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Structure and properties of chitosan derivatives modified calcium polyphosphate scaffolds

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ABSTRACT

Organic and inorganic composite material is becoming a solution on making the mechanical and degradation properties of biomaterial more suited. Porous calcium polyphosphate was immersed into different concentrations of carboxymethyl chitosan before immersing 10% alginate dialdegyde. After freeze-drying, the scaffolds were performed in physiologic saline. At stated day, the weightloss, Ca^{2+} concentration, pH value and morphology were measured. The biocompatibility of the composite was demonstrated by extract and direct contact tests. As the results showed, the degradation rates of composites were faster, and the compressive strength became bigger because of the cross-linked network formed by Carboxymethyl chitosan (CMC) and alginate dialdehyde (ADA). The pH value of composite was higher than that of calcium polyphosphate (CPP) due to the organic part of composite's pH was in slight alkaline. From the SEM, the cross-linked network structure could be observed clearly. Because the glycosaminoglycans-like chains in CMC molecules, which are typically presented in extracellular matrix (ECM), extractions of composite material gave the cells good adhesion and growth condition. All the results testified the composite scaffold was a good candidate for bone repair.

1. Introductions

Bone defect repair or reconstruction is becoming a major issue in orthopedic surgery. Many inorganic scaffolds have been studied in order to repair or substitute the injured parts because of the similarities of their components with bone in recent years. However, unmatched ratio between the mechanical and degradation properties limited these materials' applications. How to make the mechanical and degradation properties more suitable is a focus in bone tissue engineering. Composing inorganic and organic materials may be a good way to resolve this problem [1].

Calcium polyphosphate (CPP), as a kind of inorganic polymer bio-ceramics for bone tissue engineering, has become research point for its biodegradation and biocompatibility. CPP has the nominal stoichiometry of $Ca(PO_3)_2$, with a theoretic calcium to phosphorus molar ratio at 0.5, and contains long polymeric chains of PO_4 units linked by P–O–P bonding [2]. Due to its different crystallization and polymerization degrees sintered at different temperatures, the degradation rate of this material could be controlled [3]. A great number of experiments in vitro and vivo have shown that CPP surface could give cells good adhesion and growth condition [4–7], and new bone could be promoted growth rapidly in the CPP macroporous rods [8].

Chitosan (CS), a polysaccharide constituted of *N*-glucosamine and *N*-acetyl-glucosamine units, has recently attracted much attention from researchers around the world [9–11]. The electrostatic interactions between CS and anionic glycosaminoglycans (GAG), poly(γ-glutamic acid) [12] or other electronegative molecules are due to the cationic nature of CS. And GAG was linked with a large number of cytokines/growth factors, which made it become an important component of proteoglycan and typically presented in the extracelluar matrix (ECM) [13]. Because of the presences of amino and hydroxyl groups, chitosan could be easily chemically modified [14]. Carboxymethyl chitosan, as an important water-soluble chitosan derivative, is produced by the reaction of chloroactic acid and CS in alkaline medium at 50–60 °C. Because of the carboxymethylation, the opposite ionizable groups, carboxyl and amine, could coexist in the same polysaccharide chains. This conversion does not only provide carboxymethyl chitosan (CMC) super solubility, but also brings some special chemical, physical and biological properties [15,16].

However, the calcium phosphate ceramics are fragile [17]. To improve the properties of the scaffolds, chitosan was added into calcium phosphate, and these composite scaffolds showed good

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mechanical properties and excellent biocompatibility with osteoblast and mesenchymal stem cells [18,19]. Therefore we added some CS in order to enhance CPP. But CS is non-water-soluble that it could not cover CPP completely in our former researches. So the soluble-CMC was put as a bridge between CPP and CS in order to improve the bondings on the interface in this paper. We assumed that with the organic polymer addition and cross-linked network structure formation, the mechanical properties and biocompatibility of the composite scaffolds could be improved compared with CPP, and it will give us a way to continue further research on the inorganic/organic composite scaffold.

2. Experimental

2.1. Materials

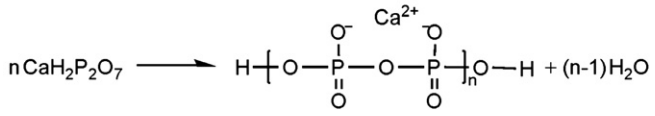
CS (with a MW of about 1.0 × 105 and a degree of deacetylation, Zhejiang Aoxing, China). Sodium alginate (low viscosity grade, 495 cps at 25 °C, Zhejiang Jinyan, China). Carboxymethyl chitosan (with a MW of about 3.0×10^5, Zhejiang Aoxing, China). Diphenyl tetrazolium bromide (MTT) was obtained from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals of the analytical reagent were used and obtained from Kelong Co. (Chengdu, China).

2.2. Synthesis of porous CPP scaffold

The process of producing CPP powders was followed as Kai Qiu made. Calcium carbonate was added into the 15% phosphoric acid (w/v), with Ca/P 1:2. After sitting for 12 h, a rotary evaporator was used to evaporate solvent, and the calcium phosphate monobasic monohydrate was collected, washed by ethanol several times and dried into powders. All the $Ca(H_2PO_4)_2 \cdot H_2O$ powders were thermally treated at 500 °C for 10 h and heated to 1200 °C for 1 h resulting to melt. The molten CPP was poured directly into the ice to avoid crystallization during cooling. After ball milling and screening, the amorphous CPP was produced. The porous CPP scaffolds were fabricated by mixing the stearic acid as the porogen with the amorphous CPP powders and press-forming as a cylinder of 10 mm diameter and 10 mm thickness. After sintered at 800 °C for 3 h, the β-CPP porous scaffolds were obtained. The reactions were expressed as the following equations:

$$Ca(H_2PO_4)_2 \cdot \chi H_2O \longrightarrow Ca(H_2PO_4)_2 + \chi H_2O$$

$$Ca(H_2PO_4)_2 \longrightarrow CaH_2P_2O_7 + H_2O$$



2.3. Periodate oxidation of sodium alginate

A total of 10.8 g sodium periodate were added into 2.5% sodium alginate solution and magnetic stirred in dark at room temperature for 1 day to produce alginate dialdehyde (ADA). The reactions were followed as the following equation:

After being neutralized by ethylene glycol, ADA was purified by precipitation with the addition of 5 g sodium chloride and 600 ml ethanol. The products were dissolved in distilled water and re-precipitated. The process was repeated three times. Then the precipitates were dried under vacuum at room temperature.

2.4. Synthesis of porous composite scaffolds

CMC powders were first added into water to form 0.1% (w/v) CMC solution. Then calcium polyphosphate scaffolds were immersed into the CMC solution. After blotting up the solution on the scaffold surfaces, the composite scaffolds were immersed into the 10% (w/v) ADA solution to be cross-linked. Followed by the freeze-drying, the process was repeated but the concentrations of CMC solution were changed to 0.5%, 1%. Then the scaffolds were immersed into 1% CS solution before freeze-drying. In order to obtain a comparison, some the composite scaffolds were not immersed into the CS solution.

2.5. Scaffolds degradation in vitro

In vitro degradation, scaffolds were measured by the weightloss over time. Seven time points (1st, 2nd, 3rd, 5th, 10th, 20th and 30th day) were selected as the degradation periods. The scaffolds were incubated in 10 ml physiological saline. At each time, the scaffolds were rinsed in distilled water and freeze-dried. The percentage of weightloss was calculated by the following equation:

$$\text{weightloss}(\%) = \frac{W_0 - W_t}{W_0} \times 100\%$$

Where W_0 is the initial weight of the scaffold and W_t is the weight of the dried scaffold at time t. The pHs of the degradation fluids were tested before 5 ml of them was changed. And the scaffolds after being weighed were collected for compress test. The compressive strengths of these materials were tested at a strain speed of 2.0 mm/min on an electronic mechanical testing machine (Instron 4302, USA).

2.6. Scanning electron microscopy (SEM)

For SEM observation, some of the scaffolds before and after degradation were sputter-coated with gold and examined by SEM (JSM-5900LV, JEOL, Japan). The scaffolds after degradation were observed on 30th day.

2.7. Cell culture

The MG-63 cells purchased from Key Laboratory of Transplant Engineering and Immunology of Ministry of Health, Sichuan University were used in this study. These cells were resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Hyclone Co., USA), and cultured at 37 °C in 5% CO_2. After disinfected in 75% ethanol and washed in phosphate buffered saline (PBS), all the scaffolds were immersed in physiological saline for 24 h at 37 °C in 5% CO_2. The MG-63 cells were seeded into a 96-well culture plate with a cell density of 2×10^4/well, and the plate was incubated at 37 °C in 5% CO_2 for