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Short communication

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 post-operatively 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.

1. Introduction

Micro-computed tomography (μCT) 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 $2 \times 2 \times 4\ mm^3$ 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

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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.

2. 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.

2.1. 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 μm isometric) 3D volume images were generated of both limbs using a μCT scanner (MicroCT40, Scanco Medical AG, Bruttisellen, CH. Tube Settings 70 kVp and 114 μA, 300 ms integration time).

2.2. 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 μCT scanner's built-in analyses routines.

2.3. 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.

2.4. 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).

2.5. Single Core Method

A single cylinder, 1.5 mm (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.

2.6. Triplet Core Method

Trabecular bone was isolated using three cylindrical cores at specified locations within the medial compartment (McErlain et al., 2008). Cylinders (0.75 mm diameter x 0.45 mm 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.

2.7. 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 0.315 mm posteriorly and 0.315 mm laterally (Fig. 2b). *BV/TV* values were recalculated for the new core locations and were averaged within each limb.

3. 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.

4. 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

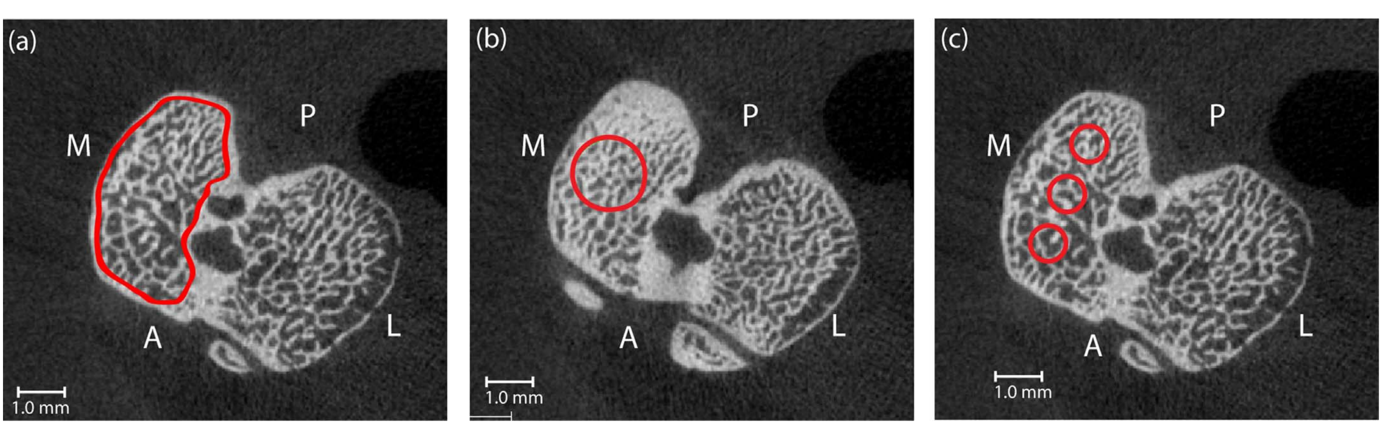

Fig. 1. Micro-CT axial slice image of the same tibia for all three methods with anterior (A), posterior (P), medial (M), and lateral (L) labeled accordingly: a) Whole Compartment Method, b) Single Core Method c) Triplet Core Method. The anterior and posterior cores were shifted to accommodate for the curvature of the tibia.

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