Extension of Limb Viability up to 72 Hours with Hypothermic *ex-vivo* Perfusion in a Porcine Limb Model

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INTRODUCTION: Limb loss has a drastic impact on quality of life and the socio-economic consequences are extraordinary. Acute management of severe limb injury is often limited to amputation. Gold standard preservation with cold ischemia, only extends limb viability to 6 hours. We present an alternative treatment option where the amputated extremity is placed on a perfusion apparatus to prolong viability up to 72 hours until the patient is stabilized, the residual limb is optimized, and there is access to necessary operative resources.

METHODS: We modified exisiting *ex vivo* perfusion technology specifically to fit extremities and promote aerobic cellular respiration. We connected the perfusion device to amputated forelimbs obtained from young male pigs immediately post-mortem. For 72 hours, the control limb was preserved following the current gold standard approach while the experimental limb was perfused using our limb perfusion prototype. Our prototype consists of a peristaltic pump, limb chamber support, oxygenated chamber, leukocyte filter, fluid cooling machine, and liquid flow regulator. A buffered extracellular solution, called STEEN, is used as perfusate at 8°C, and exchanged every 6 hours. Perfusion rate, pressure, O2 delivery and consumption, acid-base status, and blood gas parameters were monitored every 2hrs. Both superficial and deep muscle was biopsied every 6 hours to evaluate fiber morphology and cellular changes.

RESULTS: The prototype we developed from an *ex vivo* perfusion protocol achieved extended survival of porcine limbs for up to 72 hours compared to the current gold standard for cold storage. The rate of muscle fibrosis is significantly lower in the study group (1.4-2.7%) compared to the control group (7.7-31%), supporting our hypothesis that this *ex vivo* perfusion apparatus extends muscle viability ($p<0.001$). Furthermore, in the study group, in both superficial and deep muscles-the rate of muscle fibrosis remained stable at a low level of 1.4-2.7 % for the entire 72 hours. Perfused muscles appeared healthier compared to controls ($p<0.05$) (fig. 1A). Cross-sectional area analysis of muscle fiber size showed sustenance of myofiber area in the study group compared to the apparent shift to smaller fiber diameter in the control group (Fig. 1B). Several parameters, including sodium, carbon dioxide, and pH, remained stable throughout the experiment. Together, these findings suggest that tissue storage time can be extended which would be beneficial in replantation, vascularized composite allotransplantation, and the treatment of battlefield injuries that may result in amputation.

DISCUSSION AND CONCLUSION: Current methods limit limb viability for a time window of approximately 6 hours. We optimized perfusion technology specifically for extremities. This method is easy to assemble, effective, transportable and can be effortlessly carried on any airplane, including military or civilian aircraft. This study demonstrates that muscle morphology and tissue integrity can be preserved up to 72 hours using our perfusion apparatus and paves the way for improved therapeutic interventions in trauma and transplantation of human limbs.

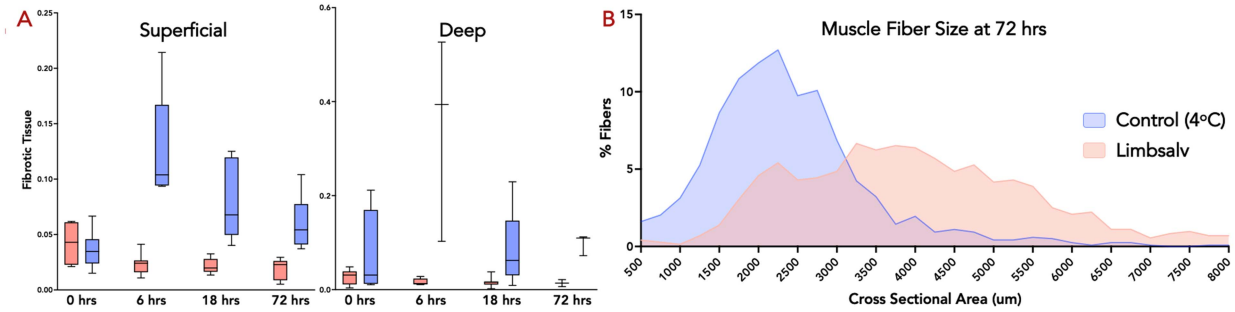

Figure 1. (A) Quantification of fibrotic area demonstrate significant reduction of fibrotic tissue in limbsalv perfused muscles up to 72 hours. Statistical difference was determined by 2-way ANOVA. * $p < 0.05$, **$p<0.02$ and ***$p<0.001$. **(B)** Quantification of fiber size (normalized to total muscle area) shows maintenance of myofiber areas in limbsalv perfused muscles 72 hours after perfusion compared to the reduced fiber size in controls at 72 hours.