

Chitosan Scaffolds Prepared by Supercritical Assisted Phase Inversion for Tissue Engineering Applications

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Abstract

The aim of this study was to evaluate the possibility of preparing chitosan porous matrixes using supercritical fluid technology. Supercritical immersion precipitation technique was used to prepare scaffolds of a natural biocompatible polymer, chitosan for tissue engineering purposes. Chitosan is a cationic polymer derived from chitin comprising copolymers of $\beta(1\rightarrow4)$ -glucosamine and N-acetyl-D-glucosamine. The physicochemical and biological properties of chitosan make it an excellent material for the preparation of drug delivery systems and for the development of new biomedical applications in many fields from skin to bone or cartilage.

Immersion precipitation experiments were carried out at different operational conditions in order to optimize the processing method. The preparation of a highly porous and interconnected structure of a natural material, chitosan, using a clean and environmentally friendly technology constitutes a new processing technology for the preparation of scaffolds for tissue engineering using these materials.

Introduction

Chitin is widely found in shells of crustaceous such as crabs and shrimp and it is the second most abundant polymer after cellulose. Chitin forms strong inter- and intramolecular hydrogen bonds, which is difficultly broken by common solvents, therefore its solubility in common solvents is rather constrained. For this reason, limited utilization of this natural resource has been reported. Up to date, the majority of uses of chitin are mainly related to chitosan, which is a cationic polymer derived from chitin comprising copolymers of $\beta(1\rightarrow4)$ -glucosamine and N-acetyl-D-glucosamine. The physicochemical and biological properties of chitosan make it an excellent material for the preparation of drug delivery systems and for the development of new biomedical applications in many fields from skin to bone or cartilage.[1] Chitosan is usually processed from a diluted acetic acid solution, thus limiting its processability.

Chitosan has been processed in several forms to be used in tissue engineering applications, namely, membranes,[2] particles[3], fibers and 3D fiber meshes[3, 4]. Chitosan may also be used as a drug delivery carrier and various studies have been reported in literature.[5, 6]

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Chitosan sponges and scaffolds are also described in literature as deliver systems able to carry active agents or biomolecules and growth factors.[7] The preparation of these systems normally involves freeze-drying or lyophilizing a chitosan gel solution.[8, 9] Recently, it has been reported the use of supercritical fluid assisted phase inversion method for the preparation of chitosan membranes from a dilute acetic acid solution.[10] Nonetheless, the proposed process is very time consuming and energy intensive. In this work we report the possibility of producing 3D chitosan scaffolds from organic solutions. Different solvents were tested in order to evaluate the effect of the solvent on the morphology of the materials obtained.

Materials and Methods

Supercritical assisted phase-inversion process

The phase inversion experiments were carried out in an apparatus especially for this purpose[11].

Briefly, in each experiment a small amount (ca. 2 ml) of the polymer solution is loaded in a stainless steel cap with 2 cm diameter, which is placed inside the high pressure vessel. The vessel is heated in by means of an electric thin band heater (OGDEN) connected to a temperature controller, that maintains temperature within ± 1 K. Carbon dioxide is pumped into the vessel using high pressure piston pump (P-200A Thar Technologies) until the operational pressure is attained. The pressure inside the vessel is measured with a pressure transducer. The system was closed for 45 minutes to allow the occurrence of phase separation. Afterwards the system is flushed for another 45 minutes, with a stream of carbon dioxide at very low flow rate (5g/min), in order to ensure complete drying of the scaffolds. The flow is regulated by a flow meter (Siemens, SITRANS FC MASS FLO MASS2100).

Scaffold characterization:

Scanning Electron Microscopy – SEM: Samples of the scaffolds prepared were observed by a Leica Cambridge S360 Scanning Electron Microscope (SEM). The films were fixed by mutual conductive adhesive tape on aluminium stubs and covered with gold palladium using a sputter coater.

Results and Discussion

In this work, the possibility of preparing chitosan scaffolds for tissue engineering applications using supercritical assisted phase inversion technique was evaluated. The solubility of chitosan in different organic solvents has been reported in literature, more specifically chitosan is soluble in carboxylic acids, such as formic or acetic acid and it has also been reported to be soluble in HFIP, 1,1,1,3,3,3- hexa-fluoro-2-propanol. This study was carried out as the selection of a proper combination of the organic solvent and the antisolvent for a particular polymer is essential for the success of the process.

Phase behaviour data on the vapour liquid equilibrium is reported in the literature for the binary systems formic acid + carbon dioxide and acetic acid + carbon dioxide[12] (figure 1).

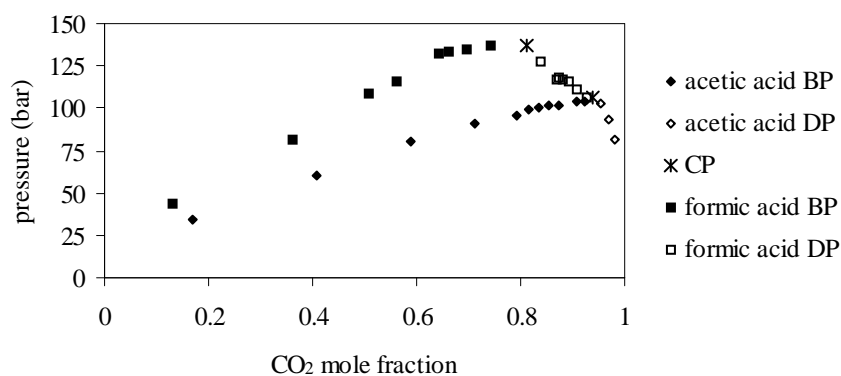


Figure 1: Phase diagram of the systems acetic acid + carbon dioxide and formic acid + carbon dioxide

The experimental conditions were chosen in order to guarantee that the solvent and anti-solvent are in a single phase, therefore are in the area of complete miscibility between the two. A summary of the experiments performed is listed in table 1:

Table 1: Summary of the experiments performed

experiment #	solvent	chitosan concentration (wt%)	Temperature (K)	Pressure (bar)
1	formic acid	2%	333.15	150
2		3%	323.15	150
3		3%	333.15	150
4	acetic acid	2%	333.15	150
5	HFIP	2%	333.15	150

Experiments performed with formic acid, caused the precipitation of a thin membrane with small porosity and interconnectivity. Changes in polymer concentration or temperature did not induce any major effect on the final matrix. In order to be able to compare the results obtained, precipitation of chitosan from acetic acid or HFIP was performed in the same conditions. Figure 2 shows representative images of the chitosan scaffolds obtained.

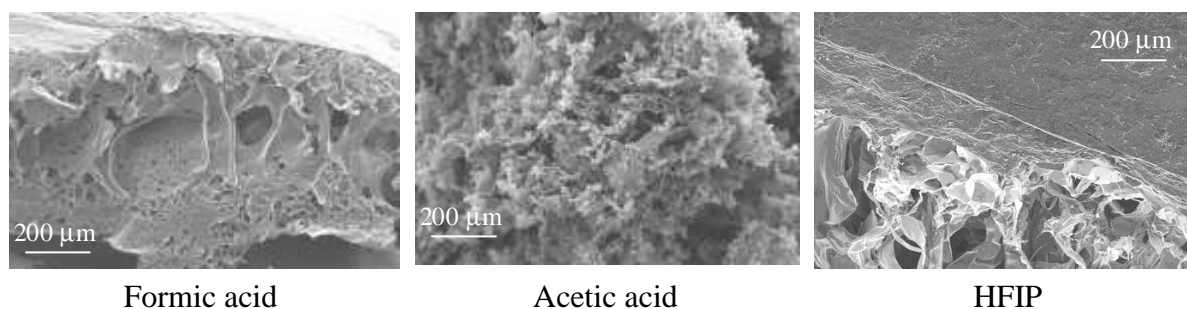


Figure 2: SEM images of the cross-section of chitosan scaffolds precipitated from different solvents

As it can be observed that completely different morphologies are obtained from the precipitation with different solvents. Chitosan processed from an acetic acid solution presented a more homogeneous structure while chitosan scaffolds precipitated from an HFIP

solution presented a compact membrane on the upper layer and a structure similar to leaves on the bottom.

The supercritical assisted phase inversion process relies on two mechanisms that take place at the same time, namely the diffusion of carbon dioxide into the polymer solution and the extraction of the organic solvent by the supercritical fluid. The high solubility between the organic solvent and the anti-solvent will favour the process, as this means a higher affinity of the solvent to the carbon dioxide, which will cause the phase separation and the precipitation of the polymer with a porous structure.

The complexity of the mechanisms involved in the process is related with the interactions between the components of the ternary mixture, i.e., polymer-solvent, polymer-non-solvent and solvent-non-solvent interactions.[13]

Conclusions

In this work we evaluate the feasibility of processing chitosan 3D scaffolds for tissue engineering applications. Different solvents were tested and we can conclude that the morphologies of the scaffolds obtained dependent greatly on the casting solvent used. The interactions between the three components of the system (polymer + solvent + non-solvent) can explain the differences obtained.

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