Surface characterization and *in vitro* biocompatibility study of surface-sulfonated chitosan membrane

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INTRODUCTION

Chitosan, a kind of glycoaminoglycan, has good biocompatibility and biodegradation, and can be applied as scaffold material in tissue engineering. In addition, blending same low molecular weight glycoaminoglycan such as chondroitin sulfate or keratin in scaffold, can improve cell adhesion and cell proliferation[1-2].

Therefore imitating these low molecular weight glycoaminoglycan in structure, this study tried to immediately sulfonate the hydroxy groups at C6 of chitosan. At the same time, protection strategy was used to hold the amino groups at C2 of chitosan which was known as the major positive functional group of chitosan with special biological character in cell culture.

Surface characterization and in vitro biocompatibility of modified chitosan were evaluated.

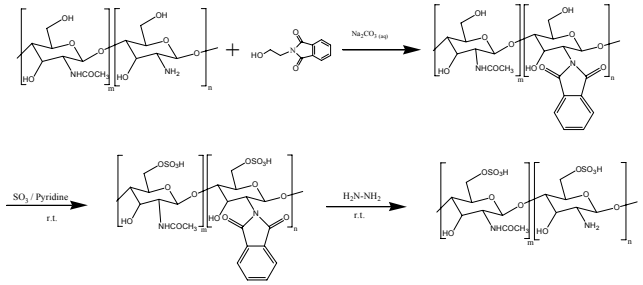
MATERIALS AND METHODS

Preparation of chitosan membrane :

Chitosan was dissolved in 1% acetic acid aqueous solution to form a 2 wt% polymer solution. The polymer solution 40ml was placed in a glass petri dish, and dried. To remove the deposited chitosan membrane from petri dish, chitosan membrane was neutralized by NaOH/ ethanol solution.

Amino group protection and sulfonation:

Protection strategy of the C2 amino groups and sulfonation of C6 hydroxy group were illustrated in scheme 1:



Scheme 1. Amino group protection and chitosan sulfonation

Surface characterization and *in vitro* biocompatibility evaluation:

Surface characteristics of modified chitosan membrane were analysed by ATR-FTIR, Energy Dispersive Spectrometer (EDS), and SEM. Zeta potential at the surface of the modified chitosan membrane was analysed by Electro Kinetic Analyzer (EKA) with streaming potential method[3]. In addition, *in vitro* biocompatibility of modified chitosan membrane was evaluated.

RESULTS AND DISCUSSIONS

Surface characteristics of modified chitosan membrane

The surface chemistry of the chitosan membranes was evaluated through the ATR-FTIR spectra shown in Fig. 1. In the lower wavenumber region around 1020– 1160 cm^{-1}, it is noticed that the band shape of sulfonated chitosan was clearly different from the unmodified chitosan. This could be attributed to the formation of O-sulfonate groups on the membranes[4] in addition to the C-O absorption band associated with the chitosan molecule itself.

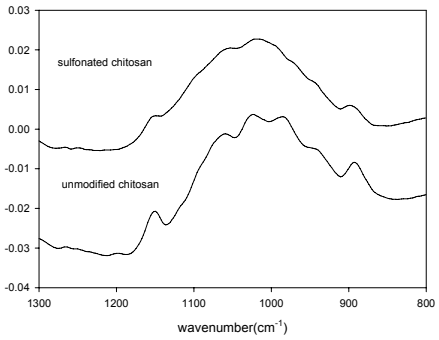
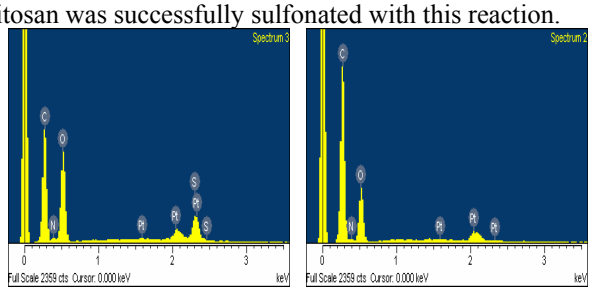


Fig 1. ATR-FTIR spectra of sulfonated chitosan and unmodified chitosan

The surface composition was analyzed by EDS shown in Fig 2. The sulfur peak appearing in the spectra of sulfonated chitosan is noted. This finding indicated the chitosan was successfully sulfonated with this reaction.



(a) (b)

Fig 2. The EDS spectra: (a)sulfonated chitosan, (b) unmodified chitosan.

Zeta potential at the surface of modified chitosan membrane:

The surface zeta potential of chitosan membranes were analysed by EKA with streaming potential method. Through the protection strategy, rhe surface-sulfonated chitosan membrane not only has the negative sulfate groups, but also preserves the positive amino groups. Therefore, the surface-sulfonated membrane has zwitterionic feature. When solution pH is lower than the pKa of –O-SO3H, the surface zeta potential of membrane is positive, and then switch to negative as pH is higher than the pKa of $-NH_3^+$.

In vitro biocompatibility evaluation:

In vitro biocompatibility of surface-sulfonated chitosan membrane was evaluated by 3T3 fibroblast cell culture, and implied the improvement of cell proliferation.

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