

# A novel extracorporeal kidney perfusion system: a concept model

Michael Szajer, Gaurang Shah, Dilip Kittur, Bruce Searles, Lu Li, David Bruch and Edward Darling

Department of Cardiovascular Perfusion, Department of Surgery, State University of New York Upstate Medical University, Syracuse, NY, USA

The number of patients awaiting kidney transplantation has more than doubled in the past decade while the number of available donor organs has seen only a modest increase, leading to a critical shortage of organs. In response to this extreme shortage, the criteria for accepting organs have been modified to include marginal donors such as non-heart beating donors (NHBD). In these kidneys, determining viability is important for success of transplantation. Therefore, a study was undertaken to develop a system that would allow the extracorporeal assessment of function and compatibility of the donor organ before the patient is exposed to the risks associated with surgery.

Following bilateral nephrectomy, the kidneys of 10 pigs (~30 kg) were connected to a commercially available hypothermic pulsatile kidney perfusion apparatus. This system was modified to allow for normothermic pulsatile

renal perfusion using the potential recipient's blood, via vascular access. These kidneys were perfused with the animal's blood for a minimum of two hours while various parameters were monitored. Perfusion pressures were kept between 60 and 90 mmHg, which correlated to flows between 70 and 150 mL/min. A decrease in perfusion pressure with a concomitant rise in flow over the two-hour period served as a good predictor of a viable and compatible graft. The modified kidney preservation system allows the normothermic, pulsatile extracorporeal perfusion of donor kidneys with the ability to monitor resistance to flow and urine production. This model also allows observation of the kidney for signs of hyperacute rejection. Further research needs to be conducted in order to determine if the system represents a methodology to increase the pool of available donor organs. *Perfusion* (2004) 19, 305–310.

## Introduction

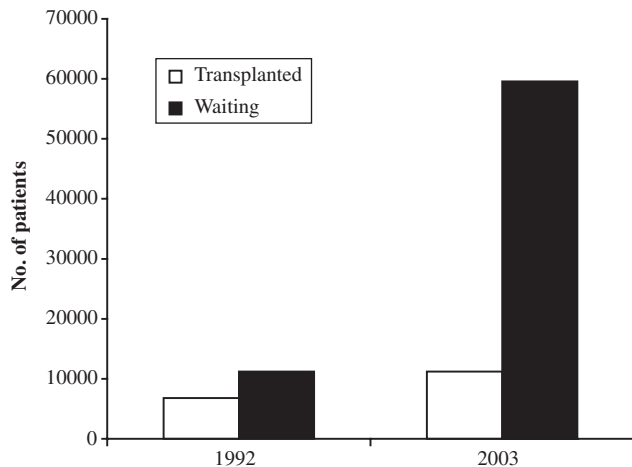
Kidney transplantation, the most frequent whole-organ transplantation worldwide, has currently been established as routine treatment for end-stage renal disease.<sup>1</sup> From the years 1992 to 2003, the number of patients awaiting kidney transplantation increased more than 250%. During the same time period, however, the number of available donor kidneys saw only a relatively modest increase, creating a severe shortage of transplantable organs (Figure 1). Organ shortage continues to represent a significant problem in transplantation. At present, in the USA, there are more than 88 000 patients waiting for transplantation. Out of these 88 000 patients, 56 625 patients (65%) are waiting for kidney transplantation (UNOS Database). Consequently, there is an urgent need for new innovative strategies to increase the number of successful transplants that can be performed using available kidneys.

This critical shortage of organs has resulted in the death of over 3500 patients in the year 2002 alone.<sup>2</sup> Despite this desperate need for organs, not all donated kidneys are used. According to United Network for Organ Sharing (UNOS), in 1998, 10.1% of initially accepted kidneys were unfortunately discarded. The most common reasons for the refusal of kidneys offered for transplantation were questions of quality, size/weight of the organ, the donor's age, positive serologic tests for infections, elevated creatinine levels and abnormal biopsy findings.<sup>1</sup>

Strategies to increase the supply of kidney grafts are being investigated. Particularly, there is interest in enlarging the pool by modifying existing criteria for acceptance, and by finding ways to reduce the number of wasted donor organs. Sources such as marginal cadaver donor, non-heart beating donors (NHBD), and suboptimal histocompatibility matches are being considered for possible transplantation. These organs pose the threat of delayed graft function requiring temporary hemodialysis treatments

Address for correspondence: Edward Darling, Department of Cardiovascular Perfusion, 750 East Adams St, Syracuse, NY 13210, USA.  
E-mail: darlinge@upstate.edu

Presented at the 2003 Boston Meeting, Boston, Massachusetts, 10–13 October 2003 and the Annual Seminar of the American Academy of Cardiovascular Perfusion, San Antonio, Texas 23–26 January 2004.



**Figure 1** The number of patients on the kidney transplant waiting list and the number of transplants performed in the USA in 1992 and 2003. Data are from the registry of the UNOS.

and decreased long-term survival of the graft due to numerous factors, including warm-ischemic injury, reperfusion injury following prolonged cold ischemic storage and hyperacute rejection. The difficulty in predicting the viability and/or tissue match of these kidneys has inhibited their widespread use.<sup>3-7</sup>

A system that would allow evaluation of marginal kidneys could help alleviate the shortage by establishing the viability of some kidneys that would normally be considered too risky and discarded. It is hypothesized that kidneys of questionable viability could be evaluated extracorporeally by interfacing the donated kidney with the potential recipient. Perfusing the kidney in this manner could allow for both visual inspection of the organ, and also assessment of various parameters of flow and resistance over time.

Specialized kidney preservation perfusion apparatus are commercially available which promote organ preservation through pulsatile flow of cold oxygenated crystalloid solution through the kidney during the transport phase. In this paper, we report the adaptation of one of these devices to allow the interface of patient and donor organ for extracorporeal assessment of function. In this model, kidney assessment of both viability and compatibility could be accomplished before either discarding the kidney or making the commitment to high risk transplant surgery.

## Methods

### Animal preparation

The protocol was approved by the Committee for the Humane Use of Animals at SUNY-Upstate

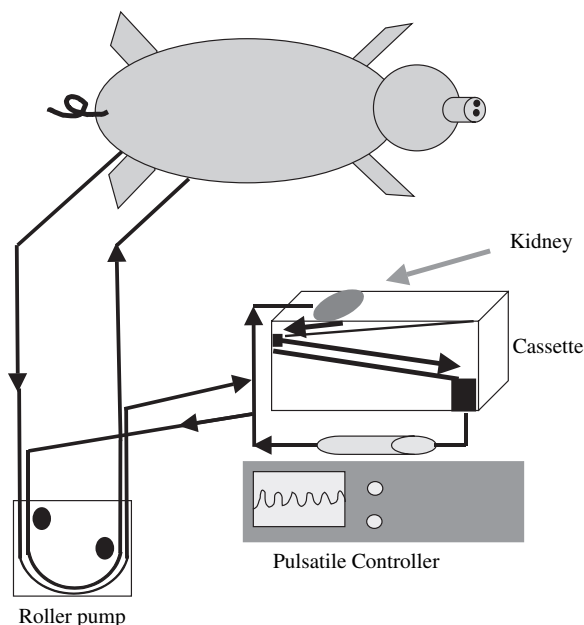
Medical University. All animals received care in compliance with the 'Guide for the Care and Use of Laboratory Animals' (NIH Publication 85-23, revised 1985).

Ten healthy, Yorkshire pigs (~30 kg) were anesthetized and instrumented as previously described.<sup>8</sup> Briefly, anesthesia was induced with intravenous (IV) sodium pentobarbital (50 mg/kg) and intubation was performed. Animals were ventilated using a Galileo ventilator (Hamilton Medical, Reno, NV). Continuous anesthesia with sodium pentobarbital (6 mg/kg/min) was delivered using a Harvard pump (Model 907, Harvard Apparatus, Mills, MA), while bolus infusions of pancuronium bromide were given to maintain paralysis. Electrocardiogram (ECG) monitoring was performed using a pacemaker/defibrillator system (Zoll Medical, Burlington, MA). A right carotid artery cutdown was performed and a 2-mm catheter placed to measure systemic artery pressure and for acquisition of arterial blood gas samples. A 7.5 French dual-lumen catheter was placed into the adjacent internal jugular vein for maintenance of IV fluids. Pressure was measured using Argon transducers (Model 049-992-00A, CB Sciences Inc., Dover, NH) leveled at the right atrium and recorded using a 16-channel PowerLab/16 s (AD Instruments Pty Ltd., Milford, MA) interfaced with a Dell Dimensions XPS R400 computer (Dell Inc., Dallas, TX).

Once instrumented, each pig underwent bilateral nephrectomy and the kidneys were flushed with 200–300 mL University of Wisconsin (UW) solution (Barr Laboratories Inc, Ponomo, NY) until the effluent was clear, followed by 15 K units of superoxide dismutase (SOD) (Sigma, St Louis, MO). Then the renal artery was cannulated (15 Fr blunt tip needle) and the explanted kidneys were connected to the extracorporeal circuit.

### Perfusion system

Figure 2 shows a schematic representation of the animal–extracorporeal system interface. The extracorporeal circuit consists of a standard roller pump with double-track tubing inserts and the RM3 Renal Preservation System with the 'MOX' 100 DCM Disposable Cassette (Waters Instruments, Inc., Rochester, MN) (Figure 3). To provide access to the animal, the cassette was modified by inserting two 1/4" × 1/4" luer connectors in the tubing between the heat exchanger and the bubble trap (Figure 3). A 3/16" ID PVC tubing served as the arterial blood inflow and was incorporated into circuit by passing through a roller pump and into the luer connection. Another 3/16" ID PVC tubing



**Figure 2** Schematic overview of the extracorporeal circuit. The roller pump provides continuous inflow of arterial blood from the animal while simultaneously returning an equal amount of blood from the circuit back into the animal.

was connected to the remaining luer connection and passed through the same roller pump back to the venous circulation of the animal (Figure 4). This arrangement established a zero-balance circulation (flow of 50–90 mL/min) supplying the pulsatile renal perfusion system with a continuous supply of oxygenated blood from the animal and returning an exactly equal volume of venous effluent from the organ to the animal's venous system.

The entire system was primed with 1 L lactated Ringer's crystalloid solution, 12.5 g mannitol, 25 mEq sodium bicarbonate, 5000 units heparin and warmed to 37°C. Following systemic anticoagulation (150 units/kg) with an activated clotting time (ACT) of >250 s, the arterial inflow line of the circuit was connected to a 10 Fr cannula in the femoral artery and the venous return line of the circuit to a 10 Fr cannula in the femoral vein. While the kidney was being prepared for extracorporeal circulation, blood was introduced into the circuit by actuating the RM3 pump and increasing flow, followed by establishing roller pump flow. Once blood was recirculated throughout the system, the pumps were stopped and the kidney was placed in the cassette, the renal artery cannulated and perfusion initiated (Figure 5).

### Perfusion management

Two DLP 6000 pressure monitors (Medtronic, Inc., Minn, MN) were used to measure negative line

pressure of the inflow (–5 to –30 mmHg) and positive line pressure of the outflow (15–30 mmHg). The target parameters were pressures of 60–90 mmHg, and flows of 100–150 mL/min. The kidney perfusion time was two hours. Replacement volume (lactated Ringer's) was added to the circuit as needed to maintain an adequate level in the cassette. ACTs were maintained at >250 s.

### Analysis

The animal's vital signs (pulse, blood pressure, respiration, oxygen saturation) were monitored continually throughout the procedure. The extracorporeal circuit was monitored for clot formation during the procedure. The kidney was monitored for urine output, macroscopic appearance (color and consistency) and a biopsy of the kidney at the one and two hours time-points was performed.

### Results

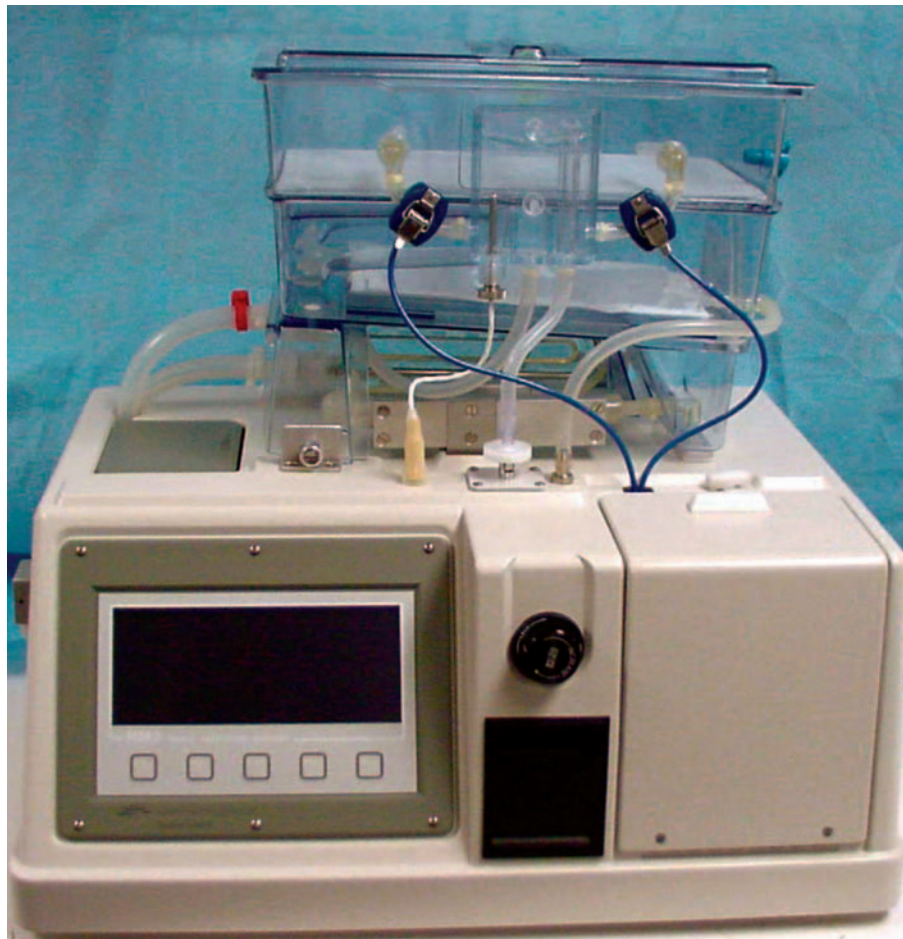
There was no evidence of a physiological reaction with the initiation of extracorporeal flow and the vital signs of each of the 10 pigs remained stable during the course of extracorporeal kidney perfusion.

There were no technical complications regarding the modification of the cassette. The roller-pump exchange system easily transferred oxygenated blood into the extracorporeal circuit and removed venous blood back to the animal and provided a static isovolemic environment. The RM3 maintained pulsatile flow to the kidney with the roller-pump exchange system operating. In one perfusion experiment, there was evidence of fibrin and clot in the collecting chamber of the cassette.

The perfused kidneys produced variable amounts of urine during the two-hour extracorporeal circulation with an average of 30 cc/hour. Kidney color remained pink, consistency remained firm, and biopsies at both one-hour and two-hour time-points showed no gross evidence of cellular damage.

### Discussion

Machine preservation via perfusion with hypothermic crystalloid solutions has been shown to improve the function of kidneys that have sustained warm ischemic damage and also to extend the period of safe preservation.<sup>6</sup> With minor modification, these devices may have a further



**Figure 3** A commercially available kidney preservation system. The cassette (top) houses the kidneys while the controller (bottom) provides pulsatile flow and resistance measurements.

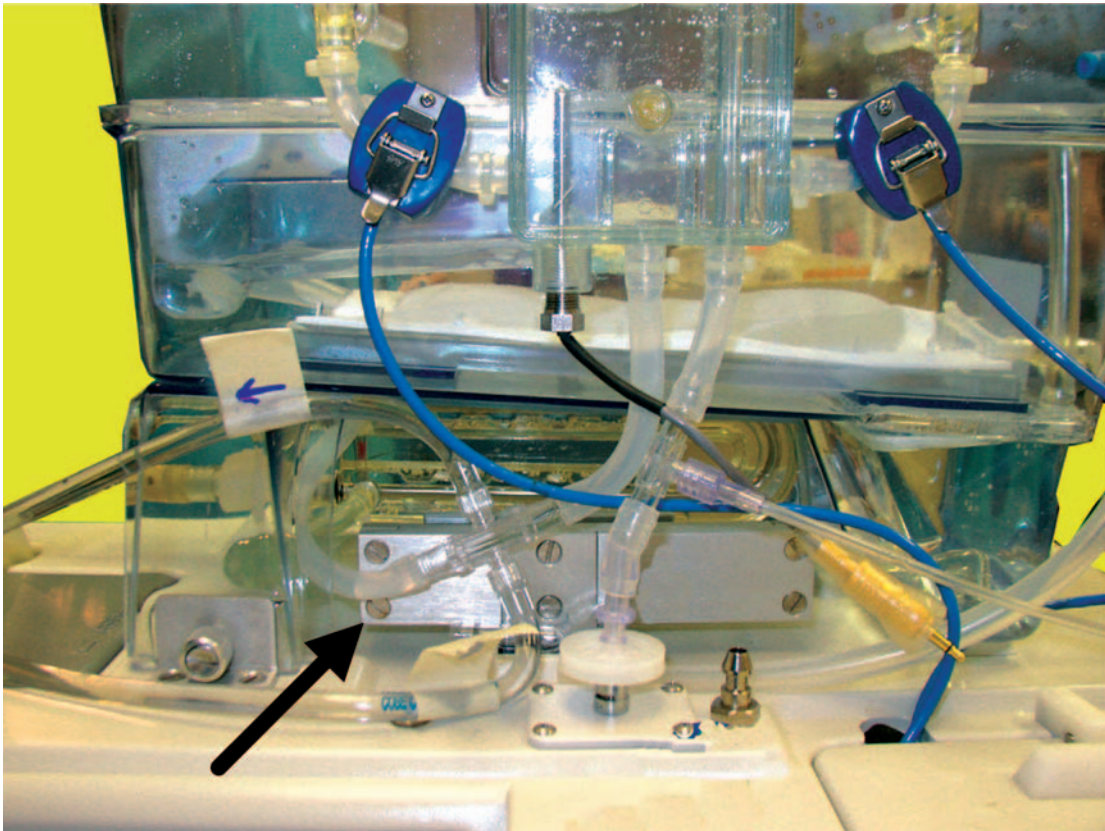
role in providing much needed organs by allowing *in situ* evaluation of questionable kidneys by an extracorporeal connection to the potential recipient.

The model we have described appears to allow evaluation and assessment of the kidney and could have a role in identifying organs previously discarded. A clinical application of this procedure would involve patients who are undergoing hemodialysis and have previously inserted AV fistulas to facilitate long-term dialysis treatments. These fistula sites could provide a convenient, minimally invasive site for vascular access to the perfusion system described here.

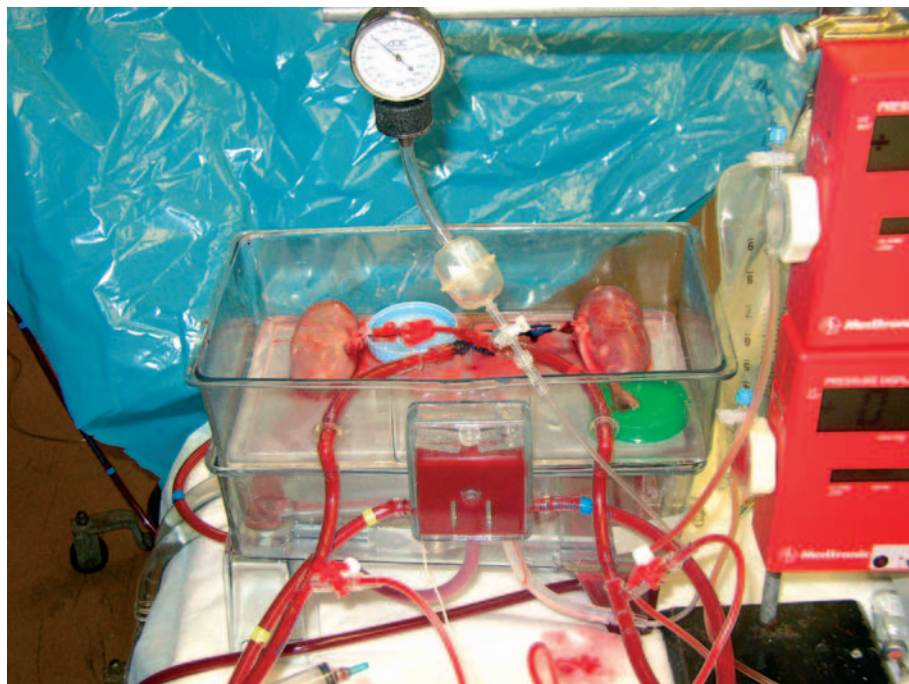
Prior to clinical application of this model, several limitations and considerations must be addressed. First, the use of the RM3 cassette in this manner is an off-labeled use. While the manufacturer's recommendation does not include patient or blood contact with the device, the rigid plastics used (butyrate and

acrylic) and the silicone and vinyl tubing are generally considered biocompatible and are tested for sterility, cytotoxicity and endotoxicity (personal communications, Waters Instruments, Inc). In this study, we observed no adverse reaction from blood contact with this system. Secondly, while this study demonstrated that this model is conceptually feasible, we provide only a basic assessment of the kidney. Further detailed analysis of the optimal perfusion parameters and donor kidney function must be carried out. Finally, because this procedure would require anticoagulation, considerations on management and the risks of bleeding must be ascertained.

Strategies to address the critical organ shortage for patients who are waiting for kidney transplant must be explored. Identifying organs which would be ordinarily discarded because of questionable viability is an area which could greatly expand the pool of available organs. The model presented in



**Figure 4** The modification of the cassette was accomplished by cutting in a double-luer connector (arrow) to allow for inflow and outflow communication with the animal.



**Figure 5** View of the kidneys in the cassette. Renal artery is cannulated while renal vein is open to drain directly into the cassette.

this manuscript represents a possible avenue to evaluate kidneys from marginal donors. Further animal studies using this model that more fully

evaluate functional parameters are necessary before clinical application, but the early results show promise.

## References

- 1 Gridelli B, Remuzzi G. Strategies for making more organs available for transplantation. *N Engl J Med* 2000; **343**: 404–10.
- 2 United Network for Organ Sharing. *Annual report*. Richmond, VA: 2003.
- 3 Cooper D, Gollackner B, Sachs D. Will the pig solve the transplantation backlog? *Am Rev Med* 2002; **53**: 133–47.
- 4 Baalpur S, Strong A, Hoernich N *et al.* Machine perfusion for kidneys: how to do it at minimal cost. *Transpl Int* 2001; **14**: 103–107.
- 5 St. Peter S, Imber C, Friend P. Liver and kidney preservation by perfusion. *Lancet* 2002; **359**: 604–13.
- 6 Kozaki K, Uchiyama T, Nemoto H *et al.* Usefulness of continuous hypothermic perfusion preservation for renal grafts from non-heart beating donors with prolonged warm ischemia time. *Transpl Proc* 1997; **29**: 3586–87.
- 7 Balpur S, Buckley P, Snowden C *et al.* The trouble with kidneys derived from the non-heart beating donor: a single center 10-year experience. *Transplantation* 2000; **69**: 842–46.
- 8 Darling E, Searles B, Nasrallah F *et al.* High-volume, zero balanced ultrafiltration improves pulmonary function in a model of post-pump syndrome. *J Extra Corpor Technol* 2002; **34**: 254–59.