Novel fabrication method of porous poly(L-lactic acid) scaffolds using liquid–solid extraction

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Porogen leaching is the most widely used method to prepare porous scaffolds for tissue engineering. Several porogen materials have been used until now, such as salt [1], hydrocarbon [2], glucose [3], gelatin [4], paraffin [5, 6], sugar [7], ammonium bicarbonate [8–10] and ice particulates [11, 12]. Among them, ice particulates may be the best. It can be removed completely, while other porogens were usually residual. Moreover, the process by freeze-drying to leach out the solvent needs much time. In this paper, PLLA scaffolds were fabricated by liquid–solid extraction method with ice particulates as porogen instead of freeze-drying.

Ice particulates were firstly prepared by spraying distilled water into liquid nitrogen from a nozzle, and then ice particulates with special sizes were achieved by sieving. PLLA was dissolved in chloroform, and the mixed solution was pre-cooled to $-30\,^{\circ}$C. Then ice particulates were added to the pre-cooled PLLA solution and stirred to gain uniform mixture. The mixture was poured into a copper mold and subsequently put into liquid nitrogen. To remove the solvent, the solidified mixture was taken out from the mold and subjected to liquid–solid extraction with alcohol at $-60\,^{\circ}$C for 12 h. Vacuum drying was used to remove the ice and alcohol for another 12 h, finally the material was held at room temperature to evaporate residual alcohol. In contrary, a PLLA scaffold was prepared by freeze-drying.

The morphologies of PLLA scaffolds coated with a thin film of gold by vacuum-deposition were observed by scanning electron microscope (SEM, SIRION, 5 kV). The porosity of the scaffolds was determined by Archimedes method.

The SEM morphologies of the scaffolds are shown in Figs 1 and 2. The scaffolds in Fig. 1 were prepared by liquid–solid extraction using ice particles with different sizes. Fig. 1e and 1f show the morphologies of the scaffolds as shown in Fig. 1c and 1d at a higher magnification. Fig. 2 shows the scaffold prepared by freeze-drying with the same amounts and sizes of materials as the one shown in Fig. 1d.

From Fig. 1, it can be seen clearly that the scaffolds fabricated by liquid–solid extraction had two kinds of pores with different sizes. The sizes of the bigger pores correspond to the ones of the ice particulates, while the sizes of the smaller one are less than 100 μm. Thus the bigger pores may be formed by ice particles while the smaller ones were created by chloroform. That is to say, the size of the bigger pores can be controlled by using ice particulates with different sizes. It also can be seen that all scaffolds had an interconnected pore structure, which is needed by tissue engineering. Comparing Fig. 1d with Fig. 2, no obvious difference appeared between the scaffolds prepared by liquid–solid extraction and the ones using freeze-drying.

According to the morphology of scaffolds, the formation process of scaffolds is as follows. At first, ice particulates existed in the solution. During freezing in liquid nitrogen, phase separation happened in chloroform solution and a porous structure with big and small pores generated. During liquid–solid extraction, chloroform was extracted and its space was occupied by absolute alcohol. During the vacuum-drying, ice sublimated and absolute alcohol evaporated, the spaces originally occupied by ice and alcohol became porous. The space where chloroform occupied had smaller pores, while the space occupied by ice particulate had bigger pores.

In order to guarantee formatting scaffold, the extractant must have several characters: firstly, it cannot

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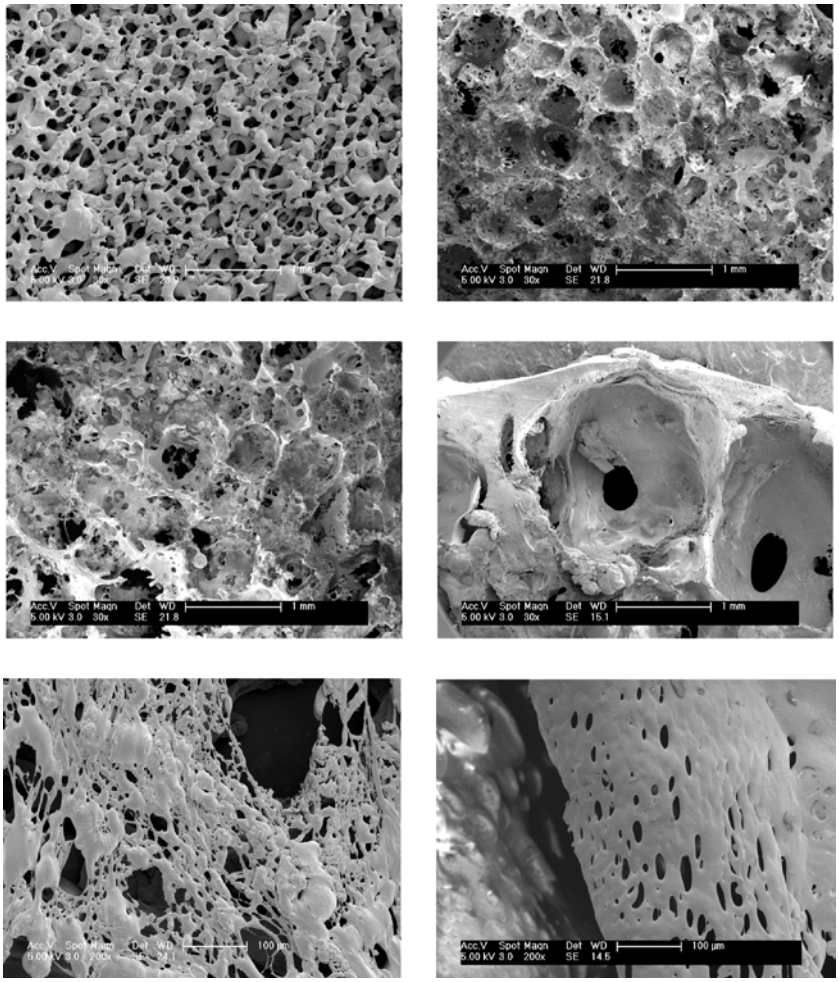

Figure 1 Morphologies of the scaffolds prepared by liquid–solid extraction using ice particulates with different sizes. (a) Ice sizes less than 300 μm, (b) ice sizes between 300 and 500 μm, (c) ice sizes between 500 and 800 μm, (d) ice sizes about 1.5 mm, (e) ice sizes between 500 and 800 μm, 200× and (f) ice sizes about 1.5 mm, 200×.

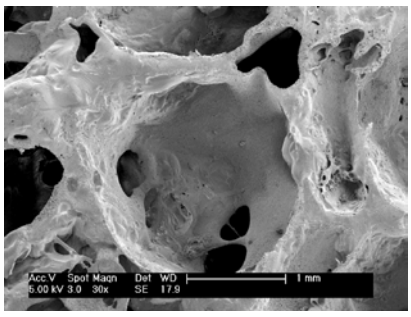

Figure 2 Morphology of scaffold prepared by freeze-drying (SEM).

dissolve PLLA; secondly, its freeze point must be low enough to ensure the extraction process, which is not liquid–liquid extraction but liquid–solid extraction; thirdly, it must dissolve chloroform; finally, the extractant should be ridden easily. Therefore, absolute alcohol with the characteristics mentioned above was chosen as an extractant. Alcohol is a good solvent for chloroform and ice, a good non-solvent for PLLA. So PLLA cannot be dissolved during the liquid–solid extraction. The freeze point of alcohol is much lower than the freeze point of chloroform, which can ensure alcohol in liquid state during the extraction at a temperature under the freeze point of chloroform, that is, a liquid–solid extraction is processed. The temperature was low enough and ensured that PLLA was rigid enough to avoid the porous structure from collapsing, so the porous block remained. In addition, alcohol can evaporate rapidly at room temperature.

The porosity of the scaffolds are listed in Table I, all samples were prepared by liquid–solid extraction using ice particles with sizes about 1.5 mm.

From the table, it can be shown that the porosity increases with the increase of ice mass fraction and the decrease of polymer concentration. When ice mass fraction is high enough and polymer concentration is low, the porosity can be higher than 80%.

Compared to freeze-drying, liquid–solid extraction needs less time. The fabrication of the scaffold shown in Fig. 1d needs 24 h, while it took 48 h to prepare the scaffold shown in Fig. 2. Liquid–solid extraction

TABLE I Porosity of the scaffolds

	Sample no.		
	1	2	3
Quality of ice (g)	2.0	2.0	2.0
Quality of PLLA (g)	1.0	0.33	0.22
Volume of chloroform (ml)	2	2	2
Porosity (%)	61.4	79.7	85.6

is a liquid–solid transformation process, while freeze-drying was a gas–solid transformation process. Obviously the speed of the former is much faster than that of the latter. In the vacuum-drying process, alcohol can dissolve ice and ice can be taken out with alcohol evaporation. So the existence of alcohol made the extraction of ice more easily and rapidly. Thus liquid–solid extraction needs less time to prepare the same scaffold.

In conclusion, using ice particles as porogen, a novel liquid–solid extraction method has been developed to prepare porous PLLA scaffolds. The scaffold prepared by this method has the same morphology as the one prepared by freeze-drying. The pores of the scaffolds are interconnected. The pore structure and porosity can be controlled using different ice particles. Compared to freeze-drying method, the present method needs less time.

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