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Short communication

Bioactive cellulose grafted soy protein isolate towards biomimetic calcium phosphate mineralization

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ABSTRACT

The current study is a new approach to prepare an environmentally friendly and bioactive material from cellulose grafted soy protein isolate (SPI) for biomimetic calcium phosphate mineralization. Neat cellulose was oxidized using periodate then chemically modified by reacting with SPI followed by soaking in doubly concentrated simulated body fluid (2xSBF) solution. Scanning electron microscopy/energy dispersive X-ray spectroscopy, X-ray diffraction and transmission electron microscopy suggested the formation of uniform hydroxyapatite rod-like nanocrystals with ~50 nm diameter. The chemical composition of the prepared materials was further investigated using infrared spectroscopy and thermogravimetric analysis. The cytotoxicity of cellulose/SPI/calcium phosphate hybrid was evaluated using animal fibroblast baby hamster kidney cells (BHK-21). The cytotoxicity results suggested cellulose/SPI/calcium phosphate hybrid as potentially useful scaffold for regenerative therapies. The current article suggests a green strategy for preparing a new biohybrid material for tissue engineering.

1. Introduction

Development of functionalized green materials from renewable resources such as biopolymers has received increasing interest in biomaterials. Recently, natural polymers were investigated as a growth modifier reagents during biomimetic calcium phosphate mineralization instead of synthetic polymers (Kong et al., 2006; Salama, 2015; Schweizer and Taubert, 2007). Moreover, sustainable materials such as functionalized polysaccharides (Rodríguez et al., 2011), animal proteins (Bleek and Taubert, 2013) and plant proteins (Zhang et al., 2013) have been used to control calcium phosphate mineralization. In addition, biopolymers based hybrid materials were suggested as useful materials for bone tissue engineering, in spite of the difference in the chemical structure (Chiono et al., 2010; Salama and El-Sakhawy, 2014). Cellulose, a renewable and sustainable raw material, is a promising candidate in biomaterials (Salama, 2016; Salama et al., 2014). The fascinating structure of cellulose and the similarity of the mechanical properties of hard tissue and cellulose suggested the cellulose as a biomaterial for bone tissue engineering. Although, cellulose shows low bioactivity towards biomimetic inorganic mineralization due to the presence of only hydroxyl groups, several trials have been carried out to enhance its bioactivity. Grafting with functional monomers (Salama, 2015) or blending with bioactive polymers (Ogiwara et al., 2015; Salama and El-Sakhawy, 2016) are some reported trials. Moreover, increasing the ionic groups in cellulose would enhance heterogeneous nucleation of calcium phosphate and control the crystal growth, in analogy with hydroxyapatite nucleation in human bone(Ogiwara et al., 2015). It was reported that specific cleavage of the C_2—C_3 bond in the cellulose units generates two aldehydic groups. These active groups can further undergo Schiff base formation with amine containing materials. Cellulose based Schiff bases were prepared for different applications such as sensors (Kumari and Chauhan, 2014), metal removal (El-Menshawy et al., 2008), oil—water stabilizer (Visanko et al., 2014) biomaterials (Wang et al., 2015).

Proteins have been developed as growth modifiers during in-vitro biomimetic mineralization (Cheng et al., 2008; Schweizer and Taubert, 2007). SPI, extracted from soy bean, is mainly composed of Glycinin (7S) and β-conglycinin (11S). These two components, containing 20 different amino acids, provide various polar functional groups such as carboxyl, amine and hydroxyl groups (Liu et al., 2016). These functional groups are active and can undergo further reactions to improve protein properties. There are few reports where plant proteins, especially SPI, have been utilized as a bioactive material for biomimetic calcium phosphate mineralization(Zhang et al., 2013). However, SPI suffers from drawbacks like solubility in acidic or basic media which hampers its applications in biomaterials.

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The purpose of the current article is to design an environmentally friendly biomaterial from sustainable macromolecules. Oxidized cellulose functionalized with SPI has been reported aiming to enhance calcium phosphate nucleation under biomimetic mineralization. The mineralized calcium phosphate crystals were characterized. Moreover, the cytotoxicity test of oxidized cellulose/SPI/calcium phosphate hybrid was investigated as a biomaterial for tissue engineering.

2. Material and methods

2.1. Preparation of cellulose grafted SPI/hydroxyapatite hybrid

SPI was prepared by stirring 150 gm of defatted soy flour in one liter distilled water for 30 min. After that the pH of the suspension was brought to 9.0 with 0.2 N NaOH with continuous stirring for additional 2 h. The insoluble portion was removed through centrifuge while soluble soy protein portion was precipitated by changing the pH to 4.5 by 0.1 N HCl. SPI was washed with excess distilled water to remove undesirable carbohydrates and then dried in air.

Oxidation of microcrystalline cellulose (purchased from Acros) at the C2-C3 position was carried out with $NaIO_4$. 1 gm cellulose was treated with 0.83 gm of $NaIO_4$ at 40 °C for 6 h with continuous stirring in the dark. After adding an excess amount of ethylene glycol, the filtrated oxidized cellulose was washed with excess amount of distilled water. The aldehyde content of oxidized cellulose was determined as reported in the literature (Kumari and Chauhan, 2014). 2% SPI was dissolved in aqueous acetic acid solution at pH value of 3.8. Then, 1 gm of oxidized cellulose was stirred with 100 ml SPI solution for 5 h at 37 °C. The modified cellulose product was filtered, washed with deionized water, and dried at room temperature.

The graft yield of SPI introduced into oxidized cellulose was determined by measuring the weight gain of oxidized cellulose and reported as 27%. The in-vitro bioactivity of cellulose grafted SPI was investigated through immersing 1 gm of the sample in 100 ml double concentration simulated body fluids (2xSBF), as showed in our previous work (Salama and El-Sakhawy, 2014) for 7 days, to accelerate the calcium phosphate mineralization. SBF was renewed after centrifugation every 24 h (5000 rpm for 10 min) and the pH was checked on samples regularly. Finally, the samples were washed with double distilled water for 3 days and dried at room temperature for further analysis.

2.2. Characterization

Fourier transform infrared spectroscopy (FT-IR) was done on a Mattson 5000 FTIR spectrometer using KBr discs in the range of 4000–500 cm^{-1}. Scanning electron microscopy was carried out on Model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 30 K. Transmission electron microscope (TEM) images were taken with a JEOL JEM-2100 electron microscopy at 100k × magnification, with an acceleration voltage of 120 kV. Thermogravimetric analysis was performed on a PerkinElmer TGA7 thermogravimetric Analyzer under nitrogen from 20 to 800 °C with a heating rate of 10 K min^{-1}.

2.3. Cytotoxicity tests

Animal fibroblast baby hamster kidney cells (BHK-21) prepared in R&D sector, VACSERA, Egypt was cultured in Minimum essential medium with Earls salt (MEM-E), supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/ml penicillin G sodium, 100 units/ml streptomycin sulphate, and 250 ng/ml amphotericin B. The cells were then incubated for 3 days in a humidified 5% CO_2-containing balanced-air incubator at 37 °C. The cytotoxicity was measured using the MTT assay method. Plates were microscopically examined to detect the crystal formation in the treated cell cytoplasm. Dye was removed by phosphate buffer saline flushing. Crystals detected in the treated cells were dissolved using dimethyl sulphoxide (BDH-England) added as 0.05 ml/well for 30–45 min. Optical density (OD) was measured using ELISA reader (Dynatech, USA) at a wavelength of 570 nm. The mean optical densities of test and control wells were recorded. The optical densities is proportional to the number of residual living cells in culture.

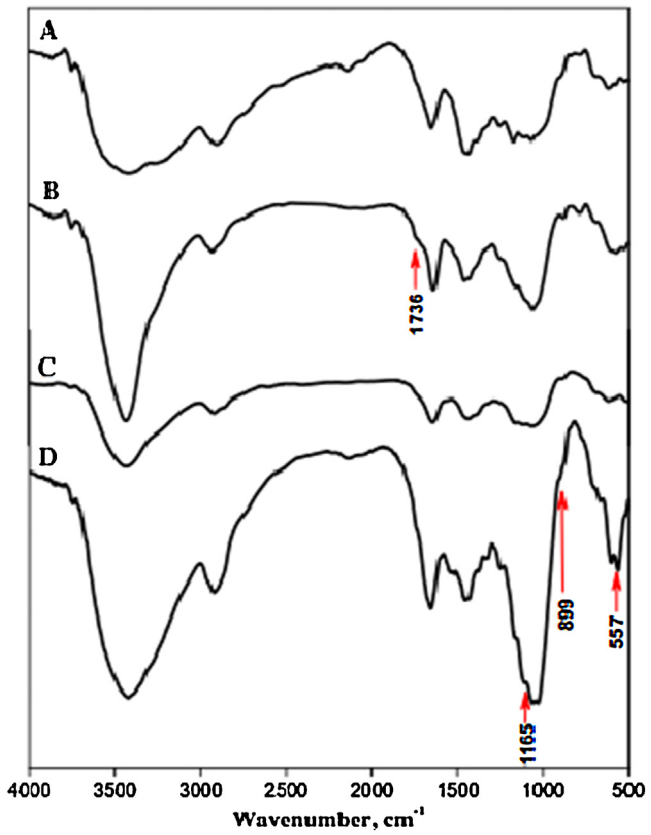

Fig. 1. FTIR spectra of neat cellulose (A), oxidized cellulose (B), cellulose grafted SPI (C) and cellulose grafted SPI/calcium phosphate hybrid (D).

3. Results and discussion

Neat cellulose was subjected to periodate oxidation to generate (−CHO) functional groups. The aldehyde content for oxidized cellulose was 44% which is close to the results obtained elsewhere (Kumari and Chauhan, 2014). These aldehyde groups can form Schiff bases upon reaction with free amino groups present in SPI. It has been reported that the calcium phosphate mineralization onto organic templates is tightly controlled by the existence of electronegative amino acid groups on the material surface. These function groups presented in the protein can form a bind with Ca^{+2} ions which initiate the nucleation, crystal formation originates and deposition of hydroxyapatite (Midha et al., 2016).

3.1. FT-IR

FTIR spectra was used to confirm the oxidation of cellulose, cellulose grafted SPI formation and biomimetic calcium phosphate mineralization. From Fig. 1, neat cellulose shows bands at 3435, 2894, 1427, and 1055 cm^{-1}, which can be assigned to the OH, CH_2,