Published in final edited form as:

J Biomech. 2016 October 3; 49(14): 3559–3563. doi:10.1016/j.jbiomech.2016.08.024.

Comparison of Micro-CT post-processing methods for evaluating the trabecular bone volume fraction in a rat ACL-transection model

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Abstract

Trabecular bone volume fraction assessments are likely sensitive to the analysis method and selection of the region of interest. Currently, there are several methods for selecting the region of interest to analyze trabecular bone in animal models of post-traumatic osteoarthritis. The objective of this study was to compare three published methods for determining the trabecular bone volume fraction of the medial tibial epiphyses in ACL transected and contralateral ACL intact knees. Micro-computed tomography images of both knees were obtained five weeks postoperatively and evaluated using three methods: (1) the Whole Compartment Method that captured the entire medial compartment, (2) the centrally located Single Core Method, and (3) the Triplet Core Method that averaged focal locations in the anterior, central, and posterior regions. The Whole Compartment method detected significant bone loss in the ACL transected knee compared to the ACL intact knee ($p < 0.001$), with a loss of $15.2 \pm 3.9\%$. The Single Core and the Triplet Core Methods detected losses of $7.5 \pm 10.5\%$ ($p = 0.061$) and $14.1 \pm 13.7\%$ ($p = 0.01$), respectively. Details regarding segmentation methods are important for facilitating comparisons between studies, and for selecting methods to document trabecular bone changes and treatment outcomes. Based on these findings, the Whole Compartment Method is recommended, as it was least variable and more sensitive for detecting differences in the bone volume fraction in the medial compartment.

Keywords

Knee; Osteoarthritis; Bone; Micro-computed Tomography; Ligament

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CONFLICT OF INTEREST

The authors have no financial or personal relationships that could bias this work.

INTRODUCTION

Micro-computed tomography (μ CT) is commonly used to evaluate trabecular bone architecture in animal models of post-traumatic osteoarthritis. Defining an appropriate region of interest (ROI) is a vital step for trabecular bone analyses (i.e. volumetric bone fraction). There are several approaches for selecting the ROI, and it is unknown how these may affect trabecular bone indices. Detailed comparisons of previously used ROIs would guide ROI selections in future studies, and would facilitate comparisons between studies.

Current methods isolate ROIs that vary in location, size, and shape. Volumetric shapes include strips (Batiste et al., 2004), cylinders (Boudenot et al., 2014; Iijima et al., 2015; Intema et al., 2010; McErlain et al., 2008; Sniekers et al., 2008), and cubes (Florea et al., 2015). Sizes vary from small $2 \times 2 \times 4\text{mm}^3$ cubes (Florea et al., 2015) to large anatomical contours that include the medial or lateral compartments of the tibia (Hamann et al., 2012; Maerz et al., 2016; Mohan et al., 2016). ROI location and number varies from a single load bearing location (Batiste et al., 2004; Boudenot et al., 2014) to multiple locations throughout the compartment (McErlain et al., 2008). The methods can broadly be categorized into 1) single load bearing location (strips, cores or cubes), 2) multiple load bearing locations (triplet cores) and 3) large contours (whole compartment or quadrants). We chose a representative method from each of these categories.

The study objective was to measure the bone volume fraction of ACL transected and ACL intact knees using three ROIs: 1) the “Whole Compartment Method” (Bouxsein et al., 2010; Hamann et al., 2012), 2) the “Single Core Method” (Boudenot et al., 2014), and 3) the “Triplet Core Method” (McErlain et al., 2008). We hypothesized that these methods would detect similar differences in the bone volume fractions within the medial tibial compartment following ACL transection compared to the contralateral ACL intact (control) knee five weeks post-operatively in the rat. Additionally, we compared the bone volume fraction of the anterior and posterior regions of the Whole Compartment Method (Hamann et al., 2012) and the individual cores of the Triplet Core Method. Finally, we performed a sensitivity study of core location for the Triplet Core Method.

METHODS

The animals used for this analysis were a subset from a larger study evaluating changes in joint arthrosis following treatment with an extracellular matrix blood composite (Proffen et al., 2016). Twelve week old Lewis rats ($n=10$) underwent unilateral ACL transection surgery to induce joint arthrosis under an IACUC approved protocol. All animals were euthanized five weeks post-operatively and the hind limbs were harvested. The soft tissues were dissected from the tibiae and femurs, leaving the capsule intact. The specimens were fixed in formalin.

Micro-computed Tomography

The tibiae were aligned with the longitudinal axis of the scanner tube. Limbs were set in 2% agarose to prevent movement and dehydration during scanning. High-resolution ($30\mu\text{m}$ isometric) 3D volume images were generated of both limbs using a μ CT scanner

(MicroCT40, Scanco Medical AG, Brütisellen, CH. Tube Settings 70kVp and 114 μ A, 300ms integration time).

Bone Volume Fraction

For all methods, the trabecular bone volume fraction BV/TV (BV=Bone Volume; TV=Total Volume) was calculated for the ROIs using the μ CT scanner's built-in analyses routines.

ROI Selection Methods

All ROIs were limited to the trabecular bone of the medial tibial epiphyses. The medial compartment was chosen for analysis because osteoarthritic damage usually first appears there (Hamann et al., 2012). A single operator performed all three methods in the ACL transected and the contralateral ACL intact tibiae.

Whole Compartment Method

Manual contours followed the peripheral corticocancellous junction and the medial border of the tibial spine (Fig. 1a). The contours were drawn every sixth slice and projected to fill intervening slices, which were adjusted manually. Additionally, the medial tibial plateau was divided into two quadrants, anteromedial and posteromedial (Hamann et al., 2012) to perform regional analysis (Fig. 2a).

Single Core Method

A single cylinder, 1.5mm (diameter) by 0.45 mm (depth) was identified in the central weight-bearing region (50% distance between the anterior and posterior margins)(Fig. 1b) of the medial compartment (Boudenot et al., 2014). Cores were drawn on the first and last slices and projected and adjusted to fill intervening slices.

Triplet Core Method

Trabecular bone was isolated using three cylindrical cores at specified locations within the medial compartment (McErlain et al., 2008). Cylinders (0.75mm diameter x 0.45mm depth) were identified in the anterior, central, and posterior weight bearing regions (Fig. 1c). The cores were localized on the sagittal midline of the medial compartment, 25%, 50%, and 75% of the distance from the anterior to posterior margins; the cores were drawn on the first and last slice and projected to fill the intervening slices. BV/TV values for the triplet cores were averaged within each animal limb. Comparisons in BV/TV were also made between cores and across limbs to compare the regional variations between cores.

Triplet Core Method Sensitivity Analysis

A sensitivity analysis was performed to test the variability in BV/TV measurements of core placement for the Triplet Core Method. Each core was shifted 0.315mm posteriorly and 0.315mm laterally (Fig. 2b). BV/TV values were recalculated for the new core locations and were averaged within each limb.

Statistical Methods

Repeated measures analysis of variance (ANOVA) was used to compare estimates of average bone volume fractions between methods and between knees. The repeated measures model consisted of two within-subject fixed factors, sampling method (Whole Compartment, Single Core, and Triplet Core Methods), knee (ACL transected vs. contralateral ACL intact), and their interaction. Additionally, two-way repeated measures ANOVA was performed within samples obtained from the Whole Compartment Method and the Triplet Core Method to compare average bone volume fractions between quadrants (anteromedial vs. posteromedial) and core locations (anterior, medial vs. posterior), respectively; and knees (ACL transected vs. contralateral ACL intact). For the Triplet Core Method sensitivity analysis, a paired t-test was conducted to compare the original triplet core average to the shifted triplet core average.

RESULTS

Significant differences in average BV/TV values were found between sampling methods ($p<0.001$) and between knees ($p=0.004$). However, there was no evidence that the mean differences between ACL transected knees and contralateral ACL intact knees were dependent on the sampling method ($p=0.09$). Bone loss was detected in the ACL transected knee compared to the contralateral ACL intact knee, with a mean loss of 15.2% (95% CI: 12.4%, 17.9%, $p<0.001$) for the Whole Compartment Method (Fig. 3a), 7.5% (−3.0%, 18.0%, $p=0.061$) using the Single Core Method (fig. 3b) and 14.1% (4.2%, 23.8%, $p=0.01$) using the Triplet Core Method (Fig. 3c). In both ACL transected and contralateral ACL intact knees, the anteromedial quadrant resulted in significantly lower absolute estimates of BV/TV compared to the Whole Compartment Method ($p<0.001$), while the posteromedial quadrant produced significantly greater absolute estimates ($p<0.001$) (Fig. 4a). The Single Core Method had the lowest absolute BV/TV values of all methods. When comparing BV/TV values between cores within a given knee with the Triplet Core Method (Fig. 3c), the BV/TV values of the posterior cores were significantly greater than those of the anterior and central cores within each knee ($p<0.001$). However, a significant core by knee interaction was observed ($p=0.007$) with only the posterior cores showing a significant difference between the ACL transected knee and contralateral ACL intact knee ($p<0.001$).

The core sensitivity analysis showed BV/TV percent differences ranged from 0.14% to 83% between the individual original cores and the shifted cores; however, the difference between the average of original triplet cores (mean=52.7±12.52%) and the average of the shifted triplet cores (mean=51.45±13.55%) was not significant ($p=0.34$) (Fig. 4b).

DISCUSSION

Whole Compartment and Triplet Core Methods revealed significant mean loss in epiphyseal trabecular bone in the ACL transected tibiae compared to the contralateral ACL intact tibiae, while the Single Core Method revealed a nearly significant bone loss, indicating that the bone changes occur between knees with osteoarthritis progression.

The core based methods showed larger standard deviations in BV/TV values (Fig. 3b, 3c) suggesting higher variability when compared to the Whole Compartment Method (Fig. 3a). This was most likely due to the smaller sizes of the cores compared to the ROI of the Whole Compartment Method. Furthermore, we observed that the anteromedial quadrant BV/TV was significantly lower than the posteromedial quadrant value demonstrating there is a significant effect due to location selection (Fig. 4a). It has been reported that trabecular bone changes are similar in the tibia and femur in post-traumatic osteoarthritis models (Lockwood et al., 2014; Sniekers et al., 2008). The higher BV/TV value in the posterior quadrant of the medial compartment is likely due to higher loading in the posterior region in the rat model (Hildebrand et al., 1991). The Triplet Core Method exhibited a similar pattern in which the BV/TV increased from anterior to posterior and revealed that there is a significant interaction between the knee and core location (Fig. 4b). Although trabecular variations of the tibial epiphysis have yet to be fully characterized, both the Whole Compartment and Triplet Core Methods may be suitable for associating bone architecture with joint loading and unloading. However, when the ACL transected and contralateral control knees were compared within the central core of the Triplet Core Method, no significant differences were observed. A similar observation was made using the larger Single Core Method. Though BV/TV values were closer to significance ($p=0.054$ for central core of the Triplet Core Method; $p=0.061$ for the Single Core Method), the cores might not be as sensitive to changes as in the anterior and posterior regions.

High variability was also demonstrated in the core sensitivity analysis. The BV/TV percent difference ranged as little as 0.14% and up to 83% when minimally shifting individual cores laterally and posteriorly by half the core diameter within knees. Although the Triplet Core Method introduced greater variability within samples, it may still be suitable for detecting localized differences when targeting specific areas of bone architecture. Alternatively, a strip that is equal to the diameter of the cylinders and length that includes the anterior, center, and posterior regions of the articulating surface may be a better way to capture localized bone structure changes in the weight-bearing region, as was done in a rabbit model (Batiste et al., 2004).

Comparatively, the Whole Compartment Method provides larger volumetric ROIs, which may be beneficial for detecting global differences in the compartment. The results with this method identified bone loss in the ACL transected knee, suggesting a global response of the bone to joint unloading, which agree with previous reports of bone loss in the medial compartment of the tibial epiphyses with ACL transection (Maerz et al., 2016; Mohan et al., 2016; Proffen et al., 2016). Previous studies suggest that decreases in BV/TV correspond to unloading of the ACL transected limb or due to an inflammatory response that affects bone resorption (Jay et al., 2012; Maerz et al., 2016; Proffen et al., 2016). A limitation of our analysis is that we did not include a sham operated group; however, we previously found that the BV/TV values for the contralateral knees of the ACL transected group were equivalent to the BV/TV means of the sham group using the Whole Compartment Method (Proffen et al., 2016), and therefore the data presented here are from both knees of the ACL transected group only. Another limitation was that scanning was performed at 30 μm . However, we have shown that there is no difference in BV/TV values when scanning was performed at 30, 18, 10, and 8 μm (Supplemental Data).

While our study was limited by the small sample size, two of the three methods showed significant BV/TV losses in the surgical tibiae compared to the ACL intact tibiae. Thus, details regarding the ROI selection methods are important when making comparisons of bone structure changes across studies as the absolute values within each knee are different. We recommend using the Whole Compartment Method because 1) it captured the global response to injury, 2) it produced BV/TV values with the lowest standard deviations, and 3) it was more sensitive to change than the Single Core Method.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The research was supported by the National Institute of Health [RO1-AR054099, RO1-AR056834 and P20-GM104937 (COBRE Bio-engineering Core)], and the Lucy Lippitt Endowment.

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Fig. 1.

Micro-CT axial slice image of the same tibia for all three methods with anterior (A), posterior (P), medial (M), and lateral (L) labeled accordingly: a) Whole Compartment Method, b) Single Core Method c) Triplet Core Method. The anterior and posterior cores were shifted to accommodate for the curvature of the tibia.

Fig. 2.

a) Division of the medial compartment into Anteromedial (AMQ) and Posteromedial (PMQ) quadrants. b) Triplet Core Method Sensitivity Analysis: Original Triplet Cores (green), Shifted Triplet Cores (blue). The red arrow denotes the length and direction of the lateral-posterior shift. The posterior core was shifted in only the lateral direction to avoid including subchondral bone.

Fig. 3.

a) Whole Compartment Method mean of BV/TV (intact= $51.1 \pm 2.9\%$, surgical= $35.9 \pm 4.8\%$; $p < 0.001$). b) Single Core Method mean of BV/TV (intact= $42.1 \pm 13.1\%$, surgical= $34.7 \pm 11.6\%$; $p = 0.061$). c) Triplet Core Method mean of BV/TV (intact= $59.8 \pm 10.9\%$, surgical= $45.7 \pm 10.1\%$; $p < 0.01$).

Fig. 4.

a) Means of BV/TV for quadrants: Anterior medial quadrant (AMQ) (intact=41.4±3.9%, surgical=29.1±3.7%; $p<0.001$) and posterior medial quadrant (PMQ) (intact=63.1±3.4%, surgical=48.3±9.1%; $p<0.001$). b) Mean of BV/TV for cores in the anterior, central, and posterior locations. Anterior (intact=47.4±12.7%, surgical=39.1±12.9%; $p=0.123$), Central (intact=54.1±14.6%, surgical=43.5±9.1%; $p=0.054$), Posterior (intact=77.9±7.8%, surgical=54.4±13.6%; $p<0.001$).