University of Wisconsin (UW) solution for 3 hours prior to transplant. Experimental group (n=24) flaps were perfused with MP/HBOC for 17 hours at a subnormothermic temperature of 21°C. Flaps were monitored daily for clinical evidence of viability and biopsied per protocol with an end point of 17 hours for ex vivo only, 14 days for autotransplants and 60 days for allotransplants. The allotransplanted animals were placed on systemic triple immune suppression and maintained at therapeutic levels for the duration of the study. Histologic analysis was blinded and reviewed by an expert veterinarian pathologist at conclusion of the study.

RESULTS: Twenty-four porcine myocutaneous flaps are designated to experimental groups and 24 to the control group. We anticipate results will be similar to previous porcine myocutaneous flaps exposed to 14 hours of CSP (n=4) or MP/HBOC (n=4). Results indicated significantly attenuated markers of IRI, significant apoptosis on TUNEL staining, and endothelial damage in the CSP group when compared to subnormothermic MP/HBOC.

CONCLUSION: If VCA can be preserved for up to 17 hours or more and be protected from ischemic damage following allotransplantation, the achievement will have a profound clinical application in VCA as well as solid organ transplantation. Based on promising preliminary data, we believe efficient tissue oxygenation promoted by subnormothermic (21°C) MP/HBOC in VCA will (1) extend graft preservation times and improve donor access across geographic spans, (2) enable increased efficacy of ex-vivo targeted graft manipulation and (3) ensure graft quality and viability prior to transplantation.

External Volume Expansion (EVE) Increases Vascularization of Subcutaneous Scaffolds

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**INTRODUCTION:** External Volume Expansion (EVE) has been shown to promote angiogenesis, adipogenesis and expansion of subcutaneous tissue in skin. In this study we evaluate the effects of EVE on an acellular scaffold implanted subcutaneously to investigate whether EVE promotes vascularization and recellularization of the scaffold.

**METHODS:** 36 wild-type mice (n = 18 per group) underwent either EVE through a previously optimized protocol or no EVE (control) for five days before receiving a subcutaneous graft of an acellular matrix (0.5cc). Grafts were collected at 6 weeks (n = 8 per group), and 12 weeks (n = 10 per group) after surgery and analyzed through histology (H&E and CD 31 staining).

**RESULTS:** At macroscopic observation grafts placed in an site previously stimulated with EVE showed a better preserved morphology. Recipient site preparation with EVE significantly improved vascularization of the acellular grafts compared to controls (+60%, p<0.05) and significantly enhanced proliferation/migration of adipocytes inside the graft.

**CONCLUSION:** EVE can be effectively used to improve vascularization and recellularization of subcutaneous acellular grafts. Further research in this field might lead to innovative reconstructive therapies that do not rely on autologus tissue (fat).

Directional Freezing and Vitrification of Whole Limbs for Future Transplantation and Organ Banking

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**INTRODUCTION:** According to the World Health Organisation, less than 10% of humanities needs for transplantable organs are being met. No data is available for vascularized composite tissue allotransplantations (VCA), yet these cases are further complicated by the need for an instantly available and compatible recipient. VCA containing skin, fat, blood vessels, bone, bone marrow and nerve are the ultimate tool available to date in reconstructive surgery. Widespread use of this tool
is hindered in part by the availability of limbs which can survive a limited amount of time disconnected from the body. Cryobiology is the scientific field which investigates biology at low temperatures. Cryobiologists have long sought to cryopreserve biological samples ranging from single cells to complex organs and whole animals. Vitrification is the transformation of a substance into a non-crystalline amorphous solid using rapid heat removal. Directional freezing is a technique, consisting of highly controlled and cell-friendly ice crystal morphology that significantly reduces mechanical damage. Extremely efficient heat removal and controlled ice crystal propagation make it suitable for freezing both small and large volumes alike. Combining Directional freezing and VCA may open the door for “Organ Banking”. If composite tissue could be frozen and then thawed without damage, non transplanted tissue and organ waste would be reduced, potentially enabling better donor and recipient availability and match.

METHODS: We used directional freezing and vitrification on a syngeneic heterotrophic rat hindlimb transplant model and monitored the animals and transplanted limbs for up to 72 hours. Cell and tissue samples were taken for culture and histology.

RESULTS: Immediately upon thawing the donor hindlimb blood vessels and tissue felt similar to the recipient’s. Following revascularization reperfusion was clinically evident by color and bleeding from distal sites to the anastomosis. Limb survival was noted up to 72 hours post op. Tissue samples including muscle, skin and blood vessels where taken at 24, 48 and 72 hours from transplantation. Histology demonstrated viable myocytes, intact intimal lining of blood vessels and all skin layers.

CONCLUSION: Whole limb freezing and reimplantation using directional freezing and vitrification is feasible in small animal models. Based on our current achievements we intend to develop protocols for limb cryopreservation in large animals, aiming to advance the field of cryobiology towards the ambitious goal of human organ banking.

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INTRODUCTION: Ischemia and reperfusion injury remains one of the major limiting factors for the success of both replantation and vascularized composite allotransplantation. Compared to cold storage of preserved/procured organs, normothermic ex-situ perfusion is a novel approach that prolongs viability of the limb by maintaining physiologic cellular metabolism avoiding the deleterious effects of both hypoxia and cooling. This study aims to develop an ex-situ normothermic limb perfusion protocol to preserve the viability and function of amputated limbs for over 24 hours.

METHODS: A total of 23 swine limbs were perfused using an oxygenated colloid solution at 38°C containing washed RBCs. The first 13 limbs were used to optimize the perfusion protocol. The subsequent 5 limbs (Group A) were perfused for 12 hours and the following 5 (Group B) as long as muscle contractility/peripheral perfusion were present. Electrolytes were balanced by partial perfusate exchanges. Limb viability was compared in the 2 groups by muscle contractility, compartment pressure, tissue oxygen saturation, indocyanine green (ICG)-angiography and thermography.

RESULTS: Perfused limbs were able to retain physiological parameters and function for 12 hours in group A and up to 44 (24–44) hours in group B. Limbs in group A had lower final weight increase (0.54%±0.07 VS 14.11%±16.27) (p=0.008) and compartment pressure compared to group B (16.23 ± 7.10 VS 24.75 ± 7.79) (p=0.175). Final myoglobin and CK mean values were lower in group A compared with group B (875 ± 291.4 ng/mL VS 1010.6 ± 323.6 ng/mL and 53344 ± 14850.34 U/L VS 71881 ± 20475 ng/mL). In group B thermography and tissue oxygen saturation were significantly higher than in group A (respectively 35.37±0.69°C VS 33±1.44°C (p=0.01) and 69.31±9.3% VS 58.69±8.4% (p=0.048)).

Extended Normothermic Ex-Situ Limb Perfusion Preserves Limbs Viable and Functional Up to 44 Hours

Presenter: Edoardo Dalla Pozza, MD