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Short communication

Hydroxyapatite-fucoidan nanocomposites for bone tissue engineering

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ABSTRACT

Hydroxyapatite (HAp) is the promising biomaterials to construct the article bone from the last two decades. In the present study, we have developed hydroxyapatite-fucoidan (HApF) nanocomposite for bone tissue engineering and subsequently characterized by different analytical techniques for bone graft substitute. The chemical characterization suggested that the prepared nanocomposite HApF have amorphous nature, crystal size between 41 and 153 nm was observed. The biochemical characterization inferred that the prepared nanocomposite were non-toxic and mineralization effect of HApF was observed two times higher then HAp. Hence, HApF is the promising biomaterial and could be used for bone tissue construct.

1. Introduction

Bone is hierarchical-structured tissue is mainly made up of Hydroxyapatite (HAp), collagen and other non-collagenous proteins. The goal of tissue engineering is functional recovery of damaged tissue *in vivo* and *in vitro* reconstruction of tissue while realizing exquisite tissue-specific functions [1]. Currently, cell and biomaterials-based scaffolds therapy to regenerate tissues are being studied [2]. An ideal scaffold can be characterized by biocompatibility, biodegradability, cytotoxicity, microstructure and mechanical properties [3]. Several biomaterials have been used and checked in preparation of artificial organ in the past decades [4]. A major and widely used class of biomaterials for bone repair is HAp [5] due to its composition and structure close to mimic the natural bone mineral, biocompatibility, osteoconductivity and osteoinductivity. Thus, HAp has been considered to be an appropriate material to build bone tissue engineering scaffolds.

Additions of biocompatible polymers with HAp have been regarded as the proper candidates for bone tissue-engineered scaffolds. Fucoidan is an anionic polysaccharide comprising fucose and sulfate ester groups as an enhancing component of cellular activities for bone reconstruction [6]. In general, fucoidan can induce Fibroblast Growth Factor-2 activity, assist febrile collagen matrix formation, enhance fibroblastic proliferation, and stimulate *in vitro* and *in vivo* angiogenesis [6]. Although a great number of studies on the different pharmacological properties of fucoidan are available, there is little information on the fucoidan-based system used in bone tissue engineering scaffold and wound healing [7–9,6,10].

In the present study, the bioactivity of nano HAp and fucoidan composite was examined in an *in vitro* biomimetic process and the influence of fucoidan on bioactivity of nHAp was investigated.

2. Materials and methods

2.1. Preparation of HAp-fucoidan composites

Calcium nitrate and diammonium hydrogen phosphate were separately mixed with deionized water with a molar ratio of 1:0.67 in order to maintain the Ca/P ratio of 1.67, which is the stoichiometric molar ratio of HAp and fucoidan added to Ca and P solutions. The mixture was stirred 700 rpm for 24 h at 25 °C. During the mixing processing, the pH of the Ca, P containing solution and Ca, P and fucoidan containing solution were maintaining 9.1–9.3 by adding aqueous solution of 27% NH_3. Each prepared solutions were precipitated and filtered by vacuum filter. The samples, HAp and HApF were dried at 50 °C for 24 h and kept in desiccator for the further analysis.

2.2. Characterization

Thermal gravimetric analysis was achieved by the use of Pyris 7 TGA analyzers, Perkin Elmer Inc., USA with scan range from 50 to 900 °C at constant heating rate of 10 °C min^{-1} with

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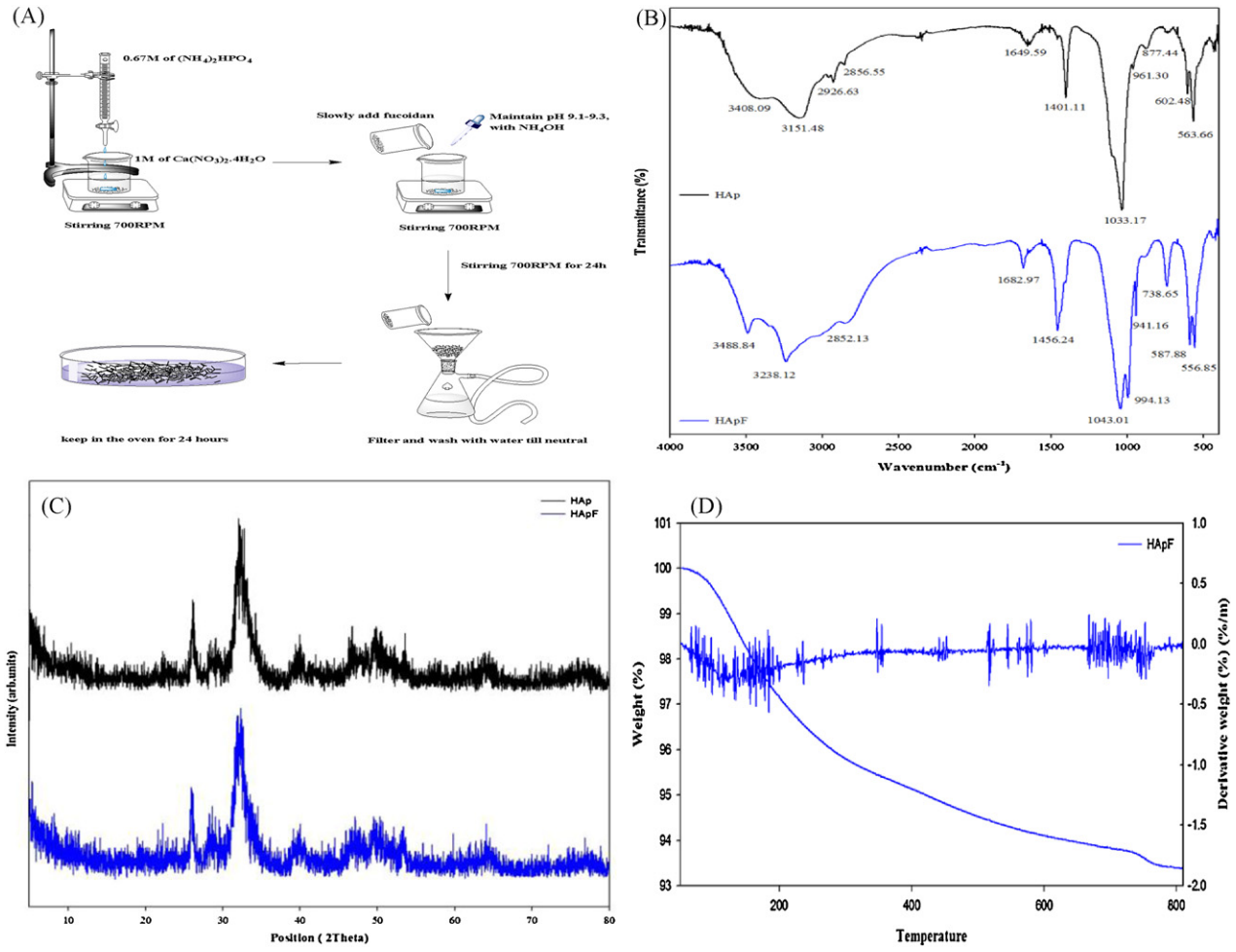

Fig. 1. (A) Schematic diagram of hydroxyapatite-fucoidan nanocomposite preparation method, (B) Fourier transform infrared spectrum of HAp and HApF composite, (C) XRD spectrum of HAp and HApF and (D) Thermal gravimetric analysis of HAp nanocomposite.

continuous nitrogen flow. The stretching frequencies of the composites samples were examined by Fourier Transform Infrared Spectroscopy, Perkin Elmer (USA) and spectrum GX spectrometer within the range of 400–4000 cm^{-1}. The phase and crystallinity were evaluated using X-ray diffractometer (PHILIPS X'Pert-MPD diffractometer, Netherland) and Cu-Kα radiation 1.5405 Å over a range of 5°–80° angle, step size 0.02, scan speed 4° min^{-1} with 40 kV voltage and 30 mA current. Morphology and chemical composition of HAp and HApF nanocomposites were obtained by field emission scanning electron microscopy (FE-SEM JSM-6700F, JEOL, Japan) equipped with an *in situ* energy dispersive X-ray (EDX) spectrometer. The cell cytotoxicity of the composite samples was studied by MTT assay using MG63 cells. Mineralization effect and expression levels of ALP mRNA were measured using RT-PCR and also performed for GAPDH independently as an internal control as per previous literature [8].

3. Result and discussion

The prepared composite HAp and HApF are in pure white and slight yellow in color, respectively. The slight yellow color in HApF is because of fucoidan addition to the composite. Both the materials are free flowing in nature. The preparative procedure of HApF nanocomposite has been shown in Fig. 1(A). We have performed FT-IR analysis to find out the chemical interaction between HAp and fucoidan, are shown in Fig. 1(B). The results showed that both phosphate and carbonate groups were present in HAp and HApF. In HAp, the wavenumbers at 563 and 1033 cm^{-1} are depicted for phosphate groups whereas, at 1043 and 556 cm^{-1} for HApF, respectively [11,12]. In addition, the peaks at 1401–1682 cm^{-1} are represented for carbonate groups of synthesis HAp [13]. Fig. 1(C) shows typical XRD patterns of HAp and HApF composite materials. The XRD patterns of HAp and HApF have shown similar characteractic peaks at 26.09°, 32.09°, and 46.75° [11,14]. From results, it can be observed that both the materials are in amorphous in nature. Thermo gravimetry analysis was performed to know about the thermal stability of the composite materials. A small weight lose was observed in HApF composite, which is depicted in Fig. 1(D). FE-SEM and TEM were used to investigate the morphology and microstructure of the synthesized HAp and HApF. The results are shown in Fig. 2(I and II). From the results, it was measured that the crystal size of HAp and HApF are 29-159 nm and 41-153 nm, respectively. The calcium phosphorous ratio of HAp and HApF were found to be 1.67 and 1.63 (Fig. 2(III)). The small deviation in calcium phosphorous ratio in HApF may be due to the addition of fucoidan.

In order to investigate cytotoxicity of composite samples, MTT assay was performed with different concentration of HAp and HApF. MTT result indicated that all the samples did not exhibit any toxic effect on MG63 cells even at the highest tested concentration (1000 μg/ml) for 24 h (Fig. 2 (IV)). The effect of HAp and HApF on the maturation of osteoblasts was examined by determining alkaline phosphatase activity in MG-63 cells. The results