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Radiation synthesis of gelatin/CM-chitosan/β-tricalcium phosphate composite scaffold for bone tissue engineering

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ABSTRACT

A series of biodegradable composite scaffolds was fabricated from an aqueous solution of gelatin, carboxymethyl chitosan (CM-chitosan) and β-tricalcium phosphate (β-TCP) by radiation-induced crosslinking at ambient temperature. Ultrasonic treatment on the polymer solutions significantly influenced the distribution of β-TCP particles. An ultrasonic time of 20 min, followed by 30 kGy irradiation induced a crosslinked scaffold with homogeneous distribution of β-TCP particles, interconnected porous structure, sound swelling capacity and mechanical strength. Fourier Transform Infrared Spectroscopy and X-ray Diffraction analysis indicated that β-TCP successfully incorporated with the network of gelatin and CM-chitosan. *In vivo* implantation of the scaffold into the mandible of beagle dog revealed that the scaffolds had excellent biocompatibility and the presence of β-TCP can accelerate bone regeneration. The comprehensive results of this study paved way for the application of gelatin/CM-chitosan/β-TCP composite scaffolds as candidate of bone tissue engineering material.

1. Introduction

Bone defects resulting from tumors, diseases, infections, trauma, biochemical disorders, and abnormal skeletal development raise significant health problems. Various biomimetic tissue engineering approaches in terms of structure and composition have been developed to replace autografting and allografting [1]. Porous scaffolds, which are similar to the structure of natural bone, could induce the formation of bone from the surrounding tissue, or to act as a template for cell growing and bone tissue regeneration [2]. Meanwhile, composites with similar components of natural bone are widely used to fabricate porous scaffolds [3].

As it is well known, the extracellular matrices (ECM) of hard tissue are composed of organic and inorganic phases. The inorganic phase consists primarily of calcium phosphate such as hydroxyapatite (HA), while the main composition of organic phase is type I collagen and small amount of ground substance including glycosaminoglycans (GAGs), proteoglycans and glycoproteins [4,5]. Herein, gelatin, CM-chitosan and β-TCP were adopted to concoct the organic–inorganic composites.

Collagen is a major organic component of ECM. Gelatin, a hydrolysis derivative of collagen, is widely used in biomedical field due to its lower antigenicity and more stable physicochemical properties [6]. Chitin and chitosan have been extensively studied for tissue engineering applications because of its excellent biocompatibility, biodegradability and osteoconductivity [7,8]. However, acetic acid or organic solvents should be applied for the processing of chitin or chitosan, which would impart certain cytotoxicity to the final product [9]. CM-chitosan, a water-soluble derivative of chitosan, has the merits of chitosan and has improved biocompatibility over chitosan [10].

HA has extensively proved its osteoconductivity as the major calcium phosphate constituent of native bone [11]. However, its poor biodegradability prevents the ingrowth of natural bone and may leads to deformity after an extended period [12]. By contrast, β-tricalcium phosphate (β-TCP), a high temperature phase of tricalcium phosphate with 1.5 Ca/P mole ratio, has 10 times higher degradation rate than that of HA. It is capable of promoting osteogenesis and accelerating bone regeneration through a process of dissolution and absorption [13]. Therefore, considering its biomimetic composition and structure, a gelatin/CM-Chitosan/β-TCP composite porous scaffold was designed for bone tissue engineering application.

In the preparation of inorganic/organic composites, the pretreatment procedure would significantly influence the compatibility of the components, resulting in rather different physicochemical properties. Ultrasonic treatment has been frequently used to improve the distribution of inorganic particles in organic phase. However, the impact of

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ultrasonic treatment was rarely discussed in the field of bio-composites for bone tissue engineering.

To prepare hydrogels and scaffolds, several crosslinking method have been developed, for example, thermal heating, ultraviolet irradiation, chemical crosslinking and radiation crosslinking. The merit and demerit of each method were discussed in our previous work [14]. Among them, radiation crosslinking technique can prepare hydrogels and scaffolds without any cytotoxic additive, which may serve as promising biomedical material [15]. In our previous work, gelatin/CM-chitosan hybrid hydrogel with open and interconnected porous structure, which had potential to be applied as wound healing material, was prepared by radiation crosslinking [14].

In this study, gelatin/CM-chitosan/β-TCP scaffolds were prepared by radiation crosslinking and lyophilizing. Effect of ultrasonic treatment on β-TCP distribution and morphology of the scaffolds were studied. Physical properties of the scaffolds such as the porous structure, compressive strength, structural stability and swelling behavior were investigated. Besides, the biocompatibility of the scaffolds was preliminary evaluated by *in vivo* implantation into the mandible of beagle dog. The scaffold is expected to be a novel biodegradable bone tissue engineering material according to the bionic principle.

2. Materials and methods

2.1. Materials

Gelatin (product G1890, 300 g Bloom, porcine skin, Type A) was purchased from Sigma Chemical Co. Ltd. CM-chitosan powder (degree of deacetylation 96.5%; Mw 70,000) was purchased from Qingdao Honghai Co. Ltd., China. β-TCP (average diameter of 5 μm) was obtained from Shanghai Rebone Biomaterials Co., Ltd, China. Other reagents used here were all of analytical grade.

2.2. Preparation of gelatin/CM-chitosan/β-TCP composite scaffolds

The gelatin/CM-chitosan/β-TCP composite scaffolds were typically prepared as follows. Firstly, gelatin and CM-chitosan powders with a weight ratio of 2:3 were mixed homogeneously by deionized water at 50 °C with a concentration of 10%. Next, β-TCP was dispersed by deionized water to form a suspension with a concentration of 10%, and then stirred at room temperature for 0.5 h and treated by ultrasonication (40 kHz, 250 W) for certain time intervals. After that, the β-TCP suspension was poured into the gelatin/CM-chitosan solution under agitation. In the end, the mixture was mixed by an ARE-310 hybrid mixer (Japan Thinky Co., Ltd.) for 10 min to form a homogenous polymer solution. According to our previous work [14], to get promising physical properties, the total polymer concentration was fixed to 10 wt.%. Thus prepared solutions were filled into test tubes (inner diameter of 10 mm) and subjected for γ-irradiation with 30 kGy using a ^{60}Co radiation facility, which was performed at room temperature at a dose rate of 20 Gy min^{-1}. The hydrogel was denominated with the β-TCP fractions in the solid part, and Table 1 showed the composition of the hydrogels.

Table 1
The composition of the gelatin/CM-chitosan/β-TCP hydrogels.

β-TCP fraction in the solid part	Composition of the hydrogel			
	Gelatin (wt.%)	CM-chitosan (wt.%)	β-TCP (wt.%)	Total solid content (wt.%)
0%	4	6	0	10
5%	3.8	5.7	0.5	10
10%	3.6	5.4	1	10
20%	3.2	4.8	2	10
30%	2.8	4.2	3	10
40%	2.4	3.6	4	10

2.3. Characterization of the scaffolds

2.3.1. Swelling behavior in deionized water and phosphate buffer solution

The hydrogels were cut into cylinders with diameter of 10 mm and thickness of 5 mm, then the samples were immersed in beakers containing 150 mL deionized water or phosphate buffer solution (PBS) (0.15 mol L^{-1}, pH = 7.2) at 37 °C. After soaking for desired time interval (described in Figs. 3 and 4), the samples were withdrawn from the solution, gently removed surface solution by filter paper. The degree of swelling was calculated using Eq. (1). Six parallel samples were measured to achieve an average value.

$$\text{Degree of Swelling} = \frac{m_2}{m_1 \times 10\%} \times 100\% \quad (1)$$

where m_1 and m_2 represent weight of the sample before and after swelling, respectively.

2.3.2. Porosity

Cylinders of the hydrogels with the size of Φ 10 × 5 mm were immersed in deionized water for 24 h and then lyophilized. Liquid displacement method was used to determine the porosity [16]. Briefly, the specimen was put into a conical flask, and then the conical flask was evacuated to let the dehydrated ethanol sucked in the porous scaffolds. The system was kept in sealed condition for 48 h until the scaffold was saturated with ethanol. The porosity of the sample was calculated using Eq. (2):

$$p = \frac{m_4 - m_3}{\rho V_1} \times 100\% \quad (2)$$

where m_3 and m_4 represent the weight of the sample before and after immersing in ethanol, and V_1 is the volume of the sample, ρ is the density of dehydrated ethanol. The scaffolds were measured triplicate to get an average.

2.3.3. Mechanical properties

The compressive strength of the samples was tested by universal material testing instrument (Instron 5843). Φ 10 × 10 mm cylinders of the hydrogels and lyophilized scaffolds were prepared. The compressive test was conducted with a constant strain rate of 1 mm min^{-1} until 90% reduction in specimen height. The compressive strength was calculated from the stress–strain curve. Compressive modulus was calculated as the slope of the initial linear portion of the curve. The samples were measured triplicate to get an average.

2.3.4. Scanning electron microscope

The morphology of the scaffolds was observed with Scanning Electron Microscope (SEM) (HITACHI S-4800, Japan) at an accelerating voltage of 1 kV. Hydrogels were immersed in deionized water for 24 h and then lyophilized before observation.

2.3.5. Fourier transform infrared spectroscopy

The hydrogels were lyophilized and ground to a fine powder. Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed using a Nicolet Magna-IR 750, with a Nicolet NicPlan IR microscope attachment (resolution 2 cm^{-1}, scan 64 times) and a MCT/A detector with a range of 700–4000 cm^{-1}.

2.3.6. X-ray Diffraction

Powder X-ray Diffraction (XRD) measurements were performed using a Philips X'Pert Pro diffractomter with a 3 kW ceramic tube as