Sol-gel derived nano/macroporous scaffolds for bone regeneration

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Statement of Purpose: Development of optimal scaffolds is still a challenge in tissue engineering and regeneration, since many materials and structures have been proposed but few have reached clinical efficiency.
This work reports on the preparation and characterization of silica-based sol-gel derived monoliths, with potential applications as scaffolds for bone regeneration. These exhibit a nano/macro bimodal pore size distribution, including pores of both ~100's of micrometers (μm) and a few to 10's of nanometers (nm) in size. Macropores in excess of 100 μm are required for bone cell in-growth, vascularization and nutrient delivery to the centre of the regenerating tissue, whereas nanopores are thought to be useful for the rapid crystallization of hydroxycarbonate apatite (HCA) and cell adhesion. Sol-gel derived bioactive glasses exhibit high specific surface area, high osteoconductive properties, significant degradability and also an inherent nanoporosity (up to ca. 5 nm), so that the principal challenge in this work lies in introducing additional macropores, with sizes above 100 μm. The coral-like morphology (with interconnected macropores) formed during phase separation by spinodal decomposition (the technique employed in the present work) has been claimed to lead to materials mechanically stronger than those obtained by other techniques, such as foaming or fugitive phase burnout [1] or dissolution, where very high pore volumes are required for interconnection of the (spherical) pores.

Methods: Silica-based bioactive compositions (70% SiO_2 – 30% CaO and 77% SiO_2 – 19% CaO – 4% P_2O_5, in mol%) were synthesized using the sol-gel/phase separation technique, where interconnected macroporosity is formed by polymer-induced spinodal decomposition of a gelling sol simultaneously with the sol-gel transition. Two different polymers were used: poly (ethylene oxide), PEO, with an average molecular weight, Mw, of 100,000 and Pluronic P123 (poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide), $(EO)_{20}$-$(PO)_{70}$-$(EO)_{20}$, with a Mw of 5,800). The precursors of SiO_2, CaO and P_2O_5 were tetramethoxysilane (TMOS), calcium nitrate tetrahydrate, and triethyl orthophosphate, respectively. Gelation was obtained at 40 ºC and the gel was aged at room temperature for 1 day. Solvent exchange was then performed by immersing the wet gel in 1N ammonia (NH_4OH) aqueous solution for 1 day, at 40ºC. The resulting gels were dried at 60 ºC and heat treated at 700 ºC (for 2 h, when the added polymer was PEO and 4 h, when the added polymer was P123). The characterization of the heat treated scaffolds in what concerns morphology, texture, structure and homogeneity have been performed by high resolution scanning electron microscopy (SEM), transmission electron microscopy, energy dispersive X-ray spectroscopy, nitrogen adsorption, mercury intrusion porosimetry, infrared (FTIR) and Raman spectroscopies and X-ray diffraction. In-vitro bioactivity tests have been carried out by soaking the scaffolds in simulated body fluid (SBF) and making a human osteoblastic cell culture on their surface.

Results/Discussion: The SEM images of Fig. 1 are typical of our 70% SiO_2 – 30% CaO monoliths, which exhibited the two elements of hierarchical pore structure obtained by the sol-gel/phase separation technique. Fig. 1a shows the interconnected macroporous network, with pores of ca. 100 μm in size within a coral-like sintered gel skeleton, and Fig. 1b shows the porous texture exhibited by that skeleton consisting of nanopores of 10´s of nm. The specific surface area (obtained by N_2 adsorption) was found to be 173 m^2g^{-1} in this case, the total pore volume was 0.7 cm^3g^{-1} and the total porosity was ca. 65 vol%.

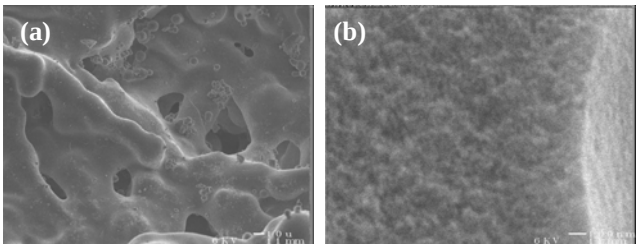

Figure 1. SEM micrographs of a heat treated scaffold, showing hierarchical interconnected pore morphology.
(a) magnification of 400 X, scale bar: 10 μm;
(b) magnification of 65,000 X, scale bar: 100 nm.

The bimodal pore size distribution typical of these scaffolds was also revealed by Hg porosimetry.
The scaffolds showed bioactivity after soaking in SBF within ca. 5 h, as verified by the formation of an HCA layer on their surface, detected by FTIR and Raman spectroscopies and SEM; bone cells became attached to these scaffold materials after two days of cell culture, using a cell seeding density of ~10^5 cells.cm^{-3}.

Conclusions: Sol-gel derived nano/macroporous silica-based monoliths have been successfully prepared using a polymerization-induced phase separation that acts as a precursor for interconnected macroporosity, whereas the nanopore structure of the gel skeletons was tailored by solvent exchange. The tendency of bone cells to attach and proliferate on these materials, as well as their bioactivity and pore morphology, revealed their potential for bone scaffolding applications.

References: [1]. Lofton C.M., Milz C.B., Huang H. and Sigmund W.M., J. Eur. Ceram. Soc., 2005; 25: 883-886.
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