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3D Image Registration Is Critical to Ensure Accurate Detection of Longitudinal Changes in Bone Microstructure and Mineral Density Measurements in Rats by *In Vivo* Micro Computed Tomography

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Introduction

In the recent decade, *in vivo* micro computed tomography (μCT) scanners have become available to monitor longitudinal bone changes in rodents.[1-2] With an isotropic image voxel size up to 10.5 μm, changes in geometry and microstructural properties of rodent bone in response to either disease or treatment can be visualized and quantified over time. In order to detect longitudinal changes, it is critical to understand the precision of *in vivo* μCT measurements. Although reproducibility of *ex vivo* μCT at various volumes of interest (VOI) and resolutions have been reported,[3-4] influences of movement and repositioning of animals, which are associated with *in vivo* scans, have not been well studied. Nishiyama *et al* reported that by 3D image registration, reproducibility can be significantly improved in microstructural measurements at 12.5 μm resolution (precision ~1-5% in rats).[5] However, in their study the follow-up scans were within 2 days where changes to the bone microstructure are negligible. The first objective of the current study was to investigate the short-term reproducibility and long-term precision of bone microstructure and density measurements in the rat tibia by *in vivo* μCT scans at the highest achievable voxel size (10.5 μm). The second objective was to test whether a 3D image registration technique can improve short- and long-term precision.

Methods

3-month-old female SD rats were used in this study. Rats in the short-term reproducibility group underwent baseline and follow-up scans within the same day (n=15) and those in the long-term precision group were scanned at day 0 and day 14 (n=16). Rats were anesthetized and the right tibiae were scanned by an *in vivo* μCT system (VivaCT 40, Scanco Medical; 10.5 μm voxel size). During the scan, the rat tibia was fixed by a customized holder to ensure minimal motion effect (Fig. 1). A total of 296 μCT slides, corresponding to a 3.1 mm region below the growth plate, were acquired. Between short-term repeated scans, animals were retrieved from the scanner with their tibiae removed from the holder and then replaced for the next scan.

First, 2 mm bone segments starting from the distal ends of the bone were analyzed for each scan. Bone volume fraction (BV/TV), trabecular thickness (Tb.Th*), spacing (Tb.Sp*), number (Tb.N*), structure model index (SMI), connectivity density (Conn.D), bone mineral density (Tb.BMD), and tissue mineral density (Tb.TMD) were evaluated by 3D standard microstructural analysis. A second set of analyses were performed for registered image pairs of both short-term and long-term groups after a 3D image registration procedure. A mutual information-based 3D image registration scheme (ITK, NLM) was applied to the trabecular bone compartments of baseline and follow-up scans to determine the rigid body transformation matrix between the two image coordinates. Then, the VOI of the first scan was located in the 2nd scan through transformation (Fig. 2). All trabecular measurements were calculated for the registered VOIs.

To evaluate the short-term reproducibility, individual coefficient of variance (CV) was calculated, and the root mean square average of the %CV (RMSCV) was derived for each parameter for unregistered and registered image pairs. To evaluate the long-term precision,

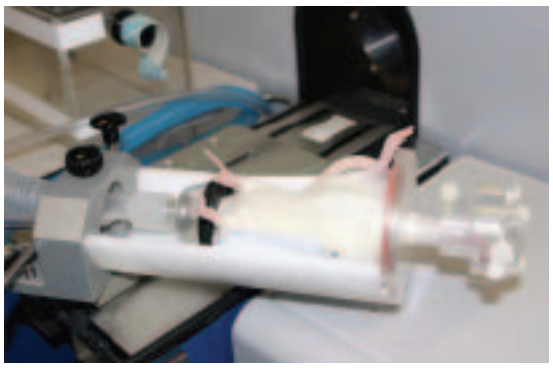

Figure 1. *In vivo* μCT scan of a rat under anesthesia with the right tibia held by customized jig.

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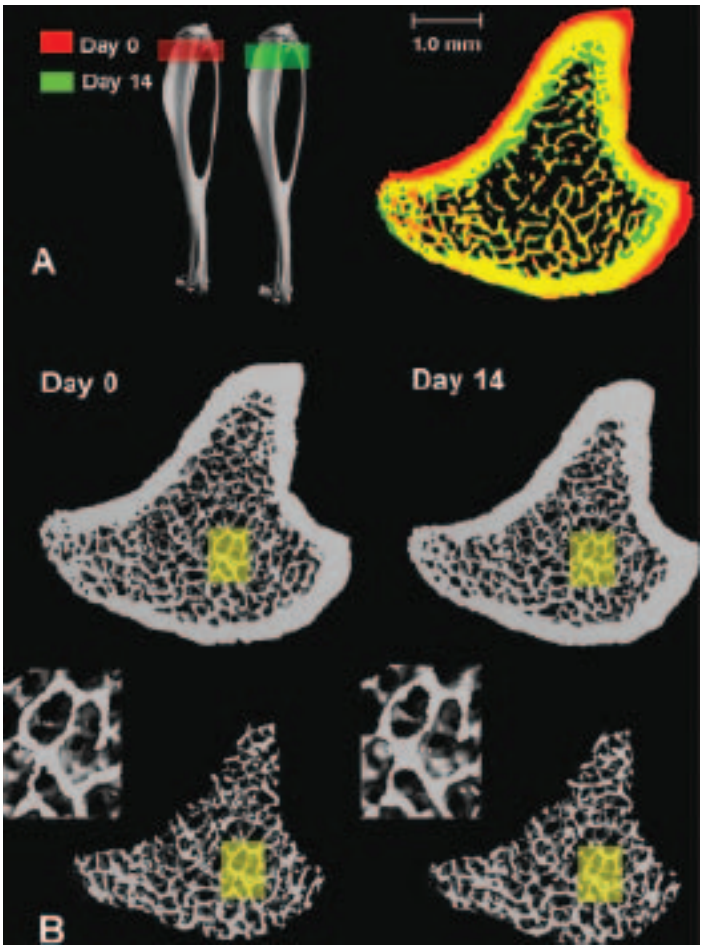

Figure 2. (A-B) Overlapped registered bone structure of day 0 (Red, also shown in B left) and day 14 (Green, also shown in B Right). Yellow: common bone area.

percent change of each parameter between day 0 and day 14 was evaluated for registered and unregistered image pairs. Paired Student's t-tests were performed with $p<0.05$ indicating a significant difference.

Results

Short-term precision: Prior to image registration, reasonable reproducibility was found for most parameters. The precision errors associated with BV/TV, Tb.N*, Tb.Th*, Tb.Sp*, Tb.BMD, and Tb.TMD ranged between 0.85% and 2.65% (Table 1). Precision error was higher for SMI and Conn.D measurements (4.29% and 7.49%). Precision errors of most measurements decreased with registration. However, improvement in reproducibility of microstructural parameters by 3D registration did not reach statistical significance.

Long-term precision: Prior to image registration, by using the growth plate as reference, comparisons between VOIs at day 0 and 14 suggested significant increase in BV/TV, Tb.N*, Tb.Th*, Conn.D, and Tb.BMD and decrease in Tb.Sp* and SMI (Table 1). However, 3D registration indicated new bone generated from the growth plate in 14 days, which was pushed from the growth plate toward the mid shaft region (Fig 2). Percent change in each parameter from registered comparisons were significantly different from that calculated based on unregistered scans. Registered results suggested that in 14 days the registered trabecular VOI had a significant increase in BV/TV and Tb.BMD, primarily caused by increased Tb.Th* and Tb.TMD. In addition, there was a significant decrease in Conn.D, as compared to an increase based on unregistered results.

Discussion

We tested short-term and long-term precision of microstructural and density measurements of an *in vivo* μCT scan protocol of the rat tibia. For the short-term study, reasonable reproducibility can be achieved by standard scan and analysis procedure for most measurements. 3D registration tended to reduce precision errors but improvements were not significant. This result differs from that reported by Nishiyama *et al* which demonstrated improved short-term precision by 3D image registration.[5] However, by using a customized jig to minimize motion artifact and reposition errors during *in vivo* scans, reproducibility of most trabecular microstructure and

Table 1. Short term RMSCV and long term % difference of registered and unregistered scans.

	Short-term RMSCV Without Registration	Short-term RMSCV With Registration	Long-term % Difference Without Registration	Long-term % Difference With Registration
BV/RC	2.65%	2.37%	10.48%*	6.30%*
Tb.N*	2.49%	2.01%	7.70%*	−1.30%
Tb.TH*	1.20%	0.97%	4.15%*	10.07%*
Tb.Sp*	1.76%	1.33%	−8.02%*	1.82%
SMI	4.29%	4.01%	−6.05%*	−0.87%
Conn.D	7.49%	7.01%	10.07%*	−12.51%*
Tb.BMD	1.14%	1.01%	7.90%*	5.49%*
Tb.TMD	0.85%	0.75%	1.31%	3.22%*

* indicates significant difference between baseline and followup scans.

density measurements in our study were within 3%, a similar level of registered precision reported by Nishiyama *et al.* This may explain the minimal improvement by adding 3D image registration in our study.

In the 14-day study, 3D registration had a significant impact on the accuracy of all measurements. Due to the continuous growth in rodents, without image registration the differences between the baseline and follow-up scans were driven by changes due to bone growth instead of local remodeling. In the current study, unregistered comparison results exaggerated the changes in trabeculae and wrongly suggested significant improvements in every structural and density parameter. After image registration, the comparison results indicated thickened and more mineralized trabeculae caused by local bone remodeling over 14 days.

Significance

Our results suggested that 3D image registration is a critical step to ensure accurate detection of longitudinal changes in rodent trabecular bone microstructure by *in vivo* μCT imaging.

References

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