

PLLA/PLA scaffolds prepared via Thermally Induced Phase Separation (TIPS): tuning of properties and biodegradability

V. La Carrubba, F. Carfi Pavia, V. Brucato, S. Piccarolo

Dipartimento di Ingegneria Chimica dei Processi e dei Materiali, Università di Palermo, Viale delle Scienze ed. 6, 90128 Palermo, Italy.

URL: www.dicpm.unipa.it

e-mail: lacarrubba@dicpm.unipa.it

ABSTRACT: Foams for tissue engineering applications were prepared via thermally induced phase separation (TIPS). Poly-L-Lactic Acid (PLLA) and blends of PLLA with PLA in different proportions were used (100/0, 90/10, 75/25, 50/50, 0/100 PLLA/PLA wt/wt) starting from ternary systems where dioxane was the solvent and water the non-solvent. Morphology was evaluated by Scanning Electron Microscopy (average pore size and interconnection) and the void fraction was measured by means of Hg porosimetry. Foams apparent density was also evaluated (porosity ranges from 87% to 92%). Biodegradability was estimated in a body mimicking fluid. Results show that structure and morphology (in terms of average pore size and distribution, interconnectivity and mechanical properties) depend upon the combination of the operating conditions adopted for the process (polymer concentration, solvent/non-solvent ration, demixing temperature and time). Furthermore, by blending PLLA with PLA it is possible to tune the biodegradability of the foam, thus addressing the possible applications in different fields of soft tissue engineering.

Key words: Tissue engineering, Scaffold, TIPS, PLLA/PLA blends

1 INTRODUCTION

Tissue engineering is an interdisciplinary field that applies the principles of engineering and life science toward the development of biological substitutes that restore, maintain and or improve tissue function¹. The production of an engineered tissue starts by the design and the formation of a structure able to support the migration and growth of cells that will originate the new tissue². Those structures are characterized by an interconnected pore network able to guide, after the degradation of the scaffold, the implanted cells to form a new tissue showing a well integrated structure.

A crucial aspect of the tissue engineering regards the scaffold biodegradability. Biodegradable materials, like polymers can decompose naturally but their degradation products will remain inside the human body. For bioresorbable materials, they will degrade after a certain period of time after implantation, and non-toxic products will be produced and eliminated via metabolism. For the chemical degradation, two different modes are defined, they are (1) hydrolytic degradation or hydrolysis which is mediated simply by water and (2) enzymatic degradation which is

mainly mediated by biological agents such as enzymes. Scaffolds should be biodegradable allowing extracellular matrix (ECM) to occupy the void space when the biomaterial is degraded. The rate of degradation, however is determined by factors such as configurational structure, copolymer ratio, crystallinity, molecular weight, morphology, stresses, amount of residual monomer, porosity and site of implantation³.

PLA is a biodegradable thermoplastic polyester that can be produced through ring-opening polymerization of lactic acid. Since lactic acid is a chiral molecule, it exists in two forms, D-PLA and L-PLA. PLLA is the result of L-PLA polymerization. While PLLA is semi-crystalline and it shows a high mechanical strength, poly(D,L-lactic acid) (PLA) is essentially amorphous, or, at most with a low crystallinity, owing to intrinsic structural reasons.. PLA is a methylated version of PGA but is less hydrophilic and, therefore, it degrades slowly. Poly(lactic acid) PLLA degrades to form lactic acid which is normally present in the body. This acid then enters tricarboxylic acid cycle and is excreted as water and carbon dioxide. No significant accumulation amounts of PLLA degradation have been reported in any of the vital organs⁴.

In this paper a preliminary approach was attempted to produce a PLLA/PLA scaffold via Thermally Induced Phase Separation (TIPS) with different weight ratio in order to tune scaffold mechanical properties, morphologies and degradation kinetics.

2 MATERIALS AND METHODS

2.1 Materials

The molecular weights of PLLA (ResomerTM, kindly supplied by Boehringer Ingelheim Pharma KG) and of PLA (Natureworks) were calculated via their intrinsic viscosities by utilizing the Mark-Houwink Constants reported in literature⁵. The W_v determined were $8,12 \cdot 10^5$ for PLA and $13 \cdot 10^5$ for PLLA.

2.2 Foam preparation and characterization

An homogeneous ternary solution of polymer (pure PLLA, PLLA/PLA 95/5 wt/wt and PLLA/PLA 90/10 wt/wt), dioxane and water was prepared, with a constant dioxane to water weight ratio of 87/13 or 84,5/15,5 based on previous literature studies on the same system^{6,7}. The concentration of PLLA was chosen to be 4% wt/wt. The solution, initially kept at 60 °C, was hot poured into an aluminium disc-shaped sample holder, with a diameter of 60 mm and a thickness of 2 mm. The temperature was then suddenly lowered to a value within the unstable region for 30 min, by pool immersion of the sample holder into a thermostatic water bath. Then a quench by pool immersion in an ethyl alcohol bath at a temperature of -20 °C for 5 minutes was performed in order to freeze the as-obtained structure.

The foams obtained were then subjected to washing in deionised water for 24 hours and drying at 20 °C under vacuum overnight, in order to completely remove any remaining solvent trace.

Foams were analysed by scanning electron microscopy (SEM) with a Philips 505 Microscope on sample cross section fractured in liquid nitrogen and gold stained.

Wide Angle X-ray Diffractometry measurements were carried out by means of a Bruker-AXS D8 Advance diffractometer with Cu-K α filtered radiation, by a focusing multilayer (Goebel Mirror), using the Bragg–Brentano geometry with 2 θ angle ranging from 5° to 35°, with a step angle and a step time of 0.18 and 10 s, respectively.

A differential scanning calorimetry (DSC) analysis was performed by recording the heating scans at 10 °C/min of films of pure PLA and blends with various PLLA/PLA weight ratios (90/10, 75/25, 50/50). Films were prepared by solvent evaporation

starting from homogeneous dioxane solutions.

2.3 Weight loss

To evaluate weight loss under body fluid-like condition, pure PLLA foams were washed in deionised water for 24 h, dried, weighed and kept in a well filled by various liquids (water, DMEM, DMEM supplemented with fetal bovine serum). The liquid into the well was changed every two days. After 30 days, the foams were extracted from the medium, rinsed in deionised water to remove the medium, dried, kept at 20 °C under vacuum for 24 hours and finally weighed. Then they were placed in a well for a further degradation step. After 60 days, the same protocol was followed to perform the dry weight measurement. The weight loss (%) of the crystalline residues after the hydrolysis was evaluated based on the weights of the foams in a dry state before and after the hydrolysis.

3 RESULTS

3.1 Weight loss of PLLA scaffolds

Table 1. % of weight loss of the hydrolysed foams

Days	0	30	60
Medium	Weight loss, %	Weight loss, %	Weight loss, %
Water	0%	0%	0%
D-MEM	0%	1.1%	1.3%
D-MEM/Serum	0%	22.1%	24.6%

In table 1 the weight loss percent of samples held in several kinds of liquids for 30 and 60 days are shown. Various samples (10 mm large and wide, 2 mm thick) were cut from a scaffold prepared at the demixing time of 30 minutes and at the demixing temperature of 30 °C, in order to check the reproducibility of the measurements. It is easy to notice that in the samples filled by water and in D-MEM, a modest weight loss has occurred; in the sample filled by D-MEM supplemented with fetal bovine serum during the first 30 days the foam weight loss is definitely highly remarkable, whereas the further weight loss from 30 to 60 days is definitely much smaller (few percent). A possible explanation of the observed behaviour (negligible weight loss for samples in pure D-MEM versus significant weight loss for samples in D-MEM supplemented with serum) stems from the observation that some enzymes reasonably contained in the bovine serum exhibit a remarkable catalytic activity in degrading PLLA.

This observation suggests the likely occurrence of a “selective hydrolysis”, which attacks mainly the amorphous region leaving almost unaffected the

