Enhancing the Biomechanical Properties of Chitosan Scaffolds for Tissue-Engineered Heart Valves

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Introduction:

Previous tissue engineering approaches to create heart valves constructs have been limited by the structural immaturity and mechanical properties. Tissue engineered heart valves constructs have been successfully implanted at the pulmonary position in lambs where the construct is subjected to smaller loads compared to the aortic position. However, the constructs still lack the mechanical integrity needed to withstand systemic pressures. Therefore, several studies aim to improve the biomechanical properties and tissue formation of the engineered heart valve via mechanical conditioning of the cell-seeded scaffold in a bioreactor system. Chitosan is a biocompatible, biodegradable linear polymer, and due to its particular properties, it is currently being used in a number of tissue engineering applications. It offers a wide variety of mechanical properties depending on the molecular weight (MW), degree of deacetylation (DD), and molecular architecture [1]. Our research seeks to enhance the biomechanical properties of chitosan-based heart valve tissue engineered scaffolds. In this study, our approach involves embedding various weight percentages of high molecular weight (HMW) chitosan fibers in a porous heart valve scaffold made out of medium molecular weight (MMW) chitosan. Cell attachment and viability is also investigated throughout this study.

Materials and Methods:

Chitosan fibers were made out of 1.5 wt% HMW chitosan dissolved in 0.2 M acetic acid by extruding the solution through a 26-gauge (i.e. 0.45 mm diameter) teflon catheter directly into 10 vol% ammonia solution. The gelled chitosan fibers were then air-dried at room temperature.

Test scaffolds were made out of 2 wt% MMW chitosan dissolved in 0.2 M acetic acid. Chitosan fibers (2-10 mm length, 40 μm diameter) were blended into the solution at four different weight percentages (0, 20, 30, and 40 wt %). Scaffolds were generated by freezing the fiber-containing solutions in 60 mm Petri dishes at -70°C, followed by lyophilization for 24 hours, and rehydration in 1% ammonium hydroxide an then washed with Phosphate Buffer Saline (PBS). Pore sizes ranged from 180 to 240 μm. Each scaffold was cut into 2x5x20 mm rectangular samples. Mechanical properties of the samples were evaluated by monotonic tensile testing. All samples were tested in a fully hydrated condition. The maximum load, ultimate tensile strength (UTS), maximum strain, and modulus of elasticity were evaluated.

To evaluate the biocompatibility of the fiber material, HMW chitosan films were cast with a different thickness in culture wells. The desired volume (i.e. 50 µl, 150 µl, or 250 µl) of 1.5 wt% HMW chitosan was distributed homogenously in 35 mm culture wells and left to dry overnight. After drying, the films were neutralized by 0.2 M sodium hydroxide, and then washed with PBS. The films were sterilized by immersion in 70% ethanol overnight and then washed with sterile PBS. The films were then seeded with valvular interstitial cell (VIC's) at a density of 8000 cell/cm^2. The cells were cultured in M199 medium that containing Fetal Bovine Serum (FBS) and Gentamycin. Cell attachment, viability and growth was then followed by phase contrast microscopy and Calcein AM fluorescence at regular intervals.

Results and Discussion:

Tensile testing results showed that addition of 40 wt% chitosan fibers (2-3 mm length) improved scaffold tensile strength approximately 3 fold from 45±18 KPa to 139.5±40 KPa (Figure. 1), and the Modulus of Elasticity from 0.07±0.021 MPa to 0.42±0.07 MPa. Statistical analysis (student t-tests-paired two samples for mean) confirmed the significance of these improvements ($p<0.004$). The values for the ultimate tensile strength and the modulus of elasticity were substantially lower than those reported in the literature for the aortic valve. Stradins and colleagues [2] reported, based on their experimental results, that the tensile strength of aortic valve cusps ranges from 0.32±0.04 MPa to 1.74±0.29 MPa, while the modulus of elasticity ranges from 1.98±0.15 MPa to 15.3±3.8 MPa respectively.

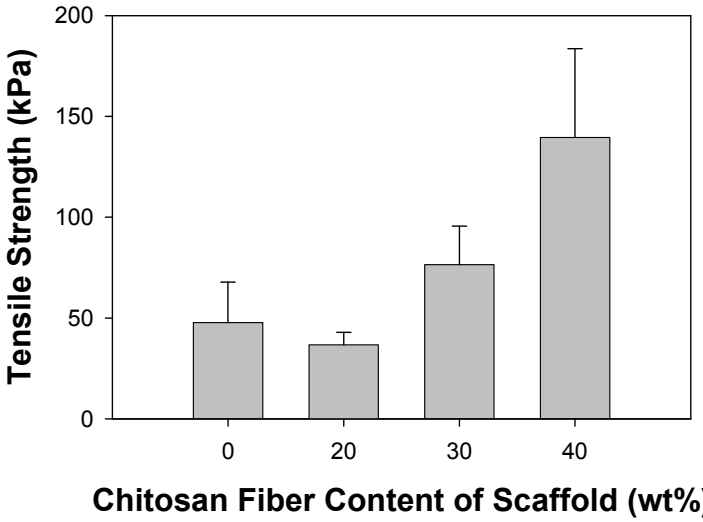
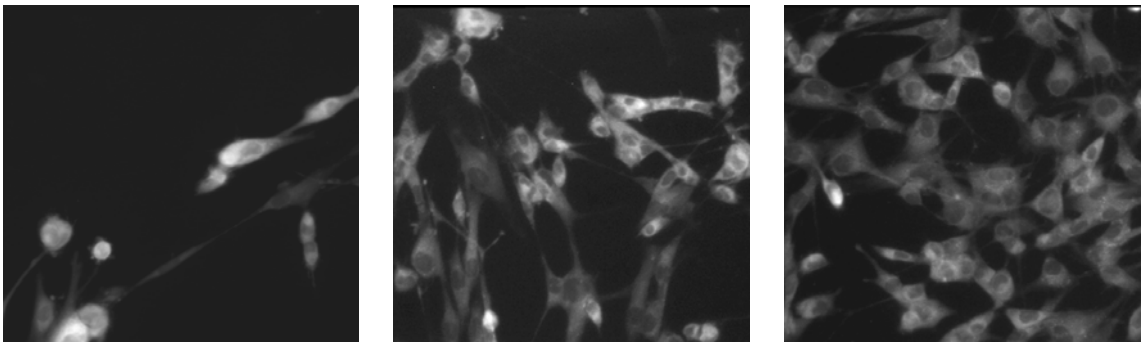


Figure. 1: Effect of chitosan fiber content on scaffold strength.

Figure 2 shows Calcein AM fluorescence microscopic images after 3 days of VIC's cultured on 3 chitosan cast films with increasing thickness. The thicker films show greater cellular attachment than the thinner ones, and in each condition, most of the cell attachment occurred at the periphery of the wells. Attachment of cells at the center of the well was either minimal or absent. Since the cast films are known to be thicker near

the container walls (a result of the fluid surface tension phenomenon), this observation suggests that the difference in cell adhesion may reflect a film thickness-induced change in film microstructure of chitosan membranes between the center and periphery of the well. This thickness effect makes the chitosan more amorphous towards the periphery. The reduced crystallinity may alter either the amount or conformation of adhesion proteins adsorbed onto the film surface, thus enhancing the cellular attachment.



(A) (B) (C)

Figure. 2: Phase contrast microscopic images at 4X magnification of Chitosan membranes with different thicknesses depending on the added volume as follow: (A): 50µl, (B): 150 µl, and (C) 250 µl

These findings of biomechanical properties and the cell studies show that fiber reinforcement of porous chitosan scaffolds can significantly improve baseline scaffold properties.

Acknowledgements

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References

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