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ORIGINAL ARTICLE

An automated and portable low-flow pulsatile perfusion system for organ preservation

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Abstract While machine preservation reduces the incidence of delayed graft function in renal transplant recipients, it is only used in 10 % of kidney transplantations. The performance of our portable, low-flow-pulsatile organ perfusion system was examined in a canine kidney autotransplantation model. Grafts were stored for 72 h by simple cold preservation in University of Wisconsin (UW) solution, or by high or low-flow machine preservation. After preservation, the grafts were autotransplanted and the animals were followed for 15 days. Graft function was better in machine-preserved kidneys. Tissue biochemistry indicated that machine preservation resulted in higher levels of adenine nucleotides and better histological integrity than the cold storage. While histology and biochemistry of machine-preserved groups were similar, electromicroscopy of high-flow grafts showed mild accumulation of intravenous debris and endothelial swelling. This study shows that a simplified machine perfusion technique is effective for organ preservation.

Key words Preservation, machine perfusion · Perfusion, preservation · Organ preservation, machine perfusion · Kidney, machine perfusion

Introduction

Compared to simple cold storage, machine preservation reduces the incidence of delayed graft function in renal transplant recipients. However, machine preservation is only used in 10 % of kidney transplantations due to the many logistic difficulties, especially the size of the preservation machine [10]. The current practice of machine preservation employs a method developed 30 years ago that requires a high-flow perfusion rate, hypothermia (6°–9°C), and oxygenation. Thus, the machine preservation system needs a high-flow pump, a refrigeration system, and an oxygenator.

It has been suggested in previous studies that low-flow perfusion using ice for cooling would simplify the machine perfusion system [11]. Recently, we have devel-

oped an automated and portable, low-flow pulsatile organ perfusion system [19, 21, 22]. The performance of the system was examined in a 72-h canine kidney preservation and autotransplantation model.

Materials and methods

This study, performed according to the principles of laboratory animal care, used adult mongrel dogs of either sex weighing 18–23 kg. After an overnight fast, animals were anesthetized with sodium pentothal for induction and maintained with 1% halothane, 50% N_20/O_2, and positive pressure mechanical ventilation. Prior to kidney removal, the dogs were given 12.5 g of mannitol and hydrated with 50 ml/kg of warm (38°C), lactated Ringer's solution. After careful dissection, the left kidney was removed and transferred to a sterile basin containing iced saline solution.

Grafts were either stored by simple cold preservation (group 1, $n = 6$), high-flow machine preservation (group 2, $n = 6$), or low-flow machine preservation (group 3, $n = 6$). Group 1 grafts were flushed with 200 ml of cold heparinized (5 U/ml), lactated Ringer's solution, followed by 200 ml of University of Wisconsin (UW) Solution (ViaSpan DuPont, Wilmington, Del.) and stored in UW solution at 0°–4°C for 72 h. Grafts for machine preservation (groups 2 and 3) were flushed with 200 ml of cold, heparinized (5 U/ml) Ringer's solution containing 5% albumin and connected to the machine preservation system. Both the high-flow and low-flow systems were primed with 500 ml of modified albumin perfusate [3]. Graft temperature in both machine systems was maintained between 0° and 4°C. The flow rate in group 2 grafts (high flow) was 0.9 ± 0.04 ml/g per minute, at a pulse rate of 60 pulses per minute (ppm), which corresponds to flow rates used in clinical practice with machines such as the "MOX-100" (Waters, Rochester, MN). The flow rate in group 3 grafts (low flow) was 0.07 ± 0.006 ml/g per minute, at a pulse rate of 1 ppm, which was chosen to be proportional to the reduction in metabolic rate at 2°C [8, 13]. Grafts were maintained on hypothermic machine perfusion until autotransplantation (72 h). At the end of the 72-h preservation period, animals were returned to the operating room and anesthetized as described above. The preserved graft was autotransplanted into the right pelvis using a side-to-end anastomosis of the iliac artery to the renal artery and a side-to-end anastomosis of the iliac vein to the renal vein. After reperfusion, immediate contralateral nephrectomy was performed. Prior to closure, a ureterocystostomy was performed. Animals were returned to the animal care facility, followed for 15 days, and then sacrificed.

Equipment

Peak perfusion pressure was the same for both the high and low-flow groups and controlled at 35 ± 5 mmHg. Perfusate flow rate was modified by adjustments in pulse rate and stroke volume, using a homeostatic perfusion apparatus (HPA). The HPA consisted of a monitoring and control circuit, a compliance chamber, and a perfusion pump (Justec, Holbrook, NY), as previously described [19, 21, 22]. The monitoring and control circuit regulates pulsatile perfusion, such that the peak pressure is programmed at a fixed level. The size of the compliance chamber determines the stroke volume, which was constant in both groups for the duration of the experiment.To maintain hypothermia, the perfusion circuit and graft were packed in ice in a 16-l icebox. Oxygenation was by diffusion from room air.

Measurements

Grafts on machine preservation were assessed for perfusate flow rate, oxygen delivery (DO_2), oxygen consumption (VO_2), and perfusate lactate levels. Graft weight change during preservation was determined for all grafts. Renal blood flow (RBF), mean arterial pressure (MAP), and urine output (UO) were monitored for 1 h after reperfusion. Renal arterial and venous blood samples were taken 1 h after reperfusion for blood gas analysis (Radiometer) and results were corrected for dog hemoglobin [17]. Post-transplant renal function was assessed with serial serum creatinine measurements. Wedge biopsies were taken of the renal cortex immediately after graft nephrectomy, after the grafts were flushed, after 72 h of preservation, and at necropsy. Biopsies were prepared for adenine nucleotide, histological, and electron microscopic analyses. Tissues for adenine nucleotide analysis were immediately frozen in liquid nitrogen. Adenine nucleotide levels were determined by an HPLC method described previously [9]. Tissues for electromicroscopy were fixed in 2.5% glutaraldehyde and tissues for histopathology were fixed in 10% formaldehyde, as previously described [18].

Statistical analysis

All measurements are reported as group mean values with standard errors. An independent sample nonparametric Mann-Whitney U-test was used for inter-group comparisons (SPSS, Chicago, Ill.). A two-tailed P-value below 0.05 was considered significant.

Results

Preservation studies

Perfusion preservation

Determinations made in groups 2 and 3 during machine perfusion are shown in Table 1. The reduction in pulse rate in group 3 compared to group 2 resulted in a decrease in perfusate flow in group 3 to 14% of the level in group 2. The increase in weight of group 3 grafts was significantly less than the increase in group 2 grafts. Oxygen delivery (DO_2) and oxygen consumption (VO_2) were significantly less in group 2 than in group 3. Perfusate lactate levels in group 2 grafts were significantly less than group 3 levels.

Tissue biochemistry

Results of biochemical analyses of kidney biopsy tissue are shown in Table 2. Machine perfusion maintained higher levels of high-energy adenine nucleotides and adenine nucleotide precursors than cold storage. Adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine (ADO) levels were higher after flushing with UW (group 1) than after flushing with the albumin solution (groups 2 and 3). Despite the higher ATP and ADP levels in group 1 at the onset of preservation, ATP and ADP levels in group 1 were significantly

Table 1 Perfusion parameters, oxygen metabolism, perfusate lactate level, perfusate pH, and kidney weight after 72 h of machine preservation of kidneys perfused at either a high-flow rate (group 2–60 ppm) or low flow (group 3–1 ppm)

Measure	Group 2 High flow	Group 3 Low flow
Perfusate flow (ml/g per minute)	0.875 ± 0.038	0.069 ± 0.006*
DO_2 (μl/100 g per minute)	136.7 ± 13.8	30.5 ± 3.9*
VO_2 (μl/100 g per minute)	53.9 ± 4.8	18.9 ± 2.9*
Perfusate lactate (mg/dl)	34.8 ± 1.49	27.0 ± 3.39*
pH	6.892 ± 0.033	6.997 ± 0.048
Weight increase (%)	36 ± 5.3	14 ± 3.8*

* $P < 0.05$ versus group 2

lower than in groups 2 and 3 at the end of preservation. While both hypoxanthine and inosine (adenine precursors) increased during machine preservation, only hypoxanthine increased during cold preservation. No differences in tissue biochemistry were noted between the high and low-flow machine preservation systems.

Histology

As shown in Figs. 1 and 2, there was no difference in the histological findings of groups 2 and 3. However, the histology of grafts preserved in UW solution showed severe degeneration of the proximal convoluted tubuli (Fig. 3). No such degeneration was seen in group 2 or 3 grafts. Electromicroscopy demonstrated that high flow (group 2) caused mild accumulation of intravenous debris, mild separation of the basement membrane, and endothelial swelling (Fig. 4). Except for minimal intravascular debris, none of these changes was seen in electromicroscopic studies of group 3 grafts (Fig. 5).

Transplantation Studies

Transplantation

Warm ischemia times were similar in all groups (34.8 ± 1.2 min, 33.7 ± 3.5 min, and 43.2 ± 6.2 min, in groups 1, 2, and 3, respectively). Urine output, renal blood flow, mean arterial pressure, oxygen delivery, and oxygen consumption measured after 1 h of reperfusion were also similar in all groups (UO was 0.38 ± 0.15 ml/g per minute, 0.51 ± 0.17 ml/g per minute, and 0.57 ± 0.18 ml/g per minute in groups 1, 2, and 3, respectively; RBF was 2.90 ± 0.54 ml/g per minute, 2.36 ± 0.84 ml/g per minute, and 2.43 ± 0.47 ml/g per minute in groups 1, 2, and 3, respectively; MAP was 124 ± 4.2 mmHg, 103 ± 7.3 mmHg, and 106 ± 5.3 mmHg in groups 1, 2, and 3, respectively; DO_2 was 22.8 ± 8.9 ml/100 g per minute and 32.9 ± 15.6 ml/100 g per minute in groups 2 and 3, respectively; and VO_2 was 2.7 ± 0.7 ml/100 g per minute and 3.1 ± 0.5 ml/100 g per minute in groups 2 and 3, respectively).

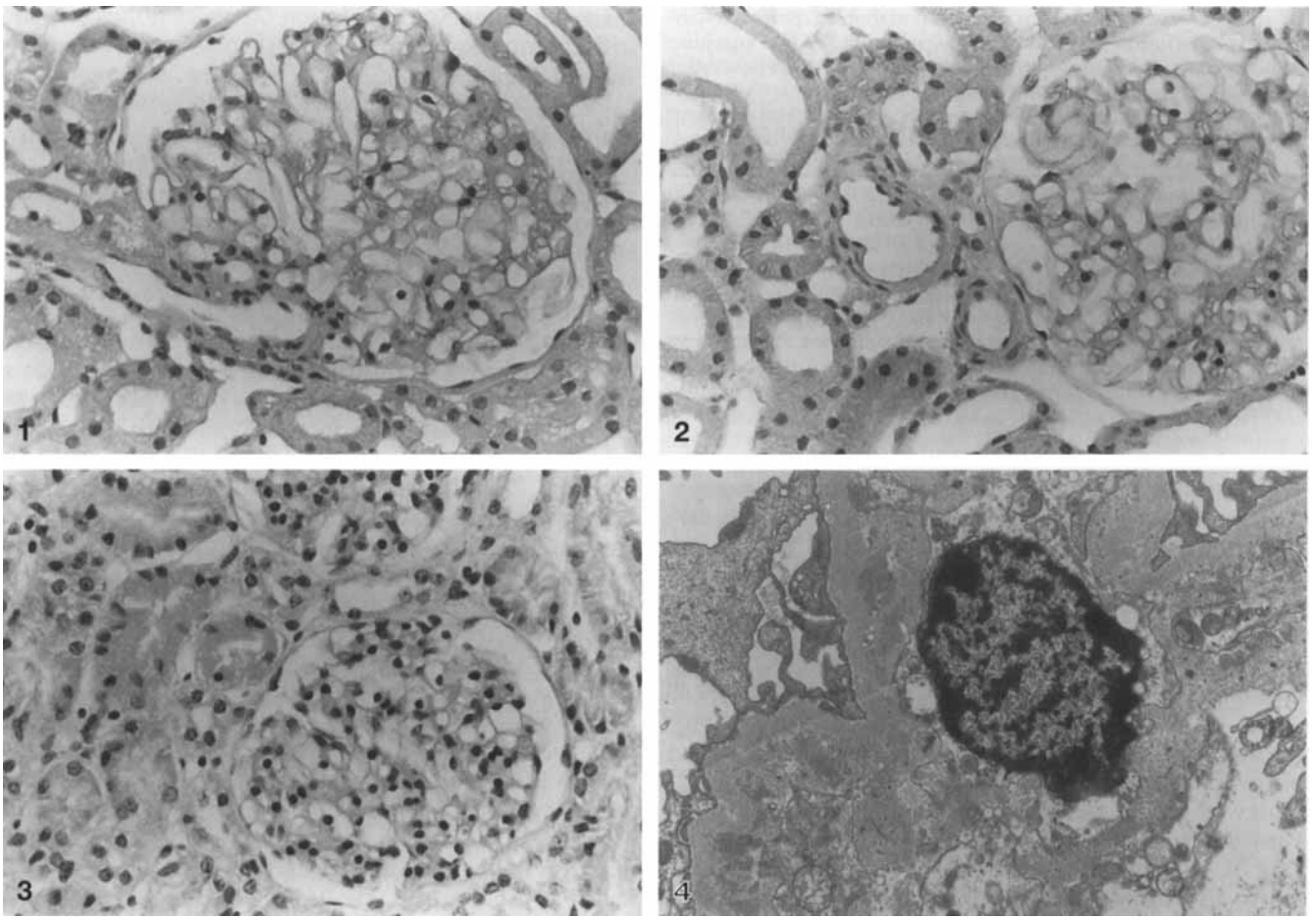
Outcome

Mean serial serum creatinine levels for each group are shown in Fig. 6. The peak creatinine level of each group was calculated by averaging the highest creatinine level of each animal in the group (14.0 ± 1.5 mg/dl, 6.2 ± 1.1 mg/dl, and 5.8 ± 0.9 mg/dl in groups 1, 2, and 3, respectively; $P < 0.05$ between group 1 and groups 2 and 3). After an initial period of renal insufficiency, all animals in group 1 survived for 15 days. However, one animal in group 1 had persistent renal insufficiency and was sacrificed on day 15. In group 2, one animal died from intussusception 8 days after transplantation with good renal function. Similarly, one group 3 animal died from wound dehiscence 8 days after transplantation with good renal function. There was no statistical difference in animal survival between groups.

Table 2 Adenine nucleotides and catabolite levels in renal cortex tissue after initial flush with University of Wisconsin (UW) solution, after initial flush with albumin solution, and after 72 h of preservation using the different methods. All values expressed in nmol/g wet tissue weight

	After flush with UW solution at 0 h	After flush with albumin solution at 0 h	After 72-h cold storage in UW solution	After 72-h high-flow preservation	After 72-h low-flow preservation
ATP	750 ± 97.3*[1]	375 ± 72.3	30.7 ± 5.9*[2]	314 ± 7.5*[5]	222 ± 43.9*[6]
ADP	818 ± 45.5*[1]	226 ± 216	134 ± 14*[2]	312 ± 153	381 ± 15.6*[6]
AMP	1357 ± 238	1560 ± 87	815 ± 117	632 ± 62.6	686 ± 129*[4, *6]
ADO	1099 ± 131*[1]	16.1 ± 2.0	34.3 ± 5.2*[2]	31.6 ± 11.1	26.9 ± 5.7
INO	143 ± 4.9	34.1 ± 3.7	254 ± 21.4*[2]	1320 ± 75*[3, *5]	1570 ± 61*[4, *6]
HX	140 ± 45.6	170 ± 12.7	1558 ± 96*[2]	1947 ± 280*[3]	1646 ± 83*[4, *6]

*[1] $P < 0.05$ between after flushing with UW and albumin group; *[2] $P < 0.05$ between after flushing and after cold preservation (group 1); *[3] $P < 0.05$ between after flushing and after high-flow preservation (group 2); *[4] $P < 0.05$ between after flushing and after low-flow preservation (group 3); *[5] $P < 0.05$ between cold storage and high-flow preservation (groups 1 and 2, respectively); *[6] $P < 0.05$ between cold storage and low-flow preservation (groups 1 and 3, respectively)



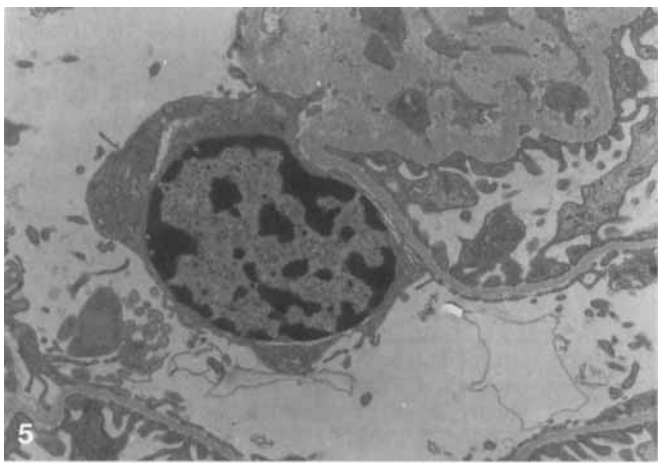


Fig. 1 Group 2, light microscopy (H&E, × 400). Group 2 renal cortex biopsies were obtained after 72 h of high-flow machine preservation at 60 ppm. Some vessels appear dilated; otherwise no consistent differences with Group 3

Fig. 2 Group 3, light microscopy (H&E, × 400). Group 3 renal cortex biopsies were obtained after 72 h of low-flow machine preservation at 1 ppm. The overall architecture is well preserved

Fig. 3 Group 1, light microscopy (H&E, × 400). Group 1 renal cortex biopsies were obtained after 72 h of cold storage in UW solution. The proximal convoluted tubuli are severely degenerated, the glomeruli are mostly intact

Fig. 4 Group 2, electron microscopy × 10.000. Group 2 renal cortex biopsies were obtained after 72 h of high-flow machine preservation at 60 ppm

Fig. 5 Group 3, electron microscopy × 10.000. Group 3 renal cortex biopsies were obtained after 72 h of low-flow machine preservation at 1 ppm

Discussion

We propose a totally portable and simple device that allows for international organ sharing with minimal risk of injury caused by increased perfusion pressure and without the need for a dedicated perfusionist.This study shows that a simplified machine perfusion technique, employing low-flow perfusion, ice cooling, and oxygenation by room air diffusion, is effective for organ preservation. Results with low-flow perfusion compared favorably to high-flow perfusion and cold preservation. Compared to machine-preserved kidneys, simple cold-preserved kidneys were associated with an increased incidence of delayed graft function. Histology suggested

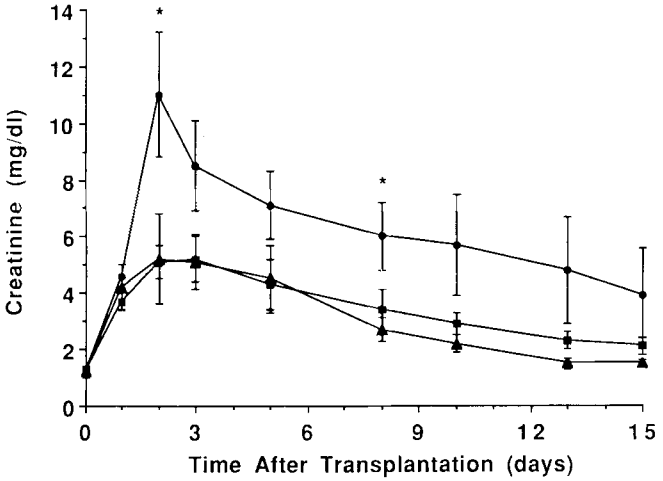


Fig. 6 Mean post-transplantation serum creatinine levels for each group. Group 1 kidneys were preserved by cold storage in UW solution for 72 h (—●—). Group 2 kidneys were preserved by high-flow machine preservation at 60 ppm for 72 h (—▲—). Group 3 kidneys were preserved by low-flow machine preservation at 1 ppm for 72 h (—■—). *$P < 0.05$ versus low and high-flow machine preservation

that machine preservation provided better graft protection than cold storage. The severe degeneration of the architecture of the proximal convoluted tubuli of cold-preserved grafts was consistent with the early renal dysfunction seen after transplantation. Andrews reported similar histological findings in UW cold-stored renal grafts [1].

The improved histology of machine-preserved grafts may be related to improved perfusion of the renal cortical microcirculation, the expulsion of red blood cells, the clearance of catabolic reaction products, and improved substrate supply [20]. Clinically, the incidence of delayed graft function of transplanted renal grafts is reduced from 16%–40% with cold storage to less than 8% with machine perfusion [2, 12]. Experimentally, machine perfusion has successfully extended the preservation of canine renal grafts for 7 days, well beyond the capability of conventional cold storage methods [15]. The potential health care cost savings that could be achieved by routine use of machine perfusion instead of cold storage using conventional technology have been documented [16].

It is our experience that the main reasons for not using machine preservation routinely are related to the limitations of current machine preservation methodology. The high-flow system is not responsive to changes in vascular resistance, possibly resulting in perfusion injury. If equipment failure occurs, the organ will warm up rapidly because cooling is dependent upon continuous, high-flow perfusion. The portable version of the MOX-100 requires frequent replacement of ice and drainage of cooling water during transport, and therefore the high-flow system requires a dedicated perfusionist.

Most of the limitations associated with the high-flow systems are overcome by the low-flow systems. A low-flow system allows for a colder perfusion temperature, uses simple and reliable cooling by immersion in ice, eliminates the need for the oxygenator, reduces the risk associated with high-pressure injury, improves user friendliness, and reduces personel requirements. Therefore, a low-flow system avoids the logistical difficulties associated with high-flow systems (current methodology) without losing the benefits associated with conventional machine perfusion.

Herrman and Turcotte first suggested the use of low-flow perfusion to avoid many of the problems associated with high-flow perfusion [11]. Grundman et al. performed a detailed analysis of different pressures using nonpulsatile perfusion and concluded that a perfusion pressure of 21 mmHg was optimal [7]. Low-pressure perfusion, however, underestimates the resistance to flow of the renal cortical microcirculation during hypothermic preservation, resulting in shunting of flow from cortex to medulla. Using microspheres injection techniques, we observed that prolonged cold storage resulted in progressive deterioration of the integrity of the renal cortical microcirculation [18, 20].

Uneven microcirculation perfusion was reported by Calne et al. in liver and kidney perfusion preservation [4]. Calne showed that nonpulsatile perfusion at both a very low flow and pressure, called "trickle perfusion", did not provide homogenous perfusion of the entire organ. To avoid this problem, Calne et al. subsequently developed a semipulsatile technique called "squirt perfusion" and preserved livers for up to 17 h [5]. Similarly, in the myocardial preservation, Manciet and Copeland documented that a mean perfusion pressure of 13 mmHg was inadequate to preserve the rabbit heart, resulting in heterogeneous perfusion associated with increased free radical release and reduced recovery of contractility [14].

The ideal perfusion system, thus, is a system that is capable of delivering the perfusate at low pulsatile pressure to avoid endothelial sloughing but that allows for adequate distribution of flow to the renal cortical microcirculation. Our new pulsatile, low-flow system overcomes the problems of previous attempts because the HPA incorporates a control circuit that continuously senses renal vascular resistance and regulates pump action accordingly through a closed feedback loop [23]. The HPA reduces the perfusate flow rate by reducing the pulse rate rather than the pressure. The HPA pump responds to changes in vascular resistance by automatic adjustments in pulse rate and, thus, allows for uniform flow distribution without the penalty of high-perfusion pressure. In the current study, we documented reduced lactate production, improved oxygen extraction, re-

duced oxygen consumption, and maintenance of high-energy nucleotide levels in the low-flow group compared to the high-flow group. Thus, both the metabolic and the histological parameters supported the use of low-flow perfusion.

Even though the current experimental model did not detect differences in outcome between machine preservation methods, low-flow perfusion caused less tissue swelling, allowed better protection of the endothelium, and was associated with improved metabolic parameters compared to high-flow perfusion.

The most intriguing application of HPA machine perfusion is its potential use in rescuing grafts from non-heart-beating donors [6, 22]. The ability of the HPA to provide adequate perfusion at lower flow rates than conventional machine perfusion may reduce additional damage by preservation. Another advantage of the HPA is the possibility of pressure-controlled perfusion. In pressure-controlled perfusion, the perfusate flow is automatically adjusted to the vascular resistance or size of the organ. Thus, organs of any size, ranging from rat kidneys to adult size human livers, can be perfused safely [24].

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