

# Successful Extension of Vascularized Composite Allograft Perfusion Cold Storage to 24 Hours in a Rat Hind Limb Transplant Model

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**Background:** Vascularized Composite Allograft (VCA) transplantation is a treatment option for complex tissue injuries; however, ischemia reperfusion injury (IRI) and high acute rejection rates remain a challenge. Hypothermic machine perfusion (HMP) using acellular storage perfusate is a potential solution. This study evaluated the University of Wisconsin (UW) Kidney Preservation Solution (KPS-1) compared to normal saline (NS) for preservation of donor rat hind limbs subjected to 24h of *ex-vivo* perfusion cold storage.

**Methods:** Hind limbs were subjected to 24-hour perfusion cold storage with heparinized KPS-1 (n=6) or heparinized NS (n=6). Flow, resistance, and pH were measured continuously. At the end of the 24h period, tissue was collected for histological analysis of edema and apoptosis.

**Results:** KPS-1 perfused limbs showed significantly less edema than the NS group, as evidenced by lower limb weight gain ( $p < 0.001$ ) and less interfascicular space ( $p < 0.001$ ). KPS-perfused muscle had significantly less cell death than NS-perfused muscle based on terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) ( $p < 0.001$ ) and Cleaved Caspase-3 (CC3) staining ( $p = 0.045$ ). During HMP, a significant decrease in pH over time was detected in both groups, with a significantly greater decline in pH in the KPS-1 group than in the NS group. There were no significant differences overall and over time in flow rate or vascular resistance between the KPS and NS groups.

**Conclusions:** Perfusion with KPS-1 can successfully extend VCA perfusion cold storage for 24h in a rat hind limb model without significant edema or cell death.