Validation of Contrast-Enhanced Computed Tomography for Imaging of Cartilage in Fracture Healing

INTRODUCTION: For the great majority of bone fractures, healing progresses initially via formation of a cartilaginous callus that subsequently mineralizes and is replaced by bone tissue. To date, early assessment of rate and extent of fracture healing by radiographs and computed tomography (CT) has been difficult due to the low radio-opacity of cartilage. A recently developed, cationic CT contrast agent, CA4+, has been shown to provide sensitive, quantitative visualization of articular cartilage [1]. Preliminary studies using murine models of fracture healing have also indicated that this contrast agent has excellent potential for measurement of the size and shape of the cartilaginous soft callus [2]. The overall goal of this study is to test the performance of the contrast-enhanced CT (CECT) method for non-destructive assessment of cartilage and mineralized tissues in fracture healing. The specific objectives are: 1) to compare, on a specimen-specific basis, the measurements of cartilage area and cartilage area to those obtained by the gold-standard method, histological analysis; and 2) to evaluate the use of CECT to identify regions of mineralized cartilage in the fracture callus.

METHODS: All animal care and experimental protocols were followed in accordance with NIH guidelines and were approved by our institution’s Animal Care and Use Committee. Closed, stabilized fractures were created in the right femora of 18 male, C57BL/6 mice, 8-10 weeks of age. Animals were sacrificed at post-operative day 9.5. Each fracture callus was imaged via micro-computed tomography (μCT), at a resolution of 6 µm/voxel, both before and after incubation in CA4+ (27 mg I/ml) for 24 hours. Calluses were then processed for undecalcified histological analysis. One transverse histological section of each callus was stained with Safranin O for identification of cartilage. For each callus, the set of pre-incubation μCT images was subtracted from the registered, post-incubation images (Amira 5.2.2, Visage Imaging, Andover, MA) [1]. The samples were divided into a calibration cohort (n=8) and a validation cohort (n=7). Three samples were excluded due to incomplete fracture or to excessive deformation during incubation. For the calibration cohort, the μCT images were examined in conjunction with the histology sections to determine the ranges of intensities in the subtracted and pre-incubation images that correspond to cartilage and partially mineralized tissue, respectively (Figure 1). Voxels containing mineralized cartilage were identified as those labeled as cartilage in the subtracted images and as partially mineralized tissue in the pre-incubation images. The intensity ranges were then used on the validation cohort to calculate cartilage area and to identify regions of mineralized cartilage for the slice in the μCT image stack that corresponded to the location in the callus from which the histological section was taken. Callus area was computed from the post-incubation images. The area measurements were carried out on individual quadrants of the section, as defined by the major and minor axes of the cortex. These measurements were compared to the corresponding histological measurements via t-tests and regression analyses that accounted for use of multiple quadrants per section (JMP 9, SAS Institute Inc., Cary, NC).

RESULTS: For the validation cohort, CECT estimated greater cartilage and cartilage areas than histology (p<0.001); however, the CECT measurements were reasonably good predictors of the histological measurements (R²>0.55, p<0.001; Figure 2). Regions identified as mineralized cartilage by CECT and by histology were in close agreement (Figure 3), as were the overall spatial distributions of cartilage, mineralized cartilage, and bone (Figure 4).

DISCUSSION: The significant correlations between CECT and histological measurements as well as the good qualitative agreement between the two methods in terms of the spatial distributions of cartilage and mineralized tissues provide solid, initial validation of CECT for the study of fracture healing. The greater callus and cartilage areas identified by CT compared to histology may have been caused by shrinkage of the callus during the dehydration step of the histological processing. This and other common histological artifacts (e.g., distortion and tearing) and limitations of the CECT method (e.g., imperfections in image registration) may also have contributed to the scatter in the data. Future work will investigate additional time-points to evaluate CECT for assessment of the full time-course of fracture repair.

SIGNIFICANCE: Non-destructive, quantitative visualization and co-localization of cartilage, mineralized cartilage, and bone in fracture calluses will enable early assessment of healing and rapid screening of therapeutic agents.

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