Custom 3D Printed Fixtures for Repeatable Torsional Testing of Mouse Tibia Fracture Callus

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INTRODUCTION: Mechanical testing of fracture callus in mouse tibia is technically challenging. 3- and 4-point bending assays are challenging given the complex shape of the tibia and variability in the callus, and don't appropriately test the complex in vivo load requirements of long bone callus. Torsional testing is the only mode which is robust to the seemingly random variations in radial symmetry commonly found in fracture callus. However, torsional testing itself is highly susceptible to perturbances in position of the fracture centroid on the torsion axis. Centering is extremely difficult to control and reproduce when using traditional fixturing methods, largely due to the intersection of test specimens' complicated external geometry and low precision of potting using manual alignment. In fact, most similar torsion studies in mouse tibial fracture have a coefficient of variance (CV) within groups of 35%-50%. The objective of this study was to design a torsional testing methodology with repeatable specimen centering, thus reducing variability in testing conditions thereby improving data reliability and reducing animal sample size.

METHODS: Distal tibial fractures were generated in isoflurane anesthetized mice at 18 weeks of age (3 male, 4 female), as previously described. Mice were administered sustained-release buprenorphine for analgesia and monitored daily post-operatively. This fracture model was approved by IACUC. Tibiae were harvested at day 25 post-fracture and scanned using micro computed tomography (µCT). Reconstructed images of each scan were imported into Dragonfly 3D and the centroid of each fracture was mathematically calculated. A holder was then designed for each bone using the following steps: [1] A contour mesh of each bone was created including a small amount of padding to ensure appropriate tolerance for fitting for the bone. [2] Each bone mesh was inserted into its own 3D modeling file in Fusion 360 containing four hemi-cylinders outfitted with slip-fit alignment pins, an alignment plate, and stabilizing clip assembly. [3] Each bone mesh was positioned with the fracture's centroid in the exact center of the holder cylinders and the bone mesh geometry was removed from the cylinders to precisely accommodate and align its bone (**Fig 1a-b**). The resultant fixtures were 3D printed using a Form 4 printer. Finally, each bone was loaded into its respective custom printed fixture (**Fig 1c**) and secured using small amounts of cyanoacrylate. A stabilizing clip was attached to the fixture to keep the fixtures rigid along the torsional axis until fixtured for testing (**Fig 1d**). Fixtured bones were scanned again using µCT to assess fit of bones into their custom holders. Fixtured specimens were then secured in a custom torsion instrument and loaded in external rotation at 0.5°/s until failure. Torque and angular displacement data were captured (**Fig 2a**), and a custom MATLab script was used to calculate ultimate torque and torque at failure. CV was calculated across the entire cohort and by sex, and approximate processing time and material burdens of these methods were recorded.

RESULTS: In total, all processing steps required to prepare one specimen for torsional testing took 1-2 hours of active effort from a trained undergraduate operator. The material burden included µCT system access, a computer with 64GB RAM and dedicated graphics, 3D data processing software, CAD software, an SLA 3D printer (or funding to order parts from a 3rd party), and a small-scale torsional testing device. Additionally, µCT scanning and image reconstruction required several hours of inactive wait time to process all 7 limbs, which is device and method dependent. Mean ultimate torque for the entire cohort (n=7) was calculated at 12.1±3.0 Nmm (CV 25%) and torque at failure at 11.2±1.8 Nmm (CV 25%). Males (n=3) had a mean ultimate torque of 13.9±3.7 Nmm (CV 27%) and torque at failure of 12.8±3.8 Nmm (CV 30%) (**Fig 2b**). Females (n=4) had a mean ultimate torque of 10.6±3.7 Nmm (CV 17%) and torque at failure of 9.9±1.5 Nmm (CV 15%) (**Fig 2b).**

DISCUSSION: This study demonstrates that we can use mathematical centering to get repeatable results with small CVs for mechanical testing of preclinical specimens. These spreads are notably small considering the difficulty of these torsion models and comparable spreads noted in literature. While our male cohort showed a considerably higher CV than our female cohort, this finding is limited by our low sample size. We intend to add specimens to this cohort before completing in depth comparative analysis to literature values. We also intend to expand on this model with data for whole tibia, femur fracture callus, and intact femur.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrates that meaningful mechanical data can be collected on fracture callus using a smaller number of mice, which supports more efficient and ethically conscious preclinical research.

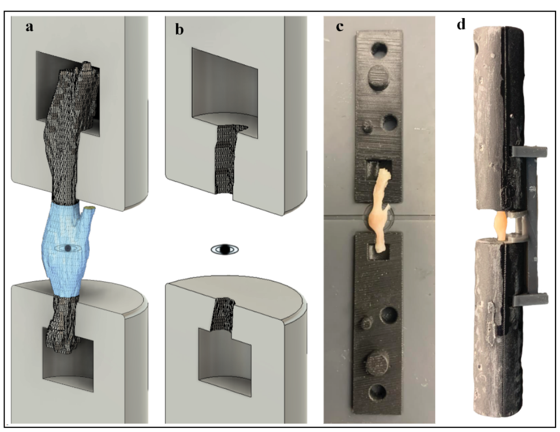

Figure 1: *(a-b) Digital design of a custom centered torsion holder. (c) Tibia being fit into custom printed fixture on alignment jig. (d) Bone fixtured for torsional testing of callus with stabilizing clip.*

Figure 2: *Results of torsional testing. [a] Representitive torque/rotaion curve. [b] Ultimate torque and torque at failure outcomes separated by sex with plotted standard deviation and individual data points.*