The Effects of Freezing on the Tensile Properties of Repaired Porcine Flexor Tendon

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Purpose When conducting complex testing of tendon repairs, it is essential that the samples are adequately preserved to prevent degradation. Freezing of samples is the most convenient method of preservation; however, there is no evidence in the literature to prove that freezing tendon before or after repair is acceptable. We aimed to prove that freezing tendons does not significantly alter the results of linear load-to-failure testing of tendon repairs.

Methods After a power study, 150 tendons were harvested from porcine forelimbs and randomized into 5 groups of 30 tendons. After division, tendons were repaired using a Pennington modified core technique with a Silfverskiöld peripheral cross-stitch. Tendons in group 1 were divided, repaired, and tested within 3 hours postmortem. Tendons in group 2 were refrigerated at 4°C for 24 hours prior to repair and testing. Tendons in group 3 were frozen at −25°C for 3 months prior to repair and testing. Tendons in group 4 were frozen at −25°C for 6 months prior to repair and testing. Tendons in group 5 were frozen at −25°C for 6 months, repaired, refrozen for 1 month, and then tested. All repairs were linear load tested to ascertain the ultimate strength and force to produce 3-mm gap in the repair.

Results Analysis of variance analysis of the results did not demonstrate any significant differences between groups.

Conclusions Freezing tendons both before and after suture repair is an acceptable method of preservation when investigating the force to produce 3-mm gap and ultimate strength of tendon repairs. *(J Hand Surg 2008;33A:353–358. Copyright © 2008 by the American Society for Surgery of the Hand.)*

Key words Freezing, repair, tendon, tensile, porcine.

THE INVESTIGATION OF TENDON REPAIRS is becoming more complex and the time taken to run experimental protocols is increasing, making the scheduling of tendon harvest and testing increasingly challenging. When performing *ex vivo* tensile testing of tendon repair techniques, it is essential to ensure that samples are adequately preserved. To limit the number of variables between and during biomechanical testing of tendon repairs, it may be more efficient to preserve harvested and repaired tendons with subsequent testing in batches.

The most common method of preserving tendon after harvest is freezing; however, with larger numbers, refreezing of samples after repair may also be required.[1] Bhatia et al[2] suggested that freezing had no effect on tendon repairs but made no mention of whether this freezing was before or after repair and provided no results to support the claim. Despite the widespread use of freezing as part of experimental protocols, there is no evidence to substantiate its use when testing repaired tendons, and researchers wishing to validate their experimental protocols must extrapolate the effects of freezing from studies using intact tendons.

Previous authors have shown that freezing tendons causes a subtle decrease in the elastic modulus[3] or the ultimate strength,[4] factors that only manifest themselves over the full extent of a stress-strain curve. As the stress-strain curve of a tendon repair only occupies a small portion of the curve for an intact tendon, these properties should not be evident. The response of the repaired tendon to freezing may be a function of the suture's interaction with the tendon rather than the viscoelastic properties of the tendon itself. In this context, the results of previous studies may not support fully the freezing of tendons, and clarity is lacking on whether it is acceptable to freeze *ex vivo* tendons before or after repair or indeed both.

The porcine tendon model is used extensively in the literature as it is readily available and similar to human flexor digitorum profundus tendon in zone II.[5,6] The deep flexor tendon[5–12] of the porcine forefoot is most commonly used, though some authors advocate use of extensor tendons.[13–15]

We aimed to investigate the effects of freezing and

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refreezing of repaired porcine deep flexor tendon on the force to produce 3-mm gap, as described by Gelberman et al,[16] and the load to failure (ultimate strength) of the repair.

MATERIALS AND METHODS

One hundred fifty profundus tendons were harvested from the central two rays of adult porcine forelimbs within 2 hours of slaughter. After harvest, the tendons were randomized into 5 groups, and each group of tendons was subjected to a different preservation protocol. During the experiments, tendons were kept wrapped in 0.9% saline–soaked swabs. The groups were as follows:

- Group 1: Thirty tendons were harvested, divided, repaired, and tested within 3 hours of slaughter.
- Group 2: Thirty tendons were harvested and wrapped in an 0.9% saline–soaked swab. They were then left refrigerated for 24 hours at 4°C. The tendons were then transected, repaired, and tested within 2 hours.
- Group 3: Thirty tendons were harvested and wrapped in an 0.9% saline–soaked swab prior to freezing for 3 months and then thawed. The tendons were then transected, repaired, and tested within 2 hours.
- Group 4: Thirty tendons were harvested and wrapped in an 0.9% saline–soaked swab prior to freezing for 6 months and then thawed. The tendons were then transected, repaired, and tested within 2 hours.
- Group 5: Thirty tendons were harvested and wrapped in an 0.9% saline–soaked swab prior to freezing for 6 months. They were then thawed, repaired, and refrozen for 1 further month. The repaired tendons were then thawed and tested within 2 hours.

To freeze the samples, 10 tendons were wrapped in surgical gauze and placed into a polyethylene bag with a small volume of 0.9% saline solution, which was then frozen in a domestic freezer at −25°C. Thawing involved warming the frozen tendons in a 37°C saline bath for 15 minutes, as previously described by Giannini et al.[4] Whenever samples were not being repaired or tested, they were stored wrapped in a saline-soaked gauze.

Tendons were divided using a scalpel at the level of the metatarsophalangeal joint, a level consistent with the literature[6,7,11] and roughly equivalent to a zone II laceration.[5,6] Prior to division, transverse lines were marked on the tendon surface using a piece of thread dipped in black ink (using the apparatus shown in Fig. 1), 2 further marks were placed 5 mm on either side of this line, and another 2 marks were placed 10 mm from this line as measured with a vernier caliper (Mitutoyo Corporation, Kawasaki, Kanagawa, Japan). The central mark was used to guide the division of the tendon, with the adjacent marks being used to guide suturing.

The core repairs were performed as described by Pennington[17] (Fig. 2) using 4-0 braided polyester with locking loops 10 mm from the cut end of the tendon (using the 10-mm ink mark as a guide). Peripheral repair was performed using the type B peripheral cross-stitch (Fig. 3) as described by Silfverskiöld and Andersson[18] using 6-0 monofilament nylon with bites 5 mm from the repair site (using the 5-mm ink mark as a guide). All repairs were performed under 2.5× loupe magnification by a single surgeon. Tensile testing was performed in a Zwick Tensiometer (Zwick GmbH & Co. KG, Ulm, Germany) with a 2.5 kN load cell and an interclamp distance of 60 mm. The tendons were secured in pneumatic clamps with coarse sandpaper grippers and then subjected to a 1 N preload followed by linear loading to failure at a rate of 10 mm/min.

FIGURE 1: Device used for marking of tendons prior to repair.

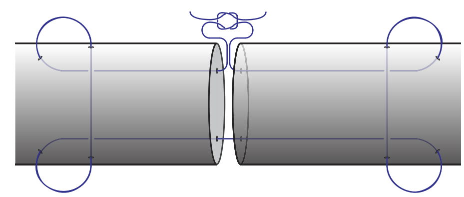

FIGURE 2: Pennington modification of the Kessler 2-strand core repair.

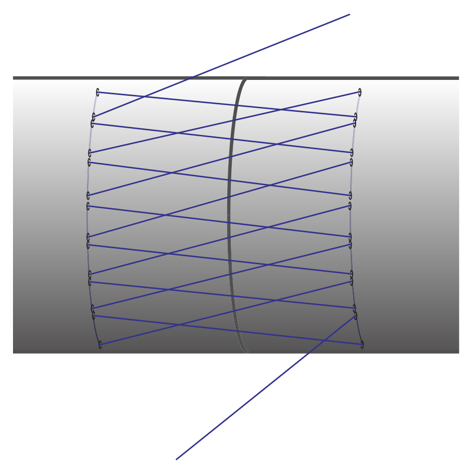

FIGURE 3: Silfverskiöld type B peripheral cross-stitch.

All tensile tests were recorded at 25 frames per second using a digital video camera linked to the tensiometer. The testing software (Zwick TestXPert 11.02) stored the