The Use of In-Life Microcomputed Tomography for Evaluating a Segmental Defect in the Rabbit Radius

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Statement of Purpose: The purpose of this study was to validate the use of in-life microcomputed tomography (μCT) as a viable means for evaluating bony healing in a well-established 1.5-cm segmental defect in the rabbit radius. Bi-weekly μCT scans were evaluated and compared to conventional analysis techniques; bi-weekly radiographs, ex-vivo μCT, high-resolution radiographs and histology. Each defect was filled with either demineralized bone matrix (DBM) or heat inactivated demineralized bone matrix (I-DBM) which served as negative control for the study.

Methods: A total of twenty (20) seventeen week old male New Zealand white rabbits were enrolled in this study and subjected to 1.5-cm critical sized radial defects in the left forelimb under approval from the Swiss Office for Animal Health, Graubunden. A titanium marker was placed in the radius 8-mm proximal to the created defect as a reference point. The animals were assigned to one of two treatment groups: DBM (n=15) and I-DBM (n=5). Each groups received 0.3 cc of either DBM or I-DBM which were combined with 2.5 ml of saline and packed into a cut-off 1cc syringe for delivery into the defect. Rabbit DBM was obtained from VTS (Veterinary Transplant Services, Kent WA). Heat inactivation of the I-DBM was accomplished by exposing the material to 100ºC for 24 hours to diminish its biological activity. I-DBM served as a negative control for this study.

Serial medial-lateral radiographs were taken of the defect site at 0, 2, 4 and 6 weeks post-op and were evaluated for healing using a 0 to 3 scale: (0) 0-25%; (1) 26-50%; (2) 51-75% and (3) 76 -100% of defect area filled with new bone. Radiographic healing was assessed by three blinded reviewers and the average score was calculated for each animal.

In-life μCT was used to assess the progression of bone healing over time. Bone volume (BV) was measured at 0, 2, 4, and 6 weeks using a Scanco Xtreme CT. The scans were performed at 60 kVp and 1 mA, with an isometric voxel size of 82 μm^3. A ROI was defined for the scan starting 5.5 mm distal from the reference pin and extending distally an additional 20 mm along the long axis of the radius. The ROI included the defect (15mm) and 2.5 mm of radius above and below the defect. Rabbits were placed into a custom holder to ensure reproducible positioning and scanned under general anesthesia. BV (mm^3) values were calculated at each time point and the reconstructed 3-D images were graded at 6-weeks for healing using the same scoring method described for the serial radiographs.

The explanted samples were stripped of soft tissue and fixed in 70% ethanol before being scanned at 70 kVp and 114 μA using a Scanco Micro CT 40 with an isometric voxel size of 20 μm over the same ROI as described in the in-life μCT scans. BV values were calculated and the 6-week reconstructed 3-D images were graded for healing using the same scoring method described for the serial radiographs.

High resolution radiographs (Faxitron X-ray Corp, model# 43885A) images were obtained prior to the samples being processed for hard-tissue histology. 60-100 micron sections were stained with toluidine blue and both the faxitrons and histology sections were scored using the same 0-3 scoring system described for the serial radiographs. For all data, significance was determined at the 95% confidence level, $p \leq 0.05$.

Results: All twenty (20) animals completed their 6-week in-life term in good health and without incident.

Comparison of in-life μCT to the bi-weekly radiographs, summarized in table 1, showed statistically lower mean scores for the in-life μCT at 4 and 6-weeks with respect to both the DBM and I-DBM groups, $p \leq 0.004$. In-life μCT also showed a significant difference between the DBM and I-DBM at 4 and 6-weeks, whereas the serial radiographs where unable to detect a difference until week 6, $p \leq 0.05$.

Six week scoring of the histology, faxitron and ex-vivo μCT demonstrated no statistical difference when compared to the in-life μCT scores, $p \geq 0.4$.

		In-Life μCT	Serial X-ray	Ex-vivo μCT	Faxitron	Histology
DBM	0 wks	0	0	N/A	N/A	N/A
	2 wks	0	0.1 ± 0.3	N/A	N/A	N/A
	4 wks	0.4 ± 0.6	1.7 ± 0.9	N/A	N/A	N/A
	6 wks	2.5 ± 0.6	3 ± 0.0	2.64 ± 0.5	2.4 ± 0.8	2.5 ± 0.8
I-DBM	0 wks	0	0	N/A	N/A	N/A
	2 wks	0	0	N/A	N/A	N/A
	4 wks	0	1.5 ± 0.9	N/A	N/A	N/A
	6 wks	1.2 ± 0.9	2.7 ± 0.4	1.6 ± 0.5	1.0 ± 1.1	1.1 ± 1.1

Table 1. Mean healing scores for each analysis techniques.

Comparison of mean bone volumes at 6-weeks demonstrated no statistical difference between the DBM in-life μCT (337.1 ± 68.5) and the DBM ex-vivo μCT (357.3 ± 73.1) values nor was a difference detected between the I-DBM in-life μCT (241.0 ± 69.9) and the I-DBM ex-vivo μCT (258.4 ± 72.2), $p \geq 0.46$. Both in-life μCT and ex-vivo μCT showed a significant difference when DBM bone volumes were compared to the I-DBM control group, $p = 0.02$.

Conclusions: By all the parameters assessed, in-life μCT demonstrated statistically comparable performance to the conventional ex-vivo analysis techniques. These data further suggest that the 3-dimensional μCT images can not only provide quantitative data earlier, but allow for a more informed analysis that can prevent misinterpretation of 2-dimensional serial radiographs that may lead to the investigator to improperly assigning the radiographs higher scores. Furthermore, in-life μCT provides a means wherein temporal changes in bone healing can be quantitatively measured within the same animal. Reducing the number of animals required for a study by eliminating the need for multiple sacrificial time points.