Mechanical Properties of Processed Chitosan Fibers for Use in Scaffold Reinforcement

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Introduction: Scaffold strength is an area of concern in current tissue engineering research. Because of superior molecular alignment, chitosan fibers have mechanical properties that allow them to reinforce porous chitosan and other hydrogel scaffolds. Previous studies have shown that annealing chitosan fibers led to a significant increase in mechanical strength. In addition, cross-linking of chitosan films with heparin has been shown to improve cell adhesion, proliferation and ECM deposition. This study sought to investigate the effect of annealing temperature and heparin crosslinking on the mechanical properties of chitosan fibers, with the ultimate goal of forming advanced composite chitosan scaffolds with superior mechanical and biological properties.

Materials and Methods Chitosan fibers were formed by extruding chitosan solution (1.5 wt% in 2% acetic acid (250 kDa, 80% deacetylated) through a 26 guage Teflon catheter into 25% aqueous ammonia. Chitosan hydrogel fiber so formed were water washed and air dried. Extruded fibers were subjected to four different treatments to examine the effects of various combinations of annealing temperature and heparin crosslinking. Chitosan fibers dried at room temperature (RT) served as the control. The test conditions were: 1) Fibers dried at RT and crosslinked with EDC-activated heparin; 2) Fibers dried/annealed at 195°C for 15 minutes and cooled at 1°C/min; 3) Fibers dried/annealed at 195°C and crosslinked with with EDC-activated heparin. Diameters of all fibers were measured after rehydrating in PBS. The feasibility of generating non-woven fibrous scaffolds was investigated by using sequential washes of acetic acid, ammonia and PBS to fuse assembled fibers. The effects of the fusion treatment on individual fiber preoperties were also examined. Tensile strength, breaking strain and elastic modulus of hydrated fibers and fusion-processed fibers were evaluated using uniaxial tensile testing at a constant strain rate of 1.0 min^{-1}.

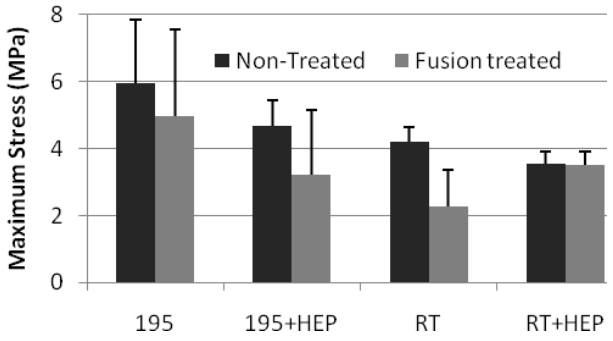
Results and Discussion Fibers, dried at RT, and rehydrated exhibited a diameter of 207 μm, a tensile strength of 4 MPa, and an elastic modulus of 12 MPa.

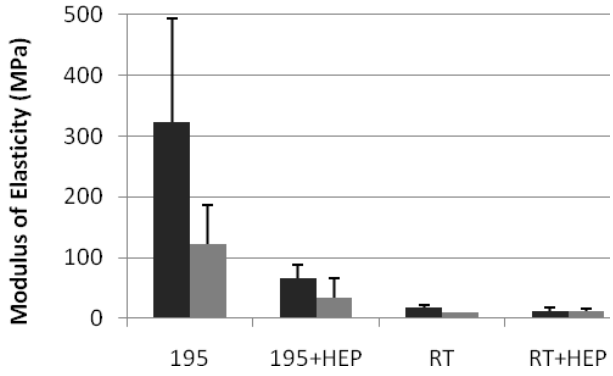
1) Heparin crosslinking of RT-dried fibers produced a 15% decrease in tensile strength, and a 27% decrease in elastic modulus. Fusion treatment of these fibers produced an insignificant change in strength and an 8% decrease in elastic modulus.

2) Annealing of the fibers at 195°C resulted in a 28% decrease in diameter, 30% increase in strength, and a 15 fold increase in elastic modulus. Fusion treatment of these fibers decreased the strength by 17% and decreased the stiffness by 50%.

3) Compared to annealed fibers, fibers annealed and cross-linked with heparin exhibited a 16% increase in diameter. Fiber strength decreased by 22% and elastic modulus decreased 4 fold. Fusion treatment of these fibers decreased strength by 31% and decreased elastic modulus by 50%.

Thus, our results show that annealing of chitosan fibers improves the mechanical properties significantly, while heparin crosslinking decreases these properties. Fusion treatment with 0.1% acetic acid increased diameter by 5%, decreased tensile strength by less than 30%, and decreased elastic modulus 50%, as illustrated in the figures below.





Conclusions This study demonstrated that physical processing of chitosan fibers improved their mechanical properties. These fibers can be used to reinforce chitosan or hydrogel scaffolds for to improved their mechanical properties. This finding establishes a foundation for engineering more versatile composite scaffolds with improved mechanical properties.