PHYSICOCHEMICAL PROPERTIES AND BIOCOMPATIBILITY OF CHITOSAN DERIVATIVES WITH ANIONIC GROUPS

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Abstract

Chitosan is widely recognized a biodegradable material with broad potential, have been proved to have biocompatibility. The aim of this study was to investigate chitosan derivatives with anionic groups as biomaterials for tissue engineering, *in vitro*. Chitosan derivatives with anionic groups were prepared by reacting chitosan with alkane sultone via ring-opening reaction. To explore changes in the physicochemical properties of chitosan derivatives, we analyzed structural changes by FT-IR, 1H NMR, and a wide-angle X-ray diffractometer (WAXD). Resulting N-sulfoalkylchitosan derivatives showed the amphoteric property due to the presence of anionic sulfonate groups. WAXD of N-sulfoalkylchitosan derivatives showed that their crystallinity were decrease with increase in the introducing sulfonate moiety on free amine of chitosan. We further examined changes in the biocompatibility of chitosan and N-sulfoalkyl chitosan derivatives based on cell growth rate. Human dermal fibroblasts (HDFs) were proliferated better to chitosan than to N-sulfoalkylchitosan derivatives.

Introduction

Chitosan has known promise as one of structural biocompatible biomaterials for a number of tissue engineering applications. It has the wound healing effect through the induction of cytokine production and activation of inflammatory cells in animals (1). It has been reported that fibroblasts might strongly adhere on chitosan film (2). But, Denuziere et al (3) and Izume et al (4) appeared that in the case of *in vitro* experiments, although chitosan is not cytotoxic, it inhibits the cell proliferation. Thus, its cytocompatibility towards fibroblasts allows its use in combination with other materials, such as collagen and glycosaminoglycans. For improvement of the cytocompatibility of chitosan, we reported neutralized chitosan as a based material for tissue engineered artificial dermis, whose low cell binding capacity could be improved by collagen coating or supplementation of bFGF and/or human fibronectin (5). The aim of this study was to investigate chitosan derivatives with anionic groups as new biomaterials for tissue engineering, *in vitro*. Chitosan derivatives with anionic groups were prepared by reacting chitosan with alkane sultone via ring-opening reaction.

Material and Methods

Chitosan derivatives with anionic groups were prepared by reacting chitosan with alkane sultone via ring-opening reaction. Chitosan (86 % deacetylation degree, 1 g) in 100 ml of acetonitrile/water (8:1, v/v) was reacted with the 0.1 to 0.5 molar ratio of propane sultone to glucosamine unit, which the mixture was stirred at 72°C for 24 h. The reaction mixture was cooled at room temperature, unreacted reagent was removed by filtration. The filtrate was precipitated in absolute ethanol. The precipitation was washed with absolute ethanol again, and then it was finally filtered, collected and freezing-dried to gain resulting chitosan derivatives. Several kinds of chitosan derivatives were code-named as CS, SC-0.1, SC-0.25, and SC-0.5, according to the ratio of propane sultone to glucosamine unit, respectively.

Structural changes between chitosan and sulfoalkyl chitosan derivatives as a consequence of sulfoalkylation were determined by infrared spectroscopy. Spectra were recorded with a Nicolet 5DX FT-IR spectrophotometer on translucent discs obtained by pressing the ground materials and KBr. The degree of substitution for sulfoalkyl chitosan derivatives was determined by elemental analysis. Elemental analysis was performed using a Foss Heraeus Analysentechnik GmbH. ^{1}H-NMR spectra were measured on a Varian VX 300 using D_2O/DCl as a solvent. The crystallinities of chitosan and sulfoalkyl chitosan derivatives were investigated by X-ray diffractograms obtained with a Rigaku Denki Model RAD-C. The X-ray source was nickel-filtered CuKα radiation (40 kV, 20 mA).

Human dermal fibroblasts (HDFs) were used as a model representation of cells a biomaterial may encounter *in vivo*, and used passage 2 and 6, and cultured in FGM-2 media. In order to biocompatibility of chitosan derivatives, each chitosan derivatives was dissolved in 0.1 M HCl to give 2 w/v% solution. Each 1 %, 5 %, and 10 % solution of chitosan and chitosan derivative solution was mixed with the same culture media as described earlier. HDFs were seeded at concentration of 3×10^3 cells/well onto each 96 well containing the mixed solution. They were incubated at 37□ in a humidified 5 % CO_2 atmosphere. The culture medium was changed 4 hours post-seeding and every 2 days thereafter. Cell development was followed by daily light microscopic observation. HDFs viabilities evaluated by MTT assay measured at 540 nm using a Bio-Rad model 450 microplate reader. All data are presented as the mean values of three counts.

Result and discussion

Chitosan derivatives with anionic groups were prepared by reacting chitosan with alkane sultone via ring-opening reaction. Figure 1 shows the FT-IR spectra of chitosan and its derivatives. There were three characteristic peaks of chitosan at 3455, 1093, and 661 cm^{-1}(16). As increasing molar ratio of propane sultone to glucosamine unit, we found some new peaks at 1350 and 1175 cm^{-1} due to asymmetric and symmetric stretch of S=O group, respectively. The intensities of two peaks are further increased with an increase in the propane ratio. A broad strong band at 1000-750 cm^{-1} due to the S-O stretch are also shown in the FT-IR spectra for the chitosan derivative.

The sulfur contents of chitosan and resulting N-sulfoalkylchitosan derivatives, CS, SC-0.1, SC-0.25, and SC-0.5, were 0.0%, 0.82%, 1.57%, and 3.18%, respectively.

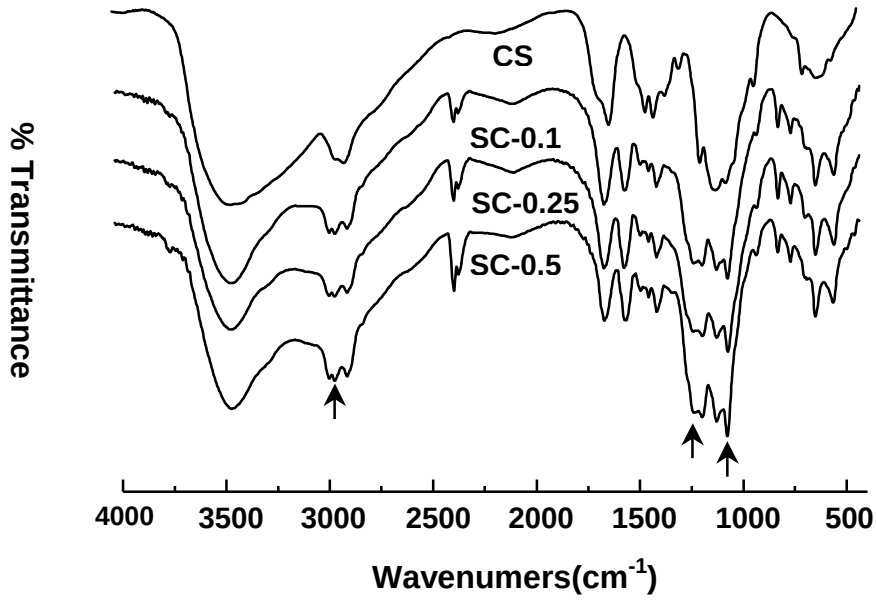

Figure 1 : FT-IR specta of chitosan and its derivatives.

Figure 2 shows the X-ray diffraction patterns of chitosan and its derivatives. WAXD of N-sulfoalkylchitosan derivatives showed that their crystallinity were decreased with increase in the introducing sulfonate moiety on free amine of chitosan. Resulting N-sulfoalkylchitosan derivatives showed the amphoteric property due to the presence of anionic sulfonate groups.
To compare the biocompatibility of chitosan with of *N*-sulfoalkyl chitosan derivatives, we evaluated the effect of the concentration and moiety of sulfonate groups on free amine of chitosan using MTT assay. Figure 3 showed that the cytotoxicity against HDFs of N-sulfoalkylchitosan derivatives as well as chitosan had dependent of their concentration. N-sulfoalkylchitosan derivatives showed that their cytoxicity was decreased with increase in the introducing sulfonate moiety. HDFs were proliferated better to chitosan than to N-sulfoalkylchitosan derivatives in general. Interestingly, SC-0.25 was very similar proliferation behavior of HDFs to chitosan. There seemed to be an optimum moiety of sulfonate groups introduced in chitosan for proliferation of HDFs. These results suggest that CS-0.25 might be a suitable for tissue engineering application.

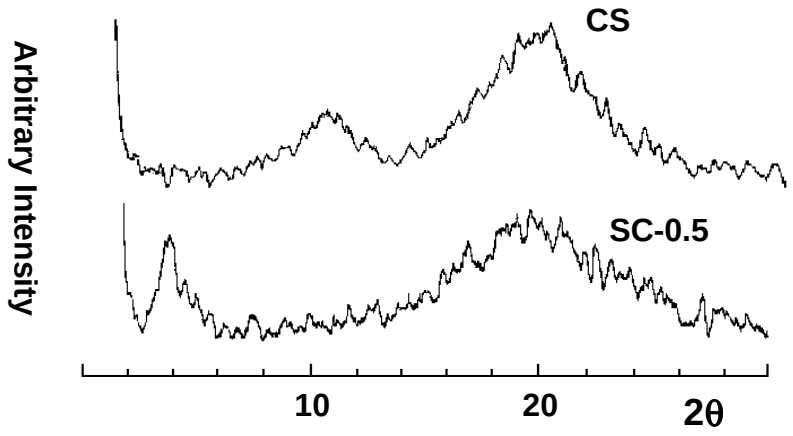

Figure 2 : XRD of chitosan and its derivatives.

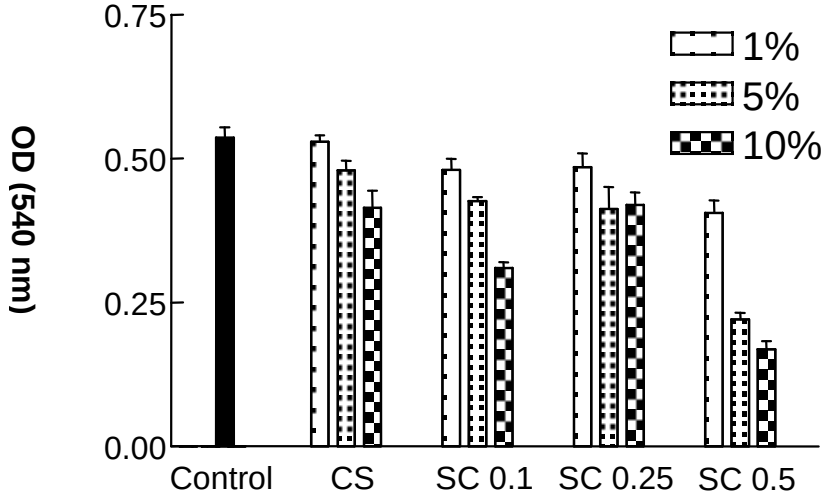

Figure 3 : The effect of the concentration and moiety of sulfonate groups introduced on chitosan using MTT assay.

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