World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:13, No:06, 2019

A Multiple Freezing/Thawing Cycles Influence Internal Structure and Mechanical Properties of Achilles Tendon

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Abstract : Tendon grafting is a common procedure performed to treat tendon rupture. Before the surgical procedure, tissues intended for grafts (i.e., Achilles tendon) are stored in ultra-low temperatures for a long time and also may be subjected to unfavorable conditions, such as repetitive freezing (F) and thawing (T). Such storage protocols may highly influence the graft mechanical properties, decrease its functionality and thus increase the risk of complications during the transplant procedure. The literature reports on the influence of multiple F/T cycles on internal structure and mechanical properties of tendons stay inconclusive, confirming and denying the negative influence of multiple F/T at the same time. An inconsistent research methodology and lack of clear limit of F/T cycles, which disqualifies tissue for surgical graft purposes, encouraged us to investigate the issue of multiple F/T cycles by the mean of biomechanical tensile tests supported with Scanning Electron Microscope (SEM) imaging. The study was conducted on male bovine Achilles tendon-derived from the local abattoir. Fresh tendons were cleaned of excessive membranes and then sectioned to obtained fascicle bundles. Collected samples were randomly assigned to 6 groups subjected to 1, 2, 4, 6, 8 and 12 cycles of freezing-thawing (F/T), respectively. Each F/T cycle included deep freezing at -80°C temperature, followed by thawing at room temperature. After final thawing, thin slices of the side part of samples subjected to 1, 4, 8 and 12 F/T cycles were collected for SEM imaging. Then, the width and thickness of all samples were measured to calculate the cross-sectional area. Biomechanical tests were performed using the universal testing machine (model Instron 8872, INSTRON®, Norwood, Massachusetts, USA) using a load cell with a maximum capacity of 250 kN and standard atmospheric conditions. Both ends of each fascicle bundle were manually clamped in grasping clamps using abrasive paper and wet cellulose wadding swabs to prevent tissue slipping while clamping and testing. Samples were subjected to the testing procedure including pre-loading, pre-cycling, loading, holding and unloading steps to obtain stress-strain curves for representing tendon stretching and relaxation. The stiffness of AT fascicles bundle samples was evaluated in terms of modulus of elasticity (Young's modulus), calculated from the slope of the linear region of stress-strain curves. SEM imaging was preceded by chemical sample preparation including 24hr fixation in 3% glutaraldehyde buffered with 0.1 M phosphate buffer, washing with 0.1 M phosphate buffer solution and dehydration in a graded ethanol solution. SEM images (Merlin Gemini II microscope, ZEISS®) were taken using 30 000x mag, which allowed measuring a diameter of collagen fibrils. The results confirm a decrease in fascicle bundles Young's modulus as well as a decrease in the diameter of collagen fibrils. These results confirm the negative influence of multiple F/T cycles on the mechanical properties of tendon tissue.

Keywords : biomechanics, collagen, fascicle bundles, soft tissue
Conference Title : ICSTBEA 2019 : International Conference on Soft Tissue Biomechanics and Engineering Applications
Conference Location : New York, United States
Conference Dates : June 04-05, 2019

Open Science Index, Bioengineering and Life Sciences Vol:13, No:06, 2019 publications.waset.org/abstracts/107911.pdf