Preconditions for warm organ preservation
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Chapter 1

General introduction
Development of organ transplantation

The development of organ transplantation has taken flight in the 1900s. It was preceded by the awareness that the body was comprised of organs and tissues as functional units. The surgeon Theodor Kocher refined the thyroidectomy-procedure in the early 1900s but noticed that patients developed a syndrome that we now call hypothyroidism. In this setting, he carried out the first organ transplantation in 1883 by transplanting preserved thyroid tissue in a thyroidectomised patient. This was followed by the first transplantation of a preserved canine kidney in 1902 by Emerich Ullman. The consequence of this remarkable event was that Alexis Carrel introduced a novel surgical technique for suturing blood vessels and performed the first canine heart transplantation in 1902. A year later, Mathieu Laboulay started experiments consisting of sheep and porcine kidney xenotransplantation in humans by connecting the renal vessels to the brachial vessels of the recipient. However, with the surgical techniques brought to perfection, it was noticed that, whereas xenotransplantation and homotransplantation resulted in perished organ function, graft survival was excellent when autotransplantation of the preserved organ was performed. This led to the concept of “transplantation immunity” as formulated by Georg Schöne and was followed by a period in which numerous attempts were undertaken to suppress the recipient’s immunity.

The first successful transplantation in 1954 by Murray and Harrison in Boston, circumvented immunological complications by transplanting a living-related kidney from identical twins. It was not until the discovery of the immunosuppressive agents azathioprine (1959) and cyclosporine (1970), however, that the basis of our current transplantation practice was established. From that time onwards, numbers of patients on the waiting list for an organ transplant continuously increased. With the demand for donor organs substantially outnumbering the availability of cadaveric organ grafts, the persistent shortage led to the inclusion of non-heartbeating donor (NHBD) organs. This development resulted in the need for novel organ preservation techniques that not only allowed transportation of organs, but also had the potential to resuscitate function of the organ.
Chapter 1

**Development of organ preservation techniques**

In 1812, the physiologist Le Gallois wrote: “If one could substitute for the heart a kind of injection of arterial blood, either natural or artificially made,...one would succeed easily in maintaining alive indefinitely any part of the body whatsoever.”

His proclamation marks the start of the idea of *ex vivo* organ preservation which was realized by the first documented attempt of normothermic (37 °C) isolated kidney perfusion using defibrinated blood by Löbell in 1849. This concept was further elaborated by the work of Ludwig and Schmidt in 1866 who devised a means to perfuse organs with oxygenated, defibrinated blood at a constant pressure.

Hereafter, oxygenated sanguineous organ perfusion took flight with the design of an air-oxygenated perfusion circuit for animal organs by Langendorf in 1895 and Brodie in 1903. However, these devices could not provide sufficient perfusate flow into the kidney and were therefore, never widely used. Furthermore, at normothermic conditions, bacterial growth posed a major problem for prolonged organ storage inasmuch that penicillin was not readily available until 1940.

The addition of oxygen to normothermically preserved organs by experimental inclusion of an animal lung into the perfusion circuit brought new possibilities for this challenging technique. Bainbridge and Evans were able to preserve organs up to 6 hours using a heart-lung preparation-technique devised by Knowlton and Starling two years earlier. Their experiments led to confirmation that 1) oxygen consumption is dependent upon the kidney’s demand and not flow, 2) an isolated kidney is capable of producing urine, 3) the lung filters unwanted substances from the perfusion circuit, and 4) *ex vivo* derived physiologic parameters are comparable to the *in vivo* work of Barcroft who is renowned for his studies on the normal physiology of animals and humans.

The normothermic preservation method presented by Brodie, Bainbridge, and Evans was, however, not clinically applicable because of the need for a donor animal to preserve the organ in.

A breakthrough in the application of clinical organ perfusion occurred when Lindbergh designed a mechanical perfusion pump in the laboratory of Alexis Carrel in order to facilitate a heart-valve operation on his sister-in-law. The all-glass Lindbergh-Rockefeller Institute perfusion apparatus (Figure 1) was the first device capable of continuous pressure, oxygenated perfusion while maintaining sterility. The device was able to preserve functioning thyroid glands and ovaries for up to 20 days in a sterile fashion without any technical failures. At the same time, however, the limitations of normothermic organ perfusion (e.g. aberrant renal tubular function and limited graft
survival) also became apparent and led to the discovery of an advantageous effect of hypothermia (4 °C) on survival of preserved kidneys.\textsuperscript{12,13} Research into organ preservation at lower temperatures revealed the need for other perfusion solutions than blood.\textsuperscript{14-16} In the 60’s, while perfusion preservation still was the standard technique, clinically-applicable advances in organ preservation were made with the finding of diluted plasma perfusion and the addition of colloids to counteract hypo-osmotic swelling of the organs.\textsuperscript{17,18} However, these perfusates were not yet reliable enough to allow for organ transportation and the demanded extended duration of organ preservation.

![Figure 1.](image)

The famous glass pulsating perfusion pump known as the Lindbergh-Rockefeller Institute perfusion apparatus.

Owing to Folkert Belzer’s experience with organ preservation in San Francisco, he was asked to develop a NHBD kidney transplant program in the midst 1960s. He soon realized that this organ transplantation program could only work if the procurement and preservation techniques could be simplified. His serendipitous discovery of cryoprecipitated plasma (CPP) as a perfusate for machine perfusion enabled prolonged preservation of kidneys at hypothermic conditions for up to 72-hours.\textsuperscript{19} The process of cryoprecipitation removed a substantial percentage of lipids from the plasma and was responsible for the favourable results. In the following period, Belzer and colleagues realized the first clinical series of human NHBD kidney preservation using the ‘Belzer’ machine perfusion pump.\textsuperscript{20,21} The Belzer machine, portable although requiring use of a
forklift and rental truck (Figure 2), provided hypothermic organ preservation using CPP as perfusate with adequate graft survival.

Figure 2.
The Belzer machine and one of the trucks with which they drove from donor to recipient hospitals.

In the late 1960s, Geoffrey Collins from UCLA chose a different approach and developed a simple electrolyte solution for static cold storage of kidneys, in which the organ is placed in a sterile bag containing the solution and is kept, without perfusion, on melting ice. The electrolyte solution was supposed to mimic the intracellular environment with a high potassium concentration. But, because osmolarity was too low, he added a large concentration of glucose to counteract cell swelling. However, the magnesium that was added to stabilize the cellular membrane precipitated in the presence of phosphates. As a result, the Euro-Collins solution was developed, omitting the magnesium and replacing glucose for mannitol with promising results. Cold storage using the simple preservation solution Euro-Collins, was then rapidly implemented in the protocols of transplantation centers worldwide. As a response, the Belzer-group now concentrated their attention on a new formulation of a simple, yet ideal perfusate to replace the CPP in machine perfusion preservation. The novel perfusate would have to be able to sustain cellular metabolism and counteract cellular injury associated with organ preservation.
In 1982, a new gluconate-containing perfusate was presented with excellent post-transplantation organ function.\textsuperscript{25} Moreover, the inexpensive, chemically stable, and easily prepared solution was capable of preventing cell swelling with the use of gluconate, a substance that did not penetrate into the cell in contrast to the previously used impermeants, glucose, and mannitol. Gluconate, therefore, rendered the solution iso-osmolar, thereby reducing the risk of the no-reflow phenomenon by avoiding a hyperosmolar interstitium.

The current gold standard in machine perfusion, University of Wisconsin solution, was modified after its clinical introduction. First, the human serum albumin was replaced by hydroxyethyl starch, making it a fully synthetic perfusate.\textsuperscript{26} In 1986, Jan Wahlberg, working with Belzer and Southard in the transplantation laboratory of the University of Wisconsin Hospital, showed that this solution was effective for cold storage of the pancreas.\textsuperscript{27} Furthermore, it also proved more effective than Euro-Collins solution for storage of kidney and liver, whereafter UW solution became the new standard in static organ preservation. Further modifications entailed the addition of adenosine as ATP-precursor and replacement of the impermeant gluconate by lactobionic acid. However, the machine perfusion variant of the University of Wisconsin solution, being the Belzer machine perfusion solution still contains gluconate as impermeant. Lactobionic acid proved ineffective as impermeant during machine perfusion preservation for reasons that are unclear up to now. Strikingly, for the next 25 years to follow, we haven’t seen such a major breakthrough again in the preservation of organs.\textsuperscript{28}

**Current developments**

Today, transplantation centers are faced with older organ donors, older recipients, and marginal or extended criteria donor grafts. In combination with expansion of transplantation indications, the number of organs transplanted is continually increasing.\textsuperscript{29} With the resulting increase in demand for donor organs and the potential of NHBD organs to enlarge the donor pool, optimization of NHBD organ preservation becomes more and more important.

We know now that the method of simple hypothermic storage encompasses preservation-mediated injury that can skew organ preservation outcome. Therefore, pulsatile hypothermic perfusion regained clinical interest and was soon shown to be advantageous for NHBD organs over simple hypothermic storage.\textsuperscript{30, 31} However, the detrimental effects of hypothermia remain when the organ is rewarmed to
Therefore, much effort is recently put into the development of subnormothermic (28 °C), normothermic, or physiological extracorporeal perfusion. With the first clinical lung and kidney preservation at normothermic conditions being realized, we are at the dawn of reconsidering the concepts of organ preservation practice. The aim of this thesis is to determine the possibilities and limitations of current methods in organ preservation, in an effort to optimize organ preservation practice.
Outline of the thesis

In 1774, the Swiss scientist John George Zimmerman (1728 - 1795) said: “An experiment differs from an observation in this, that knowledge gained through observation seems to appear of itself, while that which an experiment brings us is the fruit of an effort that we make, with the object of knowing whether something exists or does not exist”. In light of this mindset, Chapter 2 is an appraisal of the established porcine kidney autotransplantation model. Based on the experiences in our laboratory, recommendations for porcine age, sex, and housing are described in collaboration with the faculty of veterinary sciences in Utrecht. Furthermore, recommendations concerning premedication, induction and maintenance of anesthesia, and recovery from anesthesia were formulated in order to minimize influences on the hemodynamics of the pig and the transplanted kidney. Lastly, the surgical techniques and postoperative management used in our laboratory are described as a reference for future studies and experimental designs.

Besides differences in transplantation models, many different machine perfusion apparatus and preservation conditions have been used in the experimental setting. To be able to investigate hypothermic, (sub)normothermic, and organ rewarming conditions in all available perfusion apparatus, we optimized the ‘isolated perfused porcine kidney perfusion cabinet’ (IPPK). Chapter 3 deals with the influence of temperature on the viscosity of preservation solutions and on the measurement accuracy of a commonly used perfusion solution flow sensor. Hereafter, a novel, experimental machine perfusion system is presented and its system setting stability investigated, along with its capability to reproduce other perfusion conditions, and preservation capacity of porcine kidneys.

While all machine perfusion preservation devices work on electricity, thus demanding large battery-packs, Chapter 4 describes the first disposable machine perfusion device (Airdrive®) using an oxygen-driven pump system. The technical layout and capabilities of this device regarding prolonged perfusion are discussed. Furthermore, the outcome of the first ex vivo liver perfusion experiments are reported using the Airdrive® that was previously validated for kidney preservation.

Good functional outcome of transplanted grafts begins with proper washout of blood products from the grafts’ vasculature. The washout begins with the choice of preservation solution to use, being of low or high viscosity, i.e. with or without colloids, which represents a long debated issue. Therefore, Chapter 5 shows the effects of temperature on preservation solution rheology and the role the solution plays in erythrocyte agglutination. Ultimately, blood retention in rat livers was quantified by
radioactively labeled erythrocytes being trapped after washout at different temperatures and pressure settings.

After washout, exposure of the graft to (sub)normothermic temperatures in the setting of warm organ preservation poses a risk of bacterial contamination and overgrowth in preservation media. In Chapter 6, the capacity of different bacterial strains that are commonly isolated in organ preservation solutions, are tested for their capability to grow at (sub)normothermic conditions. The bacteriostatic or bactericidal efficacy of ceftriaxone and cefazolin are subsequently assessed using a broad concentration range. Finally, potential toxic effects of a high dose of cefazolin and the effect of a cefazolin washout of the graft is simulated to assess whether bacterial regrowth will occur.

Very few preservation solutions are currently capable of maintaining the metabolic demands of prolonged (sub)normothermic preservation of organs. As the endothelium is the first barrier of the organ in contact with the preservation solution after washout, Chapter 7 describes the effects of temperature and clinically used preservation solutions on the integrity and viability of an endothelial monolayer of human umbilical vein endothelial cells. In a range encompassing hypothermic to normothermic temperatures, all experimental outcomes were represented in a viability index to allow for a conclusion regarding the best match of preservation solution and temperature.

A significant proportion of vascular research, in particular transplantation-related research, is being performed in porcine models. In Chapter 8 we set out to unlock the detection of circulating endothelial cells, a promising clinical marker for vascular damage, in porcine whole blood. In the absence of specific anti-porcine endothelium antibodies, human and swine umbilical vein endothelial cells were used to assess which anti-human endothelium antibodies displayed cross-species reactivity. Hereafter, reactivity was assessed on porcine kidney endothelial cells and a method was developed to quantify circulating endothelial cells in porcine whole blood.
References


