Summary: This study investigated the room-temperature compression molding/particle leaching approach to fabricate three-dimensional porous scaffolds for tissue engineering. Scaffolds with anatomical shapes (ear, joint, tube, cylinder) were made from biodegradable poly(D,L-lactide) and poly[(D,L-lactide)-co-glycolide]. The utility of this room-temperature compression approach comes from the effect of solvent assistance, but the tendency for post-molding scaffold shrinkage is a problem unique to this method and is thus examined with emphasis in this paper. Scaffold shrinkage was found to be tolerable under normal fabrication conditions with high salt contents, which is just what the preparation of highly porous scaffolds requires. Furthermore, the resultant porosities after salt leaching were measured as well as the initial scaffold shrinkages after solvent evaporation, and the relation between them was revealed by theoretical analysis and confirmed by comparison with experimental measurements. The pores were interconnected, and porosity can exceed 90%. The effects of porosity on the mechanical properties of porous scaffolds were also investigated. This convenient fabrication approach is a prospective method for the tailoring of porous scaffolds for a variety of possible applications in tissue engineering and tissue reconstruction.

Typical poly[(D,L-lactide)-co-glycolide] (PLGA) porous scaffolds with complicated shapes (ear, joint, tube, cylinder) fabricated by room-temperature compression molding and particulate leaching (RTCM/PL) approach.

Solvent-Assisted Room-Temperature Compression Molding Approach to Fabricate Porous Scaffolds for Tissue Engineering

Dianying Jing, Linbo Wu, Jiandong Ding

1 Key Laboratory of Molecular Engineering of Polymers of Chinese Ministry of Education, Department of Macromolecular Science, Fudan University, Shanghai 200433, China
Fax: +86-21-656-40293; E-mail: jdding1@fudan.edu.cn
2 State Key Laboratory of Chemical Engineering (Polymer Reaction Engineering Branch), Institute of Polymer Engineering, Zhejiang University, Hangzhou 310027, China

Received: March 31, 2006; Revised: May 20, 2006; Accepted: May 30, 2006; DOI: 10.1002/mabi.200600079

Keywords: fabrication; polyesters; porous scaffold; shrinkage; tissue engineering
Introduction

Biodegradable polymers constitute a topic of intensive research in recent years.\textsuperscript{[1–9]} Three-dimensional porous scaffolds fabricated by biodegradable and bio-compatible polymers play critical roles in the fields of tissue engineering and tissue reconstruction.\textsuperscript{[1,2,10–12]} A number of methodologies and technologies, such as fiber bonding,\textsuperscript{[13]} porogen leaching,\textsuperscript{[14–16]} phase separation,\textsuperscript{[17–19]} freeze drying,\textsuperscript{[20,21]} gas foaming,\textsuperscript{[22,23]} compression molding/porogen leaching,\textsuperscript{[24,25]} solvent merging/porogen leaching,\textsuperscript{[26]} injection/gas foaming,\textsuperscript{[27]} three-dimensional printing (3-DP),\textsuperscript{[28]} fused deposition modeling (FDM),\textsuperscript{[29]} electrospinning,\textsuperscript{[30,31]} and phase separation/porogen leaching,\textsuperscript{[32,33]} have been applied to fabricate porous scaffolds. A feasible and practical method for scaffold fabrication should be able to form both an internal interconnected pore structure and an external anatomical shape of a porous scaffold. Most of the reports about fabrication of porous scaffolds so far focus on pore-forming methods rather than shaping methods. Scaffolds with the anatomical shapes resembling various tissues or organs are much desired.

Compression molding is widely used in the shaping of plastics with complicated geometry. On the other hand, it is well known that porogen leaching is a successful pore-forming method and can be used to fabricate porous membrane when it is combined with solvent casting.\textsuperscript{[14,34]} In the preceding paper,\textsuperscript{[35]} we reported a thermal compression molding/particle leaching approach based on a specially designed combined rigid-flexible mould to fabricate three-dimensional porous scaffolds with complicated shape. However, the processing temperature must be higher than the glass transition temperature for an amorphous biodegradable polymer or the melting point for a crystalline polymer, which might result in degradation of polyester to a certain degree. The molding process must also be carried out in a compressing machine with relatively high loading.

In this work, we examined a fabrication method of three-dimensional porous scaffolds with complicated shape by combining particle leaching with a solvent-assistant “room-temperature” compression molding at low pressure. The composites of polyester/particle/solvent have ever been used to fabricate polyester porous scaffolds in the literature.\textsuperscript{[14,23]} In our group, paste-like polymer/salt-particle mixtures containing much less solvent than that used in the conventional solvent casting/porogen leaching approach have been used to perform compression molding at room temperature in a predesigned rigid-flexible mold.\textsuperscript{[36–39]} For the resultant scaffolds, the degradation stages were summarized,\textsuperscript{[36]} the porosity and pore-size effects on degradation rate were examined,\textsuperscript{[37]} the sensitivity of the mechanical properties to the aqueous environment was determined,\textsuperscript{[38]} and pore structures using different porogens were compared.\textsuperscript{[39]} The distinguishing feature of the present paper is the focus upon the methodology research of the room-temperature compression molding/particle leaching approach (RTCM/PL). In this study, scaffolds with the anatomical shapes (ear, joint, tube, cylinder) were fabricated from biodegradable and bioreversible poly(D,L-lactide) (PDLLA) and poly[(D,L-lactide)-co-glycolide] (PLGA). Although this approach presents striking advantages, such as the ability to fabricate complex scaffolds with ease at relatively low temperature and pressure during molding, the technique might, in principle, lead to serious shrinkage during solvent evaporation after demolding. The shrinkage of the composites as well as the relation between composite shrinkage and scaffold porosity was investigated by theoretical analysis and experimental verification in this study, by which the feasibility of this convenient fabrication approach has been confirmed. The potential bottleneck of this very convenient and promising approach to fabricate porous scaffolds has thus been overcome under the indicated conditions. The morphology, pore structure, and mechanical properties of the resulting scaffolds are also examined.

Experimental Part

Materials

PDLLA ($M_n$ 336 000) and PLGA with lactide/glycolide compositions of 85/15 (PLGA85/15, $M_n$ 138 000) and 75/25 (PLGA75/25, $M_n$ 147 000) were synthesized by ring-opening polymerization from D,L-lactide (Purac Co.) and glycolide (Purac Co.) at 130 °C using stannous octoate as initiator. Sieved sodium chloride particles with particle size $d$ of 280–450 µm, 180–280 µm, 90–180 µm, and 50–90 µm were used as porogen. Chloroform was employed as solvent in scaffold fabrication.

Scaffold Fabrication

A concentrated polymer solution was prepared first. The amount of solvent was controlled according to the weight ratio of polymer to particle (usually, the solvent volume fraction $\phi_{w,\text{partc}}$ was in the range of 25–40% and the porogen volume fraction $\phi_{v,\text{partc}}$ was in the range of 30–70%). Sieved sodium chloride particles were added and mixed to obtain a paste-like mixture of concentrated polymer solution and salt particles. The two important parameters, namely, particle weight content ($\phi_{w,\text{partc}}$) and solvent volume fraction ($\phi_{v,\text{solv}}$) are defined as

$$\phi_{w,\text{partc}} = \frac{W_{\text{partc}}}{W_{\text{partc}} + W_{\text{polym}}} \quad (1)$$

$$\phi_{v,\text{solv}} = \frac{V_{\text{solv}}}{V_{\text{partc}} + V_{\text{polym}} + V_{\text{solv}}} \quad (2)$$

Here $W_{\text{partc}}$ and $W_{\text{polym}}$ denote salt weight and polymer weight, respectively, while $V_{\text{partc}}$, $V_{\text{polym}}$, and $V_{\text{solv}}$ refer to the volumetric contributions to the salt/polymer/solvent composite from particle, polymer, and solvent, respectively. (See the definitions of the important terms in this paper in Nomenclature.)
The mixtures used in our experiments were ductile and
deformable enough to be molded in a combinatory mold with a
complicated cavity shape. The combinatory mold was com-
po
di
posed of a flexible inner part used to enhance the shaping
ability and facilitate mold release and a rigid outer part used to
give the mold adequate pressure-loading capacity. The details
of the mold were described in the preceding paper. The mold
charged with the polyester/particle/solvent mixture was
then tightly compressed in a custom-designed clamp at room
temperature. The pressure was maintained for several minutes.

After demolding, the remaining solvent in the shaped com-
posite was removed by air drying for 24 h and then vacuum drying
for a further 48 h. The solvent-free mixture was then immersed in
deionized water for 48 h, during which time the water was
changed every 4 h to leach out the salt particles. The salt-free
porous scaffolds were air-dried for 24 h, vacuum-dried for another
48 h, and then stored in a desiccator under vacuum until use.

Porosity and Pore Structure

The porosity of the resultant scaffolds was determined with a
modified liquid replacement method. Briefly, a dry porous scaffold of weight \( W_s \) was put into a glass bottle con-
nected to a vacuum line and the bottle was evacuated. After
the bottle was charged with ethanol, it was connected to atmo-
sphere for a quick air charge. The scaffold was fully permeated
with ethanol by several vacuum–air charge cycles. Such an
ethanol-impregnated scaffold was put into a weighing bottle
with ethanol by several vacuum–air charge cycles. Such an
ethanol-impregnated scaffold was put into a weighing bottle
precharged with ethanol. The bottle was then weighed as
\( W_2 \). The weight of bottle became \( W_2 \) after the ethanol-impregnated
scaffold was carefully removed. The porosity of the foam can
be calculated by Equation (3)

\[
\Phi_{\text{meas}} = \frac{(W_1 - W_2 - W_s)/\rho_e}{(W_1 - W_2 - W_s)/\rho_e + W_s/\rho_p} \times 100% \tag{3}
\]

where \( \rho_e \) and \( \rho_p \) refer to the ethanol density and the density of the
associated bulk polymer, respectively.

Samples with thickness of 2–3 mm were cut from the
auricle-shaped porous scaffolds. After sputter-coating with
gold, the pore morphologies in the interior and on the surface of
the samples were observed in a scanning electron microscope (SEM, S520). Imaging was conducted at an accelerating
voltage of 20 kV.

Mechanical Test

Compressive stress versus strain was measured on an Instron
1121 testing machine at room temperature. The testing samples
were cylinders measuring 10 mm in diameter and 10 mm in
height. The testing rate was 1 mm min\(^{-1}\). Compressive modulus \( E \)
and stress at 10% strain \( (\sigma_{10}) \) were used to characterize
the mechanical properties of a porous foam.

Initial Scaffold Shrinkage

To determine the initial shrinkage from immediately demolded
composite to the solvent-free composite and to the resultant
porous scaffold, the height \( H \) and diameters at the top \( D_{\text{top}} \),
bottom \( D_{\text{bot}} \), and middle \( D_{\text{mid}} \) of the cylindrical composites
were measured after removal of solvent. The corresponding
dimensions of the resultant scaffolds after both solvent eva-
apration and salt leaching were measured again. \( H_0, D_{\text{top},0}, D_{\text{bot},0}, \) and \( D_{\text{mid},0} \) denote the height, diameters at the top,
bottom, and middle of the immediately demolded cylindrical
composites, which are equal to the corresponding dimensions
of the mold in our compression molding. The volumetric
maintenance ratio \( S_v \) is defined as the volume of the resultant
scaffold \( V \) divided by that of the immediately demolded com-
posite \( V_0 \). \( S_v \) for a cylindrical scaffold is calculated by

\[
S_v = \frac{V}{V_0} = \frac{H(D_{\text{top}}^2 + D_{\text{bot}}^2 + 2D_{\text{mid}}^2)}{H_0(D_{\text{top},0}^2 + D_{\text{bot},0}^2 + 2D_{\text{mid},0}^2)} \tag{4}
\]

The average linear maintenance ratio \( \lambda \) is defined by

\[
\lambda = \sqrt{S_v} \tag{5}
\]

In order to describe the possible inhomogeneous shrinkages at
different positions in a cylinder scaffold, four relative main-
tenance ratios with respect to the average linear maintenance
are further defined as

\[
\begin{align*}
\lambda_H &= \frac{H}{H_0} \\
\lambda_{\text{top}} &= \frac{D_{\text{top}}}{D_{\text{top},0}} \\
\lambda_{\text{mid}} &= \frac{D_{\text{mid}}}{D_{\text{mid},0}} \\
\lambda_{\text{bot}} &= \frac{D_{\text{bot}}}{D_{\text{bot},0}}
\end{align*}
\tag{6-9}
\]

Statistical Analysis

The measurements were performed in triplicate \((n = 3)\) unless
otherwise indicated. Results are given as average values with
standard errors. Student’s \( t \)-test was employed for compar-
sions, in which \( P < 0.05 \) was used as the criterion for sig-
nificant difference.

Results and Discussion

Fabrication Approach for Polyester
Scaffolds with Complicated Shape

When sieved salt particles were dispersed into a concen-
trated solution of biodegradable polymer, a paste-like
polymer/particle mixture containing a small amount of
solvent was obtained. The mixture was not fluid, but was
ductile and sufficiently deformable to be molded at ambient
temperature. Compared to the conventional solvent-casting
approach, minimizing solvent inclusion enhances viscosity
to a large extent and makes the mixture hold its shape when
discharged from the mold. It is also beneficial for avoiding the “skin effect,” which is discussed later. Using specially designed molds with complicated cavity shapes, we molded the polymer solution/particle mixture at room temperature. Porous scaffolds with complicated shape were obtained when the solvent and particles were removed by evaporation and leaching. Photographs of porous scaffolds with anatomical shapes (ear, joint, tube, cylinder) are shown in Figure 1.

The shapes of the scaffolds strongly resemble their targeted shapes. The complicated shape of the scaffold is determined by the geometry of the mold cavity. Therefore, porous scaffolds with different shapes can be fabricated using molds with different cavity shapes.

In the fabrication process, there is no necessity to remove solvent in the shaped composite before mold release. The compression molding is performed at room temperature, allowing thermal degradation to be avoided. The sedimentation of particles is negligible owing to the high viscosity of the mixtures with high content of particles and concentrated polymer solutions, and thus the composites are quite stable in processing. In addition, the approach is very simple and flexible for different polymers and porogens. Therefore, it is a practical approach for fabrication of porous scaffolds with complicated shape for tissue engineering. The polymer solution/particle composite can be molded due to its ductility and deformability, but not due to its flowability, which is different not only from “classic” solvent casting/particle leaching in tissue engineering but also from the conventional compression molding used in the plastics industry. Therefore, we denominate it as the room-temperature compression molding/particle leaching approach (RTCM/PL).

**Examination of Scaffold Shrinkage**

In general, compression molding provides products that exhibit little material shrinkage or swelling after demolding. Nevertheless, as the processing objective in our approach is a polymeric composite containing solvent other than polymer itself, the shrinkage behavior may be different from the general compression molding process. It is thus important to examine the dimensional change from the immediately demolded composite to the solvent-free composite and to the resultant porous scaffolds fabricated under different conditions. The results for cylindrical scaffolds are shown in Table 1.

When the solvent in the molded composite was removed, shrinkage was observed. Further shrinkage developed when the porogen was leached out and the scaffold was obtained. The shrinkage was found to be dependent upon the solvent volume fraction $\varphi_{v,solv}$ and the particle weight fraction $\varphi_{w,partc}$. The statistical analysis of volumeric shrinkage based upon the data in Table 1 indicates that the extent of shrinkage basically increased with the increase of $\varphi_{v,solv}$ and/or the decrease of $\varphi_{w,partc}$. The only exception in Table 1 is that there is no statistically significant difference between $\varphi_{v,solv} = 30.0\%$ and $35.1\%$, which is because the shrinkage itself is not striking at relatively low solvent

### Table 1. The shrinkage of demolded salt/polymer/solvent composites after solvent removal and salt leaching in fabrication of cylindrical porous scaffolds.

<table>
<thead>
<tr>
<th>$\varphi_{w,partc}$</th>
<th>$\varphi_{v,solv}$</th>
<th>$S_v^{a)}$</th>
<th>$S_v^{b)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(90%)</td>
<td>30.0</td>
<td>98.3 ± 3.0</td>
<td>91.4 ± 2.3</td>
</tr>
<tr>
<td>(90%)</td>
<td>35.1</td>
<td>95.0 ± 0.5</td>
<td>89.5 ± 0.5</td>
</tr>
<tr>
<td>(90%)</td>
<td>42.5</td>
<td>85.9 ± 1.7</td>
<td>81.8 ± 1.5</td>
</tr>
<tr>
<td>(90%)</td>
<td>52.8</td>
<td>79.5 ± 3.4</td>
<td>76.0 ± 3.7</td>
</tr>
<tr>
<td>85</td>
<td>37.1</td>
<td>88.9 ± 1.5</td>
<td>84.4 ± 1.8</td>
</tr>
<tr>
<td>95</td>
<td>45.9</td>
<td>90.9 ± 0.2</td>
<td>84.6 ± 0.4</td>
</tr>
</tbody>
</table>

\(^{a)}\) After solvent removal.

\(^{b)}\) After solvent removal and particulate leaching.
content. At a constant $\varphi_{w,\text{partc}}$ of 90%, the volumetric shrinkage ratio ($1-S$) was close to or below 10% when $\varphi_{v,\text{solv}}$ was 35.1% or 30.0%. Although shrinkage is relatively remarkable when compared with conventional compression molding without solvent assistance, it might be acceptable or tolerable because in our RTCM/PL approach, $\varphi_{v,\text{solv}}$ is usually less than 35% and $\varphi_{w,\text{partc}}$ is usually higher than 85% (the porosity of the resulting scaffold will, otherwise, not be sufficiently high for tissue engineering).

Another important point is the examination of possible inhomogeneous shrinkage. Four relative maintenance ratios with respect to the average linear maintenance were, by Equation (6), (7), (8), and (9), calculated and shown in Figure 2. Averaging was performed over all cylinder scaffolds ($n = 3 \times 6$) for the measurements in Table 1. For data of the composites free of solvent or for those of the resultant scaffolds in Figure 2, student’s $t$-test demonstrated significant differences among all of four dimensions with the only exception between $z_{\text{top}}$ and $z_{\text{mid}}$. According to Figure 2, $z_{\text{top}}$ and $z_{\text{mid}}$ were smaller than 100%, while $z_{\text{top}}$ and $z_{\text{bot}}$ were larger than 100%. The dashed line (100%) in Figure 2 refers to the average linear maintenance ratio instead of no shrinkage. A smaller value in Figure 2 thus indicates a more significant shrinking deformation.

The comparison among $z_{\text{top}}$, $z_{\text{mid}}$, and $z_{\text{bot}}$ reveals an interesting point that the deformation at the middle is more striking than at the two ends. Solvent evaporation immediately after demolding may lead to hardening of the salt/polymer/solvent composite and hinder further shrinking to a certain extent. In contrast to the shrinkage related to $z_{\text{mid}}$, the relatively easier evaporation at the two ends might account for the larger $z_{\text{top}}$ and $z_{\text{bot}}$. The initial shrinking along the height dimension is also relatively striking.

Another interesting point is that $z_{\text{bot}}$ was smaller than $z_{\text{top}}$. In compression molding experiments, the total amount of the feed salt/polymer/solvent composite should be slightly more than that required to fill the main body of the mold. A small part of the extra composite could be pressed into the mold to partially compensate the shrinkage of the composite near the top of the mold due to slight solvent evaporation during pressure holding. A slightly larger amount of composite near the top of the mold might be responsible for a little less deformation of this part after demolding.

It should be noted that the shrinkage differences among different dimensions were, although significant according to the student’s $t$-test, still not large because the relative maintenance ratios of $z_{\text{top}}$, $z_{\text{mid}}$, and $z_{\text{bot}}$ were all around 100% as shown in Figure 2. As mentioned above, the global linear shrinkage ratio ($1-S$) in our RTCM/PL approach was usually less than 4% (corresponding to the volume maintenance ratio larger than 90%) under the usual fabrication conditions ($\varphi_{v,\text{solv}}$ less than 35%; $\varphi_{w,\text{partc}}$ higher than 85%). Therefore, shrinkage is still controllable and acceptable. The RTCM/PL approach is feasible for fabrication of tissue engineering scaffolds.

### Solvent Contribution to Scaffold Porosity

Since serious shrinkage during solvent evaporation after demolding can be avoided under appropriate fabrication conditions, an alternative question arises: will the solvent contribute to overly high porosity in the resultant scaffolds? In principle, high porosity is beneficial for cell loading in tissue engineering. However, an overly high porosity must lead to poor mechanical properties, and should be avoided. It is thus meaningful to elucidate the relation between volumetric shrinkage and resultant porosity.

The theoretical porosity without consideration of any solvent effect, $\Phi'$, is expressed as

$$\Phi' = \frac{\varphi_{w,\text{partc}}/\rho_s + (1 - \varphi_{w,\text{partc}})/\rho_p}{100}\times 100\%$$

(10)

where $\rho_s$ and $\rho_p$ are the salt density and polymer density, respectively.

On the other hand, if the solvent contribution to the eventual porosity is considered, the theoretical porosity becomes

$$\Phi'' = 1 - \frac{V_{\text{poly}}}{V_0} = \Phi' + (1 - \Phi')\varphi_{v,\text{solv}}$$

(11)

under the approximation that the volumes of different components can be simply added, where $V_{\text{poly}}$ and $V_0$ are the volume of the skeletal polymer, and the volume of the complete polymer/salt/solvent composite, respectively.

The theoretical relation between porosity $\Phi''$ and solvent volume fraction $\varphi_{v,\text{solv}}$ under different salt weight fractions...
Fig. 3. Theoretical relation between porosity $\Phi'$ and solvent volume fraction $\phi_{v,\text{solv}}$ under the marked salt weight fraction $\phi_{w,\text{partc}}$ if all solvent in polymer/salt/solvent composites contributes to the eventual scaffold porosity.

$\phi_{w,\text{partc}}$ (95, 90, and 85%) is shown in Fig. 3, where the densities of polymer and salt are set as 1.25 g cm$^{-3}$ and 2.165 g cm$^{-3}$, respectively. When $\phi_{v,\text{solv}} = 0$, $\Phi' = \Phi$. According to Fig. 3 and also Equation (11), the deviation of $\Phi'$ from $\Phi$ is decreased with the decrease of $\phi_{v,\text{solv}}$ and also with the increase of $\Phi'$ (or $\phi_{w,\text{partc}}$). Since salt weight fraction $\phi_{w,\text{partc}}$ is usually high in fabrication of tissue engineering scaffolds due to the requirement of high porosity, the increase of porosity by solvent evaporation is thus fortunately less striking under our usual preparation conditions ($\phi_{w,\text{partc}}$ about 90%, and $\phi_{v,\text{solv}}$ in the range 25–35%).

If the volumetric maintenance ratio $S_v$ after salt leaching is known, the theoretical porosity with partial solvent contribution is described as

$$\Phi = 1 - \frac{V_{\text{polym}}}{V_0 S_v}$$  \hspace{1cm} (12)

The volume of the skeletal polymer ($V_{\text{polym}}$) can be obtained by the weight of the finished scaffold because the bulk density of the polymer is known. Then the scaffold porosity can be calculated from the volumetric shrinkage. The calculated values according to Equation (12) are shown in Table 2.

The real porosities measured by the liquid replacement method, $\Phi_{\text{meas}}$, are also shown in Table 2. The difference of each sample is defined as

$$\Delta\% = \left| \frac{\Phi - \langle \Phi_{\text{meas}} \rangle}{\langle \Phi_{\text{meas}} \rangle} \right| \times 100\%$$  \hspace{1cm} (13)

The porosity calculated from the volumetric maintenance ratio and the measured porosity are very close to each other, with the differences of them ($\Delta$) being all below 0.5%.

Table 2. The porosities calculated after consideration of shrinkage of the cylindrical scaffolds and those measured by the liquid replacement method ($\phi_{w,\text{partc}} = 90\%$).

<table>
<thead>
<tr>
<th>$\phi_{v,\text{solv}}$</th>
<th>$S_v$</th>
<th>$\Phi$</th>
<th>$\Phi_{\text{meas}}$</th>
<th>$\Delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>35.1</td>
<td>0.895 ± 0.005</td>
<td>85.8 ± 0.2</td>
<td>86.0 ± 0.4</td>
<td>0.31 ± 0.18</td>
</tr>
<tr>
<td>42.5</td>
<td>0.818 ± 0.015</td>
<td>88.1 ± 0.3</td>
<td>88.1 ± 0.3</td>
<td>0.07 ± 0.06</td>
</tr>
<tr>
<td>52.8</td>
<td>0.760 ± 0.037</td>
<td>89.0 ± 0.8</td>
<td>89.0 ± 0.6</td>
<td>0.15 ± 0.07</td>
</tr>
</tbody>
</table>

*a) The mean $\Delta$ was calculated from $\Delta$ of each sample ($n = 3$) instead of from the means of $\Phi$ and $\Phi_{\text{meas}}$.

Therefore, the relation of porosity and volumetric maintenance ratio revealed by Equation (12) is justified and the porosity can be calculated from the shrinkage of the scaffold according to the suggested formula.

The above results conclude that solvent evaporation in our RTCM/PL approach led to neither serious scaffold shrinkage nor serious porosity increase under the usual fabrication conditions.

**Pore Structure**

The SEM micrographs of the cross section of auricle-shaped PDLLA porous scaffolds with different magnifications are shown in Fig. 4. It is obvious that the pores are highly interconnected. These pore sizes are basically determined by the templates, namely, porogen particles (280–450 μm as shown in Fig. 4A, B). Some micropores with sizes less than 10 μm were also observed in the SEM micrograph with a higher magnification (the small pores in the walls of large pores in Fig. 4C), which may be attributed to evaporation of solvent. The micropores may enhance the interconnectivity and interfacial area of the porous scaffolds. The large pores in the resultant scaffolds are still predominant over the small pores, for the porosity is, as shown later, mainly determined by the salt content.

The pore structures of the porous scaffolds fabricated from PLGA85/15 and PLGA75/25 copolymers shown in Fig. 5 are similar to those of PDLLA scaffolds.

In the conventional fabrication approaches of porous scaffolds such as solvent casting/particle leaching, the external surface of the resultant scaffold is relatively less porous than the interior and sometimes acts like “skin.”[14] The SEM micrograph of the surface of an auricle-like PDLLA porous scaffold fabricated with 95% porogen particle is compared with its cross section in Fig. 6. The interconnected pore structure was also observed on the surface of the porous scaffolds. Solvent evaporation inevitably leads to a more condensed surface in the practice of particle leaching when a solvent of the polymer is

---

Table 2. The porosities calculated after consideration of shrinkage of the cylindrical scaffolds and those measured by the liquid replacement method ($\phi_{w,\text{partc}} = 90\%$).

<table>
<thead>
<tr>
<th>$\phi_{v,\text{solv}}$</th>
<th>$S_v$</th>
<th>$\Phi$</th>
<th>$\Phi_{\text{meas}}$</th>
<th>$\Delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>35.1</td>
<td>0.895 ± 0.005</td>
<td>85.8 ± 0.2</td>
<td>86.0 ± 0.4</td>
<td>0.31 ± 0.18</td>
</tr>
<tr>
<td>42.5</td>
<td>0.818 ± 0.015</td>
<td>88.1 ± 0.3</td>
<td>88.1 ± 0.3</td>
<td>0.07 ± 0.06</td>
</tr>
<tr>
<td>52.8</td>
<td>0.760 ± 0.037</td>
<td>89.0 ± 0.8</td>
<td>89.0 ± 0.6</td>
<td>0.15 ± 0.07</td>
</tr>
</tbody>
</table>

*a) The mean $\Delta$ was calculated from $\Delta$ of each sample ($n = 3$) instead of from the means of $\Phi$ and $\Phi_{\text{meas}}$.

Therefore, the relation of porosity and volumetric maintenance ratio revealed by Equation (12) is justified and the porosity can be calculated from the shrinkage of the scaffold according to the suggested formula.

The above results conclude that solvent evaporation in our RTCM/PL approach led to neither serious scaffold shrinkage nor serious porosity increase under the usual fabrication conditions.

**Pore Structure**

The SEM micrographs of the cross section of auricle-shaped PDLLA porous scaffolds with different magnifications are shown in Fig. 4. It is obvious that the pores are highly interconnected. These pore sizes are basically determined by the templates, namely, porogen particles (280–450 μm as shown in Fig. 4A, B). Some micropores with sizes less than 10 μm were also observed in the SEM micrograph with a higher magnification (the small pores in the walls of large pores in Fig. 4C), which may be attributed to evaporation of solvent. The micropores may enhance the interconnectivity and interfacial area of the porous scaffolds. The large pores in the resultant scaffolds are still predominant over the small pores, for the porosity is, as shown later, mainly determined by the salt content.

The pore structures of the porous scaffolds fabricated from PLGA85/15 and PLGA75/25 copolymers shown in Fig. 5 are similar to those of PDLLA scaffolds.

In the conventional fabrication approaches of porous scaffolds such as solvent casting/particle leaching, the external surface of the resultant scaffold is relatively less porous than the interior and sometimes acts like “skin.”[14] The SEM micrograph of the surface of an auricle-like PDLLA porous scaffold fabricated with 95% porogen particle is compared with its cross section in Fig. 6. The interconnected pore structure was also observed on the surface of the porous scaffolds. Solvent evaporation inevitably leads to a more condensed surface in the practice of particle leaching when a solvent of the polymer is
Figure 4A–C. SEM micrographs of the cross section of an auricle-shaped poly(D,L-lactide) (PDLLA) porous scaffold shown with different magnifications. Fabrication conditions: $d = 280–450 \, \mu m$; $\varphi_{w,parc} = 90\%$. 

Figure 5. SEM micrographs of the cross sections of porous scaffolds fabricated from PLGA copolyesters. (A) PLGA85/15; (B) PLGA75/25. Fabrication conditions: $d = 280–450 \, \mu m$; $\varphi_{w,parc} = 90\%$. 

Figure 6. Comparison of the pore morphology of (A) the cross section and (B) on the surface of an auricle-shaped porous PDLLA scaffold. Fabrication conditions: $d = 280–450 \, \mu m$; $\varphi_{w,parc} = 95\%$. 

used. However, the difference between surface and interior in our RTCM/PL approach was not as significant as that in the conventional solvent casting/particle leaching approach. The so-called skins were absent in the scaffolds prepared by the RTCM/PL approach. The morphology and the distribution of the pores on the surface basically resemble those in the cross section. Similar results were observed in other scaffolds with different porosities (data not shown). Disappearance of the surface skin arises from avoidance of the sedimentation of particles in the mixture containing a relatively small amount of solvent. It should be noted that high molecular weight of the polymer is also necessary to achieve an even distribution of pores, because the high adhesion force of polymer due to high molecular weight avoids an escape of the particles from the surface of the mixture when handled in the compression molding process.

**Porosity as a Function of Salt Content**

The SEM micrographs of the cross section of the auricle-shaped PDLLA porous scaffolds fabricated with various weight contents of particle (70, 85, and 97%) are shown in Figure 7. The pore density was increased and the thickness of the pore wall was decreased as the weight content of porogen increased. For the scaffolds fabricated with particle content less than 70%, the pores were not well interconnected and the particles in the scaffolds were difficult to leach out. For scaffolds fabricated with particle content higher than 80%, most pores were interconnected. The interconnectivity of the pores was improved at higher particle content. The size and morphology of the pores were both independent of the particle content.

The dependence of porosity ($\Phi_{\text{meas}}$) on the porogen weight fraction ($\varphi_{\text{w,partc}}$) is illustrated in Figure 8. All of the porosity data of different scaffolds fabricated from different conditions (different sizes and contents of particle, scaffold materials) fall in the vicinity of a fitted line according to

$$\Phi_{\text{meas}}(\%) = 0.975\varphi_{\text{w,partc}} - 0.30$$

(14)

The dashed line in Figure 8 illustrates the relationship between $\varphi_{\text{w,partc}}$ and the theoretical porosity $\Phi'$ without consideration of any solvent effect as revealed by Equation (10). It should be noted that the theoretical relation is not linear itself, but a near-linear relation may be observed in the region with high particle fractions as examined.

The experimental data is about 10% higher than the theoretical values at relatively low particle contents, which may be caused by evaporation of solvent. However, the difference becomes small as $\varphi_{\text{w,partc}}$ increases, because the content of solvent necessary to prepare the initial polymer solution/particle mixture decreases.

Figure 7. SEM micrographs of auricle-shaped porous PDLLA scaffolds with porosities of (A) 71.3%, (B) 87.0%, (C) 95.0% resulting from $\varphi_{\text{w,partc}} = 70\%, 90\%, \text{and } 97\%$, respectively.
Compressive Behaviors of Scaffolds as a Function of Porosity

Good mechanical properties are very important for porous scaffolds in tissue engineering. Therefore, it is essential to examine the mechanical behaviors of the porous scaffolds fabricated by the RTCM/PL approach. Cylindrical porous scaffolds were employed to measure the compressive modulus ($E$) and stress at 10% strain ($\sigma_{10}$). The results are illustrated in Figure 9. The compressive modulus and strength decreased significantly with increasing porosity in the experimental range of 78–92%. PDLLA scaffolds with porosity of 90% exhibited an averaged compressive strength of 0.22 MPa and an averaged compressive modulus of 5.2 MPa. $E$ and $\sigma_{10}$ were 22.6 MPa and 1.02 MPa, respectively, for a scaffold with a porosity of 78.0%, while $E$ and $\sigma_{10}$ were 1.7 MPa and 0.11 MPa, respectively, for a scaffold with a porosity of 92.6%. In the preceding paper,[35] we reported that $E$ and $\sigma_{10}$ lie in the ranges of 2.4–73.5 MPa and 0.16–3.5 MPa, respectively, for porous scaffolds fabricated with similar particle contents and molded without solvent assistance but at high temperature. Scaffolds fabricated by the RTCM/PL method were found to be slightly weaker than those prepared via thermal compression molding/particle leaching, especially at low particle content. Compared with the previous fabrication method, porous scaffolds fabricated by RTCM/PL at the same salt content and molecular weight of polymers had, due to solvent evaporation, more micropores in the walls of macropores and thus had higher porosities. This implies that the micropores also act as material defects and contribute unfavorably to the mechanical properties. Nevertheless, the mechanical properties of the scaffolds obtained via the present approach are still in the same order of magnitude as those prepared via the former approach, especially under high porosities.

Conclusion

A room-temperature compression molding/particle leaching approach has been investigated to fabricate three dimensional porous scaffolds with complicated geometry. By this approach, we have prepared PDLLA and PLGA scaffolds with complicated shapes as well as high porosities. The compressive moduli and stresses at 10% strain of the scaffolds were in the range of 2–22 MPa and 0.1–1.0 MPa, respectively, and decreased as porosity increased. Porosity is easy to adjust by porogen content, and the highest porosity reported in this paper is 95%.

The net scaffold shrinkage was found to be increased with increase of solvent volume fraction and decrease of particle weight fraction. Solvent evaporation resulted in scaffold shrinkage and porosity increase, which obeyed a certain relation between them as revealed in this paper. The shrinkage was inhomogeneous, but the absolute value of the net shrinkage was not large. The investigation demonstrated that although shrinkage is not favorable for a molding process, it is acceptable under normal fabrication conditions. We believe that this RTCM/PL approach will be very helpful for fabrication of porous scaffolds for tissue engineering or tissue reconstruction due to its simplicity, its ability to obtain complicated anatomical shape, and its avoidance of thermal degradation of polyesters due to low processing temperature.
Nomenclature

\[ \varphi_{w,\text{part}}: \] Weight fraction of particles (based just on the total weight of salt particles and polymer)

\[ \varphi_{v,\text{part}}: \] Volume fraction of particles in the salt/polymer/solvent composite

\[ \varphi_{v,\text{solv}}: \] Volume fraction of solvent in the composite

\[ D: \] Porogen size

\[ \Phi: \] Theoretical porosity with partial solvent contribution

\[ \Phi': \] Theoretical porosity without consideration of any solvent effect

\[ \Phi'': \] Theoretical porosity with assumption that all solvent eventually contributes to scaffold porosity

\[ \Phi_{\text{meas}}: \] Experimental porosity measured by the liquid replacement method

\[ \Delta: \] Relative difference between \( \Phi \) and \( \Phi_{\text{meas}} \) defined by Equation (14)

\[ V_0: \] Volume of immediately demolded polymer/salt/solvent composite

\[ V: \] Volume of the resultant porous scaffold

\[ S_v: \] Volumetric maintenance ratio defined as \( V/V_0 \)

\[ S_l: \] Lateral maintenance ratio defined as \( V/V_0 \)

\[ 1/S_v: \] Volumetric shrinkage ratio

\[ H, H_0: \] Height of the cylindrical scaffold and that of the immediately demolded composite, respectively

\[ D_{\text{top,}0}, D_{\text{top,}0}: \] Diameter at the top of the cylindrical scaffold and that of the immediately demolded composite, respectively

\[ D_{\text{mid,}0}, D_{\text{mid,}0}: \] Diameter in the middle of the cylindrical scaffold and that of the immediately demolded composite, respectively

\[ D_{\text{bot,}0}, D_{\text{bot,}0}: \] Diameter at the bottom of the cylindrical scaffold and that of the immediately demolded composite, respectively

\[ \lambda: \] Average linear maintenance ratio defined by Equation (5)

\[ 1/\lambda: \] Average linear shrinkage ratio

\[ \vartheta_{\text{top}}, \vartheta_{\text{mid}}, \vartheta_{\text{bot}}: \] Relative maintenance ratio of height for a cylinder scaffold defined by Equation (6)

\[ \delta_{\text{top}}, \delta_{\text{mid}}, \delta_{\text{bot}}: \] The relative maintenance ratios of diameters at top, middle, and bottom for a cylinder scaffold defined by Equation (7), (8), and (9), respectively

\[ E: \] Compressive modulus of scaffold

\[ \sigma_{10}: \] Compressive stress at 10% strain of scaffold

Acknowledgements: The authors are grateful for the financial support from NSF of China (Nos. 20374015, 20304012, 50533010, 20574013), the Key Grant of Chinese Ministry of Education (No. 2004C27200), and 863 Project from the Chinese Ministry of Science and Technology, Science and Technology Development Foundation of Shanghai (Nos. 04JC14019 and 052907082).


