**Introduction:** In tissue engineering applications, scaffolds serve as a biological and mechanical support for cell growth and functionality. Chitosan-based scaffolds have attractive biocompatibility and biodegradability properties but moderate mechanical properties. We previously reinforced chitosan heart valve scaffolds with embedded chitosan fibers to obtain mechanical properties comparable to the native aortic and pulmonary valves in the radial direction but not in the circumferential direction. In this research study, we seek to further improve scaffold mechanical properties by improving fiber mechanical properties without adversely affecting fibers biocompatibility and/or biodegradation rate. Effect of acetic acid (AA) concentration (in chitosan solutions), coagulation bath concentration and drying temperature on chitosan fiber mechanical properties were investigated and are reported.

**Materials and Methods:** Hydrogel chitosan fibers were formed by extruding 1.5 wt% chitosan (MW: 190-310 kDa, 75-85% deacetylated) dissolved in different concentrations of AA (1, 2, 3 and 6 wt%) through 26 gauge Teflon catheter into 10 or 25 wt% ammonia. Chitosan fibers formed from 1% AA solutions and extruded into 10 wt% ammonia and dried at room temperature (RT) were served as control. To evaluate the effect of annealing temperature, fibers were heated to different temperatures (RT, 40, 90, 140 and 195°C), held at temperature for 15 minutes and then cooled to RT at 1°C/min. Fibers were rehydrated in PBS and measured. Ultimate tensile strength, maximum strain and elastic modulus of rehydrated fibers were evaluated using uniaxial tensile testing. X-ray diffraction (XRD) studies were conducted to study fiber crystallinity.

**Results and Discussion:** Freshly extruded chitosan fibers had a diameter of 350 μm and tensile strength of 0.7 MPa and an elastic modulus of 3.6 MPa. Fiber strength increased 2-fold when 2 wt% AA used as chitosan solvent, and an additional 50% at 3 wt% AA. Further increase in AA concentration decreased fiber strength. Modulus of elasticity changed non-linearly as AA concentration increased. A 2-fold improvement in fiber stiffness was seen at 2 and 3 wt% AA followed by a decreased stiffness values at higher AA concentrations.

Increasing the ammonia (Amm) concentration of the coagulation bath improved tensile strength 62%, decreased fiber elasticity 111% and increased fiber stiffness 4-fold. XRD analysis indicated increased crystallinity of chitosan fibers extruded into 25% Amm compared to 10% Amm.

Annealing fibers at temperatures above RT increased fiber strength such that 2-fold and 3-fold improvements were achieved at 145°C and 195°C respectively. Fiber stiffness also increased as annealing temperature increased reaching a maximum of 125 MPa at 195°C. XRD data showed that the ratio of crystalline to amorphous peak heights increased as temperature increased. The figures show some of the more significant improvements in fiber strength and stiffness achieved when processing parameters were combined. The maximum strength and stiffness but the lowest elasticity were obtained when fibers were chitosan solutions in 2% AA were extruded into higher pH coagulation bath and annealed at 195°C.

**Conclusions:** In this study, chitosan fibers with greatly improved mechanical properties have been formed. These mechanically improved fibers can be used to reinforce chitosan-based scaffold using a composite material approach.

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