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 Allograft (VCA) transplantation is a treatment option for complex tissue injuries.
• Strategies to extend VCA preservation times are required to improve VCA transplant outcomes and expand the geographical donor pool.
• Hypothermic machine perfusion (HMP) using acellular storage perfusate is a potential solution.

Objective
• Evaluate the use of University of Wisconsin (UW) Kidney Preservation Solution (KPS-1) to preserve donor rat hind limbs subjected to 24h of ex-vivo perfusion cold storage.
• Assess edema and muscle cell death following 24-hours of ex vivo perfusion using KPS-1 compared with normal saline (NS).

Methods
• Brown Norway hind limbs were subjected to 24-hour perfusion cold storage with heparinized KPS-1 (n=6) or heparinized NS (n=6).
• Limbs were weighed before and after perfusion cold storage to approximate the extent of edema.
• Muscle was collected for histological analysis of edema and apoptosis using H&E, TUNEL, and Cleaved Caspase-3 (CC3) staining.

Results

Figure 1: (A) Comparison of weight (in grams) before and after 24-hour perfusion with NS/KPS. (B) Quantification of weight gain displayed as a percentage of weight gained relative to starting weight of hind limbs (n=12). (C-D) Representative images of Brown Norway hind limbs that were subjected to 24 hours perfusion with KPS solution ex vivo (top), and in vivo (bottom) following successful orthotopic transplantation. Significant differences, *p < 0.05, **p < 0.01, *** p < 0.0001.

Figure 2: Representative 10x TUNEL, and 10x Cleaved Caspase-3 staining images of rat hind limb muscle, positive/negative staining indicated by red/blue arrows, respectively. TUNEL staining of hind limbs (A) naive muscle, (B) muscle perfused with NS, and (C) muscle perfused with KPS solution. Cleaved caspase-3 staining of hind limbs (D) naive muscle, (E) muscle perfused with normal saline, and (F) muscle perfused with KPS solution. Quantification of TUNEL (G), and Caspase staining (H). Significant differences, *p < 0.05, **p < 0.01, *** p < 0.0001.

Conclusions
• KPS-1-perfused rat hind limbs did not show significant muscle edema based on histological assessment of muscle interfascicular space.
• TUNEL staining showed that muscles perfused with KPS-1 had significantly less apoptosis than those perfused with NS.
• CC3 staining was also significantly decreased in KPS-1 perfused muscle tissue compared to NS perfused muscle consistent with decreased apoptosis.
• Orthotopic hind limb transplantation could successfully be performed with limbs subjected to 24 hours ex vivo perfusion cold storage using KPS-1, but not with NS-perfused limbs due to excessive edema.
• The use of heparinized NS as a perfusion solution proved to be damaging.

Summary
• 24-hour ex-vivo HMP with KPS-1 as a perfusion solution for VCAs is feasible in a rat hind limb model and may open doorways to future preservation strategies for VCAs.
• The use of KPS-1-perfused limbs can be successfully transplanted onto a recipient animal. However, future survival studies are needed.
• NS to prepare VCA and other allografts for transplantation should be reconsidered, especially if prolonged cold storage is required.