METHODS: Immunocompromised nude-mice underwent external beam irradiation of the scalp. Five weeks later, mice either received seven deferoxamine treatments (1mg in 100ul) or saline subcutaneously to the irradiated area every other day. Laser Doppler analysis (LDA) was recorded prior to irradiation, following irradiation, and 24 hours following each treatment. Human fat grafts were then injected in the subcutaneous plane of the scalp and volume retention measured by CT scan over 8 weeks. Finally, skin and fat samples were evaluated histologically for vasculature, dermal thickness, and fat graft quality.

RESULTS: After 4 treatments with deferoxamine, a significant increase in microvasculature was observed using LDA. There was also significance with the development of microvasculature in the fat graft with LDA. Using microCT, we observed a significant increase in fat graft volume retention with the deferoxamine treated group compared to the saline treated group, and this was paralleled by improved histologic staining of skin and fat grafts.

CONCLUSION: Our results show increased microvasculature and increased fat graft volume retention with deferoxamine treatment. Deferoxamine treatment may also promote beneficial effects in dermal thickness and in quality scoring of the fat grafts, thus leading to a potential clinical application in radiation damaged soft tissue.

Stem Cells Harvested from Bone Marrow and Adipose Tissue Demonstrate Equivalent Healing but Through Different Mechanisms in a Murine Model of Irradiated Mandibular Fracture Healing

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PURPOSE: The difficulty of harvest and relative scarcity of bone marrow stromal cells (BMSCs) has limited the widespread use and clinical application of this technology, thereby necessitating inquiry into other therapies including adipose-derived stromal cells (ASCs). The goal of this study was to compare the ability of ASCs and BMSCs to heal mandibular defects and understand the mechanism through which this occurs. We hypothesize that ASCs will enhance fracture healing by improving vasculogenesis, while BMSCs will directly affect osteogenesis.

METHODS: Male Lewis rats were radiated (35Gy), and subsequently underwent mandibular osteotomy with external fixation with implantation of two million BMSCs (n=12) or ASCs (n=16) marked with Green fluorescent protein (GFP). After 40 days, union rates were evaluated using microCT. Confocal microscopy visualized the contribution of ASCs/BMSCs to the bone regenerate. Quantitative polymerase chain reaction of ASCs/BMSCs compared expression of osteogenic and vasculogenic genes. Coculture of ASCs (n=3) or BMSCs (n=3) with human umbilical vein endothelial cells (HUVECs) was performed in vitro in transwells to measure tubule formation as a marker of vasculogenesis.

RESULTS: ASC-implantation resulted in higher union rates than BMSC-implantation (union rate: 94% vs. 66%). These cells contribute indirectly to fracture healing, as GFP was not visualized at the site. BMSCs expressed osteogenic genes including osteopontin to a significantly greater degree than did ASCs, while ASCs expressed greater levels of vascular endothelial growth factor. This translated to greater tubule formation among HUVECs co-cultured with ASCs than with BMSCs (64.3 ± 7.3 vs. 23.3 ± 2.6, p=0.0008), and increased vasculogenesis in vivo in mandibles after ASC implantation.

CONCLUSIONS: ASCs heal fracture defects better than BMSCs. This effect is likely mediated by indirect modulation of vasculogenesis, rather than by a direct effect on osteogenesis. Clinicians interested in cell-based therapies for irradiated bone injury should consider ASCs as a promising option, given their abundance, ease of acquisition, and improved fracture healing.

Generation of Parathyroid Cells from Human Adipose Derived Stem Cells

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PURPOSE: Acquired hypoparathyroidism is most commonly caused by surgical removal or iatrogenic injury. Permanent hypoparathyroidism occurs in up to 5% of patients undergoing total thyroidectomy or neck dissection, which can result in profound hypocalcemia. If injury is noted intraoperatively the parathyroid gland can be minced and implanted subcutaneously to recover function. Frequently, the manifestations of hypoparathyroidism are only discovered postoperatively when symptoms of calcium and phosphorus dysregulation are noted. Medical management is required to prevent tetany, bone loss, and possibly death. Adipose derived stem cells (ADSCs) are multipotent mesenchymal stem cells which may offer an autologous treatment for this chronic condition. We hypothesize that ADSCs can be converted into functional parathyroid cells in vitro, which could be utilized for delayed subcutaneous implantation and correction of hypoparathyroidism.

METHODS: Adipose tissue was obtained from patients undergoing lipectomy (n = 5). Tissue was digested and the stromal vascular fraction obtained. ADSCs were isolated using magnetic activated cell sorting against CD90 and underwent in vitro directed differentiation over a 26-day period. Differentiated cells were stained for both Calcium Sensing Receptors (CaSR) and Parathyroid Hormone/related protein-Receptors (PTH/PTHrp-R) being verified by microscopy and flow cytometry. The physiologic response of differentiated parathyroid cells to calcium was assessed via Enzyme-Linked Immunosorbent Assay of PTH.

RESULTS: ADSCs were reliably differentiated into parathyroid cells in all patients as verified by both immunofluorescence and flow cytometry against CaSR and PTH/PTHrp-R. Furthermore, differentiated parathyroid cells exhibited a dose and time dependent release of parathyroid hormone following calcium stimulation. PTH secretion was noted at 5 minutes, peaked at 10 minutes, and returned to baseline levels after one hour.

CONCLUSIONS: A century ago, Lahey was the first to perform human parathyroid autotransplantation following a partial thyroidectomy. Failure to acutely recognize an iatrogenic injury makes autotransplantation impossible. We have consistently converted adipose derived stem cells into a parathyroid cell phenotype. Our cells demonstrate appropriate responsiveness to extracellular calcium by the release of parathyroid hormone. They may therefore represent a reliable autologous solution to hypoparathyroidism diagnosed in a delayed setting.

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Characterizing The Contribution Of Circulating Mesenchymal Cells To Pathologic Wound Healing And Heterotopic Ossification

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PURPOSE: Pathologic wound healing after injury represents dysregulation of several cellular components of the physiologic wound niche. Trauma induced heterotopic ossification (tHO) is a highly morbid class of pathologic healing characterized by endochondral formation of de novo osseous lesions in soft tissue. These lesions occur at several anatomic sites and it remains unclear which cell populations form the pre-HO niche. Identification of the specific cells which give rise to tHO is critical to the development of targeted therapeutic options. Similarities between endochondral ossification and tHO suggest a common progenitor, however, it is unclear if this population is entirely local or receives contributions from circulating cells. Here we utilized a parabiotic reporter-based model of tHO to identify and characterize contributions from circulating cells to tHO.

METHODS: A mouse model of parabiosis between wild type mice and reporters carrying the CAG-luc-eGFP L2G85 transgene to examine the presence of circulating cells was generated. These animals carry both luciferase and eGFP reporters allowing for concurrent bioluminescent and histologic analysis of circulating populations. After blood chimerism was confirmed, the wild type parabiotic mouse received a hindlimb Achilles’ tenotomy and dorsal burn. Bioluminescence imaging was used to study the timing and localization of circulating cells. Immunofluorescence was performed to identify common inflammatory, vascular, mesenchymal markers and to characterize their source and contribution to each stage of the tHO anlagen.