

# Porous polymeric structures for tissue engineering prepared by a coagulation, compression moulding and salt leaching technique

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Received 28 September 2002; received in revised form 12 November 2002; accepted 13 November 2002

## Abstract

A technique for the preparation of porous polymeric structures involving coagulation, compression moulding and particulate leaching has been developed. The technique combines the advantages of thermal processing methods and particulate leaching. A high molecular weight polymer solution in an organic solvent containing dispersed water-soluble salt particles is precipitated into an excess of non-solvent. The polymer–salt composite is then processed by thermal processing methods into devices of varying shapes and sizes, which can subsequently be extracted to give the desired porous structures. The porosities of the scaffolds could be varied between 70% and 95% by adjusting the polymer to salt ratio and the pore size could be controlled independently by varying the leachable particle size. This versatility provides for the optimisation of scaffolds used in medicine and in tissue engineering.

Compared with commonly used porosifying methods such as sintering, compression moulding combined with salt leaching, and freeze-drying, this process allows excellent control over pore size and porosity and yields scaffolds with a much more homogeneous pore morphology. We have prepared porous structures from several relevant polymers in the biomedical field: poly(D,L-lactide), poly( $\epsilon$ -caprolactone) and 1000PEOT70PBT30, a segmented poly(ether ester) based on polyethylene oxide and polybutylene terephthalate.

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*Keywords:* Biodegradable polymers; Porous structures; Coagulation; Thermal processing; Particulate leaching; Tissue engineering

## 1. Introduction

The structure and properties of three-dimensional polymeric scaffolds are of critical importance for their application in tissue engineering. It has been generally accepted, that the scaffolds need to be biocompatible, biodegradable with a degradation or resorption rate that matches the tissue replacement and possess good mechanical properties to match those of the tissues at the site of implantation [1,2]. It should be possible to process these scaffolds into a variety of shapes and sizes. Furthermore, high porosity and high interconnectivity are required to minimise the amount of implanted polymer and to increase the specific surface area for cell attachment and tissue ingrowth, facilitating a uniform distribution of cells and adequate transport of nutrients

and cellular waste products. In addition, as specific cells require different pore sizes for optimal attachment and growth [3], much research is being aimed at obtaining polymeric scaffolds with a very high porosity and simultaneous good control over pore size and morphology.

Various methods have been used for the preparation of porous polymeric structures for biomedical applications and tissue engineering. Techniques involving phase inversion processes [4] such as liquid–liquid phase separation and liquid–solid phase separation have been explored. Solvent removal by freeze-drying has frequently been used in the preparation of porous polymeric structures [5]. The morphologies and properties of the resultant scaffolds largely depend on the phase separation mechanism [6]. Liquid–liquid phase separation gives rise to isotropic pores of 1–30  $\mu\text{m}$  in diameter, depending on the process parameters and the thermodynamics of the polymer/solvent system. Solid–liquid phase separation (with crystallisation of the

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solvent) leads to ladder- or sheet-like anisotropic morphologies, which strongly depend on the quenching rate [7]. In general, freezing at a relatively high temperature induces a low nucleation rate and a low crystal growth rate, which leads to a small number of large size solvent crystals. As a result, the average pore size obtained by freeze-drying increases with increasing freezing temperature [7,8].

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 structure and increase the pore interconnectivity of the porous scaffold, particulate leaching has been used in combination with gas foaming [11], solvent casting [12,13], freeze-drying [14,15], immersion precipitation [16,17], and compression moulding [10].

From a manufacturing point of view, the preparation of porous structures from the polymer melt is a most convenient route, as it allows the rapid production of implants and scaffolds of many shapes and sizes in an economic way. A most straightforward method to obtain porous materials is by sintering of compacted powders. Especially in the preparation of porous ceramics and metals, the sintering method is often used [18]. The particles in the powder compact are bonded at elevated pressures at temperatures below the melting point of the powder. This technique can also be applied to polymeric materials [19].

More recently, rapid prototyping techniques involving the processing of polymer melts and powders, such as precise extrusion [20], three-dimensional printing techniques [21], and fused deposition modelling [2,22], 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%. More versatile is the thermal processing of polymer composites with leachable particles by extrusion and compression moulding [10,23]. These methods yield porous structures with reproducible morphologies and maximum porosities of 90%.

In this paper we describe a technique for the preparation of porous three-dimensional polymeric scaffolds with even higher porosities and a very homogeneous, interconnected pore network. First results with poly( $\epsilon$ -caprolactone) (PCL) have recently been reported [24]. The method combines the advantages of thermal processing with particulate leaching, and is applicable to a large variety of polymers. It involves precipitation of a high molecular weight polymer solution containing dispersed salt particles into an

excess of a non-solvent, followed by thermal processing and subsequent salt leaching. In this way we have prepared porous structures from poly(D,L-lactide) (PDLLA), a 1000PEOT70PBT30 poly(ether ester) block copolymer [25] and PCL, which are widely used in the biomedical field. The morphologies, porosities and mechanical properties of the resultant scaffolds were examined and compared to scaffolds prepared by other thermal processing techniques (sintering and compression moulding of mixtures of polymer particles and salt particles followed by salt leaching), and methods based on freeze-drying polymer solutions.

## 2. Experimental

### 2.1. Materials

PDLLA (L- to D-lactide ratio of 50/50,  $M_n = 116,000$ ) was prepared by ring opening polymerisation in the melt at 140–150°C with Sn(Oct)<sub>2</sub> as a catalyst [26]. 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 segmented poly(ether ester) copolymer based on polyethylene oxide and polybutylene terephthalate multiblock copolymer was prepared by a two-step polycondensation reaction of polyethylene glycol (PEG with molecular weight 1000), 1,4-butanediol and dimethyl terephthalate [27]. The resulting polymer has a  $T_g$  of –50°C, and  $T_m$  of 148°C. Poly(trimethylene carbonate) (PTMC) with  $M_n = 2.72 \times 10^5$  and  $M_w = 5.38 \times 10^5$  was prepared by ring opening polymerisation of trimethylene carbonate [16]. PTMC is an amorphous polymer with a  $T_g$  of –17°C. Commercial grade PCL (PCL, CAPA 680 with  $M_n = 7.67 \times 10^4$  and  $M_w = 1.20 \times 10^5$ ) was obtained from Solvay-Interox (UK). It is a semi-crystalline polymer with a melting temperature of 57°C and a glass transition temperature of –62°C.

Chloroform and iso-propanol were purchased from Biosolve (The Netherlands), and ethanol from Assink (The Netherlands). 1,4-Dioxane and sodium chloride were purchased from Merck (Germany). All chemicals were of analytical grade. The salt crystals were fractionated into several size ranges by sieving with standard testing sieves (ASTM–11 specifications, Fisher Scientific, The Netherlands) of mesh sizes of 106, 250, 425, 500, 710, 1000, and 1180  $\mu\text{m}$ . The sieves were mounted on an automated sieve shaker (Retsch, Germany). After size separation, the collected salt fractions were stored in a cool, dry place prior to use.

### 2.2. Sintering

Polymer particles were prepared by grinding the PDLLA and 1000PEOT70PBT30 polymers at liquid

nitrogen temperatures with a laboratory grinder (IKA A10 laboratory grinder, Germany). The obtained particles were sieved in the same manner as the salt crystals to yield polymer particle fractions with size ranges 0–250, 250–425, 425–500, 500–710, 710–1000, and 1000–1180  $\mu\text{m}$ . It was not possible to grind PCL in this manner.

Porous, disc-shaped specimens measuring 8 mm in height and 17 mm in diameter were prepared by sintering the polymer particles in stainless-steel moulds in a laboratory compression moulding press (Fonteinje, The Netherlands). A very low sintering pressure of 100 kPa was applied for a period of 2 h. The optimum sintering temperatures were close to the glass transition temperature or the melting temperature of the polymer; PDLLA was sintered at 55°C, and 1000PEOT70PBT30 at 115°C.

### 2.3. Compression moulding of mixtures of salt and polymer particles followed by salt leaching

NaCl crystals and ground polymer particles were sieved to yield fractions of different size ranges as mentioned previously. Mixtures of salt and polymer particles of varying particle sizes containing 70–95 wt% (which approximately corresponds to 60–90% by volume) of salt were prepared by mechanical mixing. The mixtures were compression moulded in a stainless-steel mould and disc-shaped polymer–salt composites measuring 8 mm in height and 17 mm in diameter were prepared. The compression moulding press was operated at a pressure of 3.5 MPa at elevated temperatures. PDLLA and salt mixtures were compression moulded at 120°C, 1000PEOT70PBT30 and salt mixtures were compression moulded at 160°C.

Porous structures were prepared by subsequent leaching of the salt particles out of the polymer–salt composites with an excess of gently stirred demineralised water for a period of 4–5 days. The water was changed every 2 h for the first 10 h, then 2–3 times a day.

### 2.4. Preparation of porous polymer structures by freeze-drying

PDLLA, 1000PEOT70PBT30 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°C 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.

### 2.5. Preparation of porous polymer structures by a process involving polymer coagulation, compression moulding and particulate leaching

The process for the preparation of porous scaffolds by polymer coagulation and particulate leaching is schematically shown in Fig. 1. With this method, leachable particles are homogeneously incorporated in a polymer matrix during precipitation of a high molecular weight polymer solution that contains dispersed leachable particles into a non-solvent.

The polymers were dissolved in  $\text{CHCl}_3$  at a concentration of 10% (w/v) at room temperature. Amounts of salt particles ranging from 70 to 95 wt% relative to the polymer mass in the solution were added to rapidly stirred polymer solutions. The salt crystals had been previously sieved to size ranges of 106–250, 250–425, 425–500, and 500–710  $\mu\text{m}$ . The rapid stirring ensured a homogeneous dispersion of the salt particles in the polymer solutions, which were then precipitated into a 10-fold excess of rapidly stirred ethanol. The solid polymer–salt composites could then be isolated from the non-solvent.

After drying and cutting into small parts of approximately  $4 \times 4 \times 4 \text{ mm}^3$ , the composite precipitate was compression moulded into discs (8 mm in thickness, 17 mm in diameter) on a hot press (Fontijne, The Netherlands) operated at a pressure of 3.5 MPa at 120°C, 140°C and 160°C for the composite from PDLLA, PCL and 1000 PEOT70/PBT30, respectively, and rapidly cooled to 15°C.

The compression moulded polymer–salt composite discs were placed into gently stirred demineralised water for a period of 4–5 days to leach out the salt. The water was changed every 2 h for the first 10 h, then 2–3 times a day. The resulting porous polymer scaffolds were vacuum-dried for a period of 24 h and stored before use.

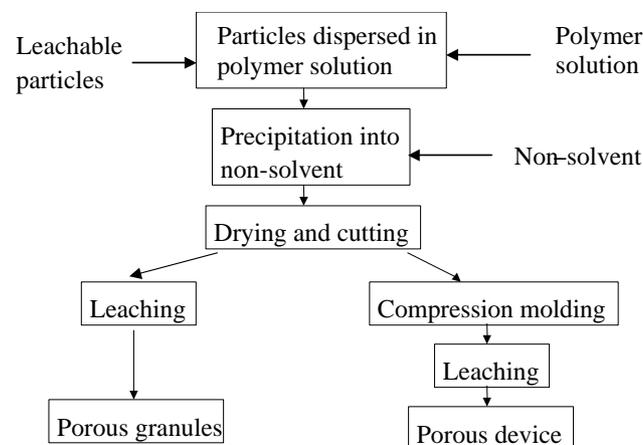


Fig. 1. Preparation of porous structures by a polymer coagulation and particle leaching process.

Alternatively, porous polymer granules can be prepared by leaching the cut composite precipitate with water to remove the salt without compression moulding.

### 2.6. Scanning electron microscopy

A Hitachi S800 scanning electron microscope was used to examine the morphology of the porous scaffolds. Cross-sections of the scaffolds were coated with gold using a sputter-coater (Turbo Sputter Coater E6700, UK).

### 2.7. Determination of the porosity of the porous polymer structures

The density and the porosity of the porous structures were determined by measuring the dimensions and the mass of the scaffold. The density ( $d$ ) of the porous scaffolds was calculated as follows:

$$d = \frac{m}{V}, \quad (1)$$

where  $m$  is the mass and  $V$  is the volume of the porous structure.

The porosity of the porous structure,  $p_o$ , was calculated as

$$p_o = 1 - \frac{d}{d_p}, \quad (2)$$

where  $d_p$  is the density of non-porous polymer, compression moulded in the same manner ( $d_p = 1.246 \pm 0.009$ ,  $1.103 \pm 0.007$  and  $1.188 \pm 0.008$  g/cm<sup>3</sup> for PDLLA, PCL and 1000PEOT70PBT30, respectively).

### 2.8. Mechanical testing in compression

The compression moduli,  $E_c$ , of the porous polymer structures were obtained at room temperature using a Zwick tensile tester equipped with a 500 N load cell at a crosshead speed of 2 mm/min. The specimens were disc-shaped with a height of 8 mm and a diameter of 17 mm. The load was applied until the scaffold was compressed to approximately 25% of its original thickness. The initial compressive moduli were determined as the slope of the linear portion of the stress–strain curve at a compressive strain of 4.5–5.5%.

## 3. Results and discussion

To compare the characteristics of porous polymer structures prepared by the process of polymer coagulation, compression moulding and particulate leaching with other common methods of preparing porous polymer scaffolds, we prepared porous PDLLA and 1000PEOT70PBT30 structures by sintering of ground

polymer particles and by compression moulding mixtures of ground polymer and salt particles which were turned into porous structures by subsequent salt leaching. These techniques are based on thermal processing of polymers allowing easy preparation of suitable porous devices of virtually any shape. Not all polymers can be processed and turned into porous structures by these methods; PCL for example is a polymer that cannot be ground into particles. Therefore, freeze-drying and casting methods involving organic solvents are much employed as well.

### 3.1. Porous polymer structures by sintering

Ground PDLLA particles were sintered at 55°C and 1000PEOT70PBT30 particles were sintered at 115°C. Although sintering is a very straightforward method of preparing porous structures (it is not necessary to use additives or solvents) the maximum porosities that can be reached are relatively low. In general porosities not higher than approximately 72% can be obtained. Table 1 shows that the maximum porosity that could be reached was approximately 60% for PDLLA, and 72% for 1000PEOT70PBT30. A main factor in determining the maximum porosity seems to be the polymer particle size employed. Furthermore, the resultant structures possess pores of irregular shapes varying from 50 to 300 μm in size as could be seen in scanning electron microscopy (SEM) images. This pore size range is equal for both polymers and is not determined by the polymer particle size. Therefore, the method does not allow the preparation of porous scaffolds in which the porosity and pore size can be varied independently.

### 3.2. Porous structures by compression moulding of mixtures of salt and polymer particles followed by salt leaching

Porous structures can conveniently be prepared by compression moulding mixtures of polymer particles and salt particles followed by leaching of the salt fraction. The porosities and pore sizes of the obtained

Table 1  
Porosities of porous PDLLA and 1000PEOT70PBT30 scaffolds prepared by sintering

Polymer particle size range (μm)	Porosity (%)	
	PDLLA	1000PEOT70PBT30
0–250	58.2	71.5
250–425	48.2	52.9
425–500	42.9	56.4
500–710	42.7	51.8
710–1000	40.6	47.9
1000–1180	39.1	47.0

Table 2  
Porous PDLLA structures obtained by compression moulding of mixtures of salt and polymer particles followed by leaching of the salt

Polymer particle size ( $\mu\text{m}$ )	Salt particle size ( $\mu\text{m}$ )	Salt content (wt%)	Salt content (vol%)	Scaffold stability during leaching	Porosity (%)
0–250	<250	90	83.8	--	
0–250	250–425	80	69.8	++	79.3
0–250	250–425	90	83.8	—	
0–250	425–500	90	83.8	±	
0–250	500–710	70	57.3	++	73.0
0–250	500–710	90	83.8	±	
250–425	250–425	80	69.8	++	81.2
250–425	250–425	90	83.8	--	
250–425	425–500	90	83.8	—	
250–425	500–710	70	57.3	++	70.1
250–425	500–710	90	83.8	±	
425–500	425–500	90	83.8	±	
425–500	500–710	90	83.8	±	
500–710	500–710	70	57.3	++	71.8
500–710	500–710	75	63.3	++	77.2
500–710	500–710	80	69.8	++	80.4
500–710	500–710	90	83.8	—	
500–710	0–250	70	57.3	--	
500–710	250–425	70	57.3	--	

The stability of the structure during salt leaching is denoted as: ++ no fragmentation, + single polymer fragment comes off, ± several polymer fragments come off, — many polymer fragments come off, -- complete scaffold disintegration.

porous structures can be varied independently by adjusting the salt to polymer ratios and the salt particle sizes, respectively. Table 2 is an overview of the results obtained after compression moulding of particle mixtures of PDLLA and salt and leaching of the salt. The table shows that it is possible to prepare porous scaffolds with a range of porosities up to more than 80%. The fact that the salt particles can be extracted, implies that the resulting pores are interconnected.

An important problem regarding this method is the structural integrity of the structure during the leaching process. Especially at high salt contents and high porosities, the scaffolds are prone to fragmentation. Table 2 also shows that when the polymer particle size used was larger than the salt particle size, the resultant porous scaffolds are damaged in the leaching process and break into several pieces. Suitable scaffolds were only obtained when the size range of the employed polymer particles was smaller than or equal to the size range of the salt particles. Irrespective of the size range of the salt particles, at salt contents below 60 wt% (data not shown) it was not possible to leach out the salt component from the compression moulded composite. And it was not possible to obtain intact porous structures at salt contents over 90 wt%.

Similar results were obtained for the preparation of porous 1000PEOT70PBT30 structures, as shown in Table 3.

Besides the weakness of the structures at high porosities, the method does not yield perfectly homo-

geneous pore structures. Fig. 2 is an SEM micrograph of a PDLLA scaffold prepared by compression moulding mixtures of polymer and salt particles followed by salt leaching. It can be seen that although the connected pores resemble the salt particle characteristics, much larger pores are also formed in the structure. It seems that the polymer and salt particles have formed aggregates that are not disrupted during the mixing process. Therefore, after compression moulding and leaching with water, a structure with a less than optimum pore morphology and decreased mechanical stability is obtained.

### 3.3. Preparation of porous structures by freeze-drying

The results on the compression moulding of polymer and salt particle mixtures and subsequent salt leaching (Tables 2 and 3) show that it is possible to obtain porous structures with interconnected pores and control over pore size and porosity. It should be noted that the maximum porosities of stable scaffolds that can be prepared in this manner are approximately 80–85%.

Among the methods developed for the preparation of porous structures, freeze-drying has often been used in tissue engineering [2,28], as it gives porous structures with even higher porosities. Furthermore, it is a suitable method when the processing of the polymer needs to be done via solutions in organic solvents. For example PCL, is a polymer that cannot be ground into particles.

Table 3  
Porous 1000 PEO70PBT30 structures obtained by compression moulding of mixtures of salt and polymer particles followed by leaching of the salt

Polymer particle size ( $\mu\text{m}$ )	Salt particle size ( $\mu\text{m}$ )	Salt content (wt%)	Salt content (vol%)	Scaffold stability during leaching	Porosity (%)
0–250	250–425	81.0	70	++	79.3
0–250	250–425	87.9	80	++	85.5
0–250	250–425	94.3	90	±	
250–425	425–500	73.2	60	++	73.5
250–425	425–500	81.0	70	++	80.6
250–425	425–500	87.9	80	++	85.0
250–425	425–500	94.3	90	–	
250–425	500–710	87.9	80	++	81.9
250–425	710–1000	87.9	80	+	80.1
425–500	500–710	73.2	60	++	75.1
425–500	500–710	81.0	70	++	78.3
425–500	500–710	87.9	80	++	85.1
425–500	500–710	94.3	90	±	
425–500	710–1000	87.9	80	+	84.4
500–710	710–1000	73.2	60	+	74.1
500–710	710–1000	81.0	70	±	
500–710	710–1000	87.9	80	±	
500–710	710–1000	94.3	90	--	
500–710	0–250	73.2	60	--	
500–710	250–425	73.2	60	--	

The stability of the structure during salt leaching is denoted as: ++ no fragmentation, + single polymer fragment comes off, ± several polymer fragments come off, – many polymer fragments come off, -- complete scaffold disintegration.

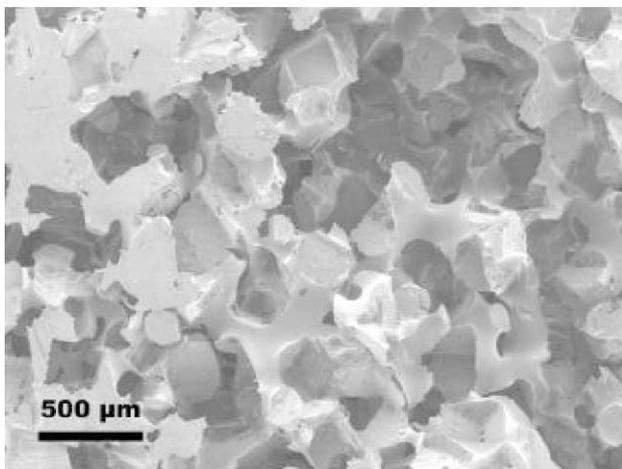


Fig. 2. SEM micrograph of an 81.2% porous PDLLA structure prepared by compression moulding and salt leaching of mixtures of PDLLA and salt particles. PDLLA particle size: 250–425  $\mu\text{m}$ ; salt particle size: 250–425  $\mu\text{m}$ .

Fig. 3 shows SEM micrographs of PDLLA, 1000PEOT70PBT30 and PCL scaffolds prepared from 10% (w/v) solutions in 1,4-dioxane (melting temperature is 11°C) by freeze-drying applying freezing temperatures of 6°C and –25°C.

The pore sizes are relatively small, with a maximum pore size of approximately 150  $\mu\text{m}$ . It can be seen that the scaffolds prepared by freezing at 6°C had a larger

pore size than those prepared by freezing at –25°C. As the specimen geometries are equal and the samples are cooled from room temperature, the higher freezing temperature has the lowest quenching rate. Although further reducing the quenching rate could increase the pore size, in our experimental set-up this is limited by the freezing point of the solution.

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 (Fig. 3F).

Although it has been shown that defect-free, porous structures with good pore interconnection can be obtained through careful control of the freeze-drying parameters (unidirectional freezing and rigorous degassing of the polymer solution is necessary to prevent cracking and void formation in the resulting porous structure) [5], the pore sizes that can be obtained in a homogeneous porous structure are limited to approximately 100  $\mu\text{m}$ .

By varying the concentration of the polymer solutions used, as shown in Table 4, the porosity of the porous polymer scaffolds could be well controlled. There is no significant effect of the freezing temperature on the porosity of the resulting scaffolds.

The poor pore interconnectivity we obtained in our freeze-drying experiments is not desired in tissue engineering, as the exchange of nutrients and cell waste

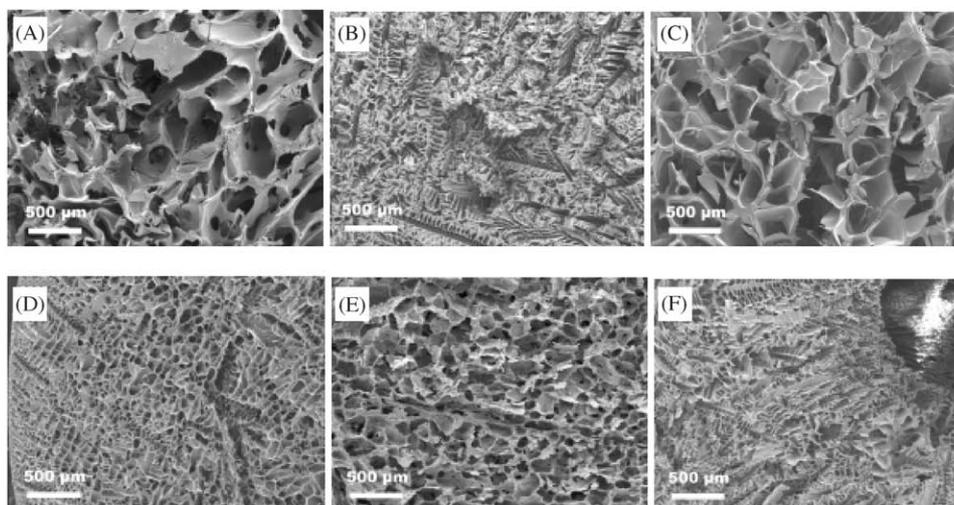


Fig. 3. SEM micrographs of PDLLA, 1000PEOT70PBT30 and PCL scaffolds prepared from 10% (w/v) solutions in 1,4-dioxane by freeze-drying. (A) PDLLA, freezing temperature: 6°C; porosity: 88.6%; (B) PDLLA, freezing temperature: -25°C; porosity: 87.6%; (C) 1000PEOT70PBT30, freezing temperature: 6°C; porosity: 88.3%; (D) 1000PEOT70PBT30, freezing temperature: -25°C; porosity: 88.1%; (E) PCL, freezing temperature: 6°C; porosity: 89.2%; and (F) PCL, freezing temperature: -25°C; porosity: 89.2%.

Table 4  
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

products is hindered and might compromise cell viability. This can be improved by combining the freeze-drying process with particulate leaching [14,15] (which simultaneously allows larger pores to be created). This method is most suitable when large fractions of leachable particles and high concentrations of polymers of a high molecular weight in organic solvents are used. We found it difficult to prepare large porous structures

in this manner, as the leachable particles tend to deposit on the bottom of the container. This deposition results in an inhomogeneous porous structure.

### 3.4. Porous polymer structures by coagulation, compression moulding and salt leaching

The process of preparing porous polymeric structures by precipitation of a polymer solution into which salt particles are dispersed and subsequent leaching of the salt particles overcomes the disadvantages of the previous methods. Now high porosity polymeric structures of which the porosity and pore size can be varied independently are easily formed. Thermal processing of the salt-containing precipitate allows the convenient preparation of porous specimens of many shapes and sizes. The obtained pore structure is very homogeneous and well-defined. Furthermore, as the polymer is precipitated during the process, the polymer is simultaneously purified and rid of additives, unreacted monomer, catalyst residues, etc. As an example, scaffolds were prepared from PDLLA that contained 1.9% residual lactide monomer, after preparation of the scaffold by the coagulation and salt leaching method no residual monomer could be detected.

Porous PDLLA, 1000PEOT70PBT30, and PCL scaffolds were prepared by this method. Coagulation of the polymer takes place when a solution of the high molecular weight polymer containing dispersed salt particles is precipitated into an excess of rapidly stirred non-solvent. In our case chloroform and ethanol were used as the polymer solvent and non-solvent, respectively. It should be noted that the dispersed salt crystals do not dissolve in the solvent and non-solvent. During

precipitation the salt remains entrapped within the coagulating polymer matrix; this yields a fibrous composite of which the extractable salt component is homogeneously distributed. After precipitation, the polymer–salt composite is dried and cut into small pieces. These granules can now be processed into a device by thermal processing techniques such as injection moulding, extrusion and compression moulding. After extraction with water to remove the salt, a porous structure in the desired shape is obtained.

In an alternative approach, the composite can be extracted with water after the drying and cutting step to give porous granules which themselves can be used as implants.

Fig. 4 shows characteristic SEM micrographs of porous PDLLA, 1000PEOT70PBT30, and PCL scaffolds prepared by coagulation of salt dispersions in polymer solutions and salt leaching of the compression moulded composite precipitate. The scaffolds feature

very regular structures with homogeneously distributed and interconnected pores, especially at the highest porosities. The shapes and sizes of the pores are in good agreement with the characteristics of the salt particles used. The morphology of the pores is therefore precisely determined by the leachable particles. Compared with the highly porous scaffolds prepared by freeze-drying, the pore structures are much more regular, and large voids or cracks are not present. The interconnection of the pores is also much greater. Porous scaffolds with a much larger range of pore sizes and porosities can be prepared than with the above-mentioned other methods.

The porosities and pore sizes of the scaffolds can easily be controlled by variation of the salt concentration and the salt particle size range. Fig. 5 shows the porosity of PDLLA, 1000PEOT70PBT30, and PCL scaffolds as a function of the salt concentration used and different salt particle sizes. The salt weight percentage

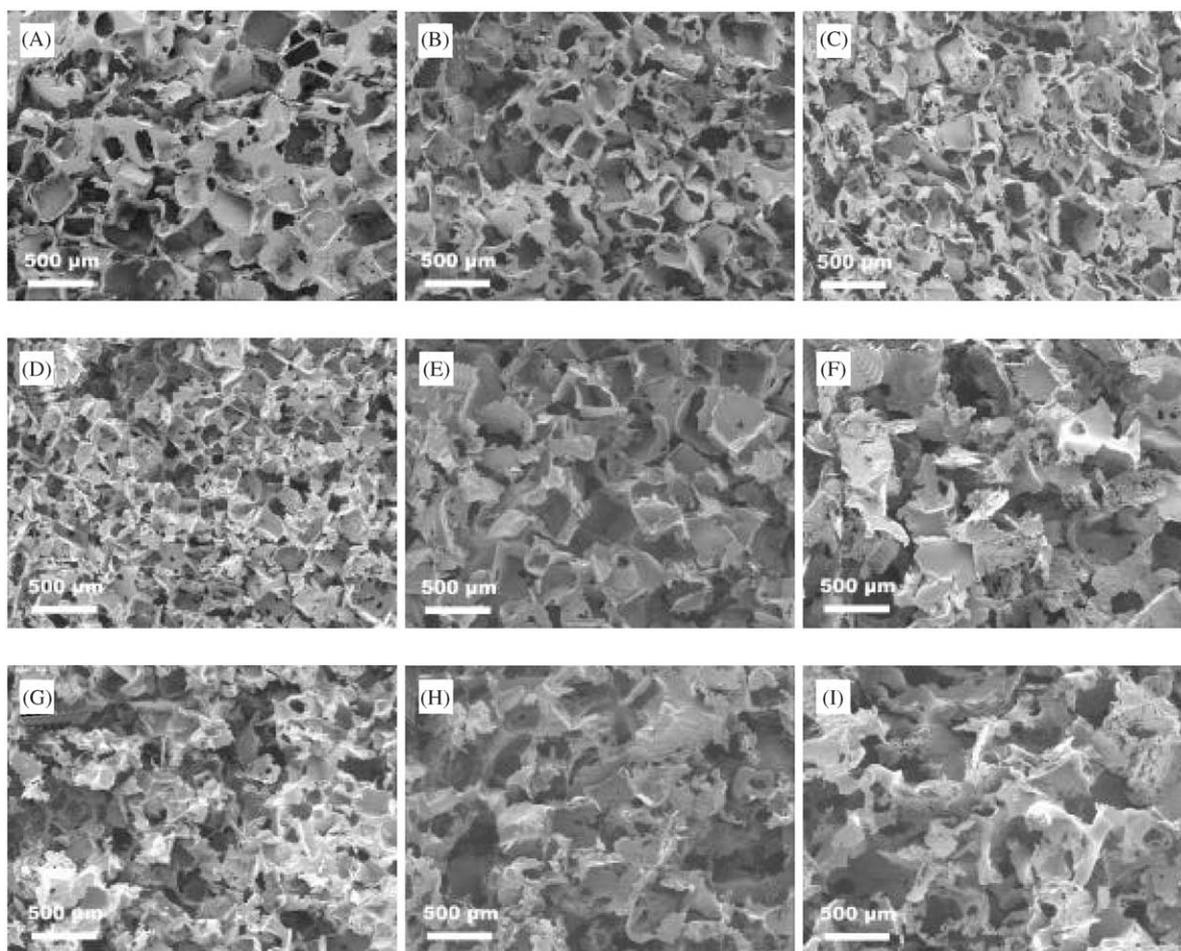


Fig. 4. SEM micrographs of porous polymer scaffolds prepared by the coagulation and leaching process. The compression moulded composites were leached with water. (A) PDLLA, porosity: 77.2%; pore size range: 250–425  $\mu\text{m}$ ; (B) PDLLA, porosity: 90.0%; pore size range: 250–425  $\mu\text{m}$ ; (C) PDLLA, porosity: 95.7%; pore size range: 250–425  $\mu\text{m}$ ; (D) 1000PEOT70PBT30, porosity: 85.8%; pore size range: 106–250  $\mu\text{m}$ ; (E) 1000PEOT70PBT30, porosity: 88.3%; pore size range: 250–425  $\mu\text{m}$ ; (F) 1000PEOT70PBT30, porosity: 93.2%; pore size range: 500–710  $\mu\text{m}$ ; (G) PCL, porosity: 91.7%; pore size range: 106–250  $\mu\text{m}$ ; (H) PCL, porosity: 92.4%; pore size range: 250–425  $\mu\text{m}$ ; and (I) PCL, porosity: 92.4%; pore size range: 500–710  $\mu\text{m}$ .

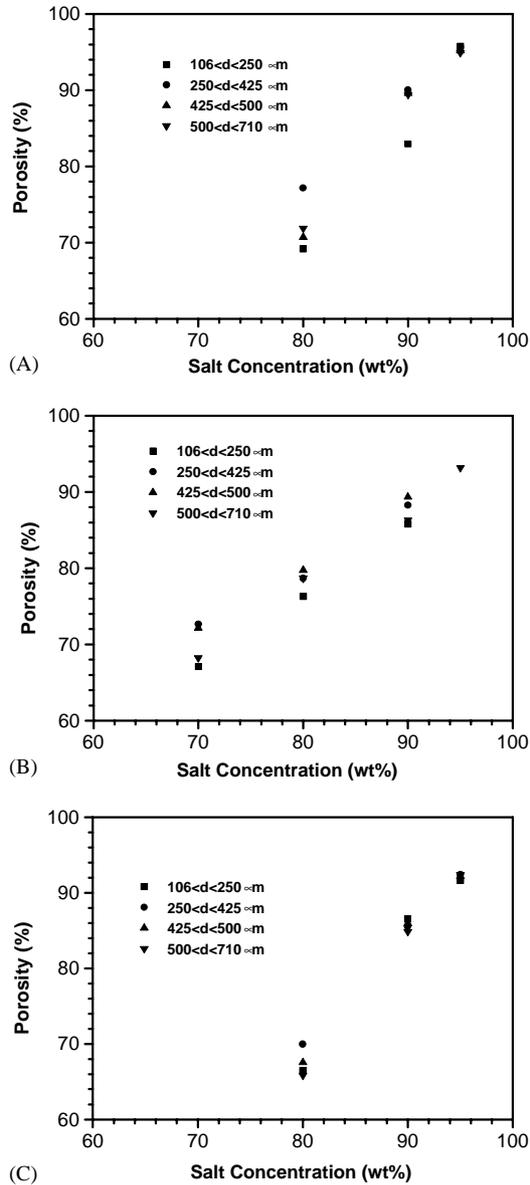


Fig. 5. Porosity of polymer scaffolds prepared by coagulation, compression moulding and salt leaching as a function of the salt content relative to the polymer at different salt particle size ranges. (A) PDLLA; (B) 1000PEOT70PBT30; and (C) PCL.

(relative to the polymer) is varied between 80–95, 70–95, and 80–95 wt% for PDLLA, 1000PEOT70PBT30 and PCL, respectively. Depending on the polymer the resulting porosities of the porous structures were between 65 and 96 vol%.

The obtained porosity after compression moulding and leaching is not much influenced by the size of the salt particles. And, as the pore dimensions are only determined by the size of the salt particles, the method allows independent control of pore size and porosity. At salt contents below 60 wt% (data not shown) it was not possible to leach out the salt component from the compression moulded composites. At higher concentrations, however, the salt particles could readily be

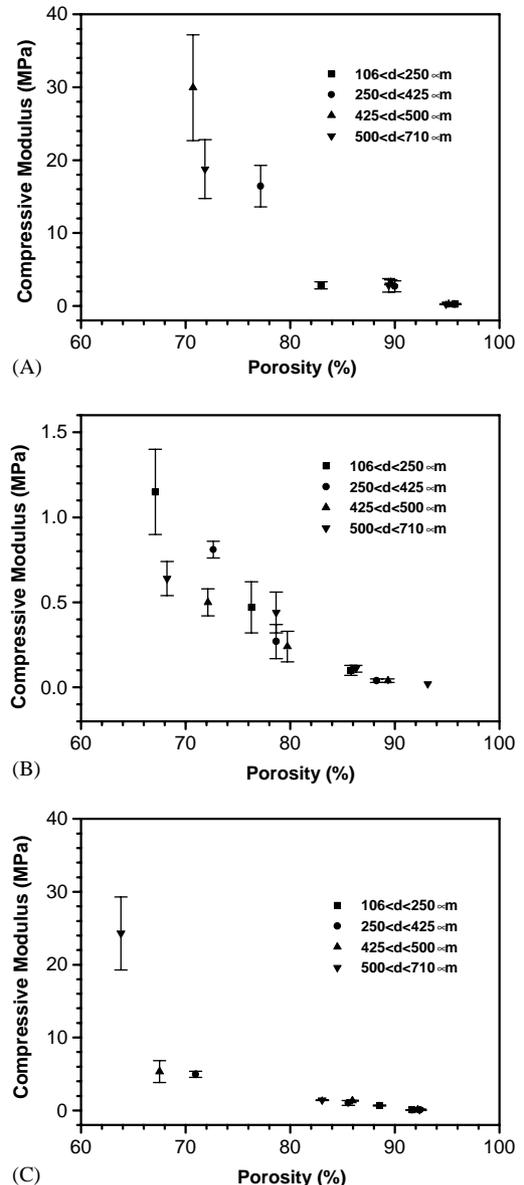


Fig. 6. Compressive modulus (in MPa) of porous polymer scaffolds as a function of porosity and salt particle size. (A) PDLLA; (B) 1000PEOT70PBT30; and (C) PCL.

extracted. This implies that at higher salt concentrations (and higher porosities) an interconnected pore network was obtained.

Fig. 6 displays relationships between the compressive modulus (in MPa) and the porosity of the scaffolds prepared in this manner. The compressive modulus of the polymeric scaffolds decreased with increasing porosity, this agrees with theory [29]. It was reported that in heterogeneous porous structures the pore size could affect the compressive modulus [30]. As in our case the dependence of the compression modulus on the pore size is negligible, it confirms the homogeneity of the pore network of the scaffolds fabricated by this method.

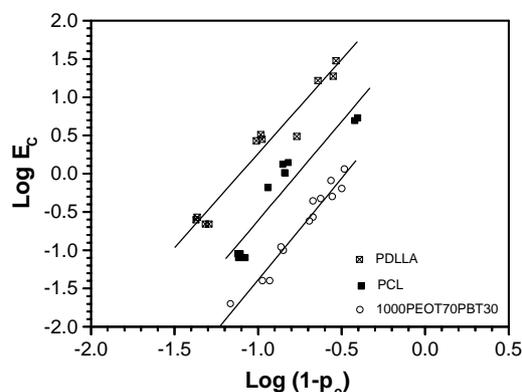


Fig. 7. Relationships between the compressive modulus  $E_c$  in MPa and  $(1 - p_o)$  for the different polymers investigated. Porous structures were prepared by coagulation, compression moulding and salt leaching.

In Fig. 7 the log value of the compression modulus in MPa is given as a function of the log value of  $(1 - p_o)$  of the different scaffolds. For the different polymers the compressive moduli could be related to the porosities of the scaffolds according to the following power-law relationships:

$$E_c = k_1(1 - p_o)^{2.42} \quad (\text{PDLLA}),$$

$$E_c = k_2(1 - p_o)^{2.66} \quad (1000\text{PEOT}70\text{PBT}30),$$

$$E_c = k_3(1 - p_o)^{2.59} \quad (\text{PCL}).$$

Theoretically, the value of the exponent varies between 1 and 3, depending on pore shape and orientation. For an isotropic porous structure with cubic pores, the exponent value is expected to be 2 [29]. The parameters  $k_1$ ,  $k_2$  and  $k_3$  are the compression moduli of the non-porous polymers. The values are 296.5, 19.0, and 236.6 MPa for PDLLA, 1000PEOT70PBT30 and PCL, respectively.

With this, it is possible to optimise the scaffold for a desired medical or tissue engineering application by variation of the polymeric material, the porosity and the needed mechanical properties.

This technique for preparing porous polymer structures is also applicable to rubbery polymers. We have been studying the use of PTMC in medical applications and tissue engineering. PTMC is an amorphous, degradable polymer with a glass transition temperature of  $-17^\circ\text{C}$ . It has only been processed into porous membranes by an immersion precipitation process [16]. In that case, pore sizes were smaller than  $20\ \mu\text{m}$ , and it was necessary to add high molecular weight PEO as a stabiliser.

We applied the coagulation and salt leaching process to high molecular weight PTMC. The PTMC polymer was dissolved in  $\text{CHCl}_3$  at a concentration of 2% on a weight per volume basis. Salt particles of  $106\text{--}250\ \mu\text{m}$  were dispersed in the solution, and the mixture was

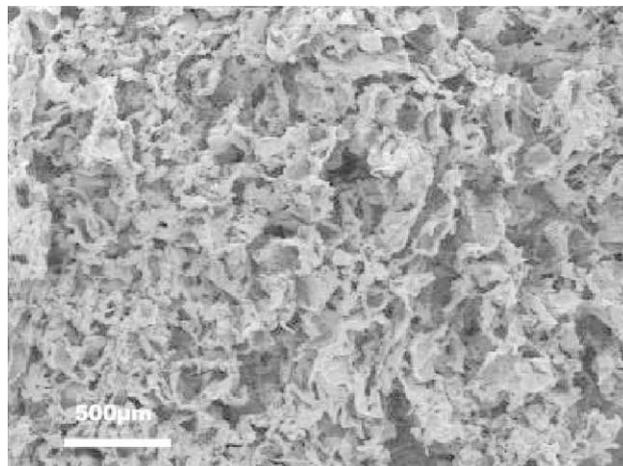


Fig. 8. Porous PTMC scaffold with a porosity of 90.0% prepared by coagulation and salt leaching. Salt particle size:  $106\text{--}250\ \mu\text{m}$ .

precipitated into a 10-fold excess of rapidly stirred isopropanol. The precipitate was recovered and dried, and the salt was leached out with water. Fig. 8 shows the resulting porous scaffold.

These results show that flexible, degradable amorphous polymers with low glass transition temperatures can be readily processed into porous tissue engineering scaffolds as well. The versatility of the method allows the preparation of optimised porous polymer scaffolds for tissue engineering, where the ingrowth of cells and tissues is determined by the nature of the polymer and the characteristics of the porous structure.

#### 4. Conclusions

We have developed a new technique for the preparation of porous polymeric scaffolds that involves precipitation of a polymer solution in which salt particles are dispersed. Following compression moulding and leaching of the salt fraction highly porous polymeric structures are obtained. This method also allows the thermal processing of polymers into porous (medical) devices that cannot be ground into particles, such as PCL.

Conventional thermal processing techniques such as sintering and compression moulding of polymer and salt particle mixtures followed by salt leaching give scaffolds with relatively low porosities. Fragile heterogeneous structures result at porosities higher than 90%.

With freeze-drying techniques highly porous structures can be obtained, but throughout the scaffold small pore sizes and poor homogeneity of the pore structure can result.

By our coagulation, compression moulding and salt leaching method, however, we cannot only obtain scaffolds with very high porosities and homogeneous interconnected pore networks from many polymeric

materials, but the porosity and pore size can also be independently controlled by variation of the salt concentration and salt particle size range, respectively. The compression modulus of the porous structures was found to decrease with an increase in porosity according to power-law relationships. This decrease was independent of salt particle and pore size.

This technique offers the possibility of optimisation of the porous structures in tissue engineering applications in relation to the specific cells and tissues and the convenient preparation of such scaffolds by thermal processing methods.

### Acknowledgements

This research is supported by the European Community, Brite-Euram project BE 97-4612. We also thank M. B. Claase and A. P. Pêgo for their kind help.

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