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Effect Of Static Cold Storage On Vascularized Composite Allografts

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Purpose: The current gold standard in organ storage is static cold storage (SCS) at 4°C in a preservation solution (UW, HTK). While kidneys can be stored for 24 hours in SCS, Vascularized Composite Allografts (VCA) have been transplanted up to 12 hours in this condition. We aimed to determine the limits of VCA viability with SCS. We used a partial heterotopic hindlimb transplantation model on rodent to assess VCA survival at various durations of SCS.

Methods: Right partial hind limbs in Lewis rats were procured and a 24G Angiocath inserted in the femoral artery. Limbs were flushed with 2ml of heparin saline (100UI/ml) at room temperature and then 5ml of ice cold HTK solution. The limbs were then immersed in HTK in a sterile bag and stored at 4°C for a period of 12(group 1), 18(group 2), 24(group 3), 48 (group 4) or 0 hours (fresh controls Group 5), before microsurgical transfer to the opposite groin. Graft weight was assessed before and after cold storage. At post-operative day (POD) 21, VCAs were harvested for histological analysis.

Results: In all groups, there was no significant difference of weight gain in SCS groups after extended SCS times. In group 1 (n=4), 3 rats survived until POD21; clinical evolution showed a limited epidermolysis (<1/3 donor skin surface) in 2 grafts. In group 2 (n=4), 2 rats survived until POD21 without complication. In group 3 (n=6), 3 rats survived until POD21; 1 showed partial superficial necrosis on 50% of the donor skin surface that led to late wound healing. In group 4 (n=4), 3 rats survived; all but one showed extended superficial necrosis over more than 2/3 of the donor skin surface; findings at end of study revealed patent arterial supply but venous thrombosis in 2 rats, inflammatory tissues around the graft and necrotic muscles. There was no difference in the VCA survival rate between the groups 1, 2 and 3 compared to the control group 5. Group 4 demonstrated delayed graft failure.

Conclusion: SCS provides a safe and simple method of organ storage but is very time limited. Cooling an organ down to 4°C aims to reduce cell metabolism 10-fold but does not make it zero. Hence, VCA hypothermic storage is limited by the remaining metabolism of the skeletal muscle. In our study, rat VCA can be stored in SCS for 24hours without significantly impacting its survival rate but an increase in muscular atrophy and epidermal necrosis correlates with longer hypothermic storage durations. We hypothesize that rat tissues are particularly resistant to SCS thus we need to scale up to a large animal model to better understand ischemic injuries on human tissues.

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Fat Grafting Depletes Profibrotic Prrx1-positive Fibroblasts In Irradiated Skin And Mitigates Radiation-induced Groin Contracture

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Purpose: Contracture is a long-term complication of radiotherapy (RT) that results from pathologic fibrosis of soft tissue in the radiation field. Fat grafting can regenerate fibrotic soft tissue by decreasing collagen density and reorganizing collagen fiber networks. Fibroblasts are the predominant cell involved in extracellular matrix (ECM) synthesis, and dermal fibroblasts possess distinct embryonic origins. We recently identified a profibrotic fibroblast in the mouse ventral dermis marked by embryonic expression of paired related homeobox 1 (Prrx1). Here we sought to investigate how fat grafting alters Prrx1-positive subpopulation distribution as well as functional contractures.

Methods: Adult Prrx-1Cre;R26mTmG reporter mice (n=9) underwent whole body lethal irradiation with 9 Gy for hematopoietic depletion. Mice were immediately reconstituted
with 2 million nucleated bone-derived cells from donor NSG (NOD.CB17-Prkdcscid/J) mice via retro-orbital sinus injection. The success of reconstitution and immunodepletion was assessed by fluorescence-activated cell sorting (FACS) analysis of peripheral blood. After 4 weeks, 30 Gy was delivered to the right hindlimb in five fractionated doses to generate limb contracture. The irradiated, contracted limb was then grafted with 200 μl fresh human lipoaspirate and limb extension was measured over the subsequent 8 weeks, at which point skin was harvested for assessment of fibroblast subtypes for FACS and immunofluorescence. A group of mice with radiation-induced groin contracture did not undergo fat grafting and served as the control group.

Results: FACS analysis indicated successful immunodepletion and engraftment by 3 weeks post bone marrow transplantation. At one month following groin irradiation, mice had developed significant right hind limb contracture with significantly reduced limb extension (**p<0.0001). Histologically this was paralleled by thickening of the dermis, and substantial expansion of the fibrogenic Prx-1-positive fibroblast subpopulation. While human fat graft volume retention was reduced over 8 weeks following implantation, this was associated with significantly improved in limb extension. The skin overlying the grafted fat showed reduced collagen density, as indicated by trichrome staining, as well as a reduction in the fibrogenic Prx-1-positive fibroblast subpopulation by immunofluorescence imaging, as compared to the control mice.

Conclusion: Here we show that fat grafting improves the extensibility of irradiated and contracted hind limbs and reverses radiation-induced skin fibrosis by both reducing the collagen content and by altering the composition of dermal fibroblast subpopulations. Specifically, fat grafting results in a depletion of the Prx-1-positive fibroblast subpopulation. Further elucidating how this profibrotic fibroblast subpopulation is involved in ventral surface soft tissue fibrosis will facilitate development of novel strategies to treat/prevent debilitating late side-effect of radiotherapy.

Morphological Changes Of Skin Related To Acellular Dermal Matrix Incorporation In Tissue Expansion

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Purpose: Acellular dermal matrix (ADM) is used to create an inferolateral sling nearly three-quarters of the time in breast reconstruction and has proven a valuable alternative to total submuscular coverage of the implant. Previous work has demonstrated decreased inflammation and fibrosis of the pocket lining the ADM sling; however there has been minimal investigation into the role of ADM in skin growth and regeneration. The present study evaluates morphologic and molecular changes mediated by use of ADM in tissue expansion.

Methods: Two tissue expanders, one wrapped in ADM, were placed subcutaneously on the back of Yucatan mini-pigs. All expanders were inflated with two weekly fills of 60cc of normal saline and skin biopsies were harvested after two weeks of expansion from each condition: control, tissue expansion (TE), and tissue expansion with ADM (TE+ADM). Three biopsies per condition were embedded in paraffin or OCT medium and stained with Russell Movat Pentachrome and Immunofluorescence of CD31, respectively. Collagen in the papillary dermis of pentachrome-stained images were analyzed using an ImageJ plug-in, Fibril Tool, that applies circular statistics to estimate average fibril orientation as the direction angle from -90 to 90 with respect to the x-axis. One-way ANOVA evaluated seventy-two measurements per condition and post-hoc analysis with Tukey’s HSD test identified significant comparisons between the groups. Number of fluorescent cells expressing CD31 (a marker of endothelial cells) were counted on 12 photographs per condition. P-values ≤ .05 were considered significant. Total deformation was calculated using a computational model and isogeometric analysis.

Results: The mean fibril orientation of TE and TE+ADM underwent -85% change (P < .001) and -15% change (P = .65), respectively, compared to control. Three times more CD31+ cells were observed in TE+ADM compared to control (P < .001), but no significant changes were detected in TE alone. Histogram of total deformation revealed more even distribution of forces in TE+ADM compared to TE and control.

Conclusions: The use of ADM in a porcine tissue expansion model appears to mitigate disarray of the collagen network in adjacent tissue, thereby creating a more extensive, yet even, distribution of stretched skin. This observation, combined with the finding of increased angiogenesis, suggests it is the incorporation of ADM that confers these protective benefits. Future studies will evaluate whether the protective