Local Mesenchymal Stem Cell Therapy for the Ex Vivo Preservation of a Porcine Vascularized Composite Allograft with 24-hour Normothermic Machine Perfusion

Alejandro Bernal, MD[1,2], Jennifer Hinshaw, BS[1], Jorge J Pena, BS[1], Cody Hinshaw, BS[1], Diandra Wood, BS[1], Kreg A Zimmern, BS[3], Nick McMahon,MS[3], Jian Ling, PhD[3], Margaux M. Salas, PhD[1], Wensheng Zhang, MD, PhD[1], Erik K Weitzel, MD[1]

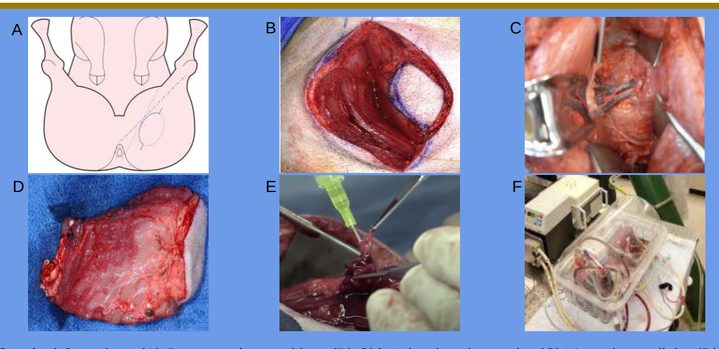
(1) 59th Medical Wing, JBSA-Lackland AFB, TX; (2) University of Texas Health Science Center at San Antonio, San Antonio, TX; (3) Southwest Research Institute, San Antonio, TX

BACKGROUND

Advances in both body armor and trauma life support have increased the survival rate of combat casualties. However, there remains vast limitations in extremity war injuries and limb salvage. As a result, the rise of vascularized composite allotransplantation (VCA) has increasingly gained traction. Since preservation research is still in its infancy, multiple factors regarding cold ischemia time and perfusion following injury through surgical intervention need to be established. Machine perfusion has become a military area of interest for organ preservation in transplantation since limitations involving (1) prolonged travel (i.e. geographical distance) and (2) time before surgical intervention are the norm in combat related injuries. Additionally, the introduction of mesenchymal stem cell (MSC)-based therapy has shown promise in VCA studies by attributing improved immunomodulation and regenerative properties. Previous VCA research models, using University of Wisconsin (UW) preservation solution under hypothermic conditions and the use of whole blood in normothermic conditions have been performed. However, limited data exist addressing the use of MSCs during an extended preservation phase of an *ex vivo* VCA study. The purpose of this study is to demonstrate the efficacy of combining local MSC infusion with fresh whole blood in an *ex vivo* porcine free flap VCA model utilizing normothermic machine perfusion (NMP) for 24 hours. We hypothesized that local intravascular infusion of MSCs during NMP will facilitate the attachment of MSCs to the VCA vasculature and lead to tissue repair as well as aid in the prevention of additional cell injury; reducing inflammatory responses during the *ex vivo* preservation phase.

METHODS

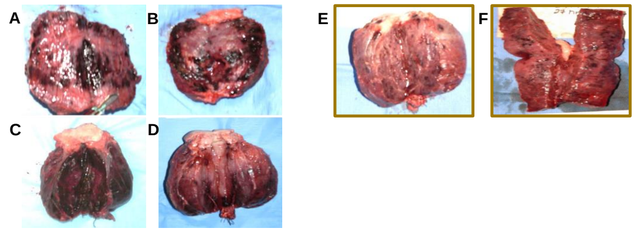
A porcine semitendinosus myocutaneous free flap was used to model VCA. Seven Yorkshire swine were randomly divided into an experimental group (n=3) and a control group (n=4). Each VCA flap averaged 200–400g, containing skin, subcutaneous tissue, fascia, muscle, and a vascular pedicle (terminal branch of deep femoral/profunda artery and vein). 5 Units of autologous whole blood was collected from the donor animal post tissue procurement. In an antegrade fashion, the harvested flap was flushed with 20 ml +/- 20 ml heparinized saline until clear to remove any formed clots. Next, the flaps underwent 3-hour cold ischemia at 4°C, preserved with UW solution administered via a one hour drip infusion. Thereafter, the free flap was connected to NMP with whole blood and specific supplements (trace elements, multi-vitamin, nutrients, antibiotics, antioxidants, and vasodilator) +/- MSCs. Flow rate was set at 5-10 ml/min for 24hrs. The flaps were monitored for various parameters in both blood perfusate and tissue biopsy within serial time points. Finally, the tracing of fluorescence-labelled MSCs in the VCA flap was assessed by an IVIS® in vivo imaging system.



Surgical Overview. (A) Preoperative position; (B) Skin Island and muscle; (C) Vascular pedicle; (D) VCA free flap; (E) Vascular cannulation; (F) Machine perfusion

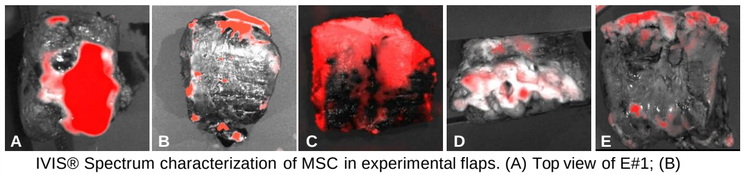
RESULTS

REDUCED TISSUE INJURY IN MSC GROUP



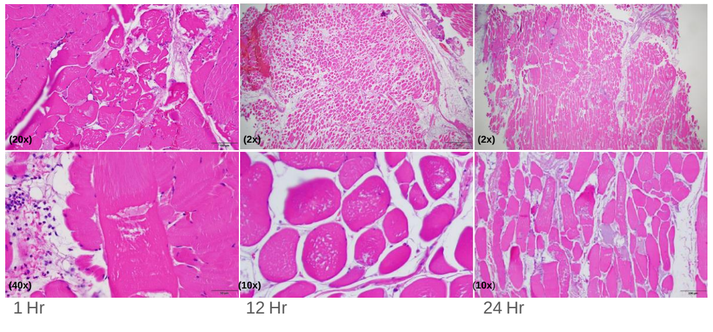
Gross anatomy post 24hr NMP. (A-D) Large necrotic areas in flaps C#1-4 representing tissue injury; (E-D) Minimal necrotic areas in flaps E#1-2 representing tissue injury

TISSUE-BOUND MSCs REPRESENTED BY FLUORESCENT



IVIS® Spectrum characterization of MSC in experimental flaps. (A) Top view of E#1; (B) L-side view of E#1; (C) L-side view of E#2; (D) Top view of E#3; (E) L-side view of E#3

HISTOLOGICAL PROGRESSION

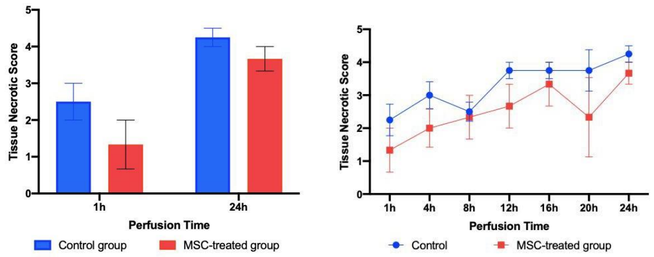


24Hr histology H&E stained tissue section. (LEFT) degeneration/necrosis with adjacent neutrophilic inflammation; (MIDDLE) myofibril degeneration with diffuse myofiber atrophy and interstitial edema; (RIGHT) degeneration/necrosis with edema.

RESULTS

Three flaps were used in the experimental group (MSC treatment), showing a slower progression of VCA necrosis after 24-hour NMP grossly and histologically compared to the control group. Patchy necrosis was visualized in skeletal muscle, but not seen in the skin. Histologically, necrotic score is defined here as a percentage of the section that was necrotic ranging from 0 = no necrosis, 1 = >5% necrotic, 2 = 5-25% necrotic, 3 = 26-50% necrotic, 4 = 51-75% necrotic, and 5 = 76-100% necrotic.

REDUCED NECROSIS IN MSC GROUP



Comparison of tissue necrosis between control and experimental groups.

CONCLUSION

- Our preliminary data suggest that the local infusion of MSCs to VCA during 24-hour NMP lead to a reduction in ischemic tissue injury and degeneration.
- IVIS images demonstrate the bondage of MSC to VCA vasculature and tissues which show minimal fluorescent illumination throughout flap and largely throughout skin.Although IVIS imaging could not be quantified as tissue flaps varied in size and due to varying biopsy collection size in both skin and muscle.
- It is therefore important for this information to continue forward with further trials on local stem cell therapy in ex-vivo VCA preservation.
- This study shows great promise for improving outcomes on delayed limb salvage surgeries in combat trauma by reducing ischemia reperfusion injury and incidence of graft rejection.

ACKNOWLEDGEMENTS

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DISCLAIMER

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