# Short-Term Vancomycin and Buffer Soaking Does Not Change Rabbit Achilles Tendon Tensile Material Properties

Olivia L. Dyer[a], Benjamin B. Wheatley[b*], Mark A. Seeley[a]

[a]Musculoskeletal Institute, Geisinger, Danville PA

[b]Department of Mechanical Engineering, Bucknell University, Lewisburg PA

Emails: Olivia L. Dyer (odyer1@geisinger.edu), Benjamin B. Wheatley (b.wheatley@bucknell.edu), Mark A. Seeley (mseeley1@geisinger.edu)

*Correspondence Email: b.wheatley@bucknell.edu

*Correspondence Address: Bucknell University, 1 Dent Drive, Lewisburg PA, 17837

Abstract Word Count: 210

Main Text Word Count: 1805

**Abstract**

*Background:* Allograft tendons are commonly used during orthopedic surgery to reconstruct tissue that is severely damaged. Soaking the tendon in an antibiotic solution, specifically vancomycin, has been shown to lower the risk of post-operative infections. While some material properties of tendon and ligament after antibiotic soaking have previously been characterized, extensive sub-failure allograft tendon material properties after soaking in antibiotic solutions have not.

*Methods:* Forty tendons were dissected from rabbits and soaked in either a phosphate buffered saline (PBS) only solution or vancomycin and PBS solution for five or 30 minutes. Immediately after soaking, quasi-static tensile experiments were performed in a materials testing system.

*Findings:* Tissue nominal stress, Lagrange strain, toe-region properties and elastic modulus were characterized. For all forty tendons, the average elastic modulus was found to be 455 ± 37 MPa, the average transition strain (from toe-region to linear elastic region) was 0.0487 ± 0.0035, and the average transition stress was 9.71 ± 0.79 MPa. No statistically significant differences in any of these material properties were found across soaking medium or soaking time.

*Interpretation:* From these results, we conclude that soaking an allograft tendon in antibiotic solution for up to 30 minutes prior to implantation does not change the tensile material properties of tendons, supporting current clinical practice.

Keywords – allograft tendon, elastic modulus, digital image correlation, mechanical characterization, optimization

## 1. Introduction

Allograft tendons are commonly used in reconstruction when tissues are severely damaged. Specifically, native tissue that no longer maintains mechanical viability is replaced with allograft tendons that exhibit the necessary material properties for tissue function. Antibiotics, such as vancomycin, are used in an attempt to reduce the risk of post-surgical infections (Banios et al., 2021; Schuster et al., 2020; Xiao et al., 2021). Recent work has shown that vancomycin soaked gauze wrapped around tendon and ligament grafts at a concentration of 5 mg/mL or greater for at least 20 minutes were able to remove bacterial contamination from the tendon grafts (Schuster et al., 2020; Schüttler et al., 2019). Additionally, soaking tendons in vancomycin does not cause tenocyte cytotoxicity if the concentration is below 12,600 μL and soaking time is less than 6 hours (Xiao et al., 2020). However, it is unclear how the sub-failure material properties of tendon – specifically characteristics of the toe and linear regions of tensile stress-strain behavior – are affected by antibiotic soaking and short-term soaking time.

The goal of the present study is to determine if the tensile material properties of hybrid New Zealand White rabbit and hybrid California White rabbit achilles tendons are altered by a) soaking in vancomycin antibiotic solution versus phosphate buffered saline (PBS) only and b) soaking for five or thirty minutes. We hypothesized that vancomycin soaking and soaking time would not affect the elastic modulus and toe-region properties of tendon. Changes to tendon graft mechanics after soaking in an antibiotic solution could alter surgical implantation procedures and *in vivo* tendon function, thus it is crucial to understand how soaking in vancomycin solution affect tendon material properties.

## 2. Materials

### 2.1 Dissection and Sample Preparation

Ten (n=10) hybrid New Zealand White rabbits and hybrid California White rabbits were acquired post-sacrifice from a local abattoir. No live animal handling was performed by the study team and all animals were bred and sacrificed as livestock prior to study team acquisition. Rabbit hind limbs were dissected and achilles tendons were harvested for materials testing using standard dissection techniques. The tendons were randomly assigned into four groups: two paired (same animal) groups were soaked in a 0.9% phosphate buffered saline (PBS) solution (pH 7.4) for five minutes or thirty minutes, and the other two paired (same animal) groups were soaked in a 0.33 g/mL vancomycin antibiotic and PBS solution for five or thirty minutes (Lamplot et al., 2021; Schüttler et al., 2019)(Figure 1A).

### 2.2 Tensile Materials Testing

After soaking and immediately prior to tensile materials testing, images of the top and side of the tendon were acquired for cross-sectional area calculations using an ellipsoidal area assumption (Pelled et al., 2012). Following testing, five width and five thickness measurements along the length of the tendon to acquired using Fiji/ImageJ (Schindelin et al., 2012). The tendons were then loaded into a custom planar biaxial materials testing system (eXpert 8000, ADMET, Inc., Norwood, USA) with a $300lb_f$ load cell and stretched at a rate of 0.05% strain/s until failure (Lim et al., 2011). Force data were acquired at 100 Hz. Charcoal powder was applied to the surface of all samples for digital image correlation (DIC) strain tracking, with a one second image acquisition rate (Figure 1B).

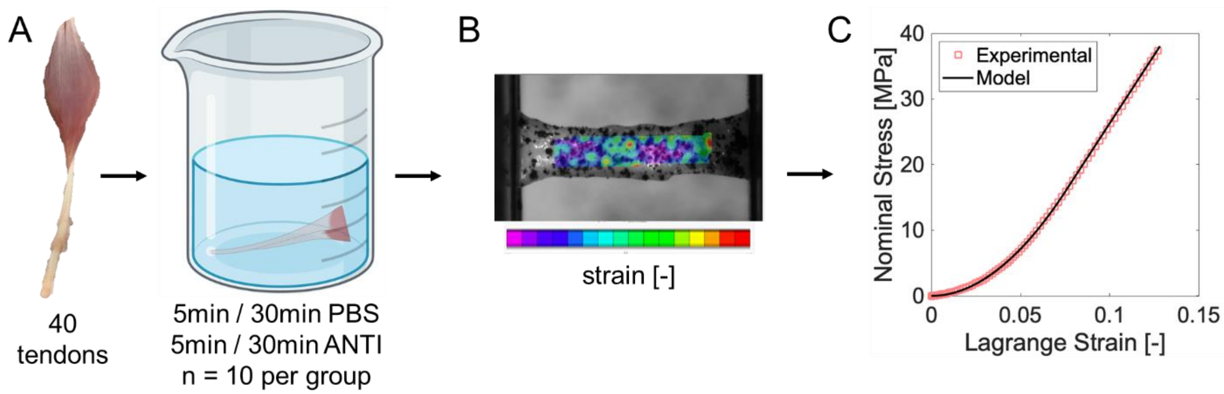


Figure 1. A) Forty dissected tendons were split into four equally sized groups (n=10) and soaked in either PBS for five or thirty minutes or soaked in a vancomycin and PBS antibiotic solution for five or thirty minutes. B) Samples were tensile tested with digital image correlation strain tracking over a mid-sample region of interest. C) A custom MATLAB script was written to fit a piecewise power law toe region and linearly elastic region to stress strain data.

**2.3 Data Analysis**

Commercial DIC analysis software was used to measure Lagrange strain of each sample over a mid-sample region of interest (ROI) (VIC-2D, Correlated Solutions, Inc., Columbia, USA) (Grega et al., 2020). Nominal stress was calculated by dividing force by average cross-sectional area. Stress and strain data were smoothed with the *sgolayfilt* smoothing function in MATLAB (Ma et al., 2009). Visual inspection was used on all stress-strain graphs to identify the end of the linear elastic region. A custom MATLAB script was written to fit a piecewise power law toe region and linear elastic region to stress-strain graphs (Equation 1) (Danto and Woo, 1993). This approach numerically extracted the elastic modulus (Equation 1, $E$ in MPa), transition strain (Equation 1 $\epsilon^*$,unitless), and transition stress (Equation 1 $\sigma(\epsilon^*)$ in MPa) by minimizing the residuals between the piecewise function (Equation 1) and the stress-strain data points for each specimen. The script tested various initial parameter guesses and used the *lsqnonlin* function to generate global minima and a best fit model (Figure 1C) (Vaidya and Wheatley, 2020).

$$\sigma(\epsilon) = \begin{cases} a\epsilon^b & ,\epsilon < \epsilon^* \\ a\epsilon^{*b} + E(\epsilon - \epsilon^*) & ,\epsilon^* \leq \epsilon \end{cases} \quad (1)$$

### 2.4 Statistics

Three two-way analysis of variance (ANOVA) tests were run to determine if the average elastic modulus, transition strain, and transition stress were statistically different across groups ($p<0.05$).

## 3. Results

Nominal stress-Lagrange strain graphs were generated for forty tendons (n=10 per group) (Figure 2). For all forty tendons, the average elastic modulus was 455 ± 37 MPa, the average transition strain was 0.0487 ± 0.0035, and the average transition stress was 9.71 ± 0.79 MPa (Table 1). There were no statistically significant differences in the average elastic modulus (p=0.12), transition strain (p=0.76), and transition stress (p=0.31) between all four groups across both soaking time and soaking solution combined (Figure 3).

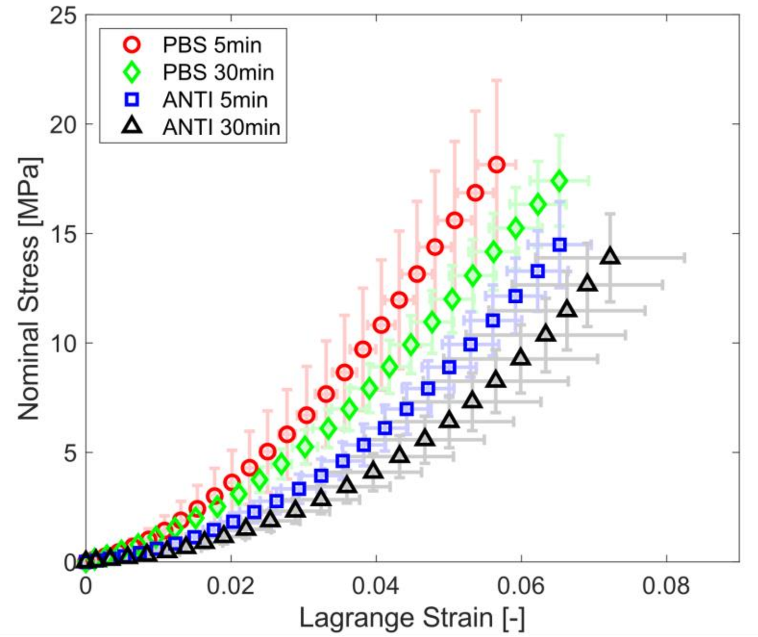


Figure 2. Average nominal stress (MPa), Lagrange strain graph of the four tendon groups. The transition into the linear region of the stress-strain curve occurs at approximately 0.05 strain for each of the four groups. Standard error bars shown.

| Solution | Soak Time (min) | Elastic Modulus (MPa) | Transition Strain (-) | Transition Stress (MPa) |
|---|---|---|---|---|
| PBS | 5 | 545 ± 83 | 0.0539 ± 0.010 | 12.4 ± 1.9 |
| | 30 | 396 ± 58 | 0.0436 ± 0.0039 | 8.55 ± 0.89 |
| Vancomycin + PBS | 5 | 395 ± 42 | 0.0516 ± 0.0049 | 9.27 ± 1.7 |
| | 30 | 483 ± 100 | 0.0456 ± 0.0079 | 8.62 ± 1.5 |

Table 1. The average elastic modulus, transition strain, and transition stress values for each of the four groups (n = 10 per group, means ± standard error).

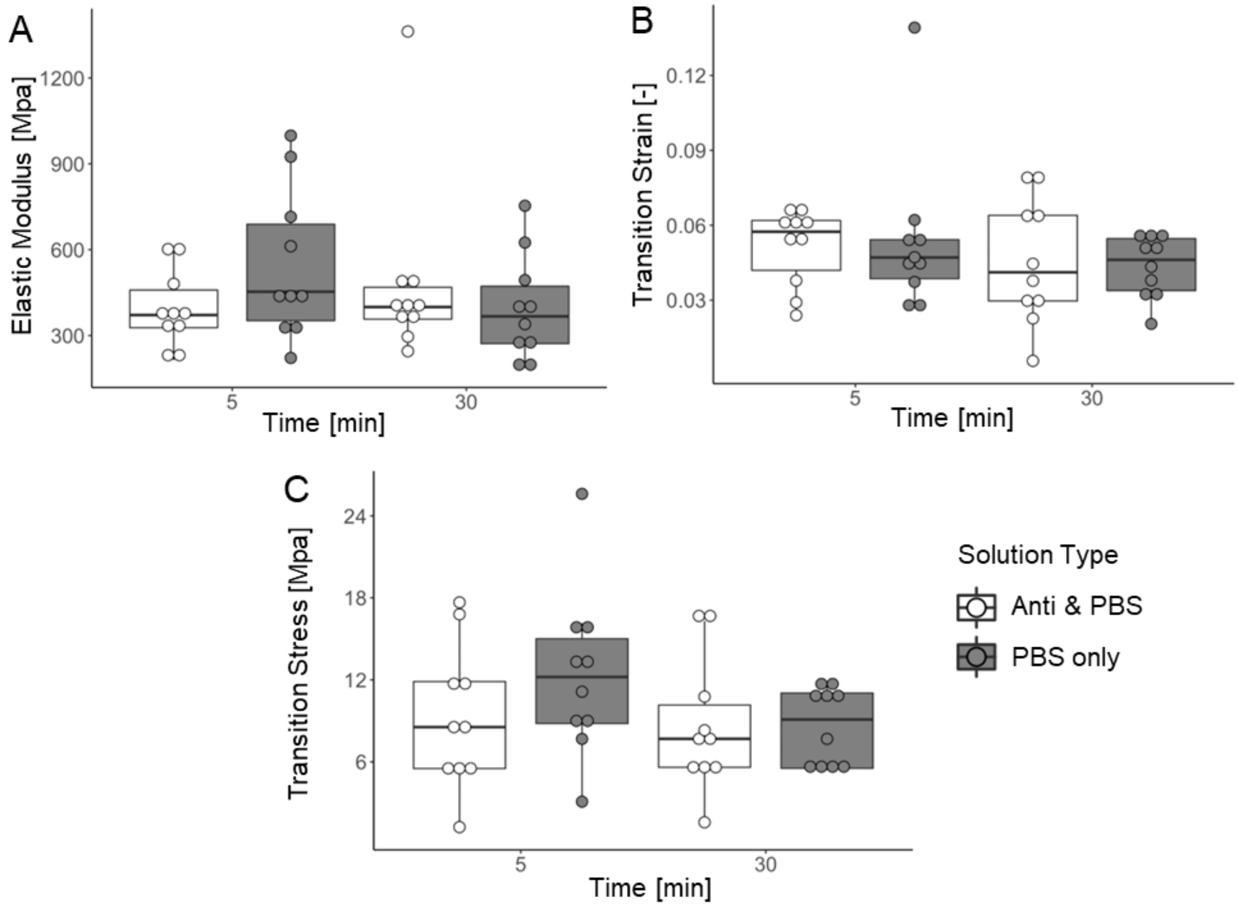


Figure 3. Bar plots for A) modulus, B) transition strain, and C) transition stresses for each of the four tendon groups (n = 10 per group). There were no statistically significant differences in any measures across the four groups (p = 0.12 for modulus, p = 0.76 for transition strain, and p = 0.31 for transition stress).

## 4. Discussion

Prior to implantation during tendon reconstruction surgery, tendon grafts were briefly soaked in solution. Prior to this work, the extent to which short-term soaking time (30 minutes or less) and soaking medium (phosphate buffered saline only or saline and vancomycin antibiotic) affected the sub-failure tensile material properties of tendon was not known. No statically significant differences were found in the elastic modulus (MPa), transition strain, and transition stress (MPa) between four group of tendons soaked in either a PBS solution only or a PBS and vancomycin solution for either five or 30 minutes (Figure 3, Table 1). Our work shows that sub-failure tensile material properties, including the elastic modulus (MPa), transition strain, and transition stress (MPa), of tendons are not altered by short-term soaking in vancomycin.

The present study was not without limitations. While rabbit tendons have been commonly used as a viable animal (Burgio et al., 2022), greater clinical impact would be achieved with human cadaver studies. Additionally, a common procedure for allograft tendons is to thaw the tissue in an antibiotic solution, which was not employed here. However, there has been extensive work showing that the material properties of tendons are not altered by a freeze-thaw cycle (Clavert et al., 2001; Huang et al., 2011; Jung et al., 2011; Lee and Elliott, 2017). Additionally, failure properties were not characterized due to the challenges of producing mid-sample failure during materials testing of whole tendons *in vitro*. Finally, with a sample size of n=10 for all groups, greater statistical power could be achieved with more samples, however it is unlikely that a greater sample size would change the conclusions of the present study.

While this work presents the first study of the effect of vancomycin soaking on tendon material properties, various studies have shown that briefly (<30 minutes) soaking tendon and ligament grafts in vancomycin solutions reduces the incidence of post-operation infection (Banios et al., 2021; Schuster et al., 2020; Schüttler et al., 2019; Xiao et al., 2021). Prior work has shown that extended soaking times and certain solutions, such as NaCl and sucrose, can affect tendon mechanics by altering tissue hydration (Safa et al., 2017). However, our work suggests that short-term soaking typically employed in a pre-surgical setting is unlikely to alter sub-failure properties such as, the toe-region properties and elastic modulus of tendon. Schüttler et al. characterized the failure properties of porcine tendons soaked in antibiotic wrapped gauze for up to 20 minutes and found no changes in those material properties (Schüttler et al., 2019), similar to our findings here. In comparison to Schüttler et al, we evaluated sub-failure properties as tendon grafts more commonly experience non-failure loads *in vivo* and properties such as tendon laxity affect muscle-tendon unit mechanics (Delabastita et al., 2018). In comparison to tendon, other studies of ligament material properties after soaking in a similar vancomycin antibiotic solution found no changes in ligament graft material properties (elastic modulus or elongation strain) (Lamplot et al., 2021). In comparison to the present study, these findings are unsurprising as both tendon and ligament are primarily composed of type I collagen fibers (Amiel et al., 1983).

While similar in composition to ligament, tendon has a highly organized structure that consists of densely packed connective tissue mainly comprised of a parallel fibers of collagen (Amiel et al., 1983; Hudson et al., 2021). Contrastingly, ligament structure consists of less organized bundles of collagen fibers (Dourte et al., 2008). These microstructural differences and the differences in connective roles in the body – tendon as a force transmitter from muscle to bone and ligament as a bone to bone stabilizer (Nordin et al., 2022) require independent mechanical evaluations (Woo et al., 2000). Future work to increase the duration of soaking time in conjunction with cell viability studies would provide further detail into the mechanobiological effects of vancomycin on biological soft tissues.

## 5. Conclusions

Soaking tendons in an antibiotic solution is a critical step in reducing the risk of post-operative infection. The present study measured the material properties of tendons soaked in PBS and vancomycin solutions for five or thirty minutes. Statistical analysis suggested no differences in tendon material properties after soaking across all four groups. These results suggest that during tendon reconstruction surgery, clinicians may soak tendons in a vancomycin and PBS solution for up to thirty minutes without altering tendon material properties.

## Acknowledgements

Funding for equipment used in this work was provided by the National Science Foundation under Major Research Instrumentation Grant No. 1828082. Authors would like to thank the Bucknell-Geisinger Research Initiative for funding to support this work.

## References


Amiel, D., Frank, C., Harwood, F., Fronek, J., Akeson, W., 1983. Tendons and ligaments: A morphological and biochemical comparison. J. Orthop. Res. 1, 257–265. https://doi.org/10.1002/jor.1100010305

Banios, K., Komnos, G.A., Raoulis, V., Bareka, M., Chalatsis, G., Hantes, M.E., 2021. Soaking of autografts with vancomycin is highly effective on preventing postoperative septic arthritis in patients undergoing ACL reconstruction with hamstrings autografts. Knee Surg Sports Traumatol Arthrosc 29, 876–880. https://doi.org/10.1007/s00167-020-06040-2

Burgio, V., Civera, M., Rodriguez Reinoso, M., Pizzolante, E., Prezioso, S., Bertuglia, A., Surace, C., 2022. Mechanical Properties of Animal Tendons: A Review and Comparative Study for the Identification of the Most Suitable Human Tendon Surrogates. Processes 10, 485. https://doi.org/10.3390/pr10030485

Clavert, P., Kempf, J.-F., Bonnomet, F., Boutemy, P., Marcelin, L., Kahn, J.-L., 2001. Effects of freezing/thawing on the biomechanical properties of human tendons. Surg Radiol Anat 23, 259–262. https://doi.org/10.1007/s00276-001-0259-8

Danto, M.I., Woo, S.L.-Y., 1993. The mechanical properties of skeletally mature rabbit anterior cruciate ligament and patellar tendon over a range of strain rates. J. Orthop. Res. 11, 58–67. https://doi.org/10.1002/jor.1100110108

Delabastita, T., Bogaerts, S., Vanwanseele, B., 2018. Age-Related Changes in Achilles Tendon Stiffness and Impact on Functional Activities: A Systematic Review and Meta-Analysis. J Aging Phys Act 1–12. https://doi.org/10.1123/japa.2017-0359

Dourte, L.M., Kuntz, A.F., Soslowsky, L.J., 2008. Twenty-five years of tendon and ligament research. J. Orthop. Res. 26, 1297–1305. https://doi.org/10.1002/jor.20646

Grega, K.L., Segall, R.N., Vaidya, A.J., Fu, C., Wheatley, B.B., 2020. Anisotropic and viscoelastic tensile mechanical properties of aponeurosis: Experimentation, modeling, and tissue microstructure. Journal of the Mechanical Behavior of Biomedical Materials 110, 103889. https://doi.org/10.1016/j.jmbbm.2020.103889

Huang, H., Zhang, J., Sun, K., Zhang, X., Tian, S., 2011. Effects of repetitive multiple freeze–thaw cycles on the biomechanical properties of human flexor digitorum superficialis and flexor pollicis longus tendons. Clinical Biomechanics 26, 419–423. https://doi.org/10.1016/j.clinbiomech.2010.12.006

Hudson, D.M., Archer, M., Rai, J., Weis, M., Fernandes, R.J., Eyre, D.R., 2021. Age-related type I collagen modifications reveal tissue-defining differences between ligament and tendon. Matrix Biology Plus 12, 100070. https://doi.org/10.1016/j.mbplus.2021.100070

Jung, H., Vangipuram, G., Fisher, M.B., Yang, G., Hsu, S., Bianchi, J., Ronholdt, C., Woo, S.L., 2011. The effects of multiple freeze–thaw cycles on the biomechanical properties of the human bone-patellar tendon-bone allograft. J. Orthop. Res. 29, 1193–1198. https://doi.org/10.1002/jor.21373

Lamplot, J.D., Liu, J.N., Hutchinson, I.D., Chen, T., Wang, H., Wach, A., Rodeo, S.A., Warren, R.F., 2021. Effect of Vancomycin Soaking on Anterior Cruciate Ligament Graft Biomechanics. Arthroscopy: The Journal of Arthroscopic & Related Surgery 37, 953–960. https://doi.org/10.1016/j.arthro.2020.10.040

Lee, A.H., Elliott, D.M., 2017. Freezing does not alter multiscale tendon mechanics and damage mechanisms in tension. Ann. N.Y. Acad. Sci. 1409, 85–94. https://doi.org/10.1111/nyas.13460

Lim, J., Hong, J., Chen, W.W., Weerasooriya, T., 2011. Mechanical response of pig skin under dynamic tensile loading. International Journal of Impact Engineering 38, 130–135. https://doi.org/10.1016/j.ijimpeng.2010.09.003

Ma, J., Goble, K., Smietana, M., Kostrominova, T., Larkin, L., Arruda, E.M., 2009. Morphological and Functional Characteristics of Three-Dimensional Engineered Bone-Ligament-Bone Constructs Following Implantation. Journal of Biomechanical Engineering 131, 101017. https://doi.org/10.1115/1.4000151

Nordin, M., Frankel, V.H., Meere, P.A., Mullerpatan, R.P., Wilke, H.-J., Leger, D. (Eds.), 2022. Basic biomechanics of the musculoskeletal system, Fifth edition. ed. Wolters Kluwer, Philadelphia, PA.

Pelled, G., Snedeker, J.G., Ben-Arav, A., Rigozzi, S., Zilberman, Y., Kimelman-Bleich, N., Gazit, Z., Müller, R., Gazit, D., 2012. Smad8/BMP2-engineered mesenchymal stem cells induce accelerated recovery of the biomechanical properties of the achilles tendon. J. Orthop. Res. 30, 1932–1939. https://doi.org/10.1002/jor.22167

Safa, B.N., Meadows, K.D., Szczesny, S.E., Elliott, D.M., 2017. Exposure to buffer solution alters tendon hydration and mechanics. Journal of Biomechanics 61, 18–25. https://doi.org/10.1016/j.jbiomech.2017.06.045

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019

Schuster, P., Schlumberger, M., Mayer, P., Eichinger, M., Geßlein, M., Reddemann, F., Richter, J., 2020. Soaking of the graft in vancomycin dramatically reduces the incidence of postoperative septic arthritis after anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc 28, 2587–2591. https://doi.org/10.1007/s00167-020-05882-0

Schüttler, K.-F., Scharm, A., Stein, T., Heyse, T.J., Lohoff, M., Sommer, F., Spiess-Naumann, A., Efe, T., 2019. Biomechanical and microbiological effects of local vancomycin in anterior cruciate ligament (ACL) reconstruction: a porcine tendon model. Arch Orthop Trauma Surg 139, 73–78. https://doi.org/10.1007/s00402-018-3006-x

Vaidya, A.J., Wheatley, B.B., 2020. An experimental and computational investigation of the effects of volumetric boundary conditions on the compressive mechanics of passive skeletal muscle. Journal of the Mechanical Behavior of Biomedical Materials 102, 103526. https://doi.org/10.1016/j.jmbbm.2019.103526

Woo, S.L.-Y., Debski, R.E., Zeminski, J., Abramowitch, S.D., Chan Saw, MS, S.S., Fenwick, J.A., 2000. Injury and Repair of Ligaments and Tendons. Annu. Rev. Biomed. Eng. 2, 83–118. https://doi.org/10.1146/annurev.bioeng.2.1.83

Xiao, M., Leonardi, E.A., Sharpe, O., Sherman, S.L., Safran, M.R., Robinson, W.H., Abrams, G.D., 2020. Soaking of Autologous Tendon Grafts in Vancomycin Before Implantation Does Not Lead to Tenocyte Cytotoxicity. Am J Sports Med 48, 3081–3086. https://doi.org/10.1177/0363546520951815

Xiao, M., Sherman, S.L., Safran, M.R., Abrams, G.D., 2021. Significantly Lower Infection Risk for Anterior Cruciate Ligament Grafts Presoaked in Vancomycin Compared With Unsoaked Grafts: A Systematic Review and Meta-analysis. Arthroscopy: The Journal of Arthroscopic & Related Surgery 37, 1683–1690. https://doi.org/10.1016/j.arthro.2020.12.212