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Gentamicin modified chitosan film with improved antibacterial property and cell biocompatibility

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ABSTRACT

Gentamicin modified chitosan film (CS-GT) was produced using a three-step procedure comprising: (i) the chitosan solution was air-dried to form a chitosan (CS) film, (ii) using citric acid to generate the amide and carboxyl groups on the surface of CS, (iii) the CS with surface carboxyl groups was modified by grafting of gentamicin. After modification, this CS-GT film has excellent hydrophilicity and biocompatibility. It is very evident that the gentamicin grafting treatment significantly improves the antibacterial properties of the CS film. Our preliminary results suggest that this novel gentamicin modified chitosan film, which can be prepared in large quantities and at low cost, should have potential application in biomedical applications.

1. Introduction

To prevent the allergic reaction risks, the surfaces of some metal-base bone substitutes are usually protected by a bioactive organic coating [1–3]. As one of the most representative material of these coatings, chitosan consists of a large number of hydroxy and amino groups, and possesses outstanding properties, such as non-toxicity, biodegradability and biocompatibility [4–6]. The above advantages make the chitosan-based material has been widely used in the field of tissue engineering [7,8]. However, its application is limited due to the hydrophobic property and common bacterial infection after surgery. Dropping antibiotics to the wound is an efficient method to solve this problem, but it is difficult to fix the amount of the antibiotic, which would cause the waste of the drugs. In addition, it is also a troublesome work to drop the drugs so frequently [9–11].

In this paper, we use the chemical cross-linking method to add gentamicin, a broad-spectrum aminoglycoside antibiotic which is effective against most species of both gram-positive and gram-negative aerobic bacteria through inhibits the bacteria protein synthesis [12–14], to the chitosan to prepare a novel gentamicin-containing antibacterial chitosan film (CS-GT). The gentamicin was grafted onto the surface of chitosan film via citric acid as a transitional object, 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) as the cross-linker. With this approach, we want to overcome the current problems of chitosan materials that used for biomedical applications.

2. Material and methods

2.1. Materials

Chitosan (≥95% deacetylated) and citric acid were purchased from Aladdin Industrial Inc, (Shanghai, China). Gentamicin sulfate (GT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). EDC was purchased from Sass chemical technology co., LTD, (Shanghai, China) and NHS was obtained from Aladdin Industrial Inc, (Shanghai, China). All cell-culture related reagents were purchased from Sigma Chemical (St. Louis, MO, USA). Phosphate buffered saline (PBS) was prepared from the tablet form (Calbiochem Corp, Germany).

2.2. Preparation of films

Firstly, the purified chitosan powder was dissolved in 0.01 mol L^{-1} acetic acid solution. Gentamicin and the citric acid were respectively dissolved in deionized water with the concentration of 10 mg mL^{-1}. Secondly, chitosan solution was air-dried to form a CS film. Thirdly, the CS film was immersed into citric acid solution to generate the formation of amide and carboxyl group. Afterwards, the film with surface carboxyl groups was transferred to the gentamicin solution containing EDC and NHS (the mass ratio

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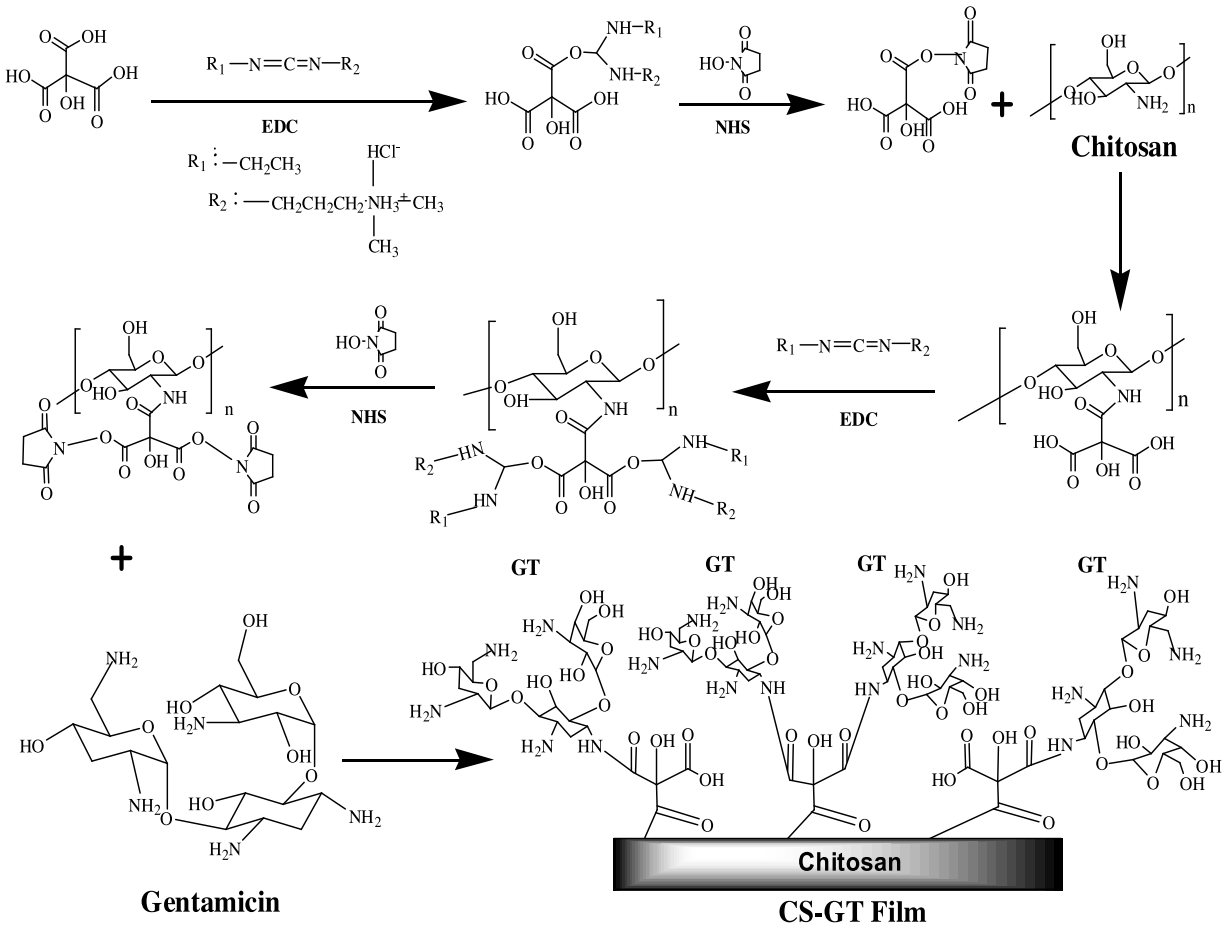

Scheme 1. The schematic illustration of possible reaction routes involved in the formation of CS-GT film.

of EDC:NHS:CS-GT is 1:1:6). The cross-linking process was carried out by stirring the mixture for about 4 h. After modification, the CS-GT film was rinsed 3 times with Milli-Q water, and air-dried again. For comparison, chitosan film without chemical modification was prepared as the control group (CS). Scheme 1 displays the schematic illustration of possible reaction routes involved in the formation of CS-GT film.

2.3. Fourier transform infrared (FTIR) spectroscope

The FTIR spectrum of the CS and CS-GT film was analysed using Fourier transform infrared-attenuated total reflectance (FTIR-ATR, Vector 33, Bruker, Germany). Before acquiring the transmission infrared spectra of the two samples, a background spectrum was collected. All the spectrums were gained obtained from 4000 to 650 cm^{-1}.

2.4. X-ray photoelectron spectroscope (XPS)

X-ray photoelectron spectroscopy (XPS) spectrometer used was a Kratos Analytical (UK) model Axis Ultra system with a mono Al Kα X-ray source ($h\nu$ = 1486.6 eV, 150 W). Binding energy was calibrated by C 1s of C—C as 284.6 eV. All spectra were obtained using a 202 μm diameter analysis area. The wide scanning was gathered with pass energy of 140.00 eV and a step size of 0.25 eV, while high-resolution region scans were operated with pass energy of 55 eV at a scan rate of 0.1 eV per step over a range of 1000 eV. Chemical analysis and quantification spectra from the individual peaks of the CS and CS-GT film were obtained. A Gaussian-Lorentzian function was applied to fit the spectra for each peak, in order to assume the kinds and quantifications of the main elements.

2.5. Surface contact angle measurements

Contact angles (CA) were tested by the sessile drop method. Measurement was carried out on Powereach JC2000D1 with an injection volume of 1 μL distilled water as medium. CA was calculated by a circle segment function intersecting with a straight baseline representing the surface (n = 5).

2.6. Swelling test

Swelling studies were performed for CS and CS-GT using PBS (PH = 7.4). Water absorption of the CS and CS-GT film was tested by swelling them in PBS at about 37 °C. Wet weight of the films was, after gently blotting the membrane surface with filler paper to remove the absorbed water, weighed immediately at different time intervals. The water absorption of the films was calculated by the following formula:

$$\text{Water absorption} = (M_t - M_0)/M_t \times 100\%, \quad (1)$$

It's worth noting that the dry materials have swelling feature after absorbing water, so it's meaningful to measure the dimensional changes of the swelled films. The thickness and surface area of the CS and CS-GT films were measured by a micrometer caliper and rules, respectively. Variations of thickness and surface area are calculated according to the following formulas:

$$\text{Thickness increase} = H_t/H_0; \quad (2)$$