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A novel comby scaffold with improved mechanical strength for bone tissue engineering

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ABSTRACT

Porous scaffold is an integral part of bone tissue engineering. However, low mechanical strength is a major challenge. A biomimetic comby scaffold was designed to get an improvement of strength in this paper. Fabrication of this bone scaffold involved first creating a pore-forming mold by using three-dimensional printing (3DP), which was cast into chitosan/nano-hydroxyapatite (CS/nHA) powders to create an inverse mold. The sacrificial mold and inverse one were then pressed by cold isostatic pressing as a whole in order to enhance the strength. Finally, the wax template was removed, resulting in a scaffold with honeycomb geometry. Compression tests were carried out to evaluate the strength of the scaffold. The behaviors and responses of preosteoblast cells on the scaffold were studied as well. The scaffold with high porosity were found to display improved compressive strength (1.62 ± 0.22 MPa) and Young's modulus (110 ± 22 Mpa) approaching the values of cancellous bone. Moreover, MC3T3-E1 cells exhibited good proliferation on the scaffold. The comby scaffold had great application potential in bone tissue engineering. This novel structural bionic approach would offer some new ideas in design and fabrication of porous scaffolds for tissue engineering.

1. Introduction

It can be envisaged that, the future of bone tissue engineering will depend on the development of the tissue specific porous 3D scaffold that will actively participate and accelerate tissue regeneration in vivo [1]. Three dimensional printing (3DP) is a versatile tool that has become popular for making scaffolds in bone tissue engineering. It can fabricate scaffolds with defined shapes, controlled and interconnected porous structures [2]. However, the rapid prototyping (RP) machines usually work with synthetic polymers and not with biocompatible materials such as natural polymers or ceramics [3]. Biopolymers, such as collagen [4], chitosan (CS) [5] and silk fibroin [6], are favorable scaffold materials for bone tissue engineering. These biomacromolecules can recapitulate natural bone extracellular matrix (ECM) architecture with the necessary biochemical and load-bearing properties for bone cells. The composite scaffolds composed of biopolymer and ceramic, which mimic the composition and functions of nature bone best, have been expected to be favorable materials for bone tissue engineering.

Chitosan/hydroxyapatite (CS/HA) porous material is a promising scaffold in bone tissue engineering. But it has a fatal disadvantage of low mechanical strength. Li [7] fabricated a porous CS/HA scaffolds via a single-step mineralization approach, which achieved nano-HA crystal layer in CS scaffold. The compressive strength of this CS/HA scaffold (porosity 50%) was 0.54 ± 0.005 MPa. The Young's modulus was 5.47 ± 0.65 MPa. Przekora [8] prepared a porous CS/HA scaffold (porosity 47.5%) via mixing of liquid phase (4% krill chitosan solution) with hydroxyapatite granules. The compressive strength of this scaffold was 1.23 ± 0.33 MPa. But the Young's modulus was only 0.46 ± 0.09 MPa. Tsiourvas [9] prepared a porous CS/HA scaffold by lyophilizing the mixture of HA nanoparticles and CS solution. This scaffold had improved strength. The compressive strength was 2.76 ± 0.51 MPa. And the Young's modulus was 0.52 ± 0.07 MPa. However, the porosity was not very high, as the volume fractions was 79–83%.

Sintering 3D printed scaffolds results in a higher mechanical strength [10,11]. However, it is not suitable for the biopolymer-ceramic composite scaffolds because the temperature is too high for biopolymers. This study aimed to fabricate a porous CS/HA scaffold with improved mechanical strength. Inspired by the structure characteristics and excellent mechanical property of honeycomb, we prepared a comby scaffold via designing a pore-forming tem-

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plate by 3D printing. Comparing with above-mentioned fabrication methods, this structural bionic approach produced regular connected porous structure and confers higher mechanical strength for CS/HA scaffold.

2. Materials and methods

2.1. Scaffold preparation

The CS/n-HA composite material was prepared by urease-catalyzed method as described in our earlier study [12]. Briefly, precipitation of CS/n-HA was obtained by stirring a mixed aqueous solution containing $Ca(NO_3)_2$, Na_2HPO_4, urea, urease and chitosan at 37 °C. Chitosan (Shanghai Bio Life Science and Technology CO., LTD) was dissolved in acetic acid (pH = 3). The terminal concentration of urease (Sigma) and urea in the solution was 50 U/ml and 1 mol/L, respectively. When urea was enzymatically hydrolyzed to form ammonia, CS/n-HA was precipitated from reaction solution. The precipitate was aged for 10 h. Thereafter, the resulting sample was centrifuged, washed by ultra-pure water, and was freeze-dried at 0.5 mmHg for 24 h.

The design of the porous comby CS/n-HA scaffold was inspired by the structure of honeycomb (Fig. 1a and b). As we know, honeycomb is an excellent nature scaffold, which has many significant advantages including high porosity and high strength. Fig. 1c illustrated the fabrication process of the scaffold. The scaffold was fabricated by an indirect approach using a sacrificial wax template. Firstly, a pore-forming template was first designed by a computer. As shown in Fig. 1d, this template consisted of regular hexagonal prisms, which was similar with the holes of honeycomb. Then, a wax template was printed by a three-dimensional printer, according to the computer design (Fig. 1e). A glass tube of 24 mm long and 24 mm inside diameter was sealed at one end with a plastic film. The wax mold was packaged using a filter paper on the side and placed into this tube. CS/nHA powder was then cast into this sacrificial mold in order to create an inverse mold. In the process, tapping the tube and vacuum pumping were used to ensure that the wax mold was filled in full of the powder. Thereafter, the wax mold filled with casting powder was taken from the glass tube and pressed under a cold isostatic pressure of 250 MPa to enhance the strength. The wax mold was dissolved using petroleum ether (50 °C), resulting in a porous comby scaffold.

2.2. Morphological and mechanical characterization

The surface microstructure of the comby scaffold was observed by an electric scanning microscope (Zeiss Supra 55, Germany). Mechanical testing for the scaffolds was performed by using a Universal Testing Machine (Zwick/Roell Z005, Germany). The porosity of scaffold was measured by the liquid displacement method.

2.3. Cell culture experiment

The 2×10^5 cells (pre-osteoblastic cell line MC3T3-E1) were suspended in culture medium and dropped onto the scaffolds pores. MC3T3-E1 cells were cultured in the normal culture media consisting of α-MEM, 10% FBS (Hyclone) and 1% penicillin/streptomycin. Cell morphology was investigated using SEM.

3. Results and discussion

The wax pore-forming template for the comby scaffold was a cylindrical mold of 20 mm height and 20 mm diameter. When CS/nHA composite powder cast into the wax mold, it formed an inverse mold. It was also a cylindrical shape, of course, with 20 mm height and 20 mm diameter. While after cold isostatic pressing and removing wax template, the resulting CS/nHA scaffold (Fig. 2a) shrunk, with 16 mm height and 15 mm diameter. Cold isostatic pressing led to a conventional shrinkage of scaffold. The reduction in diameter was greater than that in height due to

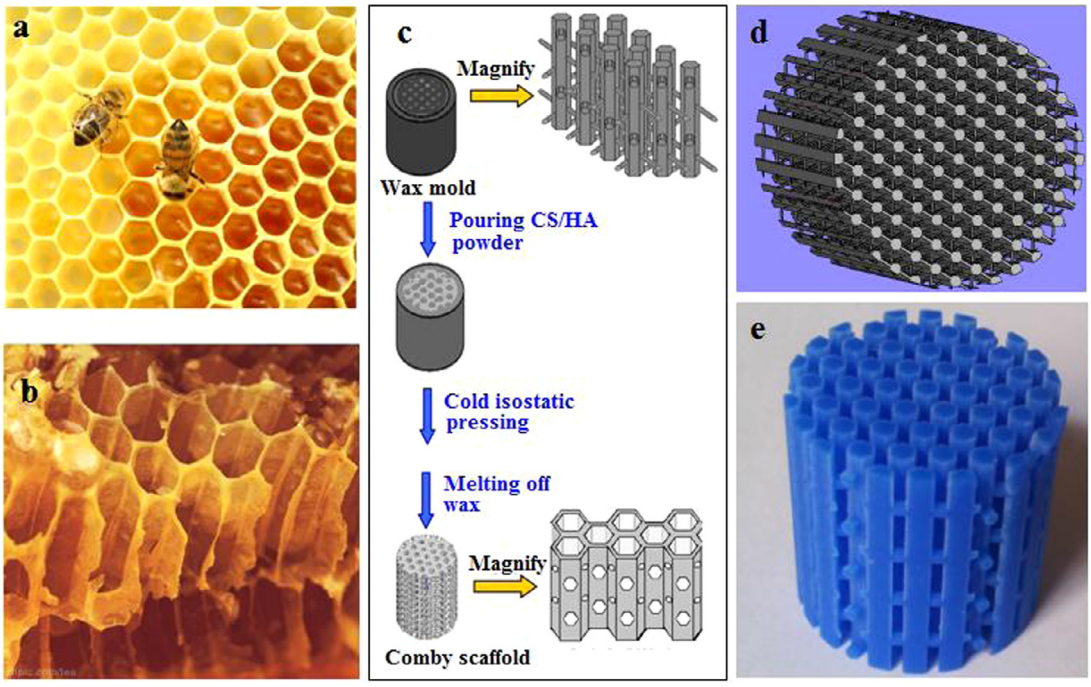

Fig. 1. Schematic diagram of bionic design of the comby scaffold. (a and b) cross and longitudinal section of honeycomb, (c) fabrication process of the scaffold, (d) computer aided design image of the pore-forming template, (e) the wax mold by 3DP.