Video Article

Preparation of Rat Tail Tendons for Biomechanical and Mechanobiological Studies

Amélie Bruneau[1], Nadia Champagne[1], Paule Cousineau-Pelletier[1], Gabriel Parent[1], Eve Langelier[1]

[1]Groupe PERSEUS, Faculté de Génie Département de génie mécanique, Université de Sherbrooke

Correspondence to: Eve Langelier at Eve.Langelier@Usherbrooke.ca

URL: http://www.jove.com/video/2176
DOI: doi:10.3791/2176

Keywords: bioengineering, Issue 41, Rat tail tendon, extraction, cross-section, optic micrometer, anchors, bioreactor, biomechanics, mechanobiology

Date Published: 7/30/2010

Citation: Bruneau, A., Champagne, N., Cousineau-Pelletier, P., Parent, G., Langelier, E. Preparation of Rat Tail Tendons for Biomechanical and Mechanobiological Studies. *J. Vis. Exp.* (41), e2176, doi:10.3791/2176 (2010).

Abstract

Rat tail tendons (RTTs) are a common biological model used in experimental in vitro studies in the fields of tendon physiology and tendinopathy. Working with those tissues is challenging because they are very fragile, and until now there was no rigorously detailed protocol for their isolation.

Faced with these challenges, we have developed methods and instruments to facilitate manipulation of RTTs and control tissue viability, sterility and integrity. This article describes the experimental procedures used to prepare RTTs for biomechanical and mechanobiological studies. Our work is divided into four main steps: extraction, cross-sectional area measurement, rinsing and loading into the bioreactor chamber.

At each step, all procedures, materials and manipulations are presented in detail so that they can be easily reproduced. Moreover, the specific instruments developed are presented: a manipulation plate used to segregate RTTs, an optic micrometer to position the tissue during the cross-sectional area measurement and an anchoring system to attach the RTTs onto a bioreactor.

Finally, we describe the results obtained after multiple tests to validate our methods. The viability, sterility and integrity evaluations demonstrate that our procedures are sufficiently rigorous for manipulations of fragile tissues such as rat tail tendons.

Video Link

The video component of this article can be found at http://www.jove.com/video/2176/

Protocol

Prior to any manipulation, you must identify the group of tendons to be used depending on the experiment you are conducting and the apparatus at your disposition. For our purposes, ventral tendons were chosen because they are smaller and thus easier to manipulate when measuring the cross-sectional area and fitting them into the bioreactor chamber.

Please note that all instruments are autoclaved or sterilized with 70% ethanol. Moreover, a spray bottle containing 70% ethanol is placed beside each work station to sterilize the experimenter's gloves before each operation.

Part 1: Extraction

After resection, the tail is carefully manipulated by its extremities to avoid damaging the tissues. Also, to conserve cell viability, all manipulations are carried out in cold saline solution.

1A) Materials:

- Cold saline solution (D-PBS)
- Crushed ice
- Surface protector
- Cutting board
- Individual manipulation plates
- 2 500 ml dishes
- 2 2L glass dishes
- Adhesive tape
- 1 Tweezers
- 1 Forceps
- 1 Tweezers stand

- 1 Pair of surgical shears
- 1 Scalpel
- 1 Pair of surgical scissors

Figure 1. Individual manipulation with orientation identification ("P" for "proximal")

1B) Work station:

1. Spread out the surface protector and place the cutting board on top.
2. Fill each two larger glass dishes to halfway with crushed ice and place them on the paper.
3. Stick a piece of tape on a corner of the manipulation plate and identify with a letter to indicate the proximal end.
4. Fill the two small glass dishes to halfway and all grooves of the manipulation plates with cold saline solution. Place them in the large glass dishes containing ice.
5. Organize all the sterile instruments on the surface protector.
6. Transfer the tail into one of the glass dish containing saline solution.

1C) Manipulations:

1. Observe the anatomy at the proximal end of the tail to distinguish the ventral side from the dorsal side. The ventral side has a larger number of tendons and a blood vessel that can be verified by gently squeezing the tail to produce tiny drops of blood.
2. Using the surgical scissors, cut the skin along the dorsal side of the tail if you want to extract the ventral tendons (or along the ventral side if you need the dorsal tendons).
3. Open the incision at the proximal end and remove the skin using forceps or your fingers and by carefully manipulating the tail ends.
4. Rinse off the blood and hair in the saline solution and transfer the specimen into the remaining glass dish containing fresh solution. Change gloves.

A transversal section viewed under light microscopy shows the six tendon groups. One tendon from each group is attached to the caudal end of each vertebra, so we cut at the intervertebral disc to expose new tendons along the cut.

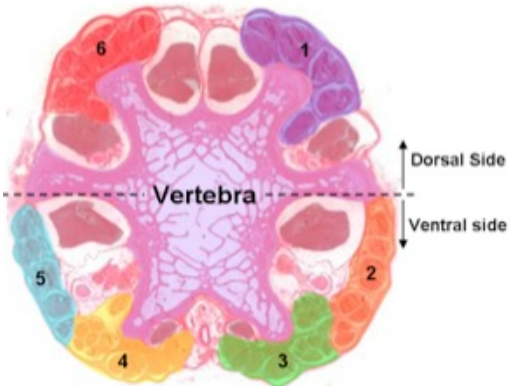

Figure 2. Transversal section of the rat tail viewed under light microscopy.

5. Cut the distal end through the cartilage 2-3 vertebrae shorter with surgical shears. Place the tail on the cutting board to cut the soft tissues using a scalpel and then immediately replace it in the solution.
6. Using tweezers, pull out one tendon from the distal tail end.
7. With a gentle hold at both ends, place the tendon into the individual manipulation plate.

Repeat the last two steps until no more tendons can be teased out and cut one vertebra shorter whenever new tissues need to be exposed.

Part 2: Cross-sectional area measurement[7]

When the tissue undergoes mechanical characterization or stimulation, its mechanical properties are described by normalizing the force inside the tendon to stress. This is why we evaluate cross-sectional area.

2A) Materials:

- Optic Micrometer
- Stereomicroscope
- Digital camera
- Edge recognition and Profile reconstruction algorithms [5,6]
- Cold saline solution (D-PBS)
- 1 20-200µL Micro volume pipette
- 1 Hex key for optic micrometer adjustments
- 2 Tweezers

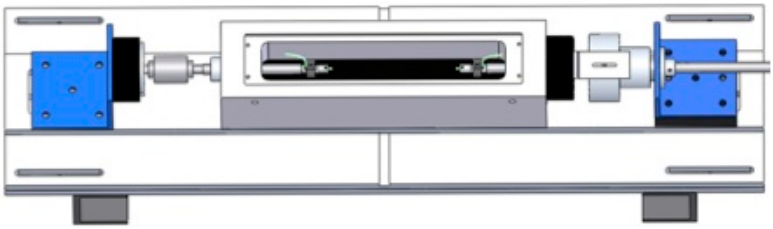

Figure 3. Optic Micrometer

2B) Work station:

1. Open the software (Digital camera and algorithms).
2. Place the optic micrometer so that there is enough room to load the tendon in the apparatus.
3. Organize the instruments and glass dish containing the extracted tendons close at hand.

2C) Manipulations:

1. Fill the measuring compartment with cold saline solution until both anchor systems are submerged.
2. Transfer the tendon into the compartment by holding the ends with tweezers.
3. Using suction via the micro-volume pipette, pull one extremity at a time into the silicon tube. Aspirate until the end extends beyond the shaft collars.
4. Tighten the shaft collars to compress the tubes and install the apparatus under the stereomicroscope.
5. At 105X magnification, stretch the tendons while observing the tissue until the creases are no longer perceptible. Stretch by additional 0.40%.

Figure 4. Tendon projection at 105x magnification before and after stretching.

6. Adjust position and focus to maintain a clear image of the tendon over a 180° rotation.
7. Take a photo at every 10° rotation without modifying tension, focus or position. Use only the rotary shaft to carry out the rotation. Due to its cone shape, these steps may be repeated at different points along the tendon.
8. Activate the edge recognition and profile reconstruction algorithms in order to analyse the database you have registered.
9. Free the tendon by loosening the shaft collars and blowing air into the silicone tubes using the micro volume pipette.
10. Reinsert the tendon onto the manipulation plate, again handling it by the ends.
11. Verify the shape of the profile reconstruction obtained and the value of the cross-sectional area.

Part 3: Rinsing

To remove contamination that may have occurred during the previous manipulations, the tissues are rinsed under biosafety cabinet.

3A) Materials:

- Cold saline solution (D-PBS)
- Multiple-groove manipulation plate
- Individual Manipulation plates
- Adhesive tape
- 2 Tweezers
- 1 Tweezers stand

3B) Work station:

1. Turn on the fan in the biosafety cabinet 15 minutes in advance and clean the inside surfaces with 70% ethanol.
2. Bring all the instruments inside the biosafety cabinet.
3. Stick pieces of tape onto a corner of each individual plate to identify the proximal end.
4. Fill all grooves with sterile saline solution.

3C) Manipulations:

1. Introduce the manipulation plate containing the extracted tendon in the cabinet.
2. Using tweezers, remove the tendon from its plate with a gentle grasp on each end.
3. Immerse and gently stir the tendon in the saline solution of each compartment of the multiple-groove manipulation plate, starting from the closest and proceeding to the furthest groove.
4. Finally, submerge the tendon in an individual manipulation plate.

Part 4: Loading into bioreactor chamber

To avoid further contamination, the following manipulations are also conducted in the biosafety cabinet.

4A) Materials:

- Bioreactor chamber [4]
- Sterile anchors
- 1 Sterile Petri dish
- Cold cell cultured Medium (DMEM)
- Cyanoacrylate
- 2 Tweezers
- Tweezers stand
- 0.5-10µl Micro-volume pipette
- 1 Ruler

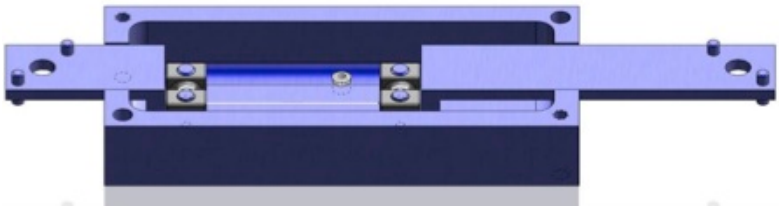

Figure 5. Bioreactor chamber

4B) Work station:

1. Since the previous step was also realized in biosafety cabinet, removal of unnecessary tools will give you more work space.
2. Bring all other instruments inside the cabinet.

4C) Manipulations:

1. Place an anchor on the manipulation plate with its spool centered over the tendon extremity and wound the tissue up and around the spool.
2. Roll the anchor over and hold the loose extremity while ensuring that the tissue stays attached to the anchor. Stop after ¾ turn.
3. Carry out the same steps with the other end.
4. Roll both anchors over equally until there is 6 cm between their centers[1].
5. Loosen the tendon to immerse it in the solution and allow the anchored sections to dry briefly to enhance its mechanical properties[2-3].
6. Pour cyanoacrylate into the Petri dish and draw 2.5µL into the micro volume pipette.
7. Apply a drop of cyanoacrylate to the wound-up tissue without dropping any glue in the saline solution. Repeat until 2 drops have been applied to each anchor.
8. With the tendon still completely immersed, wait 5 minutes for the glue to dry completely.
9. During this time, fill the chamber compartment with saline solution.
10. Rehydrate all tissue with saline solution to prevent damage.

11. By manipulating only the anchors, transfer the tendon into the bioreactor chamber and close it to prevent leakage and contamination during transfer.

Part 5: Representative Results:

The outcome of the protocol shows that when performed correctly, our tissue rigorous isolation and preparation procedure make it possible to maintain tissue sterility, viability and integrity.

First, using the simple and repetitive extraction method, we are able to extract tail tendons without damaging the collagen network as it can be observed by a microscopic analysis of H&E tainted sections realized after the extraction.

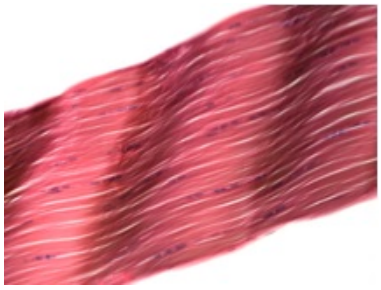

Figure 6. Tendon collagen network after extraction (5µm longitudinal section stained with H&E).

We then cultured tendons for up to ten days and conducted sterility tests. Each day, we plated used culture solution on agar and incubated it for 24 hours. Since no bacterial growth was observed, we concluded that our manipulations do not result in contamination.

With the profile reconstruction algorithm and optic micrometer, we are able to estimate the cross-sectional area within a 2% margin of error[7].

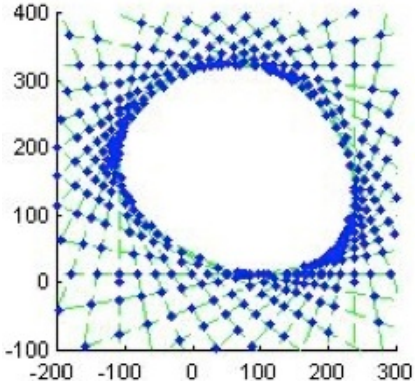

Figure 7. Profile reconstruction of a RTT.

Finally, we assessed viability using the LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells after the rinsing step and after a twelve-day culture period. Since a large majority of green fluorescent live cells were evident, we can confirm that our isolation procedures are successful in preserving live tissue. The same test was performed two hours after attaching the tendon into the bioreactor chamber. We verified that the dehydration and glue at the anchor had not spread in the tissue between both anchors.

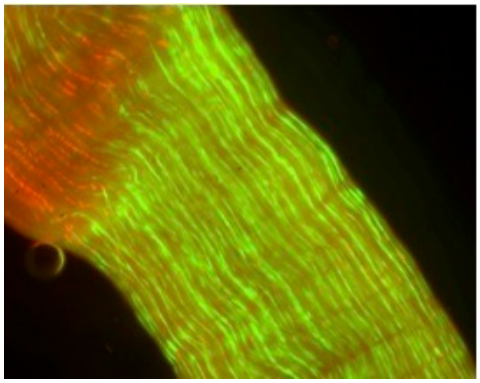

Figure 8. Tissue viability at the anchor (green = live cells, red = dead cells)

Discussion

By applying those experimental procedures, we can conduct a wide variety of *in vitro* studies on this kind of tissues. As for example, a study on tissue degeneration was carried out by the application of under-stimulation to RTTs for a period of ten days. Each day, we evaluated tissue mechanical properties in non-destructive stress relaxation tests. At the end, we were able to observe RTT stress variation and thus analyze the progression of mechanical properties.

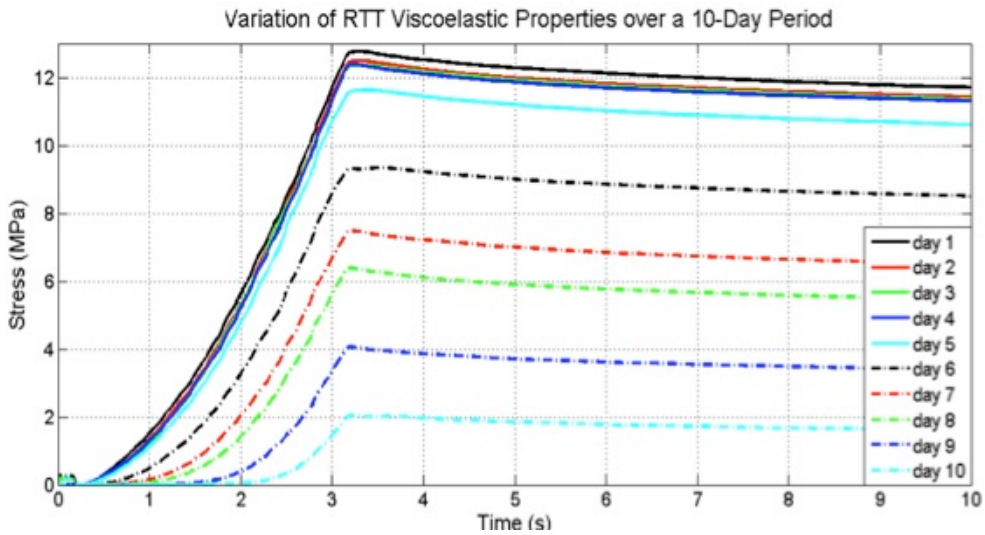

Figure 9. RTT's stress variation evaluated trough relaxation test (day 1 to 10).

Disclosures

No conflicts of interest declared.

Acknowledgements

This article was supported by NSERC Grant # 299280, IRSST as a scholarship to G. Parent and FRSQ as an undergraduate student research award to M. Cyr. We thank Yoan Lemieux-Laneville for performing the manipulations recorded on video.

References

1. Bennett, M.B., Ker, R. Dimery, N. & Alexander, R. Mechanical properties of various mammalian tendons. J. Zool. Lond. A209, 537-548 (1986).
2. Dupuis, D. PhD Thesis - Déshydratation de matrices collagéniques reconstruites in vitro: effets sur les propriétés mécaniques et histologiques, Québec, Université Laval, 114p, (2002).
3. Haut, R.C. Age-dependent influence of strain rate on the tensile failure of rat tail tendon. Journal of Biomechanical Engineering 105, 296-299 (1983).
4. Huppé, N., Lessard, J.-L. & Langelier, E. A Bioreactor Design for the Mechanobiological Study of Soft Tissue Damage and Repair in Conditions that Provide the Best Approximation of Actual Use, 31st Canadian Medical and Biological Engineering Conference, Montréal, Canada, (2008).
5. Langelier, E., Dupuis, D., Guillot, M., Goulet, F. & Rancourt, D. Cross-sectional profiles and volume reconstructions of soft tissues using laser beam measurements. J Biomech Eng, 126: 796-802 (2004).
6. Lee, T. Q. & Woo, S. L.-Y. A new method for determining cross-sectional shape and area of soft tissues. J Biomech Eng, 110: 110-114 (1988).
7. Parent, G., Cyr, M., Cousineau-Pelletier, P., Desbiens-Blais, F. & Langelier, E. Current Techniques for the Evaluation of Cross-Sectional Area in Rat Tail Tendons Generate Significant Errors, 31st Canadian Medical and Biological Engineering Conference, Montréal, Canada, (2008).

