Mechanical Property Improvements to Chitosan through Disulfide Bonds

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Statement of Purpose: Chitosan has gained popularity in the field of tissue engineering and regenerative medicine due to its widespread availability, degradation ability in vivo, and favorable immune and tissue responses in vivo. However, chitosan lacks the sufficient mechanical properties to widen its use as a biomaterial in certain tissue repair applications. For example, the mechanical properties of porous chitosan scaffolds are not close enough to the values of the native human aortic valve leaflet [1]. Significant work has been done to crosslink chitosan to improve its mechanical properties, but many crosslinking approaches diminish the mechanical properties of chitosan [2]. In the work presented here, n-acetyl-cysteine is grafted to chitosan in order to introduce sulfhydryl functional groups to chitosan polymer chains. The sulfhydryl groups are then oxidized to form disulfide bonds between adjacent n-acetyl-cysteine groups grafted to chitosan. This will result in a networked polymer with reduced crystallinity and significant improvements in mechanical properties over unmodified chitosan.

Methods: Medium molecular weight chitosan was dissolved in hydrochloric acid, and reacted with n-acetyl-cysteine (NAC) pre-activated with 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), in order to graft NAC to chitosan polymer chains. The amount of EDC was varied in order to vary the amount of NAC covalently bonded to chitosan. The result was that two polymers were synthesized with different percentages of glucosamine units substituted with NAC, to give a polymer with 6% degrees of substitution (DS) and 15% DS. Unmodified chitosan (0% DS) served as a control for all experiments. This reaction mixture was then precipitated by addition of 30% liquid ammonia, and redissolved in acetic acid. Films were cast from the polymer solutions, and mechanical testing was performed for films in a constantly hydrated state, and at a constant strain rate. Furthermore, the cell proliferation and viability of porcine aortic smooth muscle cells (PASMC) cultured on polymer films was assessed by alamarblue and calcein AM.

Results: Grafting of NAC to chitosan and subsequent disulfide bond formation resulted in a 4 fold increase in maximum tensile strength, to a value of 9.40 MPa for chitosan substituted with 15% DS NAC. Similarly, a fivefold increase in maximum tensile strain was observed for chitosan substituted with 15% DS NAC, to a maximum value of 362% of original specimen length. The elastic modulus of all polymers studied varied with higher and lower strains. Figure 1 shows the elastic modulus up to 20% strain for all polymers studied. At low strains, grafting of NAC caused an increase in elastic modulus, and thus an increase in stiffness for 15% DS NAC. Conversely, the elastic modulus of the polymers decreased for an increase in DS of NAC as strain was increased. The elastic modulus was decreased to a value of 2.03 MPa for 15% DS NAC. The specific growth rate of PASMC cultured on polymer films was also calculated for chitosan modified with 0%, 6%, and 15% DS NAC. Chitosan modified with 15% DS NAC showed a fourfold improvement in specific growth rate over 0% DS NAC, and thus a higher degree of cell proliferation.

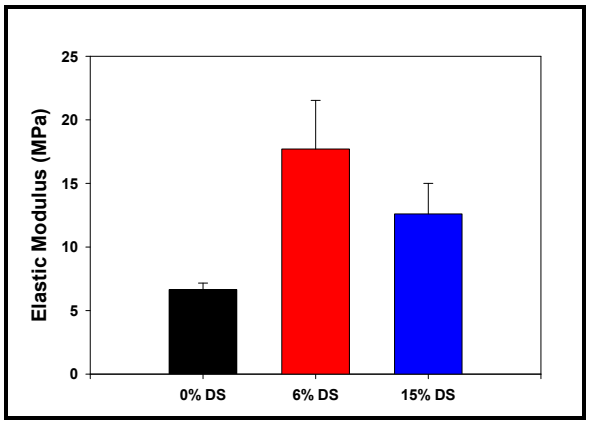

Figure 1. Elastic Modulus of Polymers at 20% Strain

Conclusion: Substituting chitosan with NAC for 6% DS and 15% DS reduces the crystallinity of chitosan, and this is seen in the increase in tensile strength for an increase in DS of NAC. Moreover, it has been reported that an increase in cell proliferation for cells cultured on polymer films is due to a reduction in crystallinity of the polymer. [3]. At low strains, the stiffness of chitosan modified with NAC is similar to the stiffness observed in the circumferential direction for a native human aortic valve leaflet [1]. Furthermore, the decrease in elastic modulus and fivefold increase in maximum tensile strain show that introducing disulfide bonds in chitosan introduces elastomeric properties. The disulfide bonding between chitosan polymer chains forms a networked polymer, which has been shown to introduce elastomeric properties in polymers [4]. The overall result of covalently attaching NAC to chitosan is a tougher, more elastic and less crystalline biopolymer, for attachment of NAC up to 15% DS.

References:

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