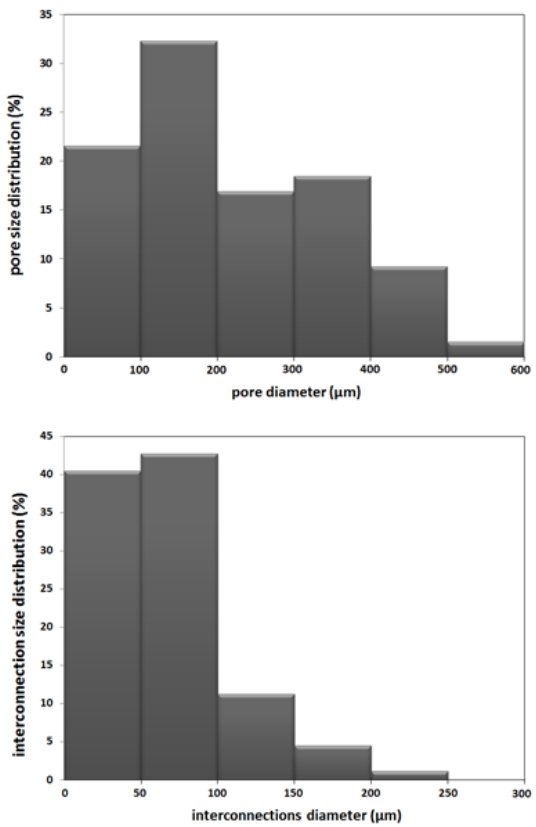

Figure 3: Pore size and interconnection distributions obtained by measurements on SEM pictures.

The foam obtained after drying is shown on Figure 1. It is then heated at 700°C in order to remove the template, the nitrates and the surfactant. The foam was weighted and geometrically measured in order to determine its apparent density (ρ_{app}). The total porosities of the foams was calculated using this apparent density and the real density of a glass containing 75 wt.% SiO_2 and 25 wt.% CaO measured by helium pycnometry (ρ_{real}=2.35g.cm^{-3}) with the following formula:

$$\text{Total porosity} = 100 \times (\rho_{real}-\rho_{app})/\rho_{real}.$$

The foam has a total porosity of 87%. The macroporosity was assessed by SEM observations. The picture in Figure 2 shows a porous and interconnected material. The foam has a structure close to ones obtained with the sol-gel foaming process, that is to say pores with spherical shape.

Pore diameters and interconnections were extracted from SEM pictures thanks to the software Image J,[19,20] 65 independent measures were realized for each parameter. This porosity study is realized instead of traditional mercury intrusion porosimetry that is unable to characterize pores bigger than 250µm,[21] which are the most interesting for cell invasion. The obtained distributions are shown in Figure 3, it exhibits a gradual porosity from a few tens until 600 µm and interconnections mainly comprised between a few micrometers and 100 µm which is suitable for cell invasion and vascularization.

The texture was evaluated by nitrogen sorption (Micromeritics, Tristar II 3020) using the BET (Brunauer Emmet and Teller) method to determine the specific surface area which is 1.5 m²/gwith no mesoporositywhich is very surprising compared with the specific surface area of the foams obtained with traditional sol-gel foaming process (around 100 m²/g). The observed difference could be due to the use of gelatin but also to the absence of HF. Indeed, Almeida et al. have shown that HF used as a catalyst for sol-gel derived bioactive glass synthesis increased the specific surface area.[22] The specific surface area of gelatin derived foams is, a priori, small, thus it was necessary to evaluate their ability to react with biological fluids in vitro and to induce the formation of a calcium phosphate layer at its surface.

To evaluate the bioactivity of the foam, pieces of foam were immersed in SBF (Simulated Body Fluid), a solution that mimics the ionic composition of blood plasma.[23] The SBF was prepared following the recommendations of Bohner and Lemaitre.[24] A first solution was prepared by dissolving NaCl, KCl, $K_2HPO_4.3H_2O$, $MgCl_2.6H_2O$, Na_2SO_4 and $NaHCO_3$ in deionized water and a second by dissolving $CaCl_2$ in deionized water. The pH was buffered with Tris(hydroxymethyl)aminomethane. The pieces were left interacting with the fluid during 1 and 7 days, and then they were removed and washed with ethanol in order to avoid further reactions. The solutions were filtered and analysed with ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy). During the interaction with SBF, the glass foam is expected to undergo a dissolution process. This is confirmed with the release of silicon from the glass to the SBF (0, 28 and 47 ppm respectively at 0, 1 and 7 days of interaction). Moreover this dissolution is the cause of the Ca increase during the first step of interaction (99 and 136 ppm at 0 and 1 day of interaction). At 7 days, the calcium content decreases to 111 ppm showing a precipitation with Ca. This precipitation is concomitant with a decrease of the phosphorus content (29, 23 and 3 ppm at 0, 1 and 7 days of interaction) which suggests the precipitation of a calcium phosphate at the surface of the glass.

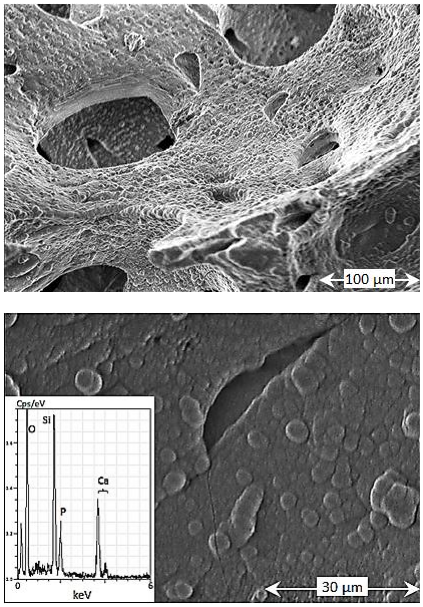

Figure 4: SEM pictures of the foam after immersion during 7 days in Simulated Body Fluidand EDX spectra (included into the lower picture).

The surface after reaction was observed by SEM to check the formation of that calcium phosphate layer. The SEM pictures (Figure 4) confirms the presence of precipitates on the surface of the foam. EDX analysis was realized to assess the nature of this precipitates, it showed the presence of phosphorus, calcium and silicon. The presence of silicon on the EDX spectra proves that X-rays from the inner part of the foam are detected so the exact composition of the precipitates cannot be determined with this technique. However, the presence of phosphorus on the surface attests that the precipitates are very likely calcium phosphates. Thus, the obtained material, compared to other materials, has a small specific surface area, nevertheless, an in vitro bioactivity test has proven that it exhibited a bioactivity process though dissolution and precipitation of calcium phosphates on its surface. As a consequence, it could be a promising candidate in the field of bone regeneration.

Conclusion

In conclusion, here we present a new process for the synthesis of glass foams with interconnected macroporosity that exhibit an in vitro bioactivity which allows considering it as a promising scaffold for bone tissue engineering. This new synthesis has three main advantages compared to other existing ones:

1) It is a template replication method in one step as the template is formed inside the solution of impregnation

2) It is a green synthesis based on the use of gelatin which is a biopolymer.

3) It is an alternative to the use of HF which is a highly hazardous product.

Consequently, we believe that this new process could be very interesting especially if the pore and interconnection sizes can be tailored by variation of the experimental parameters as it is suggested by first observations. Further experiments are in progress to improve and to better understand this new and encouraging process.

Notes and references

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