ALGINATE BASED AEROGELS FOR LIFE SCIENCE APPLICATIONS

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ABSTRACT

The present work intends to highlight the potential use of alginate based aerogel materials in tissue engineering and regenerative applications using new processing routes such as altering the hybridization of natural based polymers using starch and lignin to form hydrogels. These structures were subject to stepwise solvent exchange by ethanol followed by supercritical fluid drying. The developed aerogels can be classified as mesoporous materials with very low density and higher porosity. *In vitro* bioactivity studies of alginate-starch aerogel revealed that calcium phosphate crystals were formed after 14 days of immersion in simulated body fluid. The cytotoxicity of both aerogels was investigated using L929 fibroblast-like cell line and the results revealed that the materials are non-cytotoxic and the cells are able to adhere and proliferate on the surface of the scaffolds.

INTRODUCTION

The production of polysaccharide based aerogels with control pore size distribution still remains a challenge in life science applications. Aerogels are well known as nanoporous materials with an open structure and large specific surface area. A high mesoporosity comparable to the native extracellular matrix (ca. 80% porosity) and good mechanical properties are favorable conditions for cell growth making these materials an ideal candidate for a number of biomedical applications, including tissue engineering. The good biodegradability and stability of alginate aerogels and its versatility in processing makes it a promising component. The tuning of these parameters and their subsequent morphological analysis allow the design of the scaffold.

Aerogels processing starts with the formation of a gel from an aqueous solution. Then, gel formation is induced with a cross-linking promoter followed by liquid solvent removal namely, solvent exchange, whilst avoiding the collapse of the already existing nanoporous structure with the subsequent shrinkage and cracking of the dried gel [1]. The use of ethanol in this step before supercritical drying can be a good way to overcome this issue because is it miscible in carbon dioxide (CO_2) unlike water at mild pressure and temperature (typically, ca. 100 bar and 40°C). Supercritical drying technique is an alternative drying technique

assisted by the use of supercritical fluids, usually $scCO_2$ that overcomes the problems encountered with conventional drying methods to preserve high open porosity and superior textural properties of the wet gel in a dry form, without remnants of any liquid phase [1]. The wet gel is loaded into an autoclave/extractor, in which there is continuous flow of CO_2 above critical pressure and temperature. Depending on the nature of the biopolymer, the extent of drying resulted in significant differences in the end textural properties of aerogel network. The mesoporosity in alginate based aerogels is induced after supercritical drying [2]. Moreover, dry in supercritical condition is a process faster, easier to control and cleaner.
In this work, aerogel based biomaterials were tested to evaluated their potency as tissue scaffolds through morphological imaging and their textural properties. The biological performance was also assessed for novel therapeutic applications in tissue engineering and regenerative medicine.

MATERIALS AND METHODS

Materials

The polymers employed in our study were starch, lignin and sodium-alginate. Alginic acid sodium salt was obtained from Sigma Life science, Germany and Starch was obtained from Roquette (France). Laboratory grade Lignin was produced by enzyme hydrolysis at the TUHH, Germany which was used in the study [3]. Calcium carbonate that was used as a crosslinker was purchased from Magnesia GmbH, Germany. The anhydrous Ethanol (99.9%) that was used in the solvent exchange step was purchased from H. Möller GmbH & Co.KG. Carbon dioxide used for drying was from AGA Gas GmbH (Hamburg, Germany).

Sample preparation

Both starch and lignin aerogels were prepared by chemically crosslinking 1.5% mixture of alginate and starch or lignin with calcium carbonate under pressure which resulted in a hydrogel. The hydrogel underwent a stepwise solvent exchange procedure for 3 days with different grades of ethanol (typically 30, 60, 90 and twice 100%wt ethanol/water mixtures were used). Finally, obtained alcogels were subjected to a supercritical drying procedure at 45°C and 120 – 140 bar for 6 – 8 hours.

Characterization

The aerogels were first characterized based on their textural properties in terms of bulk and skeletal densities. The specific surface area and pore volume of the aerogels were measured by nitrogen adsorption using the BET and BJH methods. The aerogels were also characterized by Micro computed tomography (micro-CT) and scanning electron microscopy (SEM). The mechanical response of these matrices was evaluated in compression mode. In vitro bioactivity tests were performed in simulated body fluid (SBF) solution and the samples were analysed by Infra-red spectroscopy (FTIR). Cytotoxity studies and DNA quantification were performed using L929 fibroblast cell line.

Statistical analysis

Statistical analysis of the data was conducted using IBM SPSS Statistics version 20 software. Shapiro-Wilk test was employed to evaluate the normality of the data sets. Once the results obtained do not follow a normal distribution, non-parametric tests were used to infer statistical significant differences. Differences between the groups with $p < 0.05$ were considered to be statistically significant.

RESULTS

Supercritical drying has proved to be an effective process for aerogels development. In this work, alginate-based aerogels prepared by CO_2 induced gelation were characterized by different techniques regarding their morphological characteristics, mechanical properties and biological performance.

The influence of the depressurization rate on shrinkage, bulk density, surface area and pores volumes is an important operating parameter, which will ultimately determine the morphological features of the structures. As mentioned above, aerogels usually lack for macroporosity, while mesoporosity is typically high. To introduce macroporosity into hybrid aerogels, different depressurization rates, particularly in the range of 0.1 – 30 bar/min, were used. Morphological characterization by SEM and micro-CT were used to monitor the influence of the pressure release rate. Fig. 1 shows the cross section of the starch alginate aerogels (SA) obtained after fast depressurization rate.

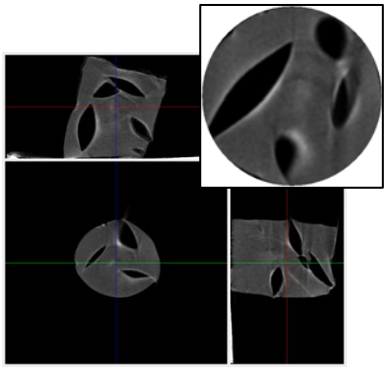

Figure 1: Micro-CT images of starch aerogel samples.

Micro-CT allows the quantification of the morphological parameters of the samples prepared. A noticeable change in porosity was observed by changing the depressurization rate. The results obtained by micro-CT demonstrated an increase from 2% up to 30% for the different conditions tested. Furthermore, the mean pore size also increased, revealing a macroporous structure interesting for biomedical applications. The SEM images are consistent with micro-CT results. It should be also noticed that all produced aerogels retain mesoporous structure showing specific surface area of 400 – 600 m^2/g and pore volume of 2 – 6 cm^3/g. Thus, both macro and mesoporosity are presented in the aerogels eventually allowing cell growth and usage these materials as carriers for active compounds.

Regarding the mechanical response of starch alginate scaffolds, the Young modulus in wet condition (0.03MPa) decreased significantly when compared with dry state (0.22 MPa). Biomedical implants are normally applied in hydrated environments, thus it is relevant to work at these conditions in order to increase their performance.

The bioactive character of starch alginate aerogels produced was tested *in vitro* by analyzing the apatite formation at the material surface after exposure to SBF solution. Fig. 2 shows the chemical changes in FTIR spectra upon immersion in SBF for 14 days.

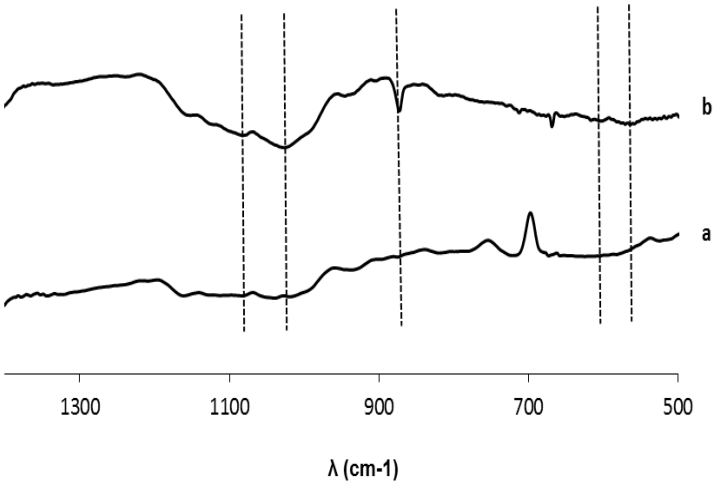

Figure 2: FTIR spectra of the aerogels a) SA; b) SA immersed in SBF for 14 days.

After CaP deposition, a characteristic peak of the phosphate (PO_4^{3-}) groups at 1020 cm^{-1} and 1080 cm^{-1} and absorption peak of carbonate groups at 878 cm^{-1} were detected. This result indicates that starch alginate aerogel is a bioactive material, being able to form hydroxyapatite crystals when immersed in simulated body fluid solution. Moreover, it was possible visualize at 570 and 600 cm^{-1} two peaks characteristic of the P–O bond. These findings suggest that the aerogels prepared may be used for bone tissue engineering applications.

To evaluate the *in vitro* biological performance of alginate based aerogels a mouse fibroblast-like cell line (L929) at a concentration $1x10^5$cells/mL was seeded on these matrices and cultured for 1, 3 and 7 days. As an example we presented the cell viability data for starch alginate which was monitored for each time point through alamarBlue assay. This assay is based on the quantitative metabolic of blue, non-fluorescent reazurin, to pink fluorescent resofurin, by living cells. Moreover cell morphology on these structures was evaluated by scanning electron microscopy. Fig. 3 summarizes the results obtained.

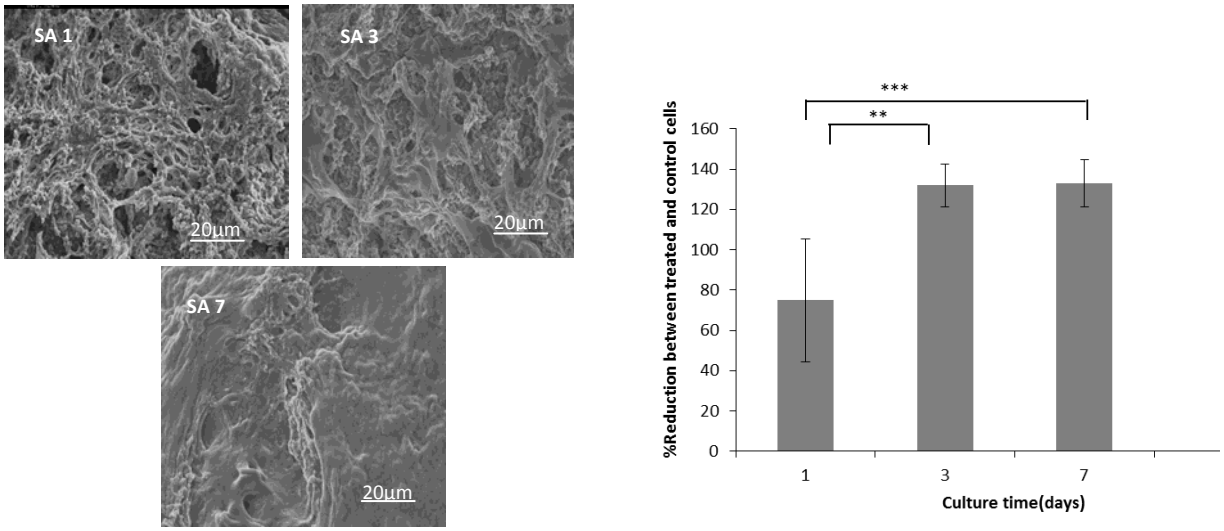

Figure 3: SEM images of cells cultured in vitro on the surface of starch alginate aerogel and reduction of alamarBlue (%) as a function of culture time.

The alamarBlue assay (Fig. 3) revealed that the L929 were metabolically active on starch alginate aerogels over the culture period under study, and there was a significant increase after 3 days of culture time in comparison with day 1. The SEM results were in accordance with the findings obtained from alamarBlue showing that the fibroblast cells were able to adhere and proliferate on these matrices and by day 7 almost all the surface of the scaffold was covered by a cell layer.

CONCLUSION

Hibrid alginate based aerogels were successfully prepared by gelation under pressure followed by supercritical drying. The textural analysis by nitrogen adsorption and SEM revealed that aerogels are both meso and macroporous. Macroporosity is found to be highly dependent on the depressurization rate of the system. The morphological analysis obtained by scanning electron microscopy and micro-CT revealed homogeneous porous structures. *In vitro* tests results revealed these materials are non-cytotoxicity and the cells have a good response. A good cell adhesion was observed on aerogel structures by scanning electron microscopy. The results obtained suggest that alginate aerogel based materials constitute a promising material to be used in biomedical applications.

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