Virtual Mechanical Testing to Track Longitudinal Healing Trajectories in Ovine Large Defect Reconstruction

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INTRODUCTION: Virtual mechanical testing is an image-based, noninvasive *in vivo* approach for evaluating bone rigidity. We previously validated a computational workflow for virtual torsion testing in intact and osteotomized ovine tibiae, including small (3 mm) to moderately large (17 mm) defects [1]. In this study, we extended the method to a new context of use: a large-defect (30 mm) limb salvage model that heals much more slowly. We also applied the validated methods to longitudinal *in vivo* scans in a 50 mm defect model, allowing for the assessment of mechanical healing progression over time. The objective of this study was to develop and validate a new approach methodology (NAM) that can capture the temporal evolution of mechanical healing and support refinement, reduction, and replacement (3Rs) of animal use.

METHODS: Thirty-three adult female sheep received either 30-mm (N=24) or 50-mm (N=9) mid-diaphyseal tibial defects with autograft stabilized by an external fixator. *In vivo* computed tomography (CT) scans were obtained at sacrifice (18 weeks) for the 30-mm defect cohort and every four weeks until sacrifice (26 weeks) for the 50-mm defect cohort. Postmortem torsion testing was performed on the 24 operated tibiae with 30-mm defects, whereas the nine animals with 50-mm defects were all reserved for histology. Animals with 30-mm defects and postmortem physical tests were used to validate the virtual mechanical test protocol. All scans were processed using Materialize Mimics (v23.0) to segment bone and callus and create 1-mm quadratic tetrahedral finite element meshes. Elementwise Young's modulus was assigned using two linear functions of bone mineral density (BMD) with distinct slopes (α_1 and α_2) and zero intercepts. Elements with BMD values below a BMD cutoff ρ_{cut} (soft callus and bone regenerate) were assigned properties using slope α_1. Elements above ρ_{cut} (cortical bone) were assigned material properties using slope α_2 = 10225 MPa-cm^3/mgHA [1]. Any elements with a negative bone mineral density were assigned a space-filling soft-tissue modulus E_s = 50 MPa, as identified in our previous study [1]. Virtual torsional rigidity (VTR) testing was performed in ANSYS (2022 R2). A k-fold cross-validation (k = 4) procedure was used to determine the optimal material model parameters (ρ_{cut} and α_1) for modelling bone regeneration in the defect site. The 30-mm cohort was randomly split into a training/testing set for the optimization (N = 14) and a validation set (N = 10) to test the optimized model. The k-fold optimization was repeated until the residual for slope and intercept of the linear regression of VTR vs physical torsional rigidity (GJ) were no greater than 2% and the root mean square error (RMSE) was less than one standard deviation of the GJ values. Approximately 1700 simulations were performed until the optimization criteria were met. The optimized material model was then applied to the animals with 50-mm defects for longitudinal analysis. Statistical analysis was performed in SPSS (v29).

RESULTS: The optimized material model for the Young's modulus of mineralized tissue in the regeneration site was a soft callus region slope of α_1 = 2640 MPa-cm^3/mgHA and density cut-off of ρ_{cut} = 1375 mgHA/cm^3. The linear regression between VTR and GJ for all optimization animals (N=24) had a strong and significant association (R^2 = 0.824, $p < 0.001$) and very strong absolute agreement (a = 1.1013, b = -0.007). The mean physical torsional rigidity was 0.246 ± 0.106 Nm^2/° while the mean virtual torsional rigidity was 0.241 ± 0.117 Nm^2/° (non-significant difference, p = 0.314). A Friedman test was run (as the data violated parametric assumptions) and demonstrated that the virtual torsional rigidity among the 50-mm defect dataset significantly differed based on weeks post-surgery ($p < 0.001$). Post-hoc analysis demonstrated that no significant changes were observed in VTR between the baseline (0 weeks post-surgery) and 8-post-surgery (p_{adj} = 1.000). The bone rigidity began to substantially increase after week 8, as indicated by the significant differences between VTR for week 8 and week 14, 20, and 26 (p_{adj} = 0.039 for each test). Callus formation plateaued starting around 20 weeks, with no significant change between weeks 20 and 26 (p_{adj} = 0.078). The 50-mm cohort also displayed substantial within-week variability in VTR beginning after 8 weeks, which suggests individual divergence in healing trajectories among the animals.

DISCUSSION: The virtual torsion test protocol reliably quantified mechanical healing and can be considered a validated surrogate for physical testing in this large-defect limb salvage model. The longitudinal analysis of the 50-mm cohort demonstrated that bone rigidity significantly increased after 8 weeks post-surgery and reached a plateau of healing by around 20 weeks, as expected in large-defect healing. Based on these results, virtual torsion testing is an innovative NAM that provides clinically relevant insights that cannot be obtained by another other method.

SIGNIFICANCE/CLINICAL RELEVANCE: Virtual torsion tests are a reliable, non-invasive, non-destructive method for measuring longitudinal *in vivo* healing trajectories in the context of limb salvage. These techniques are highly consistent with the 3Rs principles.

REFERENCES: [1] Inglis B. et al, *Sci Rep*, 12:2492, 2022. [2] Schwarzenberg P. et al, *J Orthop Res*, 39:727–738, 2021.

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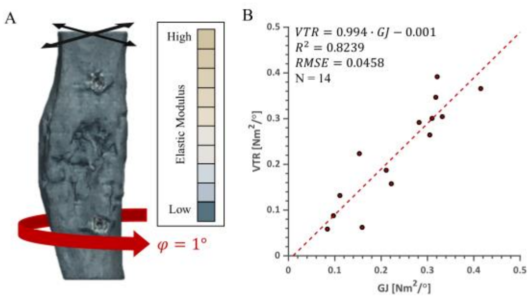

Figure 1: A) A representative image-based model from the 30-mm cohort. B) Results of validation study demonstrating strong correlation and outstanding absolute agreement between physical torsional rigidity (GJ) and virtual torsional rigidity (VTR) for the test/train subset of the 30-mm cohort that was used for computational model validation.

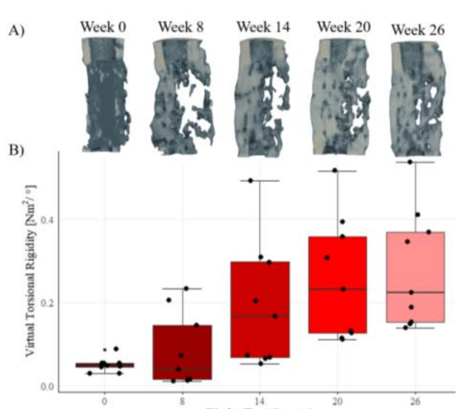

Figure 2: A) A representative image-based model from the 50-mm cohort at each scanning timepoint. B) Results of the longitudinal analysis of healing biomechanics during large defect reconstruction. Healing outcomes diverge after 8 weeks and plateau around 20 weeks post-surgery.