(p<0.01) at 1, 4, 7, 15, and 30 dpi respectively. A cardiomyocyte proliferation index generated by MEF2/PCNA staining confirmed cardiomyocyte-specific suppression in ARF:ARF heart regeneration by 46.6% (p=0.01) at 11 dpi. Tissue-specific regenerative gene expression was tracked by qPCR in ARF:ARF and WT fish. Fgf17b, vegfaa, and Twist1b were reduced by 42% (p<0.01), 43% (p<0.01), and 55% in ARF:ARF hearts at 11 dpi, reflective of decreases in myocardial regeneration, vascular regeneration, and epithelial-to-mesenchymal (EMT) transition respectively. There was no significant difference in fgfr2c expression (p=0.44), a marker of epicardial regeneration.

CONCLUSIONS: Understanding how ARF suppresses cardiac regeneration is important for promoting recovery after heart injury in humans. The timeline of recovery in ARF:ARF fish suggests that ARF does not affect the acute processes of scarring, but rather suppresses cardiomyocyte proliferation. ARF’s selective impact on myocardial regeneration, vascular regeneration, and EMT, while not affecting epicardial regeneration, elucidates that in the context of regeneration, ARF is not indiscriminately expressed in all proliferating cells, but is rather localized to cells undergoing dedifferentiation or trans-differentiation. Our findings show that ARF will require alteration in conjunction with other genes to permit regeneration.

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Extended Graft Survival After Cryopreservation and Storage Below -130°C in a Rat Orthotopic Hind Limb Model

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PURPOSE: Vascularized composite allotransplantation (VCA) is an increasingly used reconstructive option for devastating tissue defects of the face, hand, arm, and most recently the penis. Though highly successful cases have been reported, the growth of the field is restrained by the limited ischemia time that is tolerated by the graft. Long-term graft preservation would allow for transplantation across greater distances and thus improved organ sharing, better donor matching, and provide transplant teams with additional time to pre-condition recipients for novel immunomodulatory regimens. Here we present our first outcomes using cryopreservation in a rat hind limb transplant model.

METHODS: Lewis rats were used as both donors (7) and recipients (14) of orthotopic hind limb transplants. Limbs were flushed with Lactated Ringers containing Heparin, loaded with cryoprotectant formulation (10% DMSO in Culture Medium), cooled at 1°C/min to -90°C and then stored below -130°C for 1–2 weeks. Controls (N=2) were performed without cryopreservation. Intervention groups were A) controlled cooling rate frozen limbs with spontaneous nucleation (N=8) and B) similarly treated limbs with induced nucleation at -4°C (N=4). Transplants were thawed and tissue DMSO concentrations reduced by simultaneously soaking and perfusing the limbs with culture medium containing 0.5M mannitol. Preserved syngeneic hind limbs were then transplanted at the mid-thigh to the recipient animal using a non-suture cuff technique for vascular anastomosis. Recipients were monitored daily until the study endpoint of POD14. Biopsies were acquired at postoperative day (POD) 7 and endpoint. Samples were stained with hematoxylin and eosin for histopathology review. Control and treated limbs were also evaluated without implantation to determine tissue component viability using a metabolic resazurin assay.

RESULTS: Viability evaluation after re-warming demonstrated that the femoral arteries, skin and cartilage were >70% of fresh controls, while the muscle was 35–40% of controls (p<0.05). Blood flow was established in all transplanted limbs. Both control limb transplants were successful to POD14. Only one of eight limbs cryopreserved using spontaneous nucleation survived past POD7 and the recipient was euthanized on POD10. Histopathology revealed regeneration of skeletal myofibers and associated fibrosis. Two of four limbs cryopreserved using induced nucleation (50%) demonstrated gross signs of healing around the ankles and feet by POD7 and further skin and muscle regeneration between POD7-POD14.
CONCLUSIONS: This is the first demonstration of above knee cryopreserved rat limb graft survival after frozen storage. Further improvement in muscle cryopreservation is needed. From POD7 onwards the limbs grossly improved demonstrating that the cryopreserved limb tissues had the capacity to regenerate. These studies constitute critical first steps toward banking of complex VCAs to enable better phenotypic and immunologic matching of donor tissues to recipients.

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Sustained-Release Angiogenic Nanoparticle Increases Union Rates, Vascularity, and Cellularity of the Irradiated Murine Mandible Following Nonvascularized Bone Graft Reconstruction

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PURPOSE: Mandibular reconstruction secondary to oncologic resection remains limited to invasive free tissue transfer (FTT) by the detrimental effects of radiotherapy. Nonvascularized bone grafts (NBGs) represent a practical surgical alternative to FTT, but are precluded from irradiated reconstruction due to sequelae such as diminished capacity for revascularization and the destruction of osteoocompetent cells. In order to overcome these principal barriers to irradiated bone healing, our laboratory has developed an implantable, hyaluronic acid (HA)-deferoxamine nanoparticle designed to stimulate angiogenesis during the critically-important weeks following surgery. This study examines the efficacy of this novel therapy in a murine model of irradiated mandibular reconstruction with the goal of ultimately reintroducing NBGs as a viable alternative to FTT in the irradiated setting.

METHODS: Male Lewis rats (n = 33) were equally divided into three groups; control bone graft (CBG), irradiated bone graft (XBG), and irradiated bone graft with intraoperative HA-deferoxamine implantation (XHDBG). Irradiated groups received a fractionated dose of 35Gy over 5 days, comparable to 70Gy administered to head and neck cancer patients clinically. Following a 2-week recovery period, all rats underwent creation of a 5 mm critical-sized segmental defect in the left hemi-mandible and reconstruction with a NBG from the iliac crest of an isogenic donor. On post-operative day 60, all mandibles were perfused and evaluated for bony union upon dissection. Vascularity was evaluated throughout the bone graft and healing interfaces through microcomputed tomography prior to histologic analysis of osteocyte proliferation and mature bone volume. Statistical analysis was performed using ANOVA, with p values less than 0.05 considered significant.

RESULTS: Bony union rates were improved by HA-deferoxamine treatment in the XHDBG group (82%) compared to the XBG group (64%) and were similar to union rates observed in the CBG group (91%). Radiotherapy resulted in decreased vessel number, vessel volume, vessel volume fraction, and vessel thickness in the XBG group compared to CBG. Implantation of HA-deferoxamine significantly increased all metrics of bone vascularity compared to the XBG group. No significant differences were observed between the XHDBG and CBG groups. Radiotherapy-induced cell depletion at the bone graft interfaces was evidenced through a significant reduction of osteocytes in the XBG group compared to CBG. Mature bone formation was also significantly decreased in XBG in comparison to CBG. Osteocyte proliferation and mature bone formation were significantly increased in the XHDBG group compared to XBG and were not statistically different from non-irradiated control levels.

CONCLUSION: The results of this study demonstrate the ability of HA-deferoxamine implantation to significantly improve the vascularity and cellularity of NBGs in reconstruction of the irradiated mandible. Given the pre-existing status of deferoxamine on hospital formulary, this treatment represents a highly translatable method of enhanced bone healing in the setting of radiotherapy that may expand the utility of NBGs in mandibular reconstruction following tumor ablation. While further investigations are necessary, such translation would offer the practical benefits of NBGs to both surgeons and head and neck cancer patients including reduced donor site morbidity and technical demand in comparison to FTT procedures.

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Melting the Plastic Ceiling: Quantifying Resources for and Identifying Barriers to Women Seeking Academic Plastic Surgery Leadership Positions