

## Fabrication of Highly Porous Interconnected Three-dimensional Scaffolds with Micro-channels<sup>\*</sup>

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**Abstract** A novel highly porous 3-D poly( $\epsilon$ -caprolactone) (PCL) scaffold with micro-channels was fabricated by injection molding and diluent acetic acids leaching technologies. In this study, the chitosan fiber was employed to form the micro-channel in PCL matrix. The morphology, porosity and mechanical properties of the scaffolds were studied and calculated. It was found that the larger the content of chitosan fiber is, the higher the porosity would be, due to the volumetric expansion of chitosan fiber in PCL matrix during it being leached. In addition, the less the content of chitosan fiber is, the higher the compressive modulus would be.

**Keywords:** Injection molding; Micro-channel; Chitosan fiber; Poly( $\epsilon$ -caprolactone) scaffold.

### INTRODUCTION

In the past few years, considerable attention has been focused on the enhancement of the porosity and interconnectivity of polymeric scaffolds to provide an adequate space for cell seeding, growth and proliferation, especially for nutrients exchanging, waste discharging and cellular migration<sup>[1]</sup>. Techniques such as particulate leaching<sup>[2]</sup>, polymer phase separation<sup>[3]</sup>, gas foaming<sup>[4]</sup> and electrospinning<sup>[5]</sup> have all been employed to increase average pore size, pore interconnectivity and overall porosity. Although these techniques have been shown to improve the transport properties, the scaffolds which only possess pores still lack efficient transportation of oxygen and nutrients to the newly formed tissue compared with those scaffolds having micro-channels. In addition, the regeneration of some special tissues, such as tendons, ligaments, nerves and muscles (cardiac, skeletal, and smooth), requires cell alignment along one or more axes to optimize their functions. For example, for tendons and ligaments, axial alignment of cells and fibers are necessary to withstand high tensile loads. In nerve tissue engineering, cell alignment helps neurons to undergo accelerated oriented growth in a direction that is crucial for nerve regeneration processes<sup>[6, 7]</sup>. Moreover, microenvironment of scaffold has significant effect on cell behaviors, such as morphology, adhesion, proliferation, differentiation and orientation. For example,

<sup>\*</sup> This work was financially supported by the China Scholarship Council and the Wisconsin Institute for Discovery (WID), that enabled the authors to perform this research at the University of Wisconsin-Madison, the National Natural Science Foundation of China (No. 51303027) and the Scientific Research Starting Foundation, Fujian University of Technology, China (No. GY-Z13028).

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Received September 30, 2013; Revised November 13, 2013; Accepted November 14, 2013

doi: 10.1007/s10118-014-1447-4

Shen *et al.* investigated the effect of microchannels on smooth muscle cells (SMCs) morphology. The result showed that SMCs cultured in the wider microchannels (80–160  $\mu\text{m}$  wide) switch from fibroblast morphology and random orientation to spindle-shaped morphology<sup>[8]</sup>. Therefore, manufacturing scaffolds with micro-channels to instruct the regeneration of some special tissues or guide some cells to grow into functional grafts is another challenge for the tissue engineering research.

Several researchers have developed approaches to create aligned grooves or channels in polymers to allow desirable cell alignment and topographic control of cell orientation. The photo-lithography technique has been used to prepare poly-L-lactic acid (PLA) films with parallel microgrooves<sup>[9]</sup>. Microgrooved poly(D,L-lactic acid) films were produced for nerve tissue engineering by imprinting patterns onto the polymer surface from a microgrooved quartz substrate<sup>[10]</sup>. Grooved collagen membranes were fabricated by air-drying collagen hydrogels onto microgrooved templates<sup>[11]</sup>. A major limitation for the above studies with grooved substrates is that the microgrooves are fabricated on one side of the thin films (2D), on which only a monolayer of cells could be cultured. However, for clinical applications, tissue substitutes with cells well organized in 3-D structures are necessary. Therefore, Ao *et al.* fabricated an orientation-structured scaffold with microtubules by solution casting/freeze-drying technique<sup>[12]</sup>. By using this technique a kind of slender needle bundle is needed, which was first inserted into a polymer solution and then should be taken off after the polymer solution has been freeze-dried. However, the number of microtubules was small and the walls of microtubules remained thick and uneven. As a result, the porosity of the scaffold was low and the number of microtubules was too small to meet the requirements of most of tissue engineering applications. Zong *et al.*<sup>[13]</sup> made the orientated fibrous scaffolds by electrospinning technique. The limitation of the formed scaffold is the distance among fibers can only reach several micrometers, therefore, it was too small for the cells to grow enter the scaffold. In addition, Xing *et al.*<sup>[14]</sup> prepared the aligned porous structures by using freeze-drying in one direction. However, there haven't been any other studies or reports regarding the fabrication of 3-D scaffolds with micro-channels by injection molding method to the best of our knowledge.

Using chitosan as a scaffold material for tissue engineering applications has been previously reported by a number of research groups<sup>[15, 16]</sup>. However, the chitosan microfiber as a channel generator has not been demonstrated so far. Chitosan, a naturally occurring amino-polysaccharide, is biodegradable, biocompatible and nontoxic<sup>[17]</sup>. Chitosan is normally insoluble in aqueous solutions above pH 7. However, in dilute acids, chitosan becomes fully soluble due to the protonation of the free amino groups. In this study, poly( $\epsilon$ -caprolactone) (PCL) was compounded with water-soluble poly(ethylene oxide) (PEO), sodium chloride (NaCl) particles and dilute acids soluble chitosan microfibers. Utilizing injection molding to mass produce the samples and the subsequent leaching of the samples in 1% acetic acids resulted in highly porous 3-D PCL scaffolds with micro-channels.

## EXPERIMENTAL

### Materials

Poly( $\epsilon$ -caprolactone) (PCL) was chosen as the matrix material in this research. PCL (Capa® 6500,  $M_n = 50000$ ) was purchased in granular form from Perstorp UK Limited. It has a specific gravity of 1.10 and a melt flow index around 7 g/10 min (160 °C/2.16 kg). Its glass transition and melting temperatures are  $-60\text{ }^{\circ}\text{C}$  and  $58\text{ }^{\circ}\text{C}$ – $60\text{ }^{\circ}\text{C}$ , respectively.

Poly(ethylene oxide) (PEO), a water soluble polymer, with the average molecular weight of 100000 was supplied in powder form from Dow® Chemical Company. It has a specific gravity of 1.13 and a melting temperature of  $65\text{ }^{\circ}\text{C}$ .

Sodium chloride (NaCl), (reagent grade) was purchased from Fisher Scientific with a specific gravity of 2.17. It can be easily dissolved in water. Its melting point of  $801\text{ }^{\circ}\text{C}$  ensures that it will not melt at normal polymer processing conditions.

Chitosan fiber was purchased from Yoon Networks (Shanghai, China) Co., Ltd. It has a diameter of 2 denier and a length of 2 m.

The glacial acetic acid purchased from Sigma-Aldrich was diluted into 1% (g/mL) acetic acid prior to being used as a dissolvent to leach chitosan fibers.

### **Salt Reduction and Sieving**

The sodium chloride (NaCl) particles were size reduced using a Wiley mill and a screen with 1 mm diameter holes. Once the particles were size reduced, they were sieved to a size range of 150  $\mu\text{m}$  to 300  $\mu\text{m}$  using sieve screens and a wet/dry sieve shaker (H-4328, Humboldt Mfg.). This particle size range is ideal for creating pores suitable for bone regeneration<sup>[18]</sup>.

### **Compounding**

The PCL, PEO, NaCl and chitosan fibers were compounded using a twin-screw extruder with a screw diameter of 25 mm and an L/D ratio of 36. The materials were loaded into separate gravimetric feed hoppers to accurately meter the material at a constant feed rate. Before melt compounding, PCL, PEO and chitosan fiber were dried under vacuum at 37 °C, while NaCl was dried at 120 °C for 10 h. The melt compounding temperatures at different zones were independently controlled to achieve a temperature profile of 50, 80, 90, 100, and 90 °C from hopper to nozzle. The screw rotation speed was set to 100 r/min. Upon extrusion, the strands were cooled with compressed air and then pelletized for injection molding. The formulations compounded are given in Table 1.

**Table 1.** Formulations compounded in the twin-screw extruder

| Samples | PCL (vol%) | PEO (vol%) | NaCl (vol%) | Chitosan fiber (vol%) |
|---------|------------|------------|-------------|-----------------------|
| 1       | 30         | 30         | 40          | 0                     |
| 2       | 30         | 30         | 30          | 10                    |
| 3       | 30         | 30         | 20          | 20                    |

### **Injection Molding**

The pellets prepared by extrusion were fed into an injection molding machine (Arburg Allrounder 320S with a 25 mm diameter screw) to make tensile test bars as per ASTM D638. The processing parameters for injection molding process are listed in Table 2.

**Table 2.** Processing parameters during injection molding process

|                                          |     |
|------------------------------------------|-----|
| Nozzle temperature (°C)                  | 100 |
| Mold temperature (°C)                    | 25  |
| Shot volume (cm <sup>3</sup> )           | 20  |
| Injection flow rate (cm <sup>3</sup> /s) | 9.5 |
| Cooling time (s)                         | 20  |

### **Leaching**

After injection molding, the molded samples were cut into small square pieces with dimensions of 1 cm  $\times$  1 cm and leached in 1% (g/mL) acetic acid using the setup depicted in Ref. [19]. The diluted acetic acid was changed every 6 h. In our previous study<sup>[20]</sup>, for the PCL/NaCl/PEO compounded blend samples, almost all NaCl particles and PEO domains were removed out after leaching for 9 h. Therefore, the cut samples were leached 24 h in order to make sure all the NaCl particles, PEO domains and chitosan fibers were removed out in this study. The leached samples were dried by vacuum and stored in a dry state for further characterization and application.

### **Scaffold Characterization**

The morphology of the as-molded as well as leached and dried injection-molded samples was evaluated using a SEM LEO 1530 instrument with an accelerating voltage of 5 kV. All leached samples were fractured using liquid nitrogen and then sputtered with gold for 20 s prior to observation.

The universal testing instrument (Instron 5967) was used to test the mechanical properties of the molded samples at wet state. The leached and dried injection-molded parts were cut into 10.0 mm × 10.0 mm square pieces and immersed into phosphate buffered saline (PBS) for 24 h until an equilibrium state was reached before compression test. A constant crosshead moved down with a compression rate of 1.0 mm/min until a 50% deformation of the samples was reached in the thickness direction.

The porosity of the leached samples was determined by weighing them and measuring the dimensions of the weighed samples to obtain their volume. Equation (1) was then applied to determine the porosity,

$$P = \left(1 - \frac{\rho_{\text{app}}}{\rho_{\text{PCL}}}\right) \times 100 = \left(1 - \frac{m_{\text{app}} / V_{\text{app}}}{\rho_{\text{PCL}}}\right) \times 100 \quad (1)$$

where  $P$  is the porosity of the sample,  $\rho_{\text{app}}$ ,  $m_{\text{app}}$ , and  $V_{\text{app}}$  are the apparent density, weight, and volume of the porous scaffold, respectively, and  $\rho_{\text{PCL}}$  is the known density of the matrix material ( $\rho_{\text{PCL}} = 1.10 \text{ g/cm}^3$ ).

## RESULTS AND DISCUSSION

### Scaffold Morphology

Figure 1 presents the representative images of the leached PCL scaffolds with different chitosan fiber contents of 0%, 10% and 20% (vol%), respectively. For comparison, SEM images of chitosan fibers and NaCl particles were provided in Figs. 2(a) and 2(b), respectively. From Fig. 1(a), it has been found that NaCl particulates in the sample were leached out leaving many pores. And from Figs. 1(b) and 1(c), in addition to the leached NaCl particulate pores, it also has been seen that the chitosan fiber in the sample were leached out leaving many channels. Meanwhile, It can be seen from Figs. 1(a), 1(b) and 1(c), the voids left by leached chitosan fibers increased with the increasing of chitosan fiber content in the compounded blends while the pores caused by leached NaCl particulates decreased with a decrease in the NaCl particulate content.

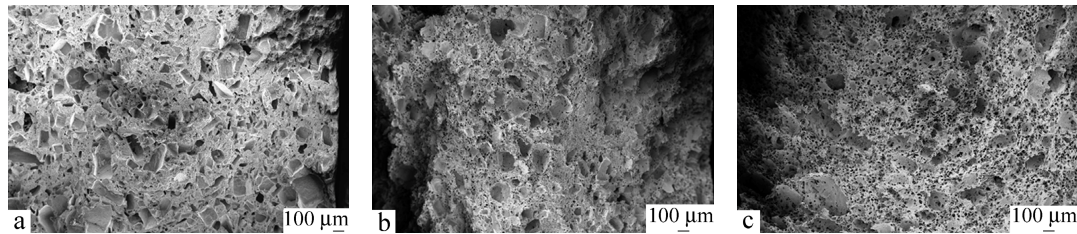


**Fig. 1** The morphology of leached PCL scaffolds with different chitosan fiber contents of (a) 0%, (b) 10%, and (c) 20%, respectively

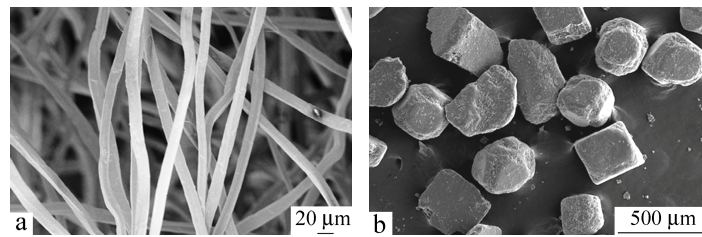


**Fig. 2** SEM images of (a) chitosan fiber and (b) NaCl particles, respectively

In order to clearly observe the morphology of pores and channels formed by leaching NaCl particulates and chitosan fibers in PCL matrix, the SEM images of leached samples at different magnification and with different chitosan fiber contents are provided in Fig. 3. As can be seen from the magnified pictures of Fig. 3(a), there are many pores (yellow circle) with a diameter of 50–250 μm formed by leaching out NaCl particulates, and some pores is smaller than the original size of NaCl (Fig. 2b), this is because part of NaCl particulate was crushed

under high shear rate conditions during compounding and injection molding processing. In addition, from the magnified pictures of Figs. 3(b), 3(c), 3(d), and 3(e), there are a lot of micro-channels (arrow) with diameters of 13–15  $\mu\text{m}$  formed by leaching out the chitosan fibers in comparison with the sample without chitosan fibers as shown in Fig. 3(a). Meantime, From Fig. 3, it can be found that the micro-channels (arrow) showed two different morphology of channel-shape and pore-shape due to the different distribution direction of chitosan fiber in PCL matrix.

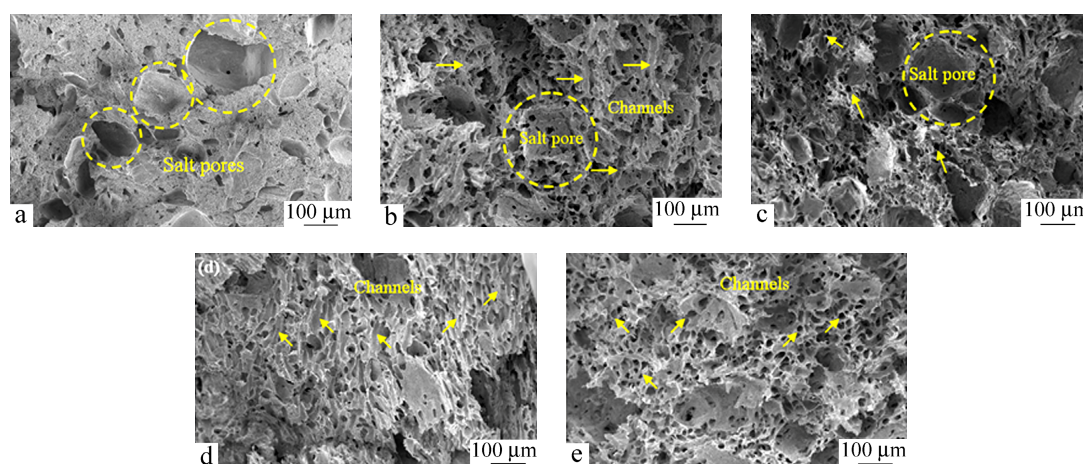


**Fig. 3** The morphology of leached PCL scaffolds with different chitosan fiber contents of (a) 0%, (b) and (c) 10%, (d) and (e) 20%, respectively

In order to clearly observe the surface features of the pores left by leached-out NaCl particles and the morphology of the PCL matrix, the magnified images are shown in Figs. 4(a) and 4(b). From Fig. 4(a), it can be found that some of micro-channels appeared inside the pores. According to the SEM image and the sizes of the NaCl particles and diameter of the chitosan fiber, it is suggested that the NaCl pores are interconnected by the micro-channels resulting from leaching of chitosan fibers. In addition, further zoom-in on the PCL matrix revealed voids of about 1 micron that are even smaller in size than NaCl particles and chitosan fibers. These voids are likely formed by the dissolution of PEO domains. Previously, it was reported that a co-continuous phase structure in PCL/PEO blends could be formed when PEO content is in the range of 40% to 70% (by volume) of the PCL/PEO content<sup>[21]</sup>. Since the PEO content used in this study is 50% by volume (of the PCL/PEO content), a co-continuous phase structure has formed resulting in interconnectivity. Therefore, the employment of chitosan fiber and PEO helped in improving the porosity and interconnectivity in PCL scaffolds.

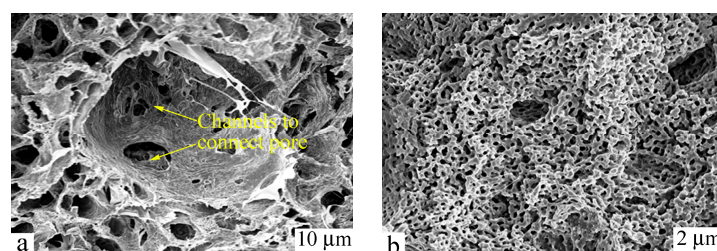


**Fig. 4** SEM surface images of leached PCL scaffolds with 20% chitosan fiber content at two different magnifications

### ***Scaffold's Porosity***

Porosity is an important factor for tissue engineering scaffolds. It provides sufficient space for cell growth and allows for the transfer of needed cellular nutrients. The porosity of the leached samples was calculated from

Eq. (1). It showed that the porosities of leached PCL scaffolds reached about 66.8%, 69.8%, and 81.3% for samples with different chitosan fiber contents of 0%, 10%, and 20%, respectively. However, according to the three original formulations of the PCL/PEO/NaCl/chitosan fiber blend composites in Table 1, the porosities should have been 70% if all of the NaCl particles, the PEO phase, and the chitosan fibers are removed. For sample 1 (chitosan fiber, 0%), the porosity (66.8%) is less than the theoretical porosity value (70%). This discrepancy might be because some NaCl particles were lost during compounding, pelletizing, and/or injection molding processes. It is also possible that some NaCl particles or fragments were not completely leached out. For samples 2 (chitosan fiber, 10%) and 3 (20%), the porosities are almost equal or more than the theoretical porosity value (70%). It might be due to much better leaching efficiency from the interconnecting chitosan fibers as well as the volume expansion phenomenon. The volume increasing of samples 2 and 3 can be measured by comparing with their original volume after leaching for 24 h. This may be because the chitosan fibers would swell before they are dissolved into diluted acetic acid. Therefore, the apparent volumes ( $V_{app}$ ) of samples 2 and 3 are larger than the apparent volume of sample 1 due to the volume expansion caused by the swelling of chitosan fibers. From Eq. (1), it also can be expressed, when the apparent chitosan fiber weight ( $m_{app}$ ) is a constant, the higher the apparent volume ( $V_{app}$ ) is, the smaller the apparent density ( $\rho_{app}$ ) would be, which results in a higher porosity. When the chitosan fiber content increases, the more volume expansion can be expected, which leads to a larger porosity. Therefore, the porosities of samples 2 and 3 were almost equal and more than the theoretical value (70%) due to the volume expansion of the samples.

### Scaffold's Compressive Properties

Figure 5 gives the stress-strain curves of the leached PCL scaffolds with different chitosan fiber contents at a wet state. It can be found that the compressive modulus of the leached PCL scaffolds was decreased with the increasing of the chitosan fiber content. The reason is that the more the chitosan fibers were blended, the higher the porosity would be, which leads to a lower mechanical property. This is in agreement with our prediction that the mechanical properties of scaffolds are strongly related to their porosity and the pore architecture.

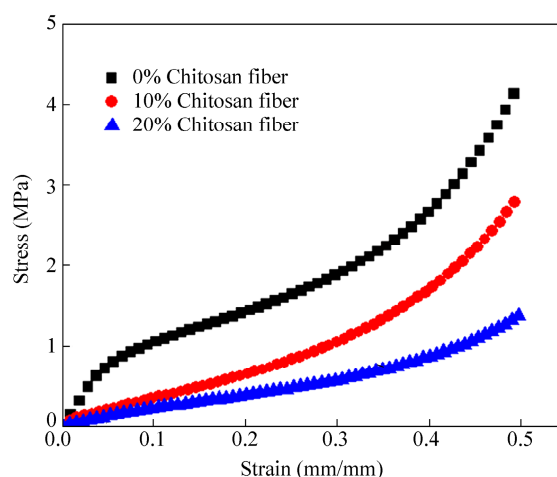


**Fig. 5** The stress-strain curves of leached PCL scaffolds with different chitosan fiber contents of 0 vol%, 10 vol% and 20 vol%

## CONCLUSIONS

In the present work, the 3-D interconnected PCL scaffolds with high porosity and micro-channels were fabricated. The porosities were increased with the increasing of the chitosan fiber content in the PCL/PEO/NaCl/chitosan fiber blends. In addition, the less the content of chitosan fiber is, the higher the compressive modulus would be.

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