Virtual Mechanical Testing for Non-Destructive Assessment of Bone Regeneration in Large Ovine Tibial Defects

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INTRODUCTION: Virtual mechanical testing is an image-based approach to evaluating bone healing that preserves the sample for other analyses, such as histology. Previously, we developed and validated the computational methods for measuring the virtual torsional rigidity (VTR) of intact and healing ovine osteotomies (1). For small defects (3 mm) and moderately large (17 mm) defects with autograft, we demonstrated that the virtual torsion tests are a reliable surrogate for destructive and labor-intensive physical biomechanical tests in ovine surgical models that progress to union within 9-12 weeks. The goal of this study was to assess the validity of virtual torsion tests in an independent dataset with a large-defect limb salvage model that heals much more slowly. Our hypothesis was that virtual torsional rigidity (VTR) would strongly correlate with the physical biomechanical data.

METHODS: Thirty-four sheep with 30-mm tibial osteotomies stabilized by external fixators were randomized into two groups based on the resorbable graft containment system used to support weak bony tissue within the ostectomy site: a resorbable copolymer poly (L-lactide-co-glycolide) mesh and a 3-D printed osteoinductive calcium phosphate coated polycaprolactone graft cage system. In-vivo clinical CT scanning was performed at intervals of 4 weeks starting intraoperatively up to euthanasia at 18 weeks post-op using an 8-slice CereTom (Samsung) mobile CT with a tube current of 4 mA and a tube voltage of 120 kVp resulting in 1.25 mm slices (reconstructed down to 0.625 mm in helical scans) with an in-plane resolution of 0.495 mm. In each group of 17 sheep, 12 animals were used for postmortem torsion testing and 5 were reserved for histology. Four animals with incomplete scans were excluded. Only the 18-week scans with corresponding physical mechanical tests were used for this validation study. All scans were processed using Materialize Mimics (v23.0) to segment bone and callus in the defect zone and create quadratic tetrahedral finite element meshes. Elementwise material properties were defined using a piecewise function according to a dual soft-hard model for mineralized and non-mineralized tissue. Elements with a bone mineral density (BMD) below 665 mgHA/cm³ were assigned a soft tissue value of 50 MPa and all other elements were assigned material properties based on a linear equation calibrated for ovine bone (2). Virtual torsion tests were performed using ANSYS (2020 R2) by rigidly fixing the distal end of the tibia and applying a 1° rotation through the bone’s long axis on its proximal end. Virtual torsional rigidity (VTR) was calculated using the formula: \( VTR = ML / \phi \) [Nm/°], where \( M \) = the calculated moment reaction, \( L \) = the working length of the bone segment, and \( \phi \) = the applied angle of twist. Torsional rigidities for the physical tests were determined by multiplying each torsional stiffness by its corresponding gauge length. Statistical analysis was performed in SPSS (v29). Pearson’s correlation was performed to evaluate the linear relationship between virtual and physical rigidity values. Additionally, a two-tailed, homoscedastic t-test was performed to assess differences in torsional rigidity between the physical and virtual tests. Normality was confirmed using a Shapiro-Wilk test.

RESULTS: The mean physical torsional rigidity was 0.563 ± 0.281 Nm/° while the mean virtual torsional rigidity was 0.471 ± 0.328 Nm/° (no significant difference between means, \( p = 0.350 \)). Virtual torsional rigidity was strongly and significantly correlated with the torsional rigidity determined from physical tests (\( R^2 = 0.756; p < 0.001 \)). With respect to this linear correlation, this virtual testing method resulted in a linear slope that closely approached unity (slope = 1.014) indicating high absolute agreement. The resorbable copolymer poly (L-lactide-co-glycolide) mesh system had a higher linear correlation between physical and virtual rigidity values (\( R^2 = 0.907; p < 0.001 \)) than the 3-D printed graft cage system (\( R^2 = 0.701; p < 0.001 \)).

DISCUSSION: Virtual torsion testing is a robust technique for non-invasive evaluation of bone healing progress in a wide variety of settings. Compared to the previous validation study, the sample set utilized in this investigation had lower resolution scans, utilized graft containment systems for relative bone location preservation, and had larger defects. Despite these differences, strong agreement was observed between physical and virtual torsional rigidities. In addition, scanned tibiae that were reserved for histology could be added to the pool of non-destructive virtual mechanical testing data.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings confirm that virtual torsion tests are an effective, non-destructive substitute for physical torsion tests in ovine osteotomy models with a wide range of scanner settings and defects sizes. Success with this large-defect animal model indicates that the technique may also have high clinical translation potential for evaluating longitudinal healing progress in limb salvage cases.


Fig. 1: Procedure for virtual mechanical testing: (a/b) segmentation of the region of interest (green defect zone) from the clinical-resolution CT scan, (b) re-alignment of test segment to the global coordinate system, (c) material properties assignment using the dual-zone material model for bone and callus, and (d) virtual torsion test.

Fig. 2: Virtual torsion testing accurately predicted the rigidity values measured in postmortem physical mechanical tests for these large defect osteotomies supported by graft containment systems.