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Nicco Krezdorn, Sotirios Tasigiorgos, Luccie Wo, Marvee Turk, Rachel Lopdrup, Harriet Kiwanuka, Thet-Su Win, Ericka Bueno and Bohdan Pomahac*

Tissue conservation for transplantation

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Abstract: Pathophysiological changes that occur during ischemia and subsequent reperfusion cause damage to tissues procured for transplantation and also affect long-term allograft function and survival. The proper preservation of organs before transplantation is a must to limit these injuries as much as possible. For decades, static cold storage has been the gold standard for organ preservation, with mechanical perfusion developing as a promising alternative only recently. The current literature points to the need of developing dedicated preservation protocols for every organ, which in combination with other interventions such as ischemic preconditioning and therapeutic additives offer the possibility of improving organ preservation and extending it to multiple times its current duration. This review strives to present an overview of the current body of knowledge with regard to the preservation of organs and tissues destined for transplantation.

Keywords: allograft preservation; graft preservation; machine perfusion; organ conditioning; organ preservation; static cold storage.

Introduction

It was only as recent as 1954 when, after numerous unsuccessful previous attempts, the first successful kidney transplantation by Murray et al. made human allotransplantation a viable treatment option for terminal organ failure [1]. In the decades that followed, the successful transplantation of all solid organs became a clinical reality [2–4]. With advancements in the fields of transplantation, immunology, microsurgery, and regenerative medicine, it became possible to extend the field further and include the transplantation of complex composite tissue allografts, making it feasible to restore the form and function of faces, extremities, and many other body parts [5–8]. Along with the growing clinical experience, insights about pathophysiological and immunological changes that take place after tissue procurement and upon transplantation have been gained. These changes determine allograft survival and function as well as recipient survival. The key players in these processes have been identified as ischemia and reperfusion (IR)-related injuries to the transplanted tissue, which can be limited by optimizing the approach to organ preservation in the time between procurement and transplantation. The currently accessible organ preservation techniques limit the available donor pool but also the geographical radius for optimal recipient matching. These limitations, among other challenges, have led to an increasing mismatch between organ demand and supply.

These opportunities and challenges also apply to other fields of surgical intervention. In cases of traumatic limb loss, for example, the success of replantation is largely limited by the ischemia time and preservation modalities of the amputated part. Despite all technological innovation, the current gold standard still is to preserve limbs on ice for a maximum of 4–6 h to prevent acute respiratory distress, limb, and multiorgan system failure [9, 10].

It is therefore of great interest to understand the underlying pathophysiological processes and the current as well as potential prospective preservation techniques in the context of transplantation.

Pathophysiology of IR

Ischemia is defined as a restriction in blood supply that causes a shortage of oxygen and glucose. Both metabolites

***Corresponding author: Bohdan Pomahac,** Department of Surgery, Division of Plastic Surgery, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA, E-mail: bpomahac@partners.org
Nicco Krezdorn: Department of Surgery, Division of Plastic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; and Department of Plastic, Aesthetic, Hand and Reconstructive Surgery, Burn Center, Hannover Medical School, Hannover, Germany
Sotirios Tasigiorgos, Luccie Wo, Marvee Turk, Rachel Lopdrup, Harriet Kiwanuka, Thet-Su Win and Ericka Bueno: Department of Surgery, Division of Plastic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

are needed for adequate cellular metabolism and tissue survival. Ischemia results in a variety of changes in the affected tissue, which depend on the duration of the ischemic episode. When blood flow is reestablished to the ischemic tissue (i.e. reperfusion), a multitude of physiological reactions occur at the local and systemic levels, and these reactions are known as "ischemia-reperfusion". IR correlates with injury to the tissue, which is why the term IR injury (IRI) has been coined.

Effects of ischemia on the cellular level

The underlying physiological effects of ischemia in tissues can be schematized as follows, although each tissue exhibits different responses and unique tolerance to ischemia.

Healthy tissue cells use mitochondrial oxidative metabolism to generate ATP from the consumption of glucose, lipids, and oxygen. When blood flow is restricted, both essential components for oxidative metabolism (glucose and oxygen) become depleted, and the cell is forced to switch to less energy-efficient anaerobic metabolism (Figure 1B). Over time, these processes lead to cellular oxygen deficit and intracellular accumulation of metabolites, such as lactic acid, which lead to acidotic changes in cellular pH, among others. All these changes result in the altered function of enzymes and mitochondrial and cellular membrane pumps. The shift in electrolytes also perturbs the oncotic equilibrium and thus leads to cellular swelling, rupture, and subsequent activation of cell death mechanisms [11–16].

Noteworthy among the metabolites forming under ischemic conditions are the reactive oxygen species (ROS).

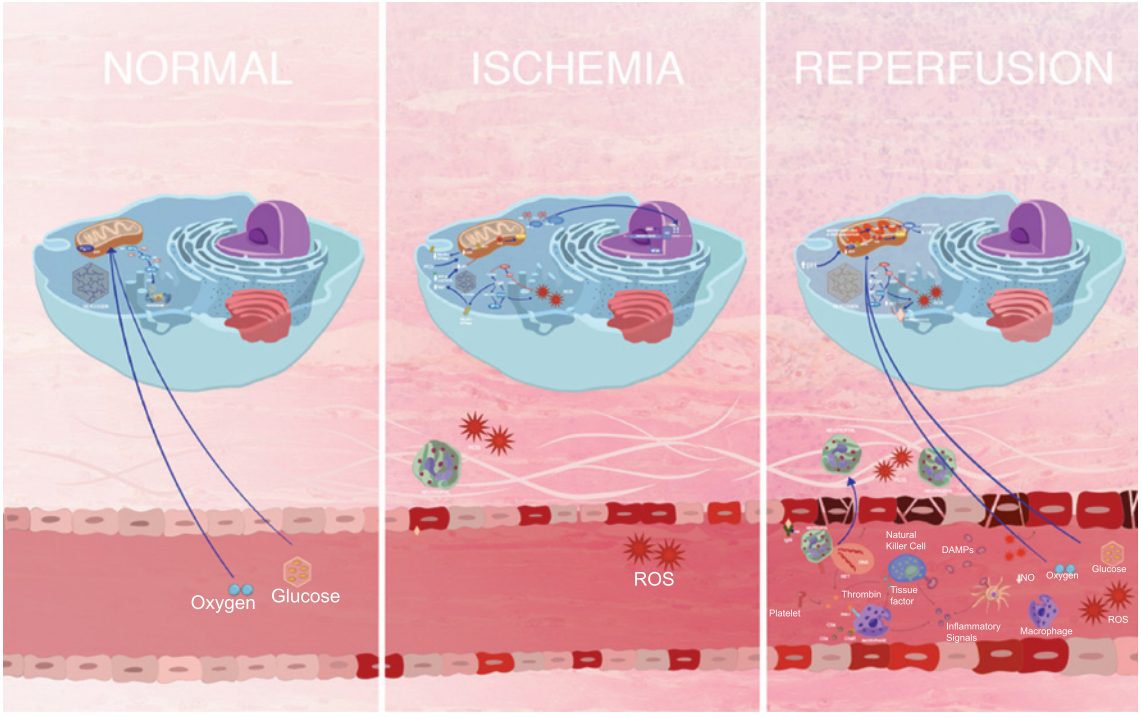

Figure 1: Overview of the pathophysiology of ischemia and IRI.
(A) Under normal conditions, oxygen and glucose are delivered to the cells and used for oxidative ATP production in the mitochondria (see Supplemental Figure 4). (B) Under ischemic conditions, energy production switches to anaerobic metabolism and cellular changes occur (for more details, see Supplemental Figure 5) that lead to the creation of ROS and subsequent cellular damages, which can lead to cell death. These changes start to attract local resident immune cells and after local inflammatory responses. On an endothelial level, ischemic stress leads to increased expression of membrane adhesion molecules and reduced cAMP leads to decreased barrier function and increased vascular permeability. (C) Restitution of oxygen and glucose upon reperfusion leads to injuries on the cellular, local, and even systemic levels. The details of the cellular changes during reperfusion are depicted in Supplemental Figure 6. Increased rates of cell swelling and injuries with expression of cell surface adhesion molecules as well as increased numbers of necrotic and apoptotic cells trigger strong local immune responses and attract even more immune cells from the bloodstream. On the vascular level, the binding of natural IgM to the adhesion molecules leads to the activation of the complement cascade through factors C3a and C5a. ROS decrease NO, which triggers more expression of adhesion molecules and negatively affects vascular tone. The release of DNA from damaged endothelial cells as well as DAMPs attract and activate both innate and adaptive immune cells. The combination of increased immune cell attraction, swelling, and activation of coagulation cascade can lead to a "clogging" of the microvasculature resulting in a so-called "no reflow" phenomenon.

These particles, which accumulate over time in hypoxic conditions, are partially released by the mitochondria [17, 18], NADPH oxidase (NOX) [19], and xanthine oxidase [20]. Higher levels of ROS, especially over longer periods of time, lead to the disruption of lipids, lipoproteins, and membranes and cause intracellular calcium accumulation, which in turn triggers even more the formation of ROS through hypoxia-induced factor-1α [HIF-1α-mediated activation of NOX and HIF2a-mediated inhibition of the ROS inhibitor superoxide dismutase (SOD); Figure 1B]. During the ischemic period, mitochondria are being damaged, especially by the formation of ROS and electrolyte changes with subsequent damage of the proteins of the oxidative chain. Acidotic pH levels during ischemia inhibit mitochondrial permeability transition pores (mPTP), which are important for mitochondrial membrane stability.

Effects of ischemia on a local level

Whereas the above-detailed changes take place within most cells affected by ischemia, there are a couple of effects of tissue-specific varying extent that ischemia imparts on different tissue types and that trigger physiological processes important in tissue damage and preservation. For example, it has been reported that endothelial cells are particularly vulnerable to ischemic conditions because of a subsequent intracellular cAMP increase and decrease of adenylate cyclase activity. This in turn weakens the endothelial barrier function and leads to increased vascular permeability [21]. With respect to endothelial cells, muscle cells show a higher tolerance for hypoxic conditions, although injury only occurs if the ischemic period is prolonged [22]. ROS that are released to the extracellular matrix from injured or necrotic cells not only damage surrounding structures and tissues but also attract local tissue resident immune cells (Figure 1B). These cells shift to proinflammatory phenotypes once they are subjected to ischemia.

Reperfusion

The restoration of blood flow that occurs during reperfusion triggers a cascade of metabolic, molecular, cellular, local, and even system reactions.

The sudden availability of oxygen causes a switch back to oxidative metabolism; however, as the mitochondrial oxidative chain has been damaged during ischemia, ROS increase drastically after reperfusion [23, 24]. This increase in ROS in turn activates the mPTP of the inner mitochondrial membrane, which simultaneously gets activated by the increased Ca^{2+} levels as well as through the readjustment of pH levels after reperfusion. This sudden overactivation of mPTP results in the massive depolarization of the inner membrane, which leads to matrix swelling and disruption of the outer mitochondrial membrane. Mitochondria-specific proteins, such as cytochrome *c*, get released into the cytosol and act as danger molecules, activating caspases and therefore initiating apoptosis (Figure 1C) [25–28]. At the local level, necrotic and apoptotic cell death triggers the activation of the innate immune system, which under normal circumstances would be essential for the clearance of detritus, regeneration, and healing. In IRI, though, injured as well as dead cells release DNA, ROS, damage-associated molecular patterns (DAMP), and ATP, all of which act as chemoattractants for neutrophils. Further ROS accumulation reduces local nitric oxide (NO) levels, which in turn deactivates adhesion molecules on cell surfaces, thus triggering the adhesion and transmigration of immune cells. Also, circulating IgM molecules can bind to surface adhesion molecules or damaged endothelium. This in turn activates the complement system and coagulation cascade. At the capillary level, this leads to the increased recruitment of immune cells and thrombosis, resulting in the occlusion of postcapillary venules – a phenomenon known as the "no reflow" phenomenon [29–32]. In the first line of response, the innate immune system gets activated. Subsequently and within the first 24 h, there is a strong activation of the adaptive immune response, mainly T cell mediated, and likely triggered by both antigen-specific and non-antigen-specific pathways [33, 34].

Mitochondrial damage with extensive ROS formation, cell damage, and/or death seems to be the leading cause of the reperfusion injury [13, 35] and in turn triggers a sterile generalized inflammatory response at the local (initially) and systemic (subsequently) levels.

Warm ischemia time (WIT)

The changes described above start to occur the moment an organ is disconnected from the body's circulation. Under normothermic conditions, the rate of these processes is unhindered and leads to the rapid deterioration of the tissue in the ischemic phase. The term "warm ischemia time" (WIT) describes the time that an organ can remain detached from the donor before reperfusion or preservation treatments begin. Experiences with organs procured from non-heart-beating donors (NHBD) provide insight

Table 1: Maximum allowable WITs per organ.

Organ	Experimental setting	Clinical setting
Lung	90 min	13–120 min
Liver	15–30 min	15–33 min
Kidney	30–45 min	21–76 min
Uterus	4 h	

Data from Piazza et al. [36] and Adachi et al. [37].

into the maximum allowable WIT that can be tolerated by specific organs (Table 1) [38].

Numerous approaches have been developed over the last century to try and limit the ischemic injury and attenuate the subsequent dependent reperfusion injury and, in this manner, better preserve tissues intended for transplantation.

Adequate tissue preservation after procurement is a key element in the transplantation of any organ for many reasons. For one, limited IRI results in increased graft function, decreased acute rejection events, decreased rates of long-term graft loss and chronic rejection, increased organ donor pools, and increased geographical radius of transportation with associated improved possibilities for donor matching [39–44].

Techniques for tissue preservation in transplantation

The physiological processes outlined in the previous section offer a variety of starting points to therapeutically intervene with the goal of tissue preservation. Figure 2 gives an overview of the most commonly used tissue preservation techniques currently available.

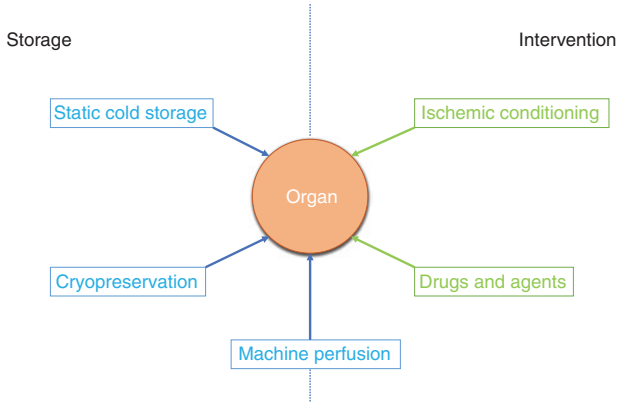

Figure 2: Tissue preservation methods for transplantation.

Static cold storage (SCS) and cryopreservation

SCS in solid organs

SCS has been the standard method for the *ex vivo* preservation of both organ and composite tissue allografts for more than 40 years [45]. As described in the literature, SCS consists of rapid vascular washout immediately after procurement followed by suspension in a bath of specialized preservation media and storage over ice to a temperature of 0 °C–4 °C [46].

The philosophy underlying SCS rests on the well-established physiological premise that a decrease in temperature results in a decrease in metabolic activity [47, 48]. By reducing reaction rates in this way, the hypothermic conditions of SCS facilitate a limitation of cellular functions to the minimum required for survival, a decrease in the depletion of essential metabolic substrates, and ultimately a prolongation of allograft viability [49, 50].

The role of hypothermia in limiting deleterious effects of ischemia has been demonstrated empirically throughout the organ preservation literature. Studies first demonstrated the role of hypothermia alone in limiting ischemic damage and prolonging ischemia time. Calne et al. for example, showed that simple cooling with only ice water preserved ischemic kidney function for 12 h [51]. Research later demonstrated the role of supplementary preservation media in both extending cold ischemic time (CIT) and attenuating deleterious side effects of hypothermia, including acidosis and edema [52]. The first SCS medium to gain widespread use was the Euro-Collins (EC) solution, which further increased the acceptable human kidney CIT to 30 h [53]. EC has since been replaced by the University of Wisconsin (UW) solution as the most prominent preservation medium and current gold standard for SCS [42, 54].

While increasing CIT is classically considered a major risk factor for the development of graft dysfunction and failure, the duration-dependent effects of SCS vary by organ. For example, the acceptable duration for the ischemic cold storage of heart allografts is 4–6 h, with longer CITs having a demonstrable adverse survival effect [55, 56]. The acceptable CITs for the kidney, liver, and pancreas, on the contrary, are significantly higher, with some literature reporting successful preservation for up to 44 h (Supplemental Table 2) [42, 57, 58]. Despite these conventions, however, there remains much heterogeneity in CIT cutoff values across all organs. Furthermore, consensus remains unreached with regard to whether CIT should be

generally considered a continuous versus a categorical risk factor for graft survival.

SCS in composite tissues

The current gold standard for storing vascularized composite allotransplants (VCA) after procurement and before transplantation is also SCS and prior flushing with a preservation solution, usually UW. Like in solid organ transplantation (SOT), there is no consensus across the literature on the most effective way to achieve optimal tissue preservation. Although there have been many successful VCA procedures completed to date worldwide, there is only limited information on the way in which the allografts were preserved before transplantation.

Most face and upper extremity transplantations performed to date have used cold preservation with the standard UW solution [5, 59–63]. Lastly, transplantation of other composite tissues, such as the penis and uterus, is of great interest and hope for future implications in clinical practice. Reports on successful transplantation in these areas used Custodiol or histidine-tryptophan-ketoglutarate (HTK) solution SCS [7, 64].

The most acceptable and widely used window of time before VCA transplantation is in the order of 4–6 h [60, 63], which is regarded as one of the major limitations to the wider application of VCA in clinical practice.

Cryopreservation

Cryopreservation is one of the earliest methods implemented in isolated tissue preservation. Cryopreservation refers to the maintenance of tissues in a living state of suspended animation at cryogenic temperatures, so that cellular functions can be slowed down while preserving the physiochemical structures of tissues [65]. The maintenance of the three-dimensional architecture of a VCA poses an extra burden in the preservation of composite tissues. Nevertheless, it has successfully been tried in isolated tissues such as bone, cartilage, skin, nerves, and vasculature.

Skin/cutaneous tissue

There is a robust amount of experimental studies across the literature, which investigate the viability of dermal-epidermal tissue segments (full- versus split-thickness grafts) when preserved with cryoprotective substances. The fundamental principle of skin storage and preservation is the maintenance of its viability and structural integrity until transplantation [66, 67]. The main options for skin tissue preservation are cryopreservation and storage in liquid medium at 4 °C [68]. Cinamon et al. reported that human cryopreserved split-thickness skin grafts at –180 °C showed better viability after 4 and 7 days of transplantation on mice when compared to a control group of glycerolized skin when assessed in terms of histological appearance [69]. Another study compared the viability of skin allografts cryopreserved with dimethylsulfoxide Me_2SO (DMSO) to that of standard glycerol cryopreserved skin and showed that the former exhibits higher viability rates [70]. Villalba et al. [71] investigated the cryoprotective effect of propane-1,2-diol on human skin tissue using tetrazolium reductase enzyme activity to assess viability and concluded that it is not a superior method of skin preservation when compared to other cryopreservant means. The tetrazolium salt assay to evaluate donor skin viability was also used by another group who showed that the viability of skin cryopreserved with 10% DMSO is comparable to that of fresh skin stored at 4 °C for 4 days [72].

Nerves and vasculature

A group from Tokyo, Japan, immersed the vascular bundle (femoral artery, femoral vein, and sciatic nerve) of a rat in 1.4 M glycerol and stored it in liquid nitrogen for 3 weeks; subsequent replantation resulted in the survival of all the experimental models, adequate function, and axonal regeneration of the sciatic nerve [73]. Komorowska Timek et al. examined the effect of cryopreservation with a Hextend and glycerol-containing solution on rat arterial grafts and concluded that cryopreserved rat arterial allografts demonstrated a satisfactory graft patency for up to 4 months after implantation [74]. Lastly, a group from France cryopreserved long nerve samples harvested from rat limbs in a mixture of various cryoprotectors (2,3-butanediol, 1,2-propanediol, polyethylene glycol, and UW solution) and assessed the viability of Schwann cells when compared to control fresh autografts. They showed an increased rate of cell survival after exposure to 50% of the aforementioned mix solution for 10 min [75].

Composite tissues (limbs and complex tissue flaps)

During the process of cryopreservation, cells are protected with the use of several cryoprotective substances. There are many studies in the literature testing different

substances/solutions for composite tissue preservation purposes and reporting different results (Supplemental Table 4) [76–85].

Machine perfusion

The optimal way to preserve organs for transplantation has been debated since the beginning of transplantation. In fact, the first organ perfusion machine was introduced in 1935 by Alexis Carrel and Charles Lindbergh [86]. Although advances have been made to perfusion machines, most of these efforts were abandoned in 1969 when Geoffrey Collins demonstrated the viability of organs after simple cold storage and later the introduction of modern preservation solutions [53, 87–89]. It is undeniable that simple cold storage is easy to implement and extremely cost-effective. However, hypothermic conditions alone cannot completely eliminate the cellular demand for energy, and there is still a slow and steady depletion of ATP and build-up of toxic metabolites and continuation of the deleterious cellular processes [90, 91]. Machine perfusion is a system that provides the tissues with continuous physiological levels of the most important components for aerobic and therefore cell protective metabolism, namely, oxygen and glucose. Typically, a machine perfusion system (Figure 3) consists of a perfusate reservoir filled with a preservation solution that gets oxygenated and pumped through the organ using organ-specific parameters of temperature, pressure, and others. A variety of sensors can provide feedback on perfusion and perfusate parameters and allow feedback mechanisms in more advanced systems.

Advantages of machine perfusion

Recently, there has been a resurgent interest in organ perfusion machines motivated by the shortage of donor organs. In the 1980s, organs were only procured from "ideal donors", that is, young individuals deceased due to brain death. However, expanding the donor selection to include donors aged 60 years with no comorbidities and donors aged 50–59 years with comorbidities (such as cerebrovascular cause of death, renal insufficiency, and/or hypertension) increased organ availability by 170% [49, 92–94]. The trade-off was a higher risk of delayed graft function and primary graft malfunction [95, 96]. The application of extracorporeal machine perfusion can further expand the pool of available organ donors by increasing the feasibility of donation after cardiac death.

Organ perfusion not only extends the life of the organ outside the body but also provides clinicians with an opportunity to assess the viability of candidate organs and provide resuscitation efforts for marginally acceptable organs, ultimately increasing the number of usable organs for transplantation. Intuitively, machine perfusion allows for the delivery of oxygen and nutrients to the organ and the transport of metabolic waste and toxins away from the organ, effectively providing a continuous flush. In addition, providing flow to the microvasculature improves endothelial gene expression and function [50, 97, 98].

Interestingly, machine perfusion has an economic advantage over SCS as well. In 2003, Wight et al. performed a meta-analysis that demonstrated that machine perfusion is more cost effective in the long run than SCS

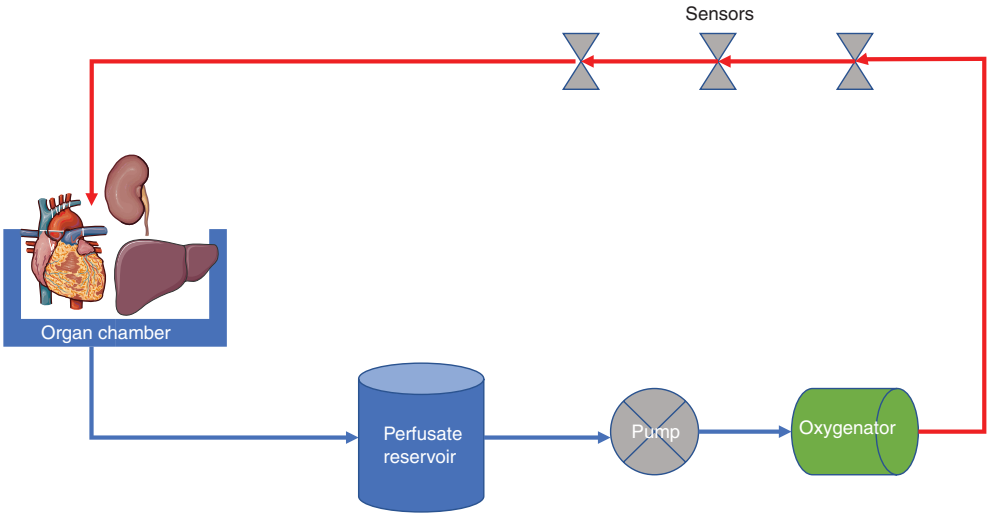

Figure 3: Basic schematic of a machine perfusion system.

due to the decrease in delayed graft function [99]. This was reinforced by UNOS in the United States in 2006 [100].

Hypothermic versus normothermic perfusion

One key distinction in machine perfusion is the perfusion temperature: hypothermic machine perfusion occurs at a temperature between 0 °C and 4 °C, whereas normothermic perfusion occurs at a more physiological temperature, which can be as low as 12 °C–15 °C [101, 102]. Hypothermic perfusion is the more traditional and better-studied machine perfusion. Its main advantage is decrease in metabolic demand; thereby, adequate oxygen levels may be provided even with minimal oxygenation of the perfusate [103, 104]. Already, hypothermic machine perfusion has been shown to decrease delayed graft function in both kidney and liver transplant models [46, 53, 88, 105]. Coincidentally, the reduction in metabolic rate that prolongs organ preservation becomes counterproductive for organ resuscitation and thorough assessment of viability. Hypothermic conditions complicate the uptake and synthesis of therapeutic reagents and furthermore alters cellular signaling cascades [45, 106]. The changes in cellular signaling that occur with hypothermic conditions can be reversed when the organ is rewarmed [45].

Normothermic machine perfusion is a more physiological form of organ preservation that entails several advantages, including (a) prolonged preservation that is not limited by hypothermic injuries, (b) providing a pretransplantation period for organ recovery from hypothermia, (c) the opportunity to measure organ viability and function before transplantation, and (d) reduction of IRI [107]. However, normothermic perfusion also introduces new challenges such as increasing metabolic function, which translates into increasing metabolic demand. From a technical standpoint, normothermic perfusion machines are complex and often equipped with additional sensors and monitors for PaO_2, $PaCO_2$, pH, and temperature [108–112]. These additions render normothermic perfusion machines less transportable and more likely to serve as a technology adapted at the transplant center.

Cellular versus acellular perfusate

Another area of potential study in the field is the properties of the perfusate used in machine perfusion. The machine perfusion system introduced by Alexis Carrel and Charles Lindbergh in the 1930s used blood as perfusate [113, 114]. Blood-based perfusates have several limitations, including gross hemolysis with prolonged perfusion, platelet activation, and clotting as well as the deterioration of the oxygen-carrying capacity of red blood cells [115]. Several of these challenges have been overcome with improved pump design, blood processing, and pharmaceutics leading to the development of normothermic perfusion systems that use a mixture of blood and crystalloid as perfusate [102, 116]. However, using blood as a perfusate creates several logistical challenges, including proper banking, storage, and matching.

Acellular perfusates are an alternative to blood-based perfusion solutions. They range from solutions similar to preservation fluids to artificial-based media with enhanced oxygen-carrying capacity [87, 89, 111]. Some acellular oxygen carriers studied in machine perfusion include perfluorocarbon, pyridoxalated hemoglobin polyoxyethylene and other stroma-free hemoglobin-based solutions, and nonprotein oxygen carrier [111, 117–120]. Although these solutions were successful in restoring organ function during normothermic perfusion, one study nonetheless showed an increasing level of lactate [121]. In addition to an oxygen carrier, acellular perfusates contain a complex cocktail of amino acids, nucleic acids, and other factors that support cellular function and normal metabolism [106]. In fact, artificial-based perfusates have evolved to even surpass blood-based solutions in terms of preservation under normothermic conditions [108].

Machine perfusion has been extensively studied in both experimental and clinical settings in SOT, and some transplant centers use machine perfusion as the standard of preservation for selected organs. Table 2 gives an overview of currently acceptable maximum preservation times for SCS and machine perfusion for various organs.

Machine perfusion of the kidneys

Machine perfusion was first studied in the kidneys during the early era of transplantation and again in the current

Table 2: Maximum accepted preservation times per organ as documented in clinical settings [7, 53, 55, 58, 60, 63, 122–125].

Organ	SCS	Machine perfusion
Lung	4–6 h	18 h
Liver	6–10 h	24 h
Kidney	30 h	44 h
Heart	4–6 h	4 h
Pancreas	12–18 h	N/A
VCA	4–6 h	N/A

resurging era; clinical application to kidney transplantation is therefore most broad. Hypothermic machine perfusion is the most widely adopted perfusion technology in kidneys, as randomized prospective clinical trials have demonstrated decreased rates of delayed graft function when compared to cold storage [105, 126, 127]. Unfortunately, improvement in 1-year graft survival has not been consistently significant between studies [105, 126–128]. The current frontier for kidney machine perfusion is studying normothermic perfusion applications for diagnostic and interventional benefits.

Machine perfusion of the liver

Machine hypothermic perfusion of the liver is currently undergoing phase 1 clinical trials after promising preclinical data demonstrating potential advantages in standard donors, extended-criteria donors, and deceased cardiac death donors [88, 107, 129]. Recruitment for a phase 3 clinical trial in normothermic liver machine perfusion has also begun [117]. The dual circulatory system in the liver presents a unique variable in liver preservation. Perfusion of either the hepatic artery or the portal vein has been tested in more than 43 studies [130]. Some believe that perfusion via the hepatic artery should allow for a better supply of oxygen to the peribiliary vascular plexus [131, 132]. This was not supported in animal studies; in fact, some studies showed a preference for portal vein perfusion [130]. Furthermore, Monbaliu and Brassil demonstrated no differences in histological outcomes between dual portal vein and hepatic artery machine perfusion versus hepatic artery alone [133]. There is a difference in target perfusion pressures depending on the route of perfusion. Perfusion of portal vein ranged from 3 to 5 mmHg, whereas low arterial pressure of 20–30 mmHg was preferred in dual perfusion models [130].

Machine perfusion of the heart

The shortage of hearts for transplantation is increasing. In fact, an estimated 43% of patients on the waitlist do not receive transplants [134]. Extending organ donation to include donation after circulatory/cardiac death has helped with increasing the supply of the kidney, lungs, and liver; however, heart transplantation after circulatory death had presented unique challenges. Beyond ethical concerns, these challenges include extending warm ischemia, difficulty in accessing the viability of the heart, and risk of occult pathology. The first heart transplantation after cardiac death was accomplished by using *in situ* hypothermic cardiac resuscitation [135, 136]. However, further investigation revealed that the heart's tolerance for warm ischemia is enhanced if the heart is then perfused under normothermic rather than hypothermic conditions [137]. Dhital et al. reported the first three successful heart transplants after circulatory death by using a perfusion machine for *ex vivo* resuscitation [122]. The PROCEED II trial compared the outcome of standard cold storage to normothermic heart perfusion in heart transplants and determined that the two methods of preservation are equivalent [138].

Machine perfusion of the lungs

Ex vivo machine perfusion of lung allografts has been feasible since 2001, when Steen et al. used an *ex vivo* system to assess lung function before transplantation after cardiac death [139]. The lung was only perfused for a little over 1 h to verify functionality [139]. Since then, *ex vivo* perfusion time in lungs has been extended up to 18 h under normothermic conditions with ventilation [123]. Both prospective and retrospective clinical trials have demonstrated that *ex vivo* lung perfusion is equivalent to standard cold storage for organ preservation [140, 141]. Finally, in August 2014, the U.S. Food and Drug Administration approved the XPS XVIVO perfusion system for preserving and resuscitating lung allografts for transplantation [142].

Machine perfusion of the pancreas

Machine perfusion of the pancreas is not as advanced as other organs and thus far only has been published in the context of animal models. The maximal perfusion time under hypothermic and normothermic conditions were 48 and 6 h, respectively [143–145]. Both perfusion failure rate and long-term survival were worse when the pancreas was perfused for greater than 24 h in a canine model [144].

Machine perfusion of composite tissues

The wider application of extremity transplantation in traumatic amputation reconstruction is significantly limited by the availability of matching donors [146, 147], time restraints [147, 148], and the extensive IRI [149–151] that follows the replantation of skeletal muscle. To overcome the aforementioned limitations, there is a growing interest in *ex vivo* machine perfusion devices such as those studied and used in SOT (Table 3) [47, 53, 137,

152–154]. Although there is no parallel experience with machine perfusion in the field of VCA, there has been a slowly progressive trend of research toward extracorporeal machines for the perfusion of amputated limbs. Distinguishing parameters between the various groups conducting research on the subject entail the experimental animals used, the composition of the perfusate, the perfusion parameters (pressure, temperature, O_2 concentration, etc.), and the maximum time of perfusion and tissue preservation before replantation. One of the first teams that used an extracorporeal perfusion machine to preserve composite tissues was Domingo-Pech et al. who extended the time of extracorporeal perfusion of six canine limbs before replantation to 24 h [155]. They used 27.5% blood in the perfusate and reported minor reversible muscle histological changes and edema, both of which were reversed with the use of peripheral vasodilators, steroids and cooling of the perfusate, and acidosis treated with bicarbonate perfusion. In another study, four porcine limbs were perfused with whole heparinized blood for up to 12 h without replantation [156]. Compared to the cold ischemia group, there was minor tissue damage observed histologically with preserved muscle stimulation. pH was normal throughout the perfusion period, and potassium was controlled with insulin and glucose. Ozer et al. perfused four amputated swine limbs for 12 h before replantation using fresh autologous plasma (10% Hct) with glucose [157]. They observed an increase in lactate levels during perfusion and pH and potassium were maintained at a normal level. Single-muscle fiber contractility was also normal. Kueckelhaus et al. designed a mobile perfusion device and used it in the 12-h perfusion of five porcine limbs after amputation [158]. The perfusate used was a low-potassium dextran acellular solution containing mostly colloid. The machine-perfused limbs did not demonstrate any hypoxic damage when compared to the control group. Lastly, Ozer et al. perfused four swine limbs for 24 h using autologous blood and then transplanted the limbs orthotopically into healthy recipients with a postoperatively survival of 12 h [159]. Their results showed intact neuromuscular electrical stimulation in the perfused group when compared to the control group and no difference in terms of single-muscle contractility.

Ischemic conditioning

Ischemic conditioning refers to the application of intermittent short episodes of temporary occlusion of the blood supply of tissues. It can be applied either before the procurement of the organ, whereupon it is called preischemic conditioning, or after replantation, where it is called postischemic conditioning. It can also be applied to a body part other than the transplant organ, in which case it is termed remote ischemic conditioning.

Multiple short episodes of ischemia applied before the organ is procured have been shown to attenuate IRI. The protective effects have been more pronounced in experimental studies than in clinical trials [160–162]. Remote conditioning, usually achieved by 5-min interval inflations and deflations of blood pressure cuffs on an extremity, does show promising results especially with regard to damage after myocardial infarctions [25, 163, 164]. Multiple studies in organ transplantation settings are currently under way [13]. Although the exact pathophysiological mechanisms are not yet entirely understood, it is assumed that the protective effect of ischemic conditioning is derived from stabilizing mPTP, particularly in early reperfusion. Further ROS production, as well as the induction of apoptosis, is reduced; the up-regulation of protective molecules such as NO, antioxidants, and heat shock proteins may also add to the protective effect [165, 166]. Postconditioning (after reperfusion) improves microcirculation and limits IRI.

Table 3: Maximum preservation times and storage solutions per storage modality.

Organ	Maximum ischemia time (h)	Storage/perfusion solution	Preservation method
Lung [123]	18	Steen	Normothermic machine perfusion (33–37 °C)
Heart [122]	4	Donor blood	Normothermic machine perfusion
Liver [133]	24	UW	Hypothermic machine perfusion (4–6 °C)
Kidney [58]	44	UW	Hypothermic machine perfusion
Face [60]	4	ILG-1/UW/HTK	SCS
Hand [63]	5–6	UW	SCS
Uterus [7]	1–2	Custodiol	SCS

UW, University of Wisconsin solution; HTK, Histidine-Tryptophan-Ketoglutarate solution; ILG-1, Institut Georges Lopez solution [7, 58, 60, 63, 122, 123, 133].

This may be achieved by acting on the endothelial level via inhibiting the endothelial-leukocyte interaction and reduced cell apoptosis. It may also block mPTP after reperfusion [164]. A variety of drugs have been found to mimic the effects of ischemic conditioning, such as cyclosporine A or morphine, some of which have shown promising results in clinical settings already [164, 167].

Pharmacological drugs and therapeutic agents

Insights into the pathophysiological processes that take place during IR have revealed a number of potential targets for therapeutic intervention aimed at prolonged and improved tissue preservation.

As HIF-1α plays a central role in orchestrating the cellular response to ischemia (Figure 1), pharmacological agents acting on the HIF pathway have been thought promising and showed the attenuation of IRI in experimental models [168]. The inhibition of the HIF-blocking PHD molecules by PHD inhibitors could increase the ischemic tolerance of organs in experimental as well as clinical settings [168].

Interfering with the microvascular effects occurring after reperfusion is another promising approach. Complement inhibitors inhibiting both pathways through the blockage of C3/C5 convertase, such as Mirococept, are currently investigated in clinical transplantation settings, after limited IRI and longer graft survival were demonstrated in animal models [169]. Other targets in this setting include the release of tissue plasminogen activator (t-pa) to prevent thrombosis by the administration of streptokinase [166, 170–172].

Other areas of interest include the stimulation of adenosine/adenosine receptor responses through CD73 and CD39 as do AMPK and MIF stimulation [173–175]. The administration of vitamins, EPO, VGEF, amino acids, statins, or activated protein C as well as different perfusion solution modalities, varying oxygen pressures, and ultrasound have been successfully tested in experimental settings but still need further investigation [16, 35].

Biological gases can also be used to attenuate IRI and therefore help preserve tissue for transplantation, namely, endogenously produced gases such as NO, CO, and H_2S. NO is constantly produced by endothelial cells by the NO synthase and helps regulate vascular tone. Furthermore, NO inhibits the expression of adhesion molecules on the (mainly endothelial) cell surface and leads to pulmonary vasoconstriction under hypoxia. In clinical studies, inhaled NO was found to successfully attenuate IRI [176, 177]. CO, as an erythrocyte-derived product, is able to limit tissue injury and inflammation likely through the stabilization of HIF-1α and the HIF pathway activation [178]. H_2S is also produced endogenously by cystathionine β-synthase or cystathionine y-lyase and has been shown to generate a suspended animation/hibernation-like state with decreased metabolic rate and hemodynamic stabilization and subsequent reduced IRI [179–181]. It showed anti-inflammatory and antiapoptotic potency in IRI models likely by stabilizing the mitochondrial membranes and acting as an ROS scavenger among others [182–184]. Neovascularization is likely promoted through the activation of VEGF signaling through H_2S [185].

Hydrogen, a soluble gas that is not produced endogenously, has been shown to effectively reduce reperfusion injury by acting as a scavenger for ROS early on, thus limiting the mitochondrial damage when administered by inhalation [186–188].

Future outlook

Looking at the advancements that have been made over the last decades, it is very likely that SCS will become mostly obsolete for the purposes of organ preservation and that machine perfusion will become the standard of care. It will be necessary to identify through thorough experimentation the ideal preservation parameters for each type of allograft. This will allow us to use dedicated perfusion devices, customized perfusion parameters, and solutions for every organ. We will see the development of various protocols combining machine perfusion with interventional protocols including ischemic conditioning as well as additive drugs and therapeutic agents according to the clinical situation. The potential implications of this are exciting and manyfold.

Going beyond simply assessing their viability, machine perfusion also provides the opportunity for clinicians to perform interventions that resuscitate marginal organs. For example, an isolated organ can receive aggressive antibiotic therapy with levels that would not be possible in the normal host due to concerns of kidney injury. In the machine perfusion model, the effect of a therapy can be more closely monitored, permitting the use of vasodilatation and other injury pathway modulators. Going further, clinicians can even improve upon the current standard of organs by applying interventions to optimize organs for transplant. One area of interest is the therapeutic approaches to reduce reperfusion injury. Hosgood et al. demonstrated that the administration

of gaseous molecules, such as NO donors and CO, can enhance renal blood flow through soluble heme-containing guanylate cyclase pathways, ultimately yielding a protective effect against reperfusion injury in porcine kidneys [189, 190]. Other targets for interventions include calcium channel blockers, lazaroids (iron-mediated lipid peroxidation inhibitor, SOD (free radical enzyme scavenger), p52 inhibitor pifithrin-α (antiapoptotic agent), and HO-1 (heat shock protein). Beyond drug delivery, multiple groups have also explored combining machine perfusion with gene therapy to improve transplantation outcomes [106]. Thus far, Brasile et al. demonstrated that kidneys could be transfected with recombinant adenovirus over the course of 24 h perfusion [110]. The addition of small interfering RNA (siRNA) to preservation solution is another approach to gene therapy that has been shown to reduce reperfusion injury through the down-regulation of caspase-3 expression in isolated kidney [191]. These interventions are not limited to the reduction of the IRI but can potentially also include modifications to counteract allograft immune reactions upon replantation. Potential targets could be MHC expression on donor cells or organ-specific resident immune cell reservoirs, which might pave the way to less and less tolerogenic organs in the future.

All of these would allow increasing the quality and quantity of the currently available allografts while also opening up possibilities to include tissues that have not been deemed unsuitable for transplantation today. They would also allow the inclusion of organs from NHBDs if it becomes possible to limit or even reverse detrimental changes that take place during the initial warm ischemia period.

We can also imagine the creation of dedicated perfusion centers at specialized institutions, where organs can be banked over days or even weeks on machine perfusion stations. These bridging intervals could be used to improve organ quality and function and/or to find the most suitable donor to maximize graft function and survival.

With preservation times of up to 36 h already today, once far-fetched concepts such as global organ sharing are becoming and will continue to become a reality as we move forward.

Summary

Although the underlying mechanisms that drive IR-related injuries are similar, every tissue shows a unique fingerprint with regard to ischemic tolerance and recovery, therefore requiring dedicated preservation modalities. Technological advances have demonstrated that machine perfusion systems have the greatest potential to increase preservation times, organ quality and function, and subsequently allograft function and survival. Combining machine perfusion with therapeutic interventions holds the promise of changing organ donation, preservation, and allocation profoundly in the not too distant future.

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Author Contributions

Nicco Krezdorn: Conceptualization; Resources; Writing: original draft; Writing: review and editing. Sotirios Tasigiorgos: Resources; Writing: original draft. Luccie Wo: Resources; Writing: original draft. Marvee Turk: Resources; Writing: original draft. Rachel Lopdrup: Resources; Writing: original draft. Harriet Kiwanuka: Resources; Writing: original draft. Thet-Su Win: Resources; Writing: review and editing. Ericka Bueno: Resources; Writing: original draft; Writing: review and editing. Bohdan Pomahac: Conceptualization; Writing: review and editing.

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Supplemental Material: The article (DOI: 10.1515/iss-2017-0010) offers reviewer assessments as supplementary material.

Reviewer Assessment **Open Access**

Nicco Krezdorn, Sotirios Tasigiorgos, Luccie Wo, Marvee Turk, Rachel Lopdrup, Harriet Kiwanuka, Thet-Su Win, Ericka Bueno and Bohdan Pomahac*

Tissue conservation for transplantation

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***Corresponding author: Bohdan Pomahac,** Department of Surgery, Division of Plastic Surgery, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA, E-mail: bpomahac@partners.org

Reviewers' Comments to Original Submission

Reviewer 1: anonymous

Feb 20, 2017

Reviewer Recommendation Term:	Accept with Minor Revision
Overall Reviewer Manuscript Rating:	N/A

Custom Review Questions	**Response**
Is the subject area appropriate for you?	3
Does the title clearly reflect the paper's content?	5 - High/Yes
Does the abstract clearly reflect the paper's content?	4
Do the keywords clearly reflect the paper's content?	5 - High/Yes
Does the introduction present the problem clearly?	5 - High/Yes
Are the results/conclusions justified?	4
How comprehensive and up-to-date is the subject matter presented?	5 - High/Yes
How adequate is the data presentation?	4
Are units and terminology used correctly?	N/A
Is the number of cases adequate?	N/A
Are the experimental methods/clinical studies adequate?	N/A
Is the length appropriate in relation to the content?	3
Does the reader get new insights from the article?	4
Please rate the practical significance.	4
Please rate the accuracy of methods.	N/A
Please rate the statistical evaluation and quality control.	N/A
Please rate the appropriateness of the figures and tables.	4
Please rate the appropriateness of the references.	2
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	4
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:
Well written overview on tissue preservation for transplantation. The paper would benefit from shortening (especially in the physiological part in the beginning). The references have to be reworked. See unclear references in the introduction (page1: line 5) and page 12 (line 10). Some references are not cited correctly: for example: "Florack G, Sutherland der, Heil J, Squifflet JP. Preservation of canine segmental pancreatic autografts: cold storage versus pulsatile machine perfusion. Journal of Surgical …. 1983."

Reviewer 2: anonymous

May 11, 2017

Reviewer Recommendation Term:	Accept with Minor Revision
Overall Reviewer Manuscript Rating:	99

Custom Review Questions	**Response**
Is the subject area appropriate for you?	5 - High/Yes
Does the title clearly reflect the paper's content?	5 - High/Yes
Does the abstract clearly reflect the paper's content?	5 - High/Yes
Do the keywords clearly reflect the paper's content?	5 - High/Yes
Does the introduction present the problem clearly?	5 - High/Yes
Are the results/conclusions justified?	5 - High/Yes
How comprehensive and up-to-date is the subject matter presented?	5 - High/Yes
How adequate is the data presentation?	N/A
Are units and terminology used correctly?	5 - High/Yes
Is the number of cases adequate?	N/A
Are the experimental methods/clinical studies adequate?	N/A
Is the length appropriate in relation to the content?	5 - High/Yes
Does the reader get new insights from the article?	5 - High/Yes
Please rate the practical significance.	5 - High/Yes
Please rate the accuracy of methods.	N/A
Please rate the statistical evaluation and quality control.	N/A
Please rate the appropriateness of the figures and tables.	4
Please rate the appropriateness of the references.	5 - High/Yes
Please evaluate the writing style and use of language.	5 - High/Yes
Please judge the overall scientific quality of the manuscript.	5 - High/Yes
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:
This is an excellent and extensive review on the current state of organ and tissue preservation with a focus on plastic and reconstructive surgery.
The authors have tried to present a most comprehensive overwiew of an increasingly important area of research with clinical relevance.
I would recommend to include one clinical example (e.g. traumatic limb amputation) to demonstrate the importance of the subject and describe the Options as of today .
Otherwise the paper should be published as is.

Authors' Response to Reviewer Comments

May 15, 2017

Dear Editors and Reviewers,
Thank you for the fair review of our manuscript entitled "Tissue conservation for transplantation" and for the opportunity to respond to the reviewer's critiques and concerns. I am pleased to now resubmit a revised manuscript.
We have carefully revised the manuscript to incorporate all of the reviewers' comments and concerns. We have prepared a point-by-point reply addressing these points and explaining the changes that were made.
Sincerely,
the authors

Reviewer: 1
Well written overview on tissue preservation for transplantation. The paper would benefit from shortening (especially in the physiological part in the beginning).
We have substantially shortened the manuscript especially in the physiological section as suggested.

The references have to be reworked. See unclear references in the introduction (page1: line 5) and page 12 (line 10). Some references are not cited correctly: for example: “Florack G, Sutherland der, Heil J, Squifflet JP. Preservation of canine segmental pancreatic autografts: cold storage versus pulsatile machine perfusion. Journal of Surgical …. 1983.”
All references have been checked, validated and reformatted accordingly.

Reviewer: 2
This is an excellent and extensive review on the current state of organ and tissue preservation with a focus on plastic and reconstructive surgery.
The authors have tried to present a most comprehensive overwiew of an increasingly important area of research with clinical relevance.
I would recommend to include one clinical example (e.g. traumatic limb amputation) to demonstrate the importance of the subject and describe the Options as of today .
Otherwise the paper should be published as is.

We added a section on the current state of treatment of traumatic limb amputation to the introduction of the manuscript.

Reviewers’ Comments to 1st Revision

Reviewer 1: anonymous

May 19, 2017

Reviewer Recommendation Term:	Accept with Minor Revision
Overall Reviewer Manuscript Rating:	N/A

Custom Review Questions	**Response**
Is the subject area appropriate for you?	4
Does the title clearly reflect the paper’s content?	4
Does the abstract clearly reflect the paper’s content?	4
Do the keywords clearly reflect the paper’s content?	4
Does the introduction present the problem clearly?	4
Are the results/conclusions justified?	4
How comprehensive and up-to-date is the subject matter presented?	4
How adequate is the data presentation?	4
Are units and terminology used correctly?	4
Is the number of cases adequate?	N/A
Are the experimental methods/clinical studies adequate?	N/A
Is the length appropriate in relation to the content?	4
Does the reader get new insights from the article?	4
Please rate the practical significance.	3
Please rate the accuracy of methods.	N/A
Please rate the statistical evaluation and quality control.	N/A
Please rate the appropriateness of the figures and tables.	4
Please rate the appropriateness of the references.	2
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	4
Are you willing to review the revision of this manuscript?	No: it should be ok by then

Comments to Authors:
The text has been changed sufficiently, however the literature is still not ok. see the copied lit. below; some with, some without pages..... please go over it again. If this done, the paper can be published.
5. Devauchelle B, Badet L, Lengelé B, Morelon E. First human face allograft: early report. The Lancet. 2006.
6. Dubernard JM, Owen E, Herzberg G, Lanzetta M, Martin X, Kapila H, et al. Human hand allograft: report on first 6 months. The Lancet. 1999;353(9161):1315-20.
7. Brännström M, Johannesson L, Dahm-Kähler P. First clinical uterus transplantation trial: a six-month report. Fertil Steril. 2014.
8. van der Merwe A, Zarrabi A, Zühlke A. Lessons learned from the world's first successful penis allotransplantation. J Mater Sci Mater Med. 2017.

Reviewer 2: anonymous

Jun 22, 2017

Reviewer Recommendation Term:	Accept
Overall Reviewer Manuscript Rating:	99

Custom Review Questions	**Response**
Is the subject area appropriate for you?	5 - High/Yes
Does the title clearly reflect the paper's content?	5 - High/Yes
Does the abstract clearly reflect the paper's content?	5 - High/Yes
Do the keywords clearly reflect the paper's content?	4
Does the introduction present the problem clearly?	5 - High/Yes
Are the results/conclusions justified?	5 - High/Yes
How comprehensive and up-to-date is the subject matter presented?	5 - High/Yes
How adequate is the data presentation?	4
Are units and terminology used correctly?	4
Is the number of cases adequate?	5 - High/Yes
Are the experimental methods/clinical studies adequate?	5 - High/Yes
Is the length appropriate in relation to the content?	5 - High/Yes
Does the reader get new insights from the article?	5 - High/Yes
Please rate the practical significance.	4
Please rate the accuracy of methods.	N/A
Please rate the statistical evaluation and quality control.	N/A
Please rate the appropriateness of the figures and tables.	4
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	5 - High/Yes
Please judge the overall scientific quality of the manuscript.	5 - High/Yes
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:
The authors haved addressed the reviewers critique accordingly. Therefore the manuscript can be accepted.

Authors' Response to Reviewer Comments

Jun 26, 2017

Dear Editors and Reviewers,
Thank you for the fair review of our manuscript entitled "Tissue conservation for transplantation" and for the opportunity to respond to the reviewer's critiques and concerns. I am pleased to now resubmit a revised manuscript.

We have carefully revised the manuscript to incorporate all of the reviewers' comments and concerns. We have prepared a point-by-point reply addressing these points and explaining the changes that were made.
Sincerely,

Reviewer: 1
The text has been changed sufficiently, however the literature is still not ok. see the copied lit. below; some with, some without pages..... please go over it again. If this done, the paper can be published.
// We have revised all references and added missing information.

Reviewer: 2
The authors haved addressed the reviewers critique accordingly. However the references should be revised for typographic and formal errors
// We revised the references for typographic and formal errors.

Reviewers' Comments to 2nd Revision

Reviewer 1: anonymous

Jun 27, 2017

Reviewer Recommendation Term:	Accept
Overall Reviewer Manuscript Rating:	N/A
Custom Review Questions	**Response**
Is the subject area appropriate for you?	5 - High/Yes
Does the title clearly reflect the paper's content?	5 - High/Yes
Does the abstract clearly reflect the paper's content?	5 - High/Yes
Do the keywords clearly reflect the paper's content?	5 - High/Yes
Does the introduction present the problem clearly?	5 - High/Yes
Are the results/conclusions justified?	5 - High/Yes
How comprehensive and up-to-date is the subject matter presented?	5 - High/Yes
How adequate is the data presentation?	5 - High/Yes
Are units and terminology used correctly?	5 - High/Yes
Is the number of cases adequate?	N/A
Are the experimental methods/clinical studies adequate?	N/A
Is the length appropriate in relation to the content?	4
Does the reader get new insights from the article?	5 - High/Yes
Please rate the practical significance.	5 - High/Yes
Please rate the accuracy of methods.	N/A
Please rate the statistical evaluation and quality control.	N/A
Please rate the appropriateness of the figures and tables.	5 - High/Yes
Please rate the appropriateness of the references.	5 - High/Yes
Please evaluate the writing style and use of language.	5 - High/Yes
Please judge the overall scientific quality of the manuscript.	5 - High/Yes
Are you willing to review the revision of this manuscript?	No: paper is done...;-))

Comments to Authors:
-