a total of 5 doses. Post-DBMT, the recipient was maintained on a tapering course of cyclosporine before complete withdrawal 28 days later. VCAs were assessed by serial clinical assessment and histopathology. Mixed chimerism in peripheral blood was monitored by flow cytometry and *in vitro* immunologic responses were assessed through mixed lymphocyte reaction (MLR) assays.

RESULTS: Two recipients were euthanized within 2 weeks of DBMT due to neutropenic sepsis and post-transplant lymphoproliferative disorder but both VCAs remained viable up to experimental endpoint. M4515 (full MHC-mismatched recipient) has been off of all immunosuppression for 3 weeks without any evidence of rejection. M3815 (haplomatched) developed mixed chimerism transiently at 6 weeks after DBMT and corresponding MLR assays demonstrated decreased anti-donor responses; immunosuppression was then successfully withdrawn for a total of 5 weeks before rejection developed. Although the rejection episode could be reversed with steroid bolus and a tapering course of FK506, recurrence occurred after another 2 weeks off immunosuppression.

CONCLUSION: As with the clinical experience with tocilizumab, vigilant monitoring is required following drug administration due to increased susceptibility to neutropenia and infections[1]. Tocilizumab appears to promote engraftment after DBMT to allow short-medium term immunosuppression-free VCA survival across haplomatched barriers in this NHP model. Continued follow-up is required to determine if similar results can be achieved across a full MHC mismatch. Further studies in our laboratory are focused on optimizing the current protocol to achieve stable engraftment and durable mixed chimerism for tolerance of VCA.

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REFERENCES:

1. Jones G, Sebba A, Gu J, Lowenstein MB, Calvo A, Gomez-Reino JJ, et al. Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: the AMBITION study. Ann Rheum Dis. 2010;69:88–96

Ex-vivo Subnormothermic Oxygenated Machine Perfusion of Swine Forelimbs Enables Prolonged Graft Preservation Prior to Transplantation

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BACKGROUND: The current gold standard in tissue preservation is static cold storage (SCS) on ice-cold (0–4°C) UW solution. While SCS slows down graft deterioration, it does not have restorative capabilities. We previously developed an ex-vivo perfusion system for subnomothermic oxygenated machine perfusion (SNMP) to resuscitate cadaveric organs. Recovered livers were perfused for 3 hours and transplanted successfully into recipient rats in 5/6 cases[1]; when scaled up to DCD human livers, SNMP demonstrated sustained and enhanced viability of liver grafts. To expand the donor pool in VCA, we investigated the utility of SNMP on preservation time and resuscitation of ischemic limbs in a swine model.

METHODS: 2 porcine forelimbs were procured and flushed with ice-cold UW on the back table through the cannulated axillary artery and veins. Warm ischemia was 45 mins and SCS was 2 hours. Before starting SNMP, the forelimbs were flushed with 1500 mL of cold Lactated Ringers. During SNMP (3 hours), the amputated forelimbs were perfused by a pressure-controlled system through the axillary artery. The perfusion solution consisted of William's E medium, which was enriched with dexamethasone, insulin and heparin. A venous outflow was prepared for sample collection. Hemodynamics of the limbs was monitored by evaluation of arterial flow and vascular resistance. Perfusion samples were collected at 30 min intervals for biochemical analysis. Lactate clearance was monitored as a marker of muscle injury. Muscle biopsies were collected at 60 min intervals for measurement of ATP production.

RESULTS: Arterial outflow and vascular resistance remained stable throughout the perfusion, between 270 and 320 mL/min and 0.23 and 0.26 mmHg/mL/min, respectively. Despite the initial increase in lactate levels from 0.2 mmol/L to > 6 mmol/L, this value remained stable during the final hour of perfusion. The increase in ATP production reflects successful resuscitation of the forelimb, increasing from a baseline of 5500 before perfusion to 7500 nmol/g protein during SNMP.

CONCLUSIONS: SNMP has the potential to both actively preserve and enhance overall preservation of forelimbs in a swine model. It may provide the crucial enabling technology for tissue preservation, transport, and eventual transplantation of VCAs.

REFERENCE:

1. Tolboom, H., Izamis, M.L., Sharma, N.et al Subnormothermic machine perfusion at both 20 degrees C and 30 degrees C recovers ischemic rat livers for successful transplantation. *The Journal of surgical research* **175**, 149–156 (2012).

Reducing Ambient Oxygen Tension Optimizes the Fabrication and Maturation of Pre-Vascularized Tissue Engineered Flaps

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INTRODUCTION: Oxygen is a potent modulator of cell function and wound repair *in vivo*. Hypoxia can enhance the production of specific extracellular matrix components and increase angiogenesis through the hypoxia-inducible factor-1 pathway. However, *in vivo*, very few cells within the body experience ambient (21%) oxygen tension. Thus, for clinically relevant tissue-engineered pre-vascularized skin flaps, hypoxic conditions can be exploited for promoting angiogenesis. We sought to identify the ideal oxygen tension in which to fabricate our novel, pre-vascularized tissue constructs containing a vascularized 1 mm diameter microchannel lined with human cells.

MATERIALS AND METHODS: Vascular networks were fabricated by sacrificing Pluronic F127 macrofibers in type I collagen with encapsulated human foreskin fibroblasts (HFF1) and human placental pericytes (HPPL) at a density of 1 x 10^6 cells/mL, respectively. Twenty-four hours following fiber sacrifice, 5 x 10^6 cells/mL of human aortic smooth muscle cells (HASMC) and 5 x 10^6 cells/mL of human umbilical vein endothelial cells (HUVEC) were seeded sequentially into the patent luminal space. Subsequently, 48 hours after fiber sacrifice, 1 x 10^6 cells/mL of human epidermal keratinocytes (HEK) were topically seeded onto scaffolds. Scaffolds were incubated at 1.5%, 5.0%, or 20.0% oxygen, underwent daily media changes, and were analyzed after 7 and 14 days in culture.

RESULTS: Macrochannels were successfully lined with HUVEC and HASMC, generating anatomically appropriate neointimal and neomedial layers by as early as day 7. The most robust cellular linings were seen in constructs incubated in 5.0% oxygen. Immunohistochemical analysis revealed CD31+ HUVEC along the luminal surface of the macrochannel, and α-SMA expressing HASMC in the subendothelial plane. Furthermore, proliferation of HFF1 was evident as early as 7 days after seeding. HEK proliferated leading to the formation of a stratified epidermal layer along the construct surface and fibroblast specific-1-expressing fibroblasts within the "neo dermis."

CONCLUSION: Hypoxic conditions promote increased angiogenesis and vascular stability in our tissue engineered, pre-vascularized skin flaps without detrimental effects on other flap cellular constituents. With a built-in vascular network, vital epidermal (HEK) and dermal (HFF1, collagen) components, these full-thickness, tissue engineered skin scaffolds hold tremendous promise as a platform to aid in evaluating cellular responses to changing oxygen concentrations in parallel to generating tissue-engineered flaps of clinically relevant sizes.

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Radiological and Histological Assessment in Perforator Flap Microvasculature Following Pretreatment with Topical Negative Pressure Therapy: An Experimental Rat Model

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BACKGROUND: Surgical delay and ischemic preconditioning have been traditionally used to precondition flaps to render flaps more vascular and resistant to changes in their microcirculation. Negative pressure wound therapy (NPWT) has been extensively in clinical