Application of Structural Rigidity Analysis to Assess Fidelity of Healed Fractures in Rat Femurs with Critical Defects


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INTRODUCTION: Approximately six million fractures occur each year in the United States, resulting in the largest (24%) total lifetime cost associated with any one type of injury. Recent Centers for Disease Control (CDC) data estimates the medical and productivity cost of fractures at over $99 billion. It is further estimated that 16% of Caucasian women aged 65 and older who are receiving Medicare benefits will sustain a hip fracture by the age of 90. As our population ages, it is only be expected that the cost and impact of fragility fractures to our society will continue to rise.

While there has been progress on the development of new therapies and products to treat and accelerate fracture healing, the assessment of fracture healing remains a subjective process. Clinicians often rely on a combination of the patient’s history and clinical findings such as pain, tenderness to palpation and motion at the fracture site over time to assess progression of healing and thereby make decisions on weight-bearing status and activity level. The problem of monitoring fracture healing is further compounded by the difficulty in developing a consistent definition that is both biologically accurate and clinically relevant.

Several studies have used micro-computed tomography (µCT) to measure bone characteristics such as volume and mineral density in a fracture callus. A recent study of murine fracture calluses showed a statistically significant correlation between measures of mineral tissue in fracture calluses (tissue mineral density, mineralized callus volume, standard deviation of mineral density, and bone mineral content) and torsional strength and rigidity as determined by direct mechanical testing. These results suggest that torsional bone strength is directly related to both the geometric and material properties of the fracture callus. Thus, any method for monitoring bone strength during fracture healing must be able to accurately account for both the material properties of bone tissue and the changes in geometry affected by the healing process.

We have previously introduced Computed Tomography based Rigidity Analysis (CTRA) to non-invasively assess the axial, bending and torsional rigidities of bones from their transaxial cross-sectional images and predict fracture risk in patients with pathological bone lesions. Given these promising results in predicting fracture risk, we hypothesize that Structural Rigidity Analysis of bones calculated from µCT data can be used to accurately and quantitatively monitor the progression of fracture healing over time in a rat model of fracture healing. To that end, we aim to validate the use of Structural Rigidity Analysis in comparison to actual mechanical testing to quantify fracture healing in a rat model of segmental critical defects undergoing human BMP-2 DNA treatment post-surgery.

MATERIALS AND METHODS: Five millimeter, critical-size mid-femoral defects were created in the right femurs of ten adult male Sprague-Dawley rats which were subjected to surgical stabilization by an external fixator. All animals received the human BMP-2 cDNA (4 x 10^3 plaque-forming units [pfu]) in an adenoaviral vector (Ad-BMP-2) at four time points. Two animals received AdBMP-2 one day post-surgery (Group 2); 3 animals received AdBMP-2 5 days post-surgery (Group 3); and the last 3 animals received AdBMP-2 10 days post-surgery (Group 4). All animals were euthanized eight weeks after surgery, the femora were harvested, and healing was evaluated with micro-computed tomography based CTRA and torsional mechanical testing. In the original study, the delayed administration of the vector resulted in progressive improvement in union and osseous filling and increasing torsional strength. Therefore, in order to represent a meaningful range of fracture healing cases, we chose specimens for this study that were subjected to vector administration over a course of 0, 1, 5 and 10 days post-surgery. The segmental defect plus the adjacent bone in the affected limb along with the homologous regions in the contralateral unaffected limb were scanned via micro-tomographic imaging (µCT40; Scanco Medical, Brüttisellen, Switzerland) equipped with a 10-mm focal-spot microfocus X-ray tube. The entire defect region was scanned at a 34-µ misotropic voxel size, beam voltage and current of 55 kVp and 145 µA respectively and integration time of 250 ms. The images were reconstructed and filtered (Gaussian filtration with σ = 1.0), and a threshold was determined using a previously described technique. In order to accurately compare each defective femur with its unaffected counterpart, the orientation of the defective femur was reversed so that it would match the contralateral unaffected limb.

The torsional (GJ) rigidity for each transaxial cross-section through the bone was calculated by summing the density-weighted area (multiplication of each infinitesimal area (pixel in this case) by its density) of each pixel by its position relative to the density weighted. Average GJ (GJ_{AVG}) and minimum GJ (GJ_{MIN}) were reported for each specimen. GJ_{AVG} represents the average torsional rigidity of the entire segment, whereas, GJ_{MIN} represents the torsional rigidity of the entire segment at its weakest cross-section. As a bone is as strong as its weakest section and not its average strength, GJ_{MIN} should provide meaningful information into the fidelity of the healed fracture. Sequential transaxial µCT slices were used to generate the average and minimum torsional rigidities of the segmental defects for all specimens and their corresponding contralateral specimens. Following µCT imaging, all specimens underwent torsional testing to failure using a previously described pure torsion testing system.

RESULTS: CTRA-based GJ_{AVG} was moderately correlated with torsional rigidity assessed from mechanical testing results [R^2 = 0.63], while the CTRA-based GJ_{MIN} was correlated to the mechanical testing based results [R^2 = 0.81]. The slopes of the two regression models were not different from one another (p = 0.67), yet the y-intercept values of the two regression models were different (p < 0.001). No significant differences were observed between the CTRA-based GJ_{AVG} and the mechanical testing based on paired t test analysis (p = 0.43). However, the CTRA-based GJ_{AVG} was statistically different from the GJ data obtained from mechanical testing results when subjected to paired analysis (p < 0.001). CTRA-based GJ_{MIN} was also well-correlated to strength [R^2 = 0.78].

Based on mechanical testing results, femurs with defect undergoing AdBMP-2 treatment at different time points regained 5% - 166% of their torsional strength when compared to their respective contralateral specimens. The CTRA-based GJ_{MIN} placed this ratio at 2% - 163%, whereas the CTRA-based GJ_{AVG} resulted in a range of 15% - 218%. The defect femurs that had regained a smaller portion of their torsional strength, in comparison to their contralateral specimens, belonged to the early AdBMP-2 administration groups (0 and 1 day post-operation), whereas the bones that regained most of their torsional strength, and in some cases exceeded the torsional rigidity of the contralateral specimens, belonged to the delayed AdBMP-2 administration groups (5 and 10 days post-operation).

Bone mineral density (BMD, g.cm^-2) and bone mineral content (BMC, g), obtained from dual energy X-ray absorptiometry, were correlated with GJ (R^2 = 0.51 and 0.48) and peak torque (R^2 = 0.32 and 0.30) assessed from mechanical testing. Polar moment of inertia (J) was also correlated with peak torque (R^2 = 0.46). These coefficients of determination are significantly different from those reported previously between CTRA-based GJ values and mechanical testing based GJ (p < 0.05 for all cases).

DISCUSSION: In summary, the results of this study suggest that structural rigidity analysis of µCT data can be used to accurately and quantitatively measure the progression of fracture healing over time in an experimental rat model. As expected, an analysis of the biophysics of bone subjected to mechanical load, minimum torsional rigidity proved a better model for measuring bone strength than average torsional rigidity. It remains to be seen whether analogous CT images in human patients could also be used to monitor fracture healing and predict non-union. Future study across multiple fracture modalities and imaging techniques involving larger sample sizes is warranted to evaluate the reproducibility and clinical utility of these promising results. However, the results of this study suggest considerable potential in the use of µCT-based CTRA to quantitatively and non-invasively assess fracture healing.