Poly (ε-caprolactone)/SBA-15 Porous Biocomposites Foamed and Plasticized with Supercritical Carbon Dioxide and Greener Additives

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Abstract: This work aims to develop highly porous biocomposites based on poly (ε-caprolactone) (PCL) and mesoporous SBA-15 silica nanoparticles (SNPs) and that may present adequate and advantageous chemical, mechanical, morphological and biological properties for potential hard tissue engineering applications. Porous biomaterials made of pure PCL and of PCL:SBA-15 (70:30 and 90:10 %w/w) were prepared using a supercritical CO_2-assisted foaming/mixing (SFM) method at constant pressure (20 MPa), temperature (40 °C), processing time (2 hours) and depressurization rate (0.37 dm^3 CO_2 min^{-1}). Additionally and in order to obtain highly porous plasticized biocomposites, four "green" additives (glycofurol, isosorbide dimethyl ether, N,N,N-trimethylethanolammonium pentanoate and tetradecyl(trihexyl) phosphonium bistriflamide) were added to PCL and to PCL/SBA-15 physical mixtures and processed using the same SFM processing conditions. All obtained biomaterials were physically, thermally, and chemically characterized. Preliminary in vitro cytotoxicity tests were also performed. Morphological and thermomechanical properties of the prepared biocomposites were strongly dependent on the additive nature and on relative PCL/SBA-15/additives compositions. In vitro cytotoxicity screening tests showed that prepared biocomposites were highly cytocompatible with SAOS-2 cells. Results demonstrated the viability of using the SFM method and plasticizer/porogenic solvents for the development of highly porous/plasticized cytocompatible PCL/SBA-15 biocomposites presenting tunable physicochemical, thermomechanical and morphological properties.

Keywords: Poly(ε-caprolactone)/SBA-15 biocomposites, supercritical carbon dioxide assisted foaming/mixing, green plasticizer and porogenic additives, tunable physicochemical, thermomechanical and morphological properties.

INTRODUCTION

Numerous conventional methods and processes have been used for the production of plasticized/non-plasticized foamed polymer-based materials intended for pharmaceutical and biomedical applications. However, these methods are usually liquid solvent-based processes that employ environmentally hazardous substances (as solvents, porogenic agents or plasticizers) and/or high processing temperatures which may lead to final material contamination and to the degradation of any involved thermo-labile substances (such as drugs, proteins, growth factors or even labile biopolymers). Moreover, required additional purification and drying steps may also degrade these substances [1-3]. To overcome these

relevant issues, several different strategies have been proposed such as the use of solvent-free processes or of alternative and safer solvents, plasticizers and porogenic agents (namely of supercritical fluids, ionic liquids (ILs) and low-toxicity/low-volatility plasticizers/porogenic agents) [1-7]

Supercritical fluid (SCF) based technologies are versatile techniques that are usually accepted as environmental-friendly alternatives to several conventional polymer processing methods, including polymer foaming and additive mixing/incorporation [3-5]. Supercritical carbon dioxide ($scCO_2$) is the most commonly SCF due to its unique and well-known advantageous properties. The contact with $scCO_2$ (or with high-pressure liquid CO_2) can temporarily swell and plasticize many polymers, since it easily promotes an increase in free-volume and in polymer chain mobility/flexibility and, consequently, it lowers polymer melt viscosity as well as glass transition and melting temperatures (T_g and T_m, respectively) [5]. Furthermore, its low critical temperature makes it very attractive for processing thermo-sensitive compounds, such as drugs and other bioactive compounds [8].

Poly(α-esters), also called poly(α-hydroxy acids), are one of the major classes of synthetic biodegradable polymers that have been studied in recent years for several pharmaceutical and biomedical applications, namely for long-term hard tissue engineering applications. This happened due to their advantageous chemical, physical and biological properties, as well as to their processability and slow in vivo degradation performances. This family of biopolymers include, for example, poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA) and poly(ε-caprolactone) (PCL) [9-10]. Amongst these, PCL is one of the most studied: it is a semi-crystalline, hydrophobic and a FDA approved poly(α-ester) for specific applications such as drug delivery and bone and dental formulations [11]. PCL can be processed using different scCO2-based techniques, including the $scCO_2$-assisted foaming/mixing (SFM) method, alone or in combination with bioactive substances (to be delivered), other polymeric or inorganic materials (e.g., to reinforce some of their mechanical properties and to mimic original bone and dental extra-cellular matrices) or other additives having specific purposes (e.g., plasticizers, porogenic/blowing/foaming agents) [5, 6].

Inorganic silica-based materials, such as mesoporous silicas, bioglasses, and silica-based composite/hybrid materials have been used in numerous biomedical and pharmaceutical applications. For example, mesoporous silica nanoparticles (SNPs) (such as MCM-41 and SBA-15 SNPs) have been used as carriers for various therapeutic agents and/or as hard (inorganic) counterparts of silica-based biocomposite materials, in order to mimic the original chemical, physical and biological properties of bone/dental hard tissues [2,5,8,15,16].

On the other hand and to promote the formation of adequate macroporous structures, to tune some of the required mechanical properties for the envisaged applications, or to change the delivery/degradability profiles of PCL, it is sometimes necessary to incorporate other additives such as liquid porogenic substances and plasticizers. Several different types of additives are currently being proposed as interesting alternatives to replace the “conventional” and usually toxic liquid porogenic agents/plasticizers. Amongst these alternatives, ILs are recently getting much attention. ILs are usually considered to be “greener” alternatives for the replacement of volatile organic compounds (VOC’s) in a wide variety of processes and innovative applications [12]. More recently, some ILs were reported and used as potential porogenic agents and plasticizers for the preparation of porous polymer-based structures [1,13]. However, the chosen and employed ILs are well-known to present relevant biological toxicities and environmental risks. Therefore, other “safer” and/or biodegradable ILs must be

selected for this type of applications, such as those ILs of the ammonium- or of the phosphonium- families. Nevertheless, other "greener" and "safer" liquid additives may as well be employed for these purposes [7].
In this work, we choose to use glycofurol (G) and isosorbide dimethyl ether (I), which are approved excipients for parenteral pharmaceutical formulations [14], alone or in combination with two "safer" ILs (N,N,N-trimethylethanolammonium pentanoate (P), and tetradecyl(trihexyl) phosphonium bistriflamide (PB)), as novel and low-volatility/low-toxicity plasticizers, porogenic and compatibility agents for the formation of porous PCL/SBA-15 biocomposites that may present adequate and advantageous chemical, mechanical, morphological and biological properties for hard-tissue engineering applications.

MATERIALS AND METHODS

Materials - PCL (pellets, $48000 \leq M_w \leq 90000$ $g \cdot mol^{-1}$, glycofurol (G) and isosorbide dimethyl ether (I) were obtained from Sigma–Aldrich. N, N, N–Trimethylethanolammonium pentanoate (P) was provided by Iolitec (Germany), while tetradecyl(trihexyl) phosphonium bistriflamide (PB) was obtained from Cytec Industries (France). Mesoporous SBA-15 type silica nanoparticles (S) were supplied by Claytec, Inc (USA). Carbon dioxide was purchased from Praxair (Spain) (99.998%, v/v).
Foam production by SFM - Porous PCL and PCL/SBA-15 biocomposites were prepared (in duplicate) by a batch $scCO_2$-assisted foaming/mixing method (SFM) and using the same apparatus and experimental procedures previously reported [16]. Temperature and pressure operational conditions were kept constant for all experiments (40 °C and 20 MPa, respectively). Processing time (2 hours) and depressurization rate (0.37 $L_{CO2} \cdot min^{-1}$) were also kept constant. SBA-15 SNPs were incorporated in two concentration ratios 10 and 30 % (w/w) and the concentration of the liquid additives was 98% (molar) over the PCL amount used.
Characterization methods – All prepared samples were characterized by various techniques including scanning electron microscopy (SEM), nitrogen adsorption, helium picnometry, mercury intrusion, differential scanning calorimetry (DSC) and dynamic mechanical thermal analysis (DMTA). Preliminary cytotoxicity tests were also assessed by LDH assay using SAOS–2 human osteogenic sarcoma cells (for biocomposite samples and for used additives).

RESULTS

SEM analysis - The influence of the CO_2, additives and SBA–15 nanoparticles on the final structure of processed PCL and PCL/SBA-15 biocomposites was observed by SEM. Some selected results are presented in Figure 1. Nevertheless, the highly macroporous morphologies of obtained biomaterials was evident for all processed samples. In addition, it was also observed that samples morphologies were quite different depending on the type of incorporated additive (or mixture of additives) and on the different amounts of loaded SBA-15 SNPs. The pore diameter for PCL/G and PCL/P biocomposites seemed to be greater if compared with the others biocomposites incorporating the PB and I liquid additives.
However and in general terms, the observed pore diameters were smaller for those samples loaded with higher SNPs amounts (30 % w/w) (and for all other tested liquid additives). This usually happens due to the presence of SNPs which will affect the foaming process by favoring heterogeneous gas nucleation: SNPs will act as nucleating agents, providing more nucleating points and a more effective contact between SNPs, PCL and $scCO_2$ (gas), thus

lowering the energy barrier for cell nucleation and increasing the nucleation rate. Therefore and for higher amounts of SNPs, more pores will be formed but having smaller diameters (as the amount of dissolved gas is constant) [2]. The biocomposites prepared with the mixture of two liquids additives (GPB and IPB) and 10 % w/w of SNPs presented more regular pore size distribution and the pores seemed to be interconnected, however appropriate techniques will be used in the near future to confirm these findings. The micro-, meso- and low-range (50nm-500μm) macro-porosities must be discussed in terms of nitrogen adsorption, mercury porosimetry and helium picnometry.

Nitrogen adsorption – In general terms, the incorporation of all liquid additives into prepared biocomposites resulted in the surface area and pore volume decrease for all processed samples. This may be due to the observed pore diameters increase and pore densities decrease. On the other hand, SNPs incorporation led to a significant increase on obtained surface areas and pore volumes, namely for those biocomposite samples loaded with the higher amount of SNPs (30 % w/w). This increase is certainly due to the extremely high surface area and pore volume of the SNPs. The average pore diameters were found to be around 0.3 nm for PCL/liquid additives, and increases after SNPs addition reaching values from ~0.4 nm to 1 nm with the addition of 10% and 30% (w/w), respectively.

Helium picnometry and mercury intrusion – By the incorporation of the liquid additives into the polymeric matrix, the real density of the biocomposites decreased for all prepared samples. This result may be attributed to the lower real density of the various used additives. This decrement was more pronounced for the biocomposites with the mixture of two liquids additives, GPB and IPB, which lowered the composites densities to ~12 and ~11%, respectively, comparatively to the density of pure processed PCL. Opposite results were obtained with the incorporation of SNPs. The real density of the prepared biocomposites was found to increase due to the higher real density of SNPs (1.8 $g \cdot cm^{-3}$). The real density of the prepared foams increased with the increasing of SNPs. Similar results were found and reported in the literature [16].

Mercury intrusion led to average pore diameters that were similar for PCL/liquid additives biocomposites without and with 10% w/w of SNPs (from 0.1 to 0.2 μm, respectively); the pore diameters increases when 30% w/w of SNPs were incorporated (from 0.3 to 0.4 μm).

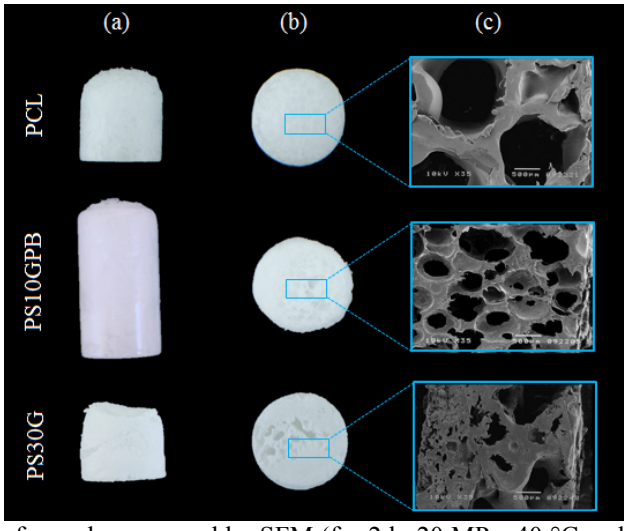

Figure 1: Selected images of samples prepared by SFM (for 2 h, 20 MPa, 40 °C and 0.37 $L_{CO2} \cdot min^{-1}$). (a) Side-view; (b) Top-view and (c) SEM micrographs for pure PCL, and for PS10GPB and PS30G biocomposites.

Melting and glass transition temperatures - The melting temperature (T_m) of pure SFM-processed PCL samples slightly decreased to ~ 62°C (supplier data: 64ºC), due to the temporary $scCO_2$ induced plasticization, chain rearrangement and crystallinity promoted during the process. Similar effects were already obtained and reported in literature [16]. As expected and in general terms, the incorporation of the liquid additives into prepared samples led to a decrease in the melting temperature of all samples (with or without the incorporation of SNPs). However this reduction was much more significant in the case of glycofurol (~11 %) and of isosorbide dimethyl ether (~8 %) and if compared with pure processed PCL. These results showed that the PCL was clearly plasticized by the 4 employed liquids, however in different extents. This is probably due to their different liquid molar volumes and to specific interactions that liquids can establish with PCL. On the other hand and in general terms, melting temperatures increased for 10% (w/w) SNPs incorporation and then decreased when the amount of SBA-15 was increased up to 30 % (w/w), which is probably due to the formation of biocomposite structures having less favorable interactions between all substances (but mostly between PCL and SBA-15 NPs) and to the formation of smaller crystallites in the final composite structure [17]. However, when two liquid additives are combined, it is possible to obtain 10% (w/w) PCL/SBA-15 biocomposites presenting much lower melting temperatures, which illustrates the important role that may be played by the different types of interactions that may be established between all substances, and by their combined effect (by adding different liquid additives and/or different relative amounts). Further studies on these effects will be performed in a near future.

The influence of liquid additive incorporation on PCL glass transition temperatures was also evaluated (not presented). It was observed that, without the incorporation of SNPs, all T_g values decreased with the addition of the different liquid additives, which again confirms the plasticizing effects of these substances. However, SNPs incorporation also affected biocomposites T_g values, which slightly increased as the SBA-15 amount was also increased up to 30 % w/w (which is probably due to limited chain mobility caused by the presence of SNPs [18]).

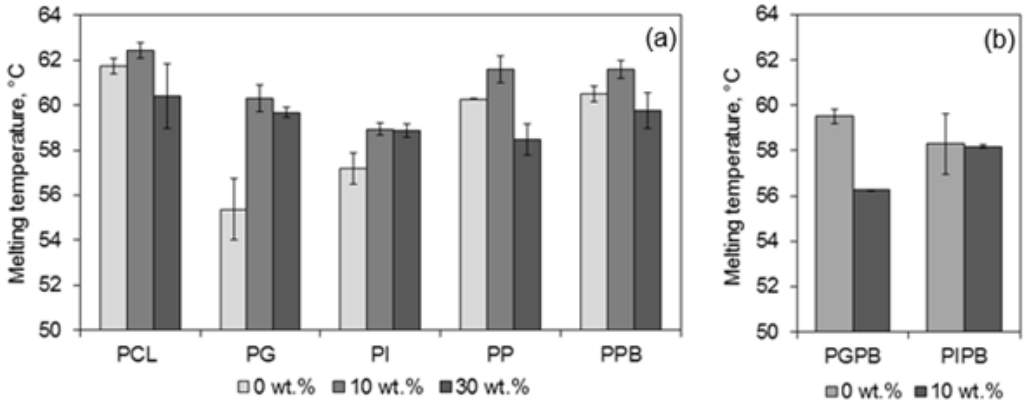

Figure 2: Melting temperatures of samples prepared by SFM (for 2 h, 20 MPa, 40 °C and 0.37 $L_{CO2} \cdot min^{-1}$). (a) single liquid additive; and (b) mixture of two liquid additives. Biocomposites were prepared with different SNPs amounts (0%, 10 and 30% w/w).

Mechanical analysis and cytotoxicity tests - The effect of the incorporation of SNPs on samples storage moduli (E'), obtained by DMTA, showed that, in general terms, a 10 % w/w SBA-15 incorporation led to a decrease in E' values while a 30 % w/w incorporation led an

increase in the E' values. These results are in line with the behavior observed for the melting temperatures and may be due to the already discussed formation of less favorable interactions between all substances for lower SNPs amounts.
Finally, preliminary cytotoxicity tests revealed good cell viability after 72 hours of cell seeding, for all tested biocomposites and liquid additives/SNPs.

CONCLUSIONS

Porous biomaterials made of pure PCL and of PCL/SBA-15 (70:30 and 90:10 %w/w) were prepared using a supercritical CO_2-assisted foaming/mixing (SFM) method. Additionally and in order to obtain highly porous plasticized biocomposites, four "green" additives (glycofurol, isosorbide dimethyl ether, N,N,N-trimethylethanolammonium pentanoate and tetradecyl(trihexyl) phosphonium bistriflamide) were also incorporated into PCL and PCL/SBA-15 physical mixtures and processed using the same SFM processing conditions. Obtained results showed that morphological and thermomechanical properties of the prepared biocomposites were strongly dependent on the additive nature and on relative PCL/SBA-15/additives compositions. In addition, preliminary in vitro cytotoxicity screening tests showed that prepared biocomposites and employed additives were highly cytocompatible with SAOS-2 cells. In conclusion, obtained results demonstrated the viability of using the SFM method and these plasticizer/porogenic liquids for the development of highly porous/plasticized cytocompatible PCL/SBA-15 biocomposites presenting tunable physicochemical, thermomechanical and morphological properties.

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