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Perspective

Freezing Biological Time: A Modern Perspective on Organ Preservation

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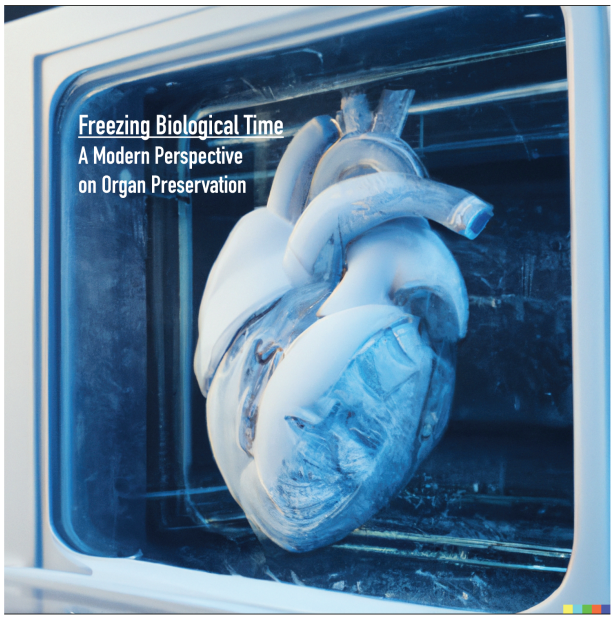
Abstract

Transporting tissues and organs from the site of donation to the patient in need, while maintaining viability, is a limiting factor in transplantation medicine. One way in which the supply chain of organs for transplantation can be improved is to discover novel approaches and technologies that preserve the health of organs outside of the body. The dominant technologies that are currently in use in the supply chain for biological materials maintain tissue temperatures ranging from a controlled room temperature (+25 °C to +15 °C) to cryogenic (−120 °C to −196 °C) temperatures (reviewed in Criswell et al. *Stem Cells Transl Med.* 2022;11(2):107–113). However, there are many cells and tissues, as well as all major organs, that respond less robustly to preservation attempts, particularly when there is a need for transport over long distances that require more time. In this perspective article, we will highlight the current challenges and advances in biopreservation aimed at "freezing biological time," and discuss the future directions and requirements needed in the field.

Key words: cryopreservation; organ transport; organ transplantation; organ banking.

Graphical Abstract



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Significance Statement

Transporting tissues and organs from the site of donation to the patient in need, while maintaining viability, is a limiting factor in transplantation medicine. In this perspective article, we will highlight the current challenges and advances in biopreservation aimed at "freezing biological time", and discuss the future directions and requirements needed in the field.

Introduction

In 1954, the first successful kidney transplantation achieving long-term organ and patient survival was performed by Joseph Murray.[1] This transplant of a kidney between identical twins was followed by further milestones in transplantation medicine including a kidney transplant between non-identical twins in 1959 and liver, heart, and pancreas transplants by the end of the 1960s.[2] As demand continues to grow for organ replacement, so too does the national and international waiting list of patients in need, and the demand for novel methods of organ transport from one geographic location to another. As of April 14, 2022, there were 106 070 patients on organ waiting lists, in the US alone (https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/#). Given the complexities associated with organ donation, including the logistics for organ transport, less than half of these waiting patients will receive organ replacements[3]. More recently, the first successful transplant of a drone-delivered kidney was reported in 2019,[4] helping to expand the opportunity for organs to reach distant patients in need, especially for those patients in geographic areas with poorly developed infrastructure.

Current Transport Logistics of Organs for Transplantation

While the number of transplanted organs have steadily increased due to improvements in immunosuppressive drugs and better donor matching, the transport of organs continues to impact the availability of organs for transplantation. Certain organs like the heart, have critical timeframes (4–8 hours typically) for transplantation that currently prevent transport over long geographical distances. In contrast, kidneys and pancreas have a longer time frame for viability outside of the body and are often transported on commercial flights. In 2020, the United Network of Organ Sharing (UNOS) figures showed that approximately 1 in 6 transplanted kidneys were shipped nationally via commercial airlines.[5] Of the 2445 shipments tracked by UNOS between 2014 and 2015, there were 28 shipment failures and 109 "near-misses" due to transportation delays, including flight delays due to weather or mechanical issues or vehicle delivery issues such as incorrect addresses and lost packages.[6] Between 2014 and 2019, approximately 7% of the organs handled by UNOS encountered transportation problems.[5] This data highlights the need for better methods of organ preservation that would allow more flexibility in transporting organs of all types to broader geographic, and perhaps global locations, to address the current critical organ shortage.

Current Challenges and Potential Solutions

Current challenges, research gaps, and emerging solutions in the field of organ preservation are provided in Table 1 (adapted from Lewis et al.).[7] Additional information on emerging technologies that may help to address limitations of the current logistics chains in the transport of viable organs for transplantation is provided in the following sections.

Emerging Technologies

The current clinical standard for organ preservation is cold storage on ice, which allows the preservation of hearts for no more than 4–8 h and kidneys for 24-36 h prior to transplant. Organ cryopreservation, resulting in weeks, months, or years of storage, would revolutionize the field of transplantation medicine. Normothermic organ storage under physiological conditions has gained a lot of clinical and commercial focus in device development but suffers from very high costs compared with hypothermic alternatives. However, transport of living cells, tissue cultures, organs on chips, and bioengineered tissues under warm physiological conditions is becoming more common,[8] and we anticipate that normothermic perfusion will become more important with time particularly as a means of assessing organs prior to transplantation in combination with the low-temperature storage strategies discussed in the following sections. Normothermic liver preservation by perfusion can lead to the expansion of the liver donor pool by increased use of livers from donors after cardiac death compared with traditional static cold liver storage.[9,10] Normothermic machine perfusion preservation of deceased donor livers reduces both posttransplant early allograft dysfunction and ischemic biliary complications.[9]

Freezing, as a method of cryopreservation, is not usually considered for organs due to ice crystal damage at the cellular level and within the vasculature. Small animal organs can be cryopreserved by vitrification, but the critical warming rates (CWR) required to avoid devitrification and ice nucleation during warming are typically an order of magnitude higher than the corresponding critical cooling rates during cryopreservation. Only one vitrified rabbit kidney has ever successfully functioned in vivo post-convection warming after more than 30 years of research.[11,12] An overview of several key technological milestones in the preservation and transportation of organs and tissues is summarized in Fig. 1.

Isochoric Supercooling, Vitrification, and Warming

While the vast majority of historical cryopreservation efforts have operated within a thermodynamic environment specified by controlled system temperature and system "pressure" (most frequently atmospheric), a growing body of new techniques seeks to stabilize ice-free states of preservation by instead controlling system temperature and system "volume." Isochoric (constant-volume) conditions dramatically change the phase equilibria of water and ice, limiting the crystallization of ice by mechanically confining its expansion from the supercooled liquid phase and consequently have been shown to alter the transport and kinetics at play in the transitions between phases.[13] Isochoric techniques can be grouped into 3 basic categories: (1) equilibrium techniques, which leverage the stable self-pressurized 2-phase equilibrium encountered in isochoric chambers to hold biologics ice-free in the portion of the system which remains liquid between 0 °C and ~−60 °C [0.1–210 MPa] (solution depending), (2) metastable supercooling techniques, which leverage the diminished

Table 1. Challenges and perspective solutions to preservation of organs and tissues (adapted from Lewis et al.[7]).

Challenge	Research gap	Emerging solutions
1. Control excessive ice formation	• Improved understanding of ice molecular structure and activity mechanisms • Need for potent, stable, small and affordable ice growth inhibitors	• Ice-free preservation • Advanced cooling and warming protocols
2. Hold CPA toxicity within acceptable levels	• Equate biochemical mechanisms of chilling injury with CPA use • Perfusion of large tissues and organs • Delineate tissue/organ type and perfusion method with CPA performance	• Novel CPA development • Nature-inspired CPAs • Advanced tissue/organ perfusion and diffusion methods
3. Limit disproportionate mechanical/thermodynamic stress	• Develop new mechanical experimentation tools that can mimic routine cryopreservation protocols • Establish databases for material properties, models for the behavior of materials, and develop rapid simulation techniques	• Advanced cooling and warming protocols
4. Control excessive chilling injury	• Resolve disparity in heterogeneous cell response to cryo-injury • Elucidate the mechanisms of chill injury (cellular dysfunction vs. death)	• Biochemical compounds that reduce cryo-injury • Cell metabolism "enhancers"
5. Avoid unacceptable levels of ischemic injury	• Better understanding the underlying mechanisms of ischemia and reperfusion-driven injury	• Novel compounds to improve oxygen diffusion and resorption
6. Ensure acceptable repair and revival procedures	• The epigenetic response of cryopreservation on cell phenotype • Cellular reprogramming as a means to modulate rejuvenation pathways	• Single cell-sequencing to understand pathways of cryo-injury • Genetic modification and/or cell therapy to protect against cryo-injury

Abbreviation: CPA, cryoprotectant agent.

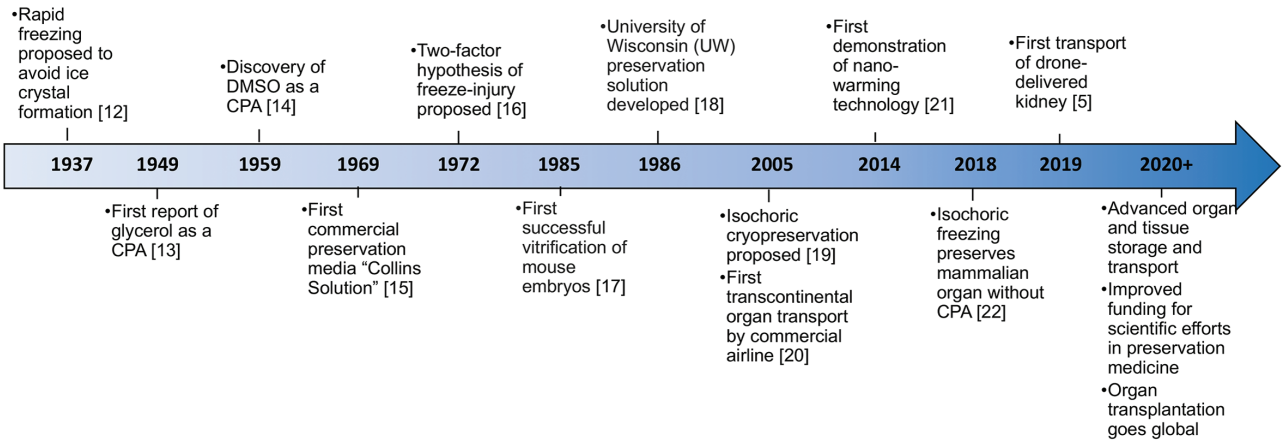

Figure 1. Milestones of organ and tissue preservation and transportation. Abbreviation: CPA, cryoprotective agent.

driving forces for ice nucleation under isochoric conditions to hold biologics in an unpressurized, supercooled, ice-free state at temperatures between 0 °C and ~−20 °C (solution dependent), and (3) non-equilibrium full or partial vitrification techniques, wherein combinations of the thermodynamic and kinetic effects governing the previous 2 techniques are leveraged to shuttle biologics into and out of a glassy ice-free state between approximately −80 °C and −196 °C with reduced cryoprotectant concentrations and reduced cooling/warming rates as compared to conventional vitrification. Early biological demonstrations have provided the first successful cryoprotectant-free sub-zero centigrade preservation of a whole mammalian organ (stable equilibrium technique)[14] and the first sub-zero centigrade preservation and revival of engineered autonomously beating human cardiac tissues (metastable supercooling technique).[15] As a whole, these recent advances in isochoric biopreservation suggest that manipulations of non-chemical thermodynamic aspects of biopreservation may provide an intriguing route forward.

Freezing and vitrification cryopreservation methods differ in cryoprotectant concentrations used and the presence or absence of ice. In successfully frozen cell samples, ice formation in the extracellular solution results in osmotic dehydration of the cells and the isolation of the cells in vitrified channels in an otherwise frozen, ice-bound, system. Relatively low concentrations of cryoprotectants are used for freezing in contrast with the high concentrations used for vitrification.[16] In successfully vitrified cell and tissue samples, ice is completely avoided. Figure 2 highlights the differences in cryopreservation methods.[17] Unfortunately, neither freezing nor vitrification has been successfully used for organ preservation to date.

Rapid Thawing and Nano-Warming

Historically, all frozen and vitrified tissues have been rewarmed by convection in a warm liquid bath. While this works well for cells in suspension in cryotubes, it is prone

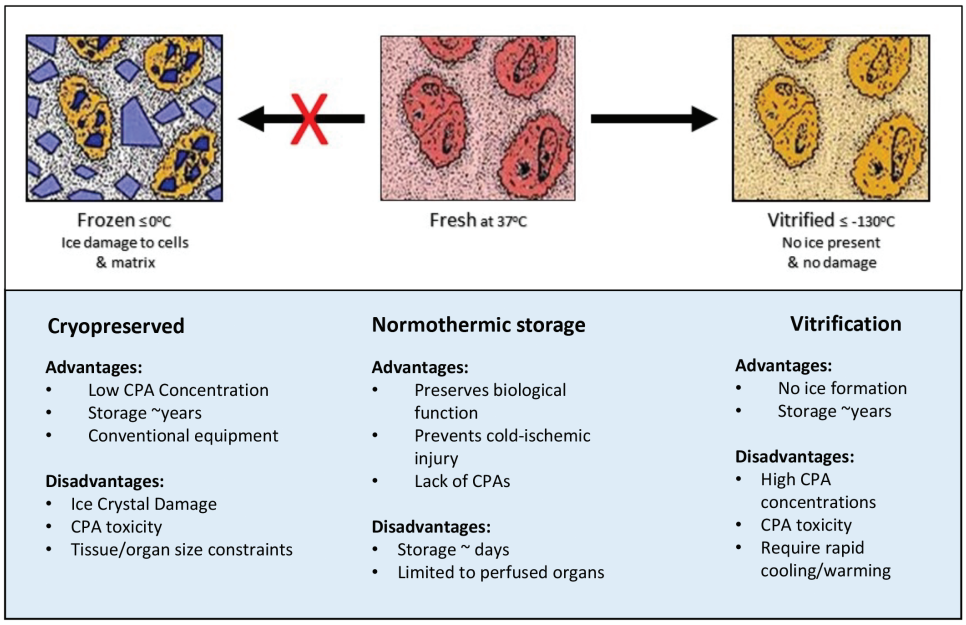

Figure 2. Cryopreservation of tissues. Top: The central image shows cells within a tissue under physiological conditions. In frozen tissues (left panel) employing low cryoprotectant (CPA) formulations, ice nucleation and growth within the cells and matrices results in cell death and structural damage. In contrast, vitrified tissues (right panel) employing high CPA formulations with rapid cooling and warming. Bottom: Comparison of strengths and weaknesses for the various preservation methodologies.

to sample contamination. The risk of contamination can be minimized by the use of dry-warming systems. A dry-warming system that compares well, if not better than the use of a liquid bath, is the SmartThaw device.[18] Studies were conducted using human prostate cancer cells and human mesenchymal stem cell samples cryopreserved using standard, controlled-rate freezing protocols and then thawed with SmartThaw. Post-thaw survival results were equivalent or improved, as compared to traditional water bath approaches, depending on the freezing media used.[18] Solutions designed to mimic the intracellular milieu that restrict passive ion fluxes across cell membranes, rather than plasma-like extracellular formulations such as culture media, are often best for hypothermic cell storage as well as cryopreservation.[19] Adherent cells may be particularly difficult to rewarm with retention of good viability. Two strategies that work well in the literature are (1) stepwise warming from cryogenic storage to a subzero temperature, −20°C to −30°C, with a hold time and then a warm bath,[20] and (2) thawing by infrared radiation.[21] These methods work well in part due to substrate warping that may occur during single-step convection warming, causing cell detachment. These warming strategies may also have benefits for bioengineered tissue constructs.

Early work using microwave heating failed due to difficulty with temperature control and the presence of hotspots. More recently nano-warming, inductive low radio frequency magnetic fields coupled with Fe nanoparticles in the vitrification formulation, created sufficient heating within 1–80 mL samples to exceed the CWR in vitrified cardiovascular tissue systems. Warming rates of >130 °C/minute were achieved with the successful maintenance of cell viability and tissue material properties.[22] Nano-warming for organ or tissue preservation is still in its infancy and prior focus has been limited to thin or highly vascularized biological systems. The application of nano-warming to small animal organs has recently shown encouraging results in vitro.[23–25] Nano-warmed hearts and kidneys performed better than convection-warmed organs,[23,24] but better post-warming functions are needed before transplant studies can be justified. However, nano-warming is ready for evaluation for vitrified natural tissues,[20] bioengineered tissue constructs,[15] and small animal organs.[21–23]

Controlling Biological Time

While new methods like those described above for advanced vitrification and warming hold promise, other methods that leverage what we know about metabolism and lessons from nature may provide insights into further areas of research that may facilitate advances in efforts to "freeze biological time."

Slowing Metabolism

With the exception of normothermic strategies for cell, tissue, and organ preservation, all approaches to biopreservation aim to stabilize biological tissues by slowing metabolic processes. Such strategies retard the cellular chemical and biochemical processes responsible for degradation during ex vivo storage and have the potential to extend storage times to hours, days, or longer depending upon the complexity of the biological tissue, method, and temperature of storage. Metabolic processes follow the "Arrhenius equation," a formula for the temperature dependence of chemical reaction rates,[26] that can be used to describe changes in the metabolic rate as a function of temperature. The blended metabolic rate for living biological materials results in an approximately 50% reduction in metabolism for every 10 °C reduction of temperature below physiological temperature. Cells, tissues, and organs can be stored in a refrigerator or on ice in the range of 0 °C–10 °C for hours or a few days depending upon the formulation

of the cold storage solution used.[27] Metabolism stops once the biomaterial is frozen or vitrified at sub-zero cryogenic storage temperatures.

Inspiration From Nature

There are many lessons that can be learned from Nature for biopreservation.[28,29] Nature employs a variety of compounds and strategies to enhance the survival of cold-blooded, ectothermic animals during cold environmental conditions. There are 2 general categories of adaptation: (1) freeze-tolerant animals that can survive freezing, but generally only in extracellular fluid compartments, and (2) non-freeze-tolerant animals that must avoid freezing. A suite of adaptations is typically required to achieve cold tolerance and these include the production of high concentrations of polyols (particularly glycerol, glucose, and disaccharides such as trehalose) and antifreeze compounds. Many freeze-tolerant insects have proteins and lipoproteins with ice nucleation activity in their extracellular fluid to prevent supercooling that may result in rapid uncontrolled freezing and lethal intracellular ice formation. Long-term preservation in the presence of ice is achieved by coupling temperature reduction with cellular dehydration, in essence, the same freezing strategy mentioned above for the cryopreservation of cells. The adaptations used by ectothermic animals to achieve cold tolerance are being investigated for biobanking of cells, tissue, and organs utilizing both freezing and vitrification preservation strategies. In principle, stabilization of viable biomaterials by lyophilization, freeze drying, or dehydration without concomitant cooling can be achieved for long-term storage at ambient temperatures. While in its infancy, the application of such ambient temperature approaches to the storage of mammalian biosystems would be tremendous breakthrough.

There are also many examples of warm-blooded, endothermic vertebrate animals that can survive high subzero temperatures. Taylor et al. referenced "at least 45 vertebrate species (including mammals) that survive days, weeks, or even months at high-subzero body temperatures in suspended animation [hibernation] without tissue damage."[28] The mechanisms of mammalian hibernation are still being investigated but include upregulation of stress response pathways and programmed suppression of metabolism. The medical implications of discoveries related to hibernation for the biopreservation of cells, tissues, and organs or for prolonged patient therapies are obvious; however, their importance may also impact the survival of the human race. Long-range space travel and human colonization of planets in distant solar systems will be achieved only when it becomes feasible to transport living beings in suspended animation.

Conclusions

Freezing "biological time" to extend function and prevent degradation is an integral factor in addressing the shortage of available organs for patients in need. While there are many technologies and methods that promise to enable extended biopreservation, including several based on lessons learned from nature, the lack of focused research funding presents a critical barrier to the development of practical organ banking and long-distance organ transport. Previous funding in the US was driven largely by the Organ Preservation Alliance (OPA) with events such as the first global Organ Banking Summit in 2015, an NSF-funded road mapping exercise, and a White House Roundtable on Organ Banking and Bioengineering.[30] These efforts resulted in support from the Department of Defense, the NIH, and most recently the funding of an NSF Engineering Research Center (ERC) (ATP-Bio), focused broadly on cryopreservation efforts. Similarly, in the European Union, the Consortium for Organ Preservation in Europe (COPE) provided funding toward general cryopreservation efforts in 2015–2019.[31] While this broad-spectrum funding has stimulated the conception of many promising new technologies, significant focused and accelerated funding is now needed to address the remaining research gaps in order to enable the translation of these technologies to the clinic and industry. In a promising early recognition of the importance of these complex-biologic preservation efforts, new lines of targeted funding have recently begun to emerge from seemingly unlikely sources, including NASA TRISH, which has recently funded proposals aimed at applying cryopreservation principles to the protection of astronaut's en-route to distant planets, and the US Department of Agriculture, which has funded proposals aimed at translating organ preservation principles to food preservation in order to reduce global food waste. These examples represent only the tip of the iceberg, however, as significantly more funding is needed to propel emerging biopreservation technologies to meaningful impacts on public health and sustainability. Extended biopreservation has the potential to transform the practical realities of life-saving organ transplantation, in addition to enabling transformative change in many of the myriad industries wherein the finite useable lifetime of biological matter proves problematic. In light of the many promising technologies that have emerged in the past decade, the most immediate barrier to realizing the promise of extended biopreservation is no longer the science—but concerted efforts and funding.

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Conflict of Interest

K.G.M.B. declared employment with Tissue Testing Technologies LLC and advisory role with LifeNet Health. M.J.P.P. holds patents relating to isochoric biopreservation technologies and ownership stake in BioChoric Inc., an entity seeking to commercialize isochoric biopreservation technologies. R.S. and M.F. declared employment with AlloSource. The other authors declared no potential conflicts of interest.

Author Contributions

T.C.: manuscript writing, final approval of manuscript. C.S.: conception and design, collection of information, manuscript writing. J.S.: conception and design, collection of information, manuscript writing. K.B.: conception and design, collection of information, manuscript writing. M.F.: conception and design, collection of information, manuscript writing. R.S.: conception and design, collection of information, manuscript writing. M.P.-P.: conception and design, collection of information, manuscript writing.

Data Availability

No new data were generated or analyzed in support of this research.

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