Triaxial mechanical testing of dog colon samples

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1 Works for me
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ABSTRACT

This protocol describes the step followed to conduct ex vivo triaxial mechanical testing (inflation, extension, torsion) of dog colon samples on our custom build testing platform.

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MATERIALS TEXT

A triaxial testing machine large enough to accommodate a 20 cm tubular colon sample is needed. The machine should allow to submerge the tissue under liquid during testing. The machine must allow to control axial stretch, luminal pressure, and circumferential twist all the while measuring axial force, outer diameter, and torque.

Collect tissue

1 1. At termination of the animal, open the abdominal cavity to expose the colon.
2. Identify a ~20 cm colon segment and isolate it with sutures or clamps at both ends.
3. Take a picture with a scale (Pic.1).
4. Dissect the segment.
5. Gently remove luminal content by squeezing it out, and clean remaining content by flushing several times with saline until completely cleaned.
6. Position on a flat surface and take another picture with a scale (Pic.2).
7. Store the segment in Ca-free Kreb at 4°C until testing.
8. Conduct testing within 4 hours.

Prepare triaxial machine for testing

2 1. Fill tank and inflation liquid bottle with Ca-free Kreb.
2. Open the 95% O_2 – 5% CO_2 line to aerate the Ca-free Kreb solution.
3. Turn on the heater (setup for 37C) and the associate pump.
4. Setup a pH-meter to monitor pH. Make sure pH = 7.4±0.1 before starting the test. Adjust pH if necessary.
5. Calibrate camera using the known diameter of the tissue holder.
6. Open main pressure valve. Open machine pressure valve to let Ca-free Kreb flow through and remove any air.

Prepare tissue for testing

3 1. Take tissue out of the storage solution and dissect connective tissues and major mesentery vessels.
2. Mount the tissue on the triaxial machine and secure it with O-rings.
3. Open exhaust bolt on the tissue mount, then open the pressure valve and let solution flow through the tissue and out of the exhaust.
4. Close pressure valve and the exhaust bolt.
5. Once bath temperature is at 37C, start the test.

Conduct testing

4 1. Stretch the tissue back-end forth up to a 40% stretch five times while maintaining tissue pressure at 0.5 kPa and 0 twist.
2. At multiples stretch steps where the tissue is not bulging, inflate-deflate the tissue back and forth four times between 0.5 and 2 kPa while maintaining the tissue at 0 twist.
3. At multiples stretch steps where the tissue is not bulging and at multiple pressure steps between 0.5 and 2 kPa, twist the tissue back and forth four times between -40 and 40 degrees.

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