Diplomarbeit

Preservation Solutions for Machine Perfusion in Organ Transplantation

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Declaration of Authorship

I hereby declare that I have written this thesis independently and without outside help, that I have not used any sources other than those indicated. All statements taken literally from other writings or referred to by analogy are marked and the source is always given.

Graz, 16.02.2022 *Dmytro Tuzov eh.*

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List of Abbreviations

ADP adenosine diphosphate

ALP alkaline phosphatase

ALT alanine aminotransferase

AST aspartate aminotransferase

ATP adenosine triphosphate

BES N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid

BH4 tetrahydrobiopterin

BSA bovine serum albumin

COR controlled oxygenated rewarming

CPP cryoprecipitated plasma

DAMP damage-associated molecular pattern

DBD donation after brain death

DCD donation after circulatory death

DGF delayed graft function

DHOPE dual hypothermic oxygenated machine perfusion

DMEM Dulbecco's Modified Eagle's Medium

DNA deoxyribonucleic acid

EAD early allograft dysfunction

ECD extended criteria donor

eGFR estimated glomerular filtration rate

eNOS endothelial nitric oxide synthase

EVLP ex vivo lung perfusion

FFP fresh frozen plasma

GGT gamma-glutamyl transferase

GLDH glutamate dehydrogenase

HBOC hemoglobin-based oxygen carrier

HbV hemoglobin-based vesicle

HEPES N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

HES hydroxyethyl starch

HIF-1α hypoxia inhibitory factor-1 alpha

HMP hypothermic machine perfusion

HOPE hypothermic oxygenated machine perfusion

HSA human serum albumin

HTK histidine-tryptophan-ketoglutarate solution

ICAM intercellular adhesion molecule

ICU intensive care unit

IFN-γ interferon gamma

IGL-1 Institut Georges Lopez-1 solution

IL interleukin

iNOS inducible nitric oxide synthase

INR international normalized ratio

IRI ischemia-reperfusion injury

KH Krebs-Henseleit solution

KPS-1 kidney perfusion solution-1

LDH lactate dehydrogenase

LTB4 leukotriene B4

MCP-1 monocyte chemoattractant protein-1

MHC major histocompatibility complex

MMP midthermic machine perfusion

MMP-9 matrix metallopeptidase-9

MP machine perfusion

MPS machine perfusion solution

NAD nicotinamide adenine dinucleotide

NADPH nicotinamide adenine dinucleotide phosphate hydrogen

NF-κB nuclear factor-kappa B

NHBD non-heart-beating donor

NMP normothermic machine perfusion

nNOS neuronal nitric oxide synthase

NO nitric oxide

NOS nitric oxide synthase

OPS organ preservation solution

PAF platelet-activating factor

PEG polyethylene glycol

PFC perfluorocarbon

PHP pyridoxylated hemoglobin-polyoxyethylene

PMN polymorphonuclear leukocyte

PNF primary non-function

PNP purine nucleoside phosphorylase

PPF plasma protein fraction

RBC red blood cell

RNOS reactive nitrogen oxide species

ROS reactive oxygen species

RT-PCR reverse transcription polymerase chain reaction

RTqPCR real time quantitative polymerase chain reaction

SCS static cold storage

SMP subnormothermic machine perfusion

SOD superoxide dismutase

TBARS thiobarbituric acid reactive substances

TLR toll-like receptor

TNC tenascin C

TNF-α tumor necrosis factor alpha

UW University of Wisconsin solution

UW-G University of Wisconsin gluconate solution

VSOP venous systemic oxygen persufflation

α-GST alpha glutathione S-transferase

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Zusammenfassung

Die Organtransplantation ist die Therapie der Wahl für Patienten mit Herz-, Lungen-, Nieren-, Leber- und Pankreasversagen im Endstadium. Bedauerlicherweise besteht weltweit ein Mangel an Spenderorganen, was zu einer hohen Sterblichkeit auf der Warteliste führt. Eine der Strategien zur Expansion des Spenderorganpools ist die Verwendung von Transplantaten minderer Qualität, welche die erweiterten Spenderkriterien erfüllen. Es sind, zum Beispiel, Organe von den älteren Spendern oder Organspenden nach Kreislauftod. Um damit akzeptable klinische Ergebnisse zu erzielen, müssen jedoch die derzeitigen Organkonservierungsverfahren verbessert werden. Die Maschinenperfusion (MP) ist ein alternatives Konservierungsverfahren zur weit verbreiteten kalten Lagerung. Sie basiert auf dem Konzept der kontrollierten kontinuierlichen oder pulsierenden Zirkulation der Perfusionslösung durch das Gefäßsystem des Organs, um kontinuierlich Sauerstoff und Nährstoffe zur Aufrechterhaltung des Organstoffwechsels zuzuführen und toxische Stoffwechselendprodukte auszuschwemmen. Rezente Studien zur Maschinenperfusion haben einige Vorteile dieser Technik gegenüber der kalten Lagerung für die Konservierung suboptimaler Organe aufgezeigt. Es gibt verschiedene Konzepte der MP, bei denen unterschiedliche Präservationslösungen als Medium verwendet werden. Diese Medien variieren in ihrer Zusammensetzung von einfachen gepufferten und mit Glukose angereicherten kristalloiden Lösungen bis hin zu nährstoffreichen Gewebekulturmedien-ähnlichen Perfusionslösungen. Derzeit gibt es keine Leitlinien für die Verwendung spezifischer Konservierungslösungen in Abhängigkeit von der Technik der MP oder dem spezifischen Organ. Ziel dieser Diplomarbeit ist es, einen Überblick und Vergleich der derzeit untersuchten Perfusionslösungen für verschiedene Konzepte der dynamischen Konservierung von intraabdominalen Organen zu geben.

Abstract

Organ transplantation is a highly effective treatment for patients with end-stage heart, lung, kidney, liver, and pancreas failure. Regrettably, there is a worldwide shortage of donor organs resulting in high waitlist mortality. One of the strategies to increase the number of available allografts is to use donor organs of substandard quality. However, to achieve acceptable outcomes, there is a need to improve current organ preservation techniques. Machine perfusion is an alternative preservation approach to widely used static cold storage. It is based on the concept of controlled continuous or pulsatile circulation of perfusate through the vascular system of the organ to continuously supply oxygen and nutrients to maintain organ metabolism, as well as to wash out toxic metabolic end products. Recent studies on machine perfusion have shown several benefits of this technique over static cold storage for the preservation of suboptimal organs. There are various concepts of machine perfusion that utilize different perfusates. These perfusates vary in composition from simple buffered and glucose-supplemented crystalloid solutions to highly enriched tissue culture media-like perfusates. Currently, there are no guidelines for the use of specific preservation solutions depending on the machine perfusion technique or the specific organ. The aim of this diploma thesis is to give an overview and comparison of currently investigated perfusion solutions for different concepts of dynamic preservation of intra-abdominal organs.

1 Introduction

1.1 *Background*

From the first successful solid organ transplantation performed in 1954 until now, allotransplantation has become the treatment of choice for patients with end-stage organ disease. This technique provides great short-term and long-term survival as well as better quality of life for patients with irreversible terminal heart, lung, kidney, liver, pancreas failure (1-3). Such progress in transplantation outcomes was possible due to the development of new immunosuppressive regimes, improved methods of organ harvesting and preservation as well as allocation strategies (2,4).

Table 1. Transplantation outcomes after deceased donation (5).

Transplantation	**Patient Survival (%)**		**Graft Survival (%)**	
	1 year	5 years	1 year	5 years
Heart	90.9	78.6	90.5	77.7
Lung (Double)	87.7	58.6	87.3	55.7
Liver	91.2	75	89.1	71.9
Kidney	96.3	83.3	93.2	74.4
Pancreas	90.9	79.6	81.8	60.1

Nowadays, the biggest challenge for transplantation medicine is the growing mismatch between the number of available organs and patients on the waiting list resulting in an increasing number of patients dying before they get transplanted (2,6). This has led to the development of different strategies to expand the donor organ pool. One of them is the utilization of extended-criteria donor (ECD) organs. Those are organs of lower quality that would have been previously considered unsuitable for transplantation and, thus, discarded. Examples are organs donated after circulatory death (DCD), organs from older donors, or organs with substantial parenchymal changes. Extensive research regarding utilization of such organs showed that although they perform less well and have higher risks of early allograft dysfunction (EAD), primary non-function (PNF), delayed graft function (DGF), and long-term biliary complications, it is possible to achieve acceptable overall performance comparing to standard-criteria donor organs and, therefore, this strategy should be used to further expand the donor organ pool (7,8).

One of the main reasons for the inferior performance of ECD organs is that they are more affected by ischemia-reperfusion injury (IRI). To counteract this injury and further facilitate

the use of ECD organs, there is an interest to improve methods of organ preservation and resuscitation. This has led to the revival of research on machine perfusion (MP) techniques as well as extensive research on the development of new preservation solutions and the use of different pharmaceutical agents to recondition donor organs (9,10).

The current standard preservation technique for intra-abdominal organs is static cold storage (SCS) with University of Wisconsin (UW) solution. Research on the use of alternative preservation solutions for SCS has shown comparable outcomes with Histidine-tryptophan-ketoglutarate (HTK) and Celsior solutions (11). With the revival of machine perfusion there is an interest in the development of new perfusates and refinement of existing organ preservation solutions to address the physiology of continuous perfusion. Currently, there is no consensus on the standard perfusion solution for MP. Furthermore, different MP techniques are being explored (e.g., normothermic, hypothermic, pulsatile, non-pulsatile, oxygenated, non-oxygenated), and they may require different perfusion solutions (12,13). The aim of this diploma thesis is to give an overview and comparison of currently investigated preservation solutions for different MP techniques.

1.2 *Ischemia-reperfusion injury*

The main principles of the development of the organ preservation solutions (OPS) are based on the understanding of hypothermic preservation and ischemia-reperfusion injury (IRI) (10). IRI describes a cascade of deleterious biomolecular events occurring at the cellular and tissue level during the state of hypoperfusion and hypoxia, as well as after the blood supply to the organ has been restored. IRI consists of two parts: ischemic injury and reperfusion injury (14). In the early days of transplantology, it was believed that ischemia was solely responsible for the most pathophysiologic changes that lead to organ failure. However, it has now been shown experimentally that reperfusion causes significant further damage to the cells and tissues, which was not seen in cells that only underwent ischemia (15,16).

IRI begins with a cessation of blood flow to the organ. Subsequent hypoxia facilitates anaerobic metabolism and leads to adenosine triphosphate (ATP) depletion. Ion pumps, such as Na^+-K^+-ATPase and Ca^{2+}-ATPase, that rely on sufficient energy supply for a proper function, start failing, resulting in intracellular sodium influx and calcium overload. This leads to cellular edema and causes enzymatic dysfunction. Furthermore, lactic acid accumulation due to anaerobic metabolism leads to an acidotic state that aggravates the impairment of enzymatic activity (17). One of the most important mechanisms of IRI is oxidative stress caused by the production of reactive oxygen species (ROS) after the

restoration of the blood supply. ROS, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) or hydroxyl radical (•OH), cause damage to the tissues via different mechanisms that are still not completely understood. These include damage to DNA and proteins, lipid peroxidation, deactivation of enzymes leading to endothelial dysfunction, inflammatory reaction with subsequent cytokine storm, and, eventually, to cell death (17,18).

ROS are normally metabolized by antioxidant systems, such as superoxide dismutase (SOD), catalase, and the glutathione peroxidase system. However, upon reperfusion, there is a huge increase in ROS production, and these systems become overwhelmed (16,19).

The main sources of ROS production during IRI comprise the xanthine oxidase system, NADPH oxidase system, mitochondrial electron transport chain, and uncoupled nitric oxide synthase (NOS) system (20). The Xanthine oxidase system is part of the purine metabolism. Under normal conditions, xanthine dehydrogenase catalyzes the oxidation of hypoxanthine to xanthine while using NAD+ as an electron acceptor. During ischemia, xanthine dehydrogenase is converted to xanthine oxidase. This enzyme uses O_2 as an electron acceptor and, thus, the reaction is accompanied by ROS production. Due to hypoxia and the subsequent inability of xanthine oxidase to perform the reaction, hypoxanthine accumulates in the tissue. Upon reperfusion and, thus, reintroduction of oxygen, the reaction takes place and causes ROS formation (20,21).

The NADPH oxidase enzyme system (NOX/DUOX) uses O_2 as a substrate for its reaction, which is accompanied by superoxide production. NOX enzymes are activated during ischemia via hypoxia inhibitory factor-1α (HIF-1α) and later upon reperfusion via Phospholipase A2, TNF-α, IL-1β, IFN-γ and Angiotensin II (17).

Under normal conditions, NOS utilizes L-arginine and O_2 to form NO and L-citrulline, using NADPH as an electron donor. An important cofactor for this reaction is tetrahydrobiopterin (BH4). IRI causes L-arginine and BH4 depletion, which results in an uncoupling of the NOS system, and these enzymes start producing superoxide through electron transfer from NADPH directly to oxygen (22,23).

Nitric oxide (NO) is one of the most important molecules involved in the regulation of microvascular circulation. Its beneficial effects include vasodilation, inhibition of platelet aggregation as well as attenuation of leukocyte-endothelial interactions (24-26). NO effects can be divided into direct and indirect effects. Direct effects are associated with low NO tissue concentrations and its direct interaction with molecules. Indirect effects unfold their action via the production of reactive nitrogen oxide species (RNOS) such as peroxynitrite (ONOO-) and occur at higher concentrations (27). NO is synthesized from L-arginine by

enzymes called Nitric oxide synthases (NOS) (28) divided into the three isoforms endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). NO, produced by eNOS and nNOS, regulates its levels through a negative feedback loop and is linked to direct effects. iNOS is associated with indirect effects (27,29). IRI results in an increase of iNOS expression and a decrease of eNOS and nNOS levels, which leads to depletion of endothelium-derived NO and causes nitrosative stress via abundant RNOS production (29).

Other important phenomena of IRI are endothelial dysfunction and leukocyte-endothelial interactions. Endothelial dysfunction is promoted by ischemia-driven ion dysregulation with subsequent endothelial cell swelling. Furthermore, it is aggravated by the depletion of a potent vasodilator NO and inflammation-powered release of endothelin-1 that causes vasoconstriction (30). Leukocyte-endothelial interaction is a three-step process consisting of leukocyte rolling, adhesion to the vascular lining, and extravasation with subsequent tissue damage. Leukocyte rolling is initially promoted by glycoproteins called selectins (P-, E-, L-selectins). IRI induces the expression of these molecules on the surface of leukocytes and endothelial cells. Adhesion is promoted by integrins and their counterpart on the endothelial wall intercellular adhesion molecules (ICAMs) and is also influenced by chemoattractants, such as platelet-activating factor (PAF) and leukotriene B4 (LTB4) (21,29). Further transmigration of the leukocytes, mainly neutrophil granulocytes (PMN cells) into the parenchyma of organs causes their contact with necrotic material and leads to their degranulation (14). PMN leukocytes promote IRI by the release of cytotoxic agents, such as ROS, proteases (Elastase, Collagenase, MMP-9) chemokines and cytokines (IL-1, IL-6, IL-12, TNF-α, IFN-γ), lipid mediators (LTB4), which results in a vascular barrier dysfunction, increased vascular permeability, prothrombotic milieu and damage to the surrounding parenchymal tissue(18,30).

Another closely connected phenomenon is called “no-reflow”. It describes a compromised blood flow in the capillaries upon reperfusion of the organ. The pathophysiology of this phenomenon has been linked to leukocytes blocking the capillary lumen, endothelial cell swelling, and edema of the surrounding tissue compressing the microvasculature (18).

Inflammation is a key element in the pathophysiology of IRI. The principal driver of this so-called “sterile inflammation” due to the absence of external pathogens is the induction of pro-inflammatory signaling cascades by ROS and intracellular calcium overload. These cascades lead to the recruitment of PMN cells, production of cytokines and chemokines, complement system activation. Activated complement factors (C3a, C5a, C5b-9) form plasma membrane attack complexes and further facilitate activation of PMN leukocytes and

macrophages (18,31). Another important mechanism of inflammatory response is the activation of danger signaling pathways. Injured cells release damage-associated molecular patterns (DAMPs), which are recognized by cells of the innate immune system, such as macrophages and dendritic cells via Toll-like receptors (TLR), and result in the activation of the immune system and promotion of the inflammatory reaction through the induction of nuclear factor-kappa B (NF-κB) that regulates transcription of DNA and cytokine production (32).

There is also a growing knowledge regarding the role of the adaptive immune system (T- and B-cells) in the sterile inflammation which may offer new potential therapeutic approaches to counteract IRI (18).

Ultimately, pathophysiologic reactions of IRI result in cell death. Cell death can occur via different mechanisms depending on extrinsic factors, such as membrane damage, cell swelling, inflammatory reaction, activation of calcium-dependent proteases, and via intrinsic death signaling mechanisms. Recognized cell death modalities include necrosis, necroptosis, apoptosis, and autophagy (18).

1.3 *Basic principles of the OPS development*

In order to prevent and counteract IRI, most organ preservation solutions include:

- Colloids (e.g., HES, dextran, PEG) and impermeants (e.g., lactobionate, raffinose, gluconate, mannitol) to reduce interstitial and cellular edema respectively (14,33). Colloids play an important role during the initial vascular flushing and also in case of continuous perfusion due to their ability to maintain intravascular oncotic pressure and improve microvascular blood flow by reducing cell volumes and extrinsic lumen compression caused by interstitial edema (14,33). Therefore, they counteract the "no-reflow" phenomenon, decrease vascular resistance, and improve flow in case of machine perfusion. Impermeants are active osmotic agents that are utilized to sustain the oncotic gradient across the cell membrane and prevent cellular swelling (14).
- Electrolytes (e.g., K^+, Na^+, Mg^{2+}, Ca^{2+}) to control osmotic balance (5). First developed organ preservation solutions (e.g., Collins, UW) had intracellular-like Na^+/K^+ ratios (i.e., high potassium and low sodium concentration) in order to counteract IRI-driven ionic shifts by minimizing concentration gradients and reduce energy consumption by Na^+-K^+-ATPase (5,14). However, since then, several newly developed preservation solutions utilized extracellular-like cation ratios (e.g., IGL-1, Celsior) or even reduced total cation concentration (HTK) and were able to achieve

satisfactory results (10,11,13). Therefore, currently, there is no consensus on the optimal Na^+/K^+ ratios for preservation solutions. The inclusion of Mg^{2+} by Collins et al. was based on the studies of ionic plasma levels in hibernating animals. They showed beneficial effects of magnesium in kidney preservation, one of the mechanisms being the prevention of intracellular potassium loss (34). The inclusion of calcium has also been a matter of debate and was connected to the "calcium paradox" observed in the development of cardioplegic solutions. Experiments have shown that complete removal of calcium from the preservation medium resulted in massive calcium uptake and myocardial cell rupture after Ca^{2+} repletion (35). This effect, however, was eliminated by adding very little amounts of calcium to the preservation medium (36). A similar effect was shown for the preservation of hepatocytes (37). Furthermore, adding slightly higher amounts of calcium to the preservation solution resulted in a significantly worse outcome in kidney preservation (38). The underlying mechanisms are not completely understood, but it appears that calcium plays an important role in the stabilization of cellular membranes (mitochondrial and plasmalemma) (37). Therefore, most preservation solutions contain little amounts of calcium; gold-standard UW solution is, however, calcium-free (14).

- Buffers (e.g., phosphate, histidine, HEPES) to stabilize pH and counteract negative effects of intracellular acidosis (5,14).
- Energy substrates (e.g., adenosine, ketoglutarate) to facilitate the restoration of metabolism on reperfusion. Hypothermia causes a significant decrease in the cell metabolism and lowers energy consumption, but it is not completely stopped and leads to ATP-precursor deficiency. Therefore, there is a need to provide energy substrates for metabolic reactions and ATP synthesis on reperfusion (5,14).
- Antioxidants (e.g., allopurinol, glutathione) to counteract oxidative stress produced by ROS (5,14). Antioxidative effects can be achieved via different pathways. For example, allopurinol acts as an inhibitor of xanthine oxidase and, thus, blocks one of the important sources of ROS production (39). Glutathione, on the other hand, is a well-known endogenous free-radical scavenger that can be added to the preservation solution to support ROS metabolization (10). Furthermore, some amino acids (e.g., tryptophan in the HTK solution) also possess high free-radical scavenging capacity (40). In addition, mannitol, which is generally used as an impermeant, possesses antioxidant properties via up-regulation of the catalase level (41).

- Other additives, such as antibiotics, hormones, vasodilators, growth factors, etc. (14)

1.4 *Principles of machine perfusion*

Though machine perfusion is not a new concept, there has been a lot of new research on MP in the last two decades because of its potential superiority over SCS for ECD organs. The main advantages of the MP compared to SCS comprise viability assessment of organs during perfusion, possible attenuation of IRI, the possibility for therapeutic interventions with the goal to resuscitate the organ, and the potential to increase preservation time to make organ transplantations similar to elective procedures (9,12).

Machine perfusion is based on a controlled continuous or pulsatile circulation of the perfusate through the organ vasculature (42). The essential components influencing the quality of machine perfusion are a pump, perfusate composition, oxygenation, control of pH, temperature, pO_2, pCO_2 and flow rates (43). The basic principle behind the physiology of MP is a continuous supply of oxygen and nutrients to support organ metabolism, as well as washout of toxic metabolic end products (44). Additionally, MP has been associated with protective effects on microcirculation through the maintenance of normal vascular tone and endothelial cell-dependent relaxation (45), as well as expression of flow-dependent, vasoprotective endothelial genes (42).

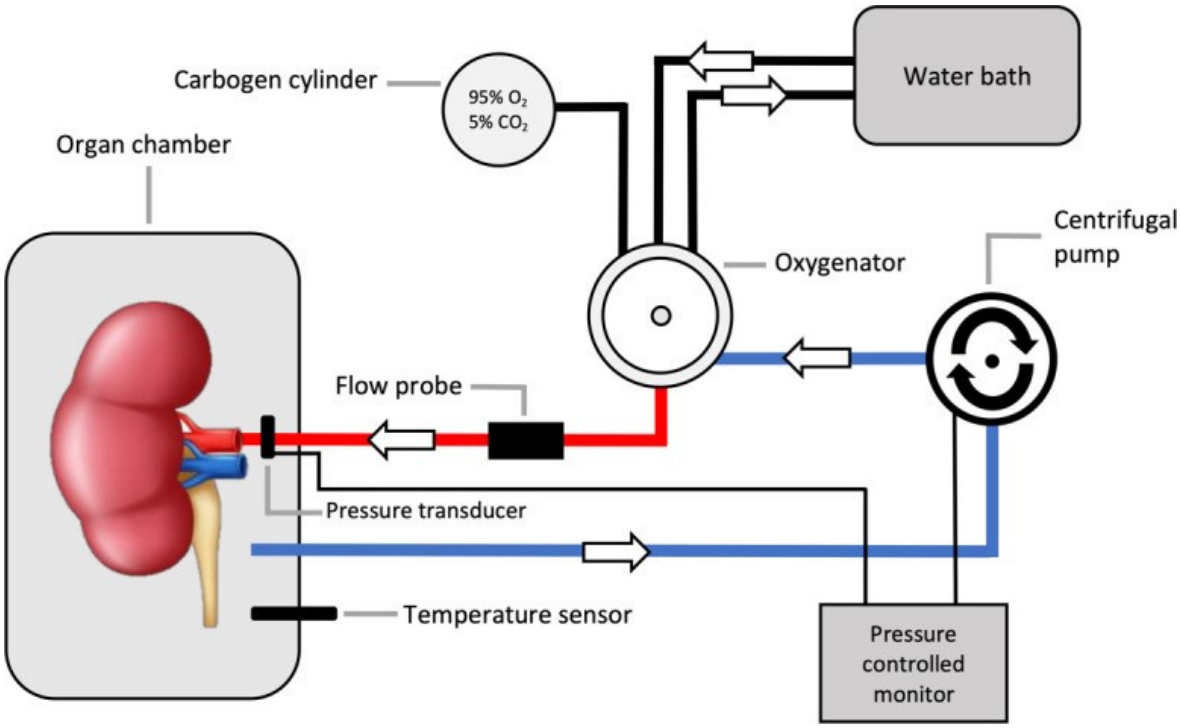

Figure 1. Schematic depiction of the machine perfusion circuit (46).

Currently, different MP techniques are under investigation. They all vary by their effects on the physiology of organ preservation. Main differences are related to the temperature of preservation, provision of oxygen, and timing of MP (47).

There has been a great heterogeneity in the reporting of studies on different MP techniques. With the goal to minimize confusion and enable reliable comparison of studies, Karangwa et al. made a proposal for a standardized nomenclature for reporting of the research on MP in liver preservation (47). They recommended the following classification of the timing of machine perfusion:

- Pre-SCS MP (MP conducted immediately after organ harvesting and followed by SCS preservation)
- Preservation MP (MP utilized for the entire preservation period with the minimally possible SCS time before and after MP)
- Post-SCS MP (MP prior to implantation after a period of SCS preservation)

Regarding the temperature of machine perfusion, they proposed the following classification:

- Hypothermic machine perfusion (HMP) – 0°C–12°C
- Midthermic machine perfusion (MMP) – 13°C–24°C
- Subnormothermic machine perfusion (SMP) – 25°C–34°C
- Normothermic machine perfusion (NMP) – 35°C–38°C

This classification is based on the change in the metabolic rate with decreasing temperature (47).

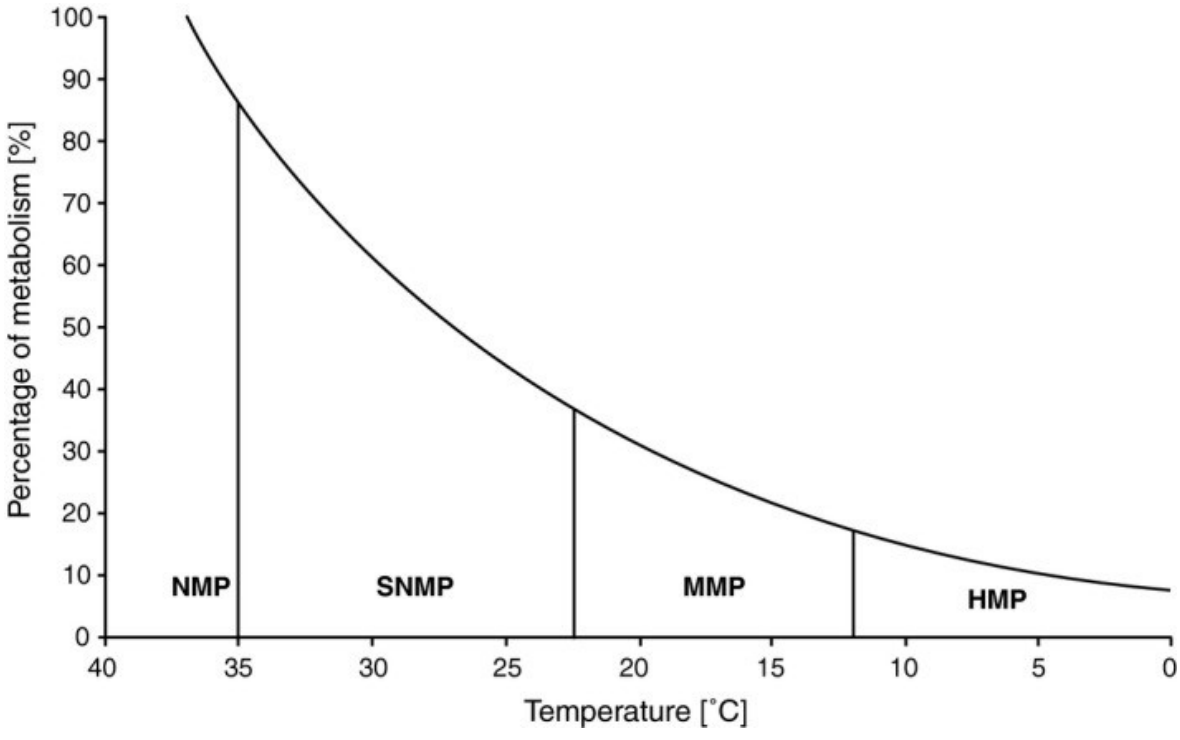

Figure 2. Graphic presentation of the change in the rate of metabolism with decreasing temperature (47). (NMP = normothermic machine perfusion, SNMP = subnormothermic machine perfusion, MMP = midthermic machine perfusion, HMP = hypothermic machine perfusion).

1.4.1 Hypothermic machine perfusion

HMP is based on the concept that aerobic energy production via mitochondrial electron transport is sustained at hypothermic temperatures (48). Therefore, this technique aims to restore ATP levels by continuously providing substrates while simultaneously decreasing the metabolic rate and oxygen demand of the tissue through the application of hypothermia (44,49). Oxygenation of the perfusate has been shown to improve outcomes of HMP (50,51). The effects of oxygenated HMP (also sometimes referred to as HOPE – hypothermic oxygenated perfusion) are the restoration of the mitochondrial redox status, decrease in ROS production, and downregulation of multiple inflammatory response cascades (52-55). An important advantage of this technique is its safety in case of a technical or logistical failure – the graft would simply return to the SCS conditions (10). However, an important disadvantage of HMP is that reliable real-time viability assessment of the graft is challenging because of the decreased metabolism (56). Therefore, surrogate parameters, such as flow and resistance dynamics, injury markers in the perfusate, the release of intracellular enzymes, pH are used. Even though some of these parameters have been shown to correlate with transplantation outcomes, their sensitivity for predicting long-term outcomes remains in question (48,56,57). Synthetic acellular preservation solutions are mainly used as perfusate for HMP. The most commonly used perfusate is the machine preservation solution developed by Belzer et al. in 1982, the so-called Belzer MPS (also known as UW-G or KPS-1) (58-60). The choice of the colloid for machine perfusion solution appears to be of the utmost importance (48).

1.4.2 Normothermic machine perfusion

NMP is based on the concept of maintaining normal metabolism during the whole time of preservation by providing oxygen and nutrients at a physiological temperature and, thus, maximally avoiding injury by ischemia as well as hypothermia (56). One important advantage of this technique is the possibility of real-time assessment of organ function. For example, synthetic function (e.g., bile production or factor V for liver), clearance function (e.g., creatinine for kidney), as well as urine output can be measured (49,61). However, there are some disadvantages of NMP. For instance, any technical failure would result in a graft being exposed to warm ischemia, which would require emergent repair. In addition,

normothermic conditions are associated with a higher risk of bacterial contamination (56). Furthermore, as the organs are fully metabolically active, the requirements on the oxygen-carrying capacity and nutrient composition of the preservation solution are much higher (62). Thus, perfusates for NMP usually contain oxygen carriers (e.g., red blood cells, hemoglobin-based oxygen carriers) as well as additional metabolic substrates like amino acids, vitamins, hormones (63).

1.4.3 Subnormothermic and midthermic machine perfusion

These techniques are based on the concept of finding a perfect balance between NMP and HMP. Karangwa et al. pointed out that most studies on these techniques for liver preservation were conducted at the temperature of 20°C - 22°C (47). Perfusion at this temperature combines benefits from lower metabolic demand while maintaining sufficient metabolism for assessment and improvement of organ function (64). One of the benefits is that, at this temperature, oxygen carriers are not necessarily needed (65). Therefore, both cellular and acellular oxygen carrier containing perfusates as well as simply oxygenated synthetic MPS can be used (56). Another closely related principle of MP is called controlled oxygenated rewarming (COR). It describes machine perfusion starting at hypothermic temperature with the slow, gradual increase of the perfusate temperature up to subnormothermic or normothermic conditions. Its goal is to avoid abrupt changes in temperature upon reperfusion that are associated with mitochondrial dysfunction and activation of the mitochondria-induced apoptotic pathway (66). Minor et al. showed that COR was associated with lower ROS release, lower enzyme leakage, and higher autophagy-related gene expression compared to SCS, HMP, and SNMP (67).

1.5 *History of machine perfusion and preservation solutions*

The fundamental idea of organ perfusion arose from the primitive concepts of extracorporeal circulation proposed by Cesar Julien Jean Le Gallois in 1812. The first documented organ perfusion experiments were carried out by Carl Loebell in the 1840s for isolated pig kidneys. Loebell used defibrinated blood as perfusate. An important milestone was the development of the first closed circulation system by Max von Frey and Max Gruber in 1885. Similar to modern perfusion systems, it contained an oxygenator, a pump, a “preheater”, as well as temperature and pressure measuring devices (68).

Another important milestone was the construction of an organ perfusion device by Charles A. Lindbergh in 1935 (69). Together with Alexis Carrel they conducted twenty-six

experiments of normothermic oxygenated perfusion of thyroid, ovary, heart, kidney, spleen, suprarenal gland and were able to keep organs alive up to 21 days. The perfusion solution was based on blood serum with the addition of "growth-activating molecules" (amino acids, hormones, vitamins) (70).

The teams led by A. L. Humphries and F. O. Belzer did fundamental experiments on the hypothermic organ perfusion during the 1960s. They were able to prolong kidney preservation and successfully reimplant kidneys to contralaterally nephrectomized dogs. Humphries et al. used diluted heparinized blood with added balanced salt solution as a perfusate, Belzer et al. used pooled plasma with the addition of magnesium, dextrose, insulin, penicillin, and hydrocortisone (43,71). The authors recognized the problem of rising perfusion pressure due to blood cell aggregation and increasing viscosity of whole blood and, therefore, favored the use of plasma as a perfusate (48,72). However, Belzer et al. showed that another important causative agent for rising perfusion pressure was blockage of the microvasculature by multiple fat emboli due to denaturation of plasma lipoproteins during extracorporeal circulation. They developed a method of preliminary denaturation of the lipoproteins by freezing, rapid thawing, and subsequent removal of the lipid components by ultrafiltering and were able to achieve completely stable hemodynamic characteristics during the whole time of perfusion (72).

Belzer et al. performed the first successful HMP-preserved human kidney transplant in 1967 (73). Furthermore, they developed the first transportable HMP device in order to minimize total anoxia of the organ during transportation (72). Belzer et al. continued to perform human kidney preservation using this technique and were able to achieve successful preservation of non-heart-beating donor organs for up to 50 hours simultaneously performing viability testing and expanding the donor organ pool. They stated that, at that time, organ preservation for up to 72 hours was the crucial time required for clinical kidney transplantation (74). At the same time, HMP using diluted blood and acellular perfusates combined with hyperbaric oxygenation were investigated for experimental liver preservation (75).

Calne et al. showed that successful kidney preservation up to 12 hours was possible utilizing a much simpler method of ice-cooling of the organ and storing it in a balanced salt solution (76). Collins et al. achieved a significant breakthrough in the development of the static cold storage (SCS) method in 1969. They were able to extend kidney preservation up to 30 hours by performing initial flush perfusion with 100-150 ml of a specifically designed synthetic electrolyte solution (so-called Collins solution) and subsequent organ storage in ice saline during transportation (77). Since then, SCS with synthetic perfusates became the gold

standard for organ preservation due to its cost-effectiveness, simplicity, and convenience for transportation, while MP required complex, expensive, and bulky equipment and was not widely available (44). Furthermore, large trials of that time showed little or no benefit of HMP over SCS on renal graft function (78-80).

Despite these developments, a small number of hospitals in the USA and Europe maintained utilization of HMP for clinical kidney preservation and experimental preservation of other intra-abdominal and thoracic organs (48). Therefore, the research on the modifications of perfusion solutions continued. The investigated alternatives to the cryoprecipitated plasma (CPP) were albumin-based perfusates (81) and plasma protein fraction (PPF) (82). However, scientists soon recognized several further problems regarding utilization of the human protein-based solutions, such as their higher cost, possible batch-to-batch variability, shorter shelf-life, the risk of immunogenicity, and the potential for spreading blood-borne disease (48,83). Thus, scientists began focusing on the development of synthetic perfusates.

2 Materials and Methods

A comprehensive literature search for all published articles regarding machine perfusion and organ perfusion solutions was performed using primarily the PubMed database. The initial search was conducted using Medical Subject Headings (MeSH) “organ preservation solutions”, “organ transplantation”, “organ preservation” and free text terms “machine perfusion”, “hypothermic machine perfusion”, “midthermic machine perfusion”, “subnormothermic machine perfusion”, “normothermic machine perfusion”, “controlled oxygenated rewarming” combined with “preservation solution”, “perfusion solution”. No specific time limits were set. After identification of specific perfusion solutions, an additional search using particular names was performed in PubMed, Google Scholar, and Web of Science database. The most recent search was conducted on January 14, 2022. At first, studies were screened regarding the relevance of their title. Only abstracts of studies with relevant titles were further assessed regarding their relevance to the purpose of this diploma thesis. Full papers of the abstracts regarded as potentially relevant and written in English or German were retrieved and underwent complete review. Relevant information was, where possible, followed to the primary source, reviewed, and cited accordingly.

3 Results

3.1 *Preservation solutions for hypothermic machine perfusion*

3.1.1 Belzer MPS (UW Machine Perfusion Solution)

The first completely synthetic preservation solution for machine perfusion was developed by the team led by F.O. Belzer in 1982. The most important change was the substitution of human-derived serum albumin (HSA) by the colloid hydroxyethyl-starch (HES) as an oncotic agent (60). Prior to the development of this perfusion solution, they experimented with multiple synthetic colloids for kidney preservation, such as dextran, HES, gum arabic, polyethylene glycol (PEG), and showed that they were all less effective than the perfusate containing HSA (84). However, the HES-based perfusate achieved acceptable outcomes and, thus, they decided to further investigate this colloid.

In the pilot study, Hoffmann et al. were able to successfully preserve canine kidneys for 72 hours by hypothermic machine perfusion with the HES-based perfusion solution, obtaining even better results than in the control group with HSA-based perfusate. In addition, they compared extracellular-like and intracellular-like modifications of ion balance in this perfusate and achieved similar outcomes (60). Other essential additives included gluconate as a major impermeant anion, glutathione as a free-radical scavenger, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) as a buffer, magnesium for membrane stabilization, insulin, dexamethasone, penicillin, calcium chloride, glucose, and high levels of phosphate (60,83). This team later made some changes to the composition of this perfusate and conducted a study on HMP-preservation of human kidneys, again achieving better outcomes than in the control group with the perfusate containing serum albumin. The modifications were the addition of mannitol as an impermeant, the addition of adenine and ribose to stimulate ATP production, the addition of sodium hydroxide for pH neutrality (83). Furthermore, the utilization of a lower calcium concentration in the perfusate was based on the previous study showing significantly better mitochondrial enzyme activity and subsequent post-transplant survival with the calcium concentration of 0.5 mM compared to 0.0 mM and 1.5 mM (38). This composition of the machine perfusion solution with the extracellular-like cation concentration persisted over the years and is now considered the golden standard MPS for the preservation of kidneys, marketed under different names, such as Belzer MPS, Kidney Preservation Solution-1 (KPS-1), UW-Gluconate (UW-G) (58). The only difference is that penicillin, dexamethasone, and insulin are no more included in the

standard composition for sale; however, they are recommended as additives by the University of Wisconsin (85). Until now, several large randomized controlled trials showed that HMP preservation of standard criteria and ECD human kidney grafts with Belzer MPS results in superior short-term and long-term outcomes compared to preservation by means of SCS (86-88).

Pienaar et al. were the first to investigate this perfusion solution for the preservation of canine livers for 72 hours and were able to achieve positive outcomes only when they utilized the intracellular-like Na^+/K^+ ratios (i.e., high potassium and low sodium concentration) (89). They linked the poor outcomes with the extracellular-like Belzer MPS to the greater sensitivity of the Na^+-K^+-ATPase to hypothermic conditions in the liver (89,90). Since then, however, there have been several clinical trials showing that HOPE preservation of DBD and DCD human livers utilizing the classic composition of Belzer MPS is safe and feasible and, furthermore, provides considerably better short-term and long-term outcomes compared to SCS (91-93). Despite the reported good outcomes of dynamic liver preservation with this perfusion solution, several prominent scientists in the field of liver preservation support the idea that its composition requires further modifications to be able to achieve maximum protection against IRI. They suggest adding further antioxidants, amino acids, vitamins, buffers, and vasodilators (94-96).

Belzer MPS is also the most widely used machine perfusion solution for experimental dynamic pancreas preservation. Leeser et al. showed that human pancreas preservation by means of HMP with UW MPS resulted in a better islet yield, viability, and insulin secretion index of islets isolated from pancreata with prolonged cold ischemia time compared to those preserved by means of SCS with UW solution. All four perfused pancreata met the criteria for islet transplantation (97). Branchereau et al. investigated the feasibility of 24-hour HMP of marginal human pancreas grafts with Perf-Gen® preservation solution, which has the same composition as Belzer MPS. They showed that after 24-hour HMP preservation, there was no edema or necrosis of pancreatic and duodenal tissue on histological examination. Additionally, the immunohistochemical stainings for insulin, glucagon, and somatostatin were normal (98). Leemkuil et al. conducted two studies on HMP preservation of human pancreas with Belzer MPS and showed a significant increase in ATP-concentration compared to SCS preservation as well as general feasibility of this technique and islet viability after isolation >90 % (99,100).

3.1.2 Polysol

Polysol was initially developed by Bessems et al. for hypothermic machine perfusion of liver grafts (101). It has similar basic constituents, same osmolality, and pH as the Belzer MPS; however, it is based on another colloid – polyethylene glycol (PEG), resulting in a lower viscosity, and it is also enriched with a mix of various amino acids and vitamins. Other modifications include the addition of buffer histidine and impermeant trehalose. It is necessary to notice that the total amount of components in Polysol is 61, compared to only 14 components in the UW MPS (101). The main concept behind the development of this MPS was to support liver metabolism under hypothermic conditions by providing a highly nutritive perfusate and, in addition, possibly resuscitate grafts damaged by warm ischemia (101). However, apart from the nutritive nature of most additional components, some of them are also known for other positive effects on organ preservation, such as antioxidant properties of several vitamins (e.g., α-tocopherol and ascorbic acid), and antiproteolytic effects of several amino acids (e.g., glutamine and glycine) (101,102).

The reason for the search for a better colloid was due to the knowledge that HES causes high viscosity of the Belzer MPS and is associated with red blood cell hyperaggregation leading to microcirculatory obstruction and altered flow dynamics (103,104). The decision to use polyethylene glycol (PEG) as the main oncotic agent was based on another study by Bessems et al., where they compared Polysol containing different colloids (HES, PEG, and dextran) to the control group perfused with UW MPS in a rat liver model (103). They showed that 24-hour HMP preservation with Polysol resulted in significantly higher bile production, ammonia clearance, and lower hepatocellular damage than in the UW MPS group. Furthermore, there was a trend in favor of the PEG- and dextran-based Polysol modifications compared to Polysol-HES (103). The authors also marked previous studies on organ preservation with the utilization of polyethylene glycol that showed its additional properties, such as protection against lipid peroxidation, restoration of ATP levels, and immunosuppressive effects (105,106).

After showing the superiority of HMP with Polysol over UW MPS for 24-hour rat liver preservation obtained under normal conditions (103) and NHBD livers (101), Bessems et al. conducted a feasibility study regarding 24-hour preservation of porcine livers (107). The control group was preserved by means of SCS with Celsior solution. This study showed significantly lower hepatocellular damage and vascular resistance in the HMP group. No differences between the two groups in ammonia clearance, perfusate pH, lactate and urea

production were observed. Furthermore, no bile was produced in both groups. The authors concluded that 24-hour HMP preservation of pig liver with Polysol solution was not feasible (107).

Polysol has also been investigated for kidney preservation. Doorschodt et al. conducted a study on 20-hour HOPE preservation of porcine kidney grafts with Polysol compared to SCS preservation with Polysol and UW solution (108). Kidney preservation with Polysol resulted in a higher microcirculatory flow, lower posttransplant creatinine and urea levels, as well as higher creatinine clearance. However, these parameters did not differ significantly between HOPE Polysol and SCS Polysol groups. The histological examination showed significantly lower tubular damage, less tissue edema and inflammation in the HOPE group (108). A further study conducted by this research team evaluated 20-hour HMP preservation of warm ischemia-damaged porcine kidney grafts with Polysol compared to SCS preservation with Polysol and HTK solution (109). The results were similar, showing the superiority of preservation with Polysol by both methods compared to preservation with HTK solution, as well as a trend towards the superiority of dynamic preservation over SCS.

Furthermore, Polysol has been investigated for perfusion under midthermic conditions. Okamura et al. conducted a study on 4-hour SMP preservation of severely steatotic rat livers utilizing Polysol as a perfusate (110). It is to notice that the temperature during perfusion was 20-24°C, which would correspond to the midthermic conditions according to the classification proposed by Karangwa et al. (47) The control group was preserved for 4 hours by means of SCS with HTK solution. The SMP preservation resulted in significantly lower hepatocellular, cholangiocellular and mitochondrial damage, lower ROS production, less microcirculatory impairment, higher bile production, and better restoration of ATP levels. The authors concluded that the SMP preservation with Polysol was able to successfully protect severely steatotic livers from IRI; however, they stated that there are more studies on this technique needed before its use in clinical transplantation (110).

The research group around M-C. J. M. Schreinemachers conducted the first clinical study on the safety of the SCS preservation of human kidneys with Polysol (111). They compared nine grafts obtained from living donors to the historical controls preserved by the SCS with UW solution. The results showed comparable renal function calculated by eGFR at 1, 6, and 12 months after transplantation between the two groups. However, the incidence of acute rejection and antibody-mediated rejection was significantly higher in the Polysol group. Due to these findings, the study was discontinued prematurely. The authors conducted a thorough search for a possible reason. After performing logistic regression analysis, in vitro

immunogenicity tests, and evaluation of the trough levels of the immunosuppressive drugs, they were not able to find an explanation; however, they concluded that these findings were unlikely to be due to confounders or "random high" of the statistical variation (111).

Another possible disadvantage of nutrient-rich preservation solutions like Polysol was studied by Bruinsma et al. for subnormothermic and normothermic machine perfusion preservation (112). They showed that bacterial growth was present in the Polysol solution during incubation at 28 °C and 37 °C, while the number of bacteria decreased during incubation at the same temperatures in the HTK, UW, and Belzer MPS groups. However, they were able to eliminate most strains with the application of antibiotic cefazolin and, therefore, advocated for the use of antibiotic prophylaxis for (sub)normothermic preservation (112).

3.1.3 Vasosol

Prior to the establishment of the specific formulation, Guarrera et al. conducted several studies investigating the addition of different pharmaceuticals for machine perfusion in kidney preservation and were able to achieve improvement of early graft function and graft survival (113,114). Based on these studies, they developed a new machine perfusion solution named Vasosol. It is based on Belzer MPS with the addition of 5 components that counteract specific mechanisms of IRI: α -ketoglutarate was added to protect mitochondria by providing energy substrate, L-arginine as a nitric oxide precursor, N-acetylcysteine as an antioxidant, nitroglycerin and prostaglandin E1 as vasodilators (96).

Most studies on machine perfusion with Vasosol, however, investigated its utilization for liver preservation. After showing the safety and reliability of this machine perfusion solution for HMP preservation on discarded human livers and in a miniature swine model (115), Guarrera et al. conducted the first prospective case-control trial for 3-7-hour HMP preservation of 20 human livers with Vasosol (96). The control group was preserved by means of static cold storage with UW solution. The major endpoints were the mean incidences of primary non-function (PNF), early allograft dysfunction (EAD), patient and graft survival at 1 month and 1 year. The results showed no difference regarding PNF, patient and graft survival. The incidence of EAD showed a trend towards the superiority of HMP preservation with Vasosol; however, it did not reach statistical significance ($p = 0.08$). Several surrogate endpoints were also measured. The mean hospital length of stay and posttransplant serum markers of liver damage and renal function (AST, ALT, total bilirubin, serum creatinine) were significantly lower in the HMP Vasosol group (96). The authors later

analyzed liver tissue and effluent collected during their Phase 1 trial using histological methods, RT-PCR, immunohistochemistry, and gel electrophoresis (116). These analyses showed significantly lower expression of the classic ischemia-reperfusion injury markers, such as pro-inflammatory cytokines (IL-8, TNF-α) and intracellular adhesion molecule (ICAM-1) in the HMP group. No differences between both groups were observed regarding early histological findings after reperfusion (116).

Guarrera et al. went further and conducted a matched cohort trial on HMP preservation and subsequent transplantation of declined human ECD liver grafts, the so-called "orphan" livers (117). These organs were preserved with 3-7 hours of hypothermic machine perfusion with Vasosol solution. They were compared to the transplanted ECD livers preserved by means of SCS. The preservation solution used for SCS was not specified in the article. The primary endpoints were the mean incidences of primary non-function (PNF), early allograft dysfunction (EAD), vascular complications, as well as patient and graft survival at 1 year. No statistically significant differences between the two groups were observed regarding the major endpoints. However, the post hoc analysis of secondary endpoints demonstrated significantly lower rate of biliary complications and lower mean hospital length of stay in the HMP group. Furthermore, there was a general trend towards the faster recovery of liver enzymes, bilirubin levels, and renal function (117).

Bae et al. investigated possible anti-inflammatory and anti-apoptotic effects of the addition of the vitamin E (α-tocopherol) to the original Vasosol formulation (118). They compared liver preservation by means of SCS with UW solution to HMP preservation with UW MPS, Vasosol, and Vasosol + α-tocopherol in a rodent DCD liver model. The results demonstrated significantly lower ALT levels in Vasosol and Vasosol + α-tocopherol groups compared to SCS preservation, as well as a trend towards the superiority of Vasosol over KPS-1. Furthermore, there were significantly lower levels of inflammatory cytokines (IL-6, TNF-α, MCP-1) and apoptotic markers (cytochrome C, caspase 3 and 7) in the Vasosol groups compared to the KPS-1 group. Generally, all measured parameters were lower in the grafts preserved by HMP with Vasosol + α-tocopherol compared to the organs preserved with the original Vasosol formulation (118).

3.1.4 Histidine-Tryptophan-Ketoglutarate (HTK)

HTK solution was initially developed in the 1980s by H. J. Brettschneider for cardiac preservation in open-heart surgery (119,120). Over time, it has been shown to be effective in the static preservation of liver (121), kidney (122), and pancreas (123) grafts for clinical

transplantation. Nowadays, HTK solution, marketed under the name Custodiol®, is used for flushing donor kidneys, liver, pancreas, and heart prior to removal and after removal from the donor, as well as for preserving the organs by means of static cold storage (124). Its advantages are low viscosity, low concentration of potassium that allows direct release into the recipient's circulation, and lower cost compared to UW and Celsior solutions (124). The essential components of Custodiol® are histidine acting as a buffer, amino acids tryptophan and α-ketoglutarate acting as membrane stabilizers and metabolic substrates, mannitol as an impermeant. An important characteristic of HTK solution is its low total cation concentration of sodium, potassium, calcium, and magnesium. Therefore, the osmotic balance is mostly provided by the buffer histidine (124). In addition, tryptophan and mannitol also act as free-radical scavengers (40,41).

Minor et al. were the first to investigate the utilization of HTK solution as a perfusate for the dynamic preservation of organs. In their pilot study, they compared 24-hour HMP preservation of NHBD rat livers with HTK solution to preservation by means of SCS with HTK and venous systemic oxygen persufflation (VSOP) (125). Superoxide dismutase (SOD) was added to the HTK solution to facilitate its antioxidant capacity. Preservation of the organs by VSOP and HMP resulted in a significantly lower portal perfusion pressure, lower liver enzyme release, higher oxygen consumption upon reperfusion, and higher bile production compared to SCS preservation. Furthermore, light and electron microscopic examination demonstrated better preservation of hepatocytes as well as non-parenchymal cells by means of VSOP and HMP. The authors concluded that long-term HMP preservation using HTK solution as a perfusate is feasible and effective despite the absence of a colloid in its original composition (125).

In their second study, this research group compared 24-hour HMP preservation of NHBD rat livers with HTK solution to dynamic preservation with Belzer MPS and SCS with HTK (126). Superoxide dismutase (SOD) was added to the preservation solution in all groups. HMP preservation resulted in a significantly better vascular conductance, lower hepatocellular damage, higher metabolic activity, and higher bile production upon reperfusion compared to SCS. Furthermore, immunohistochemical staining showed attenuation of inflammatory reaction and immunogenicity measured by the expression of ICAM-1 and major histocompatibility complex (MHC) II in the HMP groups. There were no significant differences regarding these parameters between HTK and Belzer MPS HMP groups. The only parameter that showed a significant difference was the portal venous perfusion pressure during machine perfusion, with higher values observed in the HTK group.

However, this effect disappeared upon reperfusion. The authors pointed out that there was no increase in vascular resistance during 24 hours of machine perfusion with non-colloidal HTK solution. Furthermore, tissue edema measured by dry weight/wet weight ratio showed equal values in all groups (126).

Jia et al. investigated dynamic and static preservation of rat livers with different perfusates (127). They compared saline, UW, and HTK solutions. Regardless of the perfusate, HMP resulted in better preservation of tissue morphology, lower enzyme release, and lower oxidative stress compared to SCS preservation. Dynamic preservation with HTK resulted in significantly lower vascular resistance and higher ATP levels compared to HMP with UW solution and saline. HMP with UW solution showed the least edema formation. The authors concluded that among these tested perfusates, HTK appeared to be the optimal perfusion solution for HMP of rat liver grafts (127).

After obtaining promising results with experimental liver preservation, Minor et al. conducted a study on HMP preservation of NHBD porcine kidney grafts utilizing HTK solution as a perfusate (128). Kidneys in the control groups were preserved by HMP with Belzer MPS and SCS with HTK. For machine perfusion, HTK was modified by the addition of glucose, ampicillin, heparin, and the osmolyte taurine. Perfusion with Belzer MPS resulted in lower intra-renal resistance and a twofold higher transrenal flow compared to HTK solution. However, HMP with HTK was able to keep vascular resistance stable over the whole 18 hours of dynamic preservation. Results showed significantly higher ATP levels and higher microcirculatory flow upon reperfusion in-vivo in MP groups compared to SCS. Furthermore, HMP preservation resulted in a significant reduction of posttransplant DGF, serum creatinine and urea levels. No significant differences regarding outcomes were observed between the two MP groups. The authors concluded that hypothermic machine perfusion of ischemically damaged kidneys with HTK as with Belzer MPS improves graft viability after transplantation (128). These results were further validated by another study with a similar design (129).

3.1.5 HTK-N (Custodiol-N)

Custodiol-N was developed by U. Rauen and H. de Groot based on their extensive previous research on the mechanisms of preservation injury (130). Prior to the introduction of this OPS, they made several interesting discoveries regarding the pathophysiology of hypoxic and hypothermic injury, as well as the toxicity of preservation solutions. First, they found histidine to enhance cell injury in hepatocytes (131). Second, they demonstrated the

protective effects of amino acids glycine and alanine through inhibition of hypoxia-induced sodium influx (132). Third, they showed that cold-induced oxidative injury is linked to an increase in cellular chelatable iron pool (133). Therefore, their newly developed preservation solution based on the classic HTK formulation was modified to include a non-toxic histidine derivative N-acetylhistidine as a buffer, amino acids glycine and alanine as membrane stabilizers, iron chelators deferoxamine and LK 614 to counteract ROS damage (130). Furthermore, L-arginine was added to facilitate nitric oxide (NO) production, and aspartate as a metabolic substrate for ATP synthesis (134).

In recent years, HTK-N solution was shown to be superior to HTK for SCS preservation of heart, liver, small bowel, and kidney grafts in a number of experimental animal studies. Several comparative clinical trials are on their way (135).

Stegemann et al. claimed that these modifications to the HTK solution made it particularly suitable for oxygenated dynamic preservation of organs and, thus, conducted the first experimental study on HMP preservation of liver grafts with Custodiol-N solution (134). NHBD rat livers were hypothermically perfused for 18 hours with either HTK or HTK-N solutions containing different amounts of iron chelators. The use of HTK-N resulted in a significantly lower oxidative injury and DNA fragmentation compared to the HTK solution. The addition of iron chelators to the Custodiol-N solution resulted in a significantly lower ALT, LDH, and GLDH release, better aerobic metabolic activity measured by CO_2 production, and lower expression of the apoptotic marker caspase 9 compared to HTK solution (134).

Gallinat et al. investigated the utilization of Custodiol-N solution for dynamic preservation of porcine kidney grafts (136). HTK-N solution was supplemented with dextran 40 for oncotic support and compared to gold standard KPS-1 solution. The kidneys were hypothermically perfused for 20 hours and later normothermically reperfused in vitro for viability assessment. This experiment resulted in a significantly higher renal blood flow and urine production during reperfusion in the Custodiol-N group. In addition, creatinine clearance and metabolic activity measured by oxygen consumption were found to be significantly higher, and endothelial injury marker thrombomodulin was lower in the HTK-N group. No differences were observed regarding LDH enzyme release, fractional excretion of sodium, edema formation, and vascular resistance. The ROS damage measured via the lipid peroxidation marker TBARS was significantly lower at the end of HMP in the HTK-N group; however, this effect disappeared upon reperfusion (136).

Minor et al. conducted a further study to confirm these findings in a pre-clinical autotransplantation model in vivo (137). Porcine kidneys were hypothermically perfused for 21 hours with either Custodiol-N or KPS-1 solution. HTK-N was again supplemented with dextran 40 for oncotic support. The results showed significantly better microcirculatory tissue perfusion, lower levels of ROS-mediated injury and tubular cell injury markers, lower expression of endothelial activation markers in the Custodiol-N group. In addition, kidneys preserved with HTK-N solution showed a trend towards better creatinine clearance and urea clearance function. No significant differences were observed on histological examination. The authors concluded that HMP with HTK-N solution is safe and effective for renal preservation in a clinically relevant large animal model (137).

3.1.6 Celsior

Celsior solution was initially developed by Menasché et al. for short-term perfusion and SCS preservation of heart grafts (138). Their idea was to combine protective features of cardioplegic solutions with those of organ preservation media. Celsior includes lactobionate and mannitol as impermeants, histidine as a buffer, glutamate as a substrate for ATP synthesis. Reduced glutathione, histidine, and mannitol also act as antioxidants. Furthermore, this solution was designed to counteract calcium overload and its negative effects on heart preservation. It is achieved through multiple mechanisms: low total calcium content, high magnesium content, extracellular-like composition with low potassium, and, also, a slight degree of acidosis (pH of Celsior = 7.3) (138,139).

Since then, Celsior has been shown to be equivalent to HTK and UW solution for static preservation of kidney, liver, and pancreas (11).

Compagnon et al. were the first to investigate the use of Celsior for the dynamic preservation of liver grafts (140). Rat livers were hypothermically perfused for 24 and 48 hours with Celsior via different routes (hepatic artery, portal vein, hepatic veins) and compared to SCS preservation. Celsior solution was supplemented with the colloid HES for oncotic support. Furthermore, the authors added reduced glutathione, penicillin, and streptomycin. The viability assessment was performed using normothermic reperfusion with Krebs-Henseleit buffer. The results showed lower parenchymal enzyme (AST, ALT, LDH) and non-parenchymal enzyme (PNP) leakage, as well as higher bile production during reperfusion in the livers that were perfused via the portal vein and hepatic veins. Dynamic preservation resulted in higher energy reserves and glutathione concentrations at the end of hypothermic perfusion compared to SCS; however, this effect disappeared during normothermic

reperfusion. The authors concluded that dynamic modality was superior for the preservation of liver grafts compared to SCS. Furthermore, they proposed to use portal vein as the main perfusion route (140).

Giannone et al. investigated the combined use of hyperbaric oxygenation and hypothermic machine perfusion on liver preservation (141). Rat livers were preserved for 24 hours by means of SCS or HMP with Celsior solution under normobaric and hyperbaric conditions. Dynamic preservation resulted in better cellular ultrastructure preservation determined via electron microscopy and sufficient maintenance of the baseline glycogen levels compared to the static modality with only little effect of hyperbarism. In contrast to that, markers of oxidative stress and gene expression of eNOS were significantly influenced by the hyperbaric conditions with no effect of machine perfusion on itself (141).

Catena et al. performed a study to evaluate the safety and efficacy of HMP with Celsior solution for kidney preservation in the clinical setting (142). They perfused and subsequently transplanted ten cadaveric kidney allografts from ECD donors. Primary endpoints were 1-year graft survival and incidence of DGF that were compared to the incidences from the literature on HMP. The results showed 90% graft survival and 100% patient survival. Delayed graft function was observed in one case (10%); there was no primary non-function. The authors concluded that HMP with Celsior is safe and results in comparable outcomes to those from the literature (142).

Celsior has also been investigated for perfusion under midthermic conditions. Gringeri et al. conducted a study on dynamic preservation of porcine NHBD liver grafts with Celsior at 20°C (143). They perfused grafts for 6 hours and compared them to the grafts preserved by means of SCS with Celsior solution. Dynamic preservation resulted in significantly lower AST, LDH, and lactate levels upon normothermic reperfusion, which also corresponded to significantly lower vacuolization, necrosis, and congestion rates on histological examination (143). Tabka et al. demonstrated that further supplementation of Celsior solution with Angiotensin IV for MMP of rat livers resulted in better protection against cellular injury and oxidative damage, better hepatic function, and improved endothelial function (144).

3.1.7 Institut Georges Lopez-1 (IGL-1)

IGL-1 solution was developed for SCS preservation of intra-abdominal organs at the beginning of the 2000s in Lyon, France (145). Prior to the introduction of the specific formulation of IGL-1, this team investigated different modifications of the UW cold storage solution and showed superior results with inverted cation concentrations for kidney (146)

and liver (147) preservation. Furthermore, they investigated the use of a colloid polyethylene glycol (PEG) instead of HES and showed its efficiency for the preservation of rat livers (148). Thus, IGL-1 is generally based on the UW SCS solution, but it utilizes extracellular-like (high sodium, low potassium) cation balance and uses PEG as a colloid (145). PEGs are non-toxic, water-soluble, synthetic polymers that have been shown to exert a variety of beneficial effects on biological systems, including oncotic support, induction of NO synthesis, modulation of the immune response, protection of mitochondria, stabilization of cell membrane, and others (149). Several studies have shown equivalence of IGL-1 to gold standard UW solution for static preservation of kidney (150), liver (151), and pancreas (152) grafts in the clinical setting.

Codas et al. were the first to investigate the use of IGL-1 solution for the dynamic preservation of kidney grafts (153). They compared 22-hour HMP to SCS in an NHBD pig kidney autotransplantation model. Preservation was performed using either Belzer MPS or IGL-1 solution for both modalities. The results showed significantly higher survival and faster functional recovery in both HMP groups and IGL-1 SCS group compared to SCS with Belzer MPS. The renal flow was higher, and vascular resistance was lower when HMP was performed with Belzer MPS compared to IGL-1. Histological examination revealed better preservation of morphology in perfused grafts as well as the superiority of Belzer MPS over IGL-1 for HMP. Plasma creatinine levels and tubular sodium reabsorption rates at 1 month were comparable in all groups; however, proteinuria was higher in the SCS groups. The authors concluded that dynamic preservation was superior to SCS regardless of the used perfusate. Furthermore, the less expensive IGL-1 solution was found to be compatible with both SCS and HMP and offered a similar although slightly inferior level of protection for perfused kidney grafts as Belzer MPS (153). The authors subsequently performed a longer follow-up (3 months) of the transplanted animals to determine the chronic effects of preservation (154). There were no significant changes regarding animal survival and graft function. The level of interstitial fibrosis was comparable in all groups. RTqPCR on multiple markers of chronic stress, fibrosis development, and inflammation revealed a superior level of protection in both HMP groups and the IGL-1 SCS group compared to SCS with Belzer MPS(154).

IGL-1 solution has also been investigated as a perfusate for the preservation of pancreas grafts. Prudhomme et al. conducted a feasibility study on the dynamic preservation of baboon pancreas grafts (155). They compared 24-hour HMP with IGL-1 solution using different perfusion pressures to static cold storage. No significant differences were observed

regarding morphological changes, as well as expression of pancreatic hormones and apoptosis markers between all groups. However, most organs showed significant pathological changes at 24 hours. The authors concluded that the HMP of pancreas grafts was safe and feasible for up to 12 hours of preservation (155). In their next study, Prudhomme et al. investigated the dynamic preservation of porcine pancreas grafts with IGL-1 solution (156). First, they compared 24-hour preservation by means of SCS and HMP using the measurement of injury markers. Amylase, lipase, and LDH levels, as well as immunohistochemical staining for hormones, were similar in both groups; however, macroscopic and histological examination showed more edema and necrosis in perfused pancreata beyond 12 hours of preservation. Second, they conducted pancreas allotransplantation after 2 and 6 hours of dynamic or static preservation. Recipient and graft survival, as well as allograft function measured by C-peptide and glucose levels, showed no statistically significant differences between static and dynamic preservation (156).

3.1.8 Further preservation solutions for HMP

Ecosol solution has been recently introduced by TX Innovations BV (Maastricht, the Netherlands) for static and dynamic preservation of kidney grafts (157). It is an extracellular-like preservation solution that contains polyethylene glycol (PEG) for oncotic support, antioxidants glutathione and taurine, multiple impermeants (sodium gluconate, magnesium gluconate, calcium gluconate, trehalose, raffinose, and lactobionate), as well as multiple buffers (HEPES, sodium bicarbonate, histidine, sodium citrate, and potassium phosphate), and energy substrates (glucose, pyruvate, adenine, tryptophan). Furthermore, it is supplemented with several vitamins and amino acids (157). After promising results of the first study on SCS preservation of kidney grafts with Ecosol (157), Kalenski et al. conducted a study on venous systemic oxygen persufflation (VSOP) and oxygenated HMP of porcine DCD kidneys (158). They preserved DCD grafts for 24 hours by means of VSOP, HMP, or SCS with Ecosol solution, or SCS with HTK. The control group consisted of the standard donation grafts that were cold stored in the HTK solution. The viability assessment was performed via normothermic reperfusion. The results demonstrated improved renal function and superior histological preservation in the VSOP and HMP groups compared to the DCD grafts that were statically preserved with HTK solution. Most of the measured parameters showed no difference between VSOP, HMP, and control groups. However, the authors pointed out that there was a trend towards better overall preservation quality with VSOP

over HMP. Furthermore, VSOP resulted in a superior attenuation of oxidative stress that achieved statistical significance compared to the HMP group (158).

Another novel perfusion solution named BGP-HMP was developed by Carnevale et al. for dynamic preservation of liver grafts (159). It utilizes an extracellular-like cation composition, and it is based on Good's BES buffer, impermeant gluconate, and a colloid polyethylene glycol. Further supplements include sucrose, monopotassium phosphate, magnesium sulphate, glutathione, adenosine, glycine, and antibiotics streptomycin and penicillin G (159). In their first study on dynamic preservation of liver grafts, Carnevale et al. compared 24-hour HMP of standard donation rat livers with BGP-HMP to HMP with HTK solution (160). The grafts perfused with BGP-HMP exhibited higher portal flow, lower intrahepatic resistance, higher oxygen consumption, and lower liver enzyme release during normothermic reperfusion. In addition, histological examination revealed better tissue preservation with BGP-HMP solution compared to HTK (160). In their second experimental study, they compared the 24-hour dynamic preservation of NHBD rat liver grafts with BGP-HMP solution to SCS preservation with HTK (161). The grafts preserved by means of HMP showed significantly higher portal flow and lower intrahepatic resistance during reperfusion compared to the cold stored grafts. Furthermore, histological examination showed better preservation of endothelial cells in the HMP group. On the contrary, bile production and glycogen content were higher in the SCS group. There was no statistical difference regarding ALT, AST, and LDH enzyme release between both groups (161).

The Unisol-UHK solution was developed by S. Baicu and M. J. Taylor from Organ Recovery Systems, Inc. (Itasca, USA) for universal tissue and organ preservation based on their studies on buffering capacities and different cation concentrations of preservation solutions (162,163). It is an intracellular-like solution with high potassium content, HEPES as a main buffering agent, and dextran as a colloid. Its further components are calcium, magnesium, multiple impermeants (lactobionate, sucrose, mannitol, gluconate, glucose), buffers bicarbonate and phosphate, antioxidant glutathione, and metabolic substrate adenosine (164). In their first experimental study on machine perfusion with Unisol-UHK, Baicu et al. demonstrated that this MPS was able to provide reliable pH control throughout 72-hour HMP of porcine kidneys that was superior compared to HMP with Belzer MPS (165). In their second study, Baicu et al. showed that perfusion of porcine kidneys with Unisol-UHK resulted in a superior renal metabolic function during preservation compared to HMP with Belzer MPS (164). However, the use of the Unisol-UHK was associated with significantly higher weight gain and edema formation. No significant differences were observed regarding

hemodynamic characteristics and quality of the tissue integrity preservation. The authors concluded that all grafts met the viability criteria based on hemodynamics and sustained only minimal morphological damage (164). This research group also conducted a study on the dynamic preservation of porcine pancreas grafts (166). They hypothermically perfused standard donation and DCD grafts for 24 hours with Belzer MPS or Unisol-UHK solution and compared them to the grafts preserved by means of SCS with UW solution or without preservation. The pancreata were later processed for islet isolation, and the study outcomes included parameters that described the quantity and quality of recovered islets. Dynamic preservation resulted in a significantly higher insulin content of the islets and higher islet yield from standard donation grafts compared to the SCS. Furthermore, the HMP-preserved grafts showed more homogenous digestion and better separation between exocrine and endocrine tissue components. This effect was also true for DCD grafts. There was no difference regarding islet stimulation indices between all groups. Dynamic preservation led to significantly more tissue edema compared to SCS; however, the authors hypothesized that this was one of the main reasons behind the higher islet yield and higher purity of the islets. In addition, the authors stated that there were no significant differences between the groups perfused with Unisol-UHK or Belzer MPS (166).

Several authors proposed the use of a modified HES-free UW SCS solution for the dynamic preservation of liver grafts (167,168). Dutkowski et al. stated that this choice had several reasons: first, they aimed to increase the comparability of the experiments by using the same preservation solution for both SCS and HMP; and second, UW SCS solution was the gold standard OPS for liver preservation at that time (167,169). Furthermore, their decision to omit the colloid hydroxyethyl-starch was based on the previous experiments where they observed a severe increase in vascular resistance due to the high viscosity of the UW solution. Using starch-free modification, they were able to achieve stable resistance values without a significant increase in tissue edema, especially for short-term perfusion (167,169). This research group conducted several significant studies on the dynamic preservation of rodent and porcine livers using starch-free UW solution (54,167,169,170). However, their more recent studies on human liver preservation were all performed with standard Belzer MPS (91,171,172). A research group from the University of North Carolina also conducted several studies using HES-free UW solution and achieved promising results with 5-hour and 10-hour HMP of rat livers (168,173). However, they later clearly showed the beneficial effects of additional oncotic support on the prolonged preservation of liver grafts (174).

Their more recent studies were also performed utilizing Belzer MPS as a perfusate for HMP (175,176).

IGL-2 is another recently developed preservation solution for both static and dynamic organ preservation (177). It is based on the IGL-1 solution that was modified to contain an additional buffer histidine, as well as higher concentrations of a colloid PEG and an antioxidant glutathione (177). The main rationale behind it was to enhance mitochondrial protection (149). Panisello-Rosello et al. compared IGL-2 solution to Belzer MPS for 1-hour post-SCS HMP of steatotic rat livers. Perfusion with IGL-2 resulted in significantly lower mitochondrial damage, as measured by GLDH activity; however, there were no differences regarding AST and ALT release between both groups (149). Ogbemudia et al. developed a normothermic reperfusion model to compare SCS and HMP for porcine pancreas preservation (178). They compared 6-hour SCS preservation with UW solution to HMP with Belzer MPS or IGL-2 solution. Dynamic preservation resulted in significantly higher flow rates and lower vascular resistance upon reperfusion, lower edema formation, and a more homogenous macroscopic appearance compared to SCS. Most of the measured parameters showed no difference between Belzer MPS and IGL-2 solution (178).

The studies on HMP with Aqix-RS-I solution and perfluorocarbons are discussed in the separate chapters.

Table 2. Composition of common preservation solutions for hypothermic machine perfusion.

COMPONENT	UW-G (179)	IGL-1 (145)	VASOSOL (96)	POLYSOL (101,180)	CELSIOR (138)	HTK (134)	HTK-N (134)
Sodium*	100	125	110	135	100	15	16
Potassium*	25	30	28	5	15	10	10
Calcium*	0.5	-	0.5	2	0.26	0.015	0.02
Magnesium*	5	5	5	14	13	4	8
Buffer	HEPES Phosphate	Phosphate	HEPES Phosphate	Histidine HEPES Phosphate	Histidine	Histidine	Histidine N-acetylhistidine
Colloid	HES	PEG	HES	PEG	-	-	-
Impermeant	Gluconate Mannitol	Lactobionate Raffinose	Gluconate Mannitol	Trehalose Gluconate Raffinose	Lactobionate Mannitol	Mannitol	Sucrose
Antioxidant	Glutathione Mannitol	Glutathione Allopurinol	Glutathione Mannitol N-acetylcysteine	Several vitamins Glutathione Allopurinol	Glutathione Mannitol	Tryptophan Mannitol	Tryptophan Deferoxamine LK 614
Metabolic substrate	Adenine Ribose Glucose	Adenosine	Ketoglutarate Arginine Adenine Ribose Glucose	Glucose Amino acids	Glutamate	Ketoglutarate	Ketoglutarate Arginine Aspartate
Others			Nitroglycerin Prostaglandin E1	Vitamins Amino acids			Glycine Alanine

** = mmol/L, UW-G = University of Wisconsin gluconate, IGL-1 = Institut Georges Lopez-1, HTK = Histidine-tryptophan-ketoglutarate, HEPES = N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, HES = hydroxyethyl starch, PEG = polyethylene glycol.*

3.2 *Preservation solutions for subnormothermic and midthermic machine perfusion*

3.2.1 Williams' E Medium

Despite the considerably decreased metabolism under subnormothermic and midthermic conditions, grafts might still require more nutrient support than classic machine perfusion solutions developed for hypothermic preservation can provide (181). Therefore, several research groups advocated for the use of an established cell culture medium called Williams' E Medium as a perfusate (181,182). This solution was initially developed by G. M. Williams and J. M. Gunn for long-term cell culture of rat hepatocytes for studies on carcinogenesis (183). It consists of approximately 50 components, including multiple amino acids, vitamins, inorganic salts, as well as reduced glutathione and glucose (64). Its composition makes it universal for supporting cellular function, especially under warm ex vivo conditions (181).
Berendsen et al. were one of the first to investigate the concept of subnormothermic liver perfusion without a specific oxygen carrier (184). They perfused standard donation and DCD rat livers for 3 hours at 21°C (corresponds to midthermic conditions according to the recently proposed standardized nomenclature (47)) and compared them to the standard donation livers preserved by means of SCS with UW solution and grafts transplanted after warm ischemia without preservation. Perfusate consisted of Williams' E Medium that was supplemented with insulin, penicillin, streptomycin, L-glutamine, hydrocortisone, and heparin. Subsequently, they performed orthotopic liver transplantation and a follow-up of 30 days. Animal survival was 100% in both standard donation groups and 83.3% in the MMP preserved DCD group, while no animals survived in the DCD group without preservation. This study also demonstrated that 2.5 hours of MMP was enough to restore ATP levels in the DCD livers. Liver enzymes, total bilirubin, and blood urea nitrogen levels, as well as histological examination 30 days after transplantation, showed equivalent outcomes among survivors. The authors concluded that MMP using supplemented Williams' E Medium without oxygen carriers was as effective as standard SCS for the preservation of standard liver grafts and, furthermore, was able to resuscitate ischemically damaged grafts (184). Later, this research group showed that the same protocol of midthermic machine perfusion applied at the end of preservation was able to successfully resuscitate rat livers after prolonged cold ischemic time up to 48 hours (185). They also conducted the first study on the use of MMP in human livers (64). They perfused discarded ECD human livers applying

the previously described protocol. In this study, heparin and L-glutamine were not added to the perfusate. This study demonstrated the ability of MMP to sufficiently support human liver metabolism and function. This was shown by the improvement of oxygen uptake and ATP levels, ongoing urea, albumin, and bile production, as well as stabilization of pH, lactate, and liver enzyme levels. Histological examination showed no additional morphological injury sustained during perfusion (64).

Mahboub et al. investigated the effects of controlled oxygenated rewarming after a 24-hour period of cold ischemia on rat kidney grafts (182). They perfused grafts for 90 minutes with oxygenated Williams' E Medium utilizing different strategies of gradual rewarming and compared it to a group with immediate rewarming. Creatinine, bovine serum albumin, HEPES were added to the perfusate. The results showed significantly higher fractional sodium reabsorption, lower lactate, AST, and LDH levels in the gradual rewarming groups. In addition, the RTqPCR assay showed lower expression of tubular and endothelial injury markers, as well as reduced cellular stress (182).

Several studies showed that the synthesis of important antioxidant molecule glutathione is impaired at midthermic conditions (186,187). Therefore, the authors theorized that supplementation of the perfusate (modified Williams' E Medium was used in these studies) with exogenous glutathione has the potential to improve ECD liver grafts' ability to tolerate oxidative stress (186,187).

Mahboub et al. investigated the effects of the addition of hemoglobin-based oxygen carriers (HBOCs) to the Williams' E Medium for controlled oxygenated rewarming of DCD kidney grafts in a rat model (188). Heparin, albumin, and creatinine were added to the perfusate. The control group was perfused with the simply oxygenated modified Williams' E Medium without HBOCs. The grafts were gradually rewarmed from 10 to 37°C. This study demonstrated better functional recovery of kidney grafts upon reperfusion in the HBOC group (188).

Several studies investigated midthermic and subnormothermic perfusion of rat livers using Williams' E Medium supplemented with red blood cells as oxygen carriers (65,189). Tolboom et al. showed that RBCs were not necessarily required for sufficient oxygen delivery during perfusion at 20°C (65). Oxygen extraction ratios (OER) in the study by Scheuermann et al. were below normal values during perfusion at 20°C and 30°C indicating an excessive oxygen delivery when using perfusate with RBCs at these temperatures (189).

3.2.2 Lifor

Lifor is a recently introduced preservation solution that was developed to maintain tissue and organ integrity at room temperature (190). It consists of multiple components, including amino acids, growth factors, salts, sugars, buffers, colloids, and a patented non-protein oxygen and nutrient carrier in the form of lipid nanoparticles (191). Its cation composition is extracellular-like with high sodium and low potassium content, as well as a small amount of calcium (191). The proprietary nature of Lifor precludes a detailed comparison of its composition to other preservation solutions (192). Several studies showed the suitability of Lifor and its superiority over UW solution for long-term preservation of porcine hearts by means of SMP (191,193).

Gage et al. conducted a study to compare Lifor with Belzer MPS for dynamic preservation of porcine DCD kidney grafts (190). They perfused grafts for 24 hours with Lifor and Belzer MPS at room temperature and compared them to the grafts perfused with Belzer MPS at hypothermic temperature. MMP with Lifor resulted in stable and improved perfusion characteristics compared to perfusion with Belzer MPS. Furthermore, perfusion with Lifor attenuated inflammatory response better than Belzer MPS at midthermic conditions (190).

Olschewski et al. investigated the effects of hypothermic and midthermic machine perfusion with Lifor on DCD liver grafts in a rat model (194). They perfused grafts for 6 hours at 4°C, 12°C, and 21°C and compared them to grafts preserved by means of SCS with HTK solution. The viability assessment was performed using normothermic reperfusion with Krebs-Henseleit buffer. There were two control groups that included organs that were immediately reperfused after retrieval with standard and DCD protocol. The results showed significantly lower portal venous pressure and higher bile flow upon reperfusion in the organs perfused at 21°C that was equivalent to the non-ischemic organs compared to other groups. In addition, histological examination revealed a trend towards better endothelial preservation by perfusion at 21°C. Furthermore, dynamic preservation regardless of the temperature resulted in a significantly lower ALT release compared to static preservation. However, lactate and ALT release were higher in the grafts perfused at 21°C compared to 4°C and 12°C. This fact led the authors to conclude that hepatocyte oxygen demand was not entirely met during perfusion with Lifor at 21°C (194).

3.2.3 Custodiol-MP

Recently, a further modification of HTK solution specifically designed for machine perfusion and named Custodiol-MP was introduced. Until now, there have been only two published studies regarding the utilization of this MPS: one for normothermic lung perfusion (195) and one for controlled oxygenated rewarming of kidney grafts (179). A special feature of this MPS is the possibility of flexible colloid supplementation depending on specific organ requirements. Custodiol-MP is provided in the form of a base solution and a lyophilizate containing iron chelators. The manufacturer recommends additional supplementation of glucose for nutritional support and either human albumin solution or physiological saline to obtain ready-to-use perfusate (179). The amount of added human albumin might differ depending on the requirements for oncotic support (195). Kalka et al. explained the decision to use human albumin as a colloid to be based on its wide availability compared to dextran. Furthermore, human serum albumin is known for its antioxidative properties as well as the binding of free fatty acids and other lipophilic mediators released upon reperfusion (195,196).

Von Horn et al. investigated the use of Custodiol-MP for short-term dynamic reconditioning of kidney grafts (179). First, NHBD porcine kidneys were cold stored for 20 hours in HTK solution. Then, they underwent 90 minutes of end-ischemic controlled oxygenated rewarming (COR) from 8°C to 20°C with either Belzer MPS or Custodiol-MP. Viability assessment was performed via an ex vivo reperfusion model. The results showed no significant difference regarding perfusate flow, renal oxygen consumption, glomerular protein leakage, tubular cell function, markers of renal epithelial injury, and general tissue injury measured by AST level. Histological examination demonstrated comparable renal morphology preservation in both groups. There was a trend towards higher creatinine clearance and lower inflammatory signaling activation defined through measurement of DAMP tenascin-C (TNC) in the Custodiol-MP group that, however, did not reach statistical significance. The authors pointed out that a very short time of perfusion and the use of controlled oxygenated rewarming could have possibly reduced the statistical difference of the effect that different perfusion solutions might have on graft preservation during long-term HMP. They concluded that dynamic preservation with Custodiol-MP is safe and at least as effective as gold standard Belzer MPS (179).

3.2.4 Krebs-Henseleit (KH) solution

Krebs-Henseleit buffer solution was developed by Hans Adolf Krebs and Kurt Henseleit in the 1930s for the preservation of tissue sections for their experiments on the urea cycle (197). Their concept was to create a physiologic electrolyte solution in which the inorganic salt composition would be similar to mammalian serum (197). Since then, it has been used as a bathing and perfusion medium for the preservation of various organs and tissues, such as arteries (198), hearts (199), isolated muscles (200), and livers (201). Furthermore, many studies on organ preservation used the normothermic reperfusion model with Krebs-Henseleit solution for viability assessment (126,140,194). KH solution has an extracellular-like cation composition, bicarbonate acts as a buffer, and glucose acts as an energy source (199).

Vairetti et al. conducted one of the first studies to compare MMP at 20°C without oxygen carriers to standard preservation by means of static cold storage (202). They perfused rat livers with Krebs-Henseleit solution for 6 hours and compared it to SCS with Celsior solution. KH medium was modified by the addition of 20 mmol HEPES buffer and 5 mmol glucose. Viability assessment was performed via normothermic reperfusion with KH buffer. Dynamic preservation resulted in a significantly lower ALT, AST, GGT, and LDH release upon reperfusion. In addition, ATP levels at the end of reperfusion were significantly higher in the MMP group (202).

In their second study, Vairetti et al. investigated the effects of MMP at 20°C on the preservation of fatty rat livers (203). They explained that their choice of KH medium as a perfusate was based on its similarity to Belzer MPS as well as lower viscosity due to the absence of hydroxyethyl starch. They further modified the KH solution by the addition of 5 mmol N-acetylcysteine due to its antioxidant, nutritive, and vasorelaxant properties. They perfused standard and steatotic grafts for 6 hours at 4°C, 8°C, and 20°C with modified KH medium and compared them to grafts preserved by SCS with UW solution. The same reperfusion model was used for viability assessment. Dynamic preservation resulted in significantly lower hepatocellular damage measured by AST and LDH release in steatotic grafts, with MMP being superior to other dynamic modalities. Furthermore, perfusion at 8°C and 20°C resulted in higher ATP/ADP ratios compared to SCS. The activity of an apoptotic marker caspase-3 and inflammatory marker TNF-α in fatty livers were significantly reduced by MMP compared to other modalities. Bile production was higher and biliary ALT, AST, and GGT release in steatotic grafts was lower upon reperfusion after MMP compared to

SCS. Histological examination revealed higher glycogen content and lower ROS damage in fatty livers preserved by midthermic machine perfusion in comparison to static cold storage. The authors concluded that perfusion at 20°C resulted in superior preservation of fatty livers compared to other modalities (203). They hypothesized that one of the possible reasons could be a greater susceptibility of the plasma membrane of steatotic livers to cold preservation injury (203,204). This research group conducted several other studies on the preservation of fatty livers using the same MMP protocol, which showed its beneficial effects on apoptosis and preservation of the biliary network compared to static cold storage (205,206).

Kakizaki et al. investigated the effects of short-term post-SCS perfusion at 21-25°C with oxygenated KH solution on porcine DCD liver grafts (207). There were two control groups consisting of heart-beating (HB) and DCD grafts preserved for four hours by means of SCS with UW solution and subsequently transplanted. It is important to notice that perfusion was performed via a simple dripping method instead of machine perfusion. However, the authors continuously monitored portal pressure to ensure reliable perfusion. MMP led to a significantly longer animal survival compared to the control DCD group in which no animals survived due to primary non-function. However, higher AST and ALT levels, as well as histologic examination, showed that short-term MMP was not able to completely resuscitate grafts to the level of SCS-preserved heart-beating donor grafts (207).

3.2.5 Further preservation solutions for MMP, SMP, and COR

Belzer MPS has also been widely investigated for MMP and COR of liver grafts. Ferrigno et al. modified the original UW-G formula by omitting HES, increasing calcium chloride concentration to 1.25 mM, and adding antioxidant N-acetylcysteine (208). Using this modified perfusate, they compared 6-hour MMP at 20°C to SCS with UW solution for the preservation of rat NHBD livers. Dynamic preservation resulted in significantly lower hepatocellular and mitochondrial enzyme release, higher bile flow and ATP levels, as well as better preservation of tissue integrity compared to SCS. Westerkamp et al. compared different dynamic preservation techniques and static cold storage for the preservation of rodent DCD livers using the standard composition of Belzer MPS for MP and HTK solution for SCS (209). The investigated MP techniques were 1-hour post-SCS HMP at 8°C, MMP at 20°C, and COR from 8°C to 20°C. Dynamic preservation resulted in significantly better hemodynamics and energy charge restoration, lower hepatocellular and biliary tract damage,

better metabolic, synthetic, and biliary functions compared to SCS. However, there were no statistically significant differences between different MP approaches. Research groups from Tokyo Metropolitan University and Asahikawa Medical University conducted several studies on COR and MMP of porcine DCD livers using different modifications of Belzer MPS as a perfusate (210-213). The modifications included exchanging colloid HES for dextran or polyethylene glycol and the addition of amino acids, caffeine. In their first two studies, they showed that the COR approach was associated with lower hepatocellular damage and better survival than HMP preservation of DCD livers (210,211). In their later studies, they compared MMP at 22°C to HMP using PEG-containing Belzer MPS (212,213). MMP resulted in higher oxygen consumption than HMP; however, there were no significant differences regarding most biochemical markers and hemodynamic parameters between both groups.

The research group around T. Minor conducted several studies on COR and MMP of kidney and liver grafts using Custodiol-N as a perfusate. For the studies on liver preservation, they investigated the concept of a short-term post-SCS COR of the allografts (67,214,215). First, Minor et al. showed that COR from 8°C to 20°C has a higher potential to resuscitate porcine liver grafts after prolonged cold storage than HMP or MMP (67). Then, they used the same concept to compare COR with HTK-N solution to NMP with diluted autologous blood (215). The COR approach resulted in significantly lower AST release, higher energy charge restoration, increased bile production, and less oxidative stress compared to NMP. This research group also conducted a study to investigate the COR technique for liver preservation in the clinical setting (214). They perfused and transplanted 6 human livers after prolonged cold storage and compared the results to untreated historical controls. The COR approach resulted in significantly lower posttransplant peak serum AST and ALT values. In addition, there were no cases of EAD or PNF, and 6-month graft and patient survival were 100% in the COR group; however, these parameters did not reach statistical significance compared to the control group. For dynamic renal preservation, this research group supplemented Custodiol-N solution with a colloid dextran (66,216,217). First, they compared 7-hour MMP at 20°C to HMP and SCS using the porcine DCD kidney model (216). MMP resulted in significantly higher renal blood flow and urine production upon reperfusion compared to both other modalities. Furthermore, both dynamic preservation techniques led to higher creatinine clearance and better tubular function compared to SCS. Interestingly, HMP-preserved grafts showed significantly lower TNF-α expression

compared to those preserved by MMP. Then, Schopp et al. conducted a study to investigate the post-SCS COR approach and compare it to post-SCS HMP and preservation HMP of porcine standard donation kidneys (66). COR technique led to significantly higher creatinine and urea clearance, higher oxygen consumption, better protection of mitochondria, and lower pro-apoptotic marker levels compared to both HMP approaches.

Another cell culture medium called Dulbecco's Modified Eagle's Medium (DMEM) was used as a perfusate by several authors (218,219). Both scientific groups investigated this perfusion solution for both MMP and NMP of rat liver grafts. Nösser et al. stated that NMP with DMEM in the absence of red blood cells for oxygen carriage was not feasible (218). On the contrary, MMP with simply oxygenated DMEM yielded adequate outcomes and, furthermore, the addition of RBCs to the perfusate did not lead to any significant improvement (218). Yoshida et al. supplemented DMEM with whole blood for both MMP and NMP and achieved satisfactory metabolic function upon reperfusion, as well as histopathological results that were comparable to the results obtained from the grafts reperfused immediately after harvesting (219). However, the authors noted the problem of the increased viscosity of blood under midthermic conditions that led to considerable distortion of the sinusoidal structure.

The studies on SMP, MMP, and COR with Polysol, Celsior, Steen, Aqix-RS-I solutions, and HBOC-supplemented perfusates are discussed in separate chapters.

Table 3. Composition of common preservation solutions for controlled oxygenated rewarming, midthermic and subnormothermic machine perfusion.

COMPONENT	WILLIAMS' E MEDIUM (64)	KREBS-HENSELEIT BUFFER (220)	STEEN SOLUTION (221,222)	AQIX-RS-I (223)	CUSTODIOL-MP (179)
Sodium	9.1 g/L	118.5 mmol/L	86 mmol/L	110 mmol/L	18.8 mmol/L
Potassium	0.4 g/L	4.8 mmol/L	4.6 mmol/L	5 mmol/L	8.4 mmol/L
Calcium	0.2 g/L	1.4 mmol/L	1.5 mmol/L	1.25 mmol/L	0.06 mmol/L
Magnesium	0.1 g/L	1.2 mmol/L	0.8 mmol/L	0.45 mmol/L	11.5 mmol/L
Buffer	Histidine Phosphate Bicarbonate	Bicarbonate Phosphate	Phosphate Bicarbonate	Bicarbonate BES	Histidine N-acetylhistidine
Colloid	-	-	Dextran Albumin	-	Human albumin
Impermeant	Glucose	Glucose	Glucose	Glucose	Sucrose
Antioxidant	Glutathione Amino acids Vitamins	-	-	-	Tryptophan Deferoxamine LK 614
Metabolic substrate	Multiple amino acids Vitamins Glucose Pyruvate	Glucose	Glucose	Glucose Thiamine pyrophosphate Choline chloride Several amino acids	Ketoglutarate Arginine Aspartate Glucose Pyruvate
Others	Inorganic salts Methyl linoleate			Insulin Glycerol	Glycine Alanine Phosphate

BES = N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid.

3.3 *Preservation solutions for normothermic machine perfusion*

3.3.1 Whole blood

Historically, most of the first studies on organ preservation with normothermic machine perfusion used perfusates based on whole blood (224-226). Schön et al. based their use of whole blood for preservation on the assumption that it contains all necessary substrates to maintain organ viability at this temperature (227). This research team conducted one of the first studies on normothermic dynamic preservation of standard donor and NHBD porcine liver grafts (227). Perfusate consisted of heparinized whole blood and balanced electrolyte solution at a 2:1 ratio. The liver grafts were preserved with either NMP or SCS with UW solution for 4 hours and subsequently transplanted. All NHBD grafts that underwent SCS developed primary non-function, which led to animal death, whereas NMP successfully preserved all NHBD grafts. In comparison, no difference in animal survival between NMP and SCS groups was observed in standard donor grafts. The same trend was observed regarding ALT, INR, and α-GST levels, as well as upon histologic examination. The authors concluded that NMP with whole blood was equally effective as SCS for the preservation of standard donor grafts and, furthermore, showed a considerable resuscitation potential of NHBD grafts (227).

A substantial contribution to the establishment of NMP with whole blood as a preservation technique was made by the Oxford group around P. J. Friend (61,228-234). First, they showed that their NMP protocol was able to maintain porcine liver viability for at least 72 hours as determined by physiologic perfusion parameters, ongoing metabolic and synthetic function, normal levels of hepatocellular and biliary epithelial damage markers, as well as histological examination (229). The perfusate was supplemented with antibiotic cefotaxime, vasodilator prostacyclin, as well as with total parenteral nutrition and insulin for nutritional support. Second, they compared 24-hour porcine liver preservation with NMP to standard SCS with UW solution using an ex-vivo reperfusion model for viability assessment (61). The perfusate was further modified by the addition of a bile salt (taurocholic acid) to stimulate bile production. In this study, NMP resulted in significantly lower levels of hepatocellular injury markers, better metabolic and synthetic function, and better preservation of morphology determined by histological examination compared to SCS (61). Third, they investigated the potential of NMP to preserve and resuscitate porcine NHBD liver grafts (230). Further modifications to the perfusate included the addition of calcium

chloride and sodium bicarbonate. While no livers showed signs of viability in the SCS group, NMP-preserved grafts demonstrated recovery of metabolic and synthetic function, well-preserved tissue morphology, and stable perfusion parameters upon reperfusion (230). These findings were later confirmed in an actual pig liver transplantation model (233). In addition, they were able to successfully preserve steatotic porcine livers using NMP for up to 48 hours (234). Di Francesco et al. published a case report where they successfully perfused an ECD human liver with whole blood for 4.5 hours and subsequently performed transplantation (235). There were no complications in the post-transplant period. Follow-up at 6 and 9 months showed normal organ function. The Leicester group conducted several studies on NMP with whole blood for reconditioning of NHBD kidney grafts after a prolonged period of hypothermic preservation (236-238). Prior to this, they investigated the role of leukocytes in the perfusate containing whole blood and demonstrated that leukocyte depletion resulted in an improvement of renal function, organ metabolism, and hemodynamic characteristics (239). Their leukocyte-depleted perfusate was further modified by the addition of sodium chloride, impermeant mannitol, dexamethasone, antibiotic cefuroxime, buffer sodium bicarbonate, heparin, parenteral nutrition solution, multivitamins, glucose, insulin, and vasodilator sodium nitroprusside (236-238). Their studies showed the feasibility of short-term reconditioning with NMP and its potential to reverse some of the negative effects of hypothermic preservation (236-238).

Hosgood et al. pointed out that one of the drawbacks of whole blood as a perfusate is the shortage of compatible human blood (237). In addition, several other studies of this research group showed further beneficial effects of leukocyte and platelet depletion (240). Therefore, most of the recent studies on NMP use perfusate based on red blood cells instead of whole blood (241).

3.3.2 Erythrocyte-based (RBC-based) perfusates

The most substantial amount of evidence on NMP of intra-abdominal organs exists for perfusion solutions supplemented with packed red blood cells as oxygen carriers. Except for two, all clinical trials on normothermic liver perfusion were conducted using RBC-based perfusates (241). However, there is no consensus regarding the solutions used for blood plasma replacement to provide oncotic support, electrolytes, and nutrients. Commonly used substitutes are fresh frozen plasma (FFP), human albumin solution, gelofusine, Steen solution, Williams' E medium, crystalloids like Ringer's solution. The studies on NMP with RBC-supplemented Steen solution are discussed in a separate section.

Tolboom et al. were the first to introduce a modern model for NMP preservation of liver grafts with an RBC-based perfusate (242). They showed that it was possible to successfully preserve rat liver grafts for 6 hours using NMP and achieve comparable animal survival outcomes as with standard SCS preservation. They used perfusate based on Williams' E medium, supplemented with freshly isolated rat erythrocytes and plasma, insulin, heparin, L-glutamine, hydrocortisone, and antibiotics (242).

Op den Dries et al. were the first to test NMP for perfusion and viability assessment of human liver grafts (243). They perfused four discarded human livers for 6 hours using perfusate based on RBCs and FFP, supplemented with human albumin, parenteral nutrition solution, multivitamins, trace elements, calcium glubionate, antibiotics, heparin, insulin, and sodium bicarbonate. This study demonstrated that it was possible to assess and improve human liver function and metabolism during normothermic perfusion while keeping preservation injury to a minimum (243). In another study, they perfused 12 human livers declined for transplantation and confirmed previous findings (244). Using a very similar RBC- and FFP-based perfusate composition, Liu et al. achieved the longest reported ex-situ normothermic human liver preservation of 86 hours (245). They exchanged perfusate every 12 hours for the first 24 hours and every 20 hours thereafter. The graft showed ongoing metabolic and synthetic function throughout the whole time of perfusion. Histological examination revealed well-preserved parenchyma and biliary tree (245).

Perera et al. reported the first case of a successful human liver transplantation after resuscitation of a declined graft using NMP (246). The DCD liver graft with a prolonged warm ischemia time was assessed for 2 hours on an NMP device. The perfusate was based on red blood cells; however, the authors did not specify further supplements. The graft showed stable hemodynamic characteristics with good metabolic and synthetic function during perfusion; it was re-evaluated and found to be suitable for transplantation. The total NMP time was 7 hours. The patient's recovery was uneventful, and the patient was discharged on postoperative day 10. The follow-up of 15 months showed normal graft function (246).

Ravikumar et al. conducted the first clinical trial on liver transplantation after NMP preservation (247). They perfused ten standard criteria donor livers and ten ECD grafts with an RBC-based perfusate for a mean time of 9.3 (3.5–18.5) hours and compared the transplantation outcomes to the matched control patients who received grafts preserved by means of SCS. The perfusate was further supplemented with a gelatin-based colloid solution (Gelofusine®, B Braun), glucose, amino acids, calcium gluconate, heparin, cefuroxime,

sodium bicarbonate, insulin, prostacyclin, and taurocholic acid. The results showed comparable 30-day and 6-month graft and patient survival of almost 100% in both groups. Furthermore, there were no statistically significant differences regarding PNF and EAD rate, posttransplant bilirubin, ALP and INR values, or hospital and ICU stay between both groups. However, NMP preservation resulted in significantly lower peak AST levels compared to the SCS group. The authors stated that there were no serious technical complications associated with the use of the NMP technique and concluded that normothermic dynamic preservation of liver grafts was safe and feasible (247). A similar small clinical trial conducted by Bral et al. used an identical perfusate composition (248). Most outcomes were also comparable between NMP and SCS preserved groups. However, one graft in the NMP group was discarded because of the inability to establish perfusion due to a vascular anomaly. In addition, NMP graft recipients had significantly longer ICU and overall hospital stay. The 6-month graft survival on an intention-to-treat basis was significantly higher in the SCS group (248).

Nasralla et al. conducted the so far largest multi-center randomized controlled trial to compare NMP with SCS for liver preservation (249). They used a similar RBC- and gelofusine-based perfusate supplemented with antibiotics, insulin, heparin, bile salts, prostacyclin, and parenteral nutrition solution. The primary outcome (peak AST during the first 7 days after transplantation) was significantly lower in the NMP group. In addition, graft discard and EAD rates, as well as the incidence of the post-reperfusion syndrome, were significantly lower in the NMP group. There were no differences regarding 1-year graft and patient survival, biliary complications, and length of the ICU or overall hospital stay between both groups. Furthermore, NMP allowed to significantly prolong safe preservation times (249).

A smaller randomized controlled trial on NMP preservation of liver grafts from older donors used a similar perfusate with the addition of human albumin (250). In this study, there were no differences regarding clinical outcomes, such as graft and patient survival at 6 months, peak transaminases, the incidence of biliary or vascular complications, length of hospital stay between NMP and SCS groups. However, histological examination 2 hours after reperfusion revealed better protection of grafts against ischemia-reperfusion injury in the NMP group (250).

To simplify the logistics of organ preservation by means of normothermic machine perfusion, two studies investigated a more practical concept of post-SCS NMP of liver grafts (251,252). They used the same RBC- and gelofusine-based perfusate and compared clinical

outcomes to the recipients of grafts preserved with continuous NMP. There were no statistically significant differences regarding patient and graft survival, graft function, and incidence of complications between both preservation modalities in both studies. The authors of both studies concluded that a post-SCS NMP approach is a safe and logistically beneficial alternative to continuous preservation NMP (251,252).

To specifically address the most important expected benefit of machine perfusion – the ability to expand the donor organ pool, several authors conducted clinical trials on transplantation of previously declined and high-risk liver grafts following viability assessment and resuscitation with NMP (253-255). These studies significantly contributed to the refinement of viability criteria. Watson et al. used perfusate based on red blood cells and supplemented with gelofusine, heparin, insulin, magnesium sulphate, calcium chloride, sodium bicarbonate, epoprostenol, and amino acids (253). The authors also conducted several experiments where they used Steen solution instead of gelofusine; however, they stated that due to the higher price of the Steen solution and non-superior results obtained with it, they went back to use gelofusine for oncotic support. They were able to transplant 22 out of 47 high-risk livers following viability assessment on the NMP device. One graft developed primary non-function, and three grafts developed ischemic-type biliary lesions that required re-transplantation (253). After a successful initial proof-of-concept study (254), Mergental et al. conducted a Phase II clinical trial on transplantation of declined liver grafts after viability assessment and resuscitation on NMP device (255). They used perfusate based on red blood cells, which was further supplemented with an unspecified colloid solution, heparin, calcium gluconate, sodium bicarbonate, parenteral nutrition, prostacyclin, taurocholic acid, and antibiotics. They were able to successfully transplant 22 out of 31 enrolled liver grafts with a 100% 90-day graft survival rate. In addition, most of the measured clinical outcomes were comparable with the contemporary matched controls. However, there was a significantly higher rate of EAD in the study group. The incidence of non-anastomotic biliary strictures was 18%, and, thus, the authors concluded that NMP was not able to significantly prevent ischemic biliary complications (255).

The research group around S.A. Hosgood and M. L. Nicholson conducted several studies on NMP of human kidney grafts (256-258). They used perfusate that consisted of packed red blood cells, Ringer's solution, mannitol, dexamethasone, sodium bicarbonate, heparin, prostacyclin, glucose, insulin, multivitamins, and parenteral nutrition solution. First, they published a case report of successful kidney transplantation following normothermic machine perfusion (256). Second, they conducted a clinical study on post-SCS NMP of ECD

kidney grafts (257). They perfused 18 kidney grafts for a mean time of 63 minutes and compared transplantation outcomes to the matched contemporary controls that underwent SCS preservation alone. The post-SCS NMP led to a significantly lower incidence of DGF compared to the SCS group. There were no differences regarding patient and graft survival at 12 months, as well as incidences of PNF or acute rejection (257). Third, they conducted a study on viability assessment and transplantation of declined DCD kidney grafts (258). In the first phase of this study, they found 28 out of 42 declined kidneys to be potentially suitable for transplantation based on a quality assessment score. However, there was no intention to proceed to transplantation during this phase. In the second phase, 10 out of 55 declined grafts were found suitable and recruited for NMP assessment, 5 out of 10 were subsequently transplanted. One of the transplanted kidneys developed delayed graft function, whereas the other four grafts showed normal posttransplant function (258).

Weissenbacher et al. proposed a novel concept of renal NMP that involves urine recirculation for replacement of the excreted fluid volume (259). They perfused declined human DCD and DBD grafts and were able to achieve stable perfusion for up to 24 hours. The perfusate consisted of packed RBCs, human albumin solution, cefuroxime, sodium bicarbonate, calcium gluconate, mannitol, epoprostenol, and lipid-free total parenteral nutrition with glucose.

Pool et al. investigated the effects of the use of different perfusion solutions for NMP of porcine kidneys (46). First, the retrieved DCD kidneys underwent 2-4 hours of HMP with Belzer MPS for transportation. Then, grafts were connected to an NMP device for 7-hour long perfusion. There were four different perfusions solutions used that were all based on RBCs with different media for blood plasma replacement and nutritional support. The group 1 perfusate contained serum albumin solution and Williams' E medium, the group 2 perfusate contained physiological albumin and electrolyte concentrations, the perfusion solution of the group 3 was based on the previously described colloid-free perfusate used by Nicholson et al. (257), and group 4 perfusion solution was based on the perfusate proposed by Weissenbacher et al. (259). The study outcomes included renal function parameters, cellular injury markers, hemodynamic characteristics, oxidative damage markers, expression of pro- and anti-apoptotic genes, as well as histological damage scores. The results revealed multiple significant differences regarding measured parameters between groups, with none of the perfusates being clearly superior or inferior to the others. This study demonstrated that the choice of the perfusate for MP has a strong influence on outcome markers and, therefore, can significantly affect the evaluation of machine perfusion techniques (46).

3.3.3 HBOC-based perfusates

Although dynamic preservation with RBC-based perfusates has been proven feasible and effective, there were several associated disadvantages that prompted the search for alternatives. Blood-based perfusates have a relatively short shelf-life and limited availability. Furthermore, they carry a risk of blood-borne infectious transmission, hemolytic reactions, and immunological response (260). One of the alternative approaches is the use of the so-called hemoglobin based-oxygen carriers (HBOCs). HBOCs are based on purified human or animal hemoglobins that were modified to optimize oxygen delivery and enable extended circulation time (261). HBOCs have been originally developed and tested as red blood cell substitutes for transfusion. There are several different bioengineering techniques for HBOC manufacturing. Examples are polymerization, intramolecular cross-linking, PEGylation, and liposome encapsulation (261). Therefore, there is a number of HBOC products currently undergoing clinical and preclinical trials. However, to date, none of the HBOC has gained approval for clinical use by FDA due to several associated cardiovascular adverse effects (262).

One of the first studies on the use of HBOCs for normothermic machine perfusion was conducted by Horiuchi et al. in 1992 (263). They used pyridoxylated hemoglobin-polyoxyethylene (PHP) in combination with UW solution to perfuse canine kidneys for up to 12 hours. NMP with the combination of PHP and UW solution resulted in superior preservation compared to the perfusion with UW solution or PHP alone. However, the authors stated that further research on NMP with HBOCs is required to achieve successful long-term preservation of kidney grafts (263). Later, a research group from Maastricht University conducted several studies on dynamic preservation of canine DCD kidneys at 32°C using perfusate based on a tissue culture-like medium supplemented with pyridoxylated bovine hemoglobin (264-266). In their first study, they showed that 3 hours of post-SCS warm perfusion resulted in better survival, lower rate of PNF, and lower posttransplant 24-hour and peak serum creatinine levels compared to SCS alone (264). In their second study, they investigated different combinations of static and dynamic preservation sequences and durations (265). They showed that kidney preservation with warm perfusion resulted in a significantly better renal function compared to SCS and, furthermore, that the post-SCS perfusion technique is superior to pre-SCS perfusion. In the third study, this research group demonstrated that 18-hour warm perfusion was able to successfully resuscitate kidney grafts after two hours of warm ischemia with a 5 out of 5

animal survival rate, whereas both grafts, preserved by HMP with UW-MPS, exhibited PNF, which led to animal death (266).

Laing et al. conducted the first study on the use of HBOCs for NMP in human liver preservation (260). They perfused five discarded high-risk human livers for 6 hours with HBOC-supplemented perfusate and compared them to the ones perfused with RBC-supplemented perfusate. The basal perfusate consisted of a human albumin solution, heparin, a mix of amino acids, vasodilator epoprostenol, sodium bicarbonate, calcium gluconate, and antibiotics. To that, they added either three units of packed RBCs or the same volume of Hemopure (HBOC-201), which is based on polymerized and sterilized bovine hemoglobin. Both groups had comparable perfusion parameters and were able to maintain liver metabolic function throughout the perfusion with no significant differences between groups. Furthermore, histological examination and measurement of ROS damage markers showed similar outcomes. The oxygen extraction ratio was significantly higher in the HBOC group. In addition, the authors investigated the possible cytotoxicity of Hemopure on a human hepatic cell line culture in an in vitro model of IRI (260). There was no increase in intracellular ROS production, apoptosis, or necrosis in the Hemopure group compared to standard culture media. The authors concluded that Hemopure is a feasible alternative to packed RBCs for normothermic machine perfusion of liver grafts (260).

Matton et al. conducted a similar study on the preservation of discarded human livers with HBOC-201 (267). They compared NMP using HBOC-201 plus FFP or gelofusine to NMP with RBCs plus FFP. The perfusates were further supplemented with human albumin, parenteral nutrition solution, antibiotics, vitamins, trace elements, sodium bicarbonate, calcium glubionate, heparin, and insulin. NMP with HBOCs resulted in a significantly higher ATP content and higher bile production compared to the RBC group. Other measured outcomes, such as oxygen extraction, bile composition, lactate and glucose concentrations, ALT, and histological injury, showed no statistically significant differences between all three groups. However, most of these parameters showed a trend towards better preservation with HBOC-201 compared to RBCs (267).

Aburawi et al. conducted the first feasibility study on the preservation of discarded human kidney grafts with HBOCs (268). They perfused kidneys for 6 hours with a solution based on either HBOC-201 or packed red blood cells. The basal perfusate consisted of Williams' E medium, sodium bicarbonate, heparin, insulin, calcium chloride, and dexamethasone. The authors decided not to supplement any additional oncotic agents, such as albumin, due to its negative effects on renal physiology during NMP. There were no significant differences

between groups regarding hemodynamic characteristics, urine production, creatinine clearance, oxygen consumption, ATP levels, and histological injury. Grafts perfused with RBCs exhibited higher lactate levels at all time points. The authors concluded that human kidney preservation with HBOC-supplemented perfusate was safe and feasible (268).

One of the many logistical benefits of HBOCs over RBCs is the possibility to combine different machine perfusion techniques without interruptions and without the need to exchange perfusate. The whole blood and RBC-based perfusates become viscous at low temperatures, the membranes of erythrocytes become stiff and cause subsequent hemolysis, whereas the HBOCs are not affected by the change in temperature (241). To investigate the possible benefits of an uninterrupted combination of the MP techniques, Boteon et al. conducted a study on the preservation of discarded human livers where they compared uninterrupted HMP-COR-NMP with HBOC-based perfusate to an interrupted sequence of HMP with Belzer MPS followed by perfusate exchange and NMP with HBOCs (269). The total perfusion time was 6 hours for both groups. The HBOC-supplemented perfusate consisted of human albumin solution, heparin, sodium bicarbonate, calcium gluconate, antibiotics, multiple amino acids and vitamins, and epoprostenol. There were no serious adverse effects associated with the use of HBOCs, and the Hemopure-based perfusate was found to be stable and safe at different temperatures. Most of the measured outcomes, including oxygen consumption, ATP replenishment, metabolic parameters, and markers of oxidative damage and inflammation, exhibited no significant differences between the two groups (269).

The University of Groningen conducted the first clinical trial on the use of HBOCs, the so-called DHOPE-COR-NMP trial (270). Declined DCD livers underwent 1 hour of oxygenated HMP with a subsequent 1-hour COR, followed by 2.5 hours of NMP for viability assessment. If the liver met viability criteria, it remained on NMP until transplantation. The perfusate consisted of HBOC-201, gelofusine, albumin, glutathione, heparin, insulin, sodium bicarbonate, taurocholic acid, and antibiotics. The primary endpoint was graft survival at 3 months after transplantation. Secondary endpoints were related to graft and patient survival, PNF, biliary complications, and serum biomarkers. 11 out of 16 perfused livers were transplanted. The 3-, 6-, and 12-month graft and patient survival rates were 100%, there were no cases of PNF. The outcomes were compared to the historical cohorts of the regular DBD and DCD liver transplants that were performed at the University of Groningen during about the same period. There were no statistically significant differences regarding graft and patient survival, PNF rate, rejection rate, biliary and vascular

complications, bilirubin levels, and INR values between all three groups. Peak ALT levels were significantly lower in the trial cohort compared to regular DBD and DCD transplants, and peak AST levels were significantly lower in the trial group compared to the regular DCD transplant cohort. Most interestingly, the authors stated that the introduction of this MP protocol led to a 20% increase in the number of deceased donor liver transplantations at their center during the trial running time (270).

HBOC-containing perfusates have also been investigated for dynamic preservation under midthermic conditions. Fontes et al. supplemented Belzer MPS with Hemopure for 7-hour perfusion of porcine livers at 21°C and compared the outcomes to SCS preservation with UW solution (271). Dynamic preservation with HBOCs resulted in a significantly higher animal survival rate, lower posttransplant peak serum transaminases, higher bile production, and better preservation of hepatic tissue integrity compared to SCS. Shonaka et al. conducted several studies to investigate the possible effects of the addition of HBOCs on porcine DCD liver preservation (272,273). They used human-derived hemoglobin vesicles (HbVs) as oxygen carriers and added them to UW-gluconate solution for midthermic machine perfusion. HbVs are phospholipid vesicles that encapsulate purified and concentrated hemoglobin. In the first study, they showed that the addition of HBOCs resulted in significantly higher oxygen consumption during MMP compared to perfusion with basal perfusate alone (272). However, there was no difference in hepatocellular injury. In the second study, they compared MMP with and without HBOCs to HMP with UW-G and to SCS (273). Oxygen consumption during preservation was significantly higher in both MMP groups compared to HMP; however, there was no significant difference between MMP with and without HbVs. Hemodynamic characteristics were equivalent in both MMP groups. AST levels upon reperfusion were significantly lower in the MMP+HBOCs group compared to all other preservation modalities. Furthermore, electron microscopy revealed superior preservation of mitochondria in the MMP+HBOCs group.

3.3.4 Perfluorocarbons (PFCs)

Other artificial oxygen carriers that have been investigated for use in machine perfusion are perfluorocarbons. PFCs are liquids with a high capacity for dissolving respiratory gases (274). Another important characteristic of PFCs is their biological inertness due to the extremely strong carbon-fluoride bonds, which makes them suitable for use in living organisms (275). Oxygen solubility in PFCs is approximately 25 times greater than in blood or water (274). In addition, oxygen binding by PFCs is not considerably affected by the

change in temperature, which opens a possibility to use them as an oxygen carrier at different temperatures, as well as for the sequential combination of different MP techniques or controlled oxygenated rewarming (274). Perfluorocarbons have been tested for treatment of cerebral and cardiac ischemia, anemia, for liquid breathing, as a contrast medium, and as a blood substitute for transfusions (274,275). Furthermore, they have been investigated for different techniques of organ preservation, such as flushing, static cold storage, and machine perfusion (274). Due to their lipophilic nature, PFC solutions for intravascular use must be emulsified. Early clinical trials on the first-generation PFCs reported serious adverse reactions, such as anaphylaxis, hypotension, complement activation, and thrombocytopenia, that were later linked to a specific emulsifying agent Pluronic F-68. Since then, new PFC solutions with different emulsifiers have been developed and are currently under investigation (274).

A research group led by L. Brasile conducted several studies on dynamic preservation of canine kidneys using perfusate supplemented with a second-generation PFC emulsion as an oxygen carrier (276,277). The basal perfusate consisted of a tissue culture-like medium containing more than 60 ingredients, including amino acids, vitamins, lipids, carbohydrates, colloids, plasma proteins, and a growth factor (63,276). In their first study, the addition of the PFCs to the preservation solution for perfusion at 32°C as well as static storage at 25°C resulted in lower post-transplant serum creatinine values and better histological preservation compared to the basal perfusate alone or saline supplemented with PFCs (276). The kidneys perfused with the PFC-supplemented perfusate were able to maintain normal hemodynamic characteristics during perfusion for up to 7 hours and later exhibited normal morphology upon the histological examination after the animals were euthanized (276). In their second study, in situ flushing with the PFC-supplemented perfusate at 32°C allowed successful preservation of NHBD kidney grafts for up to 8 hours (277).

Metcalfe et al. used machine perfusion at 32°C with a tissue culture medium supplemented with PFCs for oxygen carriage and albumin for oncotic support to establish a model of ex vivo viability assessment for NHBD kidney grafts (278). The hemodynamic characteristics remained stable throughout the whole 3 hours of perfusion.

The addition of PFCs was also investigated for hypothermic machine perfusion (279). Bezinover et al. perfused DCD rat liver grafts for 8 hours using UW solution with or without PFCs and compared them to the SCS with UW solution. Dynamic preservation resulted in significantly lower levels of apoptosis markers and lower intracellular fat content of the liver

tissue compared to the static preservation. Furthermore, using microarray analysis of gene expression, the authors showed that HMP resulted in a significantly lower number of genes with altered expression compared to the SCS. In addition, the authors pointed out that the addition of PFCs led to an improvement in several parameters; however, this effect did not achieve statistical significance and, therefore, requires further research (279).
The more recent studies on organ preservation with PFCs concentrated on their usage for static cold storage (280,281).

3.3.5 Steen solution

Steen solution was developed in 2001 by S. Steen in cooperation with Vitrolife AB (Gothenburg, Sweden) for normothermic ex vivo lung perfusion (EVLP) (282). In their initial studies, they proposed the combination of the Steen solution and red blood cells as a perfusate (282). However, since then, the Toronto protocol of lung perfusion with acellular Steen solution has shown very promising results and accumulated the largest amount of clinical experience with EVLP (283). This solution utilizes extracellular-like cation balance, contains glucose as an energy source, sodium bicarbonate and phosphate as buffer systems (222). Furthermore, it contains two colloids: human albumin for oncotic support and edema inhibition, as well as dextran for endothelial protection and inhibition of coagulation cascade and platelet aggregation (283).
Boehnert et al. were the first to investigate normothermic machine perfusion with acellular Steen solution for the preservation of porcine DCD livers (284). They used two models for the assessment of preservation quality: normothermic reperfusion with diluted blood and an actual orthotopic transplantation model. Post-SCS NMP was compared to SCS with UW solution. NMP duration was 8 hours in the reperfusion model and 4 hours in the transplantation model, both after 4 hours of preceding SCS. The perfusate was supplemented with cefazolin, heparin, and vasodilator and platelet aggregation inhibitor epoprostenol. Dynamic preservation resulted in a significantly lower ALT release during reperfusion, less hepatocyte necrosis, higher oxygen consumption, higher arterial perfusion of the peripheral liver parenchyma, and better preservation of the biliary tract. In addition, posttransplant serum AST levels were found to be significantly lower in the NMP-preserved group (284).
Minor et al. reported the first human kidney transplantation after preservation by combined COR and NMP with oxygenated acellular Steen solution (285). The ECD kidney graft was first cold stored for 12.5 hours and then gradually rewarmed by perfusion for 2 hours. The Steen solution was diluted with Ringer's solution at a 1:1 ratio and, furthermore,

supplemented with sodium bicarbonate, calcium gluconate, and ampicillin. During machine perfusion, the renal perfusate flow continuously increased, and there was no cellular release of potassium. The postoperative course showed immediate graft function and falling serum creatinine levels, and the patient was discharged after 16 days. The authors concluded that kidney preservation by means of COR and NMP with acellular perfusate for up to two hours is safe and feasible. However, they pointed out the need for perfusate oxygenation at a supraphysiological concentration to be able to meet the oxygen demand of the graft (285).

Despite these promising results with the cell-free perfusate, most of the studies on machine perfusion of intra-abdominal organs with Steen solution were made with the addition of red blood cells as oxygen carriers (286-289). Liu et al. conducted a study to compare the effects of different perfusates on NMP preservation of porcine DCD livers (288). The grafts were perfused for 10 hours and divided into three groups based on the used perfusion solution: whole blood, Steen solution, and Steen solution supplemented with RBCs. The control group was preserved by means of SCS with HTK solution. The viability assessment was performed with a 24-hour reperfusion model. Dynamic preservation resulted in a significantly lower hepatocellular and biliary injury, as well as higher bile production compared to SCS. In addition, the superiority of the RBC-containing perfusates over acellular Steen solution was observed during MP and during reperfusion as measured by hepatocellular and biliary enzyme release, as well as bile production. Furthermore, most parameters showed a trend towards better graft preservation with whole blood compared to Steen solution with RBCs. The authors concluded that oxygen carriers are required for a successful NMP preservation of liver grafts (288).

Selzner et al. conducted a clinical trial on liver preservation by means of NMP with Steen solution mixed with red blood cells (289). They perfused ten grafts obtained after standard donation for 8 hours and compared them to a historical control group preserved by SCS with UW or HTK solution. The perfusate was further supplemented with cefuroxime, calcium gluconate, heparin, taurocholic acid, insulins, and prostacyclin. The results showed no statistically significant difference between the two groups regarding posttransplant AST, ALT, bilirubin levels, and INR values. Furthermore, no significant differences were observed regarding clinical outcomes, such as the length of ICU and total postoperative hospital stay, as well as the rate of complications. There was a 100% patient and graft survival at 3 months in both groups. The authors concluded that NMP with Steen solution plus RBCs is safe and feasible for the preservation of human liver grafts (289).

Urcuyo et al. investigated the use of different perfusates under normothermic and midthermic conditions for dynamic preservation of porcine DCD kidney grafts (290). Their aim was to identify a perfusion technique that would enable stable kidney perfusion for 24 hours. Three techniques were tested: NMP with whole blood, NMP with Steen solution mixed with whole blood at a 6:1 ratio, MMP with acellular Steen solution at 21°C. Whole blood was supplemented with sodium bicarbonate, heparin, cefotaxime, insulin, calcium gluconate, methylprednisolone, and multivitamins. Furthermore, for the NMP with whole blood, the authors tested several vasodilators, such as verapamil and nitroprusside. NMP with whole blood resulted in a rapidly increasing intravascular resistance and, thus, perfusion of most kidneys in this group was discontinued after an average duration of 2.4 hours. Histological examination revealed severe tubular damage and glomerular necrosis. Two other perfusion techniques were able to maintain stable perfusion characteristics for at least 12 hours, with most grafts having been perfused for 24 hours. However, NMP with Steen solution mixed with whole blood resulted in a more pronounced histological damage, hyperkalemia, and acidosis compared to MMP with cell-free Steen solution. Therefore, perfusion with an acellular Steen solution at 21°C was found to be a superior technique for prolonged preservation of DCD kidney grafts (290).

The research group from the University of Toronto developed a protocol for SMP of liver grafts at 33°C using Steen solution supplemented with erythrocytes (286,287,291). Further additives included heparin, a mix of amino acids, glucose, insulin, antibiotics, vasodilator alprostadil, and antioxidant acetylcysteine. The authors also used active gaseous components, such as CO and sevoflurane, for their positive effects on endothelium (286,287). In their first experimental study, Knaak et al. compared the effects of a 3-hour SMP that was preceded and followed by SCS to preservation by means of a sole 10-hour SCS with UW solution on porcine DCD liver grafts (286). There were no statistical differences regarding posttransplant animal survival, peak AST levels, and coagulation parameters between both groups. However, SMP-treated grafts developed significantly less endothelial cell and bile duct injury. In the second study, Spetzler et al. utilized a 3-hour post-SCS SMP approach for preservation of porcine standard donation livers and compared it to 10 hours of sole SCS preservation (287). Application of the SMP protocol resulted in significantly lower posttransplant AST levels as well as better endothelial and biliary function compared to SCS alone. There was no difference regarding animal survival between groups.

3.3.6 AQIX-RS-I

Aqix-RS-I is a recently introduced cell, tissue, and organ preservation solution that was initially developed for use at normothermic conditions (292). However, it can maintain tissue viability over a wide temperature range and, therefore, can also be used for hypothermic machine perfusion (223), as well as controlled oxygenated rewarming (293). Its composition reflects physiologic ionic concentrations, osmolarity, and ion conductivity of human serum (292). Furthermore, Aqix-RS-I contains several amino acids and some other metabolic substrates (e.g., glucose and a cofactor thiamine pyrophosphate). The unique characteristic of this solution is its buffering system consisting of Good's BES buffer and the bicarbonate system that enables pH control over a temperature range of 4-37°C (292). In addition, Aqix-RS-I can carry dissolved oxygen and could also be supplemented with red blood cells (292). Initial studies investigated the potential of Aqix-RS-I for warm flushing as well as static storage of kidney grafts under normothermic conditions (292,294).

Venema et al. investigated the use of Aqix-RS-I supplemented with different colloids and red blood cells for normothermic machine perfusion of porcine DCD kidney grafts (295). First, the grafts underwent 3 hours of oxygenated HMP with Belzer MPS. Then, they were divided into five groups for a 4-hour NMP based on the used perfusate: leukocyte-depleted diluted autologous blood, AQIX-RS-I with bovine serum albumin (BSA) with and without RBCs, and AQIX-RS-I with dextran 40 with and without RBCs. The perfusates were supplemented with antibiotics, insulin, dexamethasone, mannitol, and sodium nitroprusside. The perfusate containing autologous blood was further supplemented with sodium bicarbonate, glucose, and a mix of amino acids. The results revealed significantly lower oxygen consumption and higher fractional sodium excretion, as well as a higher level of histological damage in the acellular groups. Grafts perfused with autologous blood showed significantly higher ATP levels, lower levels of ROS damage markers, higher total sodium reabsorption, and the highest creatinine clearance. The authors concluded that the addition of RBCs results in superior preservation compared to acellular groups. And furthermore, they stated that AQIX-RS-I solution with RBCs was not able to sufficiently sustain ATP production at the same level as autologous blood, which resulted in an inferior renal function and higher oxidative damage (295).

Vekemans et al. conducted an experimental study on the use of Aqix-RS-I solution for HMP of porcine livers (223). They transplanted grafts following 4-hour perfusion with Aqix-RS-I and compared outcomes to the grafts perfused with a modified Belzer MPS solution. UW

MPS was supplemented with multiple pharmacological agents to counteract the ischemia-reperfusion injury. It included several amino acids, antioxidants (catalase, superoxide dismutase, pentoxifylline, glutathione, iron chelator deferoxamine), insulin, vasodilator misoprostol, vitamin E, and multiple metabolic substrates. No statistical differences were observed regarding animal survival (33%), PNF incidence (0%), and hepatocellular enzyme release between both groups. However, the grafts perfused with Aqix-RS-I showed significantly lower proinflammatory signaling as measured by TNF-α levels and higher endothelial cell dysfunction as determined via hyaluronic acid levels.

Aqix-RS-I solution has also been used as a perfusate for controlled oxygenated rewarming of liver grafts (293,296). Von Horn et al. perfused rat DCD livers for 90 minutes using COR up to 20°C or 35°C following 18 hours of SCS preservation and compared them to the grafts that were preserved by SCS with HTK alone (293). The COR approach resulted in significantly lower liver enzyme release, higher bile flow, higher ATP levels, and lower histological damage scores compared to the SCS group. There were no significant differences regarding outcomes between COR20 and COR35 groups; however, the COR35 approach allowed for better prediction of functional recovery of the grafts upon normothermic reperfusion.

Table 4. Studies on dynamic liver preservation with common machine perfusion solutions.

	UW-G	HTK	HTK-N	Celsior	Vasosol	Polysol	Lifor	WEM	KH	Steen	AQIX	HBOC	RBC
Oxygenation	+ (93)	+ (125)	+ (134)	+ (144)	-	+ (94)	+ (194)	+ (184)	+ (203)	+ (284)	+ (223)	± (270)	± (253)
HMP	+ (297)	+ (125)	+ (134)	+ (141)	+ (96)	+ (94)	+ (194)	-	+* (203)	-	+ (223)	+ (269)	-
MMP, SMP, COR	Up to 20°C (209)	Not feasible (298)	Up to 20°C (67)	Up to 20°C (143)	-	Up to 24°C (110)	Up to 21°C (194)	+* (64)	+* (202)	+* (287)	Up to 35°C (293)	+ (271)	>20°C (299)
NMP	-	-	-	-	-	-	-	+* (242)	+* (300)	+* (284)	-	+ (267)	+ (255)
Max. duration of perfusion	24-hour human HMP (301)	24-hour rodent HMP* (126)	18-hour rodent HMP (134)	48-hour rodent HMP* (140)	7-hour human HMP (96)	24-hour rodent HMP (94)	6-hour rodent MMP and HMP (194)	6-hour rodent NMP* (242)	12-hour porcine NMP* (300)	10-hour human NMP* (289)	4-hour porcine HMP (223)	10-hour human HMP-COR-NMP (270)	86-hour human NMP (245)
Dual perfusion	+ (93)	-	+ (67)	+ (143)	+ (96)	+ (110)	-	+ (64)	+ (300)	+ (289)	+ (223)	+ (270)	+ (249)
Addition of oxygen carriers	HBOC (271)	-	-	-	-	-	N/A	RBC (242), HBOC (186)	RBC (300)	RBC (289)	-	N/A	N/A
Human MP-studies	+ (297)	-	+ (214)	-	+ (96)	-	-	+* (64)	-	+* (289)	-	+ (270)	+ (297)

*+ = feasible, - = no studies were identified, * = Modifications to the original formulation were made, N/A = Not Applicable due to presence of oxygen carriers in the basal formulation, ± = For NMP, preferably air/oxygen mixture was used aiming for hepatic venous oxygen saturation >55%, WEM = Williams' E Medium, KH = Krebs-Henseleit buffer, HBOC = perfusates supplemented with hemoglobin-based oxygen carriers, RBC = perfusates supplemented with red blood cells.*

Table 5. Studies on dynamic kidney preservation with common machine perfusion solutions.

	UW-G	HTK	HTK-N	HTK-MP	IGL-1	Celsior	Polysol	Lifor	WEM	Steen	AQIX	HBOC	RBC
Oxygenation	+ (302)	+ (128)	+ (137)	+ (179)	-	+ (303)	+ (109)	± (190)	+ (188)	+ (304)	+ (295)	+ (268)	+ (257)
HMP	+ (86)	+* (128)	+* (137)	-	+ (153)	+ (142)	+ (109)	-	-	-	-	-	-
MMP, SMP, COR	Up to 20°C (179)	-	Up to 20°C* (66)	Up to 20°C (179)	-	-	-	Up to 24°C (190)	+* (188)	+* (290)	+ (305)	+ (306)	>20°C (307)
NMP	-	-	-	-	-	-	-	-	+* (268)	+* (308)	+* (295)	+ (268)	+ (257)
Max. duration of perfusion	47-hour human HMP (309)	18-hour porcine HMP* (128)	21-hour porcine HMP* (137)	1.5-hour porcine COR (179)	22-hour porcine HMP (153)	? (142)	20-hour porcine HMP (109)	24-hour porcine MMP (190)	6-hour human NMP* (268)	24-hour porcine MMP* (290)	4-hour porcine NMP* (295)	6-hour human NMP (268)	16-hour porcine NMP (308)
Addition of oxygen carriers	-	-	-	-	-	-	-	N/A	RBC and HBOC (268)	RBC (308)	RBC (295)	N/A	N/A
Human MP-studies	+ (86)	-	-	-	-	+ (142)	-	-	+* (268)	+* (304)	-	+ (268)	+ (257)

*+ = feasible, - = no studies were identified, ? = the authors did not specify information regarding the duration of perfusion, * = Modifications to the original formulation were made, ± = room air oxygenation, N/A = Not Applicable due to presence of oxygen carriers in the basal formulation, WEM = Williams' E Medium, HBOC = perfusates supplemented with hemoglobin-based oxygen carriers, RBC = perfusates supplemented with red blood cells.*

4 Discussion

Increased utilization of ECD organs seems to be one of the promising approaches to counteract the current worldwide shortage of donor organs. However, it requires further refinement of allograft preservation and reconditioning techniques. Static cold storage is a relatively simple and cheap preservation strategy that has dominated the field of intra-abdominal organ preservation for the last 50 years. Since then, multiple clinical trials of the last decades have shown the superiority of machine perfusion over SCS for the preservation of ECD-DBD and DCD liver and kidney grafts (297,310). Dynamic preservation of pancreas grafts, on the other hand, still has not gone beyond experimental studies (311). Several research groups have even achieved expansion of donor organ pool by utilizing declined liver and kidney allografts after preceding viability assessment via NMP (255,258,270). Therefore, machine perfusion is likely to become the standard approach for the preservation of ECD organs in the future.

However, widespread clinical implementation of MP is being delayed by the great heterogeneity of investigated MP techniques and the subsequent inability to perform high-quality meta-analyses (47). The discrepancies in approaches concern temperature conditions, oxygenation, flow patterns, timing, duration of MP, and choice of the perfusion solution. Several head-to-head comparison studies have clearly shown impact of the used perfusate on study outcomes, as well as on the interpretation of viability markers (46,154). Thuillier et al. performed a multivariate analysis of variance and showed that the choice of the perfusion solution and the preservation technique had a strong individual influence on the outcomes that was further enhanced by the interaction between these two factors (154). Therefore, a better understanding of the mechanisms of dynamic organ preservation and the establishment of guidelines for the use of specific preservation solutions will facilitate the clinical implementation of machine perfusion.

The development of organ preservation solutions is based on the understanding of ischemia-reperfusion injury (10). Most solutions, therefore, contain electrolytes to counteract ion dysregulation, colloids and impermeants to prevent edema, antioxidants to protect against oxidative damage, substrates to restore cellular energy charge and metabolism, and buffers to stabilize pH. However, depending on temperature conditions of machine perfusion and associated significant changes in cellular metabolic rate, the composition of perfusate needs to be adjusted and becomes more complex with higher temperatures. For example, typical perfusate for normothermic machine perfusion of liver grafts contains oxygen carriers in the

form of red blood cells, colloids, vasodilators, buffers, antibiotics, heparin, insulin, bile salts, and total parenteral nutrition (249).

Multiple preservation solutions have been investigated as perfusates for different MP techniques, and, furthermore, a lot of research groups have modified the composition of perfusates to improve outcomes. Highly debated modifications concern the addition of different colloids, such as polyethylene-glycol, dextran, or human albumin; different oxygen carriers, such as erythrocytes, perfluorocarbons, or hemoglobin-based oxygen carriers; and also vasodilators, iron chelators, buffers, vitamins, amino acids. Moreover, there is currently no consensus regarding whether different perfusion solutions might be better suitable for the preservation of different organs. Tables 4 and 5 demonstrate that most preservation solutions have been investigated for perfusion of both kidney and liver grafts. This uncertainty comes from the fact that there have been only a few head-to-head comparison studies with different perfusion solutions. In addition to that, the bigger half of perfusates has only been studied in experimental animal models and never on human organs.

Belzer MPS, also known as KPS-1 or UW-Gluconate solution, is considered the gold standard preservation solution for hypothermic machine perfusion of kidney and liver grafts. Although it has a relatively simple composition, multiple clinical trials showed the superiority of dynamic preservation with this perfusion solution over standard SCS (297,310).

Nevertheless, some authors proposed further modifications of Belzer MPS to better address the physiology of dynamic preservation and ischemia-reperfusion injury. Guarrera et al. developed a machine perfusion solution named Vasosol. It is based on the classic formula of UW-gluconate solution and contains five further components to counteract specific mechanisms of IRI. Several case-control studies on human liver preservation showed promising outcomes after HMP with Vasosol solution (96,117). Furthermore, Bae et al. demonstrated the superiority of Vasosol over Belzer MPS in the reduction of pro-inflammatory and apoptotic signaling (118). Guarrera et al. continue their research using this perfusion solution; however, there have been no large randomized controlled trials on HMP with Vasosol so far (312).

Polysol solution was developed by Bessems et al. for HMP of liver and kidney grafts. Its basic composition is also similar to Belzer MPS, but Polysol is based on another colloid – PEG, resulting in lower viscosity and better rheologic characteristics compared to UW-G. Furthermore, it is supplemented with multiple amino acids and vitamins. Several experimental studies showed the superiority of Polysol over Belzer MPS for the dynamic

preservation of rat liver grafts (94,101,180). However, the first clinical study on the safety of SCS preservation with Polysol resulted in a significantly higher rate of acute rejection and antibody-mediated rejection in the Polysol group compared to controls preserved with UW solution (111). The authors could not identify the specific cause despite thorough examination. These results seemed to have suspended further research on this preservation solution.

HTK, an established preservation solution for SCS, has also been investigated as a perfusate for HMP. Early studies showed the feasibility of MP with HTK and its superiority over SCS; however, perfusion with HTK was associated with inferior hemodynamics compared to Belzer MPS (126,128). After several significant studies on the mechanisms of organ preservation, HTK was modified to a new solution named Custodiol-N, also known as HTK-N. Most studies on dynamic preservation with Custodiol-N investigated controlled oxygenated rewarming of kidney and liver grafts up to 20°C. Regarding HMP, several studies on kidney preservation showed the efficiency of perfusion with dextran-supplemented HTK-N solution and even its superiority over KPS-1 (136,137). However, there has been only one small clinical study on dynamic preservation with Custodiol-N, and, therefore, MP with HTK-N requires further research prior to its widespread clinical implementation (214). Recently, another modification of HTK specifically designed for machine perfusion and named Custodiol-MP was introduced. Its composition is largely identical to Custodiol-N, but there is a feature of flexible albumin and glucose supplementation depending on the requirements of a specific organ. So far, there have been only a few studies on this perfusion solution, and thus, more experimental evidence is needed prior to drawing any conclusions (179).

IGL-1 and Celsior, other widely known and established preservation solutions for SCS of kidney, liver, and pancreas grafts, have also been applied as perfusates in studies on HMP. Early studies showed comparable outcomes for dynamic kidney preservation as with Belzer MPS; however, these studies were not followed by larger clinical trials (142,153). Therefore, these perfusion solutions also require further research prior to translation into clinical practice. The same applies to recently introduced perfusates for HMP, such as IGL-2, Ecosol, BGP-HMP, and Unisol-UHK.

For NMP, most research groups seem to agree that perfusion solutions must be supplemented with oxygen carriers due to the much higher metabolic demand of organs. Perfusates that contain erythrocytes are considered the gold standard for normothermic perfusion of liver and kidney grafts. Historically, the first modern NMP models utilized whole blood as a

perfusate. However, more recent studies have shown improvement of perfusion when using leukocyte and platelet depleted blood (240). In addition to that, whole blood from donors contains high levels of inflammatory mediators due to brain death that are deleterious to the graft (288). Thus, most clinical trials on NMP utilized perfusates supplemented with packed red blood cells for oxygen carriage (297). Further additives to the perfusates vary widely among research groups, and there are not enough head-to-head comparison studies to draw conclusions regarding the possible superiority of one perfusate over another. Regarding the additives for oncotic support, the biggest amount of clinical evidence exists for a gelatin-based colloid solution called Gelofusine® (297).

A promising alternative to red blood cells are HBOCs, the so-called hemoglobin-based oxygen carriers. These are purified human or animal hemoglobins that underwent biochemical modifications to optimize oxygen delivery and enable extended circulation time. Further advantages of HBOCs over RBCs are longer shelf-life, lower risk of infectious transmission, and low immunogenicity (260). There has been extensive experimental research on the use of HBOCs for dynamic kidney and liver preservation that has shown comparable outcomes to the use of red blood cells (260,267,268). Another advantage of HBOCs is the possibility to use them for perfusion at different temperature conditions, whereas RBCs should not be used for perfusion under 20°C due to increased viscosity and subsequent hemolysis at lower temperatures. The University of Groningen conducted the first clinical trial on the use of HBOCs for combined HMP-COR-NMP of declined livers that resulted in 100% graft and patient survival at 6 months and, in addition to that, an expansion of donor organ pool by 20% (270).

Another alternative to RBCs are perfluorocarbons (PFCs). PFCs are liquids with a high capacity for dissolving respiratory gases that can be theoretically used for perfusion at different temperature conditions. However, initial clinical trials on the use of first-generation PFCs reported serious adverse reactions, which significantly postponed their clinical implementation (274). Since then, there have been only a few experimental studies on dynamic organ preservation with PFCs, and, therefore, more extensive research is needed.

Lifor solution, initially developed for tissue and organ preservation at midthermic conditions, utilizes the most novel approach for oxygen carriage, which is delivered via lipid nanoparticles (192). This solution also contains amino acids, growth factors, salts, sugars, buffers, and colloids, although the proprietary nature of Lifor precludes a detailed comparison of its composition to other perfusates. There have been only a few experimental studies on dynamic preservation of liver and kidney grafts with this perfusion solution, and,

therefore, it is difficult to draw any conclusions (190,194). However, two research groups that measured oxygen consumption and lactate production stated that the oxygen demand of rat livers was not entirely met by perfusion with Lifor at midthermic temperatures (194,313). Therefore, oxygen nano-carriers present in Lifor probably would not represent a feasible alternative to erythrocytes at temperatures closer to normothermia.

Steen solution is an established perfusate for normothermic ex vivo lung perfusion in both acellular formulation and with the addition of red blood cells (283). Both approaches were investigated for perfusion of liver grafts, and the authors seem to agree that normothermic liver preservation requires additional oxygen carriers (288,289). However, for perfusion of kidney grafts, MMP and short-term COR with NMP up to 2 hours using acellular Steen solution have acquired the best evidence (285,290,304). Two small clinical trials showed the safety and feasibility of perfusion with this preservation solution, and, therefore, larger randomized clinical trials seem now warranted (289,304).

The Aqix-RS-I solution was initially developed for normothermic preservation. However, several studies have shown that it can be used for perfusion at hypothermic, midthermic, and subnormothermic temperatures as well (223,293,295). As it does not contain colloids or oxygen carriers, adding erythrocytes and albumin could be considered to improve organ preservation depending on chosen MP technique (295,307). There have been only a few experimental studies on perfusion with Aqix-RS-I solution and, thus, more extensive research is needed.

Midthermic and subnormothermic machine perfusion, as well as controlled oxygenated rewarming, are the most novel techniques in dynamic organ preservation. At the moment, there is a huge heterogeneity in investigated approaches and perfusates since these techniques are not as well researched and established as NMP and HMP. Some researchers seem to agree that oxygen carriers are not necessarily required for perfusion at these temperatures (65,218,307), whereas others advocated for their addition (188,273,276,286). On the other hand, there is a wide consensus that these MP techniques require more nutrients due to higher metabolic rate than classic HMP perfusates contain. Therefore, some authors use modified HMP perfusates supplemented with metabolic substrates, whereas others use established tissue culture media, such as Williams' E Medium or Dulbecco's modified Eagle's Medium. Common NMP perfusion solutions have also been investigated for these MP approaches.

In conclusion, there are various perfusion solutions for different MP techniques on the market, and the research on the development of new perfusates continues. The establishment

of guidelines for the use of specific preservation solutions depending on the organ or MP approach would facilitate and accelerate the widespread clinical implementation of machine perfusion. To enable this, more head-to-head comparison studies and studies on discarded human organs, as well as subsequent clinical trials, are needed.

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