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Review

# CRITICALITIES AND USEFULNESS OF *EX-VIVO* SMALL INTESTINE PERFUSION: TRANSPLANT AND BEYOND

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## Summary

performed in very select cases due to the organ's specific characteristics: high sensibility to ischemia, increased risk of rejection, and longer length of hospital stay in comparison to other solid organ transplants. With deteriorating donor organ quality and safety, and increasing indications for intestinal transplantation, innovative and safe alternatives to increase the donor pool should be evaluated. In this review, we present a summary of organ machine perfusion history, trends, recent findings, challenges and the potential benefits and applications for intestinal transplantation. We performed a literature review of organ machine perfusion studies published in the last decade (2012-2022) for intestine, lungs, heart, liver, kidneys and pancreas and collected data from the United Network for Organ Sharing (UNOS) database aiming to show the trends of intestinal transplantation in the US and highlight the benefits of different perfusion techniques.

**Key words**: intestinal transplant, intestine, small bowel, transplantation, machine perfusion, ex-situ, ex-vivo, organ preservation

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## INTRODUCTION

Dr. Richard Lillehei performed the first intestinal transplant (ITx) in a human in 1967. At that time, immunosuppression was limited and total parenteral nutrition was unavailable. The earliest attempts were associated with high morbidity. Newer medications, surgical techniques, and management protocols have improved the outcomes of ITx [1]. However, ITx remains a challenging surgery that is performed in very select cases due to the organ's specific characteristics: high sensibility to ischemia, increased risk of rejection, and longer length of hospital stay in comparison to other solid organ transplants [2,3]. With deteriorating donor organ quality and safety, and increasing indications for ITx, innovative and safe alternatives to increase the donor pool should be evaluated. In this review, we present a summary of organ machine perfusion history, trends, recent findings and its potential benefits and applications for intestinal transplantation.

## METHODS

We performed a literature review of organ machine perfusion studies published in english in the last decade (2012-2022) for intestine, lungs, heart, liver, kidneys and pancreas. We collected data from the United Network for Organ Sharing (UNOS) database to show the trends of intestinal transplantation in the US and provide our own group's experience with intestinal machine perfusion.

## INTESTINAL TRANSPLANT INDICATIONS AND TRENDS

The Intestinal Transplant Registry collected information from 1985 to 2012 from 82 centers around the world. There were 2,887 intestinal transplants performed in 2,699 patients. Most of these cases were performed in North-America, followed by Europe, South-America and Australia-Asia. The main indication for intestinal transplant in both adult and pediatric populations was short gut syndrome. However, the cause of short gut syndrome is variable according to the population with ischemia and gastroschisis being the main causes for short gut syndrome in adults and pediatric patients respectively [3].
According to UNOS center data, 3,305 intestinal transplants have been performed since 1990 in the U.S. (1,642 adults and 1,663 pediatrics). The majority of the transplants were performed in white/non-hispanic followed by black/non-hispanic and hispanic/latino patients. The age group with the most transplants was 1-5 years. As shown in (Fig. 1), ITx numbers have beendeclining since 2008. Along with this, there has been adecline in the total number of ITx per year in the USsince 2008. Since the COVID-19 pandemic there was a 51.1% decrease in deceased donor transplantation in the USA although most of these were due to a decrease in kidney transplants [4].

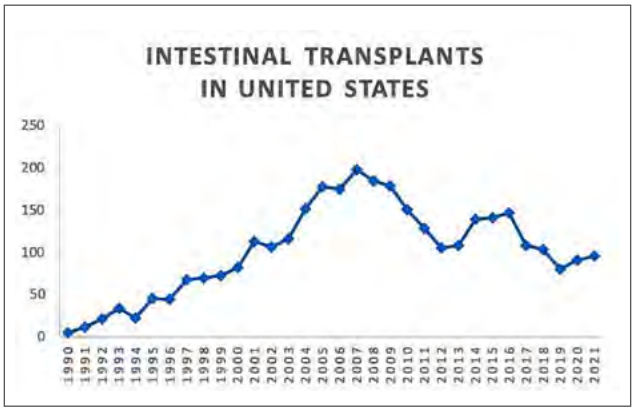


**Figure 1.** Intestinal transplant trends from 1990 to 2022 in the United States.

Along with the decrease in intestinal transplantation since 2008, the number of new patients in the waiting list for intestinal transplantation is also decreasing: 152 patients were added in 2012; 171 added in 2013; 196 in 2015; 195 in 2016; 155 in 2017. There is an increase in the waitlist from 2019 to 2021; However, the trend over the last decade has been toward fewer additions overall [5-7].

## MACHINE PERFUSION

Organ preservation developed from the primitive concept of extracorporeal circulation, which first emerged in 1812 in the monography of Cesar Julien Jean Le Gallois [95] and continued with preclinical studies in 1922 by Ernest Verney [8]. The Carrel-Lindbergh perfusion pump resulted from a collaboration between Alexis Carrel and Charles Lindbergh in 1934 [9]. Dr. Murray performed the first successful kidney transplantation in 1954, Arthur Humphries did the first machine preservation of a canine kidney followed by reimplantation in 1964 [10]. After Dr. Thomas Starzl performed the first liver transplant in 1963, he started a project of liver machine perfusion in chimpanzees with subsequent attempts of liver transplantation in 1966 [11,12]. In 1967 Dr. Belzer developed a perfusion machine that allowed perfusion of a kidney for 72 h [13] and Dr. Lillehei performed the first intestinal transplantation. In 1986 Dr. Belzer's group developed the University of Wisconsin solution which later became the standard of care for preservation [14].

## TIMELINE

1812 Le Gallois - Concept of extracorporeal circulation
1922 Ernest Verney - Artificial perfusion in canine kidney.
1934 Carrel and Lindbergh - Carrel-Lindbergh perfusion device.
1954 Murray - First kidney transplant.
1959 Lillehei - Physiology of intestinal ischemia and preservation in canine model.
1960 Belzer - Kidney perfusion device.
1964 Humphries - Machine preservation of a canine kidney.
1966 Starzl - Machine perfusion in chimpanzee liver.
1966 Lillehei - First pancreas transplant.
1967 Starzl - First successful liver transplant.
1967 Belzer - Continuous perfusion of a kidney for 72 h.
1967 Lillehei - First intestinal transplant.
1986 Belzer group - UW preservation solution.
The intestine is a hollow and colonized contaminated abdominal organ, where ischemia-reperfusion injury with the subsequent mucosal barrier damage can trig- ger bacterial translocation as well as rejection. It is one of the most ischemia-sensitive organs with, mucosal injury occurring can be observed within 1 hour of cold storage and rapidly

progressinges to subepithelial edema in 4 hours [15]. For this reason, organ transportation is a crucial intermediary step for successful ITx [16,17]. Although there have been multiple publications showing positive results in favor of machine perfusion for abdominal and thoracic organs, research on intestinal machine perfusion has been stagnant in comparison to other organs in the last decade as shown in Table I.

## CHALLENGES FOR INTESTINAL MACHINE PERFUSION AND TRANSPLANTATION

### Ischemia-reperfusion injury

The small bowel receives up to 25% of total cardiac output largely consumed in the mucosa and the submucosa to sustain its vast surface area and its high cell-turnover rate. This physiological feature of the intestine leads to the extreme vulnerability of the mucosal layer to ischemia and reperfusion injury.

Interruption of blood supply results in ischemic injury, which can rapidly damage metabolically active tissues. Paradoxically, restoration of blood flow to ischemic tissue may lead to additional damage known as reperfusion injury which frequently exceeds the original ischemic insult.

#### *Malabsorption*

It has been shown that there is an alteration of the absorptive capacity of the intestine following Ischemia-reperfusion injury [61,62]. This could lead to deficient absorption of nutrients. Sileri et al. [63] demonstrated in a rat model that ischemia-reperfusion injury of the intestine causes both acute and chronic alterations of intestinal absorptive function, which was associated with significant mortality.

#### *Bacterial translocation*

As a consequence of epithelial damage, bacterial translocation occurs when bacteria from the gastrointestinal tract passes through the epithelial mucosa to the circulation and reaching extra intestinal sites. By this mechanism bacteria can disseminate throughout the body, producing sepsis, shock, or multiple organ failure. Bacterial translocation has been reported to occur in 44% of pediatric patients undergoing small bowel transplantation. The increase in intestinal hyperpermeability occurring in ischemia-reperfusion injury of the intestine is one of the factors causing bacterial translocation [64,65].

#### *Damage to other organs*

Ischemia-reperfusion injury to the intestine results in production of molecules such as hydrogen peroxide, superoxide, and inflammatory cytokines that may harm distant organs. This leads to the development of systemic inflammatory response syndrome (SIRS), which can pro- gress to multiorgan failure [66]. Mast cells are the most abundant innate immune cells in the gut wall and they degranulate upon activation from ischemia-reperfusion injury, resulting in the release of inflammatory mediators and proteases, thereby triggering leukocyte recruitment and tissue injury [66]. Intestinal ischemia-reperfusion injury also causes pulmonary infiltration of neutrophils, which contributes to the development of acute respiratory dis- tress syndrome (ARDS) [67,68].

#### *Rejection*

Evidence shows that innate immune responses play an important role in the acute and chronic rejection of whole-organ allografts [69]. Local synthesis of complement factors within the graft, as occurs during ischemia-reperfusion injury, can also contribute to T-cell priming and shaping of the adaptive immune response that trigger graft rejection [70]. In colonized organs, such as the intestine, the crosstalk between damage associated molecular patterns (DAMPs) that arise from ischemia-reperfusion injured tissue damage and pathogen associated molecular patterns (PAMPs), that arise from the translocation of bacteria, may synergistically contribute to the development of signals that trigger alloreactivity and graft rejection [65,71].

### Preservation solutions and techniques

Folkert Belzer and James Southard from the University of Wisconsin developed a preservation solution (University of Wisconsin solution), which was the first to allow static cold preservation and transport at 4°C [11,14]. The standard of care for intestinal transplant involves *in-situ* vascular flush with either UW or histidine-tryptophan-ketoglutarate solution (HTK) solutions, followed by static cold storage and transportation. UW and HTK have been found to be comparable in terms of function. Intestinal grafts preserved in UW and HTK demonstrate no difference in graft and patient survival at 30- and 90-days posttransplant [72].

In 2007, Wei et al. [73] evaluated the potential of Polysol, a newly developed preservation solution, in cold storage of small bowel grafts, compared with the current standards, UW, Celsior and HTK and concluded that cold storage using Polysol resulted in significantly better integrity and function of small bowel grafts than UW. Polysol has also been studied for preservation of other organs in animal models with favorable results [74-76], however, Schreinemachers et al. [75] terminated a clinical trial due to early rejection of kidneys preserved with Polysol compared to UW.

A specific consideration for intestinal preservation and transplant is that compared to other allografts, this is a hollow organ. Flushing and preservation of the luminal aspect should also be considered in addition

**Table I.** 2012-2022 decade experimental studies on machine perfusion.

| Organ | Type of perfusion (normothermic *vs* hypothermic) | Model (human *vs* animal) | Type of study (clinical *vs* preclinical) | Authors |
|---|---|---|---|---|
| Intestine | Hypothermic | Human | Preclinical | Muñoz-Abraham et al. *J Gatrointest Surg* (2016) [2] |
| | Normothermic | Porcine | Preclinical | Bertacco et al. *JACS* (2016) [18] |
| | Normothermic | Rodent | Preclinical | Lysyy et al. *Transplant Proc* (2020) [19] |
| Kidneys | Normothermic | Normothermic | Clinical | Nicholson et al. *British Journal of Surgery* (2015) [20] |
| | Hypothermic | Human | Clinical | Kox et al. *Transplantation* (2018) [21] |
| | Normothermic | Human | Preclinical | Hosgood et al. *British Journal of Surgery* (2015) [22] |
| | Normothermic | Porcine | Preclinical | Hamar et al. *Transplantation* (2018) [23] |
| | Normothermic | Porcine | Preclinical | Kaths et al. *American Journal of Transplantation* (2018) [24] |
| | Normothermic | Porcine | Preclinical | Kaths et al. *American Journal of Transplantation* (2017) [25] |
| | Normothermic | Human | Clinical | Dirito et al. *American Journal of Transplantation* (2021) [26] |
| | Hypothermic | Rodent | Preclinical | Kron et al. *Transplantation* (2019) [27] |
| Liver | Hypothermic | Porcine | Preclinical | Liu, et al. *Journal of Surgical Research* (2014) [28] |
| | Hypothermic | Porcine | Preclinical | Fondevila, et al. *Transplantation* (2012) [29] |
| | Hypothermic | Porcine | Preclinical | M. C. Dirkes et al. *Artificial Organs* (2013) [30] |
| | Hypothermic | Rodent | Preclinical | Giannone et al. *Scientific World Journal* (2012) [31] |
| | Hypothermic | Porcine | Preclinical | Schlegel et al. *Hepatology* (2013) [32] |
| | Hypothermic | Human | Preclinical | Jomaa et al. *Transplantation Proc* (2013) [33] |
| | Hypothermic | Human | Preclinical | Monbaliu et al. *Liver Transplantation* (2012) [34] |
| | Hypothermic | Human | Clinical | Dutkowski et al. *Hepatology* (2014) [35] |
| | Normothermic | Human | Clinical | Ravikumar et al. *American Journal of Transplantation* (2016) [36] |
| | Normothermic | Human | Clinical | Nasralla et al. *Nature* (2018) [37] |
| | Hypothermic | Human | Clinical | Guarrera et al. *American Journal of Transplantation* (2015) [38] |
| | Hypothermic | Human | Clinical | Van Rijn et al. *British Journal of Surgery* (2017) [39] |
| | Hypothermic | Human | Clinical | Van Rijn et al. *Liver Transplantation* (2018) [40] |
| | Hypothermic | Human | Clinical | Schlegel et al. *Journal of Hepatology* (2019) [41] |
| Pancreas | Hypothermic | Porcine | Preclinical | Hamaoui et al. *Surgical research* (2018) [42] |
| | Hypothermic | Human | Preclinical | Branchereau et al. *Cryobiology* (2018) [43] |
| | Hypothermic | Human | Preclinical | Leemkuil et al. *Transplantation Direct* (2018) [44] |
| | Hypothermic | Human | Clinical | Doppenberg et al. *Transplant Int* (2021) [45] |
| | Hypothermic | Porcine | Preclinical | Prudhomme et al. *Transplant Int* (2021) [46] |
| | Normothermic | Porcine | Preclinical | Kuan et al. *Artif Organs* (2017) [47] |
| Heart | Normothermic | Human | Clinical | Van Suylen et al. *Transplant Direct* (2021) [48] |
| | Normothermic | Porcine | Preclinical | Li et al. *Artificial Organs* (2017) [49] |
| | Normothermic | Porcine | Preclinical | Zhou et al. (APM 2021) [50] |
| | Normothermic | Human | Clinical | Messer et al. *J Heart Lung Transplant* (2016) [51] |
| | Hypothermic | Porcine | Preclinical | Michel et al. *Ann Transplant* (2015) [52] |
| Lungs | Hypothermic | Human | Clinical | Van Leeuwen et al. *Transplant Direct* (2021) [53] |
| | Normothermic | Porcine | Preclinical | Olbertz et al. *Int J Artif Organs* (2019) [54] |
| | Normothermic | Porcine | Preclinical | Sommer et al. *Am J Transplant* (2018) [55] |
| | Normothermic | Porcine | Preclinical | Steinmeyer et al. *Respiratory Research* (2018) [56] |
| | Normothermic | Human | Clinical | Divithotawela et al. *Jama Surg* (2019) [57] |
| | Normothermic | Porcine | Preclinical | Kalka et al. *Int J Artif Organs* (2021) [58] |
| | Normothermic | Human | Clinical | Urban et al. *Am J Transplant* (2021) [59] |
| | Normothermic | Human | Clinical | Shamaa. *Am J Transplant* (2022) [60] |

to preservation of the intravascular component. Intraluminal preservation solutions before SCS have been introduced in an attempt to reach and protect the enterocytes and theoretically prevent fluid and electrolyte shifts. The luminal membrane can be used for the uptake of nutrients and electrolytes and the intestinal lumen provides direct access [76].

Hypothermic machine perfusion (HMP) is another mo- dality of cold preservation. Compared to SCS, HMP pro- vides continuous perfusion with cold preservation solu- tions via a perfusion machine, it can remove metabolic waste in a timely manner and provide some metabolic substrates.

To limit the damage induced by oxygen deprivation, organ preservation uses hypothermic conditions to facilitate a

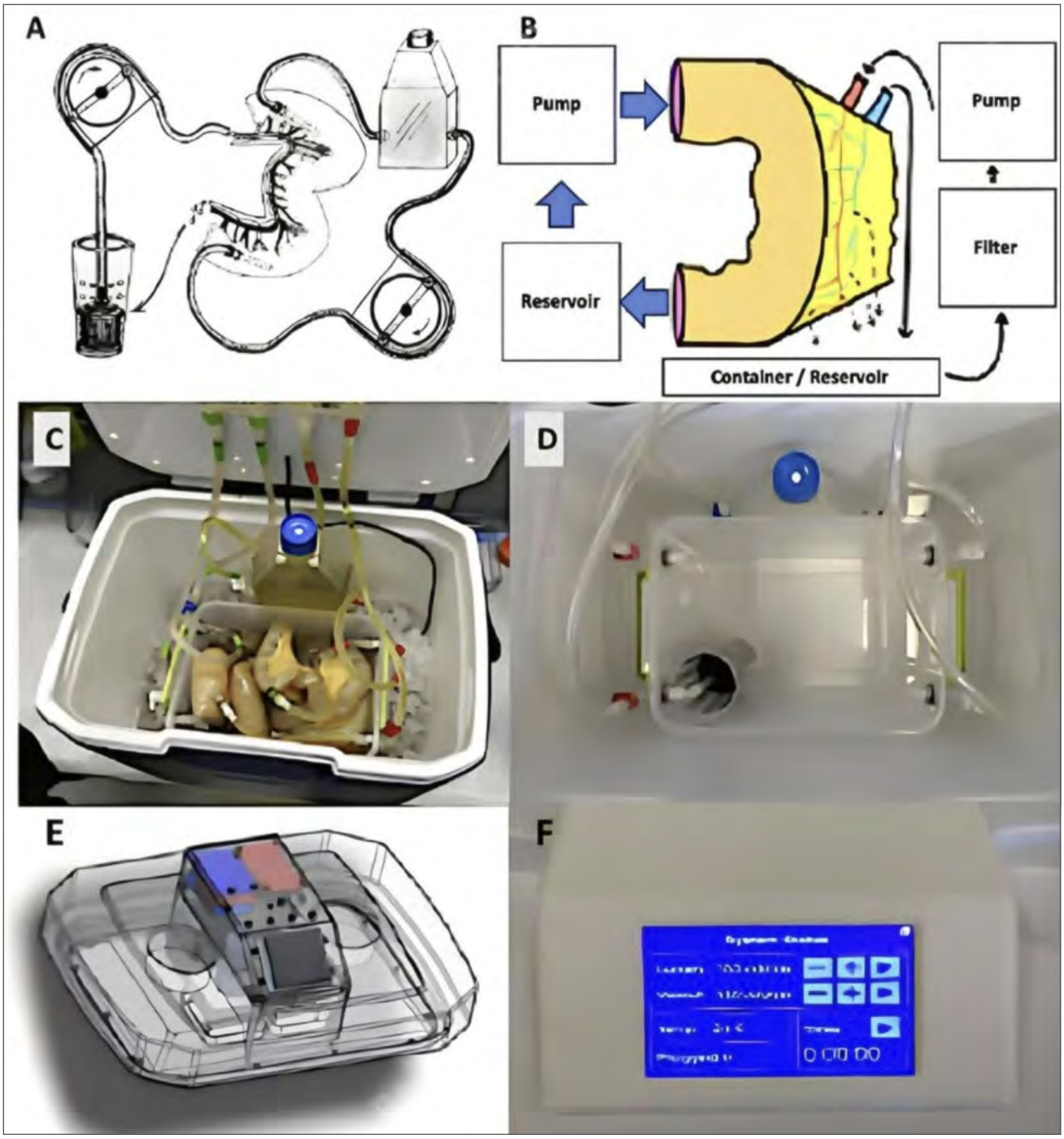


**Figure 2.** Intestinal Perfusion Unit by Munoz-Abraham, et al. [2]; A,B) blueprint showing luminal and vascular circulation and intestinal perfusion; C) interior of perfusion device with a human intestine being perfused; D) reservoir and filter inside Intestinal Perfusion Unit; E) blueprint of intestinal perfusion unit lid and pump location; F) temperature and flow velocity control (Patent: Geibel, Rodriguez-Davalos, Patron-lozano, et al; Yale University, Perfusion systems and methods of perfusing at least a portion of a small intestine. United States Patent US20160066564A).

regulated metabolic suppression. This reduces the energetic costs of preserving transmembrane electrochemical gradients and suspends the activation of apoptotic biochemical pathways. However, it can also cause certain cellular damage including a maladaptive redistribution of membrane lipids and subsequent loss of membrane integrity. Hypothermic preservation should be carefully balanced between its beneficial and detrimental effects. The

temperature at which damaging effects are minimized and protective effects maximized has shown to be around 4°C to 10°C [77,79].

HMP can better protect organ function and decrease the rate of delayed graft function [21,80]. Our group has previously provided proof of concept that hypothermic *ex-vivo* machine perfusion is feasible and can preserve intestinal grafts for extended periods with favorable pathologic results [2]. To our knowledge, this is the first and only prototype to date that has experimented with machine perfusion for both the luminal and vascular components in a human model. Previous efforts focused on perfusing solely the intestinal lumen in animal models [81,82]. A single report of *in vitro* human intestine preservation by pulsatile vascular perfusion was described in 1979 by Toledo et al. [83]. Similarly, the first attempt at hypothermic intraluminal perfusion in a rat model was described in 2003 using UW solution [84]. Despite these attempts, dual luminal-vascular perfusion has not been previously reported.

Although hypothermia reduces cell metabolism, it does not stop entirely. Continuous oxygenation during cold organ preservation might be very useful to support the remaining metabolic demand. For this reason, intraluminal gaseous insufflation has been attempted with oxygen, carbon monoxide, hydrogen and nitrogen. Oxygen insufflation was found to improve tissue energetics [84,85]. Nakao et al. [86] used CO supplementation to the UW intraluminal solution in a rodent model and demonstrated ameliorated ischemia-reperfusion injury. Hydrogen-enriched preservation solutions (UW and lactated Ringers) have shown to significantly ameliorate graft damage, reduce graft oxidative stress, maintain immune homeostasis and limit proinflammatory molecular responses [87]. Hypothermic oxygenated perfusion (HOPE) is a technique with an additional oxygen supply based on HMP and has been reported to be excellent for organ preservation [35,88].

Normothermic perfusion (NMP), in which whole blood is the main element of perfusion, has shown positive outcomes in the preservation of various organs (see Table I). Depending on the device, either rotary or peristaltic pumps circulate an erythrocyte-based preservation solution through the circuits as oxygenation/ventilation is performed on the circulating fluid. NMP also offers the capacity to perform a functional assessment of procured organs under near physiologic conditions, typically between 34°C and 39°C, this feature makes NMP not only an invaluable tool for *ex-vivo* functional evaluation, but also a highly attractive means of expanding the eligible donor pool. NMP Has been studied in animal models for abdominal multiorgan transplant where pathology of the small intestine showed the villi were preserved at 1 h, but at 6 and 12 h it showed significant damage [89].

## DISCUSSION

The organ supply from deceased donors has been insufficient to meet the growing demand despite an increase in organ donation. This has culminated in an ongoing significant organ shortage that affects patients worldwide. The consequences of the shortage include increased time spent on transplant waitlists, greater morbidity and mortality while awaiting transplant and cost increases to medically manage patients that may be best served by surgical treatment. Numerous interventions have been proposed to meet organ demands, including education programs, economic incentive strategies to encourage organ donation, applying the principle of opt-out consent, expanding the donor pool through utilization of marginal or expanded criteria donors, and improving organ procurement and preservation strategies [90].

For decades, the gold standard organ preservation strategy was static cold storage (SCS), but successes in developing machine perfusion devices, initially created to preserve kidneys, have prompted investigation into using these technologies to preserve other transplantable organs and deliver drug therapies [91].

Another function of machine perfusion is the capacity to expand the available donor pool. Organs initially classified as unfit for transplantation due to one or more risk factors can be functionally assessed ex vivo and reclassified as suitable for transplant. Machine perfusion devices can provide real time data on the function of the organ and allow surgeons to reevaluate organs. In some studies, organ preservation with machine perfusion has been associated with superior patient survival, fewer adverse events, and improved short and long-term functional outcomes compared to SCS.

The concept of treating isolated organs ex- vivo is an approach for personalized and targeted therapy that is being widely studied. Xu Jing et al. [92] reviewed multiple therapeutics that have been studied for different organs through machine perfusion including gene therapy, stem cell therapy and drug therapy with multiple agents, among other strategies. The use of these technologies is being studied to increase the organ pool and include marginal grafts; it will also allow reassessment of the organ's viability for transplantation. Additionally, the prediction of organ function during machine perfusion is being studied and will likewise be possible by detailed perfusate analysis including metabolomics and proteomics, leading to safer use of marginal grafts.

Nanoparticles have properties that make them good candidates for treatment of *ex-vivo* organs in machine perfusion. When compared to other therapies, nanoparticles have long-lasting effects and slow-release profiles that extend beyond the period of perfusion [93]. Endothelial targeted nanoparticles have been delivered in an NMP setting. With

all of the benefits of this system, NMP is emerging as a device to deliver therapies within an opportunistic window in an attempt to reduce the damage of ischemic injury [94]. Any drug that is typically delivered systemically can also be introduced during NMP for more concentrated, short term treatments. However, when treating ischemia-reperfusion injury, a more prolonged, protective action is desired, as its effects can persist long after the initial period of perfusion and after transplantation.

Regarding normothermic perfusion, there is an opportunity for different models to trial intestinal preservation mimicking the *in vivo* state. As previously discussed, the small bowel is a hollow organ and it receives its nutrients from the vasculature and from the direct absorption of nutrients by enterocytes. As proven in multiple clinical trials for the treatment of sepsis and shock, bacterial translocation is diminished when enteral feeding is used. By providing a preservation solution that would allow for enterocyte feeding and blood for the vasculature, the deleterious effects of cold preservation and bacterial overgrowth could be diminished.

In general, intestinal machine perfusion development is behind other organs mostly because with the current volumes, the industry does not consider it would be a lucrative investment to develop an intestinal specific pump, however, it has been described that customized pumps using cardiac pumps or ECMO machines could be adapted for this purpose to reduce high costs with commercial pumps [96]. Additionally, machine perfusion companies could implement a modular approach, in which one device is able to perfuse different organs. Although further research is needed to determine the cases that would benefit the most from machine perfusion and assess which perfusion techniques provide the best outcomes for each specific type of tissue/organ, in our group's experience and based on the available literature we believe machine perfusion has a role in the future of organ transplantation, and specifically for intestinal transplantation because of the characteristics and challenges described in this review.

### *Conflict of interest statement*

JPG, MIRD, RPL: hold the rights to the following patent: Geibel, et al; Yale University, Perfusion systems and methods of perfusing at least a portion of a small intestine. United States Patent US20160066564A.

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### *Authors' contributions*

SAMA, MIRD: designed the project, critical revision of the manuscript, and final approval of the version to be published; AFHM: project design, data acquisition, manuscript preparation, critical manuscript revision; BLAO: data acquisition; RPL: manuscript preparation, critical revision of the manuscript; FDA: critical manuscript revision; JPG: critical manuscript revision; DCM: critical manuscript revision; PNM: critical manuscript revision, final approval of the version to bepublished.

### *Ethical consideration*

The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki. Written informed consent was obtained from each participant/patient for study participation and data publication.

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