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Generation of Loaded PMMA Scaffolds Using Supercritical CO_2 Assisted Phase Separation

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Poly (methyl methacrylate) (PMMA) is a polymer largely used in tissue engineering applications. It has a good degree of compatibility with human tissues. A relevant biomedical application of PMMA is represented by the production of scaffolds to be used as controlled release devices for pharmaceutical products. Loaded PMMA scaffolds were prepared using a supercritical fluid-phase separation process, in which CO_2 acts as the non-solvent. Supercritical carbon dioxide (SC-CO_2) phase separation process has several advantages with respect to traditional scaffold formation processes (foaming, electrospinning, phase separation, etc); for this reason, in this work we tested this method to produce PMMA scaffolds loaded with two different pharmaceutical compounds: Cefuroxime and Diclofenac sodium, respectively, an antibiotic and an anti-inflammatory drug. We prepared PMMA scaffolds using two different procedures: dissolving PMMA and Diclofenac sodium in acetone or suspending Cefuroxime in the organic solution formed by PMMA and acetone. The effect of the drug concentration on scaffold morphology and pore size was analyzed. The obtained scaffolds, produced in correspondence of various drug loadings, were characterized by SEM, to study the morphology and pore size, and by EDX to analyze the drug distribution in the scaffolds. Some drug release rate analyses were also performed, observing very different release behaviors, depending of the solution/suspension process adopted. The results obtained confirmed the feasibility of the loading process and the advantages with respect to traditional methods: efficient encapsulation of the drug and absence of burst effects.

1.Introduction

Poly (methyl methacrylate) (PMMA) is a polymer largely used in biomedical applications. It has a good degree of compatibility with human tissues [Vecsei et al., 1981; Goodwin et al., 1997]. A relevant biomedical application of PMMA is represented by the production of porous scaffolds to be used as controlled release devices for pharmaceutical products [Wahling et al., 1978; Henry et al., 2000]. The drug release from a scaffold is controlled by different mass transfer mechanisms, such as diffusion, erosion, swelling or osmosis. It depends also on the material properties (composition, porosity, roughness, wettability and water uptake) and on the drug properties, such as its solubility and molecular weight. Therefore, a modification of these parameters changes the release profile (time and rate) of the drug [Padilla et al., 2002].
Many methods for fabrication of tissue-engineered scaffolds and drug delivery devices from biodegradable polymers involve the use of organic solvents [Park et al., 1997; Jang and Shea, 2003]. Park et al. (1997) studied the formation of biodegradable scaffolds of poly(L-lactide) (PLLA) using an air drying phase separation technique. PLLA was dissolved in methylene chloride-ethylacetate mixtures and cast on the knitted polyglycolic acid (PGA) meshes, followed by an air-drying process. The use of the three-component polymer solution (PLLA-methylene chloride-ethylacetate) generated porous substructures in the PLLA scaffolds during the solvent evaporation. Flurbiprofen and tetracycline were incorporated in the structures by adding the drugs in the PLLA solution. The drug release kinetics mainly depended upon the hydrophobic-hydrophilic properties of the drugs and the porosity of the membranes. The release rate could be further controlled by loaded drug contents. Jang and Shea (2003) presented an approach to fabricate scaffolds capable of controlled delivery characterized by the assembly and subsequent fusion of drug-

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loaded microspheres using a gas foaming/ particulate leaching process. DNA-loaded microspheres were fabricated from the copolymers of lactide and glycolide (PLGA) using a cryogenic double emulsion process. Scaffolds fabricated by fusion of these microspheres had an interconnected open pore structure, maintained DNA integrity, and exhibited sustained release for 21 days. Control over the release was obtained through manipulating the properties of the polymer, microspheres, and the foaming process.
Supercritical CO_2 (SC-CO_2) phase separation offers an attractive alternative process to obtain solvent free scaffolds. Usually, the phase separation of polymeric solutions is obtained by liquid non-solvent addition [Van de Witte et al., 1996]. The polymer solution is immersed into a coagulation bath filled with a non-solvent: the solvent diffuses out the casting film, while the coagulant (non-solvent) diffuses into it. The contact between the solvent and the non-solvent causes the phase transition and polymer precipitation, generating porous structures. Substituting the liquid non-solvent with the SC-CO_2 there are several advantages: indeed, SC-CO_2 can dry the membrane rapidly without the collapse of the structure due to the absence of a liquid-liquid interface, the process does not require additional post-treatments and it is easy to recover the organic solvent. Some works on porous structures generation using SC-CO_2 have been proposed in the literature [Cardea et al., 2006; Reverchon et al., 2007; Reverchon et al., 2008; Reverchon et al., 2010; Cardea et al., 2011; Cardeaet al., 2013]. The possibility of obtaining different scaffold morphologies, modulating cell and pore size by process parameters, has been demonstrated [Cardea et al., 2006; Reverchon et al., 2007; Reverchon et al., 2008; Reverchon et al., 2010].
Since SC-CO_2 phase separation process has several advantages with respect to traditional phase separation processes, in this work we tested this method to produce PMMA scaffolds loaded with two different drugs: Diclofenac sodium (DS) and Cefuroxime, respectively, an anti-inflammatory and an antibiotic. The effect of different organic solvents and of the drug concentration on membrane morphology and cell size was analyzed. Some preliminary release rate experiments were also performed.

2. Experimental section

2.1 Materials

PMMA (molecular weight 120,000), acetone (purity 99.8 %), and chloroform (purity 99.5 %) were bought from Sigma-Aldrich; CO_2 (purity 99 %) was purchased from S.O.N. (Società Ossigeno Napoli, Italy), DS and Cefuroxime were kindly supplied by Novartis Pharma (Napoli, Italy). All materials were processed as received.

2.2 Scaffold preparation

Scaffolds were prepared in a laboratory apparatus equipped with a 316 stainless steel high-pressure vessel with an internal volume of 80 mL, in which SC-CO_2 contacts the polymer solution in a single pass. PMMA was dissolved in the solvent; drug was loaded into the PMMA solutions by dispersing it at various polymer/drug weight ratios. The solution (or dispersion) obtained was placed in a formation cell (steel caps with a diameter of 2.5 cm and heights of 300 μm) spreading it with a glass stick to control the thickness of the film. The cell was rapidly put inside the preparation vessel to avoid the evaporation of the solvent. The vessel was, then, closed and filled from the bottom with SC-CO_2, up to the desired pressure using a high-pressure pump (Milton Roy–Milroyal B, France). We operated in batch mode for 45 min; after this period of time, a micrometric valve was opened and the operation was performed in continuous mode; i.e., with a constant CO_2 flow rate at 1.5 kg/h. Pressure and temperature were hold constant and the phase-separated structure was dried for 45 min. Then, the vessel was slowly depressurized for 30 min.

2.3 Scanning electron microscopy (SEM).

PMMA scaffolds were examined by cryofracturing them with a microtome (Bio-optica S.p.A, Italy, Mod. Microm HM 550 OMVP), sputter coating the samples with gold, and viewing them by scanning electron microscope (SEM) (mod. LEO 420, Assing, Italy) to determine pore size and scaffold structure. Sigma Scan Pro 5.0 software (Jandel scientific, San Rafael, CANADA) and Origin 7 software (Microcal, Northampton, USA) were used to determine the average diameter of the pores and to calculate pore size distributions. It was measured approximately 350 pores for each scaffold.

2.4 Energy Dispersive X-Ray analyzer (EDX)

Drug dispersion in the PMMA scaffolds was measured using an Energy Dispersive X-Ray analyzer (EDX mod. INCA Energy 350, Oxford Instruments, Witney, UK). Before the evaluation of the elemental composition, the samples were coated with Chromium (layer thickness 150 Å) using a turbo sputter coater (mod. K575X, EmiTech Ashford, Kent, UK).

2.5 Solvent Residue Analysis

Acetone and chloroform residues were measured by a headspace (HS) sampler (model 7694E, Hewlett Packard, USA) coupled to a gas chromatograph (GC) interfaced with a flame ionization detector (GC-FID, model 6890 GC-SYSTEM, Hewlett Packard, USA). Solvents were separated using two fused-silica capillary columns connected in series by press-fit: the first column (model Carbomax EASYSEP, Stepbios, Italy) connected to the detector, 30 m length, 0.53 mm i.d., 1 µm film thickness and the second (model Cp Sil 5CB CHROMPACK, Stepbios, Italy) connected to the injector; 25 m length, 0.53 mm i.d., 5 µm film thickness. GC conditions were the ones described in the USP 467 Pharmacopea with some minor modifications: oven temperature from 45 °C to 210 °C for 15 min. The injector was maintained at 135 °C (split mode, ratio 4:1) and helium was used as the carrier gas (5 mL/min). Head space conditions were: equilibration time, 30 min at 95 °C; pressurization time, 0.15 min, and loop fill time, 0.15 min. Head space samples were prepared in 20 mL vials filled with internal standard DMI (3 mL) and 500 mg of NaCL and water (0.75 mL), in which samples of loaded PMMA scaffolds, generated by SC-CO_2 phase separation, were suspended.

2.6 In vitro drug release

In vitro release assays were performed to determine the kinetics of drug release from scaffolds. The drug loaded scaffold was immersed in a glass vial containing a physiological saline solution (pH 7.2) as releasing medium (1000 mL). The sealed vial was placed in an oven at 37 °C and shaken at 200 rpm. At predetermined time intervals, a sample was filtered and the concentration of drug was assayed using a UV spectrophotometer (Varian, mod. Cary 50 Scan, Palo Alto, USA).

3. Results and discussion

A drug can be loaded in a scaffold using two different techniques: dissolving it in the organic solvent used to solubilize the polymer or generating a suspension of the drug in the organic solution formed by polymer and solvent. These two strategies could give different results in terms of scaffold formation, characteristics and drug release kinetics. In the following, it will propose different solvents and/or solutes to explore the various characteristics of loaded PMMA scaffolds. All experiments were performed at the same process conditions; i.e. operating at 200 bar, 45 °C and with a scaffold thickness of 300 µm.

3.1 PMMA-Diclofenac Sodium

DS is soluble in acetone; therefore, we prepared solutions containing 80 % of acetone and 20 % of mixture formed by PMMA and DS. We performed experiments at percentages of dissolved drug in the mixture of 25 % w/w PMMA. To study the effect of the state of the drug in the scaffold, we performed experiments using chloroform as solvent too. DS is not soluble in chloroform, therefore we prepared solutions containing 80 % of chloroform and 20 % of mixtures formed by PMMA and DS (25 % w/w).

Figure 1 shows the comparison of PMMA scaffolds, containing 25 % of DS, prepared using: a) acetone, in which drug is soluble, and b) chloroform, in which drug is not soluble. In both cases, a cellular structure was obtained; this result indicates that nucleation and growth of droplets of the polymer-lean phase with further solidification of the polymer-rich phase happened during the supercritical phase separation.

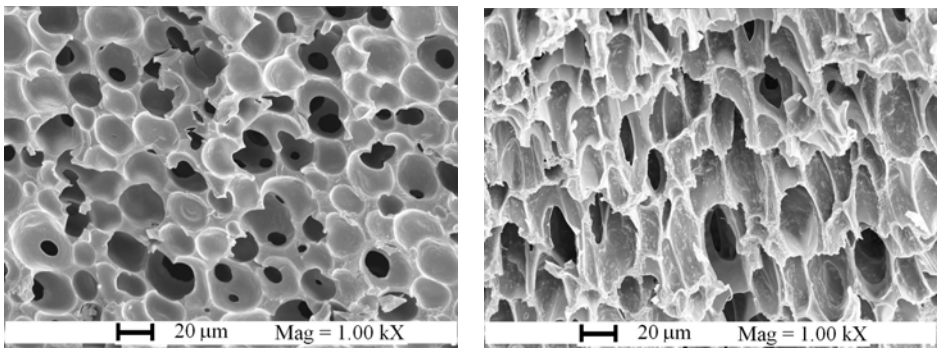

Figure 1: Comparison of scaffolds obtained operating at 200 bar, 45 °C and 80 % (w/w) of solvent, starting from a) dissolved drug (on the left) and b) dispersed drug systems (on the right).

Moreover, it is clear a change in scaffolds morphology: using acetone, surface and cells wall are smooth (Figure 1a); when chloroform is used, DS is initially dispersed in the polymeric solution and remains in this form: it is physically suspended in the polymer matrix, as is shown in Figure 1b. Solvent residue analyses

were also performed and values of acetone and chloroform lower than 5 ppm were found; i.e., as expected, SC-CO_2 phase separation allowed to completely remove the organic solvent of the starting solutions.

Qualitative and quantitative analyses on PMMA loaded scaffolds were also performed; in particular, DS distribution along the scaffolds was evaluated by EDX analysis. In Figure 2, element maps of PMMA/DS composite scaffolds obtained at 200 bar and 45 °C are reported. The drug (sodium atoms - green map) is uniformly distributed along the scaffold section and completely overlaps the presence of polymer (carbon atoms - red map); this result is extremely important because confirms that the supercritical phase separation process allows to obtain polymer/drug composite scaffolds characterized by a homogenous distribution of the active compound. This result was observed both with solubilized and suspended drug.

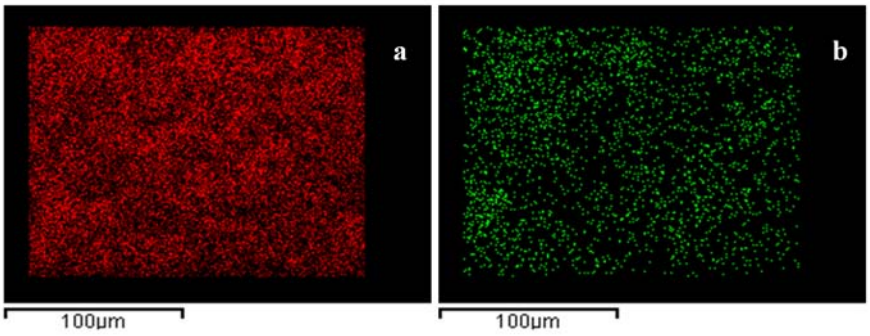

Figure 2: EDX analysis of PMMA/DS composite scaffolds obtained at 200 bar and 45 °C; a) red: carbon map, b) green: sodium map.

Since DS is largely soluble in water, PMMA scaffolds loaded with this drug were tested for drug release analysis. Figure 3 reports three release rate curves related to untreated DS, PMMA scaffold obtained starting from DS suspension and PMMA scaffold obtained starting from DS dissolved. It is possible to put in evidence that, untreated DS dissolves completely in 10 minutes. Using porous PMMA scaffolds as the controlled release device, we observed a DS prolonged release for 80 hours. No burst effect was observed: i.e., no initial fast release of the drug was obtained. It means that no drug is present on the outer layer of the scaffold, but it is (correctly) dispersed inside it.

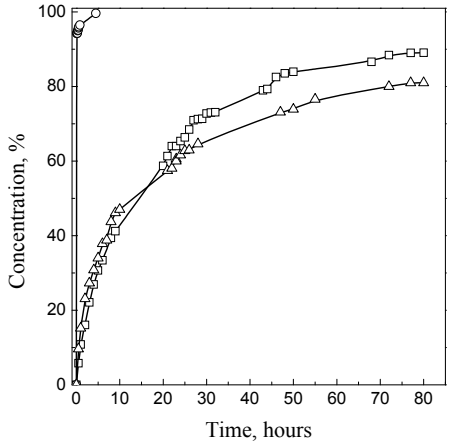

Figure 3: Release curves of: (∘) untreated DS, (□) PMMA scaffold obtained using DS suspension and (▲) PMMA scaffold obtained using DS in solution.

During the first 20 hours, the two scaffolds release curves nearly overlap. This evidence means that, at this stage of the process, drug release is governed by the same mass transfer mechanism, independently if the drug is dissolved or dispersed in the matrix. Between 20 and 80 hours, the scaffold containing the drug in dispersed form releases the drug more rapidly than the scaffold containing dissolved drug. This result can be explained considering that, when the DS is dissolved into the polymeric matrix, drug release is slowered by the polymer matrix more than in the case it is dispersed. Drug granules are more easily

dissolved than the uniformly dispersed drug (even if in this last case it is in an amorphous form inside the polymer).

3.2 PMMA-Acetone-cefuroxime

Cefuroxime is not soluble in acetone; therefore, we prepared a suspension containing 80 % of acetone and 20 % of mixtures constituted by PMMA and cefuroxime. In this case, we performed a set of experiments varying the percentage of dispersed drug from 10 to 30 % w/w PMMA. In Figure 4, examples of SEM images of scaffolds obtained at 10 and 30 % w/w of cefuroxime in PMMA are reported. Increasing the percentage of cefuroxime, the mean diameter of cells increases.

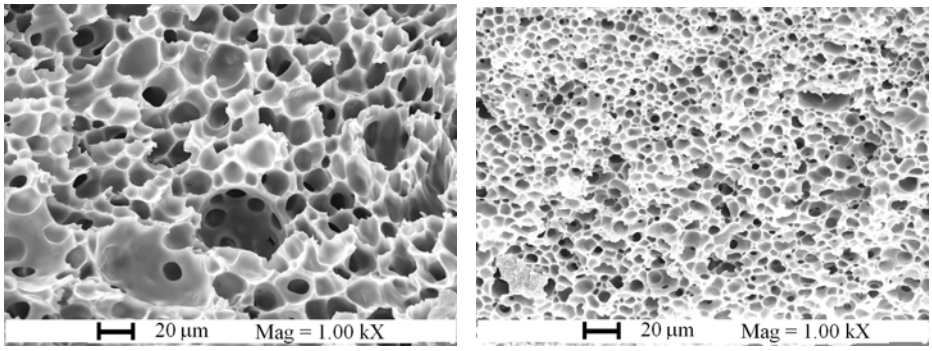

Figure 4: SEM images of PMMA scaffolds prepared using 80% w/w of acetone containing different percentage of cefuroxime: a) 30% (left), b) 10% w/w (right).

These data were quantitatively confirmed by pore size analyses reported in Figure 5; indeed, increasing the percentage of cefuroxime from 10 to 30 % w/w, the cells mean diameter increases from 5 to 15 µm. This result can be explained considering that, since cefuroxime is not soluble in acetone, increasing the drug percentage, the amount of polymer in the starting solution decreases. It is known from the classical theory on phase separation that, when the polymer concentration in the starting solution decreases, the mean cells size increases because the polymer lean-phase/polymer rich-phase ratio increases too.

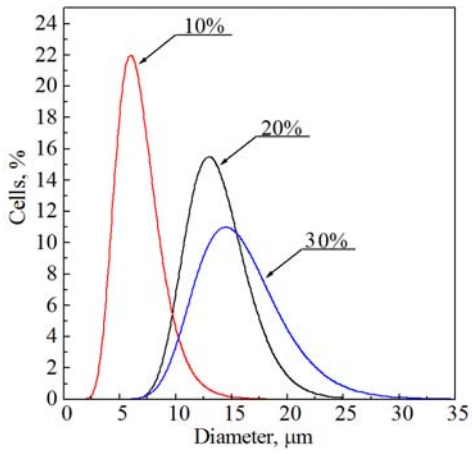

Figure 5: Pore size distribution of PLLA/cefuroxime composite scaffolds obtained using different drug concentrations: 10, 20 and 30% w/w.

Solvent residue analyses were performed and, also in this case, a residue of acetone lower than 5 ppm was found. Qualitative analysis on cefuroxime distribution in PMMA scaffolds was also performed by EDX. In Figure 6, element maps of PMMA/cefuroxime composite scaffolds obtained at 250 bar and 45 °C are reported. The drug (sulphur atoms - green map) is uniformly distributed along the PMMA scaffold section and completely overlaps the presence of polymer (carbon atoms - red map); this result is similar to the one previously observed for the system PMMA/DS. This result was observed for each percentage of cefuroxime tested.

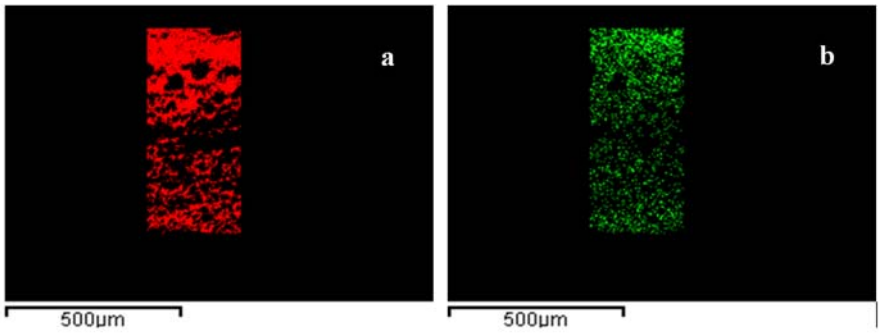

Figure 6: EDX analysis of PMMA/DS composite scaffolds obtained at 200 bar and 45 °C; a) red: carbon map, b) green: sulphur map.

4. Conclusions

Loaded PMMA scaffolds were produced using the supercritical CO_2-assisted phase separation method. The results confirmed the feasibility of the process starting both from ternary homogeneous solutions (polymer-drug-solvent) and from polymer-solvent solutions with suspended drug; moreover, evident advantages with respect to the traditional methods were obtained: short processing times, solvent-free structures formation, homogeneous encapsulation of the drug and absence of burst effects.

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