Electrospinning of Chitosan and its Correlation with Degree of Deacetylation and Rheological Property

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Statement of Purpose: Electrospinning is a spinning technique used to produce nanofibrous structure of biopolymers which is similar to natural extracelluar matrix (ECM) for tissue engineering and scaffolding. The nanofibrous structure has very high surface area with certain porosity which helps the cells to attach, proliferate, and migrate. The cells on artificial ECM then help the tissue to grow and therefore, promotes wound healing [1,2]. Chitosan can be used as a component in the extracellular matrix to promote wound healing because of biological properties such as biocompatibility, biodegradability, and its antibacterial, haemostatic and wound healing properties [3,4]. A potential application of chitosan nanofibers is in the treatment for post surgical adhesions. Around 80% of patients suffer from adhesion after abdominal surgery. Even after careful surgical procedure, high chances of post surgical adhesions are possible. Various methods have been proposed to prevent and reduce the post surgical adhesion like pharmacological agents and use of barrier films [5,6]. One potential limitation of using a barrier film is that it can slip away; exposing the two damaged wound areas which may come in contact with each other forming an adhesion [7]. This presents an opportunity to improve the efficiency of barrier product which promotes wound healing and increasing the effectiveness of film placement which further reduces the chance of post surgical adhesion to occur as a result of unsecured movement of barrier film. One method is by electrospinning a nanofiber layers of biopolymer such as chitosan on the barrier film.

Methods: The materials used in this study include two different types of chitosan and three different solvents. Low molecular weight chitosan (CS-Sigma) obtained from Sigma-Aldrich Company, with the reported viscosity of 20-300cp, deacetylation of 75-85%, and molecular weight of approximately 50,000 - 190,000 Daltons based on viscosity. The second chitosan sample was obtained from Vanson Company with a (medium viscosity chitosan) viscosity average molecular weight of 1.0×10^6 was confirmed using intrinsic viscosity and the Mark Houwink Sakurada equation, $[\eta] = kM^a$ and deacetylation of 70% measured by acid base titration method [8]. Deacetylation of chitosan was carried according to method described elsewhere. To achieve higher degree of deacetylation, the same process was repeated twice [9]. Different degree of deacetylation (DD) of chitosan solution were prepared in three different solvent (formic acid, acetic acid, and TFA) of different concentration (1-7 wt%). Electrospinning of these solutions were carried out using typical horizontal setup. The rheological studies were used to determine the zero shear rate viscosity (η_o) of the chitosan solutions to see the effect of viscosity on fiber formation through electrospinning process. Scanning electron microscopy (SEM) was used to examine the quality of electrospun sample and determine the fiber diameter, fiber diameter distribution and to further inspect the morphology of chitosan nanofiber web with the polysaccharide film (SEPRA film).

Results: Initial trials of commercial chitosan in acetic acid resulted in bead formation. The 70% DD was un-dissolved after 120hr of stirring. The rheological studies were used in order to know the electrospinnability of chitosan-acetic solution. According to results, the nanofibers formation starts from concentration 3wt% for DD-81% with some bead formation. For DD-90%, the nanofibers formation was possible from 3wt% to 4wt% because of high DD (see Figure 1). Successful electrospinning of chitosan were possible in TFA solution with higher concentration upto 7wt% without bead formation having average fiber diameter of 290±8 nm. The optimum concentration of chitosan was selected in order to electrospin chitosan on Seprafilm™ to see the effect of DD. The results showed nearly defect free chitosan nanofibers electrospun onto Seprafilm™.

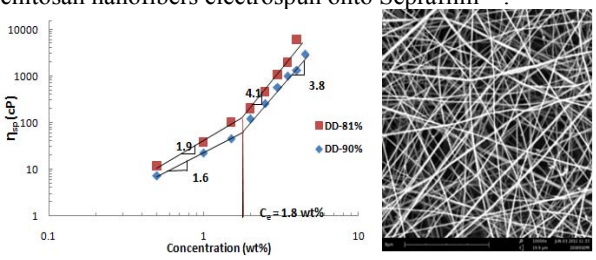

Figure 1. Plot of specific viscosity (□sp) versus concentration for the chitosan of (DD-81%) and (DD-90%) in 90% acetic acid as solvent. The entanglement concentration is 1.85 wt% and it is determined by the change in slopes. SEM of electrospun chitosan-TFA at 6wt% on Seprafilm™.

Conclusions: From the SEM results, below the entanglement concentration (1.8 wt%), only beads had formed and above this concentration, the electrospinning was not possible because of the viscous nature of solution. The average fiber diameter increased with the increased in chitosan concentration for DD-90% and also it showed larger distribution of fibers. Chitosan-TFA solutions of 6 wt% was been successfully electrospun onto the Seprafilm™ showing nearly defect free chitosan nanofibers with different degrees of deacetylation. This could be an excellent example of biomedical product that could be use as a barrier film with chitosan nanofiber layer on it for the treatment of post surgical adhesion with fast wound healing rates.

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