Acellular Hypothermic Ex-Situ Perfusion Extends Allowable Ischemia Time up to 24 hours in a Rodent Whole Limb Transplantation Model

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Introduction

The standard limb preservation method for hand transplantation is static cold storage (SCS). This is time limited up to 6 hours and prevents transportation to wider geographic areas.

Ex-situ perfusion is a method of preservation which deliver oxygen and other nutrients to maintain and extend the survival of transplanted organs.[1] This method represents the shift of paradigm from metabolic suppression during SCS to metabolic support with continuous perfusion.

It has been used in solid organs and resulted with lower rates of graft dysfunction prior to transplantation. [2-4] In hand transplantation, this method is relatively new with promising outcomes.

Objective

The purpose of this project is to investigate ex-vivo perfusion as a way to extend limb preservation in a rodent model.

Methods

All animals received humane care in accordance with NIH Guide for the Care and Use of Laboratory Animals. Protocols were approved by IACUC.

Procedure

- Five male Lewis rats (300-325g) were used. Following general anesthesia, the right hind limb was amputated and the right iliac artery cannulated using 24G IV cannula.

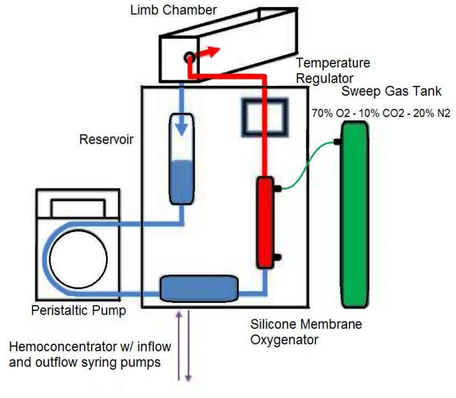
Perfusion

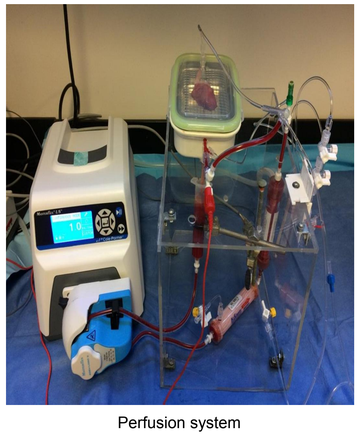
- The limb was attached to the system consisting of a pump, hemofilter, membrane oxygenator, and heat regulator. They were perfused with HTK solution containing 10% albumin for 24 hours at 10-15˚C and then transplanted to recipient animals.

Data Collection

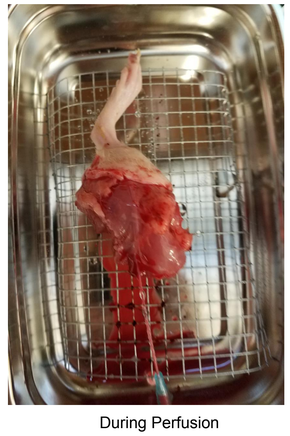
- Lactate and electrolyte levels were recorded every three hours. Hemodynamic variables were measured every 60 minutes. Weight was recorded before and after perfusion.

Ex-Vivo Perfusion Circuit



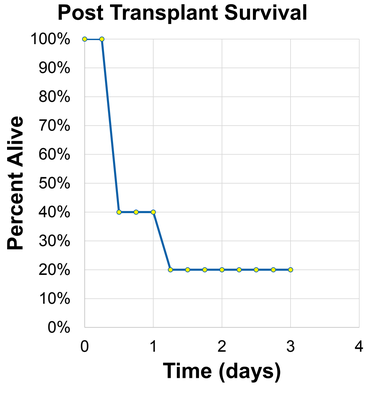


Perfusion system

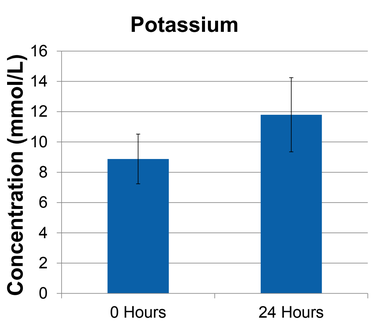


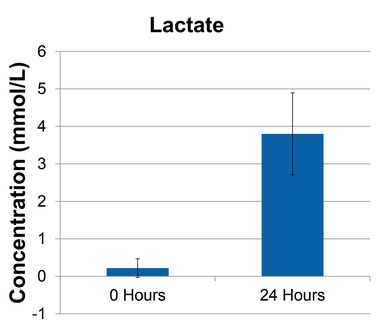
During Perfusion

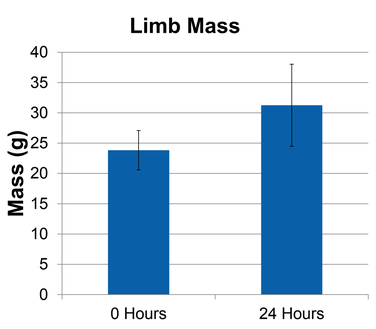
Day 1



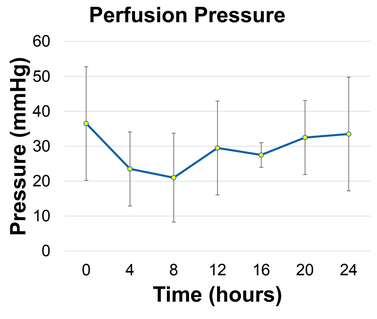
Results

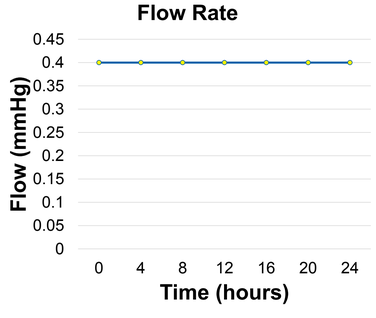


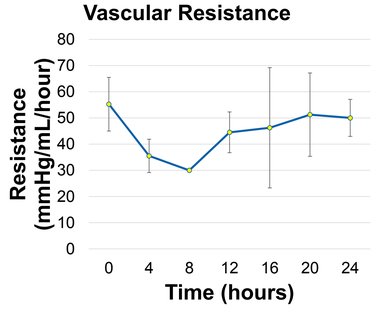




Lactate levels gradually increased from 0.22 ±0.245 mmol/L to 3.8 ±1.09 mmol/L . Potassium levels increased from 8.88 ±1.64 mmol/L to 11.8 ±2.45 mmol/L. Limb allografts gained an average of %31.12006 in mass.







Perfusion pressure remained around 28.47 ± 11.39 mmHg throughout the 24 hours. Flow rate did not change and was maintained at 0.4 ml/hour for all experiments. Vascular resistance fluctuated throughout; the average value was 44.54 ±9.17 mmHg/mL/hour.

Conclusion

The increase in lactate shows the ongoing metabolic activity and is evidence that the limbs viability can be extended using an ECMO system. However, the increase in potassium suggest that the limb is slowly degrading. Further studies are necessary to apply this technology in a clinical setting. Ultimately this method could be used to expand the pool of donor allografts for patients who need hand transplantation.

Future Directions

The authors will aim to extend survival outcomes, measure function using electromyography, and finalize neural regeneration.

References

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