

Craniofacial Regeneration in Wounds Compromised by Radiation Therapy

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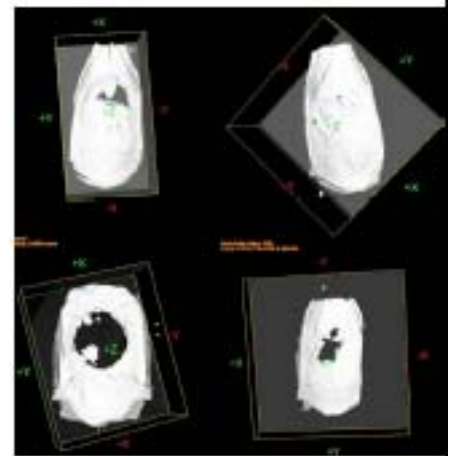
Introduction: The etiologies of craniofacial defects that require bone grafting are related to ablative tumor surgery, ischemic conditions (osteoradionecrosis), infectious causes (osteomyelitis and periodontitis), traumatic injury, or developmental/congenital causes. These defects are more likely to be in wounds complicated by microbiologic contamination, infection, and radiation therapy. One practical obstacle for successful regeneration in the craniofacial region is the exposure to radiation that is characteristic of patients with head and neck malignancies. Radiation therapy complicates reconstructive surgery by increasing apoptosis and decreasing the vascular supply to the surgical field.

The consequence is poor healing, scarring, fibrosis and a surgical site that is extremely difficult to reconstruct. Although protein therapy, gene therapy, and stem cell therapy may heal critical sized craniofacial defects, none of these approaches successfully heals clinically relevant, critical-sized bone defects previously treated with a therapeutic-equivalent dose of radiation. A potential strategy for overcoming the negative effects of radiation includes the use of anabolic dosing of parathyroid hormone (PTH), which effectively augments bone formation during stem cell directed osteogenesis. Based on this observation, we hypothesized that combining anabolic PTH treatment with gene therapy approaches would successfully overcome the negative effects of radiation for bone tissue engineering.

Methods: Three weeks before surgery, rats received a therapeutic equivalent 12 Gy radiation dose to the calvaria. Syngeneic dermal fibroblasts were transduced *ex vivo* using an adenoviral vector coding bone morphogenetic protein-7 (BMP-7). Nine mm diameter bony defects were created and implanted with a BMP-transduced cell-gelatin scaffold, covered by either LD-SAM flap or no flap. For anabolic PTH groups, animals were given daily subcutaneous injections of either

recombinant human PTH [1-34] (60 mg/kg) or vehicle (0.9% sodium chloride). Regenerated defects were harvested at 6 weeks and analyzed by micro-CT, histology, and levels of hypoxia.

Results: Pre-operative radiation therapy significantly limited tissue regeneration even when treated by BMP-7-transduced cells, (12.64 ± 2.52% in control; 18.87± 2.85% with PTH injection). However, the combination of flap transfer, significantly increased percent bone volume/total defect volume (37.09.16 ± 8.00% of flap transfer versus 61.48± 20.03% of flap transfer combined PTH, p<0.05). Bone mineral density was also significantly increased by the addition of PTH treatment in both the cell graft and combination of flap groups. Histology revealed the regeneration pattern of multiple bone nodule



formation. Flap and PTH also improved regional hypoxia status significantly.

Conclusion: The combination of a LD-SAM flap, transduced cell graft and anabolic dosing of PTH markedly increases bone formation by restoring a well vascularized environment with a responsive mesenchymal stem cell population, and can overcome the negative effects of pre-operative radiation therapy in rat cranial defects.

Fig. 1 Micro ct scans of bone regeneration in craniofacial defects. Upper left: no radiation, BMP transduced cells. Upper right: no radiation, BMP transduced cells, PTH. Lower left: XRT, BMP transduced cells. Lower right: XRT, BMP transduced cells, PTH.