Video Article

Ex vivo Mechanical Loading of Tendon

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URL: http://www.jove.com/video/209
DOI: doi:10.3791/209

Keywords: Developmental biology, issue 4, tendon, tension

Date Published: 5/28/2007

Citation: Asundi, K., Rempel, D. Ex vivo Mechanical Loading of Tendon. *J. Vis. Exp.* (4), e209, doi:10.3791/209 (2007).

Abstract

Injuries to the tendon (e.g., wrist tendonitis, epicondyltis) due to overuse are common in sports activities and the workplace. Most are associated with repetitive, high force hand activities. The mechanisms of cellular and structural damage due to cyclical loading are not well known. The purpose of this video is to present a new system that can simultaneously load four tendons in tissue culture. The video describes the methods of sterile tissue harvest and how the tendons are loaded onto a clamping system that is subsequently immersed into media and maintained at 37°C. One clamp is fixed while the other one is moved with a linear actuator. Tendon tensile force is monitored with a load cell in series with the mobile clamp. The actuators are controlled with a LabView program. The four tendons can be repetitively loaded with different patterns of loading, repetition rate, rate of loading, and duration. Loading can continue for a few minutes to 48 hours. At the end of loading, the tendons are removed and the mid-substance extracted for biochemical analyses. This system allows for the investigation of the effects of loading patterns on gene expression and structural changes in tendon. Ultimately, mechanisms of injury due to overuse can be studies with the findings applied to treatment and prevention.

Video Link

The video component of this article can be found at http://www.jove.com/video/209/

Protocol

1. Supplement media CO_2-independent media with Fetal Bovine serum, l-glutamine, antibiotics, and ascorbic acid
2. Aliquot 40 ml into 50 ml centrifuge tubes (1 for each tendon)
3. Aliquot 10 ml into 15 ml centrifuge tubes (1 for each tendon)
4. Sterilize surgical tools, autoclave sheets, tweezers and torque wrench in autoclave
5. Sterilize clamps and force micrometer tips by soaking in 70% ethanol overnight
6. Harvest tendons from the rear paws of the rabbit. Rabbit should have been euthanized no more than 2 hours ago.
7. Place tendons in 15 ml centrifuge tube filled with media
8. Lay out a sterilized autoclave sheet to establish a sterile field
9. Measure cross-sectional area of tendon
    1. Place micrometer tips on micrometer, align properly
    2. Grip tendon by the ends
    3. Fit thinnest tendon section in groove
    4. Have assistant place 50g weight
    5. Wait 30 seconds, read thickness (CSA = thickness x 1.3)
10. Place tendon back in 15 ml centrifuge tube
11. Repeat with each tendon to be loaded
12. Remove actuator from base
13. Cut two 15mm by 40mm strips and one 3mm by 5mm piece from sterile gauze for each tendon
14. Roll each strip tightly and place between the grooves of the distal clamp
15. Remove tendon from centrifuge tube and place on clamp, the distal end of the tendon on the distal clamp and the fibrocartilage region of the tendon on the proximal clamp
16. Place 3x5 gauze piece on top of the distal end of the tendon, and secure the clamp
17. Secure proximal clamp, ensuring tendon remains aligned between the clamps and with enough slack that it is not under tension when clamps are tightened. Tighten clamps to 1N-m
18. Place 50ml centrifuge tube with media in base
19. Place actuator in base
20. Repeat with each tendon to be loaded
21. Place base over water bath
22. Loading Protocol
    1. Precondition tendons

2. Apply 0.5N load, measure tendon length (this is the gauge length)
3. Apply cyclic loading

23. Remove actuator from base
24. Release tendon from clamps
25. Process tendon

Discussion

The video presents a new system for the simultaneous loading of up to four live tendons in tissue culture. Typical loading durations are 2 to 48 hours. After loading, the tendons are removed and processed for gene expression. The system allows for the investigation of the effect of loading pattern on changes in genes associated with degenerative changes in the tendon. These experiments can provide some insight into mechanisms of injury associated with repetitive loading. They also provide insight into loads that can cause tissue damage. Ultimately, the results may be used to prevent and treat overuse injuries to tendons and ligaments.

References

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