

# A Novel Method for the Repair of Critically Sized Bone Defects: Utilization of a Muscle Derived Stem Cell-Seeded Scaffold Augments Proliferation, Migration and Osteogenic Induction

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**Purpose:** Contemporary surgical reconstruction of large craniofacial defects, commonly suffered during trauma and lifesaving decompressive craniectomies, has seen tremendous evolution with the development of custom alloplast implants and rigid fixation elements. Although these implants and fixation devices are often capable of providing coverage and stabilization to smaller or less complex defects, they remain prone to infection, extrusion, migration and failure with larger complicated wounds. Within this study, we aim to assess the functionality and osteo-inductive capacity of an easily deliverable osteo-enriched scaffold construct containing hBMP-2 and traceable muscle derived stem cells (MDSCs). We hope to determine a pragmatic regenerative application of this system to civilian and military patients suffering from complex craniofacial defects which current methods cannot address.

**Methods:** Utilizing a murine model, C57BL/6 (n=60) mice received two identical 5mm full-thickness craniectomy defects using a standardized micro-drill core bit. At 8 weeks, defects were imaged using a mini-CT, laser scanning confocal microscopy and tissues collected for downstream assays including: focused osteo-induction gene and proteome arrays. Concurrently, in vitro studies utilizing baculovirus Premo Fucci® transduced MDSCs (fluorescent correlation to cell cycle stage) were monitored using a FV10i-LIV® live cell confocal imaging system. Quantitative data was extracted from confocal multi-sequence 3D-imaging of daily and real-time cell migration assays as well as cell cycle proliferation kinetic studies. Additionally cell-to-cell interaction and correlative osteo-differentiation characteristics were analyzed following scaffold substrate and hBMP-2 variation.

**Results:** While all groups depicted some form of healing, defects treated with scaffolding enriched with hBMP-2 and isolated MDSCs showed significantly higher rates of healing and reduced defect volume mm<sup>3</sup>. MDSCs re-isolated from the healing wound construct showed significant up-regulation of osteo-induction pathway genes, while imaging and proteome assays validated relative expression and healing levels. In vitro studies indicated that MDSCs more readily migrate, proliferate and differentiate when added to scaffolding and/or hBMP-2 vs. fibroblast controls. Subsequent, downstream gene and proteome arrays of in vitro defect modeling indicated significant MDSC lineage differentiation when compared to controls (p-value < 0.05).

**Conclusion:** A wide spectrum of research in bone healing has been reported in both human and animal model systems. Each study applying a range of osteo-enhancing factors to bone defects.

The conclusion of many of these studies is that there is more than one variable promoting osteogenic healing within a bone wound bed. Our study provides a unique mechanism for the delivery of therapeutic hBMP-2 enriched scaffolding, while employing the intrinsic capacity of the MDSC niche to induce intra-defect bone regeneration.