© Ann Transplant, 2010; 15(3): 35-43

Original Paper

Received: 2010.08.23
Accepted: 2010.09.01
Published: 2010.09.30

Novel portable hypothermic pulsatile perfusion preservation technology: Improved viability and function of rodent and canine kidneys

Gleb P. Tolstykh[1], Jerry F. Gelineau[1], Lisa M. Maier[2], Leonid Bunegin[1]

[1] Department of Anesthesiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas, U.S.A.
[2] Paragonix Technologies, Inc., Massachusetts, U.S.A.

Summary

Background: A reliable, portable, cost effective device for perfusion preservation of donor organs remains elusive. A portable, organ perfusion device design for hypothermic, machine perfusion (HMP) that successfully supported rodent kidneys for 24 hours was evaluated in canine kidneys.

Material/Methods: Freshly recovered rodent and canine kidneys were subjected to 24 hours of HMP or static storage (SS). Organ perfusion and cell membrane integrity were examined in HMP and SS rodent kidneys. Canine kidney function was evaluated in an isolated organ preparation. Oxygen consumption (OC), renal vascular resistance (RVR), and glomerular filtration rates (GFR) were compared.

Results: Perfusion pressure during HMP averaged 16 mmHg with oxygen delivery roughly 4 fold greater than the canine kidney's metabolic requirements. Following 24 hours of preservation, RVR was significantly elevated while OC and GFR were significantly lower in the SS organs compared to the HMP stored or freshly recovered kidneys.

Conclusions: This organ preservation technology appears to provide an excellent preservation environment for kidneys such that post-transplant delayed graft function is minimized. Additionally, compared to current machine perfusion systems, the preservation system described in this work is significantly reduced in size, weight, and complexity, such that total portability may be possible.

Key words: **organ preservation • machine perfusion • canine • rodent • kidney function**

Full-text PDF: http://www.annalsoftransplantation.com/fulltxt.php?ICID=881173

Word count: 3591
Tables: 2
Figures: 5
References: 28

Author's address: Leonid Bunegin, Department of Anesthesiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229, U.S.A., e-mail: bunegin@uthscsa.edu

Background

In the United States alone, the current number of patients waiting for an organ transplant exceeds 106,000. According to the Organ Procurement and Transplantation Network (OPTN), over 85,600 patients are currently registered for a kidney transplant. For many of these patients, transplantation is the only available treatment. Based on previous years' transplantation rates, an estimated 17,000 patients will receive a kidney transplant this year. In order to minimize the kidney shortage dilemma, expanded donor criterion and improved preservation techniques have finally been met with clinical acceptance [1].

The fundamental principles governing *ex vivo* organ preservation require the reduction of metabolism generally through profound hypothermia, delivery of basal metabolic substrates, and removal of metabolic wastes at low perfusion pressures while maintaining ionic, osmotic, and oncotic gradients [2–5]. Hypothermic machine perfusion (HMP) is the perfusion of donor kidneys at low temperatures while providing nutrients to all parts of the organ. Preservation methods based on HMP techniques have solidified the notion that delivery of necessary nutrients by perfusion of cadaveric kidneys is superior to static cold storage (SS) [6–10]. A meta-analysis of 15 comparable human trials, in which cold storage was tested against HMP, provided evidence that HMP of kidneys results in a 20% reduction in delayed graft function following transplantation [9]. The benefits associated with machine perfusion increased with longer preservation periods. Perfusion of kidneys prior to transplantation results in superior graft survival and less frequent return of transplant patients to hemodialysis treatment [11]. More recently, a large international trial of over 330 human transplant kidneys compared SS to HMP [10]. The use of HMP showed benefits over static cold storage as a method of preservation in all donor categories with preservation times less than 24 hours. Specifically, transplantation of machine perfused-organs resulted in 1) improved rates of kidney function and 1-year organ survival, and 2) reduced risk and duration of delayed kidney function and graft failure.

Despite a much reduced metabolic rate during hypothermia, approximately 10% of the organ's metabolism continues with measurable oxygen utilization. Indeed, provision of oxygen, either via direct oxygenation or by oxygen extraction from air-equilibrated perfusates is an important and desired feature of HMP [12,13]. Since irreversible structural damage to kidneys mostly occurs during warm reperfusion, the provision of oxygen is desirable since it facilitates recovery of oxidative phosphorylation and maintenance of energetic homeostasis during preservation [12].

The benefit of active oxygenation is that lower perfusion pressures can be achieved and still provide sufficient flow for fulfilling metabolic demand for oxygen. Porcine kidneys perfused for 20 hours using a system with pulsatile perfusion and oxygenation resulted in better kidney functional recovery following transplantation [13]. Low-flow oxygenated HMP is possible and has been shown to be advantageous in pre-damaged kidneys, improving graft viability after transplantation [14,15]. Short-term oxygenated HMP has been shown to recondition kidneys stored on ice and improve post-ischemic graft function upon warm reperfusion compared to ice-stored or non-oxygenated HMP-perfused kidneys [16].

While significant progress has been made in defining the principles governing tissue preservation and storage prolongation, development of a technology in a configuration that is simple to use, highly portable and cost effective remains lacking [17]. Current perfusion devices rely on conventional pump configurations for circulating the preservation fluid through the organ. In the presented device, energy for circulating the perfusate through the organ is extracted from the controlled expansion of compressed oxygen, thus requiring no electrical power [18].

To date, this technology has been tested in several animal models [18–20]. Experiments with rodent kidneys conclusively demonstrated that after 24 hours of storage using the technology, tissue integrity was maintained at a normal level, and the ability to make urine and clear inulin were within normal levels [20]. Kidneys stored in the conventional manner for a similar time failed to produce urine, could not clear inulin and had substantial areas of dead and deteriorating tissue. This method of perfusion appears to intrinsically limit barotrauma to the organ capillaries, thus minimizing the risk of pressure induced edema. Further, oxygen consumption following preservation was on average 7.8 µl O_2/g/min by HMP preserved kidneys compared to 3.6 µl O_2/g/min by statically stored kidneys [20]. Furthermore, the technology has successfully supported canine hearts for up to 14 hours [19]. Graft function

was immediate following transplantation and was comparable to pre-recovery levels [19].

The objective of this work was to examine flow distribution and cell membrane integrity following 24 hours of HMP in rodent kidneys and to evaluate canine kidney function following 24 hours of HMP using this new technology. During 24 hours of preservation, renal vascular resistance (RVR) and oxygen consumption (OC) were monitored. Following preservation, functional assessments of HMP, SS, and freshly recovered kidneys were made.

Material and Methods

Organ preservation device description

The organ preservation device consists of 4 parts; a fluid filled container which functions as the organ chamber, an interface plate having a return-flow check valve, an outflow check valve to which the organ is attached, an oxygen permeable membrane, and a lid, which combine to form three stacked hermetically sealed chambers (Figure 1). The recovered organ is attached to the central outflow check valve on the interface plate via the arterial vessel and both are lowered onto the lip of the container previously filled with preservation solution. This forms the organ storage compartment. Additional fluid is added to fill the interface plate to its rim. An oxygen permeable membrane is then placed onto the interface plate, forming the perfusion compartment. A domed lid secures the membrane, forming a gas filled cavity which functions as the pumping and gas exchange compartment. A fluidics device functions as a pump actuator. The fluidics actuator operates at low gas flow and pressure and can be configured for pulsatile operation with output pressure limitation. Temperature is maintained between 4–7°C within a Styrofoam chest by cryo-gel cooling packs.

During operation, the upper chamber is pressurized with 100% oxygen resulting in the expansion of the oxygen permeable membrane. Preservation solution is simultaneously oxygenated and forced through the central check valve into the attached organ. After perfusion through the organ, the preservation fluid exits through the organ's vein into the organ storage chamber until the pressure in the organ storage chamber equilibrates with the pressure in the upper chamber. A feedback loop from the upper chamber to the fluidics actuator interrupts pressurization

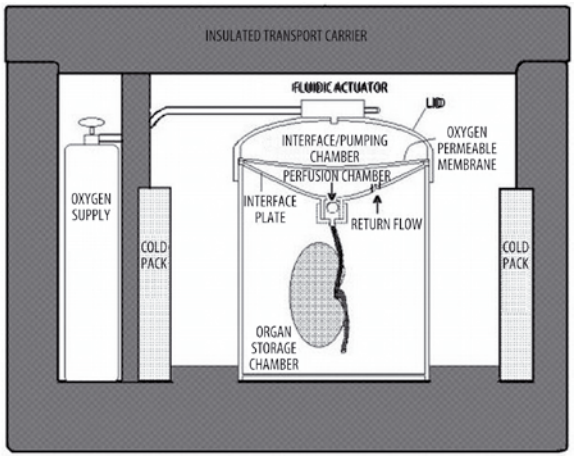

Figure 1. Schematic of the Organ Preservation Device.

allowing the chamber to be vented. The resulting pressure differential between the upper and organ storage chambers forces fluid in the storage compartment up through the return check valve for removal of CO_2 and the start of the next cycle.

Kidney recovery protocol

The experimental protocol was reviewed and approved by the Institutional Animal Care and Usage Committee. Animal usage complied with the "Principles of Laboratory Animal Care" (NIH publication No. 86-23.) In separate experiments, adult Sprauge-Dawley albino rats weighing about 350 to 500 grams and adult canines weighing 25 to 30 Kg and were anesthetized with 25 ml/kg of Sodium Pentobarbital by an intravenous injection. Experimental animals were intubated and ventilation with 40% oxygen to maintain normal arterial blood oxygenation. Animals were then placed in a supine position and a midline incision was made in the lower abdominal cavity so that both kidneys were exposed. Following heparinization, catheters were inserted into the descending aorta above, and the inferior vena cava just below the kidneys. The aorta and inferior vena cava were crossed clamped above and below the catheters and an infusion of cold University of Wisconsin Solution (UWS) at 4°C was initiated. Infusions continued until all blood was cleared from the organs. During infusion, cold saline, at 4°C, was poured over the kidneys and the excess removed by suction. The aorta and inferior vena cava were ligated at the cross clamp then cut, as were the ureters. The kidneys were quickly dissected free and placed on ice for catheterization of the ureters. Rodent ureters were catheterized with a 3/4 inch 24 gage catheters and canine ureters were catheterized with a 2 inch, 18 gage catheters.

Preservation protocol

Perfusion storage

Rodent kidneys (n=6) were attached via the aortic catheter to the organ preservation device modified to support rodent organs and immersed into cold (4°C) freshly prepared University of Wisconsin Solution. Canine kidneys (n=4) were similarly attached to the organ preservation device via the renal artery cannula. The fluidics actuator was pressurized with 100% O_2 at 2.5 to 3.0 psi and adjusted to perfuse at 70 pulses/min. The preservation device was placed into an insulated transport case into which 3 cold packs (Rubbermaid Blue Ice bricks 7.0"1.5"×6.75" stored at –20°C for 12 hours) had been previously placed. Temperature, perfusion pressure were continuously monitored. The pO_2 of the preservation solution flowing into and out of the organ and perfusion flow were measured at 15 minute intervals. Oxygen consumption was calculated by; (1) multiplying the difference between the pO_2 of the delivered UWS and the pO_2 of the organ outflow by 0.00006 the oxygen solubility factor per ml of solution at 4 degrees centigrade, (2) multiplying the result by the organ perfusion in ml/min. Renal vascular resistance was calculated as the quotient of the perfusion pressure and flow.

Static storage

Rodent (n=6) and canine (n=4) kidneys were prepared identically as for the perfusion preservation except that no pressurization of the fluidic actuator was initiated resulting in no perfusion or oxygenation during the preservation period. Only temperature was continuously monitored.

Rodent organ perfusion and viability protocol

In order to evaluate the extent of perfusion (n=3), 3ml of 2% Evans Blue dye was injected into the storage medium prior to termination of preservation and permitted to circulate through a pair of kidneys for 15 minutes. Kidneys were removed and sectioned to examine the extent of staining.

In another group of rodent kidneys (n=3), tissue viability was evaluated by the method of Patel et. al. [22]. HMP stored kidneys, and kidneys stored in a 4°C UWS for 24 hours without perfusion were injected, via the aortic catheter, with fluorescence dye probes containing fluorescein diacetate (FDA) and ethidium bromide (EtBr), each

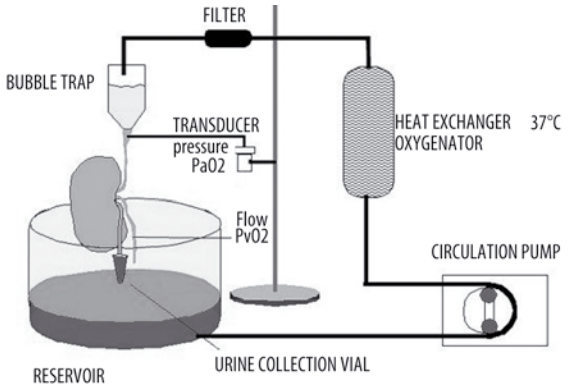

Figure 2. Schematic of the isolated organ setup for measuring organ function.

at 2×10^{-5} g/ml. Following a 5 minute equilibration period, organs were flushed with cold normal saline to remove the dye probes remaining in the vascular and interstitial spaces. Kidneys were sectioned longitudinally to expose the cortex and medulla and examined under 440 nm light.

Organ function measurement protocol

Following the preservation period, canine kidneys were removed from the preservation device and connected to a Langendorff device, Figure 2. Freshly recovered canine kidneys (n=4) were similarly attached. Canine kidneys were perfused with a 50:50 mixture of warm (37°C) oxygenated (100% O_2) K-H solution containing inulin (15 mg/100 ml) and autologous blood. Perfusion was initiated slowly and incremented at 5 minute intervals until a mean arterial pressure of 150 mmHg was achieved (Table 1). Urine, arterial and venous samples were collected from each kidney after 90 minutes in triplicate for inulin clearance and urine output measurement. Inulin was measured using the method of Waser as modified by Brown and Nolph [21]. The partial pressure of oxygen in the blood/K-H perfusate entering the renal arteries and exiting the renal veins was measured on a TruPoint Irma™ blood gas machine. Organ perfusion was measured by collecting the outflow from the renal veins during a 15 second time interval and corrected to flow/minute. Renal vascular resistance was calculated by dividing the perfusion pressure measured at the renal artery by the renal vein outflow in ml/min. Glomerular Filtration Rate was calculated as the product of the urine inulin concentration and urine flow divided by the arterial plasma inulin concentration [3];

Table 1. Comparisons between freshly recovered, perfusion preserved, and cold ischemically stored canine kidneys in an isolated organ preparation.

Parameter	Control (freshly recovered)	Perfusion Preservation (24 hrs)	Cold Ischemic Preservation (24 hrs)
Kidney Wt. (g)	56.2±2.6	55.3±8.3	53.4±10.7
Temp (°C)	37.0	37.0	37.0
Perfusion Pressure (mmHg)	150	150	150

Table 2. Preservation data for rodent and canine kidneys.

Parameter	Rodent		Canine	
	HMP	SS	HMP	SS
Temp (°C)	7.3±0.7	7.7±0.3	5.0±0.2	4.5±0.4
Perfusion Pressure (mmHg)	5.83±3.30	-------	16.0±12.8	-------
UWS Flow (ml/min)	5.32±0.60	-------	37.8±9.8	-------
Delivery O_2 (ml/min)	0.11±0.04	-------	1.2±0.3	-------
V O_2 (ml/min)	0.02±0.01	--------	0.29±0.1	--------
RVR (mHg/ml/min)	1.13±0.64	--------	0.43±0.53	--------

$$GFR = \frac{U_{in} * V_{ur}}{P_{in}}$$

where: $\mathbf{U_{in}}$ is the inulin concentration in the urine (mg/ml)

$\mathbf{V_{ur}}$ is the urine flow (ml/min)

$\mathbf{P_{in}}$ is the inulin concentration in the plasma (mg/ml)

Statistical analysis

Group means were compared using analysis of variance for repeated measures, followed by Newman-Keules post-test. Critical values for rejecting the Null Hypothesis were at $p<0.05$. Data are represented as means ± standard deviations.

Results

Rodent kidneys

During HMP, pO_2 of the UWS perfusing the kidneys increased to 280 mmHg by one hour then continued to rise reaching 325 mmHg within 5 hours and 350 mmHg by 24 hours. Organ temperature during perfusion preservation averaged 7.3±0.7°C. The pO_2 of the perfusate exiting the organ rose to 250 mmHg and essentially stayed the same for the entire 24 hour perfusion period. Oxygen delivery to the HMP kidney was more than 5 fold greater than calculated metabolic need at the storage temperature. During cold static storage organ temperature averaged 7.7±1.3°C. Organ temperatures were not statistically different. Preservation data is summarized in Table 2.

Figure 3 shows the extent of Evans Blue staining in HMP stored rodent kidneys compared to a freshly recovered reference organ. All parts of the HMP stored organs were stained blue with the most intense staining in the cortex followed by the medulla, with the papilla staining the least. Fluorescence probe analysis resulted in HMP kidneys fluorescing green while SS kidneys had a mottled red fluorescence pattern that was most intense in the medullular and papillar structures. (Figure 4).

Canine kidneys

During HMP, pO_2 of the UWS perfusing the kidneys increased to 275 mmHg by one hour then continued to rise steadily leveling off at 524 mmHg by 12 hours. The pO_2 of the perfusate exiting the organs rose to 224 mmHg and essentially stayed the same for the entire 24 hour perfusion period. Oxygen delivery was greater than 4 fold the calculated oxygen requirement at the preservation temperature. Organ temperature during

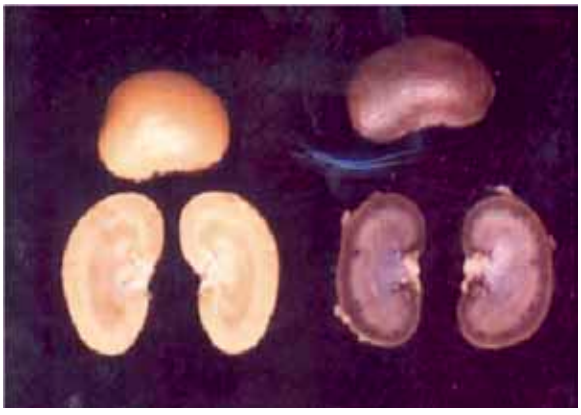

Figure 3. Right organ depicts Evans Blue staining of rodent kidney indicating the distribution of preservation solution during HMP. Left organ depicts an unstained freshly recovered reference kidney.

HMP averaged 5.0±0.2°C. During SS organ temperature averaged 4.5±0.4°C. Organ temperatures were not statistically different. Observations made during HMP and SS are summarized in Table 2.

Temperature during function measurements on the Langendorff was 37.0±0.1°C for all kidneys. Perfusion pressure for all kidneys was set at 150 mmHg (Table 1). Renal vascular resistance for freshly recovered kidneys was 2.8±0.4 mmHg/ml/min, 3.4±0.1 mmHg/ml/min for HMP preserved kidneys, and 5.4±0.4 mmHg/ml/min for SS kidneys. RVR differences between the freshly recovered and HMP kidneys were not statistically significant, while SS kidneys demonstrated a statistically higher RVR ($p<0.05$) (Figure 5).

Oxygen consumption during function testing by freshly recovered kidneys was 5.5±0.4 ml O_2/min, 3.7±0.6 ml O_2/min by HMP preserved kidneys, and 2.1±0.3 ml O_2/min by SS kidneys. GFR was 14.3±4.6 ml/g/min in the fleshly recovered kidneys. In HMP preserved organs, GFR was 18.4±4.3 ml/min and 7.4±1.8 ml/min in SS organs. There was a statistical difference ($p<0.05$) noted between freshly recovered and HMP kidneys in oxygen consumption but no significant differences in GFR. However, SS kidneys showed a significantly lower ($p<0.05$) oxygen consumption and GFR than either freshly recovered or HMP preservation groups (Figure 5).

Discussion

The organ preservation device presented in this work could represent a transformational shift in machine perfusion technology when the technology is applied in clinical medicine. The device harnesses the energy resulting from the

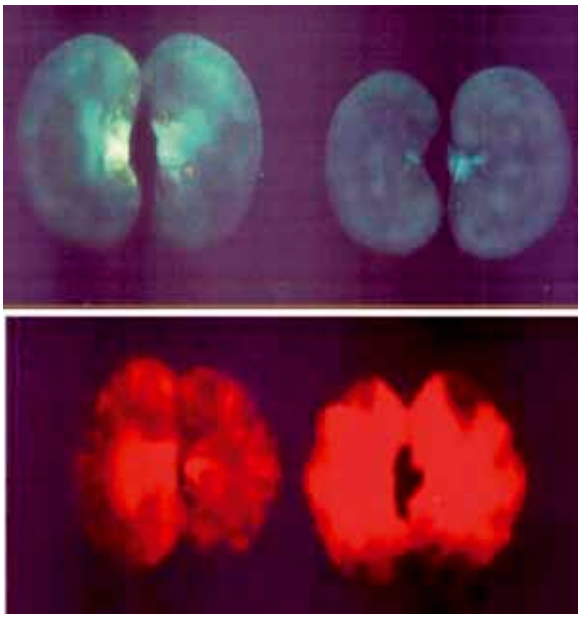

Figure 4. Membrane integrity as demonstrated by FDA/EtBr fluorescence probe. Green fluorescence (upper panel kidneys following 24 hours of HMP) indicates continued membrane integrity, red fluorescence (lower panel kidneys following 24 hours of SS) indicates membrane integrity failure.

expansion of compressed oxygen to drive preservation solution through the organ and simultaneously oxygenate the perfusate. Oxygenated perfusate allows for lower perfusion flows reducing perfusion pressure subsequently minimizing edema and pressure induced injury to the vascular endothelium. Hypothermia can easily be maintained between 4 and 7°C for as long as 24 hours with appropriately engineered insulation and current cold pack technology. Oxygenated perfusate offers yet another advantage, in that, sufficient oxygen can be delivered to support the organ's metabolism at temperatures somewhat higher than 4 °C, reducing the possibility of cold related injury to the organ tissue [23–26].

The perfusion device appears to have provided adequate perfusion flow to all parts of the rodent kidney in the proportions expected during normal perfusion. The most intense staining was seen in the cortex which normally receives the greatest portion of blood flow. Less staining was noted in the medulla, and the papilla. This observation indicates that sufficient perfusate flow can be delivered to all parts of the organ to support metabolic oxygen and substrate needs, as well as, waste product removal.

The test used to determine kidney cell viability is based on the loss of selective permeability

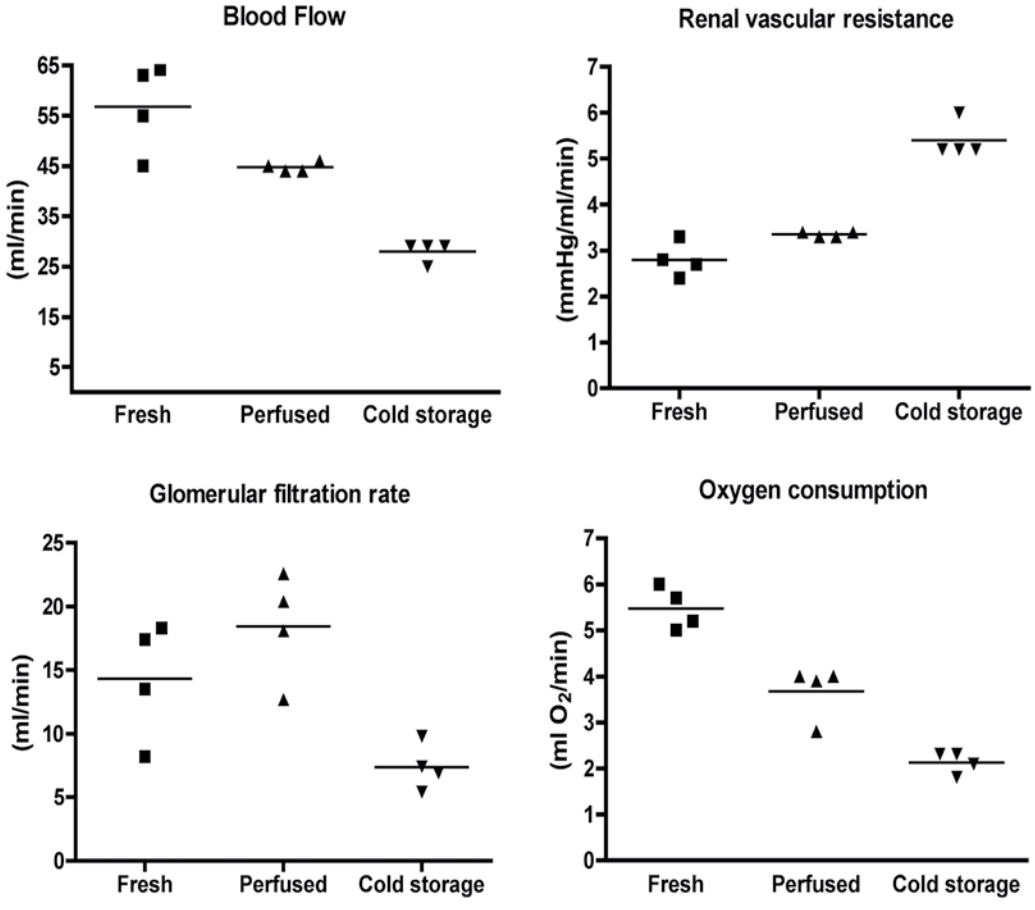

Figure 5. Comparisons between freshly recovered, perfusion preserved, and cold ischemically stored canine kidneys in an isolated organ preparation.

in the membrane of the dead/dying cells [22]. Ethidium Bromide quickly passes through the disrupted permeability barriers and intercalates between adjacent nucleotide base pairs in the DNA. The resulting EtBr-DNA complex fluoresces red when excited with 440–460 nm wavelength light indicating cell membrane damage. In viable cells where the membrane is intact, the lipid insoluble EtBr is unable to penetrate the cell. Fluoresceine Diacetate is lipid soluble and freely penetrates the intact cell membrane. FDA is immediately hydrolyzed by non-specific esterases into fluoresceine which fluoresces green under 440–460 nm light. Green fluorescence, therefore, implies intact cellular membranes and cell viability. In the 24 hour perfusion preservation kidneys, green fluorescence was observed across the entire cross-section of the organ indicating little or no cellular membrane damage. These organs apparently suffered minimal hypoxic injury during the preservation period. By contrast, kidneys stored by static hypothermia fluoresced mostly red. While portions of the cortex may have been spared, presumably due to the availability of oxygen via direct diffusion from the storage medium, deterioration in the medulla, papilla, and pelvis appears to have been substantial, possibly a consequence of longer diffusional distances.

In order to maintain tissue viability, it is essential that the organ receives sufficient oxygen to support metabolism, and perfusion at low enough pressure so as to minimize edema formation and disruption of the endothelial lining of the capillaries. Hypothermia effectively lowers oxygen requirement allowing the fluidics based preservation device to supply 4 to 5 times the organs metabolic oxygen requirement at the storage temperature. Also perfusion pressures were well below 25 mmHg, the pressure previously shown to be the critical limit for edema formation.

Functional evaluation of canine kidneys on the Langendorff apparatus showed both freshly recovered and HMP organs to have lower RVRs than SS organs. Both oxygen consumption and GRF in SS organs were significantly reduced and were consistent with reduced organ viability.

Advances in organ transplantation can be attributed to significant developments in surgical techniques, immunosuppressive agents, and to a limited extent, improved organ preservation solutions. While the limiting factor to expansion of organ transplantation still remains the organ donor rate, the methods used for preserving ischemia intolerant organs significantly influences the availability of good quality organs. Organs, such kidneys, that are tolerant of ischemia, can be transported readily using current cold preservation methods. In these organs, however, the potential for post-transplant PNF and DGF are significantly higher than in organs oxygenated and hypothermically perfused prior to transplantation. Perfusing organs in an attempt to recover ischemic insults sustained during transport can require as much as 24 hrs along with significant personnel and resources. The ability to perform HMP during transport potentially reduces or eliminates the development of ischemic injury resulting in an organ that is in an improved condition. Extended preservation time can confer several additional benefits. Firstly, it allows for better tissue matching between donor and recipient; secondly, it provides more time for preparation of the surgical team and the patient, thirdly, the organ can be reallocated to another patient in the event of problems or complications, and finally, HMP-perfused organs translate into a lower overall transplant-related health care costs. Analyses of the long term medical and economical benefits of HMP kidney storage in comparison to cold storage showed that HMP of kidneys was a cost-saving method of kidney preservation [11], resulting in lower hospitalization costs than the utilization of static cold storage of kidneys [27].

The use of HMP could significantly expand the donor pool with 'expanded criteria donors' and 'non-heart-beating donors'. With the inclusion of these types of donors (made possible by using machine perfusion), as well as improvements in the quality of heart-beating donor kidneys, the number of transplantable kidneys could be increased by as much as by 40% with a reduction of delayed graft function by 35%. These changes could potentially lead to an estimated annual savings to the U.S. healthcare system of greater than $1.5 billion [28].

Implementation of HMP techniques have not been readily adopted mostly due to the lack of portable, easy-to-use, low cost donor organ perfusion technology. Current systems utilize pumps for effecting circulation through the organ. This implies the need for electrical power which in turn necessitates battery packs inherently limiting operation time, as well as imposing significant weight to the system. Hypothermia, in these devices, is maintained with crushed ice, placing limitations on the duration of effective hypothermia and imposing still more weight. Oxygenation is also not practical, in that, the inclusion of gas exchangers and gas cylinders further exacerbates the weight and bulk situation. In order to providing enough oxygen to the perfused organ in devices that perfuse with non-oxygenated preservation solution, perfusion flows on the order of 100 ml/g/min or more may be necessary resulting in a perfusion pressure that often exceeds 30 mmHg, increasing the potential for significant edema development and barotrauma.

Conclusion

In summary, the presented technology addresses three main components that make HMP a superior preservation technique compared to static cold storage. **First**, using HMP, waste product removal and blood washout are achieved by prolonged perfusion. **Second**, oxygen is supplied to the organ to support the level of metabolism at hypothermia. **Third**, low pressures are used for perfusion, minimizing the potential for endothelial damage. **Fourth**, the system is small and highly portable. Taken together, this technology may play a direct role in significantly impacting the supply of transplantable organs and the cost of transplantation.

Acknowledgement

The authors wish to thank Shirley Caballero for her invaluable assistance in the conduct of the experimentation and collection of the data.

References:

1. Tuttle-Newhall JE, Krishnan SM et al: Organ donation and utilization in the United States: 1998–2007. Am J Transplant, 2009; 9: 879–93
2. Southard JH, Belzer FO: Organ preservation. Annu Rev Med, 1995; 46: 235–47

3. D'Alessandro AM, Southard JH, Love RB, Belzer FO: Organ preservation. Surg Clin North Am, 1994; 74: 1083–95
4. Kumano K, Wang W, Endo T: [Effects of osmotic agents for cold kidney preservation]. Nippon Hinyokika Gakkai Zasshi, 1994; 85: 925–31
5. Collins GM, Green RD, Halasz NA: Importance of anion content and osmolarity in flush solutions for 48 to 72 hr hypothermic kidney storage. Cryobiology, 1979; 16: 217–20
6. Hoffmann RM, Stratta RJ, D'Alessandro AM et al: Combined cold storage-perfusion preservation with a new synthetic perfusate. Transplantation, 1989; 47: 32–37
7. Wight J, Chilcott J, Holmes M, Brewer N: The clinical and cost-effectiveness of pulsatile machine perfusion versus cold storage of kidneys for transplantation retrieved from heart-beating and non-heart-beating donors. Health Technol Assess, 2003; 7: 1–94
8. Schold JD, Kaplan B, Howard RJ et al: Are we frozen in time? Analysis of the utilization and efficacy of pulsatile perfusion in renal transplantation. Am J Transplant, 2005; 5: 1681–88
9. Wight JP, Chilcott JB, Holmes MW, Brewer N: Pulsatile machine perfusion vs. cold storage of kidneys for transplantation: a rapid and systematic review. Clin Transplant, 2003; 17: 293–307
10. Moers C, Smits JM, Maathuis MH et al: Machine perfusion or cold storage in deceased-donor kidney transplantation. N Engl J Med, 2009; 360: 7–19
11. Wszola M, Kwiatkowski A, Latek M et al: Long term medical and economical benefit of machine perfusion (MP) kidney storage in comparison to cold storage (CS). Ann Transplant, 2009; 14: 24–29
12. Fuller BJ, Lee CY: Hypothermic perfusion preservation: the future of organ preservation revisited? Cryobiology, 2007; 54: 129–45
13. Maathuis MH, Manekeller S, van der Plaats A et al: Improved kidney graft function after preservation using a novel hypothermic machine perfusion device. Ann Surg, 2007; 246: 982–88; discussion 89–91
14. Minor T, Sitzia M, Dombrowski F: Kidney transplantation from non-heart-beating donors after oxygenated low-flow machine perfusion preservation with histidine-tryptophan-ketoglutarate solution. Transpl Int, 2005; 17: 707–12
15. Manekeller S, Leuvenink H, Sitzia M, Minor T: Oxygenated machine perfusion preservation of predamaged kidneys with HTK and Belzer machine perfusion solution: an experimental study in pigs. Transplant Proc, 2005; 37: 3274–75
16. Koetting M, Frotscher C, Minor T: Hypothermic reconditioning after cold storage improves postischemic graft function in isolated porcine kidneys. Transpl Int, 2010; 23: 538–42
17. Pirenne J: Time to think out of the (ice) box. Curr Opin Organ Transplant, 2010; 15: 147–49
18. Bunegin L, Gelineau JF: The application of fluidics technology for organ preservation. Biomed Instrum Technol, 2004; 38: 155–64
19. Calhoon JH, Bunegin L, Gelineau JF et al: Twelve-hour canine heart preservation with a simple, portable hypothermic organ perfusion device. Ann Thorac Surg, 1996; 62: 91–93
20. Tolstykh GP, Gelineau JF, Bunegin L: Perfusion preservation of rodent kidneys in a portable preservation device based on fluidics technology. Transplantation, 2002; 73: 1508–10
21. Brown P, Nolph KD: Chemical measurements of inulin concentrations in peritoneal dialysis solution. Clin Chim Acta, 1977; 76: 103–12
22. Patel BC, Courtney JM, Evans JH, Paul JP: Biocompatibility assessment: application of fluorescent probe response (FPR) technique. Biomaterials, 1991; 12: 722–26
23. Goujon JM, Vandewalle A, Baumert H et al: Influence of cold-storage conditions on renal function of autotransplanted large pig kidneys. Kidney Int, 2000; 58: 838–50
24. Hauet T, Goujon JM, Tallineau C et al: Early evaluation of renal reperfusion injury after prolonged cold storage using proton nuclear magnetic resonance spectroscopy. Br J Surg, 1999; 86: 1401–9
25. Brenner P, Hinz M, Huber H et al: Effects of prolonged cold storage time in xenotransplantation. J Heart Lung Transplant, 1999; 18: 1211–17
26. Massberg S, Gonzalez AP, Leiderer R et al: *In vivo* assessment of the influence of cold preservation time on microvascular reperfusion injury after experimental small bowel transplantation. Br J Surg, 1998; 85: 127–33
27. Buchanan PM, Lentine KL, Burroughs TE et al: Association of lower costs of pulsatile machine perfusion in renal transplantation from expanded criteria donors. Am J Transplant, 2008; 8: 2391–401
28. Taylor MJ, Baicu SC: Current state of hypothermic machine perfusion preservation of organs: The clinical perspective. Cryobiology, 2010; 60(3S): S20–35