

COMPARISON OF PRESERVATION SOLUTIONS TO REDUCE CELL DEATH IN PIG MYOCUTANEOUS FLAPS DURING COLD STORAGE. C Aber¹, Y Luo¹, (M.D.), C Blades¹, N Limon de la Rosa¹, Z Dumanian¹, Y Wong¹, (M.D.), P Arrowsmith², E Farkash², (M.D., Ph.D.), N Navarro-Alvarez¹, (M.D., Ph.D.), and C.A. Huang¹, (Ph.D.), ¹University of Colorado Anschutz Medical Campus, Aurora, CO, ²University of Michigan School of Medicine, Ann Arbor, MI.

Vascularized composite allografts (VCAs) are transplants composed of multiple tissue types and are studied for their potential in limb replacement. Preservation of VCAs can be challenging because different tissue types exhibit varying properties, such as differing levels of immunogenicity or ischemic tolerance. The standard method of preservation is static cold storage (SCS), where, following procurement, the limb is flushed with a cold preservation solution and packed with ice within insulated containers before transport and transplantation.

Purpose: This study compares the effectiveness of two different organ preservation solutions [Belzer UW® Cold Storage Solution (UW) and Custodiol® HTK (Custodiol)] alone versus in combination with the necroptosis inhibitor Necrostatin-1 (Nec-1) to determine which results in the least cell death in myocutaneous porcine flaps during SCS.

Methods: Myocutaneous rectus abdominus flaps from two Yucatan recipient pigs (35506, 35507) and five Sinclair donor pigs (4822, 4823, 26337, 26117, 27085) were flushed with Custodiol or UW preservation solution containing 40 µg/mL Necrostatin-1 (Nec-1) or dimethyl sulfoxide (DMSO). The flaps then underwent ischemia for 72 hours at 4°C. Paraffin-embedded slides of skin and muscle from the samples were used for Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) staining. Apoptotic nuclei were manually quantified using Aperio ImageScope software (Leica Biosystems), a pathology slide analysis platform. Statistical analysis was done through GraphPad Prism to conduct Welch's unpaired t-test to determine significant differences among sample groups.

Results: Statistical significance was observed only between UW and UW+Necrostatin-1 in the epidermis ($p = 0.0151$). In the dermis, comparison of UW + Nec-1 to UW, Custodiol, and Custodiol + Nec-1 trended toward significance ($p = 0.0940, 0.0796, \text{ and } 0.0617$, respectively). In muscle, comparison of UW + Nec-1 to Custodiol + Nec-1 trended toward significance ($p = 0.0657$).

Conclusions: UW + Nec-1 significantly offers superior preservation compared to UW in epidermal tissue. Although not statistically significant, trends favored UW + Nec-1 over UW and Custodiol across epidermal, dermal, and muscle tissues. More data is needed to support these findings.