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A new method for rat accessory hepatic transplantation – the cervical approach

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Abstract Current methods for accessory liver transplantation in the rat require a high degree of microsurgical expertise and long training before success is achieved. We present a simpler method of arterialized accessory liver transplantation using the cervical vessels for revascularization of the transplanted liver with the cuff technique, which is useful for studies of liver preservation, reperfusion injury, and liver regeneration. After classical 70 % hepatectomy is performed on the graft, the right common carotid artery is anastomosed to the donor aorta, the distal right external jugular vein is anastomosed to the donor portal vein, and the proximal right external jugular vein is anastomosed to the donor supradiaphragmatic inferior vena cava. The skin is not closed over the cervically transplanted liver (CTL). This method was used 30 times for periods of up to 6 h with a 90 % success rate. CTL structure and function, as revealed by histology, bile flow rates, biliary bilirubin concentrating capacity, membrane potential, enzyme activity and distribution, have shown the CTL to be a structurally normal and metabolically active graft. In conclusion, the cervical approach to arterialized accessory liver transplantation is simple, and should prove useful for studies of liver preservation, reperfusion, regeneration, physiology, and toxicology.

Key words Liver transplantation, rat, cervical · Accessory liver transplantation, rat

Introduction

Both orthotopic and accessory arterialized hepatic transplantations in the rat are procedures that require highly skilled surgeons trained in microvascular techniques [8, 20]. Using the cuff technique [22] on the cervical vessels of rats, accessory cardiac, renal, pancreatic, and splenic transplantations have been successfully performed [15, 21]. We have used the simpler cervical approach with the cuff technique in order to develop a new technique for arterialized accessory liver transplantation in the rat.

The stimulus for the development of the cervically transplantet liver (CTL) was the need for a simpler method of rat liver transplantation that enabled close graft monitoring for a specific period of time for studies of graft preservation, early reperfusion events, and liver regeneration.

Materials and methods

Male Wistar rats weighing approximately 200–300 g (donors) and 300–400 g (recipients) were housed and maintained under controlled conditions in our laboratory animal facilities. Commercial pellets and water were available ad libitum. The donor and recipient animals were prepared simultaneously to reduce the transplant ischemia time.

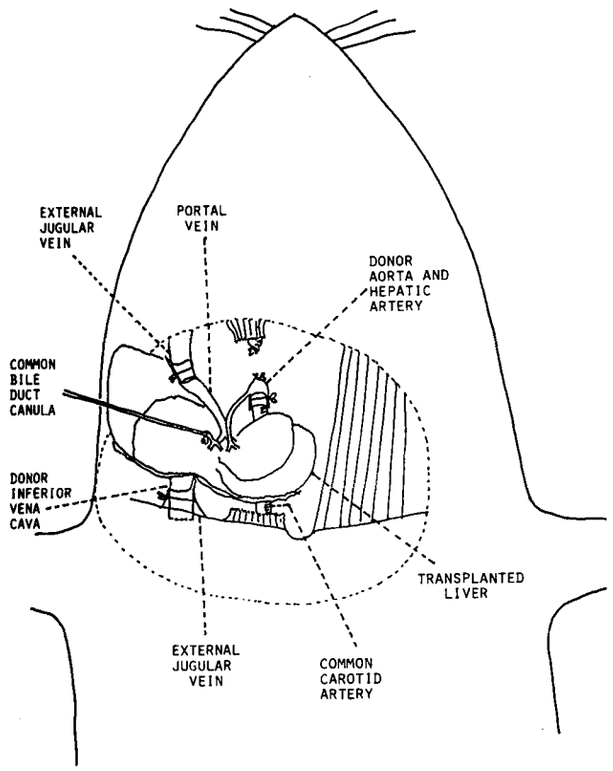

Fig. 1 Schematic diagram showing the positioning of the heterotopic reduced cervical liver transplant (CTL). The juguloportal, carotico-aortic, and jugulocaval anastomoses using the cuff technique are illustrated. The common bile duct is stented

Donor preparation

The donor was anesthetized with ether and the abdominal cavity was opened with a wide cruciate incision. The infrahepatic vena cava and portal vein were both mobilized by ligation of their main branches. The common bile duct was opened distally and cannulated with a length of PE 50 tubing (0.965 mm external diameter polyethylene tubing, Intramedic, Clay Adams, Becton Dickinson, N.J., USA). Next, the branches of the coeliac artery were ligated and the portal vein was divided and cannulated with PE 50 tubing. A total of 1000 U of heparin was administered via a femoral venous catheter. A classical 70 % hepatectomy [16] was performed and the portal vein was gently flushed with 10 ml of Hartmann's solution at 4 °C.

The abdominal aorta was mobilized by ligating its lumbar branches and the superior mesenteric artery; then it was divided at the level of the superior mesenteric artery, cannulated with PE 50 tubing, and flushed with 5 ml of the same perfusate. The liver was then excised, the infrahepatic vena cava ligated, and the supradiaphragmatic inferior vena cava prepared using a cuff of polyethylene tubing (external diameter 2.1 mm). It was then transferred directly to the recipient.

Recipient preparation

The recipient was anesthetized with ether, and a left femoral venous catheter of PE 10 tubing (0.61 mm external diameter polyethylene tubing, Intramedic, Clay Adams, Becton Dickinson, N.J., USA) was placed to enable anesthesia to be maintained with intravenous propofol (Diprivan, ICI – PHARMIA, Belgium) at 0.3–1.0 mg/kg per hour. Postoperatively this was reduced to 0.06–0.2 mg/kg per hour for sedation. A right femoral venous catheter of PE 50 tubing was inserted for venous access and the right femoral artery cannulated with PE 50 tubing for continuous blood pressure monitoring. The neck was opened widely with a transverse incision at the level of the jugular notch with midline vertical extension. The right external jugular vein was dissected along its length from its junction with the axillary vein to its division close to the submandibular salivary gland with careful ligation of all its branches. The right common carotid artery was mobilized from its distal bifurcation to as far proximally as could be safely achieved without undue stretching. The right external jugular vein was then occluded proximally and distally with atraumatic vascular clamps and divided approximately one-third of the distance between the clavicle and the right submandibular salivary gland. Just enough length was left on the proximal end of the jugular vein to allow the donor vena caval cuff to be fixed at the thoracic inlet, thus preventing outflow obstruction. The right common carotid artery was clamped proximally and divided distally. Cuffs prepared from polyethylene tubing (2.1 mm external diameter for the veins and 0.9 mm external diameter for the artery) were then applied to the vessels and held with 7/0 silk ligatures.

Transplantation

The reduced liver graft was placed upside down on the recipient cervical region with its hilum facing posteriorly. The suprahepatic inferior vena cava was anastomosed to the proximal right external jugular vein, the portal vein to the distal right external jugular vein, and the donor aorta to the common carotid artery using the cuff technique. The proximal right external jugular vein clamp was first removed, followed by the distal right external jugular vein clamp and the common carotid artery clamp. CTL reperfusion was rapid, with recoloration taking 10–20 s and bile flow 3–5 min. With two surgeons working simultaneously, the procedure took 1.5–2 h. The recipient rat remained sedated with propofol and the skin was not closed. The CTL was maintained for a mean of 3.72 h (range 1–6 h) according to various experimental protocols (Fig. 1). The CTL remained under constant visual surveillance during all perfusions. After transplantation, the CTL initially became hyperemic but then attained the normal brown color of rat liver. The CTL remained soft, its edges sharp, and there was no swelling. With any complication causing impaired CTL perfusion, the liver became dark and mottled within 10–15 min, and then harder and more swollen. These changes were even more immediately apparent in the case with outflow block.

Liver histology was obtained from the CTL after varying periods of perfusion. The CTL was stained with hemotoxylin-eosin-safranin (HES), hemotoxylin-phosphotungstic (HPT), and periodic acid-Schiff (PAS) stains.

Bile was collected during each CTL perfusion, its volume measured, and then it was immediately frozen at – 20 °C. Bile acids were estimated semiquantitatively with gas chromatography and mass spectrometry using a Hewlett Packard 5971 mass spectrometer coupled to a Hewlett Packard 5890 series II gas chromatograph [2] on hourly bile specimens collected after 2 and 3 h of perfusion of the graft. For control, bile was collected during the 1st h after common bile duct cannulation of a male Wistar rat kept under identical conditions and anesthetized with propofol.

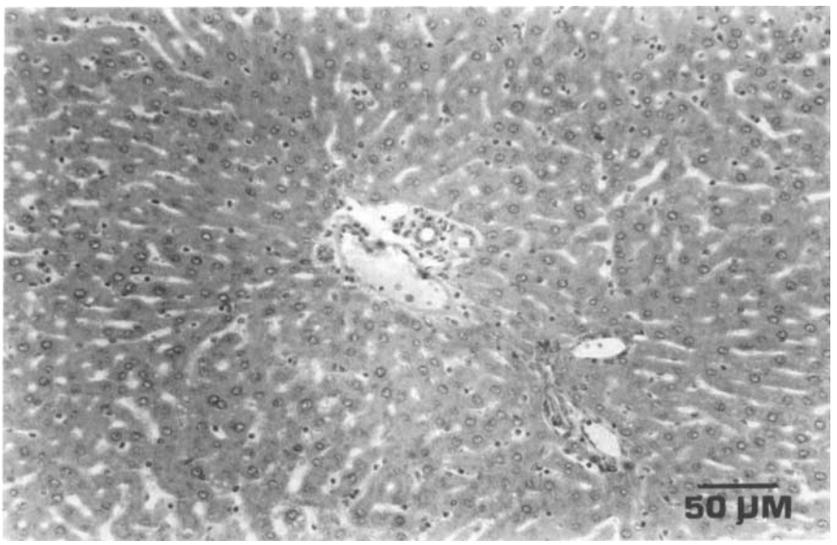

Fig. 2 Histological examination by PAS staining of CTL 6 h after revascularization

Total and direct bilirubin concentrations were estimated on specimens of bile collected from the CTL over a 4-h period with simultaneous recipient classical partial hepatectomy [16]. Total and direct bilirubin concentration measurements were performed using a Technicon Smac II system [10].

Assessment of the membrane potential was based on a method described by Corabœuf et al. [7]. Pyrex microelectrodes with a diameter of 0.5 µm were filled with 3 M KCl. The electrodes used had a tip potential of less than 5 MV and a resistance of between 10 and 20 megohms. These electrodes were inserted in the liver at the rate of 10 µm/s by a micromanipulator. The potential was amplified by an impedance reducer and amplifier (Medistor, Seattle, Wash., USA). Each descent of the electrode measured the potential of between 50 and 100 cells, thus allowing an average value to be obtained with its standard deviation at any given time.

Hepatic enzyme activity and metabolic zonation

Using a previously described technique, the activities and zonal distribution of glucose 6 phosphatase (G6P), succinate dehydrogenase (SDH), and NADPH dehydrogenase (ND) were measured in the CTL after 3 h of perfusion [26]. The metabolic zonation was expressed as the ratio of periportal to perivenular activity.

Statistics

Statistical analysis was performed using Student's *t*-test for paired variables. A *P* value below 0.05 was considered significant.

Results

The procedure was performed 30 times. In the first 20 cases a slightly different technique was used [3], in that the transplantation utilized both right and left external jugular veins respectively for portal inflow and venous outflow of the graft. Complications occurred in 6 of the first 20 cases (4 in the first 10 transplants and 2 in the second 10) and consisted of recipient hypotension ($n = 1$), pneumonia ($n = 2$), outflow obstruction ($n = 1$), bleeding from the hepatic artery ($n = 1$), and hemothorax ($n = 1$). However, it was noted that in 20 % of the recipients the blood pressure began to decline after 4 h of CTL perfusion (and 6 h of recipient anesthesia), despite adequate fluid replacement. After demonstrating that bilateral jugular vein and unilateral carotid artery ligation combined with propofol anesthesia but without the above-mentioned procedure may result in hypotension associated with histological changes of cerebral infarction (personal data), the technique was changed to avoid bilateral jugular vein ligation. The last ten transplants were performed using this modified technique and had a 90 % success rate, the only complication being one case of portal vein thrombosis. In all cases the CTL was closely observed, and in uncomplicated cases it had the appearance of normal rat liver throughout the experimental period.

Sections taken from uncomplicated CTLs and stained with HES or HPT stain showed essentially normal liver histology. Figures 2 and 3 show the comparative histology between native liver and CTL with HES staining after 6 h of CTL perfusion. The major difference was the markedly decreased intrahepatic fat droplets around centrilobular veins in the CTL compared to the native liver. A mild, diffuse, neutrophilic infiltrate was also seen in both the native liver and the CTL, but there was no evidence of hepatocyte necrosis. PAS staining showed decreased liver glycogen, related to fasting under anesthesia and occasional Kupffer cells containing PAS-positive material; again, the changes were seen equally in both livers. By contrast, the CTL in a rat hypotensive with pneumonia functioned poorly for 4 h and

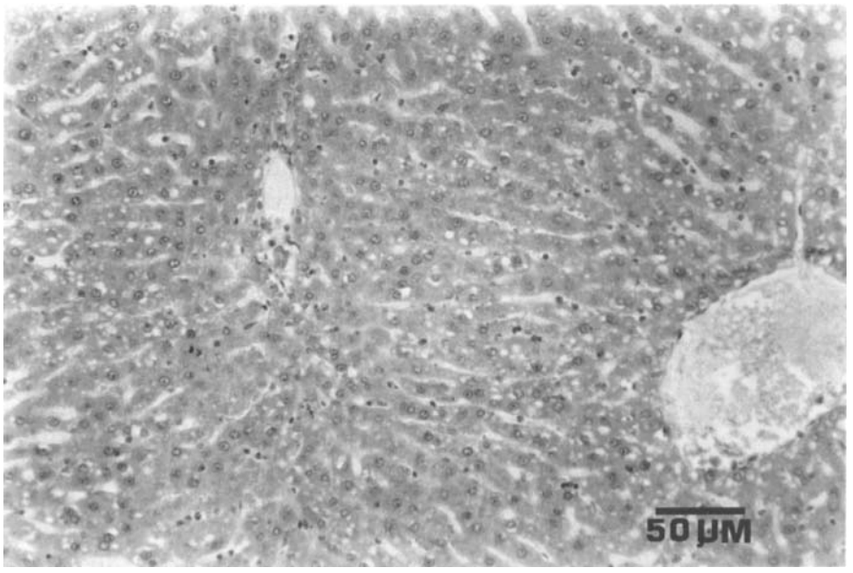

Fig. 3 Histological examination by PAS staining of the native liver of the CTL recipient shown in Fig. 2, 6 h after revascularization of CTL

showed marked vacuolization on PAS staining and red (instead of blue) staining of the cytoplasm on HPT staining.

The bile flow rate was measured in seven CTLs over a 5-h period. Bile flow increased during perfusion to reach a maximum at the end of the 2nd h and then stayed relatively constant (Fig. 4).

Total biliary bile acid concentrations during the 2nd and 3rd h after CTL revascularization showed values that were 48 % and 22 % respectively, of the control total biliary bile acid concentrations, illustrating a decrease in total bile acid excretion over time. By contrast, the total and conjugated biliary bilirubin concentrations excreted by the CTL after recipient partial hepatectomy increased over time (Fig. 5).

The membrane potential of the CTL was maintained at the same value as normal rat liver for 3 h after revascularization, the duration of this experiment. When the hepatic artery of the CTL was clamped for 30 min, the membrane potential decreased to a lower level of – 24 mV after 15 min, where it was maintained for 15 min, rising back to normal levels upon unclamping (Fig. 6).

The maximal enzyme activity of G6P, SDH, and ND for control and CTL was similar in the periportal zone, the perivenular zone, and the 5 hepatic zones in between. The metabolic zonation (PP/PV ratios) for G6P was 1.74 (control) and 1.76 (CTL); for SDH 1.90 (control) and 1.56 (CTL); and for ND 0.62 (control) and 0.64 (CTL). There was no significant difference in enzyme activity and distribution between the control and CTL groups.

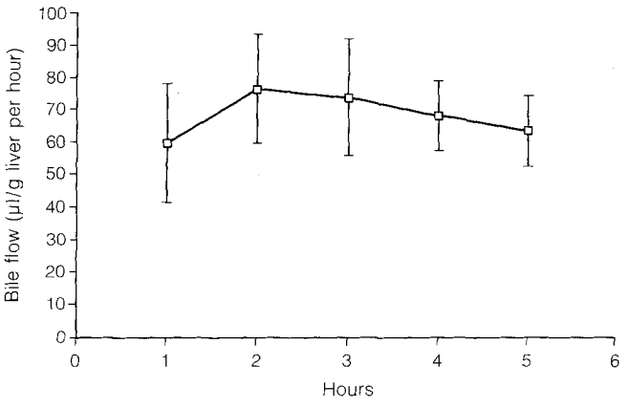

Fig. 4 Bile flow rate (mean ± standard deviation) versus time for CTL

Discussion

Cervical reduced hepatic transplantation has previously been attempted without complete revascularization and free biliary drainage. Sigel et al. [24] placed reduced size liver grafts in the cervical region of dogs, but in this model the portal vein was anastomosed to the common carotid artery and the common bile duct ligated. The grafts became rapidly engorged and subsequently atrophied. Rat liver has previously been connected to the cervical vessels for use as a biological filter for antibodies [12], but catheters were used and the liver remained in situ in the donor animal.

Histological studies of the CTL showed minimally abnormal findings that were also reflected in the native liver. Viability of the CTL hepatocytes was confirmed by

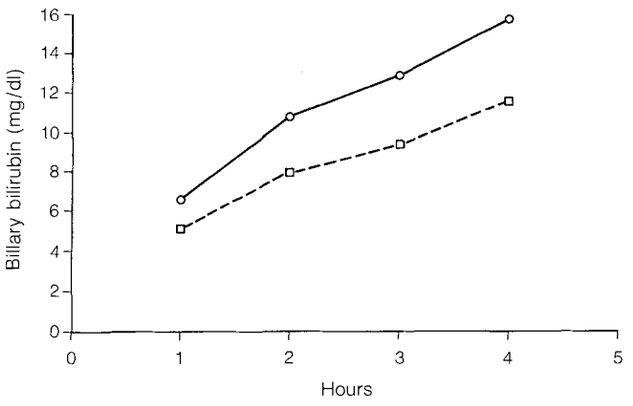

Fig. 5 Total (-○-) and conjugated (- - -□- - -) biliary bilirubin concentration versus time produced by CTL

HPT staining even after 6 h of perfusion, as the cytoplasmic color change from normal red to ischemic blue was not seen.

The rate of bile flow produced by a liver is an important indicator of liver function and is dependent upon a number of factors, including portal bile acid concentration, liver perfusion, and liver ATP content. The isolated, perfused rat liver (IPRL) without added portal bile acids exhibits largely bile acid-independent flow [9], as only traces of bile acids are secreted in the bile. The maximal bile flow rate obtained with IPRL has been 68–75 μl/g liver per hour [5, 18] for the 1st h and 60 μl/g liver per hour at 4 h (data corrected to per gram of liver) [11]. It has also been shown with IPRL that bile flow is a function of perfusate flow. When perfused with blood, maximal bile production occurred between 20 and 40 ml/min of perfusate flow and markedly decreased if flow was less than 10 ml/min [6]. Bile flow also correlates with liver ATP levels, as metabolic inhibitors cause a parallel decrease in bile flow and liver ATP [25], and liver ischemia has shown a direct relationship between bile flow and liver ATP [17].

Using a nonarterialized technique of orthotopic rat liver transplantation (OLT), which resulted in 1 h of cold ischemia for the graft, Xu et al. [28] found the bile flow rates ranged from 84 to 96 μl/g liver per hour during the first 3 h after transplantation with an intact enterohepatic circulation (data corrected to per gram of liver) [11]. The bile flow rates of the CTL at least match those of IPRL for similar bile acid-independent flow and approach those of the OLT model, without the beneficial choleretic effects of portal bile acid, thus indicating normal CTL perfusion and cellular ATP levels.

Bile acid excretion of the CTL decreased over time. As the CTL enterohepatic circulation had been interrupted, this is consistent with excretion of the liver bile acid pool, the size of which decreases with each hour of bile secretion. Conversely, bile flow increased during the 2nd hour of perfusion and remained high, showing an increase in bile acid-independent bile flow, which may have been due to the propofol anesthesia.

The normal biliary bilirubin concentration in the rat ranges from 3.8 to 6 mg/dl [1]. Classical partial hepatectomy in the rat [16] has been shown to increase the plasma concentration of bilirubin by 37.5 % and to decrease the Tm (apparent maximal biliary secretion rate) for bilirubin by 80 % during the first 24 h [23]. In the presence of an increased bilirubin load caused by simultaneous recipient partial hepatectomy, the CTL was able to actively increase its conjugation and excretion of bilirubin during 4 h. Maximal biliary secretion of bilirubin in the rat has been shown to depend on the activity of UDP glucuronosyl transferase for conjugation, an energy-dependent step [27]. This emphasizes the continuing metabolic activity of the CTL and its preserved excretory capacity.

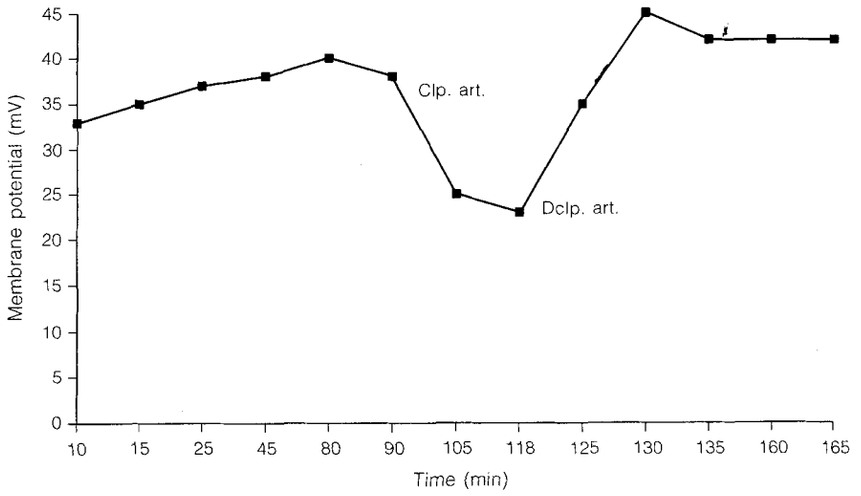

Fig. 6 Membrane potential of CTL versus time during 3 h of perfusion. The right common carotid artery was clamped *(clp. art.)* at 90 min and then declamped *(dclp. art.)* at 118 min

The membrane potential of normal rat liver in situ is -42.5 ± 0.1 mV [14] and is maintained by the activity of Na + K + ATPase, an ATP-dependent cell membrane-based enzyme. In contrast, the membrane potential of IPRL is often less than -35 mV [13]. The hepatocyte membrane potential is reduced by hypoperfusion and ischemia and has a direct relationship to cellular activity [19], making it an excellent test of hepatocyte function and viability. The sensitivity of this test is illustrated by clamping the hepatic artery, which causes CTL hypoperfusion and an immediate decrease in the membrane potential.

Enzyme activity and metabolic zonation [26] were not significantly different between the control liver and CTL. We have previously found that enzyme activity steadily declines with ischemia, reaching very low levels after 2 h of warm ischemia (personal data). The CTL showed normal enzyme activity and distribution 3 h after revascularization, demonstrating its continuing good function.

In the spectrum of rat liver transplantation techniques, the cervical approach is technically less demanding than orthotopic liver transplantation, with or without arterialization, or than intra-abdominal accessory liver transplantation [20]. As less training is required to achieve satisfactory results, this technique may well increase the opportunities for research into liver transplantation.

A disadvantage is that the CTL is an acute model, as the skin is not closed over the graft. However, for acute studies such as those of liver preservation and acute reperfusion events, which require close graft monitoring, the cervical approach has many advantages. Serial biopsies may be easily obtained, membrane potential measurements are unaffected by respiratory movement, and nuclear magnetic resonance signals are not only more easily obtained but are uncontaminated by adjacent muscle, which is frequently the case in the intra-abdominal position. Another advantage is that the CTL is arterialized, an important point when studying preservation/early reperfusion phenomena, as some mechanisms of graft injury may be oxygen-dependent.

The cervical approach is also useful in the investigation of liver-liver interactions, such as the factors affecting bile flow rates and composition and liver regeneration. It has been shown that the increased liver mass provided inhibits liver regeneration in a recipient subjected to a partial hepatectomy 14 h before the cervical procedure but not after 18 h. This shows that there is a restriction point between 14 and 18 h post-partial hepatectomy after which liver regeneration will continue despite an increased total liver mass [4].

In conclusion, the CTL has been shown histologically and functionally to be well perfused and metabolically active, and it should prove useful in the study of acute phenomena such as reperfusion injury and functional recovery, hepatic physiology, regeneration, and toxicology.

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