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Biodegradable polycaprolactone/cuttlebone scaffold composite using salt leaching process

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Abstract−We prepared biodegradable polycaprolactone/cuttlebone scaffold composite by salt leaching process. In the first step, a co-continuous blend of biodegradable materials, polycaprolactone (PCL) and cuttlebone (CB), and an amount of sodium chloride salt particles were mixed using a stirrer. Next, the extraction of mineral salts using de-ionized distilled water was performed using a biodegradable PCL/CB scaffold with fully interconnected pores. Finally, the durable morphology of the scaffolds was fabricated by freeze-drying process at −53 °C for 24 hrs in a vacuum. In addition, the quadrilateral pres ranged from about 250 to 300 μm in diameter. Scanning electron microscopy (SEM) and mercury intrusion porosimeter techniques were carried out to characterize the pore morphology. By increasing the CB and sodium chloride salt particle content, the number of interconnected pores, material properties, and pore morphology were dramatically changed. The average compressive strengths (load at 50% strain) of the different porous PCL/CB scaffolds were found to decrease from 133 to about 79 (load at 50% strain, gf) with an increase in porosity. The values of the porosity increased as the sodium chloride salt volume fraction increased

Key words: Polycaprolactone (PCL), Cuttlebone (CB), Scaffold, Biocompatibility

INTRODUCTION

Polycaprolactone (PCL) is a typical biodegradable polymer with excellent biocompatibility [1-4]. Due to its orderly and compliant chains, PCL shows relatively strong crystallization and a low melting temperature. It is very hydrophobic and degrades at a slower rate than polylactic acid or polyglycolic acid. Hence, PCL has been widely used as a bone graft substitute and drug delivery system (DDS) [5-9].

In addition, cuttlebone (CB) has been widely used as an artificial bone because of its similar chemical composition with human bone. CB composed of calcium has some unique properties such as excellent osteoconductivity and bioactivity [10,11]. CB has been used to prepare porous hydroxyapatite (HA) and β-tricalciumphosphate (β-TCP). CB is an inexpensive, readily available, morphologically complex natural material [12]. Recently, the highly sinterable HA and β-TCP using recycled CB have been successfully synthesized [13].

Scaffolds of organic and inorganic matter have been lately developed with the aim to increase mechanical scaffold stability. To obtain as many requirements of bone graft substitute materials as possible, the combination of the advantages of organic and inorganic matters seems to be a promising choice as demonstrated by the increased amount of research effort worldwide [14-17].

The regeneration of specific tissues aided by synthetic materials mostly depends on the porosity, pore size, pore shape, pore distribution (interconnection between the pores) and morphology, as well as the mechanical properties of the scaffolds. In general, a high porosity and high interconnectivity between pores are necessary to allow cell growth and flow transport of nutrients and metabolic waste. Thus, besides the choice of a suitable material, the first stage of tissue engineering begins with the fabrication of a porous three-dimensional scaffold. A number of processing techniques based on textile technologies, particulate leaching, phase separation processes, gas foaming, particle aggregation and solid freeform fabrication have already been developed with more or less success to produce porous biodegradable polymeric scaffolds for tissue engineering applications [18-20].

To improve the interconnectivity between pores, other novel scaffold preparation methods have been proposed by combining salt particulate leaching with other fabrication techniques, such as phase separation, emulsion freeze-drying, gas foaming, or rapid prototyping [21-26].

The purpose of this study was to evaluate the feasibility of a preparation method combining organic/inorganic matters and salt particulate leaching techniques for the preparation of porous scaffolds. Sodium chloride salt and chloroform are used as water soluble porogens (polymeric pore generator) to form porous polycaprolactone/cuttlebone scaffolds. The porous scaffolds were fabricated using different volume percentages of sodium chloride salt particles by controlling the size and shape. This paper focuses on the microstructure and material properties of scaffolds, such as by measuring their chemical analysis, porosity and compressive strength depending on the composite's mass ratio.

EXPERIMENT

1. Materials

Cuttlebone (CB) was divided by processing of cuttlefish living

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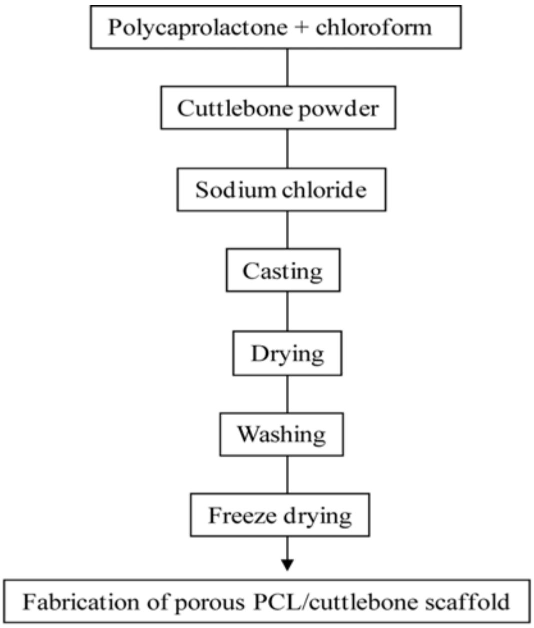

Fig. 1. Schematic diagram showing the fabrication of biodegradable PCL/cuttlebone scaffold.

in the west coast of Korea. The protein was stirred in NaOH for 20 hrs. Then, it was ground and exposed to gamma irradiation at 25 kGy with a ^{60}Co source (at a rate of 10 kGy/hr) in air at room temperature for sterilization. Polycaprolactone (PCL, $C_6H_{10}O_2$, M_w 80,000) was purchased from Sigma-Aldrich (St. Louis, USA). The CB powder and sodium chloride (NaCl, DC Chemical Co., extra pure, pore-forming agent) powders had an average particle size of about 200-300 nm and 200-300 μm diameters, respectively.

2. Preparation of Biodegradable PCL/CB Scaffolds

Fig. 1 shows an experimental schematic diagram for making a biodegradable PCL/CB scaffold. First, PCL and chloroform as a solvent in the vessel were used as the starting materials.

To increase the biocompatibility during blending, CB powder was added with a particle size of about 200-300 nm in diameter. The compositions of seven composites are given in Table 1. The weight fractions of the composites described in this paper therefore stand for those of green bodies. Mixtures of PCL/CB/sodium chloride were injected as a cylindrical rod shape (diameter=15 mm, thickness=5 mm) by Teflon, and these mixtures were compacted into pellets by using a uniaxial compaction machine.

Table 1. Characteristics of the scaffold samples prepared in this study

Sample code	Contents PCL : bone : salt (wt%)
PC-020	1 : 0 : 20
PC-210	1 : 2 : 10
PC-220	1 : 2 : 20
PC-230	1 : 2 : 30
PC-310	1 : 3 : 10
PC-320	1 : 3 : 20
PC-330	1 : 3 : 30

After drying in air, the powder mixtures were then separated from the Teflon and washed more thoroughly with de-ionized water several times using a Milli-Q system (Millipore Co., USA) for 24 hrs to eliminate the sodium chloride. The water-soluble salt particles were leached out with water to leave the remaining porous structure. This process is characterized by an easy control of the pore size, morphology, and porosity in the matrix based the amount and particle size of the added salt.

Finally, a freeze-drying process on a PCL/CB scaffold was carried out, at −53 °C in a vacuum (10^{-5} mTorr) for 24 hrs using a freeze-dryer (FD-5518, Ilshin Lab. Co., Korea).

3. Mechanical and Material Analysis of Biodegradable PCL/CB Scaffolds

The morphology and microstructure properties of PCL/CB scaffolds were examined by scanning electron microscopy (SEM, JSM-6390, JEOL, Japan) with Au coating techniques. The chemical analysis of PCL/CB scaffolds was identified using inductively coupled plasma/mass spectrometer (ICP/MS, IRIS DUO, Thermo elemental, USA). The porosity and the pore diameter of the samples were measured with a mercury porosimeter (Autopore IV 9500; Micromeritics, USA). Concerning porosity, at least three specimens were tested, and the average value was taken.

The compressive strength (load at 50% strain) of biodegradable PCL/CB scaffolds was investigated using an Instron mechanical tester (4443D2074, USA). The specimens were 20×20 mm, the thickness was 20 mm, and the head speed was 10 mm/min. The compressive strength of each sample was tested at least five times, and the average value was taken.

RESULTS AND DISCUSSION

1. ICP-MS Instrumentation

The constituents of CB powders were determined by ICP-MS. ICP-MS has an analytical technique that is able to determine the elemental composition of inorganic solid sample. The results of a quantitative analysis are shown in Table 2. From the results, we can confirm that CB powders ground from a disintegrator were obtained with the factors of biocompatibility with the constituent of low bio-inertive materials. Furthermore, the ground CB powder includes a high amount of bioactive materials such as calcium and phosphate.

2. PCL/CB Scaffold Morphology

The biodegradable PCL/CB scaffold morphology obtained in the absence of salt particulates was explored. Fig. 2 shows SEM micrographs of PCL/CB scaffolds without salt particles. After the washing and freeze-drying processes, the pore-forming agent (sodium chloride salt) was successfully removed without any processing de-

Table 2. Chemical analysis determined by ICP-MS

Minerals (mg/100 g)	Caught area
Calcium	22,341.4
Phosphorous	27.6
Magnesium	58.6
Manganese	0.5
Potassium	41.7
Sodium	583.0

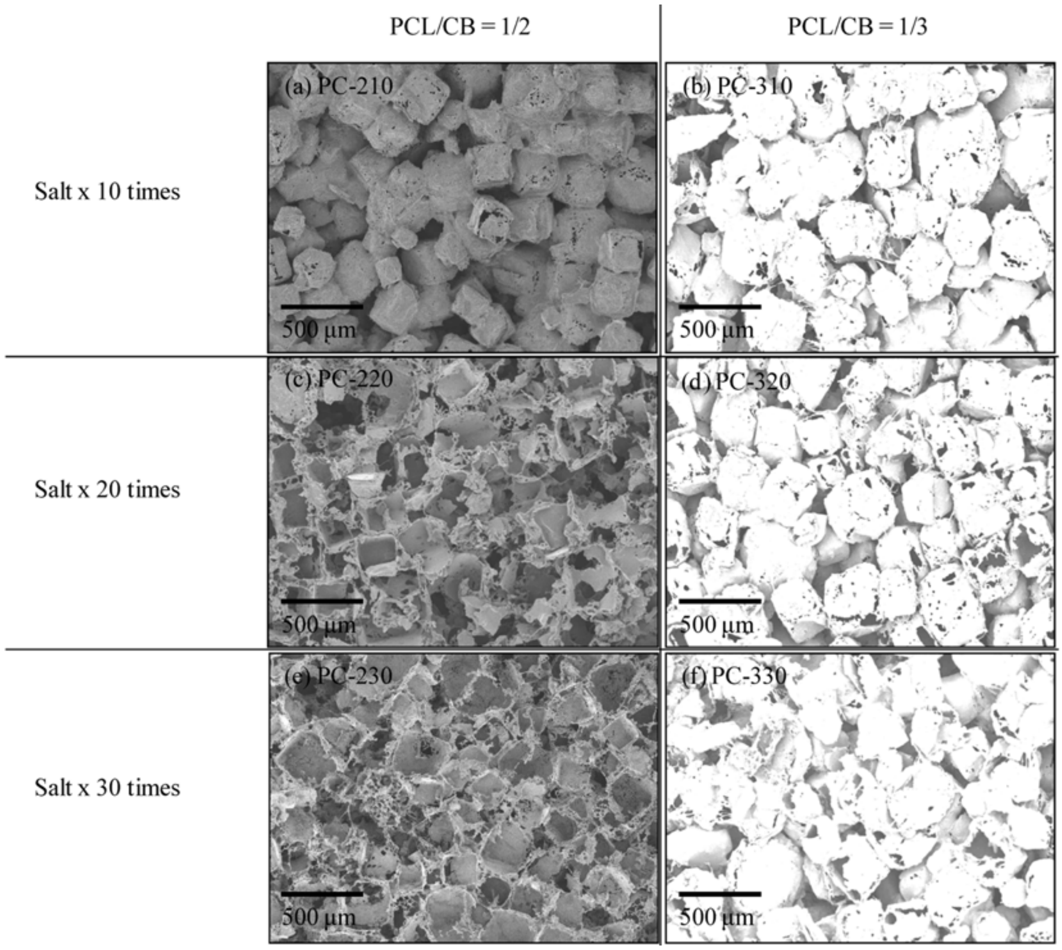

Fig. 2. SEM micrographs of porous PCL/cuttlebone (CB) scaffold depending on CB and sodium chloride salt content.

fects such as crack or swelling. The pore size was about 200-300 μm in diameter and rectangular shaped. As expected from the results, the PCL/CB scaffold in Fig. 2(a)-(f) possesses fully interconnected pores. The pore frames were about 5-10 μm thick, although the pore shapes were irregular. The amount of interconnected pores in Fig. 2(a), (c) and (e) was homogeneously dispersed and increased in the PCL scaffold due to the increase in pore-forming agent such as sodium chloride salt content. On the other hand, when increasing the CB content, the PCL scaffold changes its surface morphology, as shown in Fig. 2(a)-(b), (c)-(d) and (e)-(f). This means that the pore frame was rougher.

3. Porosity Determination and Compressive Strength

Mercury porosimetry was used to determine the porosity and pore size of these interconnected beaded wall scaffolds and showed a bimodal distribution composed of macropores interconnected by throats. These results, in combination with the interconnectivity data, suggest that the macroporous beaded-wall scaffolds have morphology suitable for tissue engineering. The PCL/CB scaffolds fabricated at varying content exhibit porosity, as shown in Table 3. The PCL/CB scaffolds obtained by a salt particle leaching technique were found to have tractable and controllable interconnected porosity within the range of 66-86%, with a typical pore size ranging from 200-300 μm. In general, to obtain a scaffold of excellent biocompatibility, the scaffolds should be porous. It has been reported that the optimum pore size diameter is within a range of 150-500 μm for bone ingrowth in the scaffolds; the new cells (such as osteoblast and osteoclast cells) grow within degrade the scaffolds, and are replaced by natural human bone [27].

Table 3. Porosity and compressive strength (load at 50% strain) of biodegradable PCL/cuttlebone (CB) scaffold depending on salt (NaCl) and CB content

Sample code	Porosity (%)	Load at 50% strain (gf)
PC-020	85.7±0.8	133±9.7
PC-210	66.0±0.4	198±3.2
PC-220	81.2±1.1	117±4.1
PC-230	84.1±0.9	110±7.8
PC-310	65.0±1.7	133±9.2
PC-320	75.6±0.4	108±5.7
PC-330	86.0±0.9	79±6.6

Furthermore, Table 3 shows the compressive strength (load at 50% strain) of the PCL/CB scaffolds depending on the CB and salt ratio. In a sample sintered at PC-330, the values of compressive strength were comparatively low, at about 79 gf, due to the interconnected pores. However, when decreasing the sodium chloride salt and CB ratio, the values of the compressive strength hardness increased due to the enhancement in the densification.

CONCLUSIONS

A salt particle leaching process was developed for the preparation of highly porous PCL/CB scaffolds. The pore-forming agent was successfully removed due to the salt particle leaching process. The diameter of the pores in the PCL/CB scaffolds was found to be about 200-300 μm depending on the mass ratio. The pore size and porosity can be precisely controlled based on variations in the range of the salt particle size and the amount of salt used. The pores in PCL/CB scaffolds are interconnected and the obtained structure is homogeneous. As the CB and sodium chloride salt content increased, the morphologies of scaffolds changed dramatically. The values of porosity and compressive strength of the PC-220 (e.g., PCL : CB : salt=1 : 2 : 20 vol%) sample were about 81% and 198 gf, respectively.

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