Preparation of Porous Biomaterial Structures by a Solvent Casting/Particulate Leaching Technique

การเตรียมโครงสร้างวัสดุชีวภาพแบบมีรูพรุนด้วยเทคนิคหล่อแบบ/กำจัดอนุภาค

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Abstract

The aim of this work was to study and develop the preparation of porous biomaterial structures for biomedical applications. The solvent casting/particulate leaching technique was used to prepare the porous structures. To prepare a porous ε-polycaprolactone (PCL) structure, PCL powder was dissolved in chloroform. Sodium chloride was later mixed into the system. The obtained solution was then cast into a glass mould and air-dried in a fume hood. After drying, salt particles were then leached out using water, leaving a porous PCL structure with high porosity. To prepare a porous hydroxyapatite (HA)/PCL composite structure, HA powder was added into a PCL/acetic acid/sodium chloride system. The porosities of the scaffolds could be conveniently controlled by varying the size and amount of leachable particles. Microstructure study showed that the pores were well distributed throughout the structures. Mechanical testing was undertaken and showed that the tensile strength of the porous HA/PCL structure was less than that of the porous PCL structure. From contact angle study, it was clearly seen that the HA/PCL sample was more hydrophilic than the PCL sample, and thus favoured cell adhesion and proliferation.

บทคัดย่อ

งานนี้มีวัตถุประสงค์เพื่อศึกษาและพัฒนาการเตรียมโครงสร้างวัสดุชีวภาพแบบมีรูพรุนสำหรับการใช้งานทางการแพทย์ด้วยเทคนิคหล่อแบบ/กำจัดอนุภาค ในการเตรียมโครงสร้างพอลิคาโพรแลคโทนแบบมีรูพรุน จะทำการละลายผงพอลิคาโพรแลคโทนลงในคลอโรฟอร์ม จากนั้นเติมเกลือโซเดียมคลอไรด์ลงไป เทสารละลายที่ได้ลงในแบบแล้วทิ้งให้แห้งในตู้ดูดควัน หลังจากชิ้นงานแห้งแล้วจะทำการกำจัดอนุภาคเกลือโดยใช้น้ำ จะได้โครงสร้างพอลิคาโพรแลคโทนที่มีความเป็นรูพรุนสูง ในการเตรียมโครงสร้างวัสดุเชิงประกอบระหว่างไฮดรอกซีอะพาไทต์กับพอลิคาโพรแลคโทนแบบมีรูพรุน จะเติมผงไฮดรอกซีอะพาไทต์ลงในระบบที่ประกอบด้วย พอลิคาโพรแลคโทน กรดอะซิติก และเกลือโซเดียมคลอไรด์ ความเป็นรูพรุนของโครงสร้างสามารถควบคุมได้ง่าย โดยการเปลี่ยนขนาดและปริมาณของอนุภาคเกลือที่เติมลงไป จากการศึกษาโครงสร้างจุลภาคพบว่าโครงสร้างมีการกระจายของรูพรุนอยู่อย่างสม่ำเสมอ การทดสอบเชิงกลพบว่าโครงสร้างวัสดุเชิงประกอบระหว่างไฮดรอกซีอะพาไทต์กับพอลิคาโพรแลคโทนแบบมีรูพรุนมีค่าความต้านทานแรงดึงน้อยกว่าโครงสร้างพอลิคาโพรแลคโทนแบบมีรูพรุน จากการศึกษาค่ามุมสัมผัสพบว่าชิ้นงานวัสดุเชิงประกอบระหว่างไฮดรอกซีอะพาไทต์กับพอลิคาโพรแลคโทนมีลักษณะชอบน้ำมากกว่าชิ้นงานพอลิคาโพรแลคโทน ซึ่งเอื้อต่อการยึดเกาะและการเจริญเติบโตของเซลล์

Keywords: Biomaterials, Porous structure, Solvent casting/particulate leaching
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Introduction

The use of porous biomaterials as tissue engineering scaffolds has been extensively studied. Porous structure implants are desirable since they allow tissue ingrowth and vascularisation (Park and Lakes, 1992; Lu and Mikos, 1996). Different pore size is required depending on the applications. For example, it should be in the range of 45-150 μm for liver tissue regeneration (Kim et al., 1998), between 20 and 125 μm for skin regeneration (Yannas et al., 1989), and in the range of 100-150 μm for bone regeneration (Spector et al., 1978). The solvent casting/particulate leaching technique was chosen to prepare the porous biomedical structures for biomedical applications due to its simplicity. Moreover, the pore size can be easily controlled by changing the size of leachable particles (Mikos et al., 1994).

In this work, the porous ε-polycaprolactone (PCL), and hydroxyapatite (HA)/PCL composite structures were prepared. PCL is one of the well-known biodegradable polymers. It has been commercially used as a surgical suture, adhesion barrier, and is being investigated for potential bone and cartilage repairs (Woodward et al., 1985; Williams et al., 2005). Hydroxyapatite, on the other hand, is the primary mineral component of bone and teeth. It has been widely used in medical applications, for example, as an artificial bone and joint, bone filler and bone formation promoter. In addition, it has also been used in dentistry as a bone cement, canal filler, tooth root and as a filler in toothpaste (Aoki, 1994). A porous PCL structure was prepared by dissolving PCL powder in chloroform. Sodium chloride (NaCl) was employed as leachable particles since it is cheap, widely available, non-toxic and easily dissolved in water. In the case of preparing a porous HA/PCL composite structure, HA particles were added into a PCL/acetic acid/NaCl system.

Material and Methods

Poly (ε-caprolactone) powder (PCL, MW = 80,000, Solvay, USA) was used as the biodegradable polymer and hydroxyapatite powder (HA, ≥ 90%, Fluka, Gillingham, UK) was used as the bioactive ceramic. Chloroform ($CHCl_3$) (99.5%) was obtained from VWR International Ltd., England, and acetic acid (CH_3COOH) (100%) was purchased from BDH Laboratory Supplies, England. Coarse sodium chloride particles (NaCl) (96%) were kindly provided by Sang Thai Salt Factory, Baan Dung, Udonthani. They were ground and sieved down to four sets of different particle sizes: 106-150 μm, 150-300 μm, 300-500 μm, and 500 μm-1.25 mm, before use. All chemicals were used without further purification.

Porous PCL Structure

PCL powder was dissolved in chloroform at various concentrations (7-13 wt.%) and stirred thoroughly at room temperature for 30 minutes. The obtained solutions were cast into glass moulds and then air-dried in a fume hood to allow complete evaporation of the solvent. After that, the moulds were removed and samples were obtained. The optimum condition, where a smooth surface PCL sample was obtained, was chosen for porous PCL structure preparation. Pre-sieved NaCl particles were added into the PCL solutions at concentrations of 70, 75, 80, and 85 wt.% and stirred vigorously for 30 minutes before casting into the moulds. After drying and demoulding, the samples were soaked in

water for 16 hours to dissolve salt particles. The obtained samples were air-dried and their physical and mechanical properties were studied.

Porous HA/PCL Structure

Acetic acid was used as a solvent in this system. HA powder was added into the premixed PCL/acetic acid solution, and the obtained solution was then stirred at room temperature for 30 minutes. After that, NaCl particles were added into the solution at the concentration of 50-90 wt.% and stirred for another 30 minutes before casting, drying, demoulding, and particle-leaching.

The properties of obtained samples were studied using several analytical techniques. Percentage of porosity (ρ_0) is calculated from the following equation,

$$\rho_0 = \left(1 - \frac{d}{d_p}\right) \times 100$$

where d is a measured density of a sample and d_p a true density of a dense material (Hou, Grijpma and Feijen, 2003). Their microstructures were studied using scanning electron microscopy (SEM, LEO 1450VP, LEO Electron Microscopy Ltd., Cambridge, UK). The samples were stuck onto the stubs and coated with gold using a sputter coater (Polaron SC500, Fisons Instruments, Uckfield, UK) before observing in the SEM microscope.

A universal tensile testing machine (Testometric Micro500, Testometric, Rochdale, England) was employed to monitor the stress (σ) versus strain (ε) responses under tension. Young's Modulus can be calculated according to the following equation,

$$E = \frac{\sigma}{\varepsilon} = \frac{Fl_0}{A_0 \Delta l}$$

where F is the force applied (N), A_0 the initial cross sectional area (m^2), Δl the change in length (m), and l_0 the initial length (m). Percentage of elongation ($\%EL$) is defined as follows,

$$\%EL = \frac{l_f - l_0}{l_0} \times 100$$

where l_f is the final length (m) and l_0 the initial length (m) of the material (Callister, 2002). Finally, a contact angle study was performed to determine their hydrophilicity. Water was dropped onto the surface of the sample and the images were captured immediately and 10 minutes after deposition.

Results and Discussion

PCL samples with different weight percentage (wt.%) of PCL in chloroform were prepared and the results are shown in Figure 1. It was found that, at 7 wt.%, the sample distorted due to its low content of PCL. As the amount of PCL increased, the samples were flatter and their surfaces were smoother. However, when the contents of PCL were too high, the solutions had high viscosities, which led to difficulty in casting. Moreover, the surfaces of samples were rougher, as can be seen in Figure 1(e-g). Therefore, the PCL/chloroform solution with 10 wt.% was chosen as the optimum condition to prepare porous PCL structures. NaCl powders (70 wt.%) of different size ranges were separately added into the solution, and the obtained porous samples are shown in Figure 2. It was found that the porous samples became more flexible, compared with a dense sample. And as the size of salt particles increased, the samples became softer and more fragile.

Figure 3 shows HA/PCL samples with different HA:PCL ratios. It can be clearly seen that, as the ratio increased, the samples became more opaque. The percentage of linear shrinkage was higher due to its high HA content. A composite sample with HA:PCL ratio of 1.0:1.0 gave the best result as the surface of the sample was smooth and had low linear shrinkage. Therefore, 1.0:1.0 HA/PCL slurry was chosen as an optimum condition for preparation of porous HA/PCL structure.

Microstructures of PCL samples prepared from different particle sizes of NaCl are shown in Figure 4. It was found that the smaller the size of salt particles, the higher the amount of salt that could be added into the PCL solution and the final samples still retained their shapes after salt removal. This is due to the fact that the bigger particles occupy more space than the relatively smaller ones, and so the highest possible amount for big particles incorporated in the sample is lower than that for the smaller particles. Moreover, it was found, as expected, that % porosity increased as the amount of salt increased, and the pores were well distributed throughout the samples.

Figure 5 shows the microstructures of HA/PCL samples (ratio of 1.0:1.0) with different amounts of NaCl. Similarly, it was found that % porosity increased as the amount of salt increased, and the pores were well distributed throughout the samples.

Mechanical properties of samples were studied using a universal tensile testing machine and the results are shown in Table 1. In the case of the non-porous samples, it was found, as expected, that the one made from pure PCL had higher Young's modulus and % elongation than the one made from HA/PCL composite. It can also be clearly seen that the Young's modulus and % elongation of porous samples dropped dramatically, as compared to those of the dense ones, due to the pores within the structures.

Contact angle was measured to study the hydrophilicity of samples. Water was dropped onto the substrates and the images were captured immediately after deposition and after 10 minutes. It was found, as shown in Figure 6, that the HA/PCL sample had higher hydrophilicity (lower contact angle: 72°) than the pure PCL sample (higher contact angle: 135°). Moreover, on the HA/PCL sample, the water drops flatten down with time (45°), while the one on the pure PCL sample retains its shape. The results found were appreciable since the higher hydrophilicity of the sample leads to better cell adhesion and proliferation on the sample.

Conclusions

Porous biomaterial structures were successfully prepared via the solvent casting/ particulate leaching technique. The porosities of these structures could be easily controlled by varying the size and amount of salt particles and the pores were well distributed throughout the structures. From the mechanical testing study, it was found that the tensile strength of porous HA/PCL structure was less than that of porous PCL structure due to the presence of HA. The results from contact angle study, however, show that the HA/PCL sample was more hydrophilic than the PCL sample, which favoured cell adhesion and proliferation.

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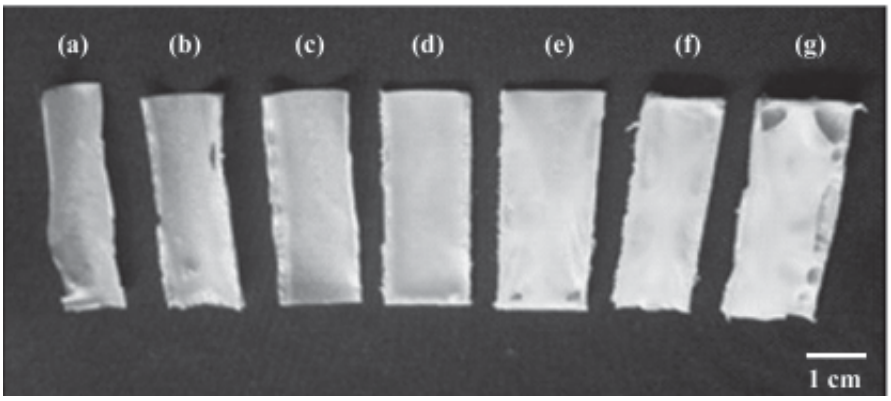

Figure 1. PCL samples with different wt.% of PCL in chloroform:
(a) 7 wt.%, (b) 8 wt.%, (c) 9 wt.%, (d) 10 wt.%, (e) 11 wt.%, (f) 12 wt.% and (g) 13 wt.%.

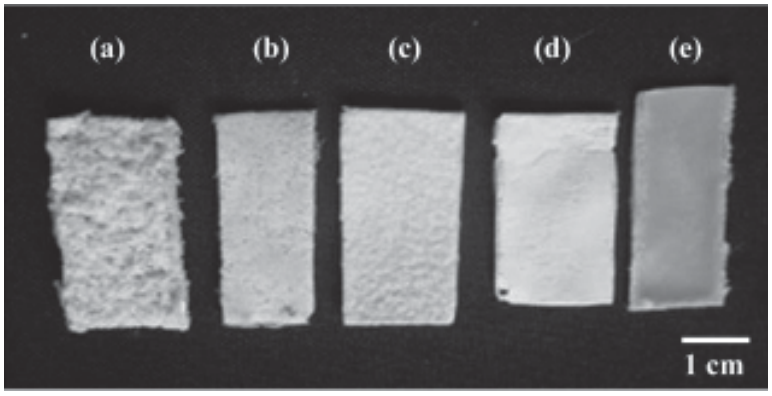

Figure 2. Porous samples with 70 wt.% NaCl in PCL/Chloroform solution, prepared using different size ranges:
(a) 500 m-1.25 μm, (b) 300-500 μm, (c) 150-300 μm and (d) 106-150 μm, compared with (e) a dense PCL sample.

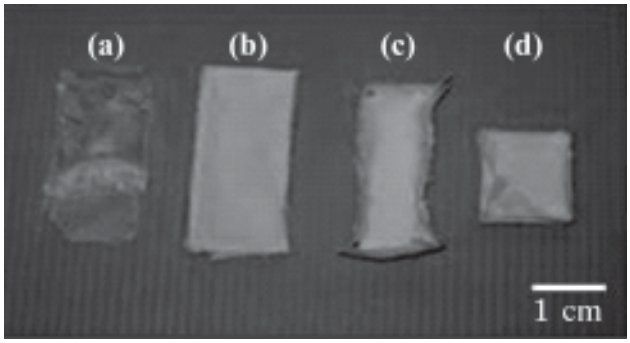

Figure 3. HA/PCL samples with different HA:PCL ratios:
(a) 0.5:1.0 (b) 1.0:1.0, (c) 1.5:1.0 and (d) 2.0:1.0.

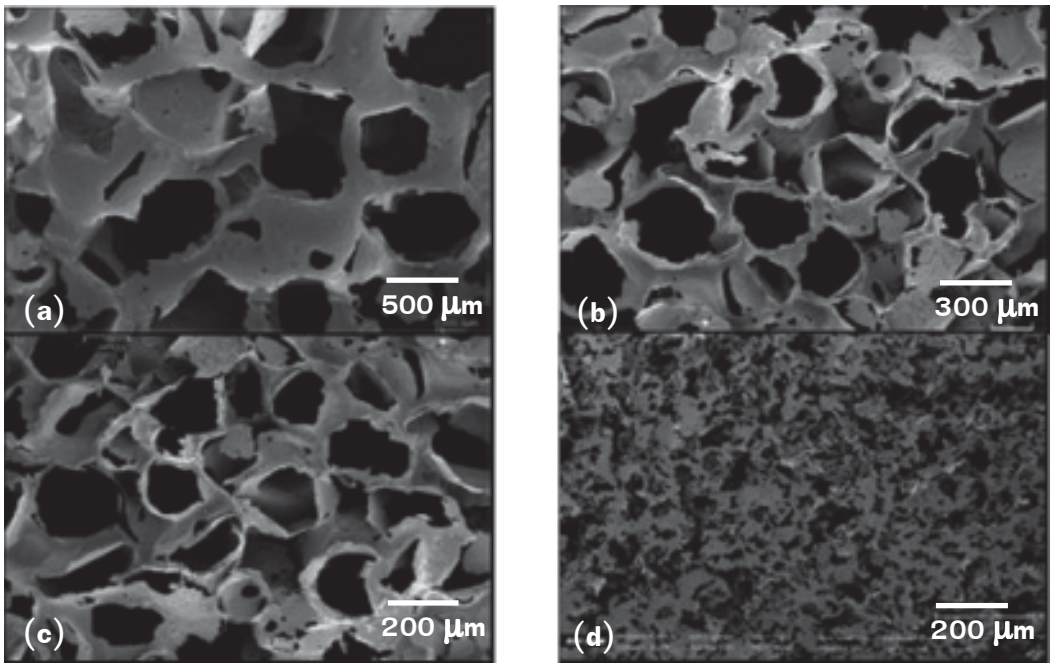

Figure 4. SEM micrographs of PCL samples prepared from different particle sizes of NaCl. The wt.% shows the highest possible amount of NaCl powder added into PCL solution and the samples still retained their shapes after salt-removal.

(a) 500 μm - 1.25 mm, 75 wt.% NaCl, porosity: 85.8%. (c) 150-300 μm, 85 wt.% NaCl, porosity: 91.4%.
(b) 300-500 μm, 80 wt.% NaCl, porosity: 88.8%. (d) 106-150 μm, 85 wt.% NaCl, porosity: 90.5%.

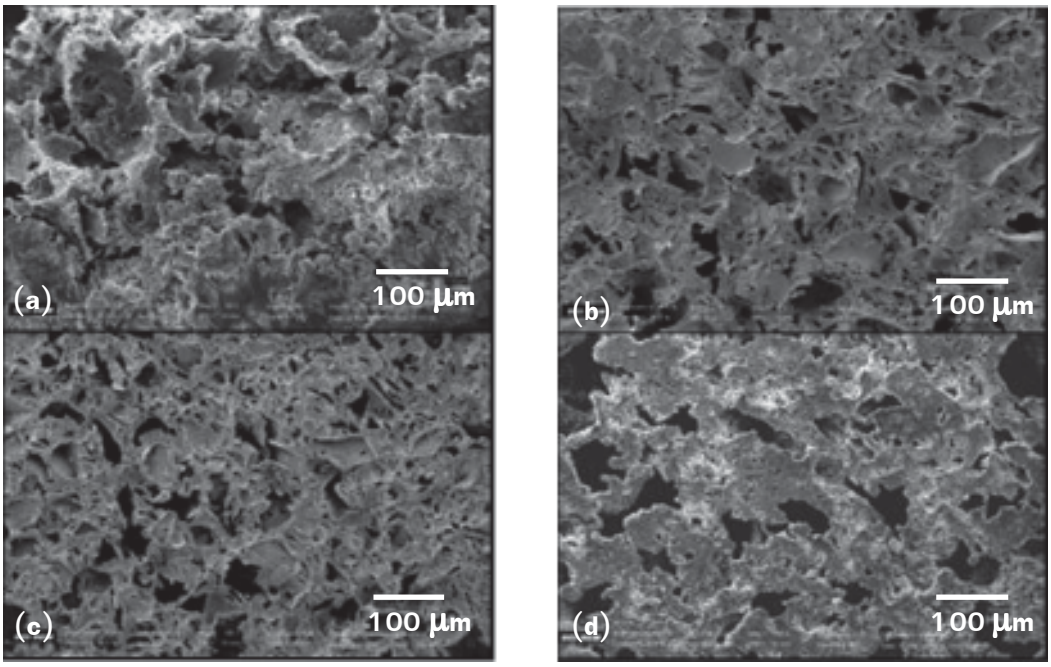

Figure 5. SEM micrographs of HA/PCL samples (ratio of 1.0:1.0) with different wt.% of NaCl (particle size 106-150 μm).

(a) 50 wt.% NaCl, porosity: 63.4%. (c) 70 wt.% NaCl, porosity: 78.2%.
(b) 60 wt.% NaCl, porosity: 73.8%. (d) 80 wt.% NaCl, porosity: 84.3%.

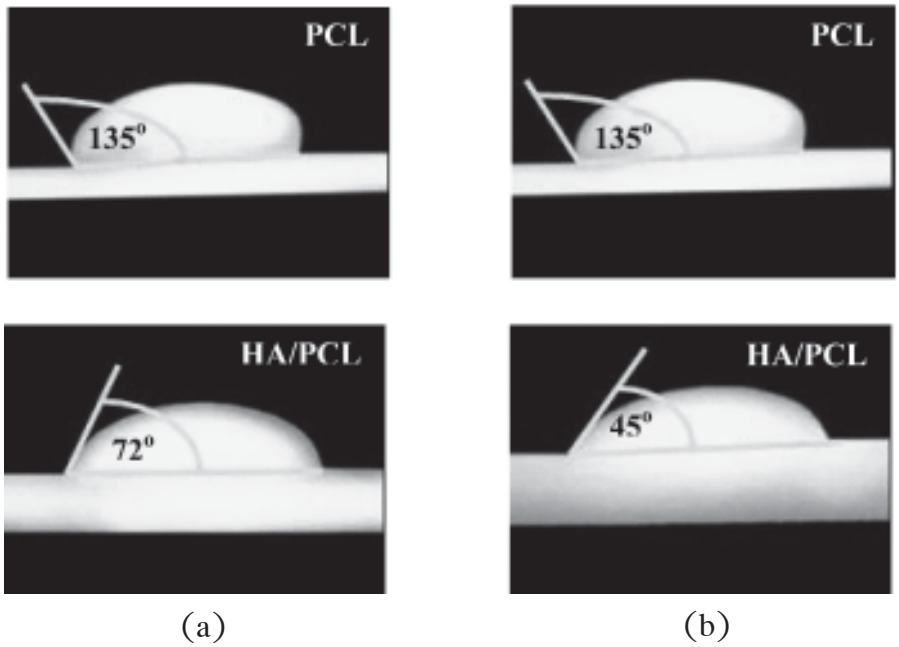

Figure 6. Water drops on PCL and HA/PCL samples (a) immediately after drop, and (b) after 10 minutes.

Table 1. Mechanical properties of biomaterial samples.

Samples	Young's Modulus/ kPa	% Elongation
Dense PCL	500	600
Porous PCL (150-300 mm, 91.38% porosity)	116.7	100
Dense HA/PCL (ratio of 1.0:1.0)	1.36	60
Porous HA/PCL (106-150 mm, 82.3% porosity)	0.77	65