

# FIBROUS BIOPOLYMER COMPOSITES OBTAINED USING A SUPERCRITICAL CO<sub>2</sub> ANTISOLVENT PROCESS

Carlos A. García-González<sup>a</sup>, Ana M. López-Periago<sup>a</sup>, Arlette Vega-González<sup>b</sup>, Pascale Subra-Paternault<sup>b,c</sup>, Concepción Domingo<sup>a\*</sup>

<sup>a</sup>*Instituto de Ciencia de Materiales de Barcelona (CSIC). Campus de la UAB s/n, Bellaterra, E-08193 Spain.  
Phone: 0034 935801853. Fax: 0034 935805729. e-mail: conchi@icmab.es*

<sup>b</sup>*Laboratoire d'Ingénierie des Matériaux et des Hautes Pressions, C.N.R.S., Institut Galilée, Université Paris  
13, 99 Avenue Jean Baptiste Clément, Villetaneuse, 93430 France*

<sup>c</sup>*Refle, Umr Cnrs 8508, Ensam/Enscpb/Univ. Bordeaux1, Site Enscpb, 16 Avenue Pey-Berland, 33607 Pessac*

This work focuses on the preparation of both biopolymers and nanometric filler-polymer composites organized in a three-dimensional network of fibers. Poly(lactic acid) (L-PLA) or the blend poly(methylmethacrylate)/poly( $\epsilon$ -caprolactone) (PMMA/PCL) were chosen as biopolymers. The preparation of these materials was carried out by a supercritical antisolvent process. Using the same technique, polymeric matrices (PMMA/PCL blends) were further loaded with *ca.* 10-20 wt% of inorganic phase (titanium dioxide) distributed throughout the composite.

## INTRODUCTION

Tissue engineering is evolving from the use of implants that repair or replace damaged parts to the use of three-dimensional (3D) scaffolds constituted by biopolymers or blends, which induce the formation of new functional tissues either *in vitro* or *in vivo* [1,2]. A correct architecture of a scaffold should be able of mimicking the natural extracellular matrix that surrounds cells in the body. Hence, required characteristics include an interconnected 3D macroporous network necessary for cell proliferation, high microporosity essential for neovascularization, and high surface area required to promote cell adhesion [3]. Polymers used in tissue engineering applications are typically biodegradable homopolymers or copolymers (e.g. L-PLA, PGA, PCL), as well as non-biodegradable materials (e.g. PMMA). Blends composed by biostable and biodegradable polymers have recently gained significant interests, since the final products can be provided with specific advantages in degradation and mechanical properties. Other materials such as nanostructured hybrid polymeric/inorganic composites with low loadings of nanosized inorganic particles can significantly improve the mechanical and physical properties of the polymer matrix [4]

Conventional techniques for polymeric scaffold formation (e.g. solvent casting, foaming, phase separation, freeze drying) involves the use of organic solvents and the residual molecules left in the polymer after processing may be harmful to the transplanted cells. Technology based on supercritical carbon dioxide (scCO<sub>2</sub>) has been recently established as an alternative to overcome some of the problems associated with the use of traditional organic solvents for pharmaceuticals and biomaterials preparation [5-6]. Supercritical antisolvent spray processes have achieved considerable success in producing polymers with different morphologies [7-10]. The aim of this work was to explore the possibilities of using the scCO<sub>2</sub> antisolvent process for the preparation of intermingled fibers of (L-PLA) or the blend (PMMA/PCL) with controlled morphology and for the formation of fibers containing ultrafine mineral particles of titanium dioxide.

## MATERIALS AND METHODS

### Materials

The characteristics of the polymers used are shown in Table 1. Blend is composed by PMMA/PCL. Nanoparticles of titanium dioxide (TiO<sub>2</sub>, P-25S, Degussa) were first chosen as the model inorganic filler. The TiO<sub>2</sub> powder was previously silanized with octyltriethoxysilane using a scCO<sub>2</sub> technique (samples labeled TsSC). A detailed description of the silanization process can be found in the literature [11,12]. Dichloromethane (DCM, Prolabo) and CO<sub>2</sub> (99.95 wt%, Air Liquide) were the fluids used.

Table 1. Characteristics of employed raw polymers ( $\chi$  = crystallinity).

Polymer	Supplier	Molecular weight [gmol <sup>-1</sup> ]	Structure	Thermal transitions [°C]
PMMA	Bonar Polymers (Colacryl 300)	300 000	amorphous	T <sub>g</sub> = 120
PCL	Aldrich	14 000	Semicrystalline, $\chi=68$ %	T <sub>m</sub> = 67
L-PLA	Biovalley	100 000	Semicrystalline, $\chi=50$ %	T <sub>g</sub> =47, T <sub>m</sub> =176

### Methods

Experiments were prepared in the apparatus shown schematically in Fig. 1. The preparation of polymer fibers was carried out using SAS (Supercritical Anti Solvent) technique, whereas the nanometric filler-polymers composites were prepared using PCA (precipitation from a compressed anti-solvent) technique.

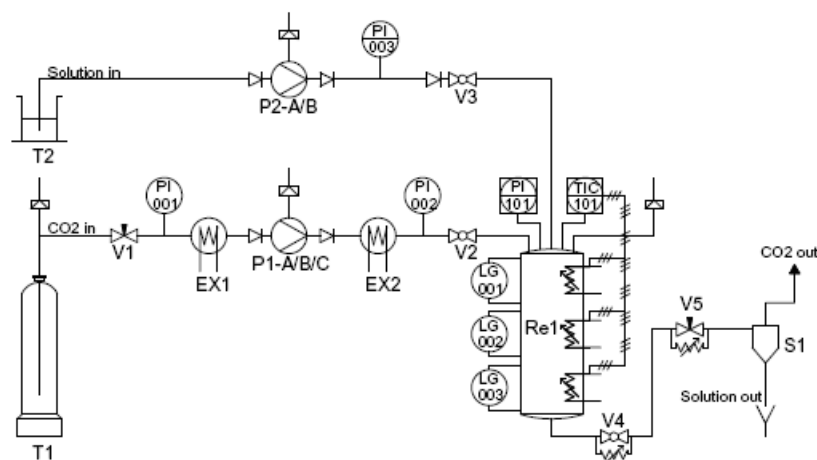


Figure 1: Experimental set-up of supercritical anti-solvent equipment

For the preparation of fibers, CO<sub>2</sub> was introduced in the vessel (Re1 in Fig. 1) until the selected pressure was reached (11.0 MPa). Then, valve (V5) was opened and the system was allowed to equilibrate maintaining the CO<sub>2</sub> flow at 100 mLmin<sup>-1</sup>. Next, the polymer solution was injected into the vessel (Re1) through a nozzle. For the preparation of nanometric filler-polymer composites, vessel (Re1) was pressurized with CO<sub>2</sub> until the pressure of 11.0 MPa. Next, the DCM suspension of filler nanoparticles and dissolved polymers was injected into the vessel through a swirl nozzle at a rate of ~2 mLmin<sup>-1</sup> by opening valve V3. In both cases, the precipitated polymer was dried using the scCO<sub>2</sub> flow. Finally, the high-pressure vessel was depressurized and samples were collected for characterization.

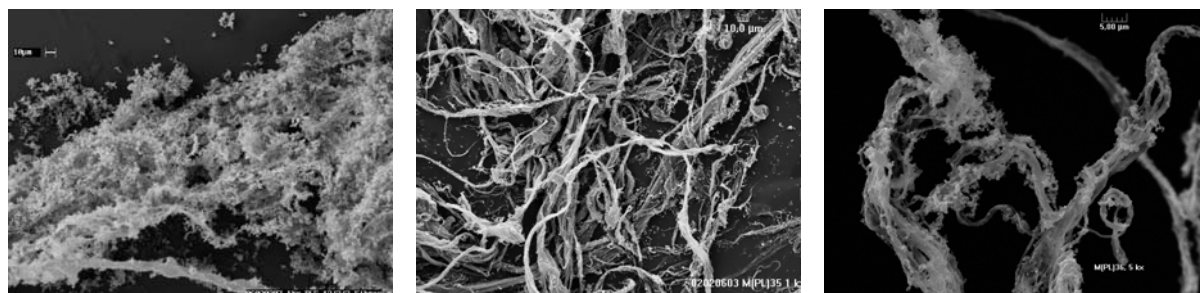
## Characterization techniques

The morphology of the polymer and composite samples was analyzed by scanning electron microscopy (SEM, Leica 5440), equipped with a solid-state backscattered probe for energy dispersive X-ray spectrometry (EDS, Princeton Gamma Tech). The specific surface area and pore volume distribution of prepared samples were determined by low-temperature N<sub>2</sub> adsorption (ASAP 2000 Micromeritics Inc.). Prior to measurements, samples were dried under reduced pressure (<1 mPa) at 323 K for 48 h. The thermal stability of the samples obtained was measured using thermogravimetric analysis (TGA, Perkin-Elmer 7) under an Ar atmosphere and at 5 °Cmin<sup>-1</sup> of temperature gradient.

## RESULTS

### (I) Preparation of L-PLA fibers

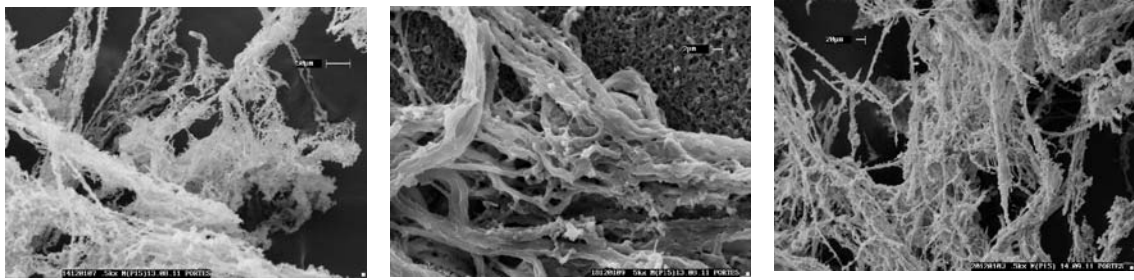
The most remarkable results on L-PLA fibers precipitation are shown here, but an extensive study can be found in the literature [13]. Variations on the liquid solution flow rate gave place to different results on the polymer morphology (Fig. 2): a large spreading in the axial direction was apparent in the micrographs of the L-PLA sample obtained at the low liquid flow rate of 1 mLmin<sup>-1</sup> giving place to a cobweb-like macrostructure. In contrast, at the high liquid flow rate of 6 mLmin<sup>-1</sup> viscous and inertial forces offer significant resistance to jet break-up. This led to the formation of a helical oscillation of the jet around its own axis. Finally, no significant effect of the nozzle design (disc or swirl) on macrostructure was observed when working at similar low liquid flow rates.



**Figure 2.** L-PLA fibers precipitated by the SAS process injecting the polymer solution separately through a disc nozzle at different liquid solution flow rate.

### (II) Preparation of PMMA/PCL blend.

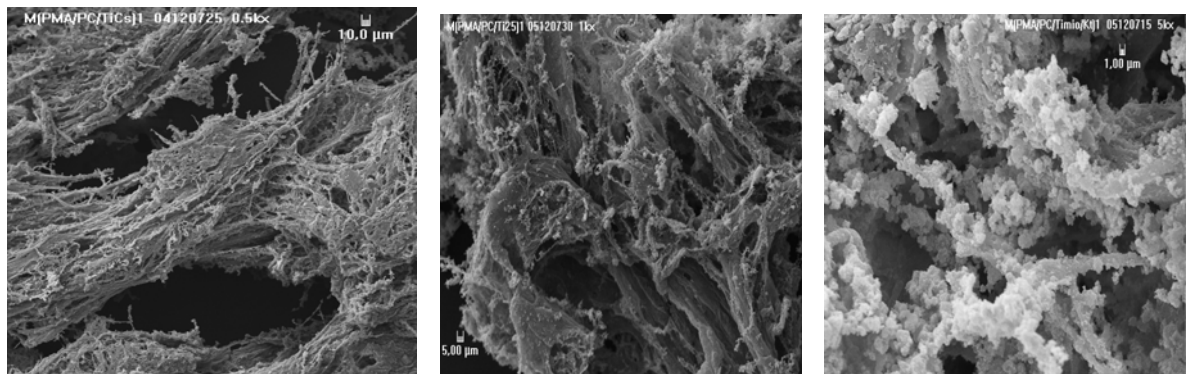
Liquid polymer solutions were prepared by dissolving different ratios of PMMA and PCL in DCM solvent [13]. PMMA and PCL are not thermodynamically miscible polymers and thus, in a solid mixture, they would exist in two phases, either segregated or blended in a metastable state. In this work, our approach to obtain a polymer blend was to precipitate the two polymers from diluted solution (0.8 wt% in DCM) rapidly using scCO<sub>2</sub> as the antisolvent. Using the SAS approach, fibers from PMMA/15 wt% PCL and PMMA/30 wt% PCL blends were obtained (Fig. 3). According to BET analysis, networks of blend fibers presented meso and microporosity and high specific surface area (200-400 m<sup>2</sup> g<sup>-1</sup>).



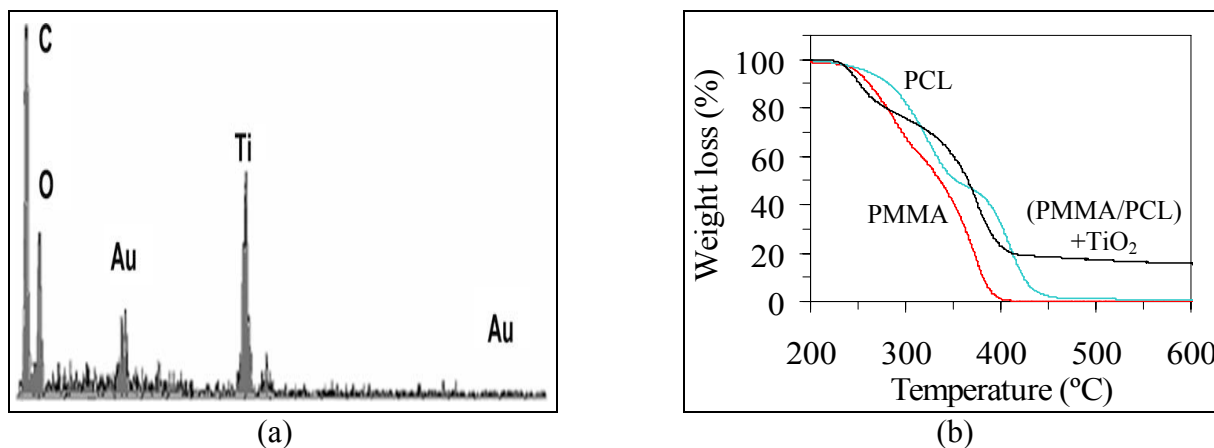
**Figure 3.** SEM images of precipitated fibers of PMMA with 15 wt% of PCL.

*(III) Preparation of nanometric filler-polymer composites (PMMA/PCL)+TiO<sub>2</sub>*

The preparation of these materials was carried out using PCA antisolvent technique [14]. In the developed process, the nonsoluble particulate filler was suspended in a polymer solution and both components were sprayed simultaneously into scCO<sub>2</sub>. Prior to suspension in the polymer solution, nanometric inorganic particles (TiO<sub>2</sub>) were silanized using scCO<sub>2</sub> as a solvent [11,12]. The silanization treatment was performed in order to improve filler dispersion and to enhance composite integrity by improving the inorganic filler-polymer interface interaction. The resulting composite was constituted by a fibrous network of (PMMA/15 wt% PCL)+TiO<sub>2</sub> and with the appearance shown in the SEM images of Fig. 4. The presence of the mineral filler was detected by EDS (Fig. 5a) and quantified after samples pirolysis using TGA analysis (Fig. 5b). The obtained thermogravimetric curves indicated that PMMA and PCL polymers vanished in the temperature interval of 250 to 450 °C. Thus, the residue above this temperature corresponded to the TiO<sub>2</sub> content of the fibers (~20 wt%) which was of the same amount as the initially added in the DCM suspension.



**Figure 4.** SEM images of precipitated fibers of (PMMA/15wt%PCL)+TiO<sub>2</sub>



**Figure 5:** TiO<sub>2</sub> content analysis of fibers of (PMMA/17wt%PCL)+TiO<sub>2</sub>: (a) EDS, and (b) TGA analysis.

## CONCLUSIONS

Fibers of L-PLA homopolymer and PMMA/PCL blends have been fabricated using the antisolvent SAS process. The percentile PMMA/PCL composition in the precipitated fibers reached the 30 % for PCL. PCA technique was used to precipitate blends of PMMA/PCL loaded with TiO<sub>2</sub> powder. In the used process, the inorganic filler was suspended in a solution formed by dichloromethane and the polymer. scCO<sub>2</sub> technology was also used to silanize the surface of the nanometric powder previous to suspension in the polymer solution with the aim to facilitate the dispersion in the hydrophobic liquid medium and to further improve adhesion between the filler and the matrix during composite formation. Networks of fibers were obtained for the system constituted by high molecular weight PMMA blended with 15 wt% PCL and loaded with 20 wt% TiO<sub>2</sub>.

## ACKNOWLEDGMENTS

The financial support of Spanish MEC projects (MAT2006-28189-E y CTQ2008-05370/PPQ) are greatly acknowledged. C. A. García-González and Ana M. Lopez-Periago give acknowledgment to CSIC for the funding support through I3P fellowship.

## REFERENCES

- [1] HUTMACHER D.W., *Biomater*, Vol. 21, **2000**, p. 2529
- [2] SHIN H., JO S., MIKOS A.G., *Biomater*, Vol. 24, **2003**, p.4353
- [3] WHANG K., THOMAS C.H., HEALY K.E., NUBER G., *Polymer*, Vol. 36, **1995**, p. 837
- [4] YEONG W.Y., CHUA C.K., LEONG K.F., CHANDRASEKARAN M.R., *Trends Biotechnol*, Vol. 22(12), **2004**, p.643.
- [5] QUIRK R.A., FRANCE R.M., SHAKESHEFF K.M., HOWDLE S.M., *Curr Op Solid State Mater Sci*, Vol. 8, **2004**, p.313
- [6] REVERCHON E., DELLA PORTA G., DE ROSA I., SUBRA P., LETOURNEUR D., *J Supercrit Fluids* Vol. 18, **2000**, p. 239
- [7] ELVASSORE N., BERTUCCO A., *Ind Eng Chem Res*, Vol. 40, **2001**, p. 795
- [8] YEO S.-D., KIRAN E., *J Supercrit Fluids*, Vol. 34, **2005**, p.287
- [9] BURG K.J.L, PORTER S., KELLAM J.F., *Biomaterials* Vol. 21, **2000**, p. 2347
- [10] SALGADO A.J., COUTINHO O.P., REIS, R.L., *Macromol Biosci* Vol. 4, **2004**, p. 743

- [11] GARCÍA-GONZÁLEZ C.A., ANDANSON J.M., KAZARIAN S.G., DOMINGO C., SAURINA J., Anal. Chim. Acta, Vol. 635, 2009, p. 227
- [12] DOMINGO C., LOSTE E., FRAILE J., J Supercrit Fluids Vol. 37, **2006**, p. 72
- [13] VEGA-GONZÁLEZ A., SUBRA-PATERNAULT P., LÓPEZ-PERIAGO A.M., GARCIA-GONZÁLEZ C.A., DOMINGO C., European Polymer Journal Vol. 44, **2008**, p.1081
- [14] GARCÍA-GONZÁLEZ. C.A., VEGA-GONZÁLEZ A., LÓPEZ-PERIAGO A.M., SUBRA-PATERNAULT P, DOMINGO, C., Acta Biomaterialia, In Press, 2009