Biomedical and Environmental Applications of Chitosan-based Nanomaterials

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ABSTRACT

Being naturally abundant resources and having many interesting physicochemical and biological properties, chitin/chitosan have been found useful in many fields. This paper describes the strategy to design a multifunctional, chitosan based nanomaterials and their biomedical and environmental applications. Different physicochemical methods including FESEM/TEM, PPMS were used to characterize the obtained nanomaterials. For each application, a series of specific characterizing methods were used for evaluating the applicability/capacity of materials.

Keywords: biomedical, environment, chitosan, nanomaterials

INTRODUCTION

Chitin, a natural polysaccharide and first identified in 1884, is the most abundant polymer after cellulose. The most important derivative of chitin is chitosan (CS), obtained by deacetylation of chitin in the solid state under alkaline conditions, resulting in a heterogeneous distribution of acetyl groups along its chains, or by enzymatic hydrolysis in the presence of chitin deacetylase (Fig. 1). A wide variety of biomedical applications in tissue engineering (1, 2), wound healing (3), drug and gene delivery (1, 4-6) was based on biocompatible, biodegradable and non-toxic CS. This paper demonstrates synthetic strategies, characterizations and the most important biomedical and environmental applications of chitosan-based nanoparticles and nanocomposites.

MATERIALS AND METHODS

Preparation of glucosamine sulphate

All the chemicals were of reagent grade used without further purification. Ferric chloride hexa-hydrate (FeCl₃·6H₂O), ferrous chloride tetra-hydrate (FeCl₂·4H₂O) and sodium hydroxide (NaOH) were purchased from Aldrich. Aluminium sulfate octadecahydrate (Al₂(SO₄)₃·18H₂O), copper(II) sulfate pentahydate (CuSO₄·5H₂O), cobalt(II) chloride hexahydrate (CoCl₂·6H₂O), cadmium chloride hemipentahydrate (CdCl₂·2.5H₂O), lead(II) nitrate (Pb(NO₃)₂), Nickel(II) sulphate hexahydrate (NiSO₄·6H₂O), 4-(2-Pyridylazo)resorcinol (PAR) were purchased from Acros.

NaOH, NH₄OH (26% of ammonia), oleic acid (C₁₇H₃₅COOH) were purchased from Aldrich. Chitin, chitosan was purchased from Nha Trang Aquatic Institute (Vietnam), OMSCS was from Aldrich. Glucosamine hydrochloride was obtained by acidic hydrolysis of chitin (with concentrated HCl) at 60–70°C. Glucosamine hydrochloride could convert into glucosamine sulphate with the help of Na₂SO₄ under room temperature (4).

Biocompatibility of the materials

In vitro biocompatibility of the materials was tested in simulated body fluid (SBF) at 37°C, for period of 0 to 7 days. SBF solutions have ionic concentrations similar to those of human blood plasma. SBF solutions were prepared according to the detailed protocol given elsewhere and denoted as 1x SBF and 5x SBF, corresponding to 1-fold and 5-fold concentrations respectively. pH of solution were buffered at the value of 7.4 using 0.01 M of tris-(hydroxymethyl)-aminomethane (CH₂OH)₂CNH₃. Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) was from Institute of Chemistry (Vietnam). Cells were cultured in RPMI 1640 (Roswell Park Memorial Institute) (Gibco) medium. This medium was supplemented with 10% Fetal Bovine Serum (Invitrogen), 100 IU/ml penicillin-streptomycine (Invitrogen), 2 mM – Glutamine (Invitrogen). Cells were grown in a humidified chamber in the presence of 9% CO₂, at 37°C.
**Evaluation of macrophage-colony stimulating factor**

Human buffy coat was obtained from National Institute of Hematology and Transfusion (Vietnam). Mononuclear cells were isolated by density gradient centrifugation using 1.077 g/ml Ficoll. Cells were cultured in RPMI 1640 medium with 1 µg/ml HGM-CSF (human granulocyte macrophage-colony stimulating factor) (MP Biomedicals). 7 to 12 week old Swiss mice were obtained from National Institute of Hygiene and Epidemiology (Vietnam). Human monocytes or mouse primary peritoneal macrophages were grown for 24 h on glass coverslips. 10⁶ cells were incubated with 0.05 mg MNPs for 2-15 h, then treated with either anti-human CD14 antibody (Bio Legend) or actins antibody (Invitrogen) for taking LSCM images.

**FT-IR Spectrometer analysis**

Infra red (IR) spectra were recorded with Nicolet 6700 FT-IR Spectrometer, using KBr pellets, in the region of 400-4000 cm⁻¹, with resolution of 4 cm⁻¹. Field Emission Scanning Electron Microscope (FE-SEM) and Transmission Electron Microscope (TEM) images was analyzed by Hitachi S-4800 and JEM-1200EX (Voltage:100kV, magnification X200,000), respectively. Dynamic light scattering (DLS) was analyzed with Zetasizer 2000 instrument (Malvern, UK).

**Confocal microscope analysis**

Laser Scanning Confocal Microscope (LSCM) images with excitation light of 488nm were collected with use of a ZEISS 510 LSCM with a 20x or 40x or 63x oil immersion objectives. The magnetic properties were measured using Physical Properties Measurement System (PPMS) from Quantum Design at fields ranging from 20 to 20 kOe at 25°C, with accuracy of 10⁻⁷ emu.

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**RESULTS AND DISCUSSION**

**Synthesis of glucosamine sulfate sodium chloride**

Glucosamine, an amino monosaccharide derived from chitin, has been demonstrated to be useful and effective in osteoarthritic therapy for nearly 40 years (7-10). Among its different derivatives including glucosamine hydrochloride, and N-acetyl-glucosamine, glucosamine sulfate (11), glucosamine sulfate is considered to be one of the most effective one (12). The synthesis of Glucosamine sulfate sodium chloride (Glu-SO₄²⁻) from chitin was performed (Fig. 2). FTIR, ¹H-NMR, ¹³C-NMR and HPLC spectra (shown in Fig. 3) confirmed that synthesized (Glu-SO₄²⁻) matched well with the standard sample. Its purity, quality and other characteristics with respect to United State Pharmacopoeia 26 were also fulfilled.

Synthesis of chitosan/hydroxyapatite for tissue engineering

The biomaterial Hydroxyapatite (HAp) has attracted many scientists thanks to its excellent physicochemical (structure, composition) and biological properties (bioactivity, biocompatibility, biodegradability). However, its mechanical (micro hardness, tensile) properties do not meet those of nature scaffolds. To overcome this limitation, HAp was combined with CS to improve mechanical properties of the resulting composites (13, 14). The HAp powder, first synthesized by coprecipitation of Ca²⁺ and HPO₄²⁻, then was immersed in chitosan solution at the weight ratio of 1 : 1, and finally dried to obtain the composite. To investigate its bioactivities, HAp/CS composites were submerged in simulated body fluid (SBF) at different testing periods. XRD spectra in Fig. 4 show that the crystallinity of HAp/CS, manifested in its typical peak of 211, increased gradually. FESEM images in Fig. 5 also confirmed that the HAp/CS has needle shapes, uniform dimensions and homogeneous dispersion after 5 and 7 days, whereas it still agglomerated at the very few days.