A Novel MicroCT Method of Analysis Employing Multiple Thresholds: LIPUS Treatment Increases Bone Formation and Resorption during Fracture Healing

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Introduction: Micro-computed tomography (MicroCT) is a relatively new technology for evaluating the structure of bone and other mineralized tissues. While it has proven to be a very powerful tool, providing both 2 and 3-D visualization, as well as tissue structural information, its true utility is limited by a lack of standardized methodology and external controls. The goal of the current study was to use a multi-threshold technique to evaluate low-intensity pulsed ultrasound (LIPUS)-mediated fracture healing. Over 10% of the 5.6 million annual fractures reported in the United States show delayed healing and nonunion (1). LIPUS has been shown to accelerate the rate of fracture healing in animal and human studies by enhancing ‘maturation’ of the calus or a so called left-shift in healing. Although the exact mechanism by which LIPUS imparts its stimulatory effect on fracture callus is still unknown, the findings of this and other studies suggest that LIPUS triggers a series of biological events that lead to faster healing of long-bone fractures (2). The application of this novel analysis technique provided insights into both LIPUS-treated and normal healing within the fracture callus. In addition, we clearly demonstrate that LIPUS promoted a timed sequence of bone formation and resorption during fracture healing.

Materials and Methods: Male Wistar rats underwent bilateral femoral intramedullary fixation with 1x25 mm titanium alloy rods under anesthesia. A closed transverse mid-diaphyseal fracture was produced by the Bonnarens and Einhorn fracture protocol (3). The fracture and fixation were confirmed by radiography. Following, all rats received 10 minutes of LIPUS (Exogen, Smith and Nephew) to one fracture side, daily for up to 7, 14 and 21 days, with the opposite side receiving sham. At harvest, the animals were sacrificed with CO2, the entire femora were carefully dissected (preserving the calluses) fixed in 4% paraformaldehyde and scanned by microCT. Multiple evaluations of each scan were run with thresholds that differentiated between pre-existing cortical bone, newly formed bone and new cortical bone (Figure 1). The pre-existing cortical bone was contoured to differentiate it from the newly formed cortical bone in the healing fracture. A paired t-test was used to test for significance.

Results: MicroCT analysis using multiple thresholds showed that LIPUS treatment significantly increased new bone formation at all time points (week 1 - 3); importantly, at week 2, there was a marked increase in cortical bone resorption. In addition, this methodology revealed the presence of a timed sequence of remodeling occurring by location from the fracture site. The microCT data shows an elevation in new bone formation followed by existing bone resorption beginning first distal to the fracture site, followed by the proximal region in a similar pattern (Figure 2).

Discussion: The process of fracture healing has been delineated into multiple stages. LIPUS is believed to influence the formation of soft callus and the process of endochondral ossification. Endochondral ossification is an intricate process involving development of a cartilage anlage which eventually becomes calcified and is replaced by bone. In this study we employed microCT with multiple threshold analysis combined with minimal contouring to analyze critical events during fracture healing. Using this methodology we were able to show LIPUS-mediated healing effects take place not only during endochondral ossification, but also at the earlier stage of perichondral bone formation and during resorption of the pre-existing cortical bone. Standard microCT methodology, which evaluates the bone volume of the entire fracture callus, does not differentiate between the multiple processes of bone formation or the fact that the opposing process of pre-existing cortical bone resorption takes place concurrently. Thereby, standard microCT data is inherently inaccurate in measuring dynamic processes and will potentially mask less significant effects. Using multiple threshold microCT analysis to assess LIPUS-mediated effects revealed many subtle changes that were previously undetected. Employing this methodology, we show that LIPUS treatment significantly increases both endochondral and periostial bone formation and, for the first time in vivo, resorption during fracture healing. This new microCT methodology can be readily applied to other hard tissue physiologies to provide quantitative information on bone formation, as well as resorption, at sites of bone turnover.

References:

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Figure 1 Standard microCT analyses of slices through the fracture callus combine all types of mineralized tissue (blue image on left). The methodology used in this study differentiates between pre-existing cortical bone (red), new bone (green) and newly formed dense bone (yellow) as shown in image on right.

Figure 2 3-D reconstruction of a 3 week fracture callus with newly deposited bone (green) and pre-existing cortical bone (red). LIPUS treatment increases resorption of the pre-existing cortical bone which occurs preferentially on the distal side (bottom) in both the LIPUS and Sham treatments.