Early and Mid Term Histological Events During Single Level Posterolateral Intertransverse Process Fusion with rhBMP-2/ACS and a Ceramic Bulking Agent in a Non-Human Primate Model: Implications for Bone Graft and Fusion Bed Preparation

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Introduction: Fusion rates after autogenous iliac crest bone grafting (ICBG) for posterolateral lumbar spine arthrodesis vary widely. Pseudoarthrosis may occur in nearly 55% of uninstrumented posterolateral lumbar fusions. Recombinant human bone morphogenetic protein-2 (rhBMP-2) on an absorbable collagen sponge carrier (ACS) (INFUSE® Bone Graft, Medtronic, Memphis TN) alone leads to consistent posterolateral lumbar spine fusions in lower order animals; however these results have been difficult to replicate in non-human primates. This has been attributed to muscle compression with rapid resorption of the sponge carrier and subsequent loss of adequate graft volume1. To date, non-human primate study has investigated the early histologic events after application of INFUSE® Bone Graft with various bulking agents in the challenging posterolateral environment. The purpose of this study was to utilize a validated non-human primate posterolateral fusion model to assess early radiographic and histologic healing patterns when combining a single dose of INFUSE® with ceramic bulking agents using different preparation techniques.

Materials and Methods: Twelve skeletally mature, rhesus macaque monkeys underwent single level posterolateral arthrodesis at L4-L5. A hydroxyapatite (HA)/Tricalcium Phosphate (β-TCP) composite bulking agent (MASTERGRAFT®, Medtronic, Memphis, TN) in two formulations was used: MASTERGRAFT® Granules (15% HA/85% β-TCP) and MASTERGRAFT® Matrix (cross linked type I bovine collagen impregnated with MASTERGRAFT® Granules).

Four treatment groups (n=3 sides/treatment/time point) were (1) ICBG alone, (2) INFUSE® Bone Graft wrapped around dry MASTERGRAFT® Matrix, (3) INFUSE® Bone Graft wrapped around MASTERGRAFT® Granules and (4) INFUSE® Bone Graft morselized and mixed with MASTERGRAFT® Granules. When used, INFUSE® Bone Graft at 1.5 mg/cc (3.0 mg rhBMP-2) was combined with 2.5 cc of MASTERGRAFT® bulking agent per side. Animals were sacrificed at 4 week and 12 week time points. Computerized tomography (CT) scans were performed immediately post-op and every 4 weeks until sacrifice. In the sagittal plane, decalcified histology sample the developing fusion mass from medial to lateral, producing over 500 histology slides for this study. Observations from decalcified histology was used to describe cell type and location within the graft or graft substitute, bone remodeling parameters (endochondral ossification, intramembranous ossification, osteoclastic resorption), measures of bone quality (woven bone, lamellar bone, Haversian systems), bone quantity and location, and residual ICBG or ceramic carrier.

Results: 4 week time point: On CT scans, between 1 and 4 weeks, the grafts appeared to consolidate toward the more medial aspects of the transverse processes (TPs), a likely effect induced by posterior muscle forces on the graft material. Histologically, the ICBG group showed unincorporated and unresorbed fragments of corticocancellous bone with new bone formation limited to the surfaces. Minimal to no residual ACS carrier was observed at 4 weeks in the INFUSE® groups. In the INFUSE® groups, the majority of de novo bone formation occurred at or between the TPs dorsal to the intertransverse process ligament. Very little new bone was noted adjacent to the posterior muscle bed. The INFUSE®-MASTERGRAFT® Granule wrap demonstrated a greater degree of cellular infiltration toward the center of the graft compared to the INFUSE®-MASTERGRAFT® Matrix wrap group that contained greatest cellular activity at the periphery of the graft. The INFUSE®-Granule mix demonstrated the greatest amount of intramembranous bone formation and bone-ceramic apposition compared to the other rhBMP-2 groups. All ceramic groups demonstrated some degree of cell-mediated ceramic degradation, a common mechanism of degradation for calcium phosphate ceramics. 12 week time point: Significant remodeling of the autogenous bone and/or ceramic bulking agents was observed between 4 and 8 weeks on CT scans. Histologically, for all INFUSE® groups, significant bone formation extended from the area of decortication along the transverse processes and along the ventral surface of the developing fusion bed, implying that direct contact of rhBMP-2 onto bleeding bone insured fusion progression. In comparison, most bone formation in the ICBG group was restricted to where previous pieces of harvested autogenous bone existed with limited bridging of bony islands observed at this time point. At the 12 week time point, developing fusion masses appeared to be undergoing remodeling and maturation through osteoclastic resorption with subsequent lamellar bone apposition onto existing woven bone. Unlike the 4 week time point, endochondral ossification was an infrequent finding. The INFUSE®-MASTERGRAFT® Matrix wrap group contained the greatest amount of residual ceramic at 12 weeks providing significant scaffolding for continued bone formation. Similar to the 4 week time period, all ceramic groups demonstrated some degree of cell-mediated ceramic degradation.

Discussion: The typical time point for examining fusion outcome in the non-human primate is 24-weeks. The current study was not designed to assess fusion efficacy. This study was designed to investigate early term (4 week) and mid term (12 week) events following rhBMP-2/ACS implantation with three formulations of ceramic bulking agents using radiographic and histologic methods. Our results indicate that the collagen carrier for rhBMP-2 is mostly resorbed by 4 weeks, thus ceramic bulking agents are important to provide longer-term scaffolding and to support continued bone formation in the mechanically challenging posterolateral environment. Use of bulking agents with more rapid resorption rates (i.e. a purely β-TCP ceramic) may limit new bone formation as the collagen carrier resorbs early on. A majority of early bone formation in the rhBMP-2 groups occurred adjacent to the TPs or along the intertransverse process ligament and not dorsally adjacent to the posterior muscles. Thus, placing rhBMP-2/ACS directly next to bleeding bone may encourage robust bone formation at early time points during the fusion cascade. Furthermore, dispersion of rhBMP-2/ACS throughout the graft (i.e. INFUSE®-Granule mix group) may also enhance short term histological bone formation. These results demonstrate that variations in technique may influence short term mechanisms of bone formation with INFUSE® Bone Graft at the 1.5 mg/cc concentration but as indicated by previous preclinical and clinical investigations, all are expected to lead to high fusion efficacy.2-6.

6. Bae et al., ORS 2007 Poster #1457, 2007