Preparation of Interconnected Highly Porous Polymeric Structures by a Replication and Freeze-Drying Process

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Abstract: Three-dimensional degradable porous polymeric structures with high porosities (93–98%) and well-interconnected pore networks have been prepared by freeze-drying polymer solutions in the presence of a leachable template followed by leaching of the template. Templates of the pore network were prepared by fusing sugar or salt particles to form a well-connected structure. The interstices of the template were then filled with a polymer solution (5–15% w/v) in 1,4-dioxane, followed by freeze-drying of the solvent. Subsequent leaching of the sugar template ensures the connectivity of the pore network. The scaffold architecture consists of relatively large interconnected pores modeled after the template and smaller pores resulting from the freeze-drying process. The total porosity of the resultant porous structures is determined by the interstitial space of the leachable template and by the polymer concentration in the freeze-drying solution. The freezing temperature also has an effect on the final morphology of the porous structures. Compared with freeze-drying and combination of freeze-drying /particulate leaching techniques, this method facilitates higher interconnectivity of the scaffolds. Porous structures have been prepared from several relevant polymers in the biomedical and tissue-engineering field: poly(D,L-lactide) (PDLLA), 1000PEOT70PBT30, a segmented poly(ether ester) based on polyethylene oxide and polybutylene terephthalate, and poly(ε-caprolactone) (PCL). The mechanical properties of the porous structures prepared by this technique depend on the nature of the polymer, porosity, and the freezing temperature. With porosities in the range of 95–97%, the compression moduli of scaffolds prepared from the different polymers could be varied between 13.0 and 301.5 kPa.

Keywords: biodegradable polymers; leachable template; freeze-drying; porous structures; tissue engineering

INTRODUCTION

Tissue engineering, which aims at developing functional substitutes for damaged or diseased tissues, has attracted much attention during the last decade.[1–3] In tissue engineering, three-dimensional biodegradable polymeric scaffolds play an important role as biologically active, temporary supports for the transplantation of specific cells and tissues. Besides biocompatibility, biodegradability, processability, sterilizability, and mechanical strength of the scaffolding material, careful design of the microstructure and morphology of the porous structures is of critical importance for their success. In general, a high porosity and a high interconnectivity of the scaffold is desired to minimize the amount of implanted polymer and to increase the specific surface area for cell attachment and tissue ingrowth. Furthermore, the pore morphology can affect the growth of cells and even alter cell function.[4] Interconnected pores larger than the dimensions of the cells are essential for allowing infiltration of the cells into the scaffold, whereas smaller pores may positively influence the exchange of nutrients and cellular waste products.[5] Therefore, an appropriate pore size range and distribution of pore sizes is beneficial to the viability and function of the cells within the tissue-engineering scaffolds.

Various methods have been used for the preparation of porous polymeric structures for biomedical applications and tissue engineering. Techniques involving phase inversion processes[6] such as liquid–liquid phase separation and liquid–solid phase separation have been explored. Freeze-drying has also been used frequently in the preparation of porous polymeric structures.[7] The morphologies and properties of the resultant scaffolds largely depend on the phase separation

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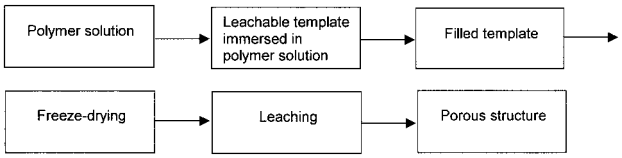

Scheme 1. Preparation of porous polymeric structures by a replication and freeze-drying process.

mechanism.[8] In methods based on the leaching of soluble particulates,[9,10] the porosity can be effectively controlled by variation of the amount of leachable particles, and the pore size of the porous structure can be adjusted independently of the porosity by using particles of different sizes. To improve the pore interconnectivity of the scaffold, particulate leaching has been used in combination with gas foaming,[11] solvent casting,[12,13] freeze-drying,[14,15] immersion precipitation,[16,17] coprecipitation,[18] and compression molding.[10]

More recently, rapid prototyping techniques involving the processing of polymer melts and powders, such as precise extrusion,[19] three-dimensional printing,[20] and fused deposition modelling,[21] have received considerable interest. Complex free-form parts can be readily produced from computer-aided design (CAD) models. However, these techniques are time-consuming and require sophisticated equipment. The maximum porosities obtained are limited to approximately 80%. A more versatile method is the thermal processing of polymer composites with leachable particles by extrusion and compression moulding.[10,22] These methods yield porous structures with reproducible morphologies and maximum porosities of 90%.

A replication technique has also been used to prepare highly porous materials with controllable pore sizes from inorganic materials,[23,24] and polymer materials.[25–27] This technique is a multistep procedure, in which first a replica of the porous structure is made from wax or another material that can easily be removed by melting or dissolution. This replica is then used as a negative casting mold; the interstices are filled with the desired polymer in the liquid phase. After hardening of the liquid polymer by curing, cooling, or precipitation, the mold forming the pore network is removed. Very recently fused sugar or salt particles[5,28,29] were used to improve the pore interconnectivity of polymer scaffolds. These porous scaffolds were fabricated either by a solvent-casting/ template or by a gas-foaming/ template process.

This article describes a simple and effective method for the preparation of highly porous polymeric structures that combines a replication technique and a freeze-drying process. The predesigned template models the pore network and ensures interconnectivity, while freeze-drying allows a very high porosity scaffold to be obtained. First, results with poly(D,L-lactide) have recently been presented.[30] In this way porous structures have been prepared from poly(D,L-lactide) (PDLLA), a 1000PEOT70PBT30 poly(ether ester) block copolymer[31] and poly(ϵ-caprolactone) (PCL), which are all widely used in the biomedical field. This technique also allows for the preparation of porous structures from many different polymeric materials, such as high melting polymers. Thermal processing, which often leads to decreased molecular weights, is not required during the procedure. This versatility provides for the optimization of scaffolds used in the biomedical field, especially in applications involving the ingrowth of cells and tissues, such as in tissue engineering.

EXPERIMENT

Materials

Poly(D,L-lactide) (PDLLA) (L- to D-lactide ratio 50/50, M_n = 116,000) was prepared by ring-opening polymerization in the melt at 140–150 °C with $Sn(Oct)_2$ as a catalyst.[32] The obtained polymer is amorphous, with a glass transition temperature (T_g) of 52 °C. The residual lactide monomer content was 1.9%. 1000PEOT70PBT30, a poly (ether ester) multiblock copolymer based on polyethylene oxide and polybutylene terephthalate, was prepared by a two-step polycondensation reaction of polyethylene glycol (PEG with molecular weight 1000), 1,4-butanediol, and dimethyl terephthalate.[33] The ratio of soft to hard segments is 70/30. The resulting polymer has a T_g of −50 °C, and T_m of 148 °C. Commercial-grade poly(ϵ-caprolactone) (PCL, CAPA 680 with $M_n = 76.7 \times 10^3$ and $M_w = 120.0 \times 10^3$) was obtained from Solvay-Interox (UK). It is a semicrystalline polymer with a glass transition temperature of −62 °C and a melting temperature of 57 °C. 1,4-dioxane was purchased from Merck (Germany). Ethanol and chloroform were purchased from

TABLE I. Porosities of Porous Polymer Scaffolds Prepared by Freeze-Drying

Polymer	Polymer Concentration (w/v %)	Freezing Temperature (°C)	Porosity (%)
PDLLA	3	6	95.7
	3	−25	95.4
	5	6	92.7
	5	−25	92.7
	10	6	88.6
	10	−25	87.6
	15	6	83.9
	15	−25	81.9
1000PEOT70PBT30	10	6	88.3
	10	−25	88.1
	15	6	84.8
	15	−25	85.9
PCL	3	6	96.6
	3	−25	96.4
	5	6	93.6
	5	−25	93.6
	10	6	89.2
	10	−25	89.2
	15	6	85.1
	15	−25	84.8

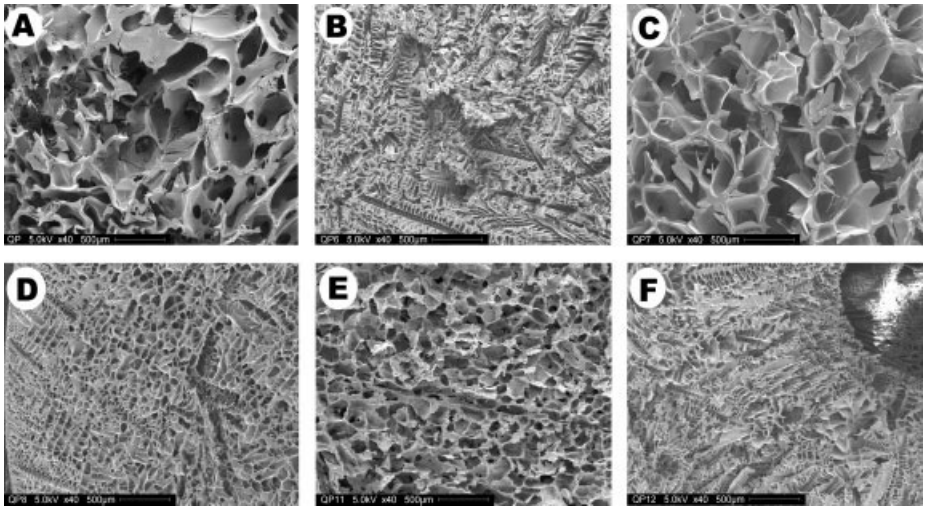

Figure 1. SEM micrographs of PDLLA, 1000PEOT70PBT30, and PCL scaffolds prepared from 10% (w/v) solutions in 1,4-dioxane by freeze-drying. (a) PDLLA, freezing temperature: 6 °C, porosity: 88.6%; (b) PDLLA, freezing temperature: −25 °C, porosity: 87.6%; (c) 1000 PEOT70PBT30, freezing temperature: 6 °C, porosity 88.3%; (d) 1000 PEOT70PBT30, freezing temperature: −25 °C, porosity: 88.1%; (e) PCL, freezing temperature: 6 °C, porosity 89.2%; (f) PCL, freezing temperature: −25 °C, porosity 89.2%.

Assink and Biosolve (The Netherlands), respectively. All chemicals were of analytical grade. The sucrose templates (27.5 × 18 × 12 mm in size, bulk density: 1.007 g/cm^3, porosity: 36.5%) were commercial food-quality sugar cubes from Van Gilse, the Netherlands. Alternatively, sugar or sodium chloride templates were prepared by fusing the sugar or salt particles (see below). These particles were sieved with standard testing sieves (ASTM–11 specifications, Fisher Scientific BV, the Netherlands) with mesh sizes of 106, 250, 425, 500, and 710 μm placed on a sieve shaker (Retsch, Germany).

Preparation of Porous Polymer Structures by Freeze-Drying

PDLLA, 1000 PEOT70PBT30, and PCL polymers were dissolved in 1,4-dioxane to make 5–15% (w/v) solutions. The polymer solutions were poured into cylindrical polypropylene containers (height: 70 mm, inner diameter: 10 mm, wall thickness: 1 mm) and subsequently frozen at 6 or − 25 °C for 20 h. After phase separation, the solvent crystals were removed by freeze-drying for a period of 2–3 days in vacuum. The highly porous cylinders could easily be removed from the polypropylene containers.

Preparation of Porous Polymeric Structures by a Combination of Freeze-Drying and Particulate Leaching

For comparison purposes, porous polymeric scaffolds were first prepared by a combination of freeze-drying and particulate leaching. PDLLA or PCL was dissolved in 1,4-dioxane at room temperature to make a 10 w/v% solution. The polymer solution was poured into cylindrical polypropylene containers (height: 70 mm, inner diameter: 10 mm, wall thickness: 1 mm), and 92 w/w% of sugar or salt particles were added to the polymer solution. Subsequently the polymer solution and sugar/salt particles were frozen at 6 or − 25 °C for 20 h. After phase separation, the solvent crystals were

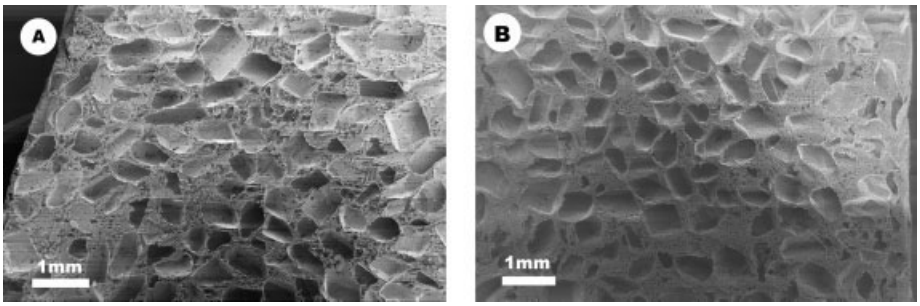

Figure 2. SEM micrographs of porous structures prepared by freeze-drying and particulate leaching. (a) PDLLA, salt size: 500–710 μm, overall porosity: 95.8%; (b) PCL, salt size: 500–710 μm, overall porosity: 96.6%.

TABLE II. Sugar/ Salt Templates Prepared by Fusing Sugar or Salt Particles.

(a)

	Relative humidity (%)	Time (day)	Porosity (%)	Stability*
Sugar	75	2	n.d.	−
Sugar	81	2	n.d.	−
Sugar	81	4	n.d.	−
Sugar	97	1	37.0	++
Sugar	97	2	36.2	++
Sugar	97	4	35.2	++
Sugar	97	10	26.5	Partially dissolved
Salt	75	2	n.d.	−
Salt	81	1	n.d.	−
Salt	81	2	43.1	+
Salt	81	4	42.0	+
Salt	81	10	40.0	+
Salt	97	1	44.7	+
Salt	97	2	42.3	++
Salt	97	4	41.8	++

(b)

	Acetone/water (v/v)	Porosity (%)	Stability*
Sugar	9/1	36.3	++
Sugar	7.5/2.5	36.4	+
Salt	9/1	44.2	++

*−: minimally stable; +: stable; ++: very stable; n.d., not determined.

removed by freeze-drying for a period of 2–3 days in vacuum. The porous cylinders could easily be removed from the polypropylene containers after leaching out the sugar or salt particles.

Preparation of Sugar or Salt Templates for Replication

To obtain scaffolds of various shapes and sizes and with controllable interconnected pore size ranges, sugar or salt templates were predesigned and prepared by either of the following methods.

1. Sugar/salt particles with desired size range were put into a plastic container and placed in desiccators with relative humidity of 75%, 81%, and 97% for 2 days. The atmosphere with 75%, 81%, and 97% relative humidity was created by using an excess of sodium chloride, ammonium sulfate, and potassium sulfate in contact with their saturated solution in an enclosed space at 25 °C, respectively.[34] Then the template composed of fused sugar or salt powder was dried and removed from the container.
2. Sugar or salt particles with certain size ranges were first soaked in a mixture of acetone and water (7.5/2.5 to 9/1 v/v), then gently packed into a plastic container with desired shape and size. After drying, the sugar or salt template could be removed from the container.

The porosity of the sugar or salt templates was calculated in the same way as the porous polymeric structures. The densities of the crystal sugar and salt are 1.587 and 2.165 g/cm^3, respectively.

Preparation of Porous Polymeric Structures by a Replication and Freeze-Drying Process

The procedure for the preparation of porous scaffolds by a replication and freeze-drying process is shown in Scheme 1. Commercially available sugar cubes or fused sugar/salt templates were used as the leachable templates. Briefly, the polymer of choice was first dissolved in 1,4-dioxane (at room temperature for PDLLA and PCL, 80 °C in the case of 1000PEOT70PBT30 copolymer) to make 3–15% solutions (w/v). The sugar templates were then immersed into the polymer solutions and the interstices of the templates were filled with the polymer solution by repeated application of a low vacuum (30 mbar). The sugar templates do not dissolve in 1,4-dioxane.

The sugar templates filled with the polymer solutions were immediately frozen at temperatures of 6 or −25 °C for 20 h. The solvent was then removed by freeze-drying at 0.04 mbar for 2–3 days. Subsequently, the structures were placed in gently stirred demineralized water for a period of 4–5 days to

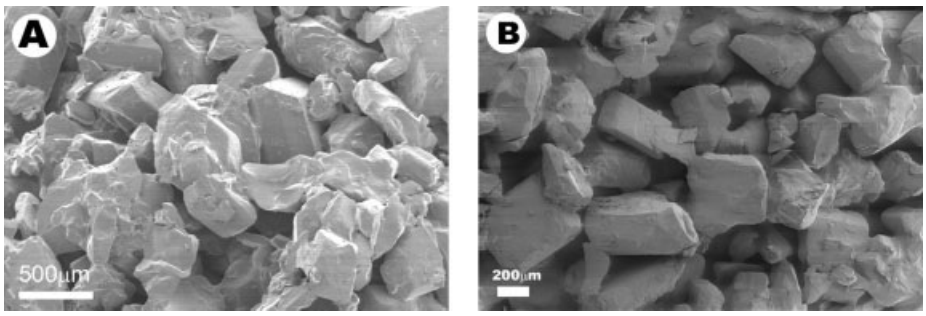

Figure 3. SEM micrographs of the fracture surface of sugar templates. (a) Commercially available sugar cube (particle size range: 100–600 μm, porosity: 36.5%); (b) sugar template prepared by fusing sugar particles (particle size range: 425–500 μm; relative humidity: 97%; time: 2 days; porosity: 38.0%).

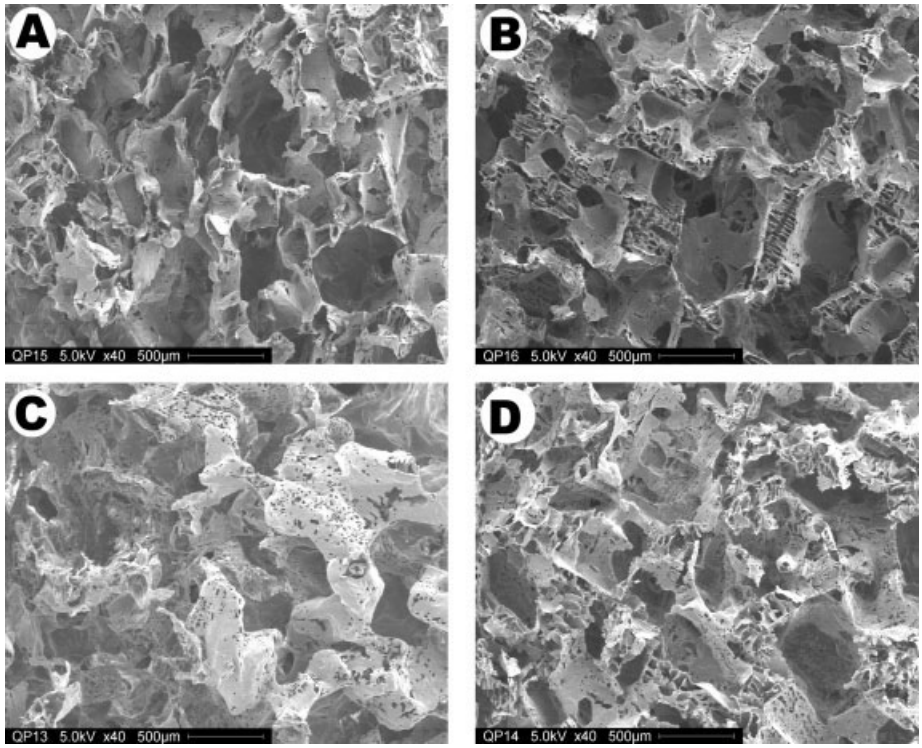

Figure 4. SEM micrographs of PDLLA scaffolds prepared from freeze-drying polymer solutions of 1,4-dioxane in the presence of a sugar template. The larger pores (approximately 100–600 μm) result from leaching of the sugar template; the smaller pores (smaller than approximately 100 μm) result from the freeze-drying. (a) Polymer concentration: 10%, freezing temperature: 6 °C, porosity: 97.2%; (b) polymer concentration: 10%, freezing temperature: −25 °C, porosity: 97.7%; (c) polymer concentration: 5%, freezing temperature: 6 °C, porosity: 98.5%; (d) polymer concentration: 5%, freezing temperature: −25 °C, porosity: 98.5%.

leach out the sugar template. The resulting polymer scaffolds were vacuum dried.

Characterization of Scaffolds

Determination of porosities of the scaffolds. The density and the porosity of the scaffolds were determined in triplicate by measuring the dimensions and the mass of the scaffold. The density (d) of the scaffolds was calculated as follows:

$$d = \frac{m}{V} \tag{1}$$

where m is the mass and V is the volume. The porosity p_o was calculated as

$$p_o = 1 - \frac{d}{d_p} \tag{2}$$

where d_p is the density of the nonporous compression moulded polymer also determined from the specimen mass and volume (d_p =1.246 ± 0.009, 1.188 ± 0.008, 1.103 ± 0.007 g/cm^3 for PDLLA, 1000PEOT70PBT30, and PCL, respectively).[35] The relative error in the porosity determinations was less than 2%.

Scanning electron microscopy (SEM). A Hitachi S800 scanning electron microscope was used to examine the morphology of the porous scaffolds. The specimens were cut with a razor blade after being frozen in liquid nitrogen for about 5 min. Cross-sections of the scaffolds were coated with gold with the use of a sputter-coater (Turbo Sputter Coater E6700, UK).

Mechanical testing in compression. The compression modulus E_c of the porous polymeric structures in the dry state at room temperature was determined in triplicate with the use of a Zwick tensile tester equipped with a 500-N load cell at a crosshead speed of 2 mm/min. The load was applied until approximately 25% of the original thickness of the specimen remained. The initial compressive moduli were determined from the slope of the linear part of the stress–strain curves at a compressive strain of approximately 5%.[35] The relative error in the measurements was less than 10%.

RESULTS AND DISCUSSION

Preparation of Porous Structures by Methods Based on Freeze-Drying Process

Porous polymeric structures of very high porosity can be obtained by freeze-drying. By varying the concentration of

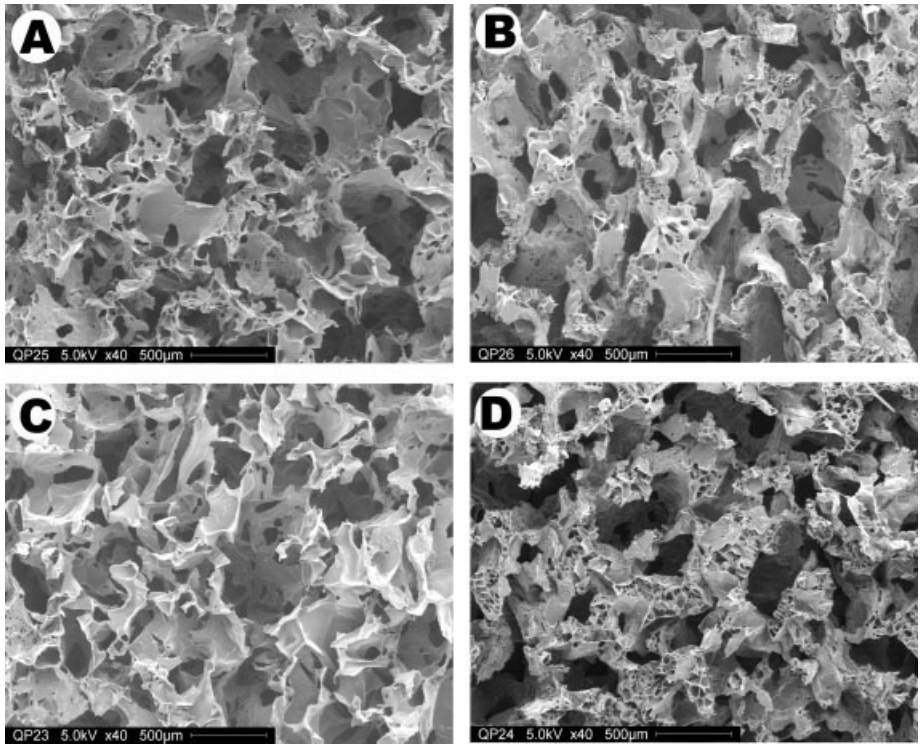

Figure 5. SEM micrographs of 1000PEOT70PBT30 scaffolds prepared from freeze-drying polymer solutions of 1,4-dioxane in the presence of a sugar template. The larger pores (approximately 100–600 μm) result from leaching of the sugar template; the smaller pores (smaller than approximately 100 μm) result from the freeze-drying. (a) Polymer concentration: 15%, freezing temperature: 6 °C, porosity: 94.0%; (b) polymer concentration: 15%, freezing temperature: −25 °C, porosity: 93.1%; (c) polymer concentration: 10%, freezing temperature: 6 °C, porosity: 96.3%; (d) polymer concentration: 10%, freezing temperature: − 25 °C, porosity: 95.0%.

the polymer solutions in 1,4-dioxane, the porosity of the porous PDLLA, 1000PEOT70PBT30 and PCL scaffolds could be well controlled, as shown in Table I. Figure 1 shows SEM micrographs of PDLLA, 1000PEOT70PBT30 and PCL scaffolds prepared from 10% (w/v) solutions in 1,4-dioxane by freeze-drying. The pore sizes are relatively small, with a maximum pore size of approximately 150 μm. All scaffolds prepared by freeze-drying show an irregular porous structure with poor pore interconnectivity. Furthermore, the pore sizes and shapes vary considerably throughout the scaffold, and large voids or defects are often observed within the structure. No cells can enter the closed pores of the scaffolds, which render them unsuitable for tissue engineering. Improved scaffolds can be obtained by combining the freeze-drying process with particulate leaching.[14,15] This method is suitable when large fractions of leachable particles and high concentrations of polymers of high molecular weight in 1,4-dioxane are used. However, the final pore architecture is still not highly interconnected. Figure 2 shows SEM micrographs of PDLLA and PCL scaffolds prepared in this manner. After leaching out the sugar/salt particles, a dense polymer layer remained in between the resulting pores. The addition of leachable particles to the polymer solution before freeze-drying does not ensure complete interconnectivity of the porous structures.

Preparation of Leachable Templates

Leachable templates with various shapes and sizes can be prepared by fusing the sieved sugar or salt particles of determined size range in an (enclosed) atmosphere with a relative humidity (RH) of 97% for about 2 days, followed by drying in ambient air. Salt cubes can be prepared in an atmosphere of RH of 75–81%, but the preparation of sugar cubes requires a relative humidity as high as 97% to obtain very stable templates. The porosity of the templates slightly decreases as the fusing time increases. The results are shown in Table II(a). Alternatively, the templates can be fabricated by compacting the soaked sugar or salt particles of certain size range in an acetone/ water mixture, as sugar cubes were reported to be prepared by compressing moist sugar particles.[36] When the acetone/water ratio is in the range of 7.5/2.5 to 9/1 (v/v), all prepared templates are stable. The porosities of the resultant templates are close to those prepared by the first method, as shown in Table II(b). Figure 3(a) shows an SEM micrograph of the fracture surface of the commercially available sugar cubes. Figure 3(b) shows the micrograph of templates prepared by fusing sugar particles of 425–500 μm. It can be seen that both consist of a fused structure in which the sugar particles are well connected. As the sugar template will be leached out with water after the freeze-drying step, this

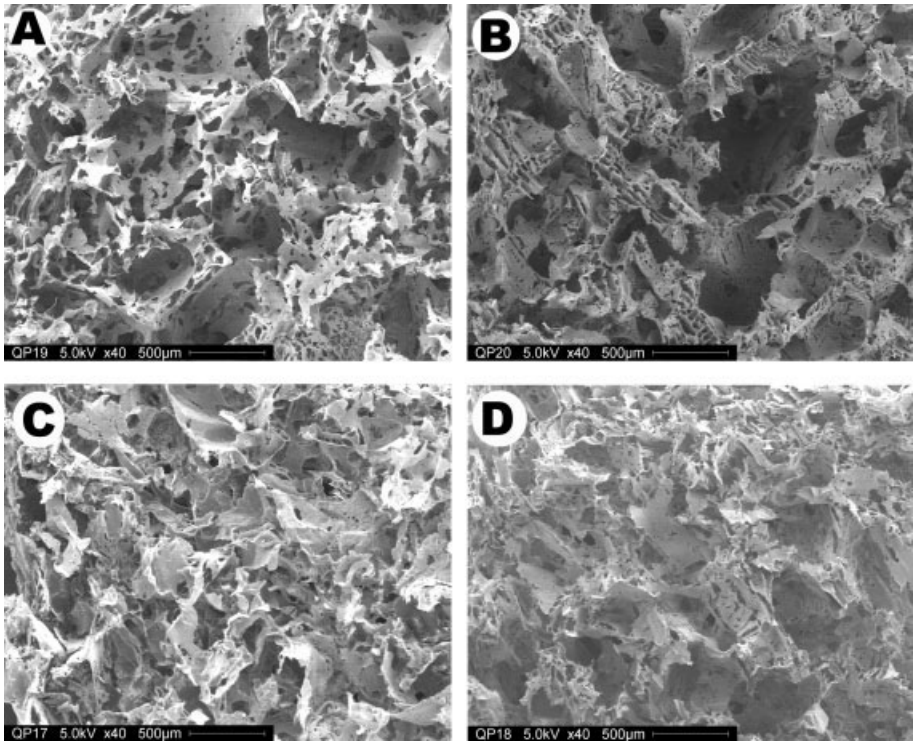

Figure 6. SEM micrographs of PCL scaffolds prepared from freeze-drying polymer solutions of 1,4-dioxane in the presence of a sugar template. The larger pores (approximately 100–600 μm) result from leaching of the sugar template; the smaller pores (smaller than approximately 100 μm) result from the freeze-drying. (a) Polymer concentration: 10%, freezing temperature: 6 °C, porosity: 96.5%; (b) polymer concentration: 10%, freezing temperature: −25 °C, porosity: 96.8%; (c) polymer concentration: 5%, freezing temperature: 6 °C, porosity: 97.8%; (d) polymer concentration: 5%, freezing temperature: −25 °C, porosity: 97.5%.

structure will reflect the connected pore network of the polymer scaffold.

Preparation of Porous Polymer Structures by Replication and Freeze-Drying Process

Typical SEM micrographs of PDLLA, 1000PEOT70PBT30, and PCL scaffolds prepared by freeze-drying a 5–15% (w/v) polymer solution in 1,4-dioxane in the presence of commercially available sugar cubes are shown in Figures 4–6.

Highly porous structures, with porosities over 97%, have been prepared. Figures 4–6 show that highly interconnected porous structures are obtained with large pore sizes of 100–600 μm. This is because the relatively large sugar particles of the template are well bound; therefore, the pores obtained after leaching of the template are open and well interconnected. At the same time much smaller pores (less than 150 μm) are obtained by the freeze-drying process. It can be seen that these pores are not well interconnected. The final pore structure of the scaffolds is determined by the size and content of the sugar particles of the template as well as by the solvent-crystal morphology after freezing of the 1,4-dioxane solution. The average size of pores obtained from the leaching of the sugar template can be controlled by varying the characteristics of the sugar particles used. The average size of the pores from freeze-drying can be adjusted to be in the range of several microns to 150 microns by changing the freezing temperature.[35]

The overall porosities are mainly dependent on the interstitial space of the sugar templates, showing a lesser dependence on the concentration of the polymer solution in the range of 3–15% (w/v) and freezing temperature, as shown in Table III. The calculated porosity of the scaffold can be obtained by the following equation:

$$p_c = 100\% - 36.5\% + \frac{100}{100 + c/d_p} * 36.5\% , \qquad (3)$$

where p_c is the calculated porosity, c is the concentration of the polymer solution (g/100 ml), d_p is the density of the polymer, and 36.5% is the porosity of the sugar template.

It can be seen that the porosity of the resultant scaffolds are close to the calculated values. However, volume shrinkage of the scaffolds occurred during drying after leaching when the concentration of the polymer solution was less than 5% (w/v); thus the resulting pores were partially deformed. On the other hand, when the concentration of the polymer solution was over 15%, it became difficult to fill the voids of the templates due to the high viscosity of the polymer solu-

tion. Hence, the concentration of the polymer solution should be preferably at the range of 5–15% (w/v) for PDLLA and PCL, and 10–15% for 1000PEOT70PBT30. Under these conditions, all porous structures prepared were physically stable. The height, breadth and width of the employed sugar templates and the resulting porous structures were measured, and found to differ by less than 3%.

Table IV shows the compression moduli of the polymer scaffolds prepared by the replication and freeze-drying process. It can be seen that the scaffolds prepared by freezing at relatively high temperature possess higher compression moduli. Scaffolds prepared from freezing at a higher temperature have larger pores after removal of the solvent crystals.[35] It has previously been observed[37] that an increase in pore size causes a rise of the modulus of both flexible and rigid foams. Furthermore, these pore sizes are closer to the average size of the pores obtained from leaching of the sugar particles. As a result, the overall pore size distribution is narrower than those obtained from freezing at a lower temperature. The homogeneity of the pore structure enhances the mechanical properties of the polymeric scaffolds.[28,38]

TABLE IV. Compression Moduli of Polymer Scaffolds Prepared by a Replication and Freeze-Drying Process.

Polymer	Polymer concentration (w/v %)	Freezing Temperature (°C)	Overall porosity (%)	E_c (kPa)
PDLLA	10	6	97.2	301.5
		−25	97.7	227.4
1000PEOT70PBT30	10	6	96.3	21.4
		−25	95.0	13.0
PCL	10	6	96.5	69.3
		−25	96.8	44.6
PCL	15	6	95.1	90.9
		−25	95.5	54.5

CONCLUSIONS

Large porous polymer structures that have very high porosities up to 98% and an interconnected pore network were prepared by a technique that combines template leaching with freeze drying. The process includes three main steps: filling the interstices of a leachable template with a polymer solution; freeze-drying of the filled template; and leaching the template out in water. The scaffold architecture is determined by the properties of the leachable template, involving the size of the particles employed in the preparation of the templates, the initial concentration of the polymer solution employed in freeze-drying, and the freezing temperature. The porosities of the resulting porous structures are determined by the interstitial space in the leachable templates and by the initial concentration of the polymer solution used in freeze-drying.

The ability to obtain a very high porosity with an interconnected pore network and tailored pore size distribution, the applicability to a wide variety of polymers, and ease of preparation of porous polymer structures with different shapes and sizes, are major advantages of this pore-forming technique. The method for the preparation of highly porous polymer structures presented here can be utilized in the biomedical field as well as in many other fields. The particle size range and distribution of which the templates are prepared can readily be optimized for a specific application.

TABLE III. Porosities of Porous Polymeric Scaffolds Prepared by a Replication and Freeze-Drying Process.

Polymer	Polymer Concentration (w/v %)	Freezing Temperature (°C)	Overall Porosity (%)	Calculated Porosity (%)
PDLLA	3	6	98.6	99.1
	3	−25	98.7	99.1
	5	6	98.5	98.6
	5	−25	98.5	98.6
	10	6	97.2	97.3
	10	−25	97.7	97.3
	15	6	94.0	96.1
	15	−25	96.2	96.1
1000PEOT70PBT30	10	6	96.3	97.2
	10	−25	95.0	97.2
	15	6	94.0	95.9
	15	−25	93.1	95.9
PCL	3	6	97.5	99.0
	3	−25	97.5	99.0
	5	6	97.8	98.4
	5	−25	97.5	98.4
	10	6	96.5	97.0
	10	−25	96.8	97.0
	15	6	95.1	95.6
	15	−25	95.5	95.6

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