Transactions of the Materials Research Society of Japan 25 [4] 887-890 (2000)

Preparation of Three-dimensional Biodegradable Porous Scaffolds for Tissue Engineering

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Three-dimesional biodegradable porous scaffold of poly(L-lactic acid) (PLLA) for tissue engineering was fabricated by a porogen leaching technique using pre-prepared ice microparticulates as a porogen. Ice microparticulates were prepared by spraying cold water in liquid nitrogen through a capillary. PLLA solution in chloroform was mixed with the ice microparticulates. The mixtures were frozen in liquid nitrogen and freeze-dried to form the three-dimensional porous PLLA sponges. Six kinds of PLLA sponges with different pore structures were fabricated by changing the weight fraction of the ice microparticulates. SEM observation of the PLLA sponges showed that evenly distributed and interconnected pore structures were formed in these sponges, and that the pores became more interconnected as the weight fraction of the ice microparticulates increased. The pore morphology was the same as that of the ice microparticulates. The decreasement of PLLA concentration resulted in increasing of the porosity of the pore walls of the sponges. The pore structures of the sponges could be manipulated by controlling processing variables such as the size and weight fraction of the ice microparticulates and polymer concentration.

Key words: poly(L-lactic acid), ice micropaticulate, three-dimensional scaffold, biodegradable, tissue engineering.

1. INTRODUCTION

Three-dimensional biodegradable porous scaffolds play an important role in tissue engineering (1-3). Biodegradable aliphatic polyesters like poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and their copolymer of poly(DL-lactic-co-glycolic acid) (PLGA) are biocompatible and among the few synthetic polymers approved by the Food and Drug Administration (FDA) for certain human clinical application, such as surgical sutures and some implantable devices. They have been most commonly used to construct three-dimensional porous scaffolds for tissue engineering (4-6). Several methods have been developed to prepare these kinds of three-dimensional biodegradable scaffolds, including phase separation (7, 8), emulsion freeze-drying (9), gas foaming (10), fiber bonding (11), and porogen leaching (12, 13). By using phase separation, porous structures can easily be obtained by adjusting thermodynamic and kinetic parameters. However, because of the complexity of the processing variables involved in the phase separation technique, the pore structures can not be easily controlled, and also it is difficult to obtain large pores. Both an emulsion freeze-drying technique and an expansion technique using high-pressure CO_2 gas often result in a closed cellular structure within the scaffold. The network of bonded PGA fibers constructed by a fiber bonding technique lacks structural stability. Compared to these methods, the porogen leaching method provides easy control of pore structure like porosity, pore size, and pore morphology. However, the problem of residual porogen remains.

In the present study, we employed pre-prepared ice microparticulates as a porogen to fabricate three-dimensional biodegradable porous scaffolds for tissue engineering. The conventional method of porogen leaching by washing with water was replaced by freeze-drying, facilitating the removal of the porogen and making removal more complete. Biodegradable sponges of poly(L-lactic acid) (PLLA) were fabricated by this method. Their pore

structures were observed by scanning electron microscopy. The pore structures of the sponges could be manipulated by controlling the properties of the ice microparticulates and the polymer concentration.

2. MATERIALS AND METHODS

2.1. Preparation of ice particulates

Ice microparticulates were prepared by spraying cold deionized water into liquid nitrogen from a distance of 10 cm at a flow rate of 33.0 μl/s through the capillary of an Eppendorf Femtotip (Hamburg, Germany). The sizes of the ice particulates were measured from their photomicrographs. The ice microparticulates were stored at –80°C until used.

2.2. Preparation of PLLA sponges

PLLA sponges were prepared by mixing a PLLA solution in chloroform with ice microparticulates and freeze-drying the mixture. Polymer solutions of different concentrations were prepared by dissolving 5.0, 3.33, 2.14, 1.25, 0.56, 0.26 g of PLLA (molecular weight of 85-160 kD) (Sigma Chemical Co., St. Louis, MO) in chloroform (5 mL) and cooled to –20°C. Ice microparticulates (5 g) were added to the pre-cooled PLLA solutions and the dispersions were vortexed and poured into aluminum molds. This process was conducted in a low-temperature room (4°C). The dispersions were frozen by placing the molds in liquid nitrogen. Subsequently, the materials were freeze-dried for 48 h under liquid nitrogen freezing and for another 48 h at room temperature to completely remove the solvent. The weight fraction of ice microparticulates, defined as the percent of the weight of the ice microparticulates to the total weight of ice microparticulates plus polymer, was varied from 50% to 95% to fabricate PLLA sponges with different pore structures.

2.3. SEM observation

The structures of the PLLA sponges were observed by scanning electron microscopy (SEM). The sponges were cut with a razor blade, and the cross-sections were coated with gold using a sputter coater (Sanyu Denshi Co., Tokyo, Japan). The gas pressure was set at 50 mtorr and the current was 5 mA for a coating time of 180 s. The samples were observed with a scanning electron microscope (JSM-6400Fs, JEOL Ltd., Tokyo, Japan), which was operated at 3 kV. For energy dispersive spectroscopy (EDS) analysis, the sponges were coated with carbon to a depth of 200 Å using a vacuum evaporator (JEE-4C; JEOL, Ltd.). And then the elementary composition of the pore surfaces of the PLLA sponges were analyzed by EDS.

3. RESULTS AND DISCUSSION

Ice microparticulates were formed by injecting cold deionized water into liquid nitrogen through a capillary. The microparticulates were almost spherical. Their diameters were measured from their photomicrographs by hypothesizing that all their shapes were spherical. The mean diameter was 237 μm ±67 μm.

Using ice microparticulates as a porogen, six kinds of PLLA sponges were prepared by mixing a PLLA solution in chloroform with ice microparticulates, freezing the mixture in liquid nitrogen, and freeze-drying. The weight fractions of ice microparticulates were 50, 60, 70, 80, 90, and 95%, respectively. SEM photomicrographs of the cross-sections of the PLLA sponges are shown in Fig. 1. All sponges were highly porous with evenly distributed and interconnected pore structures. The pore shapes were almost the same as those of the ice microparticulates. All the PLLA sponges had almost the same median pore size, which coincided with the fact that the same size of ice microparticulates was used. The sponges became more interconnected as the weight fraction of the ice microparticulates increased. The polymer concentration also had some effect on the pore wall structures: low polymer concentration resulted in more porous pore wall structures.

The EDS analysis indicated that the main elements of the surfaces of the pores of PLLA sponges were carbon and oxygen, without other elements such as sodium and chloride.

Because ice can easily be removed by freeze-drying, it has been used as a porogen material in the

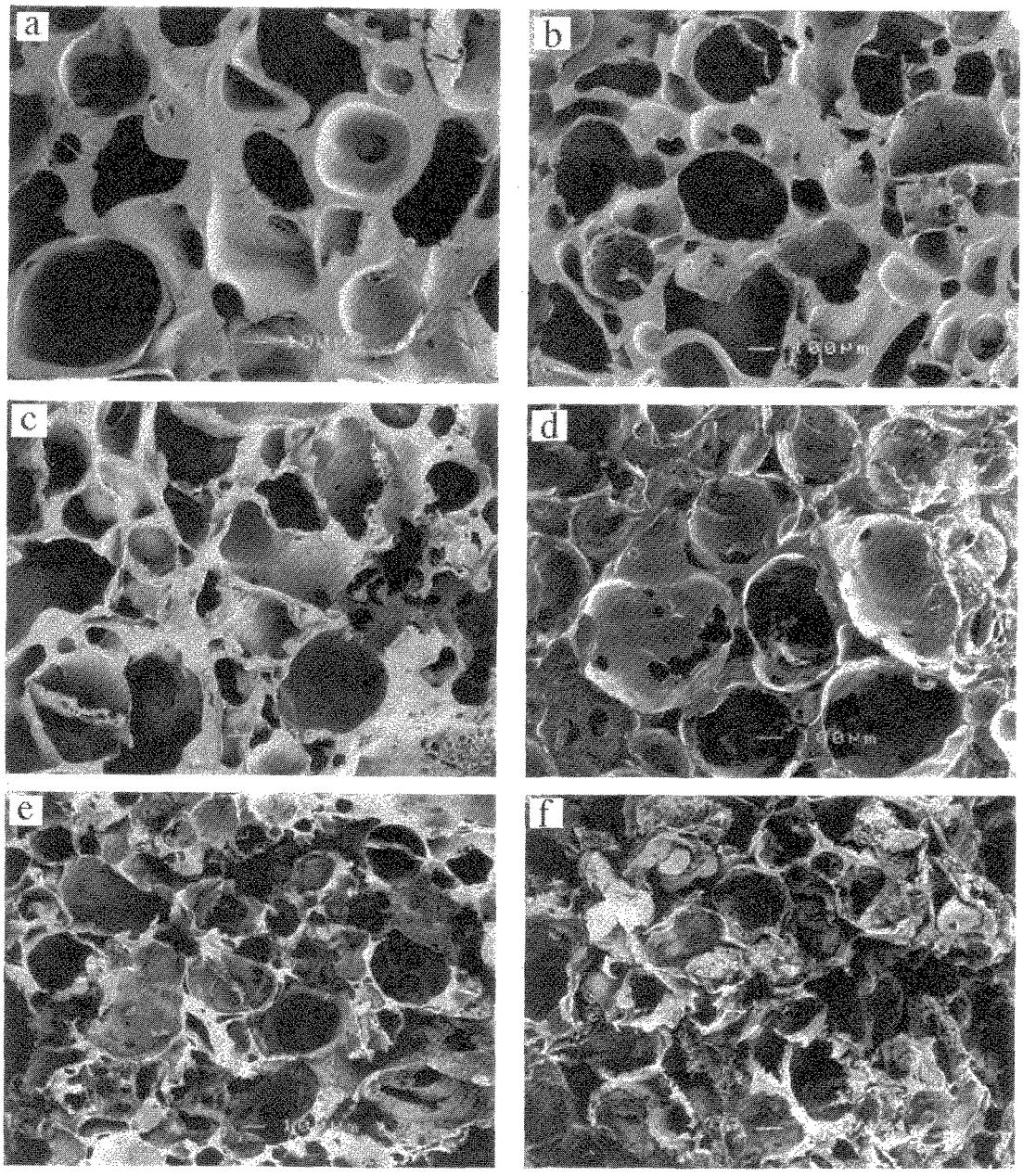

Figure 1. SEM photomicrographs of cross sections of PLLA sponges prepared with the different weight fractions of ice microparticulates at original magnification×60: (a) 50%, (b) 60%, (c) 70%. (d) 80%, (e) 90% and (f) 95%.

preparation of collagen sponges by freeze-drying aqueous collagen solution or collagen gel immersed in water (14 15). However, the ice particulates formed during freezing are influenced by several processing variables such as the temperature of freezing, making it difficult to precisely control the pore structure, including the pore size distribution and the surface area of the scaffold. Nevertheless, the pore structures of scaffolds are important in applications in tissue engineering. Therefore, precise control of pore structure is a major goal in preparation of three-dimensional biodegradable porous scaffolds for tissue engineering. In the present study, by using pre-prepared ice microparticulates as a porogen, a simple method to prepare three-dimensional biodegradable porous scaffolds with controlled pore structures was developed. The conventional method of porogen leaching by repeated washing with water was replaced with freeze-drying. The removal of the

porogen thus became easier and more complete. Porous biodegradable PLLA sponges prepared by this method had evenly distributed and interconnected pore structures. The pore structures of the three-dimensional biodegradable porous scaffolds could be manipulated simply by varying the shape, weight fraction, size of ice microparticulates and polymer concentration. Because there are no residual porogen molecules, the 3-dimensional biodegradable porous scaffolds prepared by this method should prove useful for tissue engineering.

ACKNOWLEDGMENTS

This work was supported in part by the New Energy and Industrial Technology Development Organization of Japan and in part by a Grant of the "Research for the Future" Program (JSPS-RFTF96I00202) from the Japan Society for the Promotion of Science (JSPS).

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(Received December 16, 1999; Accepted September 30, 2000)