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Liver4Life Perfusion Machine for Liver Graft Preservation before Transplantation

Keywords: Machine Perfusion, Organ transplantation, Organ preservation, Liver transplantation, *ex vivo* perfusion (EVP) technology

SUMMARY OF TECHNOLOGY

The standard of care method of preserving liver grafts before transplantation has been static cold storage (SCS) for the past 4 decades. It involves flushing the organ with a preservation solution and keeping it in ice bath at 4°C.[1] It is cost-effective and practical and has helped in the treatment for end-stage organ failure.[2] This method allows safe storage for up to 12 – 18 hours.[3] However, the preservation is far from perfect; cold ischaemia triggers a cascade of cytotoxic pathways, leading to sinusoidal endothelial cell swelling, membrane damage and injury to other components of the hepatic microvasculature.[4] This can eventually lead to early graft dysfunction and limits the time between organ retrieval and implantation.[5] Machine perfusion (MP) is an alternative to this method; a perfusate solution (typically dilute oxygenated blood) is actively circulated through the organ to maintain cellular metabolism and prevent the effects of cold ischaemia.[6] A study by Nasralla et al (2018) has demonstrated the efficacy of machine perfusion over SCS using a normothermic MP (NMP) system by showing a 50% lower level of graft injury with a 50% lower rate of graft discard and 54% longer mean preservation time.[7] Apart from its main objective of preserving liver grafts for liver transplantation, the technology has been suggested by Hefler et al (2020) to be applied outside its current realm including treating the grafts before implantation, extracorporeal liver support device as well as for research purposes namely in the domains of cancer and therapeutics.

The Liver4Life perfusion machine is a liver perfusion machine that sought to extend perfusion time beyond 24 hours that recapitulates additional core body functions that are critical to liver health.[8] It features a unique dual vascular supply with high-pressure, oxygen-rich arterial blood entering through the hepatic artery and low-pressure, oxygen-reduced portal vein blood draining the abdominal viscera. The hepatic artery is supplied with oxygen-rich blood at elevated pressure (mean arterial pressure (MAP) ≥ 65 mmHg) in a pulsatile manner. The portal vein receives blood at low pressure (around 5–10 mmHg) with a reduced oxygen content (venous blood, non-pulsatile). Vena caval pressure is continuously kept at physiological levels close to 0 mmHg (0–2 mmHg) to prevent liver congestion. An oxygenator provides oxygen to the hepatic artery and to the portal vein while removing CO2 from the perfusate. Parenteral nutrition and ursodeoxycholic acid are injected into the portal vein line of the perfusion machine to mimic the function of nutrients and bile salt transport *in vivo.* An integrated dialysis unit for physiologic electrolyte balance and removal of metabolic waste products from the blood It has an algorithm to automatically adjust the dialysate flow, controlling the concentration of red blood cells (haematocrit) on the basis of continuous measurements. Automated insulin and glucagon administration was used to maintain physiological blood glucose levels (targeted range of 3.5–6.5 mmol/l) in response to continuous glucose measurement. Continuous movement of the liver, in an effort to mimic diaphragm oscillations, is also integrated into the system.[8]

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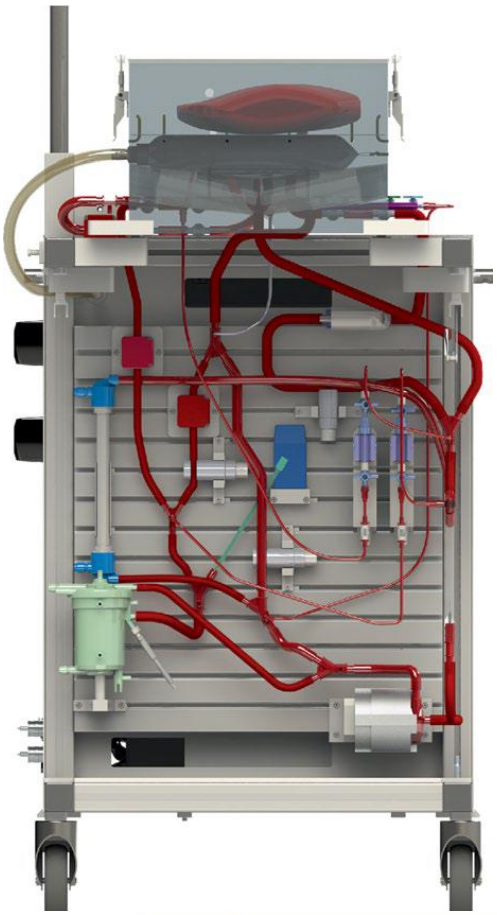

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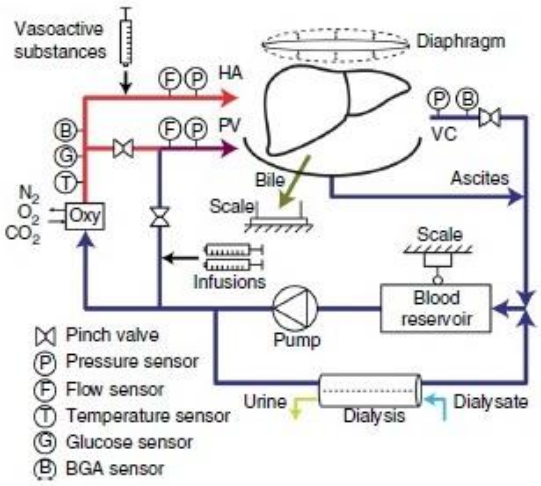

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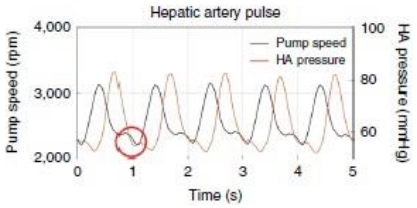

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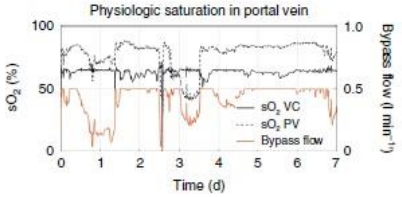

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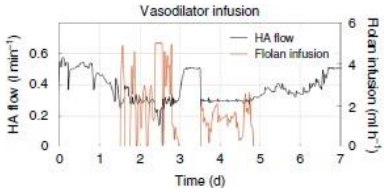

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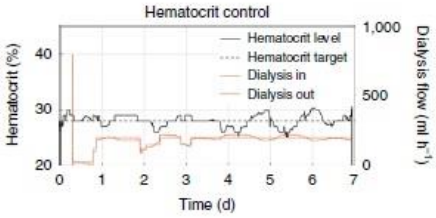

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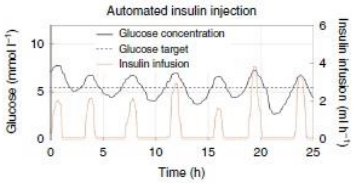

G

	Commercial perfusion machines	LIVER4LIFE Long term perfusion machine	
Liver chamber	X	X	Safe liver storage
Pump Continuous flow in PV Pulsatile flow in HA	 X X	 X X	Wave and pulse shape of flow and pressure of commercial machines not reported
Pressure/flow sensors HA PV Vena cava	 X X X	 X X X	Continuous pressure and flow monitoring
Oxygenator Gas supply Heat exchange Individual gas-supply for pH control in blood	 X X	 X X X	pH control with individual O_2, N_2, and CO_2 gas supply to oxygenator
Blood gas analysis HA PV Vena cava	X	 X X X	Monitoring of blood gases and other critical parameters during long term perfusion
Physiologic PV saturation with one oxygenator		X	Prevention of hyperoxygenation Reduction of vasoconstriction
Online glucose sensor		X	Real-time glucose monitoring
Feedback controlled infusions Insulin/glucagon		X	Automated correction of blood glucose level within predefined limits
Feedback controlled dialysis system		X	Metabolic waste removal Acid-base balance Control of sodium & electrolytes Hematocrit control
Liver movement		X	Prevention of pressure necrosis
Continuous response evaluation to vasoactiva, insulin and glucagon		X	Continuous viability assessment

Figure 1. The Liver4Life liver perfusion machine; A) the machine B) the diagrammatic representation of the machine C) The mean arterial pressure within the hepatic artery that carries oxygen-rich blood in a pulsatile manner D) the hepatic artery haemodynamic which is controlled by an automated infusion of vasoconstrictors and vasodilators E) the haematocrit level and dialysate flow F) the blood glucose level controlled by automated insulin and glucagon

administration and G) the differences between this machine and commercially available perfusion machine. (Source: Eshmuminov D, Becker D, Borrego L, Hefti M, Schuler M, Hagedorn C, Muller X, Mueller M, Onder C, Graf R, Weber A, Dutkowski P, Rudolf von Rohr P, Clavien P-A). An Integrated Perfusion Machine Preserves Injured Human Livers For 1 Week. Nat Biotechnol. 2020;38:1-10)

The details of the machine including the physiological adjustment that were incorporated into the machine are depicted in Figure 1. The machine was first tested using pig livers in which five major hurdles were identified: 1) the control of glucose metabolism, 2) prevention of haemolysis, 3) removal of waste products, 4) control of perfusate oxygenation and 5) simulation of diaphragm movements. These major hurdles were overcome using 70 healthy pig livers before the initiation of the human study.

INNOVATIVENESS

Novel, completely new	
Incremental improvement of the existing technology	√
New indication of an existing technology	

DISEASE BURDEN

Liver disease accounts for approximately 2 million deaths per year worldwide, 1 million due to complications of cirrhosis and 1 million due to viral hepatitis and hepatocellular carcinoma.[9] In terms of global mortality, cirrhosis is currently the 11th most common cause of death globally and liver cancer is the 16th leading cause of death; combined, they account for 3.5% of all deaths worldwide.[10] Cirrhosis is within the top 20 causes of disability-adjusted life years and years of life lost, accounting for 1.6% and 2.1% of the worldwide burden.[10] About 2 billion people consume alcohol worldwide and upwards of 75 million are diagnosed with alcohol-use disorders (AUDs) and are at risk of alcohol-associated liver disease.[11] Approximately 2 billion adults are obese or overweight and over 400 million have diabetes; both of which are risk factors for non-alcoholic fatty liver disease and hepatocellular carcinoma.[12,13] The global prevalence of viral hepatitis remains high, while drug-induced liver injury continues to increase as a major cause of acute hepatitis. Liver transplantation is the second most common solid organ transplantation, yet less than 10% of global transplantation needs are met at current rates[10]. Though these numbers are sobering, they highlight an important opportunity to improve public health given that most causes of liver diseases are preventable. Table 1 and 2 below illustrated the burden of liver diseases according to its global mortality and morbidity. Liver cirrhosis, the final common pathological pathway (inflammation occurs first, followed by fibrosis, and eventually extensive scarring causes the liver to shrink and hardened) of liver damage arising from a wide variety of chronic liver disease and a well known risk factor for liver cancer, can ultimately lead to end stage liver disease (ESLD), with liver transplantation being the definitive treatment for it. In Malaysia, 460 cirrhotic patients were identified in a cross sectional study done at a local university hospital from April 2006 to May 2009. The most common etiology for Malays and Chinese were Hepatitis B infection; alcohol consumption was the predominant cause in Indians.[14]

Organ transplant has been practised in Malaysia since 1970s. However, it took two decades for Malaysia to officially recognise the importance of a structured effort relating to organ donation when the National Transplant Centre was established in 1997 at Hospital Kuala Lumpur. Since then, numerous types of organ transplantation have been performed in Malaysia.[15] Table 3 shows the number of transplant surgeries that has been carried out in Malaysia since 1975 whereas Table 4 shows how many was performed in 2020. As of today, there are still 8 patients

waiting for liver transplant surgery (Table 5). There are 2 public hospitals currently performing liver transplantation in Malaysia namely Hospital Selayang and Hospital Tunku Azizah, Kuala Lumpur (paediatric).

Table 1. Global mortality related to liver disease and liver cancer (Source: Global Health Estimates 2015: Death by Cause, Age, Sex, by County and by Region, 2000-2015. Geneva, World Health Organization; 2016)

	Cirrhosis and the liver				Hepatocellular Carcinoma
	Global rank	Deaths (000s)	% of total deaths	CDR (per 100,000 population	Deaths (000s)
World	11	1,162	2.1	15.8	788
East Asia & Pacific	13	328	2.0	14.4	547
Europe & Central Asia	17	115	1.2	12.7	78
Latin America & Caribbean	9	98	2.7	15.6	33
Middle East & North Africa	8	77	3.5	18.2	24
South Asia	10	314	2.5	18.0	38
Sub-Saharan Africa	16	179	1.9	17.9	42

CDR, Crude Death Rate

Table 2. Global morbidity related to chronic liver disease 2015 (Source: Global Health Estimates 2015: Disease burden by Cause, Age, Sex, by County and by Region, 2000-2015. Geneva, World Health Organization; 2016)

	DALYs (000s)	Rank	% DALYs	DALYs per 100,000 population	Rank	YLLs (000s)	% YLL	YLLs per 100,000 population
World	41,486	16	1.6	565	12	40,986	2.1	558
WHO African Region	7,242	18	1.2	732	17	7,195	1.3	727
WHO Region of the Americas	4,890	17	1.8	496	10	4,826	2.7	489
WHO South-East Asia Region	15,581	13	2.2	808	10	15,450	3.0	801
WHO European Region	3,608	>20	<1.3	n/a	12	3,502	1.7	385
WHO Eastern Mediterranean Region	3,409	17	1.4	530	11	3,371	1.8	524
WHO Western Pacific Region	6,518	19	1.3	351	11	6,407	2.0	345

DALY, disability-adjusted life years, YLL, years of life lost

Table 3. Number of transplant surgery according to organ in Malaysia from 1975 to 2020. (Source: National Transplant Resource Centre, Malaysia)

Organ	Number
Kidneys	2278 (618 cadaveric; 1660 living-related)
Liver	170 (88 cadaveric; 71 living-related; 11 living non-related)
Heart	28
Lungs	6
Heart & Lungs	6

Table 4. Number of transplant surgery according to organ in Malaysia in 2020. (Source: National Transplant Resource Centre, Malaysia)

Organ	Number
Kidneys	154 (41 cadaveric; 113 living-related)
Liver	24 (17 cadaveric; 7 living-related)
Heart	0
Lungs	0
Heart & Lungs	1

Table 5. Number of patients in the waiting list according to organ. (Source: National Transplant Resource Centre, Malaysia)

Organ	Number
Kidneys	5,012
Liver	8
Heart	7
Lungs	0
Heart & Lungs	0

CURRENT OPTIONS FOR PATIENTS

The main challenge for organ preservation is to maintain the viability and function of the organ in the absence of an adequate blood supply, metabolic waste removal, and physiological stimulation. Apart from this, the ischaemia-reperfusion injury (IRI) is also an important risk factor for both acute rejection and long-term graft outcome.[16] According to Salehi et al (2018), ischemia occurs as a consequence of the shortage of oxygen and glucose. In turn, cells switch to the less energy-efficient anaerobic respiration in response to oxygen deficits, intracellular accumulation of metabolites such as lactic acid, and acidic changes in cellular pH. ATP becomes rapidly depleted within the cells resulting in a shift to adenosine monophosphate (AMP) as the predominant nucleotide. Elevated levels of reactive oxygen species (ROS) during ischemic time led to the disruption of lipids, lipoproteins, and cellular membranes as well as the accumulation of intracellular calcium. Consequently, additional ROS is generated through the hypoxia-induced factor-1α-mediated pathway. ROS generation and electrolyte imbalance damage mitochondria and the proteins of the oxidative chain. When blood flow is re-established to the ischemic tissue (*i.e.,* ischemia-reperfusion), a multitude of physiological reactions occur. ROS are widely recognized as important mediators of post-reperfusion induced organ injury.

There are basically two modes of organ preservation prior to being used worldwide: the static cold storage (SCS) and machine perfusion (MP). **Static cold storage** offers a simple and effective way to preserve and transport organs and is the most used method. However, several limitations are associated with SCS, including tissue damage induced by prolonged hypothermic preservation, difficulty in assessing donor organ function and viability, inevitability of ischemia-reperfusion injury, and limited opportunity for organ repair.[17] Recently, the growing use of marginal organs from extended criteria donors has led to an emergence of MP or *ex vivo* lung perfusion (EVLP) to assess donor lung function[18,19]. In addition to being an excellent graft assessment tool, EVLP has also shown potential for enabling graft repair, reconditioning, and immunomodulation[20], which inspired similar research and clinical applications in other organ systems particularly the liver.[21] Over the last decade, machine perfusion technology has been investigated to improve the quality of marginal liver grafts based on the encouraging experimental data and results from the clinical experiences.[22] The most profound impact of

normothermic machine liver perfusion (NMLP) is derived from this technology's unique ability to assess viability during storage.[23]

The main difference between MP devices is the temperature at which the organ is perfused. With **hypothermic machine perfusion** (HMP), the graft is kept as cold as 4°C, similar to SCS.[24] The suggested benefit over SCS is improvement of microcirculation by mobilization and dilution of metabolic waste. HMP does not provide supplemental oxygen, whereas its oxygenated alternative is referred to as hypothermic oxygenated perfusion (HOPE). HOPE typically perfuses at a slightly higher temperature (10–12°C), though both use preservation solutions, such as University of Wisconsin (UW) solution or Vasosol (a modification of UW solution containing additional vasodilatory and antioxidant agents), as the perfusate.[25] The oxygen supplied in HOPE is only that dissolved in the perfusate (i.e., there is no additional oxygen carrying molecule). A disadvantage of the hypothermic approach is that the low metabolism at these temperatures makes assessment of functional viability more challenging. **Sub-normothermic machine perfusion** (SMP) maintains the graft at a warm, but still sub-physiologic temperature, typically between 25°C and 34°C.[26] Similar to HOPE, the graft is perfused with an oxygenated preservation solution. It essentially attempts to find the middle ground between HOPE and NMP. At this temperature, the graft may still benefit from lower metabolic demand, while retaining enough metabolic function to allow for viability testing. **Normothermic machine perfusion** (NMP), by contrast, aims to target an environment as close to physiologic as possible. The graft is kept between 35–38°C and the perfusate contains erythrocytes, either from stored blood products (for human grafts) or diluted whole blood, in the case of animal models. NMP seeks to minimize cold ischemic time and maintains a liver that is fully metabolically active, which allows for accurate functional viability assessments, such as via glucose metabolism and bile production. A recent study has shown that human livers can be stored at −4°C with supercooling with a new multi-temperature perfusion protocol, effectively extending the *ex vivo* life of the organ by 27 hours as depicted in Figure 2. Sub-zero preservation has the potential to extend the organ storage limits, as the metabolic rate halves for every 10°C reduction in temperature thereby reducing organ deterioration rate. Supercooling has the major advantage that it allows preservation at high sub-zero storage temperatures, while avoiding phase transitions and consequent lethal ice-mediated injury, as well as toxicity of most common cryopreservatives.[27] A recent systematic review and meta-analysis of machine perfusion vs SCS on liver transplantation outcomes found that MP to be superior to CS on improving short-term outcomes for human liver transplantation, with a less clear effect in the longer term.[28] Jia et al (2020) also added that hypothermic machine perfusion but not NMP conducted significantly protective effects on early allograft dysfunction (EAD) and biliary complications.

As of today, *ex vivo* perfusion (EVP) technology is already commonly used for kidneys in some health care system.[29] Driven partly by urgent need, and partly by the continued convergence of engineering with medicine, improved generations of these machines are emerging for other organs, too, including livers, lungs, and hearts as depicted in Table 6. Most devices, for organs other than the kidneys, are being used in a limited capacity and experimental in nature. Most studies of EVP technologies have been small, single-centre trials focused on proving the safety and feasibility of the method, and on its ability to resurrect extended-criteria (suboptimal) organs to achieve similar outcomes as acceptable organs on SCS. It is worth noting that the global perfusion systems market is expected to reach USD 183.8 million by 2025 driven by the increased incidence of vital organ failure.[30] Table 7 shows selected currently available liver machine perfusion systems that are in the market.

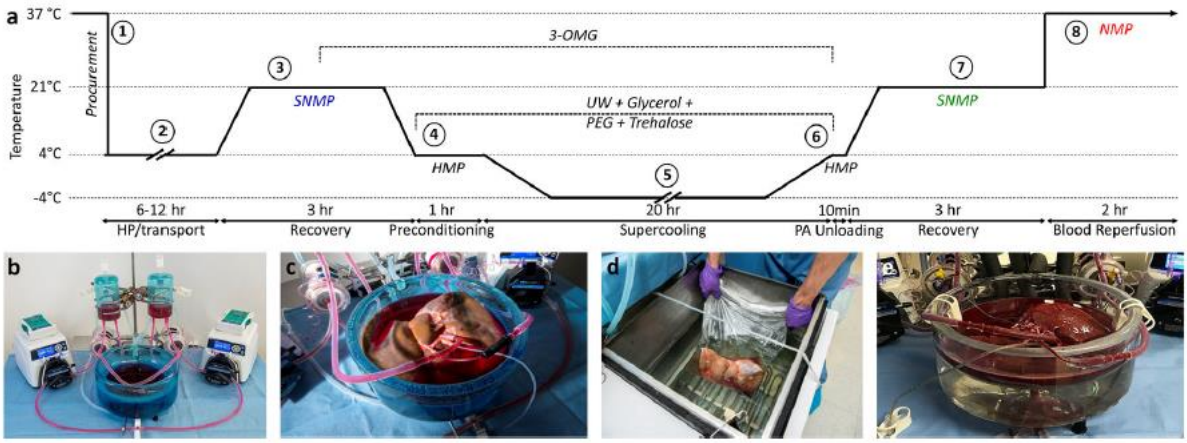

Figure 2. The study protocol of de Vries et al (2019) which involve 8 steps; 1) Human livers were procured in standard fashion and 2) transported under hypothermic preservation (HP) conditions. 3) Upon arrival, the grafts were recovered from the incurred warm and cold ischemia under 3 hours of subnormothermic machine perfusion (SNMP). The perfusate was supplemented with 19.42 g/L (200 mM) 3-O-methyl-d-glucose (3-OMG) during the last hour of perfusion. 4) At the end of SNMP, the perfusion temperature was lowered gradually, which was followed by hypothermic machine perfusion (HMP) with University of Wisconsin solution (UW) supplemented with 50 g/L (1.43 μM) 35kD polyethylene glycol (PEG), 37.83 g/L (100 mM) trehalose dihydrate and 125.7 g/L (1.36 M) glycerol. 5) Following preconditioning with the protective agents, the livers were supercooled and stored free of ice at −4°C for 20 hours. 6) After supercooling, the protective agents were gradually washed out, and 7) the livers were recovered by SNMP, identical to pre-supercooling conditions except addition of Trolox to the perfusate and absence of 3-OMG and cooling at the end of SNMP. 8) Three livers were additionally reperfused with non-leukoreduced red blood cells and plasma at 37°C as a model for transplantation. (**b**) Machine perfusion system. (**c**) Liver during SNMP recovery. (**d**) Liver in supercooling basin of the chiller. (**e**) Normothermic reperfusion with blood of the supercooled liver. (Source: de Vries RJ, Tessier SN, Banik PD, Nagpal S, Cronin SEJ, Ozer S, Hafiz EOA, van Gulik TM, Yarmush ML, Markmann JF, Toner M, Yeh H, Uygun K. Supercooling extends preservation time of human livers. Nat Biotechnol. 2019;37(10):1131-1136)

Table 6. Selected companies which are developing *ex-vivo* perfusion system and their stage of development (Source: Senior M. Beating the organ clock. Nat Biotechnol. 2018;36(6):488-492)

Company name	Production Description	Stage of development
XVIVO Perfusion	XPS normothermic EVP machine for lungs, non-portable	CE-marked and FDA approved for initially unacceptable donated lungs
Organ Assist	KidneyAssist, LiverAssist, LungAssist: variable-temperature devices, mobile (on wheels) but non-portable	CE-marked
Organ Recovery System	LifePort Kidney Transporter, LifePort Liver Transporter; hypothermic machine preservation technology, portable	Kidney Transporter FDA approved (2003) and CE-marked (2004), globally commercialized; Liver Transporter pending approval
TransMedics	Organ Care System (OCS): normothermic, portable devices for lung and hearts	CE-marked and used in transplant centres in Europe, Canada, and Australia. Lung system received FDA premarket approval in April 2018 for standard double-lung transplants. Liver system initiating trials.
Lung Biotechnology (United Therapeutics)	Lung repair centres (Silver Spring, Maryland) and planned for Jacksonville, Florida	Repair centres involved in a clinical trial where organs are sent in on ice, perfused using Toronto EVLP system for up to six hours, and recooled for shipment to the recipient.
OrganOx	The metra: portable, normothermic *ex vivo* perfusion device for livers	CE-marked; US/Canada trials on-going
Tevosol	Portable, warm perfusion technology for lungs, hearts, livers, and kidneys	Early development
XOR Labs	Standardized and scalable EVP machines for use at any temperature	Early development

Table 7. List of selected commercialized Liver Perfusion system (Source: Salehi S, Tran K, Grayson WL. Advances in Perfusion Systems for Solid Organ Preservation. Yale J Biol Med. 2018;91(3):301-312)

Brand	Unique features	Status
LifePort Liver Transporter (Organ Recovery System)	• Uses preservation solution • Hypothermic perfusion • Monitors: temperature, flow rate, vascular resistance, and • pressure • System is reusable, perfusion kit is single use • Small, lightweight, transportable	NCT0348445[31,32] - Study is ongoing
Liver Assist (Organ Assist)	• Uses preservation solution • Temperature controllable 10-38°C • Oxygenates solution • Outputs flow, temperature, and pressure readings • Allows for sampling of perfusate and bile	Hypothermic (HOPE-ECD-DBD trial)[33] - Multicentre randomised controlled trial investigating the specific effects of Hypothermic oxygenated machine perfusion (HOPE) on extended criteria donor (ECD) organs in donation after brain death (DBD) transplantation - Completed in September 2020 but not published yet Combo (Hypothermic & Normothermic)[34] - Prospective clinical trial to evaluate sequential hypothermic and normothermic machine perfusion (NMP) as a tool to resuscitate and assess viability of initially declined donor livers to enable safe transplantation - Sixteen livers underwent DHOPE–COR–NMP - During NMP, all livers cleared lactate and produced sufficient bile volume, but in 5 livers bile pH remained <7.45. The 11 (69%) livers that met all viability criteria were successfully transplanted, with 100% patient and graft survival at 3 and 6 months. Introduction of DHOPE–COR–NMP increased the number of deceased donor liver transplants by 20%. Normothermic[35] - First series of five transplants with rejected livers following viability assessment by normothermic machine perfusion of the liver - The median (range) total graft preservation time was 798 (range 724–951) min - The transplant procedure was uneventful in every recipient, with immediate function in all grafts. - The median in-hospital stay was 10 (range 6–14) days - All recipients are well, with normalized LFTs at median follow-up of 7 (range 6–19) months
OrganOx metra (OrganOx)	• Uses blood • Normothermic perfusion • Maintains oxygen in perfusion • Measures pO2, pCO2, pH, temperature, glucose, bile production • Console is reusable but has a sterile disposable portion for single use • Large, but transportable	Birmigham VITTAL study[36] - To determine if a declined liver is viable using normothermic machine liver perfusion - Thirty one livers were enrolled and assessed by viability criteria based on the lactate clearance to levels ≤2.5 mmol/L within 4 hours. The viability was achieved by 22 (71%) organs, that were transplanted after a median preservation time of 18 h, with 100% 90-day survival - During the median follow up of 542 days, 4 (18%) patients developed biliary strictures requiring re-transplantation - This trial demonstrates that viability testing with NMP is feasible and in this study enabled successful transplantation of 71% of discarded livers, with 100% 90-day patient and graft survival COPE European Trial[37] - Two-armed, randomized controlled trial to test efficacy of machine perfusion against conventional cold storage in liver transplantation

		- 220 liver transplantations, with 120 to machine perfusion and 100 to SCS - 50% lower level of graft injury, measured by hepatocellular enzyme release - 50% lower rate of organ discard - 54% longer mean preservation time (11 h 54 min versus 7 h 45 min; *P* < 0.001) Edmonton Trial[38] - Non-randomized pilot study was performed at a single liver transplant site to evaluate outcomes of livers transported from distant retrieval sites in SCS with delayed initiation of NMP (back-to-base) compared with locally procured livers with immediate initiation of NMP after retrieval (local NMP) - The 30-day patient and graft survival in the back-to-base and local NMP groups were both 100% (primary outcome: safety). Despite significantly prolonged mean cold ischemia time (6 versus 3.2 hours; *P* = 0.001), the back-to-base livers demonstrated no difference in graft function, incidence of complications, or graft and patient survival. - the back-to-base approach was safe, did not compromise the overall benefit of NMP, and offers a practical alternative to portable normothermic ex situ machine transport Ongoing Trials[39] - USA multi-centre RCT (NCT02775162) - Toronto trial (NCT02478151)
OCS Liver (Transmedic)	• Uses donor blood + solution mix • Normothermic Perfusion 34-37°C • Maintains oxygen in perfusion • Measures lactate in perfusate and bile production for evaluation • Enables ultrasound assessment and blood sampling • Console is reusable, but perfusion set is one-time use • Large but transportable	OCS Liver PROTECT Trial[40] - two-armed, multicentre, prospective, randomized, controlled pivotal trial to evaluate the effectiveness and safety of the machine perfusion to preserve and assess donor livers intended for transplantation - 300 patients, with 153 patients randomized to transplantation using the OCS Liver and 147 patients randomized to the control group, which used cold storage methods - Primary effectiveness endpoint ○ significantly lower incidence of early allograft dysfunction (EAD) compared to control (17.3% OCS vs. 30.5% Control p=0.009) across both the donors after brain death (DBD) and donors after circulatory death (DCD) cohorts in the trial - Secondary effectiveness endpoints ○ able to maintain a near physiologic functioning state and monitor the condition of the liver outside of the human body; patient survival at 30-days post-transplant was high and non-inferior to control (99.3% OCS vs. 99.3% Control p=0.0004) ○ significantly lower incidence of ischemic cholangiopathy complications at 6 months post-transplantation (1.4% OCS vs. 8.5% Control p=0.005) - Safety endpoint ○ Average liver graft-related serious adverse events (SAEs) per patient observed using the OCS Liver was non-inferior to Control (0.046 OCS vs. 0.075 Control p<0.0001). ○ Out of 155 donor livers, including both DBD and DCD, were instrumented on the OCS Liver, of which 152 were successfully transplanted, yielding a 98.1% utilization rate

POTENTIAL IMPACT OF TECHNOLOGY

Clinical impact

Before a human study can be carried out, several substantial adjustments to the machine had to be made to successfully transfuse human livers.[8] First, in contrast to pig liver perfusion with autologous fresh donor pig blood, the perfusate used for human livers consisted of preserved packed human blood products with altered electrolyte levels (for example, high potassium (>10 mmol) and low pH (<7.0)). The electrolyte levels and pH were corrected through an integrated dialysis before starting perfusion. Second, pig and human livers disclosed differences in the sensitivity toward glucagon, with the need to adjust glucagon levels (0.11 U ml−1 in human versus 0.01 U ml−1 in pigs). Third, the vasodilator nitroprussiat used in pigs is not approved for human use in Switzerland and was therefore replaced by epoprostinolium (Flolan, GlaxoSmithKline). Fourth, the final human protocol includes ursodeoxycholic acid, which is commonly used in the clinic, in place of taurocholic acid derivatives for bile flow stimulation. Ten human livers that had been declined for transplantation by all hospitals in Switzerland, then in all Europe owing to poor quality were used in the human trial. They were connected to the ex vivo perfusion machine through the hepatic artery, portal vein, vena cava and bile duct. The perfusion was conducted using a blood-based perfusate, reconstituted from packed erythrocytes, fresh frozen plasma, albumin, and platelets at 34°C owing to protective effects at this temperature. To assess the livers during machine perfusion, several parameters were measured. In the perfusate, hepatocellular enzymes (alanine aminotransferase (ALT) and AST), signalling proteins (damage-associated molecular patterns (DAMPs)), uric acid, the pro-inflammatory cytokine interleukin-6 (IL-6), the anti-inflammatory cytokine interleukin-10 (IL-10) and clearance of ammonia and lactate were measured. We also measured liver tissue energy sources, such as adenosine triphosphate (ATP) and glycogen in tissue were also measured. Daily liver tissue samples for histology were also obtained.

For injury markers and DAMPs, six out of the ten human livers (Liver 1 to 6) tested showed a decrease in injury and inflammation markers and DAMPs (ALT, AST, IL-6, IL-10 and uric acid) whereas the other four livers failed to show any improvement. The six livers (Liver 1 to 6) were also successfully maintained viable for 1 week (the rest failed to reach the objective and showed on-going cell death and signs of liver failure). Histological analysis of the livers before insertion into the perfusion machine revealed cirrhosis (n=1), fibrosis grade 2-3 (n=3), macrosteatosis (>25%, n=1; >50%, n=1), inflammatory infiltrates (n=4) and necrosis (n=1). Under haematoxylin and eosin staining, injuries were observed in Liver 1 to 6 until the second day of perfusion and declined continuously until day 7. After 1 week of perfusion, liver biopsies did not show evidence of substantial cell death in Liver 1 to 6. A PET-CT demonstrated preserved metabolism with absence of relevant non-perfused areas in places of contact with the silicon mat after 1 week of perfusion. Livers 1 to 6 generally had endothelial cells with intact structure as assessed by immunohistochemistry of von Willebrand factor and mRNA level detection of intercellular adhesion molecule 1 (ICAM1). In terms of liver function assessment, all perfused livers demonstrated considerable biological functions during ex vivo perfusion. They continuously consumed oxygen with increasing cellular ATP synthesis as compared to baseline values. Also, BUN production, lactate clearance and maintenance of albumin level were present in all livers. Nine livers showed ammonia clearance and synthesis of coagulation factor V and seven of ten livers produced bile continuously with effective bilirubin clearance. The perfusate was never exchanged during the entire course of perfusion in all cases. Additional blood products were not added except for liver 5, where there was substantial loss of the perfusate owing to a leak in the oxygenator. As for the haemodynamic response to vasoactive substances, with a flow of 1 l/min, the portal pressure could be maintained in physiological range (<10 mmHg) in each liver except in two livers (livers 1 and 3) that had pre-existing fibrosis and cirrhosis (portal vein pressure 12–

15 mmHg), a typical feature observed in the clinic with patients suffering from injured parenchyma. For livers 1 to 6, flow rates in the hepatic artery remained constant with infusion of vasoactive substances. The hepatic artery response to vasoactive substances is therefore a valuable sign of organ viability. Figure 3 and 4 illustrate the parameters which were measured in this study.

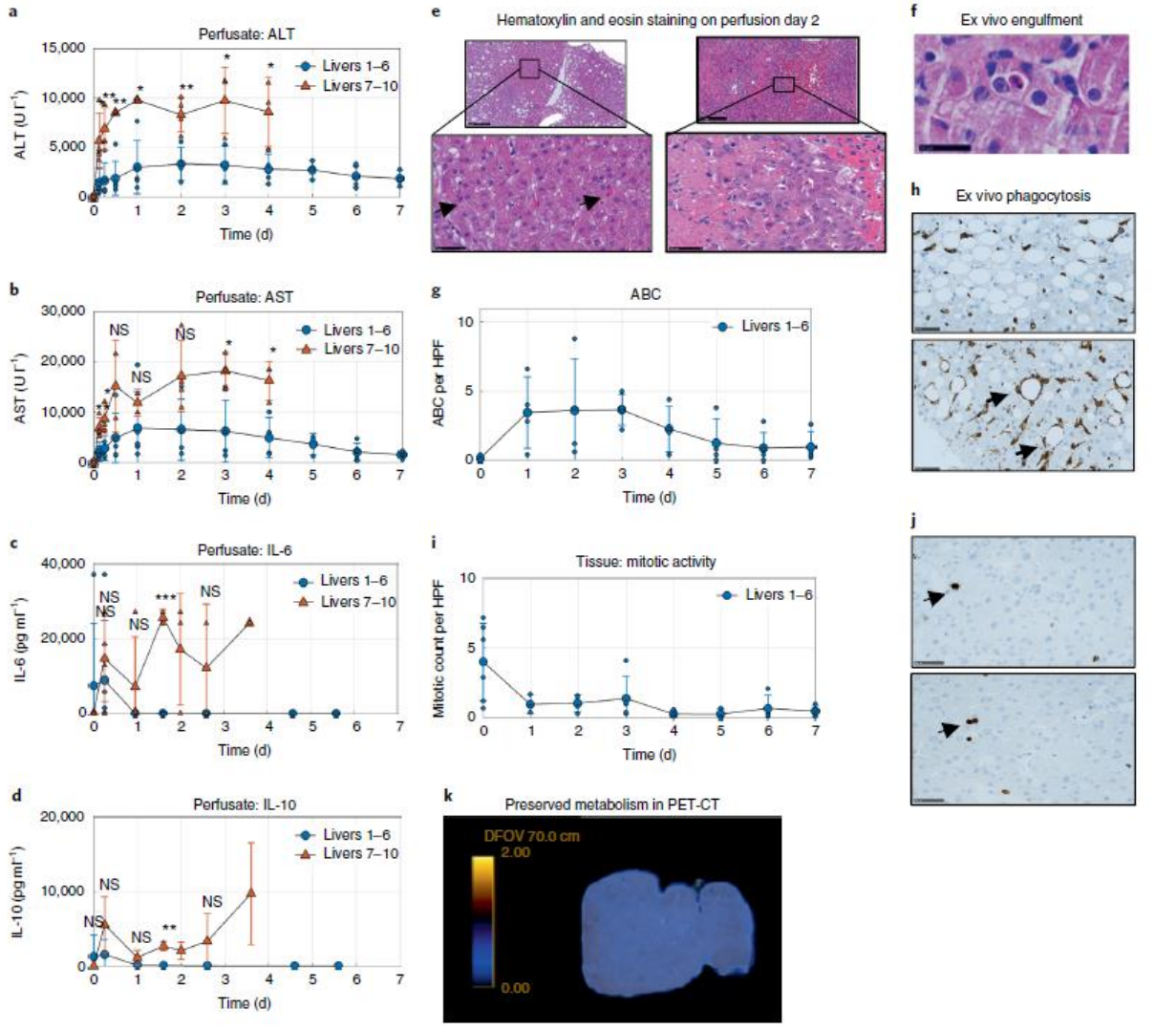

Figure 3. Injury markers in perfusate and tissue during ex vivo human liver perfusion (*n* **=** 10 livers). **a**, **b**, Release of injury markers into perfusate with ALT (**a**) and AST (**b**) levels. Human livers 1 to 6 (blue line, *n* = 6 livers) and human livers 7 to 10 (red line, *n* = 4 livers). **co**d, Cytokine release shown for pro-inflammatory IL-6 (**c**; *n* = 9) and anti-inflammatory IL-10 (**d**; *n* = 9. **e**, Haematoxylin and eosin staining on day 2. Left, representative haematoxylin and eosin staining showing apoptotic bodies (black arrows) in livers 1 to 6. Right, representative haematoxylin and eosin staining showing massive cell death in livers 7 to 10; the higher magnification shows some hepatocytes that are still viable. Scale bars: slide overviews, 250 µm; higher magnifications, 50 µm. **f**, Representative image showing engulfment of an apoptotic body by hepatocytes (black arrow) in livers 1 to 6. Scale bar, 50 µm. **g**, Apoptotic body count (ABC) seen on haematoxylin and eosin staining (per HPF) in livers 1 to 6, **h**, Top, representative slides showing phagocytosis with CD68+ immunohistochemistry for liver macrophages (livers 4 and 5). Bottom, before reperfusion, fat vacuoles are not phagocytized. Phagocytosis of released fat vacuoles by macrophages (lipopeliosis) on day 4 in a steatotic liver (black arrows). Scale bar, 50 µm. **i**, Mitotic count (pH3+ hepatocytes) per HPF in livers 1 to 6. **j**, Representative slides from two grafts demonstrating nuclei of pH3+ hepatocytes (seen only during mitosis) on day 4 (black arrows) from livers 1 to 6. Scale bars, 50 µm. **k**, Representative image demonstrating radioactive glucose uptake in PET-CT as a sign of preserved metabolism after 1 week of perfusion in a human liver (standardized uptake value $(SUV)_{max}$ 1.15, SUV_{mean} 0.64). Injected dose of 22 MBq of radioactive glucose and uptake time of 78 min. Notably, there was no sign of necrosis on areas of contact with the silicon mat in the PET-CT images (*n* = 1, liver 6). (Source: Eshmuminov D, Becker D, Borrego L, Hefti M, Schuler M, Hagedorn C, Muller X, Mueller M, Onder C, Graf R, Weber A, Dutkowski P, Rudolf von Rohr P, Clavien P-A). An Integrated Perfusion Machine Preserves Injured Human Livers For 1 Week. Nat Biotechnol. 2020;38:1-10)

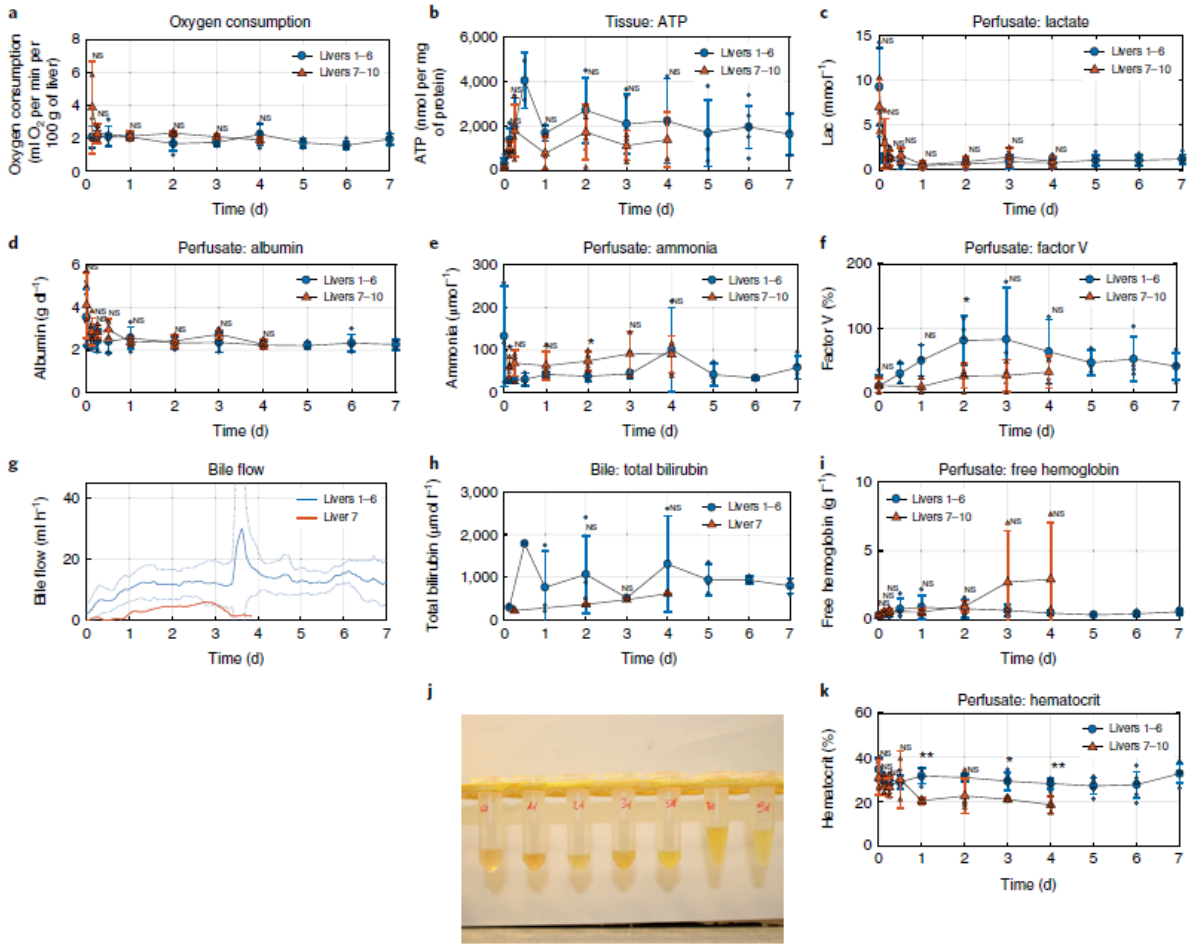

Figure 4. Liver function during human liver perfusion (*n* **=** 10 livers). **a**, **b**, Oxygen consumption (**a**), and ATP levels (**b**). Human livers 1 to 6 (blue line, *n* = 6 livers), human livers 7 to 10 (red line, *n* = 4 livers). No significant difference in liver functions was observed between the groups. **c**, Human blood products have high lactate at delivery (0 time). Lactate was cleared shortly after perfusion start by all the livers. **d**–**f**, Livers maintained a physiologic albumin level (**d**), cleared ammonia (**e**) and produced coagulation factor V (**f**). **g**, Bile flow was present constantly in livers 1 to 6, while in livers 7 to 10 only one liver disclosed bile flow (data shown are mean (solid blue lines) with s.d. (dotted blue lines) for livers 1 to 6). **h**, Clearance of bilirubin into bile. **i**, Haemolysis rate. Free haemoglobin in livers 1 to 6 was maintained at a low level (*n* = 4 human livers) or was reduced during perfusion (*n* = 2 human livers). Livers 7 to10 showed, although not significantly, increasing levels of free haemoglobin. Small error bars for livers 1 to 6 were not plotted on the presented scale after day 4. **j**, There was no macroscopic sign of haemolysis on a representative image of the daily centrifuged perfusate (plasma) from livers 1 to 6. **k**, Constant haematocrit level for 7 d without exchanging perfusate. (Source: Eshmuminov D, Becker D, Borrego L, Hefti M, Schuler M, Hagedorn C, Muller X, Mueller M, Onder C, Graf R, Weber A, Dutkowski P, Rudolf von Rohr P, Clavien P-A). An Integrated Perfusion Machine Preserves Injured Human Livers For 1 Week. Nat Biotechnol. 2020;38:1-10)

Safety

To test *in vivo* reperfusion injury and viability after a 7-d perfusion, the investigators transplanted three livers in recipient pigs of similar size.[8] As controls, five livers that had been kept only for 2–3 hours with static cold storage before the procedure. The aim of these experiments was to confirm feasibility of vascular anastomoses and to investigate the early phase of reperfusion after long-term perfusion. All transplant experiments were terminated 3 hours after transplantation under general anaesthesia because survival experiments with cessation of general anaesthesia were prohibited by the local animal protection authorities. Therefore, the

main endpoints of these transplantation experiments remained early markers of reperfusion injury, including release of transaminases and histology. These results indicated no difference between release of liver enzymes and histology as compared to controls livers that had been transplanted after standard cold storage without long-term preservation in the machine perfusion system.

Organizational

The usual organizational issues related to the procurement, preservation and transplantation of solid organs being faced by any transplantation programs are postulated to occur as well. The team will also need to be well-trained with clear protocols to be followed.

Cost

No specific cost was mentioned in the article. However, a cost-utility study[41] which was carried out using OrganOx metra, a portable device intended to preserve and maintain the donated liver in normothermic conditions for up to 24 hours between the point of retrieval and transplantation, was found to be more cost-effective than the current practice of static cold storage. The total costs per patient were £37,370 versus £46,711 and the total effectiveness per patient was 9.09 QALYs versus 10.27 QALYs for SCS and OrganOx metra groups, respectively. The estimated ICER was £7,876 per each QALY gained. Results from the probability sensitivity analyses showed that use of OrganOx metra has 99% probability of being cost-effective at a £20,000 willingness-to-pay threshold. OrganOx metra led to the utilisation of 54 additional livers with patients experiencing lower rates of early allograft dysfunction and adverse events.

Societal & Ethical Issue

There was no retrievable evidence with regards to societal & ethical issues obtained on this item.

Conclusion

Static cold storage can preserve liver grafts between 12-18 hours; machine perfusion can increase the duration to up to 27 hours depending on the type of MP that are being used. The development of a perfusion machine which can preserve human livers especially those of low quality which would normally be rejected for a significantly longer period, to allow sufficient time for the organ to repair and regenerate will help in overcoming the common problems that the whole world is facing especially in the procurement and preservation of organs, thus providing more patients with liver graft. Further studies on humans to test the *in vivo* perfusion and viability of grafts is needed to ensure the desired outcome can be achieved, reducing the rate of acute rejection and improving long-term graft outcome.

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