Abstract

The reliability of Machine Perfusion (alternative to static cold storage) for the preservation of liver for transplantation has been well investigated in experimental models, by taking into account the temperature, oxygenation, flow, pressure, and settings of the machine or proposed circuit. Machine Perfusion is considered by many researchers as a valid method for preserving organs. While circuits or machines for preservation have been described, no agreement has been reached concerning how these devices should be developed. The machines proposed to date are considered here to identify the technical and functional features necessary for a machine to have multifunctionality and adaptability to cater all the needs of preservation according to the type and features of the liver to be transplanted, including marginal livers. *The need to establish a uniform method for the use of this machine is also emphasized, in order to achieve a clinical protocol for its use .*

Introduction

In case of organ transplantation, particularly the liver, preservation method between retrieval from the donor and transplantation into the recipient is of greatest importance. Since the early times, researchers have maintained livers using a specialized perfusion system (1–4) under conditions close to physiological conditions. Attempts to develop a suitable machine were later abandoned (5–7), for 2 main reasons: technological limitations in developing a machine and the introduction of hypothermia in clinical practice. Simple cold storage (SCS) is universally used for liver preservation as it reduces liver metabolism by approximately 95% and allows organ preservation for 10–12 h. During this time, which can vary due to several parameters, including the ages of donor and recipient and the basic conditions of the liver, the liver is still subjected to hypothermic injury as well as hypoxia due to biological changes, such as damage to sinusoidal endothelial and Kupffer cells; microvascular circulation and peribiliary vascular plexus; change in ATP content, mitochondria and hepatocytes membrane properties; free radicals production; and electrolyte disturbances, that following the organ replacement, can lead to graft failure, and are generally defined as ischemia/reperfusion injury (8–10).

The technological innovation accompanying the evolution of surgical techniques along with new scientific knowledge, drugs, and optimal structuring of the organizational support necessary for transplantations, has substantially improved transplant surgery outcomes and greater public confidence, with transplantation indicated as the best therapy in cases of irreversible liver failure. These factors have, in turn, significantly lengthened waiting lists (11), with an average patient stay of approximately 1.5–2 y accompanied by a mortality rate of 6–7%. Thus, increasing availability of organs is necessary and urgent in order to cope with increasing demand for marginal livers (12). Donation after circulatory determination of death (DCDD) and steatotic livers are considered unreliable for transplantation with current conservation techniques, as their applicability necessitates preservation of the features that are partially impaired by steatosis or the initial period of warm ischemia**.** This has given new impetus to research on machine preservation techniques, and many researchers have tested the machines, especially in hypothermia (13–15), and in the resuscitation of

livers now considered marginal (16, 17). It is this very ability to expand the overall number of livers, making it usable even those currently deemed unavailable, the main objective of the use of machine perfusion.

These investigations postulate the validity of preservation by machine perfusion (MP) compared to SCS, quantified with well-defined laboratory parameters, histopathology, microcirculation state, bile production, ATP content (15, 18), and mitochondrial viability (19–21). This has led to the use of the hypothermic perfusion method for the kidneys (22), and, in case of the livers, Guarrera et al. (23) performed the first clinical series in 2010. However, along with establishing good outcome of preservation by perfusion, some issues still remain to be clarified before it is introduced to clinical practice to replace SCS. Among these are temperature, oxygenation, suitable perfusion solution, and especially the technique characteristics and technology of the machine. We discuss these aspects, with some reflection on the cost, to determine the features of an ideal MP system.

Hypothermia, subnormothermia, and normothermia

Perfusion Systems used to date have been characterized by considering temperature. Preservation by hypothermic machine perfusion (HMP) (temperature range from 2–6°C) has generally positive effects on organ viability and function due to the continuous supply of nutrients and/or oxygen, monitoring of the status of the organ, the removal (flushing) of catabolites and free radicals produced as a result of hypothermia (15), and the possibility of resuscitation of steatotic (17) and NHBD (24, 25) organs. HMP, mainly used for the kidney, has demonstrated very positive results in clinical practice (22). Although MP results in substantially better liver performance, this method has not yet been adopted in clinical practice due to the lesions after hypothermic preservation.

Currently, only one clinical series of liver transplantation has been published (23), but it did not confirm whether the benefits of perfused livers were exclusively due to HMP. The studies conducted

thus far have demonstrated that HMP eliminates some negative aspects, but HMP does not preserve the liver from all damage (26, 27). Particularly, HMP substantially supports the functionality of hepatocytes and ensures greater production of ATP, but does not eliminate sinusoidal endothelial cell damage during perfusion (28, 29) and reperfusion (23). Monbaliu et al. (30) compared preservation by HMP and SCS in pig livers, and observed similar degree of ischemia-reperfusion injury.

Since the early development of perfusion machines (31), performing perfusion in normothermia (temperature range from 35–38 °C) has been considered to limit the damage caused by hypothermia. The basic concept of perfusion in normothermia is to completely maintain the physiological level of functionality, thereby minimizing the production of catabolites and free radicals underlying the damage caused by hypothermia (32). Subsequent studies in model animals have demonstrated the possibility of limiting the damage from hypothermia and expanding the usability of marginals livers. Early studies in this area were conducted with mice, whose reliability was questioned because of very small liver volume, different organ anatomy, greater diversity of microcirculatory system, and faster metabolism, and these results were not fully transferable to larger livers (10, 18). These data were previously obtained in larger animal models, by Schon et al. in 2001 (33), who transplanted livers perfused at normothermia in a porcine model, and by Butler et al. in 2002 (34), who maintained viable pig livers perfused at normothermia for 72 h. Other studies have subsequently confirmed that normothermic perfusion not only better preserves livers but also revitalizes NHBD livers (DCDD) (35–39). However, theoretically, normothermic perfusion has greater possibility of infection, so there is a need for the administration of antibiotics and for an oxygen carrier as well (40, 41), which could limit the possibility of clinical application. The only limitation to the use of normothermic machine perfusion is the cost due to a more complex organization.

The third approach is subnormothermic perfusion (temperature range 20–24 °C). This has advantages derived from the absence of hypothermia in terms of decreased cellular damage and preserved mitochondrial function, and the possible disadvantages of normothermia are also decreased. Subnormothermic perfusion has been performed in mice with positive results concerning

both storage and the postoperative assessment of the liver (42–45), and in porcine animal model with hyperbaric oxygen support, which confirmed good mitochondrial function after perfusion (21).

Especially, as reported by Vairetti et Al (46) better morphology, higher content of glycogen, lower production of oxygen free radicals by the sinusoidal cells, bile production, as well as better mitochondrial performance are the elements that characterize the subnormothermic preservation in steatotic livers compared to those stored in hypothermia. These results confirm both the possibility of use of steatotic livers if properly preserved and the effectiveness of subnormotermia in decreasing damage from storage. Similarly, Gringeri et Al (47), also reported that porcine NHBD livers, preserved with subnormothermic perfusion show a minor liver damage compared to those stored in hypothermia, confirming that the subnormothermic perfusion makes usable livers also not considered available with hypothermic preservation methods .

Oxygenation of the perfusion solution

At 4 °C, hepatic metabolism requires oxygenation of 0.27 µmol/min/g liver, implying that hypothermic perfusion needs oxygen. The higher the operating temperature of the machine, more oxygen is consumed. Perfusion pressure, minimum oxygen partial pressure required, is inversely proportional to normalized flow (ml/min/g liver). Fujita et al. (48) demonstrated good organ viability at an oxygen saturation of 95%, while a total lack of oxygen caused cellular damage, especially to endothelial cells. The damage in SCS is caused by ischemia by preservation under limited oxygen supply. To confirm this, many studies have investigated the outcomes of livers supplied with oxygen during hypothermic and/or subnormothermic perfusion (20, 25, 49, 50).

The major problem in oxygen administration is how to ensure that oxygen is transported and perfused into liver cells (51–53). Henry et al. (15) highlighted the lack of clarity regarding whether HMP requires active oxygenation or diffusion alone can maintain usable levels of oxygen, and Olschewski et al. (43) pointed out the lack of a specific oxygen transporter that causes liver cell damage due to

oxygen deficiency in perfusion tests at 21 °C. For effective result, the liver must be perfused by blood (40, 34), or by a fluid with an artificial carrier (41). Majority of arterial oxygen is transported in the form of oxyhemoglobin, and a small percentage (0.3%) is dissolved in the plasma. Additionally, at atmospheric pressure, the partial pressure of oxygen in arterial blood (PaO_2) corresponds to 100 mmHg, which is equivalent to 97% hemoglobin saturation. The need for a carrier may be obviated if the proportion of dissolved oxygen is increased and therefore readily available.

According to Henry's Law, P = kC, where P is the pressure of the gas, C, its concentration, and k, Henry's Law Constant of the gas, increasing partial pressure of a gas increases its solubility in a liquid. Thus, if hyperbaric oxygen is incorporated into MP to increase the pressure of administration to 3 times of atmospheric pressure, PaO_2 will approach 2000 mmHg. Under these circumstances, the plasma is charged with dissolved oxygen (21). Presence of oxygen also decreases endothelin secretion, stress indicator of endothelial cells (50), thus improving microcirculation. Finally, under hyperbaric conditions, oxygen is available without any organic or synthetic carriers, and it is transported to the peripheral areas of organs, ensuring a more uniform perfusion, and in turn, reducing oxidative stress (54). Hyperbaric oxygenation can also resolve problems related to the working pressure in the portal vein: overly high pressure is known to damage the sinusoidal endothelium, while overly low pressure is not able to guarantee perfusion in the peripheral areas (55). In our previous study, we obtained oxygenated perfusion with a flow in the portal vein of 0.20 ml/min/g, thereby maintaining a low pressure.

Perfusion solution

Liver preservation process induces substantial method-dependent metabolic changes, mainly related to temperature and the presence of oxygen. Perfusion solution can counter the damages caused by hypothermic preservation. Among the main factors are free radical production, the reduction of cellular ATP content, osmotic changes, and alteration of mitochondrial structure and function (9) as well as

effect on microcirculation. Many perfusion solutions are currently available for liverpreservation. Guibert et al. (9) compared the Euro-Collins, UW (Viaspan), Celsior, Custodiol, and IGL-1, and Dutkowski et al. (54) compared UW-gluconate, HYK, Polysol, Celsior, IGL-1, and Modified UW.

Corrective measures to be adopted in HMP are related to antioxidants, buffers, electrolytes, and nutrients. Bessem et al. considered polysol solution to be best equipped than UW-G (56). A solution designed for HMP, however, may not be appropriate for normothermia or subnormothermia, and therefore, more studies are needed on the solution that can best adapt to these conditions. In normothermia, addressing remediate damage is not necessary, but nutrients and oxygen are particularly important, while subnormothermia with oxygen presents a reduced need for antioxidants but dependence on storage time. A short perfusion time results in release of less metabolic catabolites into the circulation, while a long perfusion time (>6 h) requires a filter or dialysis system to purify the solution and a system to integrate antioxidants during the casting (57). These findings indicate that having appropriate perfusion solution for each type of thermal preservation condition is desirable, as the types of damage that the solution needs to counteract differ depending on storage time and temperature.

The machine

We have clarified some terms related to the systems used to date or proposed for liver perfusion:

a) Perfusion circuit is an assembly of components used to obtain an organized system of perfusion but not included in a single transportable container a with centralized function-control system, not energetically autonomous.

Conversely, b) Perfusion machine is structured and organized to realize a complete apparatus for perfusion with components assembled in a transportable container equipped with a central external control and management of the functions, energetically autonomous (batteries).

To our knowledge, currently, some perfusion machines relevant to liver preservation are available in the market (the Airdrive, produced by Doorzand Medical Innovations, Amsterdam, the Netherlands the more recent Organ Assist's Liver Assist; the Liver Transporter, Organ Recovery Systems, *and the OrganOx recently used al the king's College of London*), but none of them works in Hyperbaric condition. For the first clinical series (23), the authors used a modified Medtronic Portable Bypass System.

The difficulties in achieving positive results with MP have arisen due to logistical and transportation-related problems and a lack of appropriate technology. While the technological problems are being overcome, many issues still remain to be defined leading to the belief that the search for an ideal machine is not yet finished.

Many researchers have investigated liver perfusion using home-made machines in different settings and provided descriptions of various new prototypes (Table 1). Analysing the perfusion systems based on the studies conducted in animal models illustrates how they differ with respect to the type of flow (single or dual), operating temperature, oxygenation, perfusion pressure, time of perfusion, and other factors representing the multiplicity of needs. The proposed systems are accompanied by different modes of perfusion, but all of them aim to ensure organ function and preservation from damage, from hypothermia- or possible normothermia-related adverse effects, excessive pressure in the sinusoids, excessive production of free radicals, alteration of microcirculation, or imperfect whole-organ perfusion. These factors suggest that it is not right to ask whether an ideal machine exists to preserve livers by perfusion. Instead, we should ask if a unique mode of preservation by perfusion exists, that can be used for any type of liver. According to Vekemans et al. (18), the ideal preservation method should extend shelf life, reduce the incidence of primary graft nonfunction after transplantation, allow assessing organ viability before transplantation, and facilitate a more extended use of marginal organs. Monbaliu and Brassil (10) further listed the benefits of MP compared to SCS. It is therefore necessary to better define both the technical features of MP (Table 2) and its use in order to establish their future use. The technical features should enable

differential use of the machine depending on the type of the liver to be preserved, because conservation needs vary depending on liver condition (good or marginal) and storage time—shorter preservation time if removal and transplantation occur at the same location, or longer preservation time if the liver must be transported from one location to another. The machine must be able to cope with both situations using technology making its application multi-functional and universal. Thus, the machine should be equipped with the following characteristics (Table 2):

a) Portability
b) Energy autonomy
c) Double circuit (venous and arterial)
d) Output or reperfusion filtering system
e) Flow and pressure control
f) Hyperbaric oxygenation
g) Temperature control
h) External central function control
i) Autodiagnostic system and malfunction alarm
j) Control system for administering drugs and fluid testing

The features listed (Table 2) allow modulation of the use of the machine, with possible changes to temperature, stream type, perfusion pressure, type of oxygenation, and perfusion solution, according to the requirements of the type of the liver.

Cost-effectiveness of the system

The costs for new system for liver preservation have not been investigated yet. Wszola et al. (58) and Groen et al. (59), however, have investigated the costs of the kidney conservation, and indicated that

even if machine unit costs higher than that of SCS, because of lower wards occupancy and lower incidence of complications due to reduced reperfusion injury, the overall cost per life-year is minor for organs preserved with MP. Thus, it is reasonable to predict a similar result for MP of livers, even without accounting for the lives saved and the expansion of the pool of available livers that this technology could lead to.

Conclusion

Some studies (25, 29) argue that a short period of hypothermic oxygenated perfusion after cold ischemia can reverse cold damage in DCDD livers, steatotic livers, and livers that have suffered prolonged (>6 h) cold storage. By contrast, Reddy et al. (26) believe that 1 h of warm ischemia followed by 4 h of hypothermic preservation makes the liver non-viable by causing serious injury and loss of cellular function, while 1 h of cold preservation results in less severe injuries, and is not associated with major graft dysfunction. Thus, no agreement on the modalities of perfusion is evident. Two other aspects must also be considered. First, in general, withdrawals from donors are multiorgan, and eliminating cold preservation for liver prior to a multiorgan withdrawal could be harmful to other organs. Second, the time needed to determine injuries from hypothermia, the minimum time of reperfusion in normo- or subnormothermia necessary to repair any damage, and the maximum preservation time beyond which vitality of the liver is affected needs to be clarified. By analyzing the studies on various MP models, preservation methodology can be evaluated differently depending on the characteristics of the liver.

MP is the method to make even steatotic or DCDD livers usable with a good safety record, and thus promises a significant increase in the pool of available livers, thereby shortening waiting time and especially deaths. However, some aspects of MP related to the machine technology and indications for the different types of preservation must be defined in more detail before MP can be extended to all livers, because all livers cannot be indiscriminately allocated to the same mode of the machine. HMP

is considered better than SCS by many researchers but does not exclude the possibility of damage and therefore should be limited as much as possible. Normothermia is superior and appropriate method for the resuscitation of marginal livers (60), while subnormothermia appears to be the best method to maximize advantages of perfusion while minimizing its disadvantages. Ideal MP for livers to be transplanted, whether DCDD and/or steatotic or simply taken from a beating-heart donor, should be multifunctional and adaptable to different storage conditions, and thus able to precisely define the various modes to use the proper one for each liver. An attempt to define the methodology for the use of machine perfusion is therefore an important goal for the immediate future. *Since there are still no clinical evidence such as to establish a protocol for clinical use of the machines - the only published clinical series is to Guarrera, while only not yet published data refer about a double transplant took place at the King's Hospital in London Colelge after normothermic preservation of the liver in a new machine developed at the University of Oxford, (http://www.ox.ac.uk/media/news_releases_for_journalists/ 130315.html) is not yet possible to encode what are the parameters of use of the machine in order to obtain an optimum result both in terms of organ preservation and of actual success of the transplant.*

The machine must therefore still be tested, possibly on porcine model looking for standardize , as much as possible, research protocols in order to get to define the protocol of clinical use of the machine itself. The data in the literature shows strengths and weaknesses of the different thermal levels of preservation. A multifunctional machine may therefore use the advantages of each thermal level of preservation being able to vary the rules of the preservation depending upon the characteristics of the organ, the donor and the recipient , in order to achieve the result of an expansion of the number of organs available for transplant also making sure those organs that the CSI preservation suggests unsuitable and improve the overall outcome of liver transplants.

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Table 1: Circuits and machines used in experimental models of perfusion. Here “machines” are defined as systems specifically described as being included in a single container.

Author	Circuit Features	Machine Features
Shon et al. 2001 (33)	Dual circuit, organ chamber, oxygenator dialysis circuit, pump and control unit. Normothermia, liver	
Lee et al. 2002 (16)	Hypothermia, liver	
St Peter et al. 2002 (40)	Extracorporeal perfusion circuit (Medtronic), 2 circuits in parallel Normothermia, liver	
Bessems et al. 2006 (53)	Control over pressure, flow, temperature, time, perfusion, oxygenation. Normothermia, liver	
Van der Plaats et al. 2006 (61)		Prototype (Groninger Liver Perfusion System) dual perfusion, organ chamber, oxygenator, battery pack, control unit. Hypothermia, liver
Rubbini et al. 2007 (21)		Prototype. Organ chamber, portal flow, temperature control, time of perfusion, hyperbaric oxygenation, return to the chamber and perfusion pump Subnormothermia, liver
Guarrera et al. 2010 (23)		Modified Medtronic Portable Bypass System, dual circuit, organ chamber, heat exchanger, control temperature and pump, reperfusion system, Hypothermia, liver
Xu et al. 2012 (39)		Dual circuit, centrifugal pump, heat exchanger, membrane oxygenator, bubble trap, venous reservoir, organ chamber. Perfusate outflow to chamber, than returns to centrifugal pump. Normothermic, liver
Post et al. 2012 (62)		Custom-built with components from various companies. Organ chamber, roller pump, filter, oxygenator, bubble trap, mass flow transducer, heat exchanger, pressure sensor, temperature control. Multiple organs

Table 2. Liver perfusion machine specifications

Function	Features
Portability	The machine must be included in a single container with a maximum weight of 15–18 kg or assembled on a cart with wheels. The container or the jacket may be carbon, Kevlar, or of another resistant material, yet lightweight. All parts in contact with the liver and the perfusion fluid should be disposable
Energy autonomy	Possibility of connection both to the power grid (fixed or from means of transport) and to its own batteries
Perfusion circuit	Equipped with single or double circuit. The organ is housed in a container, and the perfusion fluid is reintroduced into the circulation after filtration
Output or reperfusion filtering system	A system of filters for dialysis is connected to the inflow pumps in order to retain the catabolites
Flow and pressure control	Constancy of flow and pressure in both venous and arterial systems. The pressure should be maintained at approximately 8 mmHg (venous) and 100 mmHg (arterial) in normothermia, and between 3–4 mmHg (venous) and 30–40 mmHg in the case of dual arterial perfusion in subnormothermia. The venous flow should be between 0.15–3.0 ml/min/g liver.
Oxygenation	Hyperbaric oxygenation to 1.5 ATM with release of oxygen into the organ chamber at scheduled times or depending on consumption
Temperature	Possibility of operating in hypothermia, subnormothermia, and normothermia
External function control system	Outside of the casing of the machine, a terminal must be present for the control of the operating functions.
Autodiagnostic system and malfunctioning alarm	The machine is equipped with an automatic self-diagnosis system to run at predetermined times or on request and a warning for malfunction.
Control system for administering drugs and fluid testing	Outside the chamber, a normobaric control system is provided that can run control testing on the perfusate or enter medications.