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

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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 biodegrability 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 function[1]. 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 tissue[2]. 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 implantation[3].

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 organs[4].

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 (Resomer^{TN}, 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 literature[5]. The W_v determined were 8,12 10^5 for PLA and 13 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-Kα filtered radiation, by a focusing multilayer (Goebel Mirror), using the Bragg–Brentano geometry with 2θ 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

Table1. % 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

crystalline domains, for which the activation energy barrier turns out to be too high to be overcome.

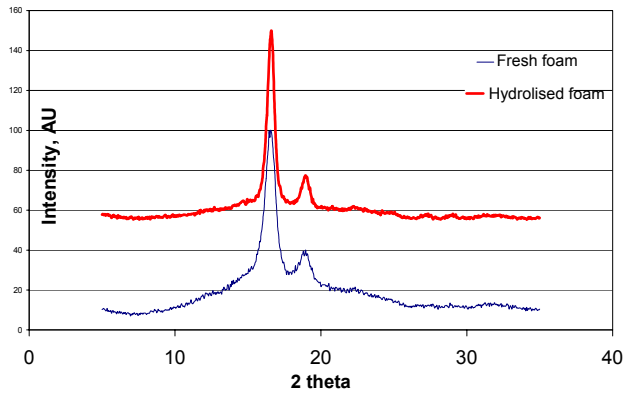

Fig. 1: WAXD patterns of fresh and hydrolised foam

Therefore, to determine the different amount of amorphous content, a WAXD analysis of the degraded foams and of the fresh sample (as prepared) was performed[7]. As shown in fig. 1, the fresh foam presents the typical pattern of a semicrystalline polymer with a crystalline content of 40 to 50 %. On the other hand, degraded foam shows two very intense and sharp peaks and a very low intensity of amorphous part, thus indicating a well-defined increase of crystalline to amorphous content ratio, in agreement with previous experimental data concerning degradation of PLLA in media with catalytic substances, such as enzymes. These data reveal that the foam weight loss depends on selective hydrolysis of amorphous regions, leading to intense degradation in the first 30 days.

3.2 Films of PLLA/PLA

In order to tune the biodegradability, an attempt to use mixtures of PLLA and PLA in various proportions, instead of pure PLLA, was made. In this way, the cristallinity of the scaffold should be lowered, and therefore the as-obtained scaffold should give rise to a faster hydrolysis and degradation.

Fig. 2 shows the DSC heating scans of various PLLA/PLA films obtained by solvent evaporation from an homogeneous solution. 90/10 PLLA/PLA blend exhibits a melting peak located around 180 °C, whereas pure PLA exhibits a peak located around 155 °C, with a melting enthalpy slightly lower than the one observed for the 90/10 blend. 75/25 sample shows a melting endotherm very similar to the 90/10, whereas the 50/50 sample exhibits a lowered ability to crystallize (lower enthalpy), related to blending. For the sake of a comparison, WAXD spectra were carried out on PLLA and PLA films and scaffolds as shown in figs. 3 and 4, respectively. It should be observed that the degree of crystallinity of PLLA and PLA does not show appreciable differences (in line with DSC results), in spite of the different structures of the polymers. This unexpected behaviour is probably related to the fact that in both cases samples were crystallized from solution, hence a very high degree of crystallinity is attained.

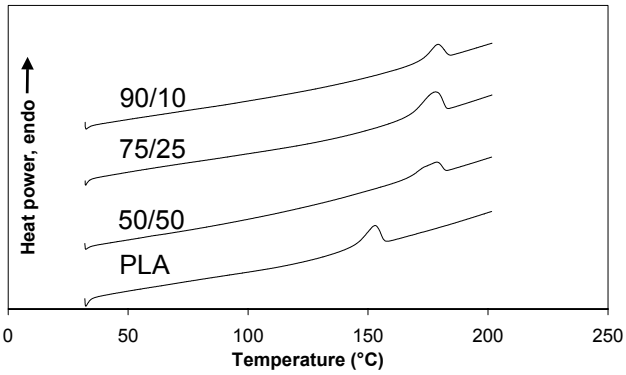

Fig 2: DSC heating scan of various PLLA/PLA films

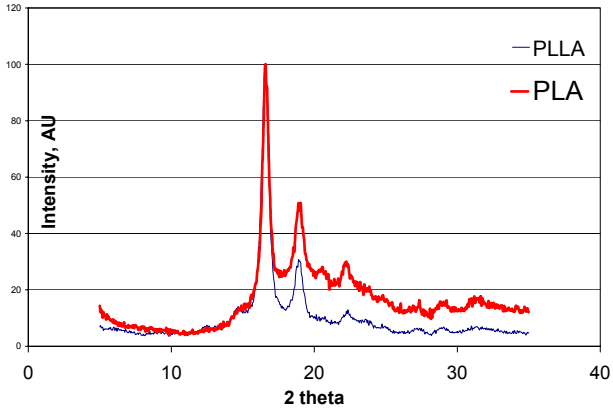

Fig. 3: WAXD of PLLA and PLA films

3.3 Scaffolds of PLLA/PLA blends

The cloud point curve of pure PLLA reported in literature clearly shows that using a solution containing the 4% of PLLA and a dioxane/water weight ratio 87/13, the metastable region is situated between 25 and 35 °C.

Using this solution with a mixture 95/5 PLLA/PLA wt/wt, instead of pure PLLA, at a temperature of 30 °C one does not observe phase separaration. On the contrary, lowering the demixing temperature to 25 and 20 °C leads to a porous structure (fig. 6). Finally, at the demixing temperature of 15 °C the structure collapses, i.e. the final thickness of the sample is drastically reduced.

Moreover, another TIPS was performed using a solution containing a different dioxane/water ratio (84.5/15.5). The results showed that the porous scaffolds were obtained when setting the demixing temperature at 35, 40 and 45 °C (see fig. 7). The scaffolds we made using pure PLLA and the same dioxane/water ratio were attained at the demixing temperatures of 56 and 52 °C.

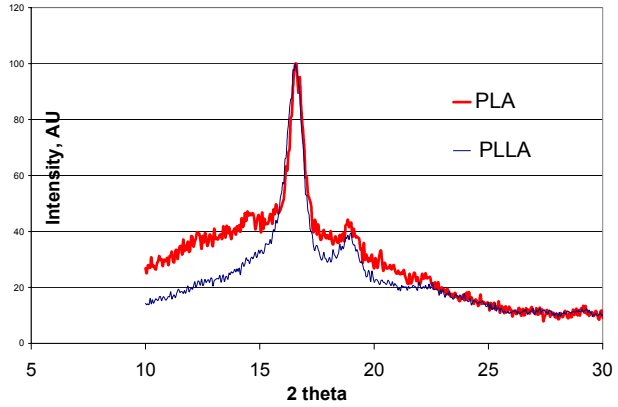

Fig. 4: WAXD of PLLA and PLA scaffolds

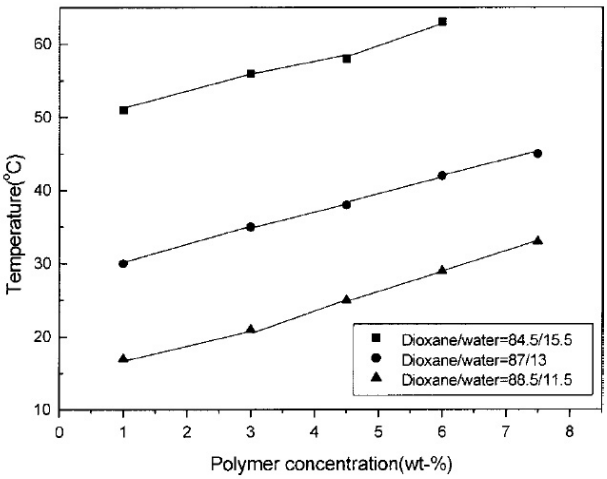

Fig. 5: Dependence of cloud point temperature on solvent composition and polymer concentration (from Hua et al, 2002[6])

Finally, a solution containing a 90/10 PLLA/PLA ratio with 87/13 and 84,5/15,5 dioxane water were utilized. The data revealed that at demixing temperature of 20, 25 and 30°C no homogeneous porous structure are obtained.

4 CONCLUSIONS

A preliminary study of the pure PLLA scaffold degradation kinetics in a biological environment has shown that two different kinds of degradation kinetics take place, a faster one involving the amorphous part of PLLA foams, and a slower one concerning crystalline regions.

The experiments about the use of blend PLLA/PLA suggested that adding the 5% of PLA to PLLA, the metastable region seems to be lowered by ~10 °C with respect to pure PLLA, regardless the dioxane/water ratio, probably due to a larger solubility of PLA in the solvent mixture with respect to PLLA. No homogeneous porous structures were obtained when using a 90/10 PLLA/PLA blend. This could be due to the fact that 90/10 foams do not exhibit adequate mechanical strength and therefore they collapse after phase separation.

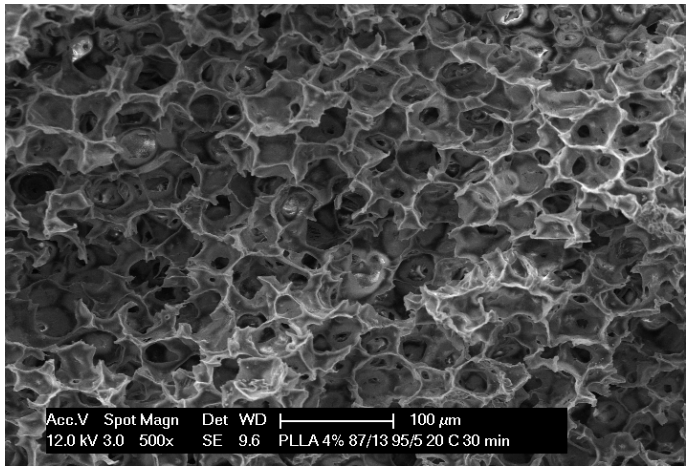

Fig. 6: SEM image of a scaffold prepared via TIPS. 95/5 PLLA/PLA, 87/13 dioxane/water. Demixing T = 20°C.

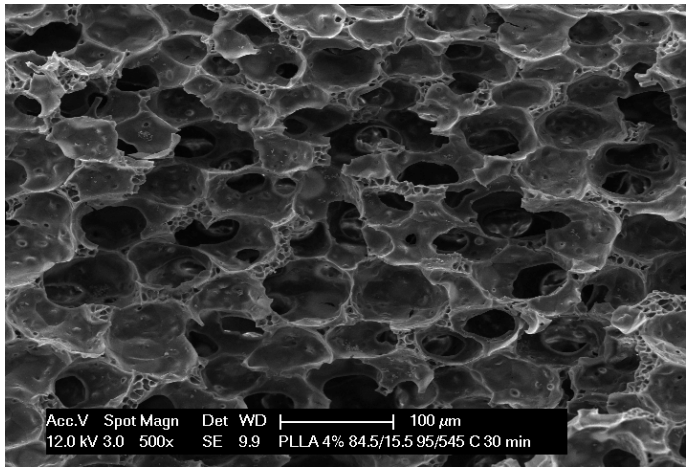

Fig. 7: SEM image of a scaffold prepared via TIPS. 95/5 PLLA/PLA, 84.5/15.5 dioxane/water. Demixing T = 45 °C.

Experiments are still in progress in order to explore the possibility to produce porous scaffolds starting from solution containing larger PLA amounts.

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