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Development of a cost-effective torsional unit for rodent long bone assessment

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INTRODUCTION

Mechanical testing mode is governed by the desired information. For instance, whereas bending provides a measure of tensile/compressive stiffness and strength, torsion provides a measure of shear stiffness and strength¹. Further delineation may be made in that four-point bending subjects a central region of the bone shaft to pure bending while three-point bending subjects a central region of the bone shaft to a combination of bending and shear. Torsion systems are commonly used in biomechanics and descriptions of their use may be found throughout the literature. Much of the smaller animal (rodent) torsion testing is accomplished with commercial testing systems and the focus of the work lies with the analysis rather than the system and experimental technique^{1–6}. However, when dealing with specimens on the scale size of rodent limbs, it is essential that care be taken to minimize pre-testing bone damage and ensure an accurate and reproducible test. For mechanical testing it is important to appreciate that data is only as reliable as the system and methods applied to obtain it.

In a previous *Technical Note* published in this journal, we detailed the development of a small-scale, cost-effective loading machine for biomechanical research⁷. The goal was to demonstrate to researchers interested in biomechanical testing that a relatively simple, cost-effective and flexible uniaxial testing platform could be constructed in-house and to aid nonengineering-oriented researchers in the development of their own system. In addition, testing capabilities were discussed which included compression, tension and bending and it was illustrated that this system could grow with the user's requirements. As we continue to use this system in biomechanical evaluations of rodent limbs and neonatal organ cultures, testing needs have grown to include torsion. In this work the development, characterization and utilization of a simple rack and pinion system for torsional testing is described. Furthermore given the small scale work, a fixture/potting system was developed to ensure that loading and premature damage did not occur during potting and test set up. The concepts and approaches discussed here are readily applicable to any axial loading machine.

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METHODS

To begin, it is important to preface that historically ‘torsion’ tests conducted in biomechanics would more correctly be categorized as ‘twist’ tests, and until recently pure ‘torsion’ tests were not conducted^{8–9}. Whereas in a ‘pure torsion’ test both ends of the specimen are equally rotated, in the ‘twist’ test one end is fixed and the other is twisted about the fixed end. Given that the convention has been to refer to these twist tests as ‘torsion’, we will continue this convention. We will use the term ‘pure torsion’ to indicate tests in which the torque is applied equal and opposite at both ends.

Torsion may be accomplished with a variety of mechanisms incorporating sliders, cams and bell cranks. In this work a rack and pinion system which utilizes a commercially-available gear set (spur gear and rack) to translate the linear (vertical) motion of the loading machine into rotational motion was developed. While the linear rack (Stock Drive Products, S1VS48-064F1324) mounts to the loading arm of the existing machine, the pinion (Stock Drive Products, S1811Y-RB-4P) mounts to the base of the loading machine platform (Figure 1). In order to utilize rack and pinion systems, it is important that the rack conform to the pressure angle (14.5°) and pitch (12) of the spur gear and this is easily accomplished if the two are purchased together. In addition, the rack should be of sufficient length to accommodate both internal and external rotation depending upon the direction of motion of the loading arm. That is, if the bones are tested such that a clockwise rotation of a right limb will produce an external rotation, a counter-clockwise rotation is necessary to produce an external rotation in the left limb for the same orientation in the fixtures. The spur gear is mounted to an aluminum shaft (19 mm diameter) and sandwiched between two bearings mounted in each of the arms of the holding bracket secured to the base of the loading machine to enable unrestricted shaft rotation. A major advantage of the rack and pinion approach is that it accommodates a larger rotation range than other approaches enabling both routine internal and external torsional analysis.

Given the existing uniaxial loading platform, the goal was to develop a torsional system that would fit within this frame and would enable torques up to 176.5 Nmm (25 oz-in) to be recorded. For simplicity, a torque cell that would interface with our existing data acquisition system (Vishay) was selected. In addition to the torque cell, a 25 mm displacement sensor was outfitted to the system to convert displacement to rotation and obtain torque-twist data in real-time. The conversion is fixed for a given pinion diameter; when the rack moved 25.4 mm, the pinion point moved 25.4 mm along its circumference. Using the ratio of the fraction of the circumference (arc length (s)) traveled by the linear displacement to the gear radius (r), the linear motion was determined and converted to degrees (θ) ($\theta = (s/r) * (180^\circ/\pi)$). For a pinion diameter of 35 mm, a 25.4 mm linear displacement resulted in a rotary motion of the pinion of 83 degrees (1.45 rads).

With the rack and pinion system in the testing machine, an aluminum calibration block was fabricated to set the gap distance between the two ends of the torsion device to accommodate the specimen length and lock down the fixtures (Figure 2). In addition, alignment was fine tuned at this time. That is, using the linear motion of the slide, the pinion was rotated slightly until the calibration block slid freely within the torsion device to ensure that the specimen would not be twisted when set into the frame.

A design goal was to develop fixtures that could accommodate specimens of various lengths always ensuring that the bone/specimen was tested on its central (mechanical or geometrical) axis in order to guarantee torsional loading of the various specimens. Again, for this type of testing, the torsion was not ‘pure’ in the sense that both ends of the specimen were rotated. That is, one end of the specimen was fixed while the other was rotated to

failure. In addition to the rack and pinion fixtures, given the small scale of rodent work, it was also necessary to develop a means by which to mount these specimens in the testing machine while ensuring that unintentional but damaging loads were not placed on them that would cause premature failure. To accomplish this task, a series of potting fixtures and a testing methodology that enabled minimization of pre-testing loading damage was developed. As shown in Figure 3, aluminum potting/testing fixtures and a slotted alignment frame with thumbscrews were fabricated that enabled the specimens to remain aligned while being potted in polymethylmethacrylate (PMMA) bone cement. The bone cement was mixed relatively thick and the specimen was lowered into the potting fixture after it was filled with cement to avoid PMMA on the shaft, which would affect the test. In addition, a square plastic template with a central hole matching the size of the specimen shaft was placed in the fixture to keep the specimen centered within the fixture. Once one end of the specimen was potted, the potting fixture was inverted and the procedure was repeated for the other end. The specimens were kept hydrated in saline, rehydrated for 60 min following potting and tested immediately thereafter. Although other methods of anchoring the bones to the fixtures were tried including cyanoacrylate, nothing performed as well as the PMMA. For example, pinning the bone ends to give the cement something to anchor to was not appropriate given the size of the bones and automotive putties were too thick to sufficiently fill the area around the specimen and ensure a rigid fixation. Given the small amount of PMMA used, exothermic setting effects were not substantial. In addition, the base of each potting fixture was drilled and tapped then plugged during testing with a set screw. Prior to potting, silicone (3M) was sprayed in the cups and following testing the set screw was used to push the cement out of the fixture. This made removal of the cement from the potting fixtures relatively problem-free. Briefly freezing the fixtures also helped to release the PMMA. Once potted, the specimen was placed in the square testing fixtures. The alignment frame was removed and plates were screwed to the top of the fixtures to secure the specimen during testing. The square shape of the testing and potting fixtures was a simple way to ensure that potting slippage during testing did not occur.

Prior to use, the system was calibrated using test samples of PMMA (plexiglas). Using paraffin wax and stainless steel Steinmann pins, a mold was made to produce uniform PMMA rods of 3.2 mm diameter, Figure 4. The 50 mm rods were cut in half and potted in the fixtures using PMMA such that a 16 mm shaft length was left exposed. Four rods were tested to failure with torque-twist recorded. From this data, a stress-strain curve was obtained and the shear modulus (G) was calculated as the slope of the shear stress-strain curve, Figure 4f. As shown in the figure, the potting technique adequately forced failure to the shaft and testing resulted in a spiral fracture. For the specimen shown, a shear modulus of 1090 MPa was obtained. This agreed nicely with previous results in the range of 1094–1100 MPa⁹. All four specimens exhibited spiral fractures. Moduli for all four specimens were within 6% of each other and the protocol and accuracy and precision of the torsional device were deemed acceptable. However, rigorous statistics on a large sample size were not completed due to torque cell damage concerns. That is, torque cell capacity (176.5 Nmm) was exceeded during testing, Figure 4e. While the recorded values were actual torque cell readouts and not interpolated data, if testing of objects on this scale is warranted a torque cell of larger capacity should be utilized.

To verify that this system works well for small-scale applications, fresh humeri and femurs from 3 month old mice were potted and tested to failure, Figure 5. To assess an appropriate loading rate range, humeri (n=4) were tested to failure under displacement control at a constant linear rate of 2 mm/sec (6.5 deg/sec) while femur (n=4) testing utilized a linear loading rate of 0.35 mm/sec (1.1 deg/sec). In both cases, a data collection rate of 10 Hz was employed. Structural properties and failure locations were determined. Strain gauges were not applied given their possible effect on bone shaft compliance.

RESULTS

From destructive testing on the mice humeri, mean $\pm$ standard deviations for stiffness, failure torque and failure twist were: 2.84 ± 3.7 (Nmm/deg); 12.00 ± 5.1 (Nmm) and, 12.01 ± 2.8 (deg), respectively. From destructive testing on the mice femurs, mean $\pm$ standard deviations for stiffness, failure torque and failure twist were: 2.90 ± 1.0 (Nmm/deg); 25.45 ± 4.0 (Nmm) and, 10.23 ± 1.2 (deg), respectively. For this work, the gage length was kept constant and the humeri and femurs were relatively the same length. Had animals of different ages and sizes been addressed, an approach to account for these discrepancies would have been to normalize the rotation angle by dividing it by the gage length¹. Typical torque-twist curves for a humerus and femur test are shown in Figure 5 and the classic spiral fracture (Figure 3f) accompanying torsional failure was highly reproducible.

DISCUSSION

The goal of this work was to design, fabricate and validate the use of a cost-effective rack and pinion torsional system and setup technique for reproducible and accurate rodent limb torsion testing. The goal was not to give the reader an extensive review. Moreover, the intent was not to suggest that the use of this system is sufficient to provide a thorough analysis of bone behavior under torsional loading. For instance, it is important to understand that the behavior of bone is governed by both its material and structural properties which together determine the mechanical properties. Whereas the system and approach developed in this work provided structural property determination (shear stiffness and strength), the geometry and material properties also contribute to the mechanical properties and a complete analysis requires evaluation of both the geometry (bone quantity) and material properties (bone quality). With the increase in researcher access to micro-computed tomography (microCT) scanners and their accuracy, geometry and density measures are becoming increasingly useful for material property determination⁵.

As with any testing machine, the major limitation is allowing for machine deformation independent of specimen deformation, which is seldom done¹⁰. However, as with most biological materials it is assumed that if the machine is relatively more rigid than the specimen¹, machine deformation is negligible and the measured deformation is the specimen deformation. An additional consideration centers around the rack. Given that the rack attaches to the arm of the platform at one end, the length of the rack is directly proportional to the compliance; the greater the rack length, the greater the compliance and the more inaccurate the results. As shown in Figure 1, the arm holding the rack attaches to the slide at three possible locations/elevations. We have developed racks of different lengths to accommodate the amount of rotation needed, while minimizing compliance. For instance, generally for 25 mm of test travel we will utilize our 50 mm rack in comparison to our 100 mm rack. The shorter rack increases system rigidity and yields a much more stable test. This is convenient when internal/external rotation is not a concern. However, if the longer rack is required we engage the rack and pinion while affixing a dial indicator with magnetic base to the rack to verify that there is nominal deflection in the rack throughout the range of testing travel. We also set up the torsional system once and conduct all tests with this setup to minimize the effects of setup variation. Although this may seem like a trivial issue, given that we have chosen to calculate twist based upon linear rack motion it is critical that rack compliance be monitored. Given our pre-existing platform, for convenience and cost reasons we chose not to measure rotation directly from a rotary variable differential transformer (RVDT). These efforts ensure that our linear measurements are accurate and our rotational calculations are acceptable.

After addressing these platform concerns, the practical limitations of this system are then determined by the bone shaft. That is, with respect to length the shaft must be long enough that the bone ends can be sufficiently potted to concentrate the torque in the central bone shaft rather than tear the bone at the growth plate. Furthermore care must be taken to ensure that bone cement does not accumulate on the bone shaft or this will significantly alter the data. Given our interest in neonatal rat organ culture modeling, we have attempted to test bones as small as 6.5 mm in length (3 day old neonatal Wistar rat femurs) unsuccessfully. In our hands, we have successfully tested rat humeri taken from 12 day old neonates (approximately 14 mm in overall length). The animal model is stressed in that bone dimensions may change with strain variation and transgenic alteration. Furthermore, loading rates from 0.3 mm/sec to 2.5 mm/sec were acceptable given the 10 Hz sampling rate (scanner limitation). Slower rates quickly became noisy while faster rates were insufficient to determine curve characteristics.

Another major issue that must be addressed with the shaft is the anatomical and mechanical axis. In testing long bones with curvature, considerable error in torsional values have been obtained when applying techniques developed for prismatic materials to inhomogenous, non-axisymmetric materials such as bone. Our system addresses the curvature with potting technique. We pot bones on the anatomical axis and ensure that the bone ends are aligned coaxial in the platform. We justify this simplification based on the practical limitation that our exposed shaft (gage length) is on the order of 1–2 mm in length and the curvature, particularly in our neonatal models is relatively nonexistent. Of greater concern is the simplification of conducting a twist versus a pure torsion test and the inaccuracies that result from this simplification. Nazarian et al., recently focused on this critical simplification and developed and verified a system to apply pure torsion to bone using a force couple approach^{8–9}. The interested reader is referred to this excellent work to further highlight the impact of this issue and the effects on testing results. However, our goal was to utilize a pre-existing linear loading platform to accommodate torsional loading. As evidenced by the work of Nazarian et al., their system, while more accurate was a single purpose system and given our sporadic torsion testing needs we felt our approach more practical. However, it is important to note that while our system would more accurately be referred to as a ‘twisting’ system than a ‘torsional’ system, given that we are using this ‘twisting’ system to determine structural properties, our relative comparisons are still accurate. As noted in the work of Nazarian et al., the coupling approach would be the superior method for material property determination⁸. Moreover, the excellent agreement between our PMMA moduli to Nazarian and manufacturer’s standards, should not be misinterpreted as a twist test yielding equally valid material property data as a pure torsion test. The PMMA molds were developed to test an ideally symmetric, homogenous material, a description not relevant to bone. This further demonstrates that it is important to understand the limitations of your testing system/approach. It is ultimately the responsibility of the user to determine which test will provide the correct information.

Our interest is in the development of cost-effective testing platforms and fixtures that effectively allow users from all backgrounds to competently conduct standard biomechanical tests. The development, fabrication and validation of this rack and pinion system enables successful torsion testing of rodent limbs and builds upon the standard testing platform previously developed⁷. The potting system enables the bones to be aligned and minimizes unintentional pre-loading damage resulting in increased testing accuracy, precision and reproducibility. As such, the system and approach developed offer a cost-effective solution to adapt any axial testing machine for torsion testing and are particularly useful for small-scale rodent limb work.

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Figure 1a.

Figure 1b.

Figure 1.

a) Schematic of loading platform with torsional fixture in place. Torsion (twist) was achieved with a rack and pinion approach to utilize the linear motion of the existing axial platform. In this type of torsional system, the specimen end attached to the rack while the pinion rotated relative to the other, fixed specimen end. b) Photograph of torsional system in loading platform.

Figure 2. Photographs of (a) setup gap distance to accommodate potted specimen, (b) square geometry of the testing fixture designed to eliminate slip, and (c) square calibration block used to lock down the fixtures and verify alignment.

Figure 3.

Photographs of (a) potting/testing fixtures. (b) Two potting chucks on the sliding alignment frame eliminate pre-load on the bone shaft prior to testing. (c) The alignment system slides into the torsional fixture (post-calibration – Figure 3) and the alignment plate removed (d). (e–f) Care taken to minimize pre-loading damage results in a highly reproducible testing procedure and the classic spiral fracture characteristic of a properly conducted torsion test. Arrows indicate location of exposed bone shaft.

Figure 4.

(a) To calibrate the system, axisymmetric, homogenous PMMA rods were fabricated using a paraffin wax mold and a 3.2 mm diameter Steinmann pin as a blank. Four rods were potted and tested to failure and torque-twist recorded (b–d). (e) Typical torque-twist curve for PMMA specimen and (f) converted stress-strain curve. Converting to stress-strain, the shear modulus was calculated as the slope of the stress-strain curve.

Figure 5. Typical torque-twist curve of a mouse humerus (top) and femur (bottom) demonstrating data curves from the extremes of acceptable loading rates with a 10 Hz data collection rate.