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Biomimetic Design of Oxidized Bacterial Cellulose-gelatin-hydroxyapatite Nanocomposites

Mao Yang[1], Wenjuan Zhen[2], Hui Chen[1], Zhihua Shan[1]

1. *National Engineering Laboratory for Clean Technology Leather Manufacture*, *Sichuan University*, *Chengdu* 610065, *China*
2. *Department of sports medicine*, *Chengdu Sport University*, *Chengdu* 610041, *China*

Abstract

Oxidized Bacterial Cellulose (OBC)-hydroxyapatite (HAp)-gelatin (Gel) nanocomposites were prepared by a biomimetic process. HAp nanocrystals were precipitated in a mixed solution of Na_2HPO_4 (pH 9.2) and Gel solution at 37 ˚C, and OBC was used to generate a three-dimensional (3D) network stent. The tensile strength of OBC-HAp-G was higher than 0.3 MPa, and the complete degradation time was approximately 90 d in Simulated Body Fluid (SBF). Fourier transform infrared spectroscopy demonstrated that a coordinate bond had formed possibly between HAp and the cellulose hydroxyl. X-ray diffraction showed that both the oxidation of bacterial cellulose and an increase in Gel content induced the formation of tiny HAp crystallites during composite fabrication. Specific surface area and porosity measurements indicated that a low Gel concentration contributed to retention of porous structure. The Ca and P contents on the surface of materials increased initially and then decreased with an increase in Gel content, as measured by energy dispersive spectroscopy. From the thermogravimetric data, the increase in decomposition temperature suggested the formation of chemical bonds among OBC, HAp, and Gel. The above results suggest that the OBC-HAp-G0.3 composite is a potential bone scaffold material.

Keywords: bacterial cellulose, hydroxyapatite nanocomposite, gelatin, scaffold material

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1 Introduction

Bone defects caused by congenital diseases, trauma, infection, and tumors are common. The autografts and allografts used for substitution and repair of bone defects are limited owing to short supply, additional complications, and immune rejection[1,2]. Therefore, the design and fabrication of a synthetic bone substitute with good mechanical properties, biological compatibility, biodegradation, and a porous structure through analysis of the micro assembly, biological function, and mechanisms underlying formation of natural tissue has been an important research direction. Bone is essentially a biocomposite of minerals and collagen. As an important mineral for bone, hydroxyapatite (HAp: $Ca_{10}(PO_4)_6(OH)_2$) nanocrystals have been widely studied and applied clinically because they show minimal rejection, good biocompatibility, and chemical bonding with bone[3,4]. Nevertheless, in most cases, it is difficult to properly balance the mechanical properties, biological function, and biodegradation of pure HAp bioceramics calcified at high temperature because of their difficult mold, fragility, and the lack of bone induction activity[5]. A new development in biomaterials is the biomimetic synthesis of HAp in a polymer matrix[6,7]. Evidence shows that gelatin (Gel), a partly denatured collagen protein, strongly drives self-assembly for the development of synthetic bone nanocomposites[8–10]. Furthermore, the Gel molecular chains contain repetitive arginine, glutamic and aspartic acid (RGD) motifs that favor deposition of extracellular matrix (ECX) and integrins, and they can thus modulate the adhesion and physiological activities of cells, as well as growth factor release, cell spreading and blood vessel growth to improve the final biological behavior of biomaterials[10,11]. Therefore, Gel is an ideal material for use in the biomimetic preparation of HAp nanocomposites.

Bacterial Cellulose (BC), which is synthesized by nonpathogenic microbial strains such as Gluconacetobacter, is a glucose-based, linear supramolecular poly-

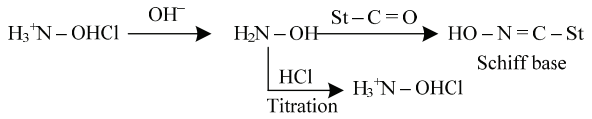
Corresponding author: Zhihua Shan
E-mail: zhihuashan@sohu.com

mer that forms a three-dimensional (3D) nanofiber network, and possesses numerous advantages for tissue engineering, including wettability, porosity, high crystallinity, biocompatibility, adaptability, and high mechanical strength and modulus[12,13]. Owing to these excellent characteristics, BC shows promise as a framework for the preparation of desired HAp composites. Although the degradation of BC by enzymes in the body is minimal, BC can be rendered more degradable by chemical modification such as oxidation[14,15]. In this study, a novel Oxidized Bacterial Cellulose (OBC) was prepared using H_2O_2 as the oxidation agent and SC-Fe_2+ (sodium citrate and Fe^{2+} complexes) as the catalyst under the desired current density. Electro-Fenton technology is a highly efficient, environmentally-friendly process for the direct production of hydroxyl radicals (·OH) (the oxidation potential can be up to +2.8 mV)[16,17]. After oxidation, the crystallinity and associated structure of BC were partially destroyed, which is expected to be helpful for degradation within the body, and large amounts of carbonyl and carboxyl groups were introduced on the surface, which could contribute to the immobilization of the Gel and HAp by formation of chemical bonds. Then, OBC-G-HAp nanocomposites (composites of OBC, Gel and HAp) were prepared by precipitating HAp nanocrystals in different concentrations of Gel solution. The microstructure, crystallinity, BET surface area, pore size, morphology and thermostability of OBC-HAp-G were analyzed by Fourier Transform Infrared (FT-IR) spectroscopy, X-Ray Powder Diffraction (XRD), specific Surface Area and Porosity (SAP), scanning electron microscopy (SEM), and Thermogravimetric Analysis (TGA), respectively.

2 Materials and methods

2.1 Materials

BC membranes were supplied by Hainan Yi De Food Co., Ltd. Sulfuric acid (H_2SO_4, 98%), sodium sulfate (Na_2SO_4, 99.5%), sodium citrate (99.5%), ferrous sulfate heptahydrate (Fe_2SO_4, 99.5%), and hydrogen peroxide (H_2O_2, 30 %) (for oxidation of BC by the Electro-Fenton method) and disodium hydrogen phosphate (Na_2HPO_4, 99.5%) and calcium chloride ($CaCl_2$, 99.5%) (for preparation of hydroxyapatite nanocrystals) were purchased from Chengdu Kelong Chemical Co., Ltd. Sodium hydroxide (NaOH, 99.5%) and hydroxyl ammonium chloride (NH_2OH·HCl, 98.5%) were obtained from Chongqing Chuandong Chemical Co., Ltd. for determination of the carbonyl content by the Schiff base reaction (see the reaction below). Gelatin (138579-66-5) was purchased from Sighter Biochemical Reagent Co., Ltd.



Percentage of carbonyl content = [BC blank sample) mL × acid normality × 0.028 × 100]/sample weight (dry basis) in g.

2.2 Preparation of OBC

BC pellicles (15 mm × 15 mm; 5 g, dry basis) and Na_2SO_4 (10 g·L^{-1}) as the electrolyte were added into an electrolytic cell equipped with magnetic force stirring and a water-bath heating device. The pH was adjusted to 3.0 using H_2SO_4. The desired current density was maintained using an electrochemical analysis system (LK98B II, LanLiKe, TianJing, China) equipped with a titanium plate as the cathode, a graphite plate as the anode, and a saturated calomel electrode as the reference. Then, 10 mL of 30% H_2O_2 and 20 mL of 0.1 mol·L^{-1} SC-$Fe2^{+}$ complexing liquid (sodium citrate and Fe^{2+} complexes as a catalyst) were added using a constant pressure dropping funnel. The temperature of the reactants was maintained at 50 ˚C under continuous stirring. Once the desired electrolysis time (3 h) was completed the current was turned off. Residual iron ions were removed by sodium citrate solution, and then the OBC pellicles were washed with deionized water until the water was colorless. The carbonyl content ((20.4 ± 0.5)%) of the OBC sample was determined by a Schiff base reaction with NH_2OH·HCl[16,19], and the carboxyl content ((18.9 ± 0.5)%) was analyzed by the electric conductivity titration method[20].

2.3 Preparation of OBC-HAp-G nanocomposite

OBC-HAp-G nanocomposites were prepared by biomimetic precipitation of HAp nanocrystals in various concentrations of Gel solution. A cycle consisted of immersing the OBC samples in 50 mM $CaCl_2$ (pH 5.8) in a constant temperature oscillating incubator (oscillation speed 100 r·min^{-1}, amplitude 20 mm) for 24 h (37 ˚C), rinsing the OBC pellicles in deionized water,