3-D composite scaffolds obtained by a supercritical fluids assisted process

L. Baldino, S. Cardea*, M.L. De Pascale, M. Scognamiglio, E. Reverchon
Department of Industrial Engineering, University of Salerno,
Via Giovanni Paolo II, 132, 84084, Fisciano (SA), Italy
E-mail: scardea@unisa.it,
FAX: 0039-089964057

ABSTRACT

One of the most important goals of tissue engineering is the design and the generation of a biocompatible porous 3-D structure, with high porosity, high interconnectivity and homogenous morphology. Nevertheless, the implantation of engineered biomaterials might cause local inflammation because of the host's immune response. To overcome this limitation, in this study, 3-D polymeric composite scaffolds loaded with an active compound have been generated by supercritical freeze extraction process (SFEP). This is an innovative process that combines the advantages of the Thermally Induced Phase Separation (TIPS) process and of the supercritical carbon dioxide (SC-CO_2) drying. Poly-l-lactid acid (PLLA)/ibuprofen composite scaffolds characterized by a 3-D geometry, micrometric cellular structures and wrinkled pore walls have been obtained; moreover, the homogeneous drug distribution inside the polymeric matrix and controlled release of the active compound have been demonstrated.

INTRODUCTION

The next generation of engineered tissues is based on the development of loaded scaffolds containing bioactive molecules in order to control the cellular function (for example, growth factors) or to interact on the surrounding tissues (for example, drugs such as anti-inflammatory agents or antibiotics) [1-2]. Drug delivery systems, due to the wide range of materials that can be processed, their various morphologies, sizes and shapes as well as different administration ways, are suitable for many therapeutic applications. One ideal strategy of tissue engineering (TE) is to enable the self-healing potential of the patient to regenerate body tissue and organs [3-4]. This goal can be achieved if the bioactive scaffold is designed in a manner that provides a support for cells to grow, inducing also their differentiation and proliferation. Moreover, implantation of engineered biomaterials might cause local inflammation because of the immune response of the host; thereby, use of anti-inflammatory agents can be required, whether steroidal or nonsteroidal. Within the last group, ibuprofen [2-(p-isobutylphenyl)propionic acid] has been widely used orally, intravenously, and even topically, for example, in postoperative conditions, where an immediately available dose might be useful or required. However, only recently specific studies on ibuprofen loading and release from porous scaffolds have been reported [5-6]. One of the most important stages of TE is the design and the generation of a porous 3-D structure, with high porosity, high interconnectivity and homogenous morphology. Various techniques have been reported in the literature for the fabrication of biodegradable scaffolds [7-9], but, these techniques suffer several limitations; particularly, it is very difficult to obtain simultaneously macro, micro and nanostructural characteristics that are required for the various TE applications. The most used method is the phase separation of a polymeric homogeneous

solution [10-13], in which polymer-poor and polymer-rich liquid phases are generated. The subsequent growth and coalescence of the polymer-poor phase forms pores in the scaffold. In the case of thermally induced phase separation (TIPS), solution temperature is lowered with respect to room temperature to induce the phase separation. It typically leads to the formation of cellular porous structures. When the temperature is low enough, frozen solvent and concentrated polymer phases can be generated due to the solid–liquid demixing mechanism [14]. During the subsequent solvent removal, the porous structure needs to be carefully preserved and freeze-drying is usually performed for solvent removal to avoid the collapse of the porous structure [12-13,15-16]. But, this method presents several disadvantages being time consuming and having problems of dense skins formation.
Supercritical fluids assisted processes have been proposed to overcome the limitation of traditional techniques in several fields [17-20] due to their process flexibility and gas-like mass transfer properties. Carbon dioxide is the most commonly used supercritical fluid; its elimination and the recovery of final products are easy (no residue is left and a dry solid product is obtained, just by controlling the pressure), leading to processes with less energy consumption. The aims of SC-CO_2 assisted techniques in TE is to modulate mass transfer properties, to obtain an efficient solvent elimination, due to the large affinity of SC-CO_2 with almost all the organic solvents, and to work with short processing times, taking advantage of the enhanced mass transfer rates [21].
In a previous work [22] our research group proposed an innovative supercritical freeze extraction process (SFEP) for the formation of PLLA porous structures suitable for TE applications, that combines the advantages of the TIPS process for the phase separation and 3-D structures formation, and of the supercritical drying, that allows complete and fast solvent elimination avoiding structure collapse. The possibility of producing 3-D PLLA scaffolds characterized by a homogeneous microstructure (suitable for cells colonization and growth) and nanostructured internal surfaces (for cells interaction and guidance for their adhesion, migration and organization) was shown.
In this study, the possibility of generating a 3-D PLLA scaffold loaded with an active compound by SFEP has been evaluated. The effects of process parameters on the scaffolds morphology, the drug distribution and the drug release have been studied.

MATERIALS AND METHODS

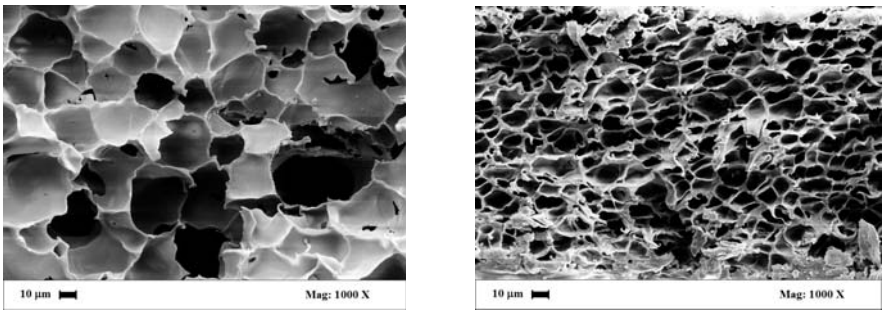
Poly(l-lactic acid) (PLLA) with a M.W. ranging between 150000-200000 (L209s) was purchased from Boehringer Ingelheim (Ingelheim, Germany), ibuprofen sodium salt (98% purity) and chloroform (99.8% purity) were bought from Sigma Aldrich (Milan, Italy), CO_2 (99% purity) was purchased form SON (Società Ossigeno Napoli, Italy). All materials were used as received.

RESULTS

We processed PLLA + ibuprofen solutions in chloroform with different polymer concentrations ranging between 5 and 15% w/w and with a fixed amount of drug, 10% w/w respect to the PLLA. TIPS was obtained at -30°C for 24 h; then, supercritical drying was immediately performed operating at different pressures, ranging between 100 and 200 bar, and different temperatures, ranging between 35 and 55°C. The time gap between TIPS and supercritical drying was minimized (about 50 s), since TIPS process is reversible and a

temperature increase of the sample can modify the morphology of the phase-separated structures.

We studied the effect of polymer concentration in the starting solution on the final composite scaffolds morphology. The first series of experiments was performed using polymer concentrations between 5 and 15% w/w; supercritical drying experiments were performed at different pressures and temperatures. An example is reported in SEM images (taken at the same enlargement) of figure 1, where PLLA/ibuprofen composite scaffolds obtained at 45°C and 150 bar at different polymer concentrations are presented. Homogeneous structures characterized by cellular morphology were generated; the presence of drug is not detectable by SEM analysis and the results obtained put in evidence the fact that the drug did not interfere with the scaffolds formation. Indeed, the composite structures are similar to those generated in a previous work, in which pure PLLA scaffolds were obtained by SFEP [22]. Increasing the polymer concentration, the pore size largely decreased; this information is qualitatively shown in figure 1, where SEM images taken at the same enlargement were reported, and it is quantitatively shown by pore size distributions in figure 2. Increasing from 5 to 15% w/w PLLA concentration, the mean pore size decreases from about 30 to 8 μm and pore size distribution shrinks.



(a) (b)

Figure 1: Sections of PLLA/ibuprofen composite scaffolds obtained starting from solutions at different polymer concentrations: a) 5% w/w, b) 15% w/w, phase separated at -30°C and dried by SC-CO_2 at 45°C and 150 bar.

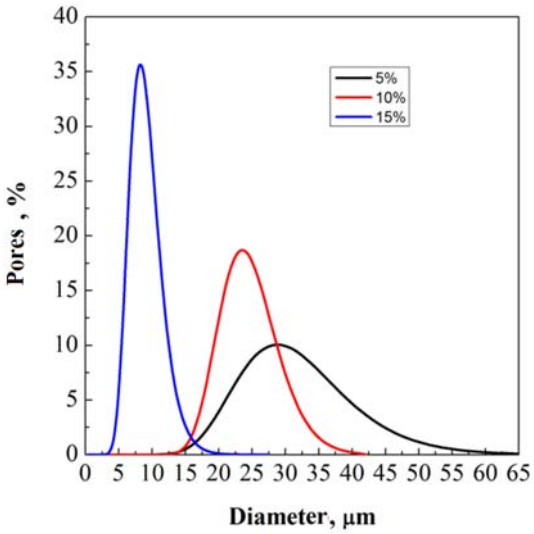


Figure 2: Pore size distribution of PLLA/ibuprofen composite scaffolds obtained starting from different polymer concentrations.

Moreover, porosimetric analysis measured a porosity ranging between 89% (for 5% w/w PLLA scaffold) to 78% (for 15% w/w PLLA scaffold). Observing SEM images produced at higher magnifications, like the one reported in figure 3, it is possible to note that, in addition to the micrometric structure, pore walls were also characterized by a nanometric substructure in which pores and fibres of about 100 nm in diameter are present.

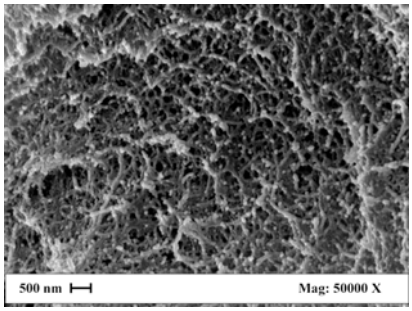
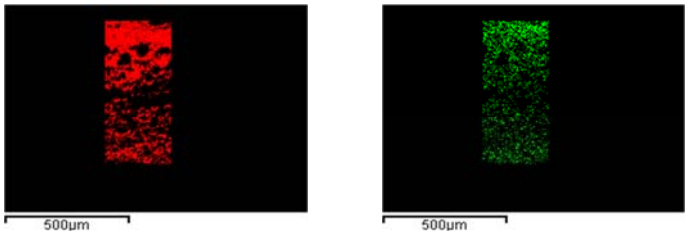


Figure 3: SEM image at higher magnification of a 15% w/w PLLA/ibuprofen composite scaffold, phase separated at -30°C and dried by supercritical CO_2 at 150 bar and 45°C.

Solvent residue analyses were also performed and values of chloroform lower than 5 ppm were found; i.e., as expected, supercritical drying allowed to completely remove the organic solvent of the starting solutions.
Qualitative and quantitative analysis on PLLA/ibuprofen scaffolds were also performed; in particular, ibuprofen distribution along the scaffold was evaluated by EDX analysis. In figure 4, element maps of PLLA/ibuprofen composite scaffolds obtained at 150 bar and 55°C are reported. The drug (green map) is uniformly distributed along the scaffold section and completely overlap the presence of polymer (red map); this result is extremely important because confirms that the SFEP process allows to obtain polymer/drug composite scaffolds characterized by an homogenous distribution of the active compound. This result was observed for each operating condition tested and at all polymer concentrations.

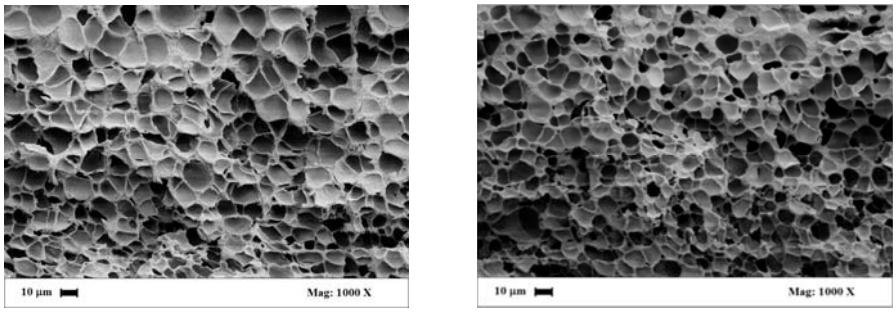


(a) (b)

Figure 4: EDX analysis of PLLA/ibuprofen composite scaffolds obtained at 150 bar and 55°C; a) red: carbon map, b) green: sodium map.

These results can be explained considering the classical theory of the phase separation process. The cellular structure suggests that a liquid-liquid phase separation occurred during TIPS [22-23]. Moreover, the presence of a nanosubstructure is usually related to a solid-liquid demixing mechanism [23-25]; therefore, an overlap of a solid-liquid demixing with the liquid-liquid demixing can be hypothesized during TIPS of PLLA/ibuprofen samples. Regarding the effect of the polymer concentration in the starting solution, it is known that a decrease of the starting polymer concentration leads to a reduction of polymer-rich phase during the phase separation [23,26-27]. Obviously, this phenomenon causes an increase of pores size and a larger porosity.

Subsequently, the effect of the supercritical drying pressure on the composite scaffolds morphology was studied. Analyzing the experiments performed at 35°C and with 15% w/w of PLLA (and 10% w/w of ibuprofen respect to PLLA), we observed that an increase of pressure from 100 to 200 bar did not affect the scaffolds morphology; indeed, a cellular structure was always obtained and this result was confirmed at each polymer concentration and temperature tested. Moreover, the pores size did not vary too. This result is qualitatively visible in figure 5, where SEM images of PLLA/ibuprofen composite scaffolds obtained at different pressures are reported, and quantitatively confirmed by pore size distribution reported in figure 6.



(a) (b)

Figure 5: PLLA/iburprofen composite scaffolds obtained at 15% w/w of PLLA, 35°C and at different pressures: (a) 100 bar; (b) 200 bar.

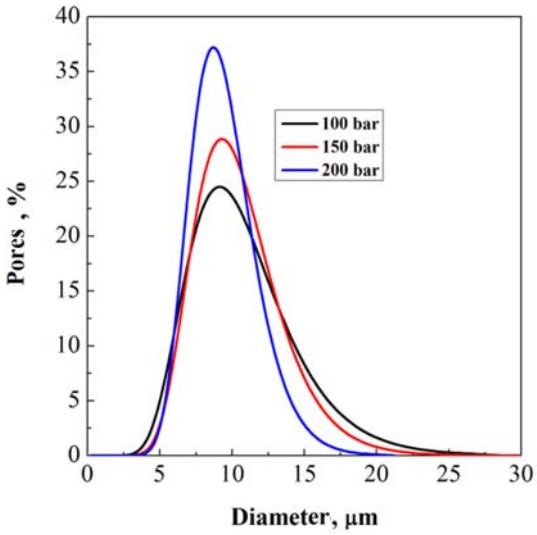


Figure 6: Pore size distributions of PLLA/iburprofen composite scaffolds obtained at 15% w/w of PLLA, 35°C and at different pressures (100, 150 and 200 bar).

Similar results were obtained in the case of supercritical drying temperature effects. The limited effects of supercritical processing parameters after TIPS was not surprising; indeed, it shows that supercritical drying preserved the solid structures and their morphology, but it was not able to modify them, that are completely formed during the TIPS step.

In the last part of the work, we focused our attention on the controlled release of the active compound, i.e., ibuprofen, from the PLLA matrix. First, the effect of the polymer concentration on the drug release was studied. In figure 7, the drug release profiles from PLLA scaffolds obtained at 150 bar and 45°C are reported. It is possible to observe as,

increasing the polymer concentration from 5 to 15% w/w, the drug release rate changes. In particular, the ibuprofen release rate increases when the polymer concentration decrease; for example, after 80 h of analysis, the 5% w/w PLLA scaffold released about 90% of drug, the 10% w/w PLLA scaffold released about the 80% of the drug, and the 15% w/w PLLA scaffold released about the 70% of the drug. Moreover, it is possible to put in evidence that the 100% of drug is released in about 160 h for 5% w/w PLLA scaffold and in about 240 h for 10 and 15% w/w PLLA scaffold. This behavior was observed for each operating condition tested. As expected, the homogenous distribution of the drug, observed by EDX analysis, avoided problems of burst effect.

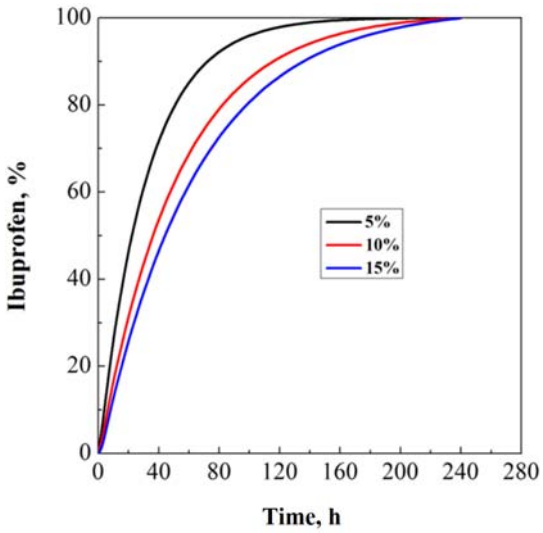


Figure 7: Ibuprofen release profiles from PLLA scaffolds with different polymer concentrations (from 5 to 15% w/w) obtained by supercritical drying at 150 bar and 45°C.

CONCLUSIONS

SFEP confirmed to be a promising process to generate polymeric scaffolds characterized by a 3-D geometry (due to low temperature process combined with supercritical drying), micrometric cellular structures and wrinkled pore walls. The possibility of generating polymer/drug composite scaffolds has been showed, assuring homogeneous distribution and controlled release of the active compound.

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