**Optimizing Mechanical and Cell Adhesion Properties of Chitosans Through Simultaneous Manipulation of Molecular Weight and Crosslinking**

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**Introduction:** Chitosan obtained by N-deacetylation of natural polymer chitin has recently sparked great interest in tissue engineering due to several desirable properties such as minimal foreign body reaction, mild processing conditions, and availability of chemical side groups for attachment to other molecules. Current difficulties with using chitosan include low strength and inconsistent behavior with cell adhesion. Covalent crosslinking of chitosan is one approach to modifying its mechanical and biological properties. The covalently stabilized chitosan obtained could have reduced crystallinity, and might thus produce a more amorphous network microstructure with the potential for elastomeric behavior. In this study, we examined the effects of both crosslinking density and chitosan molecular weight (MW) on the properties of crosslinked chitosans. Low molecular weight chitosans were crosslinked with a dicarboxylic acid (sebacic acid), and the mechanical and cell-interaction properties of the resultant materials were characterized. Specifically, monotonic tensile testing of cast chitosan-sebacic acid films was conducted and the adhesion and growth of lamb aortic smooth muscle cells (SMC) on these materials was examined.

**Materials and Methods:** 1.5 wt% Chitosan (85% deacetylated, 600 kDa, Fluka) was dissolved in 0.2 M acetic acid. Solution aliquots were reduced to molecular weights of 5, 10, 20 and 50 kDa using nitrous acid depolymerization [1]. Sebacic acid was activated in aqueous solution with 1-ethyl-3-(3-dimethylaminopropyl) carboiidide (EDC). The activated sebacic acid was then blended with reduced MW chitosan solutions for 3 hours to generate sebacate-crosslinked chitosan aggregates in solution. Activated sebacate was blended in at di-acid to chitosan-amine molar ratios of 0.01 and 0.1. Films of the crosslinked materials were prepared by casting the crosslinked solutions, air drying and then washing with 3% ammonia solution followed by PBS. Rectangular film samples were subjected to uniaxial tensile testing under hydrated conditions to determine tensile strength, elastic modulus (at 20% strain) and breaking strain for the crosslinked and non-crosslinked chitosan films. To evaluate cell interactions, films were cast into polystyrene tissue culture dishes, neutralized as described above, and sterilized with 70% isopropanol overnight. After washing with PBS, lamb aortic smooth muscle cells (SMC) were seeded onto the films in serum-supplemented MCDB 131 culture medium. The cells were seeded at a density of 5000 cells/cm$^2$. Cell adhesion and spreading were characterized by quantitative image analysis.

**Results and Discussion:** Mechanical testing showed that the more highly crosslinked films (1:10 sebacate:amine ratio) exhibited lower stiffness values as compared to 1:100 crosslinked and non-crosslinked chitosan films (Figure 1). These more highly crosslinked films also had higher values of breaking strain, particularly at chitosan molecular weights of 10 and 20 kDa. However, no significant differences in tensile strength were observed between materials. Aortic SMCs cultured on these films exhibited increasing levels of spreading and proliferation rates with increasing crosslinking density (Figure 2). Sebacate crosslinking of low MW chitosans provides a mechanism for modulating both mechanical and cell adhesion/proliferation properties. The improved cell interaction may be the result of increased protein binding to the surface due to the presence of hydrophobic domains contributed by the sebacate crosslinker. In addition, changes in chitosan crystallinity due to the steric changes produced during solution crosslinking may also influence film microstructure and serum protein binding. In addition to crosslinking effects, cell adhesion was also influenced by the MW of the chitosan molecules and showed increases with MW up to at least 50 kDa. However, high MW chitosans (i.e. 600 kDa) exhibited lower cell spreading and proliferation. Current work is aimed at more detailed characterization of the effects of MW, crosslinking density and membrane microstructure, and results will be reported.

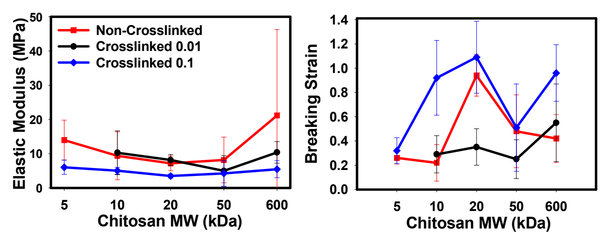


**Figure 1:** Mechanical properties of films

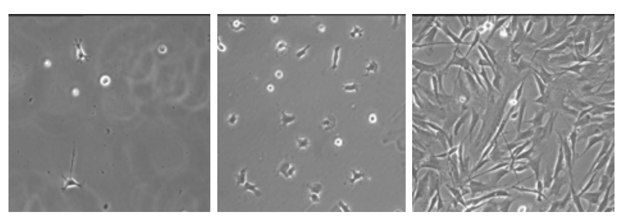

**Figure 2:** Vascular SMC on sebacate-crosslinked 50 kDa chitosan films. Images show films with crosslinking densities of 0.0, 0.01, and 0.1 respectively.

**Conclusions:** Solution phase crosslinking of reduced molecular weight chitosans produced materials with lower stiffness and higher extensibility. Cell spreading and proliferation was also enhanced in proportion to the degree of crosslinking.

**References:**

1. G Allan, M Peyron, Carbohydrate Research 277 (1995) 273-282.