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Biomechanical Uniaxial Analysis of Porcine Tendon in the CellScale BioTester® 5000

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ABSTRACT

Background: The study was aimed to evaluate whether a mechanical biaxial tester can be used in a uniaxial mode to evaluate the mechanical properties of tendons. **Materials and methods:** The study was carried out on specimens of porcine superficial digital flexor tendon (n = 9). The mechanical properties (elastic modulus, and stress at 15% strain) were measured two times consecutively in the uniaxial mode with the BioTester® 5000 (CellScale) equipment. **Results:** Values of 0.313 ± 0.096 MPa for the elastic (Young's) modulus and of 0.702 ± 0.174 MPa for the stress (at 15% strain) were measured, indicating that the porcine superficial digital flexor tendon is not a strong tendon. **Conclusions:** When suitable specimens cannot be obtained for a biaxial evaluation, tendons can be evaluated mechanically in the BioTester® 5000 employing the uniaxial mode.

Keywords: tendons, mechanical properties, porcine SDFT, uniaxial testing

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INTRODUCTION

Historically, the biomechanics of ligaments and tendons was a field of experimental physiology, which, before the 1970s, has been associated with only minor developments in spite of genuine interest from orthopedic surgeons. Today the situation is very much different, as illustrated in the number of available publications, although the outputs were estimated with rather great variability. For instance, according to such an estimation,1 over 4,000 papers have been published on the topic within the 2000–2010 decade. A PubMed search in March 2023 using "biomechanics of tendons and ligaments" as a search term resulted in 3,680 publications since 1963, while a recent search on Google Scholar using the same term and time range provided around 19,000 entries (a figure likely to be seriously affected by repetitions). Regardless, only a small number of these publications have included actual numerical data for the relevant mechanical characteristics. A variety of mechanical parameters have been reported including yield or ultimate tensile stress (strength) (henceforth, UTS), yield or ultimate load, yield or ultimate elongation (strain), elastic (or Young's) modulus (henceforth, YM), stiffness, and toughness. They were evaluated mostly in the tensile mode, but also in the compressive or shear modes. An excellent summary and analysis of the measured ex vivo biomechanical tensile properties in human and animal tendons and ligands, reported between 1976 and 2015, has placed the recorded values within the 2–230 MPa range for UTS; 1.3–3,000 MPa for YM; and 2–1,100 N/mm for stiffness.2 By any standard, these are exceedingly broad data distributions, arising from causes such as different origins of tendons, different harvesting and conditioning procedures of tendons prior to testing, sample slipping and distortion during testing, stress concentrations occurring in the tissue, and a great diversity of the evaluation instrumentation and techniques. Slippage of tendon samples from the clamps during measurements is still the major factor in preventing reliable and reproducible results to be generated by the testing machines, and a large assortment of clamping and mounting systems have been proposed including frozen clamps ('cryojaws'), or even cyanoacrylate glues.

The earliest significant mechanical evaluation of human tendons was carried out by Guillaume Wertheim,3 who presented his results in 1846 to the French Academy of Sciences. He measured the YMs and UTSs not only for tendons but also for bones, nerves, blood vessels, and muscles. Wertheim was an outstanding experimentalist, and the values published by his team for certain tissues could still be seen in reference texts 100 years after his premature death. For the mechanical characteristics of human tendons, Wertheim measured values for YM (then termed as 'coefficient d'élasticité') between 1.26 and 1.97 GPa and for UTS ('cohésion') between 41 and 102 MPa in five human plantaris tendons and one flexor longus tendon harvested post mortem. In 1936, in an introduction to his own report,4 Cronkite discussed Wertheim's results and also cited from indirect sources the work with human tendons of other early investigators including Valentin (1847), who measured values of UTS between 15 and 22 MPa for palmaris longus and plantaris tendons; Rauber (1876), who measured an UTS of 68 MPa in an unspecified tendon; and Triepel (1902), who measured an average value of 44 MPa for UTS in a plantaris tendon specimen harvested during surgery. In a major study,4 Cronkite himself carried out tensile evaluation on 294 human tendons harvested post mortem, using a mechanical tester for solid materials with a custom-made clamping system. The average UTS of the tendons in different cadavers varied from 60 to 124 MPa, and a large variation of up to 200% between the minimum and maximum values in the same body. He concluded that "it is obviously futile to establish a norm for tensile strength for tendons in general", a conclusion still valid today. Cronkite also noted that the strength of fresh specimens did not differ substantially from that of embalmed (fixed) tendons. Further work carried out during the 1960s was reviewed in studies by Harris et al.5,6 at Tulane University, in which they also reported findings on human tendons, using a custom-made optical-mechanical tester and a more advanced statistical processing of the data points obtained from 30 embalmed plantaris tendons5 and 54 unembalmed specimens removed from amputated lower limbs6. In the first study,5 the values measured for UTS were between 73 and 147 MPa (average 98 MPa), and for YM were ~1.24 GPa for wet specimens and ~2.76 GPa for dried specimens. In their second study,6 the average UTS measured for extensor tendons was 92.3 MPa, while for flexor tendons was 75.5 MPa. However, the YM was variable, and no clear pattern could be defined upon increasing stress. LaBan was the first investigator to associate the tendon's tensile strength to its collagen fibrillar organization.7 Using a custom-made tensile device combined with a microscope, he found that in a canine calcaneal tendon, no tearing of collagen fibers could be noted at a stress below 6 MPa. Within this range, the tendon displayed viscoelastic properties rather similar to those of certain synthetic polymers.

Since then, an impressive variety of measuring techniques and devices have been used to evaluate the mechanical properties of tendons. Over the last few decades, the uniaxial machines (e.g., Instron® or Zwick/Roell testers) became popular and are still used extensively. In the uni-

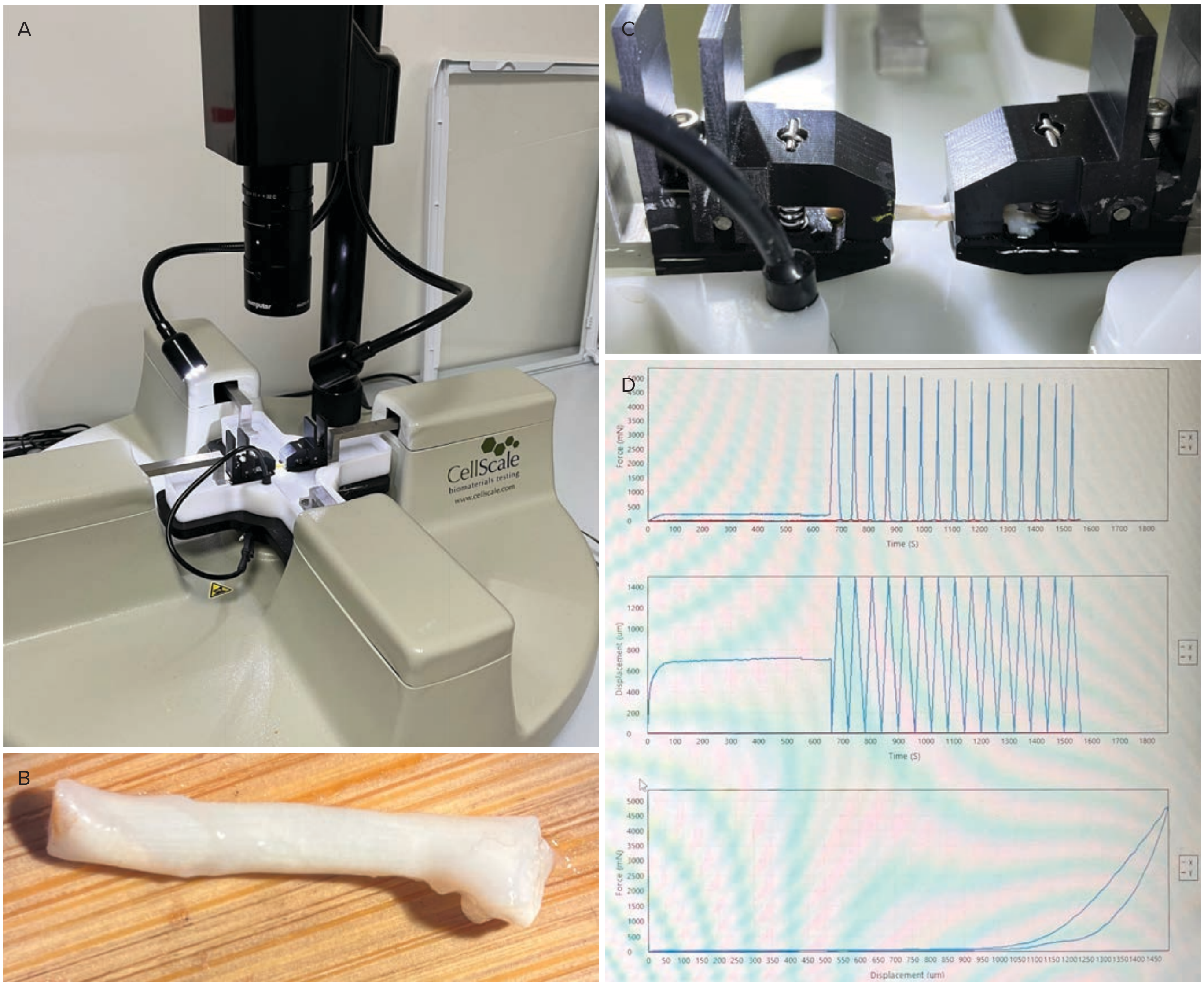

FIGURE 1. **A** – The BioTester® 5000 instrument; **B** – An excised segment of the porcine superficial digital flexor tendon; **C** – A tendon specimen mounted in the tester by clamping; **D** – Graphical output from the tester's software corresponding to the main evaluation stages

axial test, the sample is subjected to a force along one direction only until failure occurs due to stretching (or other type of force). In recent years, the biaxial testing started to be employed in many laboratories. In the biaxial test, the sample is stretched along two distinct perpendicular directions. This method is recommended for anisotropic materials such as soft biological tissues; however, it is difficult sometimes to find tendons that can be excised and fashioned into square plane slabs as required by the cruciform clamping system of the testing instrument. In such cases, the biaxial tester could be used in the uniaxial mode, and an example of such versatile machine is the BioTester® 5000 manufactured by the CellScale company (Waterloo, ON, Canada) (Figure 1A).

In the present study, we have evaluated specimens of the porcine superficial digital flexor tendon (SDFT), which are elliptical in cross-section and could not be fashioned into square slabs with a width allowing the cruciform clamping normally required for biaxial testing.

MATERIALS AND METHODS

Materials

The study was carried out on nine individual segments of the SDFT retrieved post mortem from three pigs (species Sus domesticus) of the breed White Large, 10 months old, procured from a local authorized slaughterhouse (Agro-

Ardeal S.R.L., Orheiu Bistriţei, Bistriţa-Năsăud). The animals were sacrificed for commercial purposes, and the tendons would have been discarded if not used in this study. Only the tendons from the frontal legs of the animals have been harvested, having an average length of 19.39 ± 1.49 mm and an elliptical circumference with an average diameter of 2.95 ± 0.32 mm (Figure 1B). After harvest, the tendons were stored at −20°C. Phosphate buffered saline (PBS) was supplied by Lonza (Verviers, Belgium).

Uniaxial analysis in the CellScale BioTester® 5000

The BioTester® 5000 (CellScale) included four actuators (only two actuators being used for uniaxial testing), load cells, systems of rakes with tines and hooks (BioRakes®) for specimen mounting, clamp sample mounting systems, and user interface software for simple or multi-modal testing with real-time feedback. The 23-N load cell was used in our experiments.

After harvesting, the tendon segments were cut with a scalpel into specimens of approximately 13 mm in length, which were stored in PBS at room temperature prior to measurements. The thickness of each specimen was measured in triplicate with a caliper by the same person and the values averaged for further data processing.

The specimens were clamped along the longitudinal axis between two opposite arms of the instrument. A working distance of 10 mm was set as the initial distance between the two arms. The specimens were inserted manually between the clamps (Figure 1C), preferably by the same person to minimize possible bias related to the act of insertion.

To ensure that the samples were tensioned, the evaluation started with a tensile preloading for 60 s until a force of 230 mN was attained, followed by a 10-min hold period at this force. After this preconditioning period, 15 cycles were initiated, each consisting of a stretch period (where the maximum tension was set at 15% of the initial length of the specimen), a deformation of 1% per second, and a relaxation period, as illustrated in a typical graphical output of the BioTester (Figure 1D). The series of nine specimens was evaluated two times in the tester.

By employing the LabJoy 2.0 software (CellScale, Waterloo, ON, Canada), the raw data were generated in an Excel file, ready to be processed for calculating strength and YM for each specimen.

Statistical analysis

The data were plotted as mean values ± standard deviation. For the statistical comparison of values, GraphPad® Prism software (version 6.0) was used, with application of the Wilcoxon matched-pair rank test for continuous data (for n = 9).

RESULTS

The set of nine tendon specimens was evaluated in the BioTester two times consecutively with an aim of assessing stability of the measuring technique and reproducibility of results. The results were presented as bar graphs in Figure 2. Neither the YM (Figure 2A), nor the strength (at 15% strain) (Figure 2B) sets of values showed any significant difference between the two measurements, as reflected in the high p values.

DISCUSSION

Most of what has been reported so far on the mechanical properties of porcine tendons was summarized in a recent review.8 Following the information provided in this source, the values reported for porcine digitorum profundum flexor and extensor tendons included ~0.8 to ~1.7 GPa for YM, and ~40 to ~90 MPa for UTS, while for the porcine Achilles tendon values of 248 to 409 MPa for YM and 42 to 76 MPa for UTS have been also reported.

Many investigators expressed their results in units of maximum load (N) or stiffness (N/mm). For instance, Domnick et al.9 compared porcine flexor digitorum profundus tendons with human cadaveric semitendinosus tendons in a Zwick/Roell uniaxial tester using frozen clamps to prevent slippage. There were no significant differences between the two sets regarding stiffness (porcine ~211 N/mm, human ~208 N/mm), but the maximum load was higher for porcine tendons (~1.8 kN) as compared with human (~1.4 kN). It was concluded that the use of porcine flexor tendons as grafts in human surgery is justified mechanically. To compare these data with our findings, it is necessary to know the dimensions of the samples in order to render the reported parameters into units of YM and stress.9 There is only one study published on porcine SDFT, reporting maximum loads of ~230 N and stiffness of ~56 N/mm.10 Again, these results cannot be compared with our results due to lack of dimensional details for the specimens.

When it was possible to compare our results for YM and stress with other reported values (less than 0.4 MPa for YM, and less than 0.9 MPa for stress, see Figure 2), it could be seen that the former were much lower. Nonetheless, this can be explained by the fact that the existing literature data refer to the porcine flexor digitorum profundum (deep) tendon or to the extensor tendons, which are

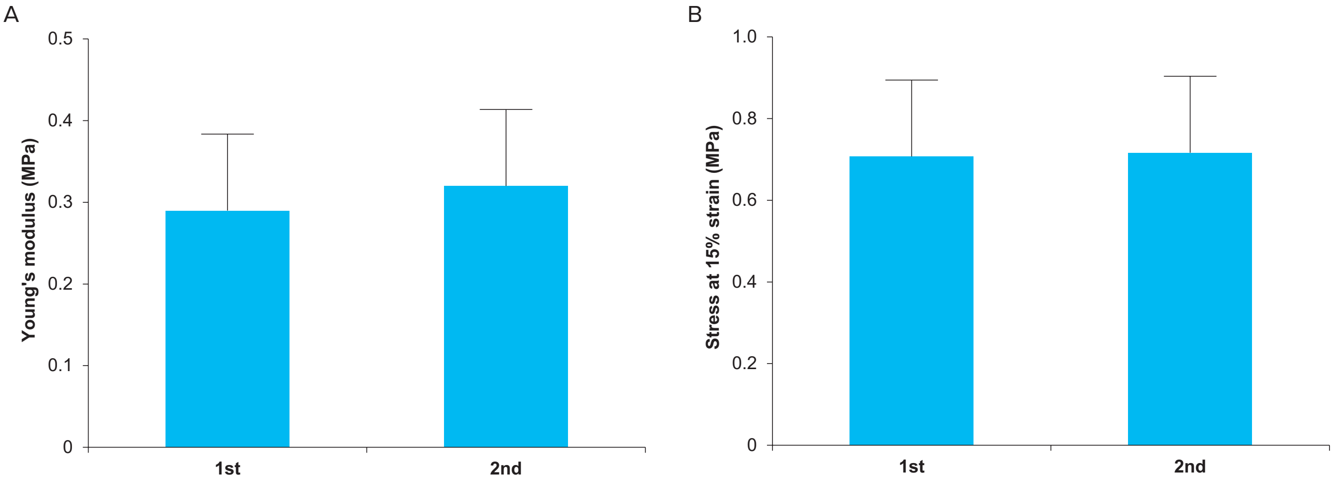

FIGURE 2. The results of two consecutive measurements of the 9-specimen set for the YM (**A**) and for strength at 15% strain (**B**)

much stronger mechanically than the superficialis tendon that was used in our study.

CONCLUSION

The porcine SDFT can be conveniently evaluated mechanically in the BioTester® 5000 (CellScale) in the uniaxial mode using an adequate clamping system. The measuring technique is stable and leads to reproducible results.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest or any financial interests that are relevant to the content, authorship, or publication of this article.

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DATA AVAILABILITY STATEMENT

The authors confirm that all relevant data are included in the published paper. Additional information can be provided upon reasonable request.

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