Towards Portable Leg Perfusion: Initial Prototype Testing of a Selective Leg Perfusion System

George Harea[1,2]; COL Ian Stewart, MC, USAF[4]; Lawrence Renna, PhD[1]; Teryn Roberts, PhD[1,3]; Dan Wendorff, BS[1]; Brendan Beely, RRT[1]; Kostyantyn Gumeniuk, MD, PhD[5]; Ivan Slichko, MD, PhD[6]; Serhii Sudakevych, MD, PhD[7]; Andriy Rusnak, MD, PhD[5]; COL John Holcomb, MC, USA, (Ret.)[8]; Teja Guda, PhD[2]; Borys Todurov, MD, PhD[7]; Andriy Batchinsky, MD[1,2,3]

[1]Autonomous Reanimation and Evacuation Research Institute and Innovation Center, The Geneva Foundation, San Antonio, Texas, USA; [2]The University of Texas at San Antonio, San Antonio, Texas, USA; [3]Department of Translational Medicine, University of the Incarnate Word School of Osteopathic Medicine, San Antonio, Texas, USA; [4]Department of Medicine, Uniformed Services University, Bethesda, MD; [5]Ukranian Military Medical Academy, Kyiv, Ukraine; [6]Shupyk National Healthcare University of Ukraine, Kyiv, Ukraine; [7]Ukrainian Health Ministry Heart Institute, Kyiv, Ukraine; [8]University of Alabama, Birmingham, AL

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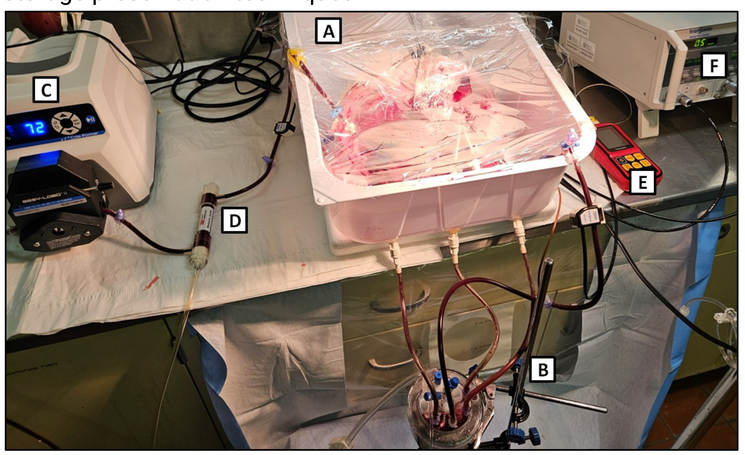
Introduction

- Drone based warfare in Ukraine continues to produce long torniquet application times and record numbers of amputations
- Tourniquets save lives but can result in ischemia if applied over 2 hours; average tourniquet application length in Ukraine is 6 hours, but can be as long as 72 hours, leading to an unprecedented incidence of tourniquet syndrome and limb loss
- We developed a prototype leg perfusion for both torniquet management and perfusion of an amputated leg

Objective and Hypothesis

- Develop a dedicated extracorporeal perfusion system suitable to mitigate the effects of ischemia reperfusion injury
- We hypothesize that a dedicated perfusion system with an integrated oxygenator maintains limb viability with less tissue damage vs. perfusion without oxygenator and vs. standard cold storage preservation techniques



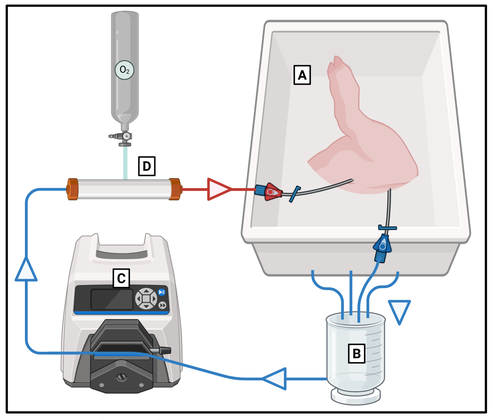


Figure 1: Image (top) and schematic (right) of limb perfusion system. Cannulated limb (A) connected to reservoir/filter (B) which feeds into the pump (C). Blood is pumped into an oxygenator (D), and after oxygenation it is perfused into the limb in the containment unit. Thermometers (E) and ultrasonic flow meters (F) are attached to system for monitoring. Schematic created using Biorender.com

Methods

- The circulation circuit consists of:
  - Cardiotomy filter from D100 oxygenator (Fig 1B)
  - Cole-Parmer Masterflex peristaltic pump (Fig 1C)
  - Two 50cm long Tygon 3/16-inch ID tubing segments
  - Prototype hollow fiber oxygenator (generic) (Fig 1D)
  - Transonic ME4PXL ultrasonic blood flow sensor (Fig 1F)

Methods (cont'd)

- Both hind limbs were explanted from swine donors and 1 L heparinized whole blood (50,000 IU/L) collected from the same donor for perfusion. A special-purpose limb containment system was developed for blood collection, treatment, and perfusion (Fig 1)
- One limb was cannulated with 9F catheters into the exposed femoral artery and vein; the other limb was placed in static cold storage
- Blood gas and chemistry analysis performed using a GEM4000 analyzer
- Study has three groups (n=6/group):
  OXY- group; no oxygenator present
  OXY+ group; oxygenator with 4 Lpm 100% O_2 sweep gas flow
  CSC group; static cold storage control
- At the start of circulation, the baseline (BL) timepoint was performed, with extracorporeal pump RPM, blood flow, temperature and blood gas data recorded. Subsequent timepoints were taken hourly for 6 hours
- Tissue biopsies were taken for histological analysis at BL, 3H and 6H
- Time dependent changes were assessed by one-way mixed model with Dunnett correction. Groups were compared by repeated measures mixed model with Tukey's adjustment (tests two-sided, alpha=0.05, SAS v 9.5)

Results

- No differences in pump RPM and blood flow rates between groups (Fig 2A-B)
- Blood temperature decreased in both groups (Fig 2A)
- At 6 hours, the OXY+ group (vs. OXY-) showed normal blood pH (Fig 2C, p=0.006), improved base excess (-15.8±2.0 vs -23.2±1.8 mmol/L, p=0.019) and improved blood oxygen saturation (Figure 2D, p=0.003)
- pCO_2 decreased in OXY- group at hour 2, and in OXY+ at hour 1 (Fig 2E)
- pO_2 increased in OXY+ group at hour 2, with no changes in OXY- (Fig 2E)
- Potassium increased from BL starting at hour 2 for both groups (OXY+ p<0.001; OXY- p<0.001; Fig 2F)
- Lactate increased starting at hour 1 for both groups (OXY+ p<0.001; OXY- p<0.001; Fig 2F)
- Sarcoplasm degeneration lower in OXY+ vs CSC (1.67 vs 2.67, p=0.038)
- Combined injury scores lower in OXY+ vs CSC (8.33 vs 13.83, p=0.040)
- Sarcoplasm necrosis lower in OXY- vs CSC (0.17 vs 1.17, p=0.022)
- Sarcoplasm vacuolation lower (0.5 vs 1.5, p=0.045)
- No observed difference in histology scores for OXY+ vs OXY-

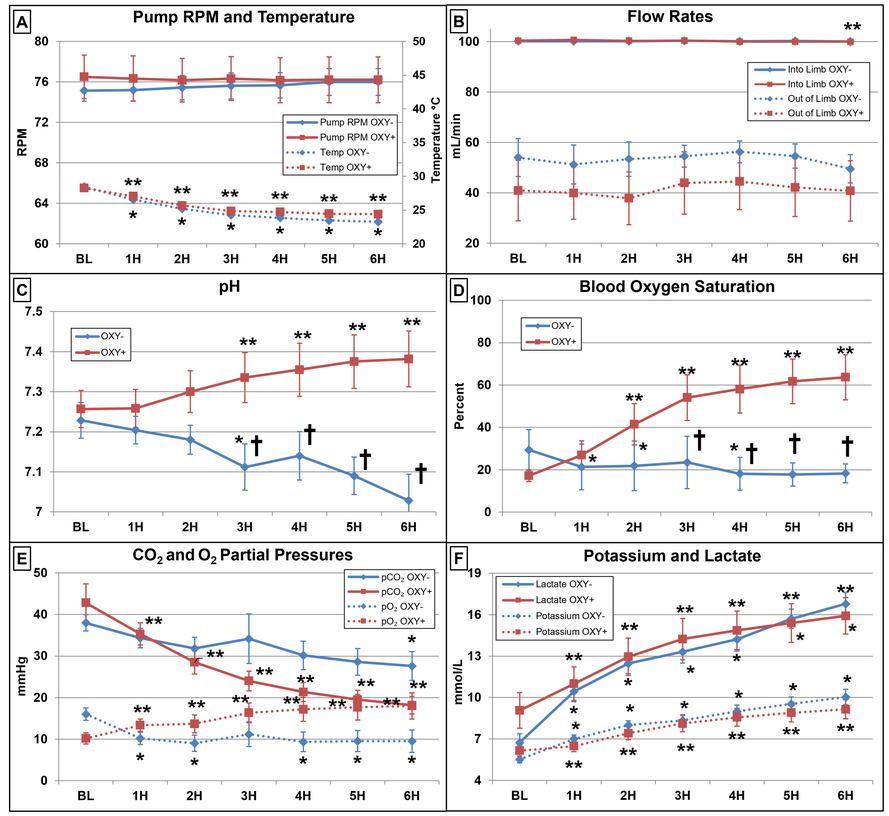


Figure 2: Results from limb circulation (n=6/group). Data shown is mean ± Std Error for all variables. *Significant difference vs. BL in OXY- group; **Significant difference between vs. BL in OXY+ group; †Significant between group differences; p< 0.05 for all tests.

Conclusions

- Explanted limbs were successfully cannulated and perfused extracorporeally; circulation was maintained at a constant flow rate without clotting
- We observed between group statistical improvements for pH, base excess and blood saturation in the OXY+ perfusion group with oxygenator versus OXY-
- We observed a reduction in tissue damage in both OXY+ and OXY- treatment groups compared to the static cold storage control group at 6 hours
- We developed a purpose-built limb perfusion system for management of ischemia reperfusion injury, showing promise for limb preservation

Acknowledgments

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