Preparation of β -glucan scaffolds by hydrogel foaming with supercritical CO₂

M Salgado¹, ARC Duarte^{2,3}, S Rodríguez-Rojo¹, RL Reis^{2,3}, MJ Cocero¹

¹ <u>High Pressure Processes Group</u>, Department of Chemical Engineering and Environmental Technology, EII Sede Mergelina, University of Valladolid (UVa), 47011 Valladolid, Spain. ² <u>3B's</u> <u>Research Group - Biomaterials, Biodegradables and Biomimetics</u>, University of Minho,

Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4805-017 Barco, Guimarães, Portugal.³ ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal.

INTRODUCTION: New materials and processing techniques are being constantly developed for the production of scaffolds for tissue engineering applications. Traditionally, foaming of polymers with supercritical fluids is one of the procedures used to create porous matrices. However, this technique cannot be applied to hydrophilic polymers which suffer degradation below their melting temperature. Foaming of hydrogels is an alternative to conventional gas foaming for the processing of hydrophilic polymers by dissolution of supercritical CO_2 in the water of the hydrogels [1]. Through a fast depressurization, a highly porous structure is obtained, which results in the foamed matrix of polymer after water removal.

In this work, β -glucan aerogels are produced by hydrogel foaming with supercritical CO₂. Among polysaccharides, β -glucans have not been widely explored yet for tissue engineering applications. Depending on their origin, they posses different structures and properties. In our study, hydrogels were created from barley and yeast β -glucans. The produced aerogels were characterized in terms of morphology, mechanical properties and degradation rate in physiological fluids.

METHODS: Hydrogels of 2.5% (w/w) yeast β glucans (L-Naturae Nutraceutical, Naturae) and 4% (w/w) barley β -glucans (Glucagel, DKSH) were foamed in a high-pressure vessel at 37 °C and different pressures in the range of 80-200 bar. The hydrogels were introduced in the pre-heated vessel and CO₂ was fed into it at the selected pressure. The system was kept at high pressure for 1.5 h and afterwards it was sudden decompressed in order to promote the foaming and at the same time freezing of the hydrogels, producing stable structures, which were further frozen at -80 °C and subsequently freeze-dried.

The structure of the aerogels was analyzed by SEM (Leica Cambridge S360) and micro-CT (SkyScan 1272, Bruker). Compression tests were performed in a mechanical testing equipment (model 5543, Instron). Degradation tests were done by

immersion of the aerogels on PBS at 37°C and weight loss of the samples was followed for 3 weeks.

RESULTS: Porosity, pore size and interconnectivity of β -glucan aerogels were greatly increased due to the foaming (Fig. 1). A highly porous structure was obtained after foaming. Scaffolds with an average pore size in the order of 100 µm and 80-90% porosity were obtained and the porosity increased with the operating pressure.

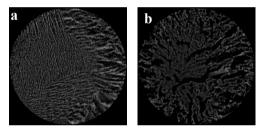


Fig. 1: Micro-CT images of non-foamed (a) and foamed at 80 bar (b) barley β -glucan aerogels.

On the contrary, their mechanical resistance diminished in comparison with non-foamed aerogels. Also, their degradation rate was higher when foamed, although they were not completely decomposed in any of the cases.

DISCUSSION & CONCLUSIONS: β -glucan hydrogels were successfully foamed with supercritical CO₂. The resulting scaffolds fulfill the morphological and mechanical requirements to be used as scaffolds in regenerative medicine.

REFERENCES: ¹ C. Tsioptsias, M.K. Paraskevopoulos, D. Christofilos et al. (2011) *Polymer* **52**:2819-26.

ACKNOWLEDGEMENTS: Authors thank MINECO (CTQ2013-44143-R) for financial support. M. Salgado thanks MECD for her FPU and mobility grants. S. Rodríguez-Rojo acknowledges MINECO and UVa for her Juan de la Cierva fellowship (JCI-2012-14992).

