ABSTRACT SUBMISSION

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Mechanisms controlling the porous structure of freeze-dried hydrogel scaffolds

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Biocompatible hydrogels are substrate of choice for tissue engineering and 3D cell culture. Freeze-drying is a common process used to shape hydrogel in the form of porous scaffolds [1]. Cell attachment and viability are strongly correlated with pore size [2]. However, the mechanisms of pore formation during freeze-drying of crosslinked hydrogels are poorly-characterized. Moreover, the well-known scenario advanced for the freezing of colloidal suspensions [3] cannot be applied to crosslinked polymer networks. This work aimed to study the formation of pores by carefully characterizing the hydrogel scaffold from the swollen state before freeze-drying to the final textured state consisting of a cellularized 3D porous scaffold.

We focused on a porous polysaccharide-based pullulan/dextran hydrogel scaffold developed for bone tissue engineering [4]. Crosslinking was carried out using STMP and scaffolds were cut in discs (diameter = 8 mm, height = 2 mm). The measured volume fraction of the polymer in the swollen state was equal to ν_{2s} = 0.012. The elastic modulus was measured by rheometry and found equal to G' = 0.9 ± 0.1 kPa in the linear domain. Thus, mesh size of the crosslinked polymer network was estimated at 32 -34 nm. During freeze-drying (performed at low cooling rate, i.e. - 0.1 °C.min^{-1}), the nucleation of ice was heterogeneous with nucleation temperature distributed around – 13.3 ± 3 °C. Furthermore, scaffolds contained very few active nuclei. Scanning electron microscopy at – 100°C together with Electron Backscattering Diffraction (EBSD) were used to explore the frozen state of the scaffolds. A polycrystalline equiaxed structure was observed with polymer surrounded ice grains of 200 µm size. This structure results from secondary nucleation. This process was induced by the fragmentation of ice needles due to the constraints exerted by the polymer network. After the drying step, ice grains become pores of 160 ± 20 µm size as assessed by X-ray tomography and image processing with Avizo®. After swelling, porosity is equal to 30 % and is independent of the solvent (pure water, NaCl solutions at 0.25, 1 and 9 g.L^{-1}, and DPBS were tested). Homogenous cell seeding (MC3T3E1 osteoblasts) demonstrated interconnectivity of the pores. Cell clusters of 53 µm were subsequently obtained. This size is similar to the scaffold mean pore size as measured by confocal and two-photon microscopy.

This interdisciplinary inquiry, involving cutting-edge technics from ice physics and materials science, provides a precise description of the mechanisms acting during the freeze-drying of crosslinked hydrogel. A better control of hydrogel scaffold porous structure is now available.

References: [1] Kang et al., Biomaterials (1999); [2] O'Brien et al., Biomaterials (2005); [3] Deville et al., Science (2006); [4] Fricain et al., Biomaterials (2013); [5] Vali, Atmospheric Chem. Phys. (2008);