YALE JOURNAL OF BIOLOGY AND MEDICINE 91 (2018), pp.301-312.

REVIEW

# Advances in Perfusion Systems for Solid Organ Preservation

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In the past, a diagnosis of organ failure would essentially be a death sentence for patients. With improved techniques for organ procurement and surgical procedures, transplantations to treat organ failure have become standard medical practice. However, while the demand for organs has skyrocketed, the donor pool has not kept pace leading to long recipient waiting lists. Organ preservation provides a means to increase the number of available transplantable organs. However, there are significant drawbacks associated with cold storage, the current gold standard. To address the short-comings due to diffusional limitations, engineers have developed cold perfusion systems. More recently, there has been a significant trend towards the development of near-normothermic systems to enhance the functional preservation of solid organs including livers, lungs, hearts, kidneys, and vascularized composite allotransplants. Here we review recent advances in the development of perfusion systems for the preservation of solid organs. We provide a brief history of organ transplantation, the limitations of existing systems, and describe research being done to develop commercially available perfusion systems to enhance organ preservation.

## A BRIEF HISTORY OF SOLID ORGAN TRANSPLANTATION

Current advanced technologies in organ transplantation are the fruits of more than a century of pioneering efforts in surgery. The desire to remove tissue from one anatomical site and use it as autografts or allografts for cosmetic, restorative, or therapeutic reasons has its root in ancient civilizations; however, only in the early twentieth century were successful transplantations of non-visceral tissues such as human skin and cornea achieved [1] due to surgical advances in vascular anastomosis [2]. That was followed by the first successful kidney transplant between identical twin brothers [3,4] and the initial liver transplant trial performed a few years later. The liver transplants failed due to overwhelming technical and hemorrhagic complications aggravated by severe portal hypertension and coagulopathy. Increased surgical experience plus improvements in immunosuppression therapies ultimately resulted in prolonged liver recipient sur-

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†Abbreviations: VCA, vascularized composite allotransplantation; IRI, ischemia-reperfusion injury; ROS, reactive oxygen species; AMP, adenosine monophosphate; UW, University of Wisconsin; HTK, histidine, tryptophan, and ketoglutarate; EVLP, *ex vivo* lung perfusion.

Keywords: Bioreactors, mesenchymal stem cell manufacturing, tissue-on-a-chip

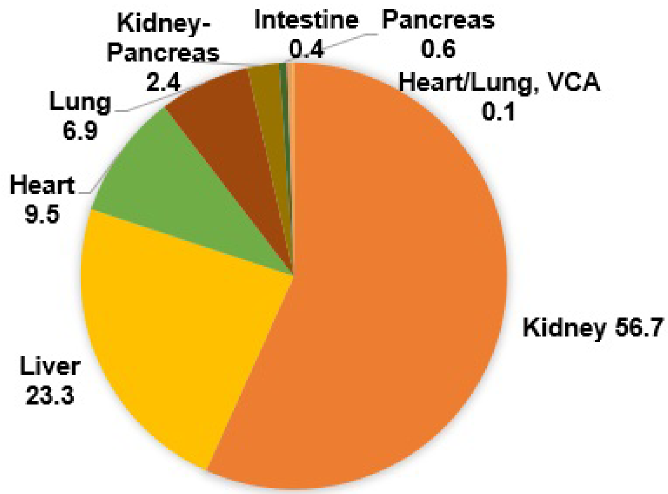


**Figure 1**. Pie chart showing the breakdown of solid organ transplants by tissue type. Data obtained from United Network for Organ Sharing (March 4, 2018) and covers the period January 1, 1988 - January 31, 2018 [11].

vivals [5]. In 1967, the first human heart transplant was performed, but its outcome and that of subsequent heart transplants were very poor, with few patients surviving to leave the hospital [6]. Several other organ transplantation "firsts" took place in this era: lung transplantation in 1963 [7], pancreas transplantation in 1968 [8], and heart-lung transplantation in 1968 [9].

Since 1968, solid organ transplantation has become widely used in the medical field and in 2016, 33,600 organ transplants [10] were performed in the U.S. with 114,756 patients still on the waiting list [11]. The relative numbers of solid organ transplants for various organs including vascularized composite allotransplants is given in Figure 1. As a result of previous and continued success in solid organs transplantation, the field of vascularized composite allotransplantation (VCA†) has grown exponentially over the last decade; though its numbers are almost negligible compared to kidney, liver, and heart transplants. VCA offers functional and aesthetic advantages over autologous tissue reconstruction and prostheses. To date, transplantations of the face [12], hands [13], lower extremity [14], vascularized knees [15], abdominal wall [16], and larynx [17], have been performed. Although VCA has made great strides, the field is still in its infancy, and challenges persist.

The field of organ transplantation is undergoing scientific and technological developments in harvesting and procurement techniques, immunosuppression regimens, tissue matching, anti-infection protocols and surgical methods, which are continually improving transplantation outcomes. However, the massive imbalance between the demand and supply of organs remains the major problem in the field. Consequently, organ preservation is a primary means to bolster the supply line for organ transplantation. The ability to deliver high quality donor organs capable of rapid resumption of their function in the recipient is a major factor in the success of organ transplantation. Efficient preservation allows staff and facilities to organize, transport organs, and perform essential laboratory tests. Therefore, methods to extend the periods over which organs can be preserved and their functionality maintained prior to transplantation is a growing research area.

## ORGAN PRESERVATION

The fundamental challenge of organ preservation is the need to maintain the viability and function of the organ in the absence of an adequate blood supply, metabolic waste removal, and physiologic stimulation. Apart from this, *ischemia-reperfusion injury* (or IRI) remains an important risk factor for both acute rejection and long-term graft outcomes [13]. Ischemia occurs as a consequence of the shortage of oxygen and glucose. In turn, cells switch to the less energy-efficient anaerobic respiration in response to oxygen deficits, intracellular accumulation of metabolites such as lactic acid, and acidic changes in cellular pH [18-20]. ATP becomes rapidly depleted within the cells resulting in a shift to adenosine monophosphate (AMP) as the predominant nucleotide. Elevated levels of reactive oxygen species (ROS) during ischemic time lead to the disruption of lipids, lipoproteins, and cellular membranes as well as the accumulation of intracellular calcium. Consequently, additional ROS is generated through the hypoxia-induced factor-1α-mediated pathway. ROS generation and electrolyte imbalance damage mitochondria and the proteins of the oxidative chain [21,22]. When blood flow is re-established to the ischemic tissue (*i.e.,* ischemia-reperfusion), a multitude of physiological reactions occur. ROS are widely recognized as important mediators of post-reperfusion induced organ injury [23].

The strategies in organ preservation can be divided into two distinct categories: (i) Suppressing metabolism to conserve ATP and minimize waste production and (ii) Mimicking physiological conditions through normothermic perfusion. Metabolic suppression of metabolism has been the most established strategy in organ preservation and includes both hypothermic preservation (for hours) and cryopreservation (for days). Recently, major emphasis has been placed on the investigation of normothermic perfusion. Cryopreservation of cells and other tissue types such as bone and cartilage for extended durations is well established, but recent evidence on a cryopreserved ovary and its successful reimplantation makes this method a feasible option for long-term solid organ preservation [24].

### *Cold Storage and its Limitations*

Static cold storage is the clinical gold standard for preservation of most solid organs. Organs are stored in chilled specialized preservation solutions that contain impermeants and colloids which prevent cellular swelling and minimize molecular changes within the cells. Each 10 °C drop in temperature of the organ results in a 50 percent decrease of its metabolic rate, until it reaches 10 to 12 percent of normal physiological rates at 4 °C [25].

Cell swelling, acidosis, and ROS production are primary side effects of hypothermia. Severe acidosis activates phospholipases and proteases causing lysosomal damage and eventually cell death [26]. Therefore, the preservation solution requires pH levels to be sufficiently controlled. The first cold storage solution was *EuroCollins* which uses glucose as an osmotic agent and phosphate for pH buffering [27]. The *University of Wisconsin (UW) solution* incorporates scavengers (glutathione, allopurinol) and adenosine as an ATP precursor. The UW solution uses HES (Hydroxyethyl starch) as a colloid to increase the oncotic pressure and also incorporates metabolically inert and osmotic substrates such as lactobionate and raffinose [28]. Another commonly used preservation solution, *HTK*, consists of histidine (H, a very potent buffer) and two amino acids, tryptophan (T) and ketoglutarate (K). Tryptophan serves as membrane stabilizer while ketoglutarate acts as a substrate for anaerobic metabolism during preservation [25]. *Celsior* is another extracellular solution and has proven to be effective in preserving abdominal organs as well [21,22]. It combines the inert osmotic control provided by *UW Solution* with the strong buffering capacity of *HTK*. Clinically it has resulted in satisfying outcomes in heart, lung, liver, pancreas, kidney, and small bowel preservation [29,30]. To date, numerous solutions exist with little consensus between transplant centers as to which is the ideal preservation solution [31].

### *Hypothermic Perfusion*

In spite of its successes, cold storage does not provide extensive organ preservation times. The slow rates of diffusion of the preservation solutions through the organ lead to ATP depletion and necrosis within tissue [32,33]. Machine perfusion can overcome this limitation by providing enhanced nutrient and oxygen delivery. Perfusion requires reliable pumps, biocompatible elements of the perfusion circuit, and oxygenation and temperature control of the perfusate [34-36]. Belzer developed hypothermic perfusion techniques for the preservation of kidneys and used whole blood as a perfusate [29]. Later, he used oxygenated micro-filtered cryoprecipitated plasma and patented the first hypothermic machine perfusion for kidneys. However, in spite of known benefits, it is technically challenging to correctly implement these machines and two large-scale studies comparing Belzer's perfusion to cold storage failed to provide superior outcomes in terms of organ function post-transplantation [30,32].

## NEAR-NORMOTHERMIC PERFUSION

Alternatively, over the last two decades several groups have examined the effects of increasing the temperature of machine perfusion to near-normothermic temperatures (20–33 °C). At these temperatures, the normal cellular and metabolic activities enable the assessment of graft viability and function prior to transplantation. Near-normothermic perfusion systems have been developed for the liver, heart, lung, and kidneys and there are ongoing clinical trials in Europe and North America [33-48]. Since these organs have distinct biophysical requirements, the organ care systems need to be customized to (i) meet each organ's specific biophysical needs, *e.g.*, breathing for lung or electrical stimulation for heart and (ii) provide specific biomarkers to assess the viability of the organ and preservation of function. Near-normothermic preservation is particularly applicable to organs from so-called "*marginal*" or non-heart-beating donors. In these cases, due to the prolonged warm ischemic times, the organ viability is negatively impacted by the subsequent cold preservation. Hence, normothermic perfusion may enhance preservation and transplantation outcomes and reduce the risk of non-functional organs. Machine perfusion systems are closely tied to transport systems and both are considered in the global market for machine perfusion organ preservation systems (Table 1). A list of perfusion (hypothermic and near-normothermic) systems being developed for clinical use is provided in Table 2.

In 1935, Carrel *et al.* created a system to perfuse various organs from cats and fowl [49]. The components of

**Table 1. Projected Global Market for Preservation Solutions and Machine Perfusion/Organ Transport Systems through 2020. Data from 2014 are provided for historical purposes.**

| Type | Amount ($Millions) | | | CAGR% |
|---|---|---|---|---|
| | 2014 | 2015 | 2020 | 2015-2020 |
| Preservation solutions | 261.0 | 316.0 | 510.0 | 10.0 |
| Machine perfusion/organ transport systems | 198.0 | 266.5 | 560.0 | 16.0 |
| **Total** | **459.0** | **582.5** | **1,070.0** | **12.9** |

that system still define what is commonly used in current perfusion systems. Their set up contained: 1) A housing chamber to maintain a sterile environment for the organ; 2) Perfusate as a medium to supply oxygen and nutrients to the organ; 3) Means to replenish the consumed oxygen in the perfusate; and 4) Phenol Red to non-invasively monitor the metabolic activity of the organ via changes in pH. Many perfusion systems of today use components from Carrel's 1935 set up as the basis for their designs.

### *Housing Systems*

Housing systems for organ preservation provide a closed, humidified, and sterile environment to protect the organ from any bacterial infections and allow for the other parts of the perfusion system loop to connect to the organ itself. Key examples of housing are provided in the previously mentioned systems such as Organ Transport Systems' LifeCradle device for *ex vivo* heart perfusion and Transmedics' Organ Care Systems that are able to be specialized for the heart, lung, or liver [50-54].

### *Perfusion Loops*

Optimization of machine perfusion requires efficient implementation of key elements including the pump, oxygenator, perfusate, reservoir, heat exchanger, sensors, stimulators, and the perfusion protocol to control how the perfusate is conditioned and transferred into the organ [53]. There is a growing body of research to study the impact of each element's performance on the effectiveness of organ preservation. For example, it was initially considered advantageous to use roller pumps that produce pulsatile wave patterns of flow [55,56], however, subsequent studies found it is most beneficial to simply use the lowest effective flow rates (*i.e.*, sufficient delivery of oxygen and nutrients) to minimize damage to the vascular endothelium [57]. More recently, atraumatic centrifugal pumps have been employed [58], though it is unclear whether they provide improved outcomes. Since many organ perfusion devices operate at a pressure and flow that is often lower than physiological levels (80 to 120 mmHg for humans) to prevent pressure related tissue injury, the other components such as the oxygenator, heat exchanger, and sensors will also need to function at decreased pressures and flow speeds [59]. For example, OrganOx's metra normothermic liver perfusion device also has several of those components: a perfusion pump that maintains the hepatic artery pressure between 60 to 75 mmHg, an oxygenator that keeps the respective partial pressures of oxygen and carbon dioxide at 12 kPa and 5 kPa, a heat exchanger to maintain the perfusate pressure at 37 °C, and continuously infuses bile salts, insulin, prostacyclin, heparin, and other nutrients into the perfusate [60].

### *Non-Invasive Measurements*

Non-invasive measurements would allow for continuous and automated feedback regarding the organ's functional metrics and enable real-time control over the perfusion protocol. Some universally employed sensors are used to measure pressure, flow-rate, temperature, and pH, as well as oxygen, glucose, and lactate concentrations. With static cold storage, there is no way to monitor the status of the organ during storage up until the transplant surgery. However, with normothermic perfusion systems, special sensors can be included to monitor organ specific functions and thus the status and functional capacity of the organ itself. For example, Transmedics' Organ Care System for the heart allows for the continuous monitoring heart rate via an electrocardiogram, and thus to check for any fibrillations of the heart during preservation and transport [61]. The Transmedics' system monitors the R-Wave of the ECG to adjust the pump speed and thus pump stroke volume as needed to keep a continuous flow of blood in the system [61]. Lung preservation systems include a method to adjust the gaseous contents of the perfusate in order to analyze the ability of the lungs to oxygenate blood [51]. Liver preservation systems allow for a method to collect and sample the bile produced by the liver during perfusion. The quantity and components of the bile produced can be analyzed to determine the health of the liver [62]. Even though the systems being developed for kidneys listed in Table 2 do not offer kidney specific measurements for kidney viability, improve-

**Table 2. A List of Commercialized Perfusion Systems.**

| Organ | Company Device | Common Features | Unique Features | Clinical Trials Status |
|---|---|---|---|---|
| **Heart** | **Organ Transport Systems**<br>Life Cradle<br>[50,51] | • Safe organ storage<br>• Maintains oxygen levels<br>• Monitors Temperature<br>• Oxygenates perfusion solution<br>• Transportable | • Uses preservation solution<br>• Hypothermic Perfusion (5°C) | Pre-clinical trials completed<br>No clinical trials reported. |
| | **Paragonix**<br>SherpaPerfusion<br>Cardiac Transport<br>System<br>[80] | | • Uses preservation solution<br>• Hypothermic perfusion (4-8°C)<br>• Monitors ischemia time<br>• Low pressure perfusion (4-6 mmHg)<br>• Can communicate with mobile devices<br>• Single-use, disposable system | No record of clinical trials |
| | **Transmedics**<br>**Organ Care System**<br>Heart<br>[52,53,81] | | • Uses donor blood + solution mix<br>• Normothermic Perfusion<br>• Monitors organ function. Such as aortic pressure, coronary flow, heart rate, blood temperature<br>• Housing enables ultrasound assessment and blood sampling<br>• Console is reusable, but perfusion set is one-time use | • NCT02323321<br>• NCT00855712 |
| **Lung** | **Organ Assist**<br>Lung Assist<br>[82] | • Safe organ storage<br>• Ventilates organ<br>• Allows for oxygenation and deoxygenation of perfusate for evaluation of lungs | • Uses preservation solution<br>• Temperature controllable: 10-38°C<br>• Pumps output up to 20 mmHg and 5L/min | No record of clinical trial |
| | **Transmedics**<br>Organ Care System<br>Lung<br>[51] | | • Uses donor blood + solution mix<br>• Normothermic Perfusion<br>• Enables ultrasound assessment and blood sampling<br>• Console is reusable, but perfusion set is one-time use<br>• Transportable | • NCT03343535<br>• NCT01630434<br>• NCT01963780 |
| | **XVIVO Perfusion**<br>Xvivo Lung Perfusion<br>System (XPS) and<br>Disposable Lung Set<br>(DLS)<br>[83] | | • Uses preservation solution<br>• Temperature controllable 15-39°C<br>• Monitors pO2 and pH of perfusate<br>• Allows for X ray<br>• XPS is reusable, but requires single use DLS | • NCT01365429 |
| **Kidney** | **Organ Recovery Systems**<br>LifePort Kidney<br>Transporter<br>[84] | • Safe organ storage<br>• Uses preservation solution<br>• Transportable | • Hypothermic perfusion (uses ice)<br>• Monitors: temperature, flow rate, vascular resistance, and pressure<br>• System is reusable, perfusion kit is single use | • NCT03024229<br>• NCT02876692<br>• NCT02826213<br>• NCT01731457 |
| | **Organ Assist**<br>Kidney Assist<br>(Transport)<br>[85] | | • Temperature control 10-38°C<br>• Transportable version only supports 0 - 4°C<br>• Oxygenates solution<br>• Outputs flow, temperature, and pressure readings<br>• Pumps output up to 20 mmHg and 5L/min | http://cope-eu.com/work%20 programme/trials.html |

**Table 2. A List of Commercialized Perfusion Systems, cont'd.**

| | | | | |
|---|---|---|---|---|
| | **Waters Medical Systems**<br>Wave or RM3<br>[59,60,86,87] | | • Hypothermic perfusion (3-10°C)<br>• Oxygenates solution<br>• Outputs 0-250 mL/min flow<br>• Monitors pressure, flow, temperature, and renal resistance<br>• Control unit is reusable, cassette is disposable/single use<br>• Can be connected to a network for online monitoring of data | • NCT02826213<br>• NCT01170910 |
| **Liver** | **Organ Recovery Systems**<br>LifePort Liver Transporter<br>[88] | • Safe organ storage | • Uses preservation solution<br>• Hypothermic perfusion<br>• Monitors: temperature, flow rate, vascular resistance, and pressure<br>• System is reusable, perfusion kit is single use<br>• Small, lightweight, transportable | No record of clinical trials |
| | **Organ Assist**<br>Liver Assist<br>[89,90] | | • Uses preservation solution<br>• Temperature controllable 10-38°C<br>• Oxygenates solution<br>• Outputs flow, temperature, and pressure readings<br>• Allows for sampling of perfusate and bile | • NCT01317342<br>• NCT02584283<br>• NCT03124641 |
| | **OrganOx**<br>Metra<br>[63,64,91] | | • Uses blood<br>• Normothermic perfusion<br>• Maintains oxygen in perfusion<br>• Measures pO2, pCO2, pH, temperature, glucose, bile production<br>• Console is reusable but has a sterile disposable portion for single use<br>• Large, but transportable | • NCT02478151<br>• NCT03089840<br>• NCT02775162<br>• NCT02740608 |
| | **Transmedics**<br>Organ Care System Liver<br>[65,66] | | • Uses donor blood + solution mix<br>• Normothermic Perfusion 34-37°C<br>• Maintains oxygen in perfusion<br>• Measures lactate in perfusate and bile production for evaluation<br>• Enables ultrasound assessment and blood sampling<br>• Console is reusable, but perfusion set is one-time use<br>• Large but transportable | • NCT02522871 |

ments can be made such as collecting the urine produced by the kidneys for biomarker analysis of viability. Various components in urine and the perfusate such as lactate and Glutathione S-Transferases have been connected to predicting the outcome of kidney transplants [63].

### *Biomimetic Stimulation*

Studies have shown that neuromuscular electrical stimulation can have an effect on helping patients recover from musculoskeletal injuries and that electrical stimulation can even have an effect on cardiac tissue culture [64,65]. Even though electrical stimulation can have an effect on promoting tissue growth and recovery in cardiac and skeletal muscle, and the Transmedics' heart Organ Care System includes an electrode for providing electrical stimulation to the heart [61], there remains a need for greater emphasis on integrating electrical stimulation into organ preservation.

## RECENT RESEARCH ADVANCEMENTS

Apart from systems described above and in Table 2, there are a number of advanced research-grade systems being developed. These are described in more detail below:

### *Kidneys*

Brasile *et al.* investigated an acellular perfusate based on cell culture media that includes emulsified perfluorocarbons as the oxygen carrier for kidney perfusion at 32 °C and showed superior outcomes compared to hypothermic perfusion and cold storage. Another theoretical advantage of perfusion under sub-normothermic conditions is that increased solubility of oxygen at lower temperatures (compared to 37 °C) would decrease the amount of oxygenation needed [66,67]. The Nicholson group, who were also pioneers in the field, used fully normothermic autologous blood perfusion for 16 hours after 2 hours of cold storage. They observed a significantly enhanced ability to concentrate creatinine and conserve sodium in the preserved kidneys [68]. Later studies comparing normothermic perfusion of whole blood versus leukocyte-depleted blood demonstrated lower initial renal vascular resistance, improved base excess, creatinine clearance, renal blood flow, and increased oxygen consumption with leukocyte-depleted blood as the perfusate [69]. This has led to other groups using leukocyte-depleted blood as their perfusates in blood-based organ perfusions.

The concept of "organ culturing" in kidneys during preservation involves repairing ischemia tissue *ex vivo*. Brasile *et al.*, after 2 hours of warm ischemia, perfused kidneys for 24 hours in the presence or absence of fibroblast growth factors, which are known to stimulate pathways leading to cell recovery after renal injury. Gene transfection of the kidneys with adenovirus expressing green fluorescent protein was performed during the 24-hour perfusion. Positive expression of this exogenous protein was revealed by histologic assessment, confirming that *ex vivo* perfusion is sufficient to allow *de novo* protein synthesis; however, the chance of recovery was low after re-implantation [55]. To achieve practical gene therapy, normothermic systems must include targeted manipulation of cytokine expression, modulation of apoptotic and costimulatory pathways, and manipulation of leukocyte recruitment signaling pathways [56].

### *Livers*

Early studies that compared the normothermic perfusion efficiency in liver preservation between heart beating donors and cold storage has led to many controversies [36,70]. Due to the dual-vessel supply, normothermic perfusion of the liver is more complicated than other organs such as kidneys. A later study by Schon *et al.* demonstrated that normothermic perfusion can be substantially effective for *ex vivo* resuscitation of warm, ischemic livers. Towards mimicking physiological conditions, they designed a complex perfusion circuit in which the liver was placed in a water bath with oscillating pressures to simulate intra-abdominal pressure changes and perfused with a mixture of whole blood and an electrolyte solution [71]. The perfusate was filtered with a dialysis system which regulated its pH and electrolyte concentrations [71]. Alternatively, a less complex system was implemented by reassembling standard cardiopulmonary bypass components including a centrifugal pump, a membrane oxygenator, and a heat exchanger. It relied on the inherent ability of a healthy liver to regulate its own acid-base status. Friend *et al.* implemented the so called *Oxford* system and demonstrated its potential of improved preservation over 24 hours compared to cold storage [72-74]. The group successfully preserved a porcine liver extracorporeally for 72 hours with the system [72-74]. However, the system was not readily portable and utilized cold storage. Later studies revealed different injury patterns caused by cold and warm ischemia. Thus, normothermic perfusion systems need to be portable and not utilize cold storage to become a realistic option for liver perfusion [65].

High temperatures in normothermic perfusion resolves the issue of low oxygen absorption in tissues, which is important in highly metabolic organs such as the liver. However, if the extended preservation time is the goal, using blood and its oxygen carrying capacity is not feasible. Laing *et al.* reported the first acellular hemoglobin-based oxygen carrier, *Hemopure*, in a discarded human liver using the Liver Assist Device which perfuses both the hepatic arterial and portal venous systems [66]. The group eliminated red blood cell constituents, bacterial endotoxins and viruses to obtain bovine hemoglobin product and mixed it with other perfusion fluid constituents such as heparin, dextrose, and human albumin. The perfusate was delivered at controlled pressure and 37 °C for 6 hours and the results compared to a control (red blood cells). The perfusion parameters remained similar in both the experimental and control groups and histologically demonstrated viability to the same extent. The oxygen consumption was increased because of the physiological and rheological properties of *Hemopure*. However, at the same time, because of its right shifted oxygen dissociation curve, *Hemopure* gives up more oxygen. Thus, within an environment free from recipient immune mediated injury, organs replenish their energy stores and attenuate IRI. The optimum temperature and so the optimum perfusate for liver perfusion still remains the main focus of research in the field [66].

### Lungs

Viability and functional assessment, which are critical tools provided only by normothermic perfusion have been the focus of the lung perfusion systems. Steen *et al.* used normothermic *ex vivo* perfusion combined with cold storage to assess lungs before transplantation. Donated lungs that failed conventional criteria for transplantation were first cooled for 3 hours and then transferred to an *ex vivo* perfusion unit where their viability and function were tested [67,75]. The lungs were then stored in cold storage for 8 hours [67,75]. The preservation and assessment of lungs from non-heart-beating donors for 6 hours has also been achieved. Extended preservation times and the possibility of functional assessments have motivated researchers to investigate organ treatment during preservation in order to expand the organ pool. Keshavjee and colleagues were able to suppress inflammation for superior post-transplant lung function in porcine lungs with adenoviral vector gene delivery and normothermic perfusion [76].

Lung preservation times need to be sufficiently long for the organ treatment to be effective. Since the common lung normothermic perfusion times are still too short for organ treatment methods to take effect, different approaches are being investigated to extend lung perfusion times even further. O'Neill *et al.* developed a normothermic perfusion platform by combining *ex vivo* lung perfusion (EVLP) with cross circulation in a clinically relevant swine model [77]. Cross circulation, where a healthy individual supports and augments the organ function of a critically ill patient, has already been developed for some reversible illness in humans. These investigators examined two different groups of swine lungs preserved by, either cold storage (18 hours) or EVLP (4 hours) followed by 36-hour of cross-circulation. During cross-circulation, the epithelium layer is replaced by adipose-derived mesenchymal stem cells following decellularization of targeted bronchopulmonary segments using micro-catheter delivery. The lungs possessed critical structural and biochemical factors for the proper attachment and function of newly delivered cells. Further assessment showed epithelial cells delivered by hydrogels had circulated across the airway surface and attached to the basement membrane while alveolar progenitors were found throughout the alveoli. Overall, they showed that their normothermic extracorporeal organ support systems which combines EVLP with cross-circulation was able to maintain both the extracorporeal and recipient lungs at a viable and stable state for 36 hours.

### Vascularized Composite Allotranplantation

VCA grafts are composed of multiple tissue types (such as skin, fat, and muscle). These types of transplants are often performed after traumatic amputations have occurred and static cold storage is still used as the current gold standard for preservation. Even though cold storage lowers the metabolic requirements of these transplants, the transplantation surgery still needs to be performed within 4 to 6 hours of amputation. This time-frame is incredibly restrictive. At the moment, there are not any commercialized perfusion systems that are designed for VCA transplants but there is a trend towards researching and developing methods for extracorporeal VCA perfusion and preservation. Two groups have made notable advancements in the field of VCA preservation.

In 2016, Kueckelhaus *et al.* developed a mobile system to perfuse porcine limbs. Their system used a peristaltic pump to deliver cool, oxygenated *Perfadex* solution into a porcine forelimb for 12 hours while taking measurements such as pressure, temperature, and blood gas analysis for oxygen concentrations. Even though their perfused limbs had a significant amount of weight gain compared to limbs stored in static cold storage, they were able to electrically stimulate their perfused limbs for longer periods of time. Histological analysis of the perfused limbs did not show hypoxic damage to the cells in contrast to cold storage limbs [70]. More recently, they compared their perfusion system to static cold storage by replanting the limb onto the donor pig after 12 hours of perfusion or 4 hours of cold storage. After replantation, they monitored the pigs for 7 days. They found that the control animals (limbs preserved with cold storage) had higher levels of potassium and myoglobin in their blood, which suggests muscular tissue damage. They also found that the expression of hypoxia-inducible factor-1 alpha and beta (HIF-1α and HIF-1β) in the perfused limbs were comparable to fresh muscle tissue, which suggests the limbs were adequately oxygenated. One of the four pigs in the control group (static cold storage) died of pulmonary complications as a consequence of IRI, while all of the three pigs in the treatment group (perfusion) survived past the 7-day mark [78].

In 2015, the Ozer group created their own porcine limb perfusion set up that perfused warm (27–32 °C) autologous blood into the limbs. After 12 hours of perfusion or 6 hours of static cold storage, the limbs were transplanted onto another porcine host. They monitored the perfusion parameters such as temperature and pressure during perfusion, collected the perfusate for blood gas analysis, and checked for muscle contraction of muscle fiber bundles using a nerve stimulator. Even after 12 hours of perfusion, they found that the muscle fibers were still able to contract [72]. More recently, they used the same set up but perfused the porcine limbs for 24 hours. Once again, they found that the perfusion group had better results than the cold storage group and the muscle fibers were able to contract upon electrical stimulation after 24 hours of perfusion, indicating the presence of healthy myocyte units [79]. In a 2017 report, the group switched models by creating a new perfusion set up and perfusing a human forelimb from brain dead adult donors for 24 hours. After perfusion, they performed histology on the muscle fibers of the limbs and found that there were no signs of necrosis, degeneration, or inflammatory cell infiltration. Also, they were able to stimulate and obtain contraction from both dissected single muscle fibers and the whole limb against gravity. Their findings indicate that they were able to use near-normothermic extracorporeal perfusion to preserve human VCA function for at least 24 hours [73].

## CONCLUSIONS

Recent studies have demonstrated advancements in preservation technologies for solid organs such as liver, heart, kidney, lungs, and VCA. These promising strategies have the potential to reduce the number of people on transplant waiting lists. There have been a number of developments leading to improvements in both research-grade perfusion systems and systems used in clinical trials. In particular, near-normothermic perfusion systems promise to mitigate the effects of ischemic-reperfusion injuries, enable longer preservation times, and provide preserved solid organs with increased functionality. Additionally, they potentially “rescue” marginal organs that would normally be rejected for transplantation. In spite of the major advances, organ care systems still only provide a limited extension of preservation times. Each of these systems can be further customized to provide biophysical cues adapted to meet organ-specific needs as well as to evaluate organ-specific biomarkers. Current trends focus on applying biophysical stimulation of the organs as well as improving techniques for non-invasive viability measurements which correlate with post-transplantation survival. In a few examples, current research suggests preservation times of 24 hours and longer might be clinically feasible. Realizing that potential would radically transform the transplantation field by not only increasing the number of available organs, but by also enabling clinicians time to “treat” the transplants to reduce rejection.

## REFERENCES

1. Leland R. Brief History and Biologie of Skin Grafting. Vol. 21. Ann Plast Surg. 1988:358–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3069030
2. Zirm ME. Eduard Konrad Zirm and the “wondrously beautiful little window”. Refract Corneal Surg. 1989;5(4):256–7.
3. Hamilton DN, Reid WA. Voronoy and the first human kidney allograft. Surg Gynecol Obstet. 1984 Sep;159(3):289–94.
4. Merrill JP, Murray JE, Harrison JH, Guild WR. Successful homotransplantation of the human kidney between identical twins. J Am Med Assoc. 1956 Jan;160(4):277–82.
5. Starzl TE, Koep LJ, Halgrimson CG, Hood J, Schroter GP, Porter KA, et al. Fifteen Years of Clinical Liver Transplantation. Gastroenterology. 1979;77(2):375-388.
6. Brink JG, Hassoulas J. The first human heart transplant and further advances in cardiac transplantation at Groote Schuur Hospital and the University of Cape Town - with reference to : the operation. A human cardiac transplant : an interim report of a successful operation performed at Groote Schuur Hospital, Cape Town. Cardiovasc J Afr. 2009;20(1):31–5.
7. Hardy JD, Webb WR, Dalton ML, Walker GR. Lung Homotransplantation in Man. JAMA. 1963 Dec 21;186(12):1065–74.
8. Kelly WD, Lillehei RC, Merkel FK, Idezuki Y, Goetz FG. Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropaty. Surgery. 1967;61:827–35.
9. Cooley DA, Bloodwell RD, Hallman GL, Leachman RD, Nora JJ, Miam JD. Cardiac Transplantation: General Considerations and Results. Annals of Surgery. 1969;169(6):892-905.
10. Susan Scutti. US organ transplants increased nearly 20% in five years. Vol. 17, CNN. 2017 [cited 2017 Jan 9]. Available from: https://www.cnn.com/2017/01/09/health/organ-donation-2016/
11. Transplant trends. UNOS. [cited 2018 Apr 5]. Available from: https://unos.org/data/transplant-trends/#waitlists_by_organ
12. Hardy MA, Furr A, Barret JP, Barker JH. The immunologic considerations in human head transplantation. Int J Surg. 2017 May 1;41:196–202.
13. Breidenbach WC, Gonzales NR, Kaufman CL, Klapheke M, Tobin GR, Gorantla VS. Outcomes of the First 2 American Hand Transplants at 8 and 6 Years Posttransplant. J Hand Surg Am. 2008 Sep 1;33(7):1039–47.
14. Fattah A, Cypel T, Donner EJ, Wang F, Alman BA, Zuker

RM. The First Successful Lower Extremity Transplantation: 6-Year Follow-Up and Implications for Cortical Plasticity. Am J Transplant. 2011 Dec 1;11(12):2762–7.
15. Hofmann GO, Kirschner MH, Brauns L, Wagner FD, Land W, Bühren V. Vascularized knee joint transplantation in man: a report on the first cases. Transpl Int. 1998 Jun 5;11(0):S487–90.
16. Levi DM, Tzakis AG, Kato T, Madariaga J, Mittal NK, Nery J, et al. Transplantation of the abdominal wall. Lancet. 2003 Jun 28;361(9376):2173–6.
17. Strome M, Stein J, Esclamado R, Hicks D, Lorenz RR, Braun W, et al. Laryngeal Transplantation and 40-Month Follow-up. N Engl J Med. 2001 May 31;344(22):1676–9.
18. Eisenhardt SU, Schmidt Y, Karaxha G, Iblher N, Penna V, Torio-Padron N, et al. Monitoring Molecular Changes Induced by Ischemia/Reperfusion in Human Free Muscle Flap Tissue Samples. Ann Plast Surg. 2012 Feb;68(2):202–8.
19. Khalil AA, Aziz FA, Hall JC. Reperfusion injury. Plast Reconstr Surg. 2006 Mar 1;117(3):1024–33.
20. Levitsky S. Protecting the Myocardial Cell During Coronary Revascularization. [cited 2018 Apr 5]; Available from: https://pdfs.semanticscholar.org/2179/d11976d4ebd67eb4fc910a562194cd56cc57.pdf
21. Pedotti P, Cardillo M, Rigotti P, Gerunda G, Merenda R, Cillo U, et al. A COMPARATIVE PROSPECTIVE STUDY OF TWO AVAILABLE SOLUTIONS FOR KIDNEY AND LIVER PRESERVATION. Transplantation. 2004 May [cited 2018 Apr 5];77(10):1540–5. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00007890-200405270-00011
22. Boggi U, Vistoli F, Del Chiaro M, Signori S, Croce C, Pietrabissa A, et al. Pancreas preservation with University of Wisconsin and celsior solutions: A single-center, prospective, randomized pilot study. Transplantation. 2004;77(8):1186–90.
23. Epstein FH, McCord JM. Oxygen-Derived Free Radicals in Postischemic Tissue Injury. N Engl J Med. 1985 Jan 17;312(3):159–63.
24. Woman has baby using ovary frozen in childhood - BBC News. [cited 2018 Apr 5]. Available from: http://www.bbc.com/news/health-38312995
25. Southard M. D JH, Belzer, M.D FO. ORGAN PRESERVATION. Annu Rev Med. 1995 Feb 28;46(1):235–47.
26. Bonventre JV, Cheung JY. Effects of metabolic acidosis on viability of cells exposed to anoxia. [cited 2018 Apr 5]; Available from: https://www.physiology.org/doi/pdf/10.1152/ajpcell.1985.249.1.C149
27. Collins GM, Bravo-Shugarman M, Terasaki PI. KIDNEY PRESERVATION FOR TRANSPORTATION: Initial Perfusion and 30 Hours' Ice Storage. Lancet. 1969 Dec 6;294(7632):1219–22.
28. Ploeg RJ, van Bockel JH, Langendijk PT, Groenewegen M, van der Woude FJ, Persijn GG, et al. Effect of preservation solution on results of cadaveric kidney transplantation. Lancet. 1992 Jul 18;340(8812):129–37.
29. Belzer FO, Ashby BS, Huang JS, Dunphy JE. Etiology of rising perfusion pressure in isolated organ perfusion. Ann Surg. 1968;168(3):382–91.
30. Clark EA, Terasaki PI, Opelz G, Mickey MR. Cadaver-Kidney Transplant Failures at One Month. N Engl J Med. 1974 Nov 21;291(21):1099–102.
31. Adam R, Delvart V, Karam V, Ducerf C, Navarro F, Letoublon C, et al. Compared Efficacy of Preservation Solutions in Liver Transplantation: A Long-Term Graft Outcome Study From the European Liver Transplant Registry. [cited 2018 Apr 5]; Available from: http://www.eltr.org/spip.php?page1/4centers-tous
32. van der Vliet JA, Vroemen JP, Cohen B, Lansbergen Q, Kootstra G. Preservation of Cadaveric Kidneys. Arch Surg. 1983 Oct 1;118(10):1166.
33. Gerhard Opelz PI. Advantage of Cold Storage Over Machine Perfusion For Preservation of Cadaver Kidneys. 1982. pp. 64–8.
34. Iyer A, Gao L, Doyle A, Rao P, Cropper JR, Soto C, et al. Normothermic Ex Vivo Perfusion Provides Superior Organ Preservation and Enables Viability Assessment of Hearts From DCD Donors. Am J Transplant. 2015 Feb 1;15(2):371–80.
35. Andreasson AS, Dark JH, Fisher AJ. Ex vivo lung perfusion in clinical lung transplantation--State of the art. Eur J Cardio-Thoracic Surg. 2014 Nov 1;46(5):779–88.
36. Opelz G, Terasaki PI. Advantage of cold storage over machine perfusion for preservation of cadaver kidneys. Transplantation. 1982 Jan;33(1):64–8.
37. Kondo T, Chen F, Ohsumi A, Hijiya K, Motoyama H, Sowa T, et al. β2-Adrenoreceptor Agonist Inhalation During Ex Vivo Lung Perfusion Attenuates Lung Injury. Ann Thorac Surg. 2015 Aug 1;100(2):480–6.
38. Hosgood SA, Barlow AD, Dormer J, Nicholson ML. The use of ex-vivo normothermic perfusion for the resuscitation and assessment of human kidneys discarded because of inadequate in situ perfusion. J Transl Med. 2015 Dec 16;13(1):329.
39. Watson CJ, Kosmoliaptsis V, Randle LV, Russell NK, Griffiths WJ, Davies S, et al. Preimplant Normothermic Liver Perfusion of a Suboptimal Liver Donated After Circulatory Death. Am J Transplant. 2016 Jan 1;16(1):353–7.
40. Selzner M, Goldaracena N, Echeverri J, Kaths JM, Linares I, Selzner N, et al. Normothermic ex vivo liver perfusion using steen solution as perfusate for human liver transplantation: First North American results. Liver Transplant. 2016 Nov 1;22(11):1501–8.
41. Kaths JM, Cen JY, Chun YM, Echeverri J, Linares I, Ganesh S, et al. Continuous Normothermic Ex Vivo Kidney Perfusion Is Superior to Brief Normothermic Perfusion Following Static Cold Storage in Donation After Circulatory Death Pig Kidney Transplantation. Am J Transplant. 2017 Apr 1;17(4):957–69.
42. Goldaracena N, Echeverri J, Spetzler VN, Kaths JM, Barbas AS, Louis KS, et al. Anti-inflammatory signaling during ex vivo liver perfusion improves the preservation of pig liver grafts before transplantation. Liver Transplant. 2016 Nov 1;22(11):1573–83.
43. Banan B, Watson R, Xu M, Lin Y, Chapman W. Development of a normothermic extracorporeal liver perfusion system toward improving viability and function of human extended criteria donor livers. Liver Transplant. 2016 Jul 1;22(7):979–93.

44. Messer S, Ardehali A, Tsui S. Normothermic donor heart perfusion: current clinical experience and the future. 2014 [cited 2018 Apr 5]; Available from: https://static1.squarespace.com/static/57d7d2ad15d5db47a2e81a13/t/5974cb61e3df28d88899f6ae/1500826467871/Tsui%2C+2014+-+heart+normothermic+perfusion.pdf
45. Kaths JM, Echeverri J, Goldaracena N, Louis KS, Chun YM, Linares I, et al. Eight-Hour Continuous Normothermic Ex Vivo Kidney Perfusion Is a Safe Preservation Technique for Kidney Transplantation. Transplantation. 2016 Sep;100(9):1862–70.
46. Mahboub P, Bozorgzadeh A, Martins PN. Potential approaches to improve the outcomes of donation after cardiac death liver grafts. World J Transplant. 2016;6(2):314.
47. Stone JP, Ball AL, Critchley WR, Major T, Edge RJ, Amin K, et al. Ex Vivo Normothermic Perfusion Induces Donor-Derived Leukocyte Mobilization and Removal Prior to Renal Transplantation. Kidney Int Reports. 2016 Nov 1;1(4):230–9.
48. Yong C, Hosgood SA, Nicholson ML. Ex-vivo normothermic perfusion in renal transplantation. Curr Opin Organ Transplant. 2016 Jun;21(3):301–7.
49. Carrel A, Lindbergh CA. The Culture of Whole Organs. Source Sci New Ser. 1935;81(21):621–3.
50. Organ Transport Systems. (2012). Technology. Available from: http://www.organtransportsystems.com/OurTechnology.html [Accessed 15 Aug. 2012].
51. Fishman R. (2018). Systems and methods for ex vivo lung care. US12099717.
52. Andover, M. (2016). TransMedics Announces The World's First Human Liver Transplantation Using The Organ Care System (OCS) Liver Technology & The Initiation of The OCS Liver PROTECT U.S. Pivotal Trial: TransMedics, Inc. Transmedics.com. Available from: http://www.transmedics.com/wt/page/pr_1456236855.html [Accessed 23 Feb. 2016].
53. Krezdorn N, Tasigiorgos S, Wo L, Turk M, Lopdrup R, Kiwanuka H, et al. Tissue conservation for transplantation. Innovative Surgical Sciences. 2017;2(4):171–87.
54. BIRNBAUM D. Extracorporeal circulation in non-cardiac surgery. Eur J Cardio-Thoracic Surg Suppl. 2004 Dec 1;26(1):S82–5.
55. Brasile L, Stubenitsky BM, Haisch CE, Kon M, Kootstra G. Repair of Damaged Organs in Vitro. Am J Transplant. 2005 Feb 1;5(2):300–6.
56. García-Valdecasas JC, Tabet J, Valero R, Taurá P, Rull R, García F, Montserrat E, González FX, Ordi J, Beltran J, López-Boado MA, Deulofeu R, Angás J, Cifuentes A, Visa J. Liver conditioning after cardiac arrest: the use of normothermic recirculation in an experimental animal model. Transpl Int. 1998;11(6):424-32.
57. Pegg DE, Green CJ. Renal preservation by hypothermic perfusion using a defined perfusion fluid. Cryobiology. 1972 Oct 1;9(5):420–8.
58. Guarrera JV, Estevez J, Boykin J, Boyce R, Rashid J, Sun S, et al. Hypothermic Machine Perfusion of Liver Grafts for Transplantation: Technical Development in Human Discard and Miniature Swine Models. [cited 2018 Apr 8]; Available from: https://ac.els-cdn.com/S0041134504015337/1-s2.0-S0041134504015337-main.pdf?_tid=41928ecf-a961-4361-ba9e-647f5bb63b19&acdnat=1523207569_a814c72fdf21cbae64af467931305646
59. Understanding Blood Pressure Readings. [cited 2018 Mar 29]. Available from: http://www.heart.org/HEARTORG/Conditions/HighBloodPressure/KnowYourNumbers/Understanding-Blood-Pressure-Readings_UCM_301764_Article.jsp
60. Ravikumar R, Jassem W, Mergental H, Heaton N, Mirza D, Perera MTPR, et al. Liver Transplantation After Ex Vivo Normothermic Machine Preservation: A Phase 1 (First-in-Man) Clinical Trial. Am J Transplant. 2016 Jun;16(6):1779-87.
61. Hassanein W. Systems and methods for ex-vivo organ care. US9516875B2, 2005.
62. Bruinsma BG, Avruch JH, Weeder PD, Sridharan G V, Uygun BE, Karimian NG, et al. Functional human liver preservation and recovery by means of subnormothermic machine perfusion. J Vis Exp. 2015 Apr;(98).
63. Maidhof R, Tandon N, Lee EJ, Luo J, Duan Y, Yeager K, et al. Biomimetic perfusion and electrical stimulation applied in concert improved the assembly of engineered cardiac tissue. J Tissue Eng Regen Med. 2012 Nov;6(10):e12–23.
64. Imber CJ, St. Peter SD, Lopez de Cenarruzabeitia I, Pigott D, James T, Taylor R, et al. Advantages of normothermic perfusion over cold storage in liver preservation. Transplantation. 2002;73(5):701–9.
65. Schön M, Kollmar O, Akkoc N, Matthes M, Wolf S, Schrem H, et al. Cold ischemia affects sinusoidal endothelial cells while warm ischemia affects hepatocytes in liver transplantation. Transplant Proc. 1998 Aug 1;30(5):2318–20.
66. Laing RW, Bhogal RH, Wallace L, Boteon Y, Neil DA, Smith A, et al. The Use of an Acellular Oxygen Carrier in a Human Liver Model of Normothermic Machine Perfusion. Transplantation. 2017;101(11):2746–56.
67. Steen MD. PhD S, Ingemansson, MD, PhD R, Budrikis, MD A, Bolys, MD R, Roscher, MD R, Sjöberg, PhD T. Successful Transplantation of Lungs Topically Cooled in the Non–Heart-Beating Donor for 6 Hours. Ann Thorac Surg. 1997 Feb 1;63(2):345–51.
68. Metcalfe MS, Mann CD, Waller JR, Saunders RN, Roehlke W, Nicholson ML. Normothermic perfusion of ischaemically damaged porcine kidneys: an evaluation of ex vivo function. Transplant Proc. 2001 Nov 1;33(7–8):3743–4.
69. Harper S, Hosgood S, Kay M, Nicholson M. Leucocyte depletion improves renal function during reperfusion using an experimental isolated haemoperfused organ preservation system. Br J Surg. 2006 May 1;93(5):623–9.
70. Kueckelhaus M, Fischer S, Sisk G, Kiwanuka H, Bueno EM, Dermietzel A, et al. A Mobile Extracorporeal Extremity Salvage System for Replantation and Transplantation. 2016;76(3):355–60.
71. Schön MR, Kollmar O, Wolf S, Schrem H, Matthes M, Akkoc N, et al. Liver transplantation after organ preservation with normothermic extracorporeal perfusion. Ann Surg. 2001 Jan;233(1):114–23.
72. Ozer K, Rojas-Pena A, Mendias CL, Bryner B, Toomasian C, Bartlett RH. Ex Situ Limb Perfusion System to Extend Vascularized Composite Tissue Allograft Survival in

Swine. Transplantation. 2015;99(10):2095–101.
73. Werner NL, Alghanem F, Rakestraw SL, Sarver DC, Nicely B, Pietroski RE, et al. Ex situ perfusion of human limb allografts for 24 hours. Transplantation. 2017;101(3):e68–74.
74. Cobert M, Peltz M, West L, Jessen ME. 174: Maintenance of Human Heart Oxidative Metabolism after 12 Hour Perfusion Preservation. J Heart Lung Transplant. 2009 Feb;28(2):S126–7.
75. Steen S, Kimblad O, Sjoberg T, Lindberg L, Ingemansson R, Massa G. Safe Lung Preservation for Twenty-Four Hours With Perfadex. Ann Thorac Surg. 1994 Feb;57(2):450-7.
76. Yeung JC, Wagnetz D, Cypel M, Rubacha M, Koike T, Chun YM, et al. Ex vivo adenoviral vector gene delivery results in decreased vector-associated inflammation pre- and post-lung transplantation in the pig. Mol Ther. 2012;20(6):1204–11.
77. O'Neill JD, Guenthart BA, Kim J, Chicotka S, Queen D, Fung K, et al. Cross-circulation for extracorporeal support and recovery of the lung. Nat Biomed Eng. 2017 Mar 6;1(3):0037.
78. Kueckelhaus M, Dermietzel A, Alhefzi M, Aycart MA, Fischer S, Krezdorn N, et al. Acellular Hypothermic Extracorporeal Perfusion Extends Allowable Ischemia Time in a Porcine Whole Limb Replantation Model. Plast Reconstr Surg. 2017;139(4):922e–32e.
79. Ozer K, Rojas-Pena A, Mendias CL, Bryner BS, Toomasian C, Bartlett RH. The effect of ex situ perfusion in a swine limb vascularized composite tissue allograft on survival up to 24 hours. J Hand Surg Am. 2016;41(1):3–12.
80. Paragonix Technologies, Inc., Announces European Conformity ("CE") for the SherpaPak™ Cardiac Transport System and SherpaPerfusion™ Cardiac Transport System. Businesswire.com. Available from: https://www.businesswire.com/news/home/20180220005551/en/Paragonix-Technologies-Announces-European-Conformity-%E2%80%9CCE%E2%80%9D-SherpaPak%E2%84%A2 [Accessed 15 Aug. 2018].
81. Nice.org.uk. OCS Heart system for heart transplant | Guidance and guidelines | NICE. Available from: https://www.nice.org.uk/advice/mib86 [Accessed 20 Feb. 2018].
82. Organ Assist - Organ Perfusion Systems - Lung Assist. [cited 2018 Mar 29]. Available from: https://www.organ-assist.nl/products/lung-assist
83. Perfusion XV. Home - XVIVO Perfusion. Available from: https://www.xvivoperfusion.com/ [Accessed 15 Aug. 2018].
84. LifePort® Kidney Transporter 1.0 Available from: https://www.organ-recovery.com/lifeport-kidney-transporter
85. Organ Assist - Organ Perfusion Systems - Kidney Assist-transport. Organ-assist.nl. Available from: https://www.organ-assist.nl/products/kidney-assist__transport
86. Wtrs.com. Waves – WTRS. Available from: http://wtrs.com/portfolio/waves/ [Accessed 16 May 2018].
87. Wtrs.com. RM3 – WTRS. Available from: https://wtrs.com/portfolio/rm3/ [Accessed 16 May 2018].
88. Organ-recovery.com. LifePort Liver Transporter | Organ Recovery Systems. Available from: https://www.organ-recovery.com/lifeport-liver-transporter
89. Management L. Organ Assist - Organ Perfusion Systems - Liver Assist. Organ-assist.nl. Available from: https://www.organ-assist.nl/products/liver-assist
90. Hassanein W. TransMedics Inc assignee. Ex vivo organ care system. US Patent US14728771. 2015-06-02
91. OrganOx. OrganOx metra. Liver transportation and liver perfusion. Available from: http://www.organox.com/