**INTRODUCTION:** The goal of this proposal is to therapeutically reverse the damaging effects of radiotherapy on bone formation and healing to enable non-vascularized grafting in irradiated bone. Utilizing a rodent model of mandibular bone grafting, we quantified metrics of diminished graft take and bone healing in response to radiation treatment. Subsequently, we utilized implantable deferoxamine (DFO)-an angiogenic stimulant, to reverse these radiation-induced detriments. We hypothesized that the addition of our proposed therapy, would evidence quantifiable degrees of remediation on the process of tissue regeneration, graft incorporation and bone healing.

**METHODS:** Male Lewis rats received a human equivalent dose of radiotherapy (7Gy/d x 5d) to left hemi-mandibles. After recovery, a circular trephine burr (6mm) was utilized to create a critical size defect just posterior to the third molar, and a bone graft was harvested from the right hemi-mandible of the same animal and secured with a custom PLA resorbable plate. Three groups (n=8/group) of animals were investigated: Control, (irradiated) XRT and irradiated + implantable deferoxamine (DFO). Mandibles were imaged at 14, 40 and 60 days with in vivo µCT, and a 60-day healing period was allowed prior to further outcomes testing. Bony union was judged clinically by 3 blinded reviewers on a scale from 0 to 4, representing the approximate percentage of robust union formation along the circular graft-recipient site interface (e.g. 1=25%, 4=100%). Statistical comparisons were conducted with ANOVA ($p<0.05$).

**RESULTS:** We observed a significant diminution of bone graft healing after radiotherapy. At 60 days, the bone volume fraction (BVF) of the XRT group decreased by 20% ($p=0.001$), and exhibited lower bony union scores when compared to control ($p=0.005$). With the addition of DFO, these findings were largely remediated. At 60 days, the BVF improved upon the XRT BVF by 12% ($p=0.025$), and was not different than control ($p=0.282$). In addition, the bony union scores of the implanted DFO group significantly improved from XRT levels ($p=0.05$), and were not different than control ($p=0.200$).

**CONCLUSION:** Implantable DFO strongly remediates the effects of radiation on non-vascularized bone graft incorporation and healing as measured by micro-densitometry and bony union analysis. These observations are promising with regards to the potential utility of this therapy to enhance bone graft incorporation in the irradiated mandible for head and neck cancer survivors.
MEK/ERK cascade, PD98059, to characterize the necessity of each pathway for osteogenesis.

METHODS: hMSCs were cultured in osteogenic media on Col-GAG or MC-GAG scaffolds. Scaffolds were untreated or treated with the DMH1 or PD98059 at 50 mM for 4 days, 14 days, 24 days, 4 weeks, and 8 weeks. Gene and protein expression were measured using quantitative RT-PCR and western blot analysis. Scaffolds were subjected to histochemical and micro-computed tomographic analyses.

RESULTS: Inhibition of the BMPR signaling pathway inhibited Runx2 and BSPII gene expression of primary human mesenchymal stem cells (hMSCs) on MC-GAG. In contrast, inhibition of the MEK/ERK axis downregulated BSPII expression on Col-GAG independent of Runx2 expression. While inhibition of the BMPR signaling pathway resulted in decreased mineralization on both Col-GAG and MC-GAG, inhibition of the MEK/ERK axis only affected mineralization on Col-GAG. When the mechanistic details were evaluated in greater detail, inhibition of the BMPR pathway reduced both Smad1/5 phosphorylation and Runx2 protein expression on both MC-GAG and Col-GAG. Inhibition of the MEK/ERK axis downregulated phosphorylation of ERK1/2 and JNK1/2 without affecting Smad1/5 phosphorylation or Runx2 expression.

CONCLUSION: Interactions between hMSCs and collagen-based materials result in mechanistic differences in osteogenesis. Activation of the canonical BMPR signaling is required for osteogenic differentiation and mineralization of hMSCs on Col-GAG or MC-GAG. The MEK/ERK cascade, intimately tied to JNK activation, is necessary for Runx2-independent osteogenesis on Col-GAG, while completely dispensable in osteogenesis on MC-GAG.

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INTRODUCTION: Over 60,000 new cases of head and neck cancer were diagnosed in the U.S. in 2016. Radiation is commonly required to reduce recurrence rates and improve survival; however, complications after radiation, including poor bone healing and osteoradionecrosis, contribute to the significant morbidity associated with this disease process. The current standard of treatment of such complications is limited to free tissue transfer. Given the significant morbidity associated with these procedures, it is important to examine the utility of cell-based therapies as a potential translational treatment to promote bone regeneration for irradiated patients. Adipose-derived stem cells (ASCs) and bone marrow derived stem cells (BMSCs) represent translational therapies that can improve osteogenesis. We recently demonstrated that ASCs are superior to BMSCs in enhancing bone healing using a segmental defect model in the rat mandible. We hypothesize that differing mechanisms of action between the two cell types contribute to the superiority of ASC’s to enhance bone healing.

METHODS: BMSCs and ASCs were harvested from male Lewis rats (n=3), plated at a density of 200,000 cells/well, and treated with osteogenic differentiation medium. Alkaline phosphatase stain was performed to evaluate osteogenic potential. Vascular endothelial growth factor (VEGF) was also measured and compared. Finally, ASCs and BMSCs were cocultured with human umbilical vein endothelial cells using a transwell system to study the paracrine effect of these two cell types on vasculogenesis. Student’s t-tests were used to compare the osteogenic and vasculogenic potential of the two groups.

RESULTS: ASCs had significantly less osteogenic potential than BMSCs (11.8 ± 0.9 vs. 16.3 ± 0.4; p<0.05). Conversely, ASCs were significantly more vasculogenic than BMSCs based on VEGF release (3.573 ± 0.4 vs. 1607.0 ± 45.0; p<0.001). These findings translated to significantly greater tubule formation in transwells treated with ASCs.

Adipose-Derived Stromal Cells Demonstrate Superiority over Bone Marrow-Derived Stromal Cells in Bone Healing by Enhancing Vasculogenesis in a Murine Model of Irradiated Mandibular Fracture Injury

Presenter: Lauren Buchman