Current Opinion in Organ Transplantation

Organ preservation solutions: linking pharmacology to survival for the donor organ pathway

--Manuscript Draft--

Manuscript Number:	
Full Title:	Organ preservation solutions: linking pharmacology to survival for the donor organ pathway
Article Type:	Review Article
Corresponding Author:	Barry Fuller UCL London, UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	UCL
Corresponding Author's Secondary Institution:	
First Author:	Barry Fuller
First Author Secondary Information:	
Order of Authors:	Barry Fuller
	Farid Froghi
	Brian Davidson
Order of Authors Secondary Information:	

Organ preservation solutions: linking pharmacology to survival for the donor organ pathway

Barry Fuller, Farid Froghi, Brian Davidson

Division of Surgery & Interventional Sciences, University College London (UCL), Royal Free Hospital, Pond Street, London

Corresponding author: Prof Barry Fuller
Division of Surgery & Interventional Sciences
9th Floor Royal Free Hospital
Pond Street
London NW3 2QG
b.fuller@ucl.ac.uk

Abstract (200 words)

Purpose of Review:

To provide an understanding of the scientific principles which underpinned the development of organ preservation solutions, and to bring into context new strategies and challenges for solution development against the background of changing preservation technologies and expanded criteria donor access.

Recent Findings:

Improvements in organ preservation solutions continue to be made with new pharmacological approaches. New solutions have been developed for dynamic perfusion preservation and are now in clinical application. Principles underpinning organ preservation solution pharmacology are being applied for cold chain logistics in tissue engineering and regenerative medicine.

Summary:

Organ preservation solutions underpin the donor organ pathway. The solution compositions allow additives and pharmacological agents to be delivered direct to the target organ to mitigate preservation injury. Changing preservation strategies provide further challenges and opportunities to improve organ preservation solutions.

Keywords: 3-5 keywords

Organ preservation
Ischaemia reperfusion injury
Organ preservation solutions

KEY POINTS

- Organ preservation solutions (OPS) are the interface between organ procurement team and the start of the organ preservation process. These solutions have been developed to pharmacologically target the multiple injuries which inevitably occur when an organ is removed from the body.
- As understanding of the overlapping injury pathways improved, additional agents have been introduced into OPS to better target specific signalling events. Whilst increased efficacy for OPS with each new addition is the aim, the stability and delivery of the agents within the base solution also need to be evaluated for optimal OPS manufacture and distribution.
- Innovations in OPS pharmacology continue to be identified. Similar philosophies are being applied in both abdominal and cardiothoracic OPS. Molecular profiling of the organ responses to preservation will help in better targeting to specific signalling pathways. The resurgence in dynamic perfusion preservation methods, alongside access to marginal organs, will provide additional impetus to identify new agents for repair and regeneration processes; these will likely require further OPS refinement and development.

INTRODUCTION

The preservation pathway enabling donor organs to be procured, transported and successfully grafted has become a cornerstone of modern transplant services over the past four decades [1–5]. A major factor within this framework, has been the application of a range of synthetic, reliably produced, regulatory compliant sterile solutions to sustain the vascular, ductular (where appropriate) and parenchymal cell compartments in broadly similar ways for all solid organ grafts (both abdominal and cardiothoracic). Organ preservation solutions (OPS) have developed over time to reflect the collective wisdom on changes which occur once organs are removed from the body, and to attempt to counteract these by pharmacological approaches. OPS are now so widely used on a global basis that they are often considered as mundane components of the donor organ pathway; equally, little attention is given to the fact that as 'pseudo drugs', their production processes must be highly validated in regulatory approved ways, with aims for continual refinement and improvement. This review will address some of these topics, and highlight areas where new concepts over the past 2 years are being proposed to improve OPS efficacy, and to expand applications beyond organ transplantation into new areas like regenerative medicine.

PATHOPHYSIOLOGY OF PRESERVATION INJURY AND THE DEVELOPMENT OF OPS

There has been a growing understanding of the biochemical consequences of organ preservation / reperfusion injury in parallel to development of OPS. The multifactorial injury pathways include failure of aerobic energy metabolism, depletion of ATP, loss of adenine nucleotide intermediates and an increasing acidification following anaerobic glycolysis (Figure 1). Following this disruption of homeostasis, intracellular ion balances change, with negative effects on mitochondria and plasma membrane solute exchangers, activation of catabolic enzyme pathways, and oxidative stress mechanisms, leading to multiple phenotypic changes [6,7]. Reperfusion further exacerbates the injuries which, if they are not quickly reversed, leading to both localised cell death and release of inflammatory markers. The ability to deliver a partial pharmacological mitigation of the changes is the underpinning goal of OPS.

INSERT FIGURE 1 (legend at the end of manuscript)

Original concepts of organ preservation were proposed alongside contemporaneous clinical and scientific knowledge on the value of hypometabolism in different physiological states,

such as cold tolerance in mammalian hibernation [8,9]. Thus, it was intuitive to apply hypothermia to the problem as a sole strategy. The available knowledge on hypometabolism was another reason why some groups developed continuous hypothermic perfusion for organ preservation in the same era [10,11]. With the benefit of hindsight this appears as a conflict of scientific philosophies, but actually this was not the case. The proposal, and subsequent demonstration, that a pharmacological approach could improve static organ preservation by Collins and his group [12], by infusing a synthetic cold solution with a defined targeted composition, was a 'game-changer' which allowed the expansion of organ procurement services in ways not possible if continuous machine perfusion had been the only available option.

SOLUTE ADDITIVES IN OPS

The innovations made by Collins *et al.* in solution design were based on their understanding of natural hypometabolic states [12]. Several putative pathways were targeted by controlling the solution ionic balances, which have subsequently been referred to as 'intracellular' ion balances with reversal of Na^+/K^+ ratios from those in plasma. Other solutes such as moderate (10mmol l^{-1}) concentrations of glucose were added. In fact, Collins made several insightful studies into OPS pharmacology, and later argued that high magnesium and sulphate were the main beneficial changes [13], more so even than the switched Na^+/K^+ cation balances. The following years saw the proposal of other OPS in which different anions (such as citrate) were balanced with the cations, and which then were used in the clinic in different settings [14,15]. However, few prospective clinical trials for head to head comparisons of OPS were reported. The citrate-based solution did progress to clinical evaluation [16], and in fact is still utilised in specific indications in some countries such as UK. Against this background, the use of OPS with low ionic strength seems counterintuitive, such as the histidine-tryptophan-ketoglutarate (HTK) solution of Bretschnieder, which has a combined cation content of only about 30mmol l^{-1}. The majority of the osmotic balance is provided by amino acid buffers. However, Collins & colleagues (1984) also demonstrated that OPS with low ionic strength were effective in experimental renal preservation as long as overall osmotic balance was maintained by inclusion of high glucose concentrations. HTK became an OPS of choice for abdominal organ preservation by some groups [17,18], and is still used clinically today. Given these differing OPS formulations, it might seem fortuitous that good clinical outcomes have been obtained using a particular OPS. One might assume that multiple intracellular signalling pathways for cell death are amenable to modulation by OPS additives in multiple combinations. However, these overlapping and sequential pathways have never been fully mapped. The concurrent partial understanding of some of

the pathways was exploited further by Belzer, Southard and colleagues in the 1990's to develop a novel OPS culminating with University of Wisconsin (UW) solution [19–21], which has remained the foremost OPS in many different organ systems. The basic ionic classes remain similar in many cases to those in Collins' solutions, although specific alterations (e.g. introduction of lactobionate as a large molecular weight anion over other possible choices such as sulphate) led to improved outcomes. Additionally, introduction of antioxidants (glutathione), and pharmacological agents (adenosine, allopurinol) provided enhanced preservation. Variations on the Belzer strategy have resulted in development other OPS such as Celsior, which is also in clinical use in some countries [22,23] with broadly similar efficacies but specific reasons for use in different organ systems. In clinical practice, for abdominal organs UW, HTK or Celsior provide similar efficacy, when reviewed largely on single-centre or registry data [24].

RECENT APPROACHES TO ENHANCING OPS PHARMACOLOGY

Whilst the basic components of OPS have remained broadly similar for several decades, the search has continued for more effective pharmacological additives and new formulations (Table 1).

INSERT TABLE 1 (legend at the end of manuscript)

Small molecule bioregulators

Interest in small molecule bioregulators (SMB – also termed gasotransmitters - notably CO, H_2S, NO) has increased significantly over the past decade in a wide range of cytoprotective physiological systems [25,26]. As gasses with reasonable aqueous solubility at their effective concentrations, they could be considered as potential OPS additives. They share several similar chemical properties including co-ordination with metals, especially iron-hemes, thiols and thiol protein targets which contribute to the signalling [26,27]; however, they have short half-lives which may limit their potential as a component of OPS. Also, whilst the pharmacological efficacy of these SMB is achieved at low concentrations, in themselves the agents are toxic at high concentrations, which could impact on safety aspects during organ procurement. CO and H_2S releasing chemicals have shown benefits in experimental OPS [28–30]. Direct pre-gassing of OPS with SBS (such as CO) has been used in experimental organ preservation in which the organs are stored in gas-tight receptacles [31].

In the past 2 years, hyperbaric pressures have been used to deliver CO in OPS. Zhou and colleagues used a pressure chamber (at 4atm) to store rabbit hearts in a modified Krebs solution using $CO:O_2$ at a 1:4 mixture for 18 hours [32]. The $CO:O_2$ mix preserved hearts showed reduced apoptosis and improved histological appearance. Hatayama and colleagues applied a mixture of $CO:O_2$ at a ratio of 4:3 and a pressure of 7atm in rat heart preservation using an extracellular-type solution and a prototype hyperbaric chamber [33]. After heterotopic transplantation followed to 100 days, $CO:O_2$ hearts functioned as well as control non-stored grafts. The $CO:O_2$ hyperbaric mixture approach has also been investigated in rat renal preservation [34]. Some efficacy was shown when the gas mixture was redesigned ($CO:O_2$ at 2:1 and 5atm) during 24h preservation using UW solution. A different approach to CO dosing of OPS has been developed by Steiger & colleagues who produced a controlled-release cartridge device which delivered a defined CO dose to HTK solution used for rat liver preservation [35]. They showed clear evidence of a molecular tissue response to CO delivery and reproducible dose delivery of the SMB.

Recent studies on H_2S in OPS have focused on utilisation of carrier molecules to deliver pharmacological doses of the SMB. Sodium hydrosulphide added to UW solution improved early graft survival in a rat renal allograft model, however, the agent showed no evidence of anti-rejection properties in this allograft model [36]. Sodium sulphide was used to deliver H_2S to HTK solution and improved the microcirculation and function of rat liver preservation after prior warm ischemia [37]. Delivery of SMB by direct gaseous persufflation is another potential option if safety considerations can be met. Combining NO with O_2 for experimental liver preservation by persufflation was reported by Porschen and colleagues [38]. Oxygen itself is not an SMB, but oxygen delivery by persufflation of UW or HTK solution can positively affect preservation outcomes. By maintaining aerobiosis, with positive impacts on mitochondria and intracellular energy balances, signalling for cell death pathways may be blocked, which in turn mitigate apoptosis, autophagy and inflammation [39]. The importance of oxygen, as an additive to OPS in dynamic perfusion, is discussed below.

Antioxidants and Anti-inflammatory agents

Problems of oxidative stress (OS), oxygen free radicals and associated inflammatory activation have long been recognised as a consequence of organ preservation [40,41], and in part UW solution was designed to counteract these [21]. A major limiting factor in improving pharmacological protection in OPS has been the complex and overlapping events during organ preservation which impact on OS. A large number of different antioxidant

effectors have been investigated during organ preservation [42,43], largely in experimental models.

One OPS which has been designed to combat OS on several fronts is the modified HTK solution, also termed TiProtec. On the base of standard HTK multiple agents (iron chelators and n-acetyl histidine to mitigate OS effects, arginine to impact NO supply) have been added. In experimental systems, improved cardiac function has been reported after cardiac ischaemia reperfusion (IR) [44]. However, human clinical trials have not been reported.

Another OPS additive with putative anti-inflammatory actions is polyethylene glycol (PEG), although it may not be viewed as a traditional pharmacological agent. PEGs are polymers with a range of molecular masses and which have been investigated in organ preservation over many years [45,46] with a recent resurgence of interest [47]. PEGs of different molecular masses may have different properties. PEG-35 has been incorporated into the OPS named IGL-1 (Institut Georges Lopez-1 solution). IGL-1 has been used in clinical liver transplantation with outcomes similar to other OPS such as HTK [48,49]. IGL-1 has been suggested to have specific benefit for preservation of fatty liver grafts but with data only in an experimental model [50].

Similar efforts to target inflammation and oxidative injury have been made in cardiac preservation, by adding a range of pharmacological agents to the respective OPS base solutions, as recently reviewed [51]. These await wider clinical evaluation.

OPS IN DYMANIC PERFUSION

Early studies in dynamic organ perfusion used diluted blood or plasma protein solutions as OPS [52]. Belzer's Machine Perfusion solution was developed as a variant of the raffinose-containing solution which led to the UW formulation, with the main differences being inclusion of gluconate as the major anion, and a different HES fraction as colloid [53,54]. This remains the most widely-used OPS for renal perfusion preservation (also known as KPS-1), and has also been used clinically in hypothermic liver perfusion [55]. The KPS-1 base was modified for liver perfusion by adding antioxidants, vasodilators and metabolic intermediates (N-acetlycysteine, L-arginine, nitroglycerin, prostaglandin E1, a-ketoglutarate) to produce Vasosol® [56,57]. The recent interest in oxygenated donor organ perfusion has refocused attention to use of red blood cells for their oxygen carrying capacity by applying OPS for erythrocyte dilution. The albumin-based Steen solution with a plasma-like ionic balance, enriched with potassium and magnesium, has been used for hypothermic

oxygenated cardiac perfusion using diluted erythrocytes [58], with the addition of cortisol, insulin, lidocaine, thyroid hormones, adrenaline and noradrenaline, and the antibiotic imipenem. Steen solution has also been applied to clinical normothermic oxygenated liver perfusion as erythrocyte diluent [59]. In another trial, oxygenation was facilitated using erythrocytes diluted using the colloid gelofusine, supplemented with gluconate, sodium bicarbonate and cefuroxime for liver normothermic perfusion [60]. Dynamic end-ischaemic reconditioning has been performed using oxygenated Custodiol-N (based on HTK) in a small clinical trial which also investigated graded rewarming of the stored livers during perfusion [61]. Adequate oxygen delivery during perfusion presents opportunities to introduce novel solutes into OPS. A cell-free bovine haemoglobin product has been tested in a human liver perfusion model [62]. Addition of a novel marine invertebrate oxygen carrier to Perfadex® OPS during static lung preservation improved early graft function [63].

HORIZONS FOR THE MARKET IN CLINICAL GRADE OPS

OPS have been variously classified for licensing over the years. In Europe the original solutions, e.g. EuroCollins and UW were generally registered as drug substances. They were subsequently classified as medical devices which were CE marked. As of 25th May 2017 the EU has issued new Medical Device Regulations which stipulate that *all* preservation solutions *must* be Class III (higher scrutiny) Medical Devices within 3 years. In addition, Notified Bodies which regulate CE marking are responsible for monitoring production. Thus, in future new OPS will need longer and more expensive registration processes. Additionally, many well-known OPS are now ‘off-patent’ and hence will become cheaper. These factors combined may mean that there will be less incentive to develop new OPS.

OPS and new opportunities in machine perfusion

Machine perfusion presents a new market opportunity for OPS. However, there are many unanswered questions concerning machine perfusion – e.g. what temperature(s), with or without added oxygen, which organs, transportable or hospital based, end ischemic or continuous? Currently, only Belzer machine perfusion solution under various brand-names is CE marked and available. Similar challenges exist for the development and licensing of new solutions in this arena, including the costs and complexity of running clinical trials.

FUTURE PERSPECTIVES AND CONCLUSIONS

It may appear that OPS development has plateaued, but emerging areas of tissue engineering and regenerative medicine require preservation solutions for product delivery. HTK variant, TiProtec has been used for effective 2-day hypothermic preservation for 'liver on a chip' technology [64]. Stem cell-derived cardiomyocytes have been hypothermically preserved for 3 days using HypoThermosol solution [65]. Porcine lacrimal gland tissues were successfully cold-preserved for 2 days using tissue culture medium [66]. UW has been modified by inclusion of 'antifreezes' such as PEG and 3-O methyl glucose for subzero non-freezing storage [67]. OPS could be modified with agents which stimulate hypometabolic pathways which have been identified in naturally cold-tolerant species as well as agents used in cryogenic storage [68], but these await proof-of-principle. The power of gene expression profiling will impact significantly on our abilities to understand both beneficial and detrimental signalling pathways during organ preservation [69] and help identify appropriate pharmacological interventions. As these technologies move towards clinical application, similar regulatory requirements to those discussed above will likely be imposed on the OPS forcing a fusion of cross-disciplinary pharmacological preservation strategies and facilitate new OPS development.

Acknowledgements

We wish to acknowledge the valuable discussions provided by Mr Mike Kappler (*Bridge to Life (Europe) Ltd.*) on commercial aspects of OPS provision. Many members of transplant services, Royal Free London NHS Trust, have contributed by discussing these concepts on OPS and organ preservation in recent years.

Financial support and sponsorship

The authors have no financial support or sponsorship to declare.

Conflicts of interest

The authors have no conflicts of interests to declare.

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TABLES AND FIGURES

Table 1. Development of OPS over the past 50 years.

Descriptions of the development of OPS over the past 50 years, as a highlight for the progressive formulations made as knowledge of cold preservation and reperfusion events increased, and pharmacological agents were included to mitigate the injuries. *PO_4 is both anion and buffer; **Lactobionate is anion with calcium chelation properties; ***N-acetyl histidine is an osmolyte and intracellular buffer.

Four OPS from different era have been selected as illustrative examples, but this does not imply enhanced efficacies between themselves or other OPS. The Collins C2 solution can be seen as the forerunner of subsequent 'intracellular' OPS formulations. By the time UW was introduced, several improvements in ionic balance were assimilated, a colloid was included and an objective decision to include pharmacological agents was taken. IGL-1 solution retained a similar pharmacological approach whilst retaining a broadly similar requirement for impermeant and colloid, but the ionic balance was switched towards the 'extracellular' milieu. The Ti-Protec solution, developed on the base of the HTK formulation has a higher fractional ion content, metabolic intermediates a-Ketoglutarate and aspartate, and iron chelators to prevent iron-catalysed oxidative stress.

Figure 1. Pathways which impact on generic cell injury during cold preservation and reperfusion

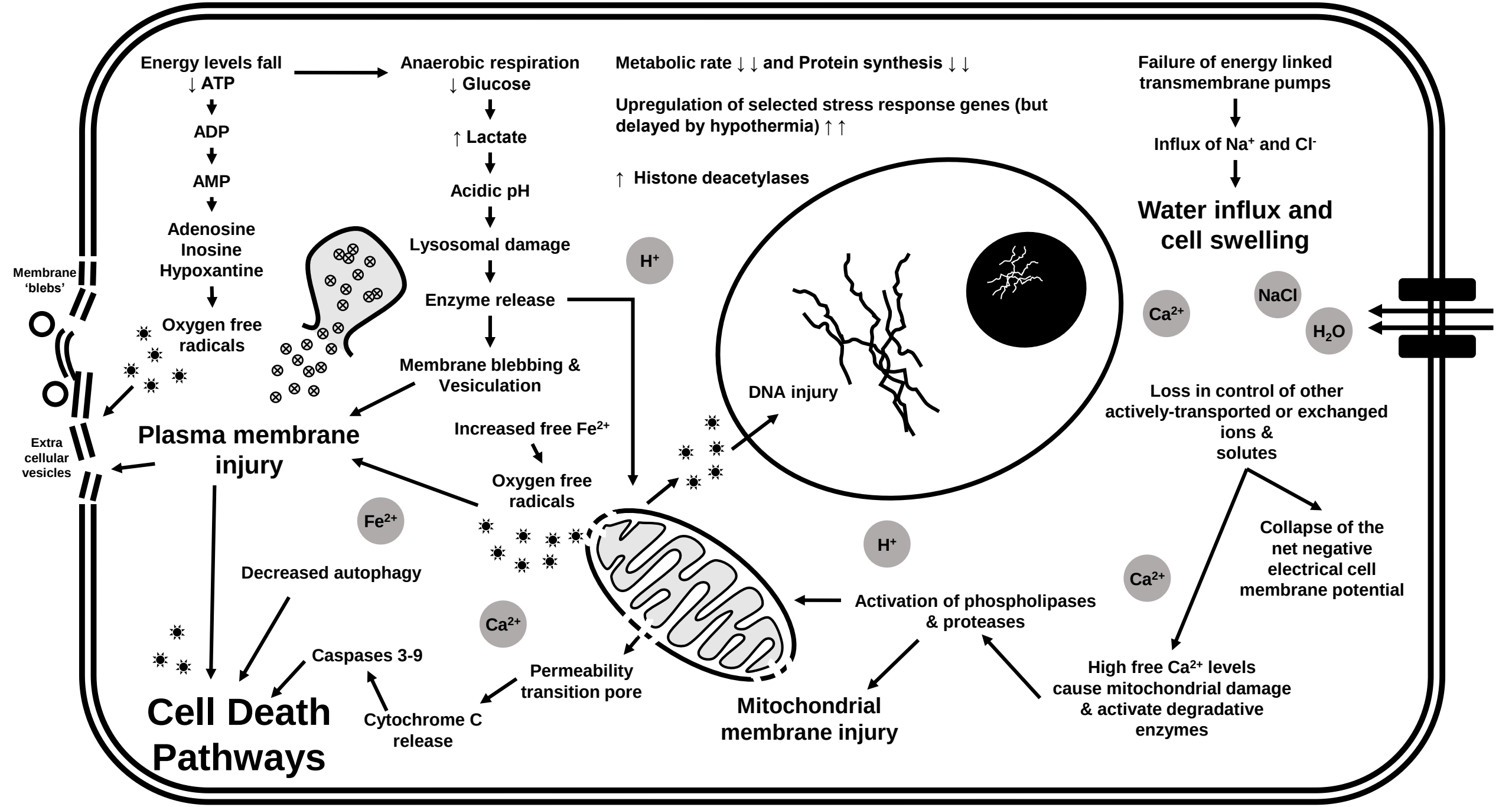
The compositions of OPS have been developed over time, attempting to mitigate these. A complex overlapping continuum of numerous failed homeostatic mechanisms contribute to both direct injury and to signalling for cell death pathways. Cooling, enhanced by infusion of chilled OPS, produces a rapid overall strong metabolic rate depression for energetically costly cell functions such as transmembrane ion pumping and synthesis of macromolecules. This has an early benefit by prolonging the time in which cells can survive under hypoxic conditions where energy metabolism is failing. However, as time passes, loss of homeostatic control results in alteration of the intracellular environment with multiple harmful consequences. ATP synthesis is greatly reduced and adenine nucleotide breakdown products accumulate, including hypoxanthine which can fuel oxygen free radical (OFR) production. Altered membrane potential resulting from changed intracellular ionic balances can lead to loss of adenine nucleotides from the cells, which can prove problematic during

reperfusion. The eventually futile switch to anaerobic glycolysis for energy production results in lactate accumulation and a diminution in intracellular pH, activating lysosomes. Membrane ion pumps fail because of both a lack of energy and inhibition from cold-induced alterations in local membrane viscosity, leading to influx of sodium, chloride and water, loss of potassium and magnesium, increases in free ionic calcium and iron pools. These collectively contribute to a pro-oxidant environment, which in turn fuel OFR injuries in early reperfusion. Cell and mitochondrial swelling gradually increase, with associated physical reorganisation of membrane bilayers, blebbing, and shedding of membrane material including extracellular vesicles. There is a release of Cytochrome C from mitochondria, activating cell death by apoptosis. Aggregate mitochondrial injury becomes a major deciding factor in successful cell recovery during reperfusion. In a similar way, cell tolerance to, and recovery from the disruption in homeostasis signal for multiple changes in gene regulation, transcription and translation with variable downstream consequences for the transplanted organ. (Figure redrawn from Fuller et al. 2010 [6])

Table 1. Development of OPS over the past 50 years

	Collins C2	UW	IGL-1	HTK – Ti-Protec
Transplantation Era	1970s – 1980s	1990s – current	2010's- onward	Future – In evaluation
ELECTROLYTES				
Cations				
Na+	10	30	120	16
K+	108	125	25	93
Mg++	30	5	5	6
Ca++				0.05
Anions				
Cl-	14		20	103
HCO3-	9			
PO4 --*	47	25	25	
SO4--	30	5	5	
Lactobionate-**		100	100	
BUFFERS				
Histidine				198
Glycine				5
Tryptophan				2
IMPERMEANTS				
Glucose	126			10
Raffinose		30	30	
Sucrose				37
COLLOIDS				
HES g/l		50		
PEG g/l			1	
OSMOLALITY	320	320		305
pH	7.3	7.4	7.3	7.0
PHARMACOLOGICAL AGENTS				
Adenosine		5	5	
Glutathione		3	3	
N-Acetyl Histidine ***				30
Allopurinol		1	1	
a-Ketoglutarate				2
Aspartate				8
Deferoxamine /L 20 iron chelator				0.5 / 0.02

Figure 1


Energy levels fall
↓ ATP
ADP
AMP
Adenosine
Inosine
Hypoxantine
Oxygen free radicals
Anaerobic respiration
↓ Glucose
↑ Lactate
Acidic pH
Lysosomal damage
Enzyme release
Membrane blebbing & Vesiculation
Metabolic rate ↓ ↓ and Protein synthesis ↓ ↓
Upregulation of selected stress response genes (but delayed by hypothermia) ↑ ↑
↑ Histone deacetylases
Failure of energy linked transmembrane pumps
Influx of Na+ and Cl-
Water influx and cell swelling
Membrane 'blebs'
Extra cellular vesicles
Plasma membrane injury
H+
Ca2+
NaCl
H2O
DNA injury
Loss in control of other actively-transported or exchanged ions & solutes
Increased free Fe2+
Oxygen free radicals
Fe2+
Decreased autophagy
Ca2+
H+
Activation of phospholipases & proteases
Collapse of the net negative electrical cell membrane potential
Caspases 3-9
Permeability transition pore
Cytochrome C release
Cell Death Pathways
Mitochondrial membrane injury
High free Ca2+ levels cause mitochondrial damage & activate degradative enzymes