Demixing Time and Temperature Influence on Porosity and Interconnection of PLLA Scaffolds Prepared via TIPS

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Summary: Scaffolds suitable for tissue engineering applications were prepared by Thermally Induced Phase Separation (TIPS) starting from a ternary solution PLLA/dioxane/water. The experimental protocol consisted of three consecutive steps, a first quench from the homogeneous solution to an appropriate demixing temperature (within the binodal region), a liquid-liquid demixing stage for a given time and a final quench from the demixing temperature to a low temperature (within the spinodal region). A large variety of morphologies, in terms of average pore size and interconnection were obtained upon modifying the demixing time and temperature, owing to the interplay of nucleation and growth processes during the residence in the metastable state. An interesting combination of micro and macro-porosity was observed for longer demixing times (above 30 min at 35 °C).

Keywords: PLLA; porosity; thermally induced phase separation

Introduction

A very actual theme that has raised a large technological and scientific interest deals with the possibility of controlling polymer foams products to be employed as scaffolds for therapeutic applications like tissue or organs restoration. This kind of research is part of the so called tissue engineering discipline, which studies the functional-structural relationships of human tissues trying to restore, maintain, improve or eventually substitute the function of living tissues partially damaged; Langer.[1]

Those structures have to be characterized by a degradable interconnected pore network guiding the cell adhesion and proliferation, thus creating a regenerated tissue exhibiting a well integrated structure. The scaffold is indeed a biodegradable synthetic 3D support emulating the extracellular matrix; Schugens, et al. [2]. Among the polymeric materials used in tissue engineering, the synthetic resorbable polylactide (PLLA), have great potential because of their well-known biocompatibility and biodegradability; Nam and Park.[3] The scaffold morphology control is a focal point of a research area within highly integrated fields involving biomedical studies, engineering applications and biological investigations. The largest part of porous structure are produced by techniques based on liquid-liquid phase separation of polymer solutions; van de Witte et al.,[4] which still seem to be the most promising route, owing to its versatility and ease of operation and to the vaste latitude of morphologies achievable.

The typical mechanism of the phase separation of polymer solutions is liquid-liquid demixing leading to a polymer rich and a polymer lean phase; Han,[5] according to a binodal demixing or a to a spinodal decomposition or to a combination of both. The former, characterized by nucleation and growth, takes place inside the metastable region (between the binodal and the spinodal curve), while the second takes

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place inside the spinodal region. The kind of morphology developed during a phase separation process is therefore crucially affected by the particular thermal/time path followed during the process.

If the prevailing mechanism is nucleation and growth, a porous structure with poorly interconnected cells is obtained, while spinodal decomposition probably leads to interconnected network; Nam and Park.[3] By changing the polymer concentration, the solvent, the cooling rate and the final temperature it was then possible to vary the phase separation path leading to scaffolds exhibiting different micro and macro structures; Ho et al. [6] and final properties.

The aim of the present work is to explore a suitable route to reliably prepare porous biodegradable foams by thermally induced phase separation (TIPS) starting from PLLA/dioxane/water systems, varying the residence time and temperature in the metastable state, so as to control the final structure in terms of complex morphology, average pore size and degree of interconnection.

Experimental Part

An homogeneous ternary solution composed by PLLA (ResomerTN, kindly supplied by Boehringer Ingelheim Pharma KG, inherent viscosity 3 dl g^{-1}), dioxane and water was prepared, with a constant dioxane to water weight ratio of 87/13, based on previous literature studies on the same system; Hua et al. [7]. The concentration of PLLA was chosen to be 4% wt/wt. The solution, stable 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 pool immersion of the sample holder, into a thermostatic water bath, imposed a rapid drop in the temperature (continuously monitored by an embedded thermocouple) to a value within the metastable region; Hua et al. [7] (from 25 to 35 °C). Holding the sample holder in the bath for a well-defined time interval (from 5 to 60 min) and, subsequently, pool immerging it in an ethyl alcohol bath, at a temperature of −20 °C for 5 minutes, completed the forming process.

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

Pore size and morphology of the foams obtained were analysed by scanning electron microscopy (SEM) using a Microscope Philips 505 on a gold stained sample cross section, fractured in liquid nitrogen. The foam's porosity was determined measuring their thicknesses and cutting 1 cm foam disks. Weighing of disks led to their apparent density evaluation and then to the total porosity estimation.

Results and Discussion

Foams prepared from 87/13 (v/v) dioxane/water solutions, at a demixing temperature of 25 °C and at demixing times of 5 and 15 minutes, exhibit a similar average pore size (10 to 15 μm approx) and a very satisfactory level of interconnection.

Figure 1a and 1b show the SEM images of foams demixed at a temperature of 30 °C and demixing times of 5 and 15 minutes. Also these foams exhibit similar average pore size (12 to 15 μm approx).

By raising the demixing time to 30 minutes, the average pore size enlarge (50 μm approx), while the interconnection appears to remain sufficiently effective (Figure 2a). The average pore size further increases by going to 60 minutes (Figure 2b), although a lower lever of interconnection is observed, with most of the pores appearing as closed-cells.

Some remarkable differences may be observed in the foams prepared at demixing temperature of 35 °C. As shown in Figure 3, these foams show a larger average pore size with respect to the foams prepared at 30 °C; as a matter of fact the pore dimensions range from 50 to 90 μm. Moreover, the interconnection seems to decrease for demixing times over 30 minutes while an interesting microporosity (1.5 μm approx.) of the larger pore walls takes place.

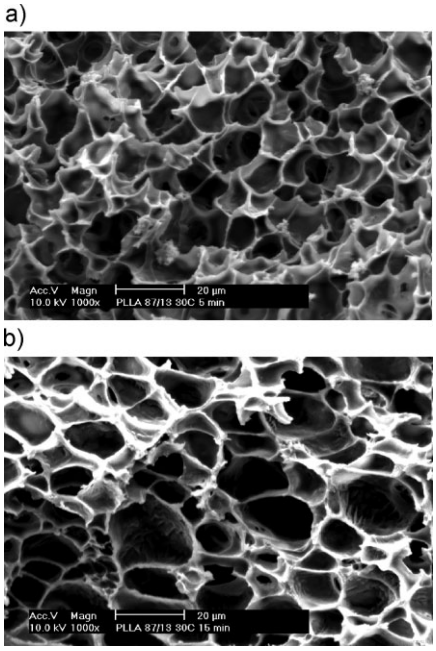

Figure 1.
SEM image of the cross section of PLLA samples at 30 °C; (a): 5 min, (b): 15 min.

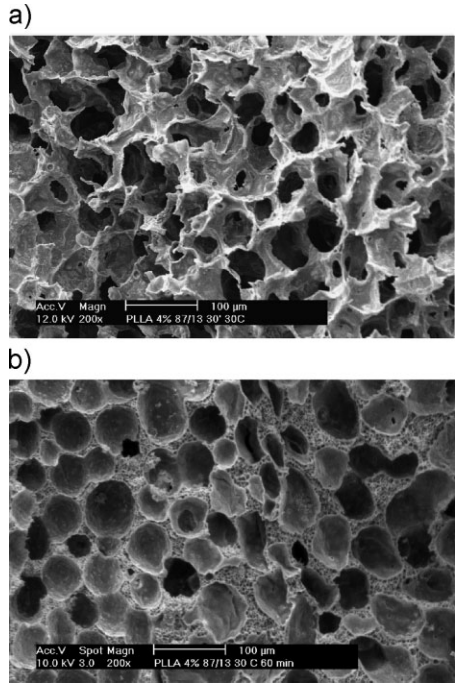

Figure 2.
SEM image of the cross section of PLLA samples at 30 °C; (a): 30 min, (b): 60 min.

The aforementioned information about pore size are summarized in Figure 4, where the average main pore size is plotted versus the demixing time for different demixing temperatures. The foams prepared with a demixing temperature of 25 °C under different demixing times present a constant pore size independent of demixing time, at least in the investigated demixing time interval. In the foams prepared at demixing temperature of 30 °C, pore diameter is almost independent of time up to 15 minutes; at 30 minutes a significant increase of pore size is observed, followed by a further increase of the average pore dimension at 45 and 60 minutes. The foams prepared at demixing temperature of 35 °C present an average pore size following the same qualitative trend exhibited by the foams prepared at 30 °C, with higher initial (short times) and final (long times) levels and a less remarkable difference between the two plateau levels (60–70 μm at 30 °C versus 40 μm at 35 °C). Pore size results can be interpreted on the basis of a balance between nucleation and growth processes. As matter nucleation is very sensitive to undercooling, the lower the temperature (i.e. the larger the undecooling), the most effective the process. At 25 °C a very fast nucleation sets in leading to a very low center to center nuclei distance; hence the growth process practically does not further affect the resulting pore size, being the process very slow due to diffusive constrains.

Upon raising the temperature to 30 °C the same argument discussed with reference to the behaviour at 25 °C holds in the early stages, although for times longer than 15 min the effect of growth starts to become detectable, being the diffusive constrains less stringent. Therefore one could notice an increase of average pore size up to a saturation level (situated around 70–80 μm).

Moreover, at 35 °C, nucleation is more sporadic and growth is faster: for that reason one observes larger pores even at short demixing times. When the demixing time increases, once again an increase of

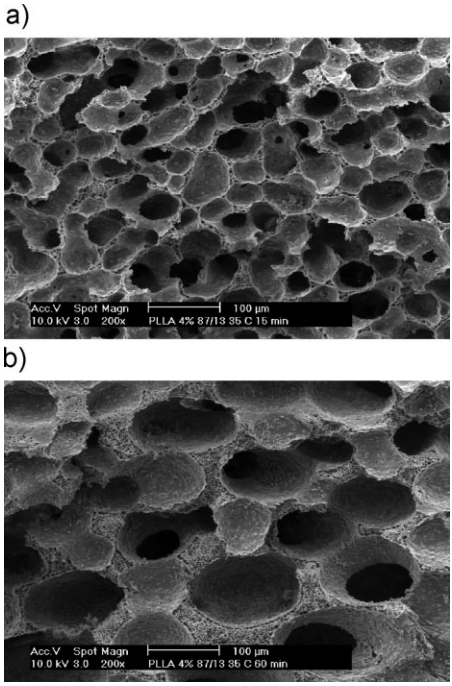

Figure 3.
SEM image of the cross section of PLLA samples at 35 °C; (a): 15 min, (b): 60 min.

the average pore size may be detected, due to enlargement of the phase separation domains, up to a new plateau (saturation level) even determined by a larger center to center average spacing.

Finally, it is interesting to notice that in the time interval 30 to 45 min, for the foams prepared at 30 and 35 °C, there are not very large differences in the average pore diameter (50–90 μm) and the level of interconnection seems to be optimal for the cell seeding.

The estimation of the apparent density of the foams has indicated that foam porosity ranges from 82% to 92%.

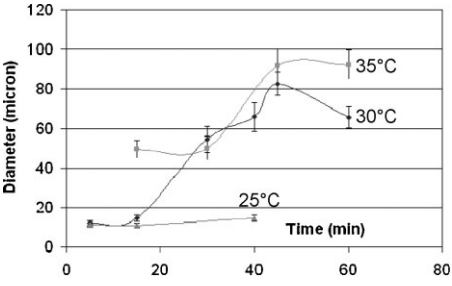

Figure 4.
Average pore size of 4% wt/wt PLLA sample prepared via TIPS from dioxane/water 87/13 v/v solutions.

Conclusion

A ternary system PLLA/dioxane/water systems was investigated in order to explore a reliable route to prepare via Thermally Induced Phase Separation (TIPS) porous, interconnected and biodegradable scaffolds with morphology and properties suitable for tissue engineering applications.

Foams with an average pore size ranging from 10 to 100 μm were obtained, by varying the residence time in the metastable region (defined by the binodal and the spinodal curve.

Pore size and interconnection are closely related to the thermal pathway followed during the phase separation, the former being linked to the balance of nucleation and growth processes (in their turn depending upon demixing time and temperature) and the latter forming during the successive stage by spinodal decomposition. By raising the demixing time, a network of micropores (1–2 micron) among micropores appears. This feature affects mechanical properties, surface area and biodegradation kinetics, so further studies on the possibility of controlling this feature are advisable.

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