An Automated MicroCT-based Methodology for Quantification of Bridging Bone in a Rodent Spine Fusion Model

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Disclosures: W. Hsu: 1; Stryker. 3B; Asahi, Medtronic, Promimic, ZSFab, Amphix Bio. 5; Inion. E. Hsu: 5; Inion, Amphix Bio. For all other authors there are no disclosures.

INTRODUCTION: Posterolateral lumbar spine fusion (PLF) procedures aim to generate new bone growth across the transverse processes bilaterally to prevent motion and stabilize the spinal segment. A common method for fusion scoring in a rodent PLF model is blinded manual palpation (MP) to assess whether sufficient new bone has formed to stabilize the transverse processes. This method is limited to binary outputs of no-fusion or fusion and does not provide an objective assessment of successful fusion or robustness of the bone regenerative response at the fusion site. To address this limitation, this study introduces a semi-automated microComputed Tomography (microCT) tool to quantitatively and objectively evaluate spinal fusion in a rodent model.

METHODS: Female Sprague-Dawley rats underwent bilateral L4-L5 PLF using sub-therapeutic doses of recombinant human BMP-2 (bone morphogenetic protein 2) on absorbable collagen sponge implants. The dosing regimen ensured that not all implants would be sufficiently osteoinductive to result in a robust fusion mass across L4-L5, thereby allowing variable fusion responses. Only female rats were used as our laboratory reported sex-based differences in response to rhBMP-2 in this rat posterolateral fusion model (1). Rats were euthanized 8 weeks postoperatively, and fusion was assessed using a standardized blinded MP scoring system. Left and right fusion beds were treated as independent specimens and categorized as fused (mean MP=1), unfused (mean MP=0), or questionable (mean MP=0-1). Spines were imaged by microCT (pixel size: 6.6 μm or 30 μm), and reconstructions were segmented in ImageJ. The thickness of the narrowest bone segment bridging L4-L5 (T_{bridge}) was determined by successively applying single-volume element (voxel) erosions until ImageJ particle analysis registered L4 and L5 as separate particles. Thus, T_{bridge}, calculated as twice the number of erosions times the voxel size, is a quantitative measure of the robustness of the bone regenerative response at the fusion site. Mean MP scores were compared to T_{bridge} values via ANOVA.

RESULTS: Among the non-fused (n=6; MP=0) and questionable specimens (n=6; MP < 1), microCT showed no instances of bone bridging L4 and L5 (T_{bridge} = 0μm). Of the 6 specimens categorized as fused (MP = 1), microCT showed that all had bone bridging L4 and L5, with a mean T_{bridge} = 460μm. When blinded observers agreed on either the presence or absence of fusion across L4-L5 (i.e., MP = 0 or MP = 1), the microCT-based method agreed with the observers, i.e., showing bridging or no bridging, respectively. When the blinded observers disagreed (0 < MP < 1), microCT identified these specimens as unfused (T_{bridge} = 0μm). ANOVA analysis demonstrated a significant difference in T_{bridge} values for the fused group compared to both the unfused and questionably fused groups (P<0.001).

DISCUSSION: As a pre-clinical model of spinal fusion, the rat PLF procedure represents a standardized and widely used tool to screen new materials for the potential for future clinical success. However, as the primary outcome measure for successful fusion, MP-based fusion scoring is a gross assessment that is subjective and lacks sensitivity. The presence of newly formed bone in the intertransverse space does not necessarily result in a full bridging of the TPs. Likewise, newly forming bone may be perceived as bone by a blinded observer, even when not yet fully mineralized. This study demonstrates that MP-based fusion scoring may overestimate the fusion response when robust bone formation is absent. Our results suggest that greater sensitivity can be achieved with the new microCT-based method relative to MP when observer scores vary.

SIGNIFICANCE/CLINICAL RELEVANCE: Supplementing manual palpation with an objective, quantitative measure of fusion quality enables more precise detection of differences in bone regeneration and osteogenesis. This novel imaging-based approach may facilitate clearer distinctions among materials evaluated in animal model spinal fusion studies, potentially lowering research costs and quickening *in vivo* validation of novel biomaterials that promote new bone formation.

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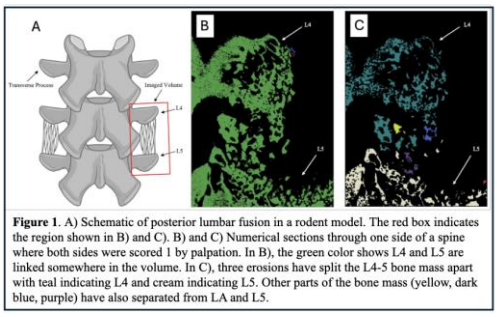

Figure 1. A) Schematic of posterior lumbar fusion in a rodent model. The red box indicates the region shown in B) and C). B) and C) Numerical sections through one side of a spine where both sides were scored 1 by palpation. In B), the green color shows L4 and L5 are linked somewhere in the volume. In C), three erosions have split the L4-5 bone mass apart with teal indicating L4 and cream indicating L5. Other parts of the bone mass (yellow, dark blue, purple) have also separated from LA and L5.

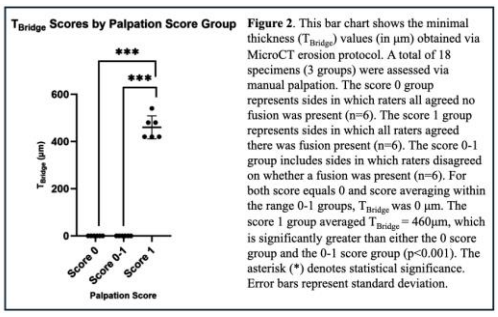

Figure 2. This bar chart shows the minimal thickness (T_{Bridge}) values (in μm) obtained via MicroCT erosion protocol. A total of 18 specimens (3 groups) were assessed via manual palpation. The score 0 group represents sides in which raters all agreed no fusion was present (n=6). The score 1 group represents sides in which all raters agreed there was fusion present (n=6). The score 0-1 group includes sides in which raters disagreed on whether a fusion was present (n=6). For both score equals 0 and score averaging within the range 0-1 groups, T_{Bridge} was 0 μm. The score 1 group averaged T_{Bridge} = 460μm, which is significantly greater than either the 0 score group and the 0-1 score group (p<0.001). The asterisk (*) denotes statistical significance. Error bars represent standard deviation.