Materials Letters 93 (2013) 72–76

Contents lists available at SciVerse ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

An innovative method to fabricate honeycomb-like poly(ε-caprolactone)/nano-hydroxyapatite scaffolds

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ARTICLE INFO

Article history:
Received 5 September 2012
Accepted 5 November 2012
Available online 20 November 2012

Keywords:
Biotemplating
Biomimetic
Poly(ε-caprolactone)/nano-hydroxyapatite composite
Biomaterials
Porous scaffold
Bone tissue engineering

ABSTRACT

The novel honeycomb-like poly(ε-caprolactone)/nano-hydroxyapatite whiskers composite (PCL/nHA) scaffolds were fabricated by an innovative biotemplating technique based on the negative NaCl mold of cane. The microstructure, mechanical properties and in vitro cytocompatibility of the scaffolds were characterized using SEM, mechanical tests and MTT assay. SEM revealed the good transformation of cane structure into PCL/nHA composite. The PCL/nHA scaffolds had a bimodal pore distribution with interconnected channels ranging from 50 to 260 μm surrounded by micropores of <20 μm. The compressive modulus and porosity were 275.1 kPa and 95.87%, respectively. The in vitro biological evaluation with MG-63 cells confirmed that the incorporation of nHA whiskers into PCL matrix significantly improved initial cell attachment and proliferation. The results suggest that the novel PCL/nHA scaffolds are promising for application in bone tissue engineering.

1. Introduction

Synthetic biopolyesters such as poly(L-lactide), poly(lactic-co-glycolic acid) and poly(ε-caprolactone) (PCL) are widely applied in various biomedical fields due to their excellent biocompatibility, good biodegradability and ease of processability. PCL has attracted special interest in tissue engineering because it has higher toughness than other biopolymers [1]. However, PCL scaffolds have one critical disadvantage in that they are hydrophobic and exhibit poor cell affinity [2,3]. On the other hand, inorganic biomaterials such as hydroxyapatite (HA), beta-tricalcium phosphate and bioactive glass have excellent biocompatibility, good osteoconductivity and even osteoinductivity, but poor mechanical properties, including low strength and high brittleness, limits their potential applications [4]. HA was considered as an essential component for bone scaffolds because of its chemical and physical resemblance to the mineral component of natural bone [5], and was usually employed to enhance the biological affinity of polymer scaffolds [3]. Therefore, biopolymer/bioceramic composite scaffolds will take advantage of the mechanical properties, processability, degradability and osteoconductivity of the individual components [6,7].

The porous structure and pore size play vital roles in the physicochemical and biological properties of scaffolds [8]. Some studies have confirmed that the scaffolds with multichannels could facilitate the cell penetration to scaffold center and promote nutrient and waste exchange outside and inside the scaffolds [8]. Thus, honeycomb-like scaffolds are attracting more attention. Several methods such as rapid prototyping [9], stainless steel needles/rings array templating [8] and unidirectional freeze-drying [10,11], have been developed to fabricate porous scaffolds with multichannel or aligned pores. However, they present some drawbacks, including low resolution, poor channel interconnectivity or small channel size (<100 μm). Therefore, the fabrication of novel honeycomb-like scaffolds is not only technically promising, but also a challenge. Recently, wood-derived honeycomb-like bioceramics received some attention because of their good biocompatibility and high structural similarity to spongy bone [12,13]. However, both the stiffness mismatch between natural bone and the ceramics and unideal degradable rate are two major concerns. If the porous structure of wood can be converted into biopolymer-based composite, it will be possible to overcome these problems.

In this study, we described a novel method of fabricating honeycomb-like PCL/nHA scaffolds from cane as a starting template. The morphology, mechanical properties and cytocompatibility of the scaffolds were characterized. The preliminary results showed that the PCL/nHA scaffolds had great potential as scaffold for bone tissue engineering.

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http://dx.doi.org/10.1016/j.matlet.2012.11.046

2. Material and methods

Materials: PCL with molecular weight of ca. 200,000 g/mol was purchased from Daigang Biomaterials Co., Ltd. (Shandong, China). The nHA whiskers with the average length of 100 nm and diameter of 20 nm were synthesized according to the reported method [14]. All chemicals used were of reagent grade. The human osteoblast-like MG-63 cells were obtained from the Medical Center of Xi'an Jiaotong University (China).

Scaffold fabrication: The honeycomb-like PCL/nHA scaffolds were fabricated according to the following procedures. Firstly, cylindrical cane-derived biocarbon samples were repeatedly infiltrated with NaCl solution, dried at 110 °C, and sintered at 780 °C for 2 h in argon to form biocarbon/NaCl complexes. Secondly, the complexes were oxidized at 600 °C for 4 h to remove biocarbon, obtaining the NaCl molds. Thirdly, the molds were impregnated with a homogeneous PCL/nHA suspension for 30 min under −0.06 MPa so that their pores were filled. The nanosuspension

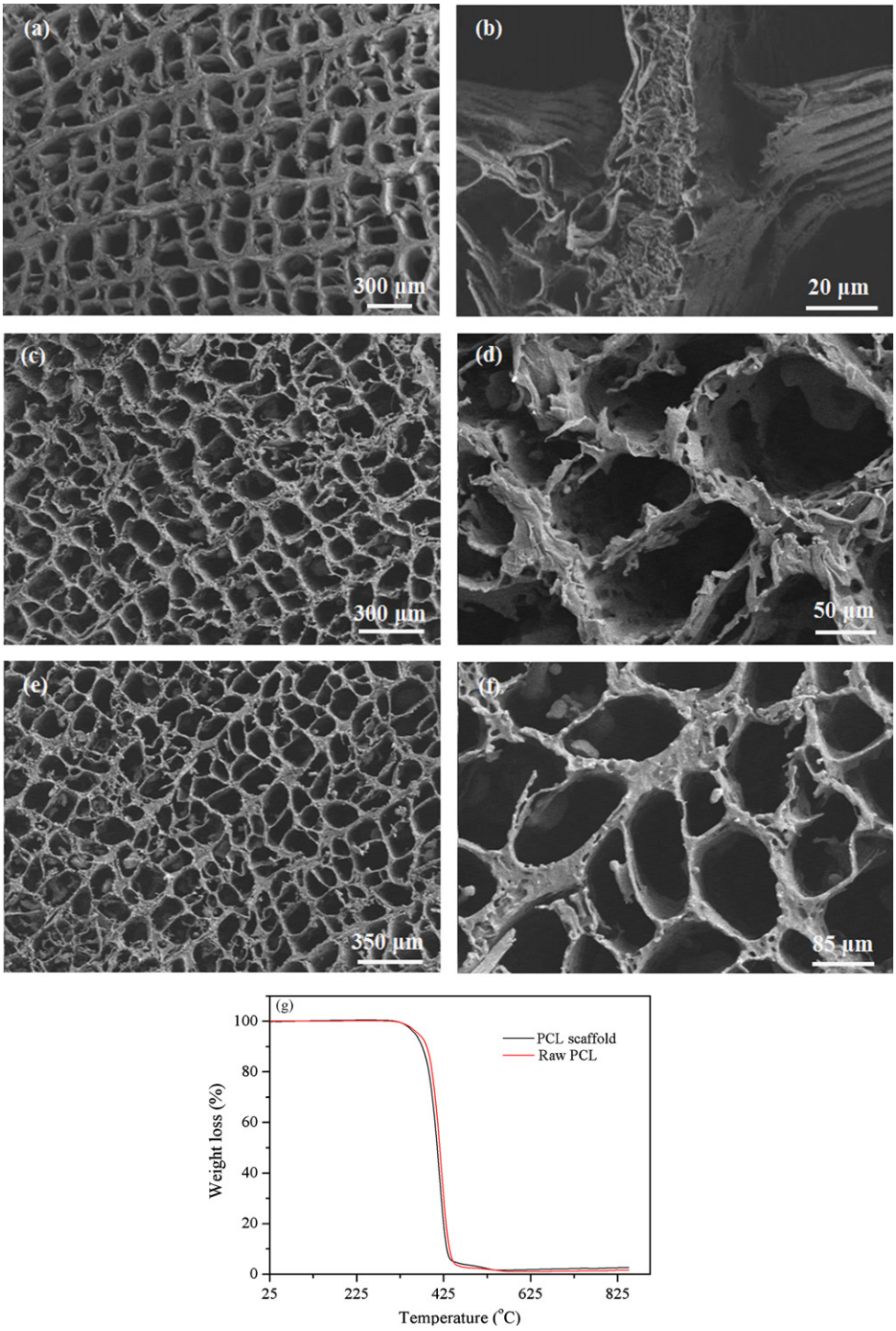

Fig. 1. SEM images of (a, b) cane, (c, d) PCL and (e, f) PCL/nHA scaffolds. (g) TG curves of raw PCL and PCL scaffold.

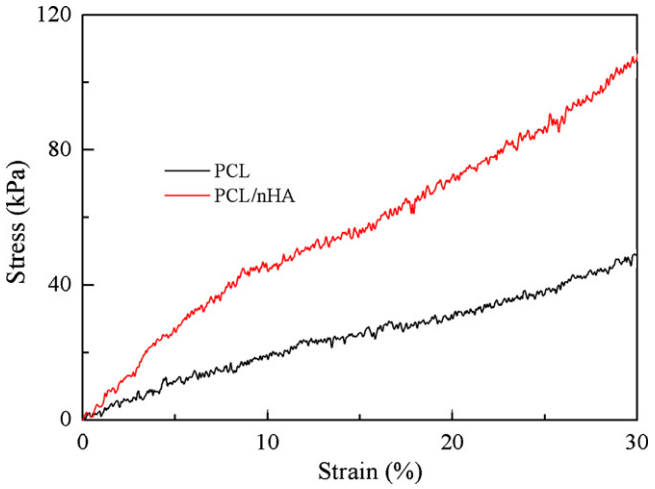

Fig. 2. Typical stress–strain curves of PCL and PCL/nHA scaffolds.

was obtained by mixing nHA and PCL in CH_2Cl_2 using a high-speed homogenizer (FJ200-SH model, Shanghai Specimen and Model Factory, China) at 20,000 r/min for 20 min. The concentration of PCL was 15%(wt/vol), and the mass ratio of nHA to PCL was 1:19. Finally, the impregnated samples were dried, and NaCl was removed by leaching with distilled water for 2 days. The water was replaced every 4 h. The PCL/nHA scaffolds were dried for further use. PCL scaffolds were prepared as control using the same process.

Characterization: The morphology of cane and the scaffolds was observed by SEM (VEGA 3 XMU, Tesan, Czech Republic). Pore size was estimated by analyzing SEM images with image-analysis software (the ImageJ software, NIH, USA). The thermogravimetric analysis (TGA) was performed on a SDT-Q600 instrument (TA, USA) under a nitrogen atmosphere. The compressive characteristics of the cylindrical scaffolds ($\emptyset$ 9 mm × 15 mm) were measured using Instron 5943 universal testing instrument (Instron Int. Ltd., USA). The compressive modulus was determined from the stress–strain curves between 10% and 20% strain range. The porosity was measured using Archimedes' principle [15].

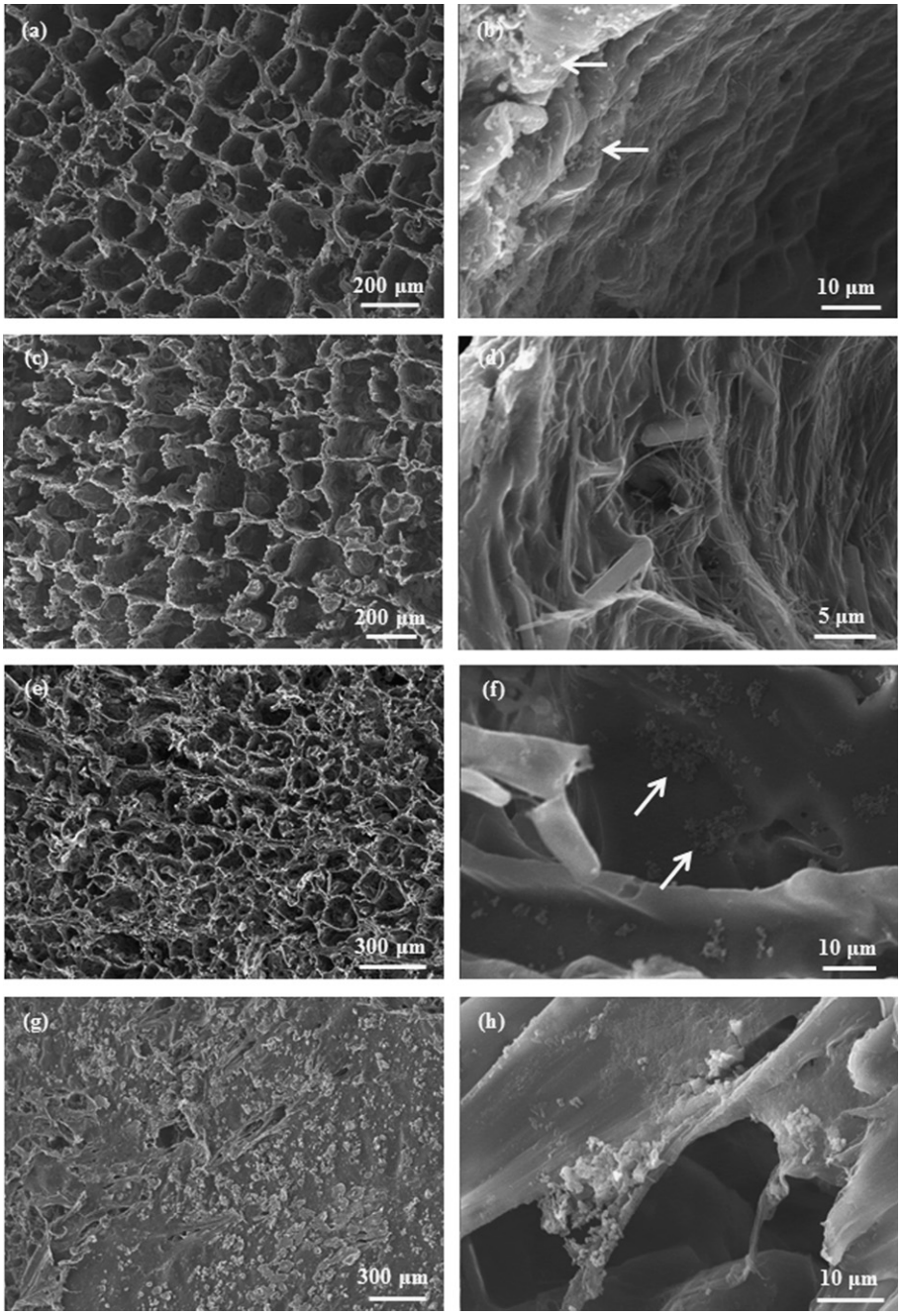

Fig. 3. SEM images of MG-63 cells on (a, b, e, f) PCL and (c, d, g, h) PCL/nHA scaffolds cultured for (a–d) 1 and (e–h) 5 days. The white arrows indicate MG63 cells.

In vitro cytocompatibility of the scaffolds (ϕ 9 mm × 3 mm) was evaluated with MG-63 cells using SEM and (3-(4,5)-dimethylthiazol-2,5-diphenyltetrazolium bromide) (MTT, Sigma) assay. Five samples per experimental condition were used to perform mechanical tests and cytocompatibility assay. One-way analysis of variance and a *t*-test were used to assess the experimental data and the results are reported as mean ± SD. Statistical significance was considered at $p < 0.05$.

3. Results and discussion

Biotemplating is a novel technique to fabricate honeycomb-like scaffolds for tissue engineering. In this study, PCL and PCL/nHA scaffolds were prepared using the method. Fig. 1(a)–(f) shows the SEM images of cane, PCL and PCL/nHA scaffolds. It can be seen from Fig. 1(a) and (b) that cane is highly porous and exhibits a unique three-dimensional interconnected multichannel structure, and the nearly cylindrical channels are surrounded by microporous struts. The SEM images in Fig. 1(c)–(f) show that PCL and PCL/nHA scaffolds exhibit the same morphological characteristics similar to those of cane. The porosity of PCL and PCL/nHA scaffolds is 95.31 ± 1.27% and 95.87 ± 0.39%, respectively, but no significant difference is found. Their parallel channels with a size range of 50–260 μm are interconnected by micropores of < 20 μm. The channel sizes are in the optimal pore size range of 100–400 μm required for bone tissue engineering application [16]. It is noted that the channel wall thickness of the scaffolds is thinner than that of cane. The reason for this phenomenon is that the woody channel walls of cane were substituted by PCL solution or PCL/nHA suspension during the cane-to-scaffold conversion, and the removal of solvent led to thin polymer-based channel walls. In addition, the TGA results (Fig. 1(g)) confirm that the PCL-based scaffolds have a very low content of residual NaCl of 0.49%, which was calculated based on the weight loss rates of raw PCL and PCL scaffold at 600 °C. Therefore, fabricating honeycomb-like polymer-based scaffolds from cane is feasible.

Fig. 2 presents the compressive stress–strain curves of PCL and PCL/nHA scaffolds. It can be seen that the stress of the two scaffolds increases with the increasing strain. They exhibit a linear elastic behavior. The smooth stress–strain curves suggest that there was no partial collapse of the porous structure during the tests. The PCL/nHA scaffold shows a much higher compressive modulus than PCL scaffold. This indicates that the incorporation of nHA into PCL had an obvious enforcement action, which involved the load transfer from PCL matrix to nHA whiskers. The compressive modulus of PCL and PCL/nHA scaffolds is 124.0 ± 7.1 kPa and 275.1 ± 11.4 kPa, respectively, which can meet the mechanical requirement of scaffolds for in vitro cell culture experiments. In addition, no plastic plateau is observed during the progressive compression because PCL has good elastic behavior.

The cytocompatibility of the PCL and PCL/nHA scaffolds was investigated with MG-63 cells by SEM and MTT assay, and the results are given in Figs. 3 and 4. Fig. 3 shows the morphology and distribution of MG-63 cells on the scaffolds after being cultivated for 1 and 5 days. It was apparent that MG-63 cells seeded into the PCL/nHA scaffolds could well adhere to both the surface of pore struts and the inside of the channels, indicating their good biocompatibility. In contrast, the MG-63 cells on the PCL scaffolds always exhibited a particle-like morphology over the 5-day culture period. After 1 day of culture, the MG-63 cells on the PCL/nHA scaffolds presented some protruding filopodia (Fig. 3(d)), indicating better cell spreading on the scaffolds. Such morphology is similar to that of the MG-63 cells on poly(L-lactide-co-glycolide) foil [17]. Moreover, the nHA whiskers were uniformly dispersed in PCL matrix and no aggregation appeared. The MG-63 cells after 5 days of culture formed flat cell sheets, covering the scaffold surfaces. The results indicated that the incorporation of nHA whiskers into PCL increased the surface roughness, and provided a more favorable substrate for the initial cells' attachment and proliferation. The results of MTT assay (Fig. 4) further confirmed that the PCL/nHA scaffolds significantly enhanced the proliferation of MG-63 cells compared with the PCL scaffolds, particularly at day 5 ($^*p < 0.05$). The possible reason is that the nHA whiskers incorporated into PCL enhanced the cell attachment and protein adsorption on the scaffolds. In the future, more biological evaluation on the honeycomb-like PCL/nHA scaffolds will be needed to confirm the potential usefulness for bone tissue engineering.

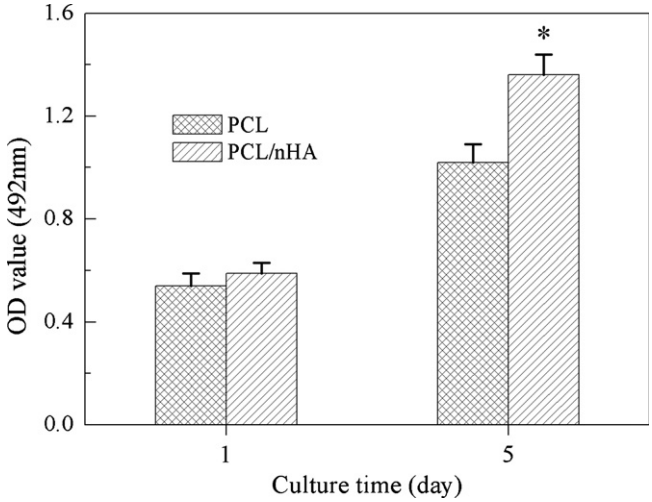

Fig. 4. MTT assay results showing the MG-63 cells on PCL and PCL/nHA scaffolds after 1 and 5 days of culture (significance level $^*p < 0.05$).

4. Conclusions

Novel honeycomb-like PCL/nHA scaffolds were successfully fabricated by an innovative biotemplating technique based on the negative NaCl mold of cane. The PCL/nHA scaffolds exhibited a bimodal pore distribution with channel sizes of 50–260 μm and micropores of < 20 μm. The scaffolds possess a high porosity of 95.87% and a compressive modulus of 275.1 ± 11.4 kPa. The incorporation of nHA whiskers into PCL significantly improved the mechanical and biological performances, but had a negligible effect on the porosity and morphology. The preliminary in vitro evaluation confirmed that the scaffolds could support the attachment and proliferation of MG-63 cells. Biotemplating is a promising method to fabricate honeycomb-like polymer-based scaffolds.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (50773062, 30830099), the Project Supported by Natural Science Basic Research Plan in Shaanxi Province of China (SJ08-ZT12, 2011K13-01-09), and the Fundamental Research Funds for the Central Universities (xjj20100115, xjj2012146).

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