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Technical examination of cryonics

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Abstract. Cryonics is the preservation of the human body in low temperatures with the hope of reviving it in the future. This comprehensive article navigates through the three stages of cryonics: Preparation, Stasis, and Revival. In the Preparation phase, from the deployment of standby teams upon legal death announcement to cryoprotectant perfusion, which replaces bodily fluids with solutions to minimize freezing damage. Vitrification is an innovative technique, countering ice crystallization and reducing the dependence on precise optimal cooling rates. The cryoprotective agents, permeable and nonpermeable, are required to assist vitrification. As a result, it is important to take into account the toxicity of cryoprotective agents. Stasis, the immersion in liquid nitrogen, secures long-term preservation. Cryostats and Dewars serve as the guardians of cryonics temperatures, maintaining a stable environment. Revival, the final stage, is determined by precise rewarming and cell repair. Nanotechnology might be applied to the warming and repairing procedures. Understanding cell volume dynamics and harnessing nanotechnology's potential increases the possibility of successful revival. This article aims to provide an examination of Cryonics.

Keywords: Cryonics, Cryopreservation, Cryoprotective Agents, Vitrification.

1. Introduction

Cryopreservation typically refers to the preservation of cells, tissues, or organisms in extremely cold environments, such as in liquid nitrogen at -196 °C, liquid nitrogen vapor at around -160°C, or deep freezers at -80 °C or -150 °C [1]. Cryonics, a form of cryopreservation, refers to the process of preserving a human body at very low temperatures with the hope of reviving it in the future when medical technology has advanced sufficiently. Since biochemical reaction rates also follow the Arrhenius relationship, a decrease in temperature can effectively slow down various life processes at the microscopic scale within living organisms. This includes accompanying processes such as metabolism, active transport, enzyme-catalyzed reactions, and passive diffusion, all of which slow down in response. In other words, lowering the temperature can "slow down" or even "completely halt" biological time, which forms the physical mechanism and theoretical basis for low-temperature preservation [1]. In 1934, Luyet founded the journal "Biodynamica," and in 1940, he published the book "Life and Death at Low Temperatures." Luyet made remarkable contributions during the nascent stage of the field of cryobiology, earning him the title of "Father of Cryobiology." He was also the inaugural president of the International Society for Cryobiology [1].

Cryonics has been continuously explored, and it is widely recognized by the public but rarely academically discussed. Cryonics is divided into three parts, preparation, stasis, and revival (wake up).

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This article will focus on the technological challenges following the order of the three parts. This article aims to provide a better theoretical basis for future people's understanding of Cryonics.

2. Preparation

Deployment and Standby: Upon notification of an impending case, a standby team from Cryonics organization is dispatched to the patient's location. This team ensures swift action after legal death is declared.

Stabilization: After legal death is pronounced, rapid cooling begins, circulation is restored, and measures like lung ventilation and medication administration are undertaken to protect the brain and prevent blood clotting. In cases where transportation to Cyro Organization's facility takes longer, to enhance cooling and prevent clot formation, the individual's bloodstream is substituted with an organ preservation solution.

Cryoprotectant Perfusion: The patient's circulatory system, including their blood or preservation solution, is replaced with a vitrification solution. This vitrification solution is then flowed through the patient's blood vessels while maintaining a cold temperature. Replacing some of the cell's water with vitrification solutions inhibits ice crystal formation during further cooling.

Cryogenic Cooldown: Following cryoprotectant perfusion, the patient is gradually cooled to the temperature of liquid nitrogen for long-term preservation [2].

The primary emphasis throughout all stages of cryopreservation is the preservation of cellular and tissue integrity while minimizing the deleterious effects of crystallizing. Cryopreservation can be divided into two distinct methods, whether intracellular freezing is permitted: conventional cryopreservation, which permits intracellular freezing, and vitrification, which excludes intracellular freezing [3]. Over time, the predominant challenge is the crystallization of ice. the major problem for cell damage is ice crystallization, water within and outside of the cell forms crystals that damage the organelles of the cell [1, 3]. Various techniques have been developed to overcome ice crystallization, such as the addition of cryoprotectants, the utilization of vitrification, and the precise regulation of cooling rates.

*2.1. Cr*yoprotective agent

Cryoprotective Agents (CPAs) are used to protect biological materials, such as cells, tissues, or organs, from damage during freezing and thawing processes. The replacement of biological fluids (blood and water) with CPAs, which are additives used to reduce ice-induced damage to cryopreserved materials and assist their post-thaw recovery, is a crucial step in preparing patients for cryostasis [2]. The induction of thermal hysteresis, prevention of ice nucleation and recrystallization, and ice are just a few of the different classes of CPAs that exist [4]. Based on whether permeable to the Cell Membrane, CPA can be divided into two major categories: penetrating/permeable CPA and non-penetrating CPA [1-3,5].

The cryopreservation process at low temperatures generally requires the use of penetrating CPAs, and sometimes nonpermeating CPAs need to be added simultaneously. Nonpermeating CPAs often contribute to promoting solution vitrification, stabilizing proteins and cell membranes, and inhibiting ice crystal growth. However, their cryoprotective effect is limited when Nonpermeating CPAs are used alone. They typically need to be used in conjunction with penetrating CPAs to enhance their effectiveness or reduce their dosage.

Nonpermeating CPAs' examples include Polyvinyl pyrrolidone (PVP), dextran, albumins, sucrose, and hydroxyethyl starch. Although these cryoprotectants are water soluble, they cannot pass through cellular membranes. They can transform a solution into a super-cooled state, which lowers the concentration of solutes (electrolytes) at specific low temperatures and provides protection [5].

Glycerol (GL), dimethylsulfoxide (DMSO), and acetamide are a few examples of permeating CPAs. They can permeate into cells to hydrate with water, preventing the formation of ice crystals, and they can control the elevation of intracellular solute concentration, reducing cooling-induced cellular damage. These protective agents are primarily neutral and of low molecular weight. Their ideal concentrations, capacity to enter cells, and impact on water molecule activity (reduction of freezing point) differ [5].

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During the preparation step, to avoid damage caused by freezing when the body or brain reaches 0 degrees Celsius, cryoprotective perfusion is used to substitute blood and the liquid components of cells with a cryoprotectant agent (CPA) solution. To accomplish this, access to the vascular system is established, and the chosen CPA solution is introduced using perfusion methods. This gradual process of increasing the concentration of CPA ingredients simultaneously lowers the patient's temperature [2].

Through years of development, the usage of CPAs of the whole body switched from DMSO to Glycerol and then M22. In recorded experiments involving the cryopreservation of organs, a solution known as M22 is used, which is infused into kidneys at a temperature of -22 degrees Celsius, hence its name. M22 primarily consists of penetrating cryoprotectants like DMSO, formamide, and ethylene glycol. The equal amounts of DMSO and formamide in the formula hark back to Gregory Fahy's discovery in the field of cryobiology, where it was observed that DMSO can reduce the harmful effects of formamide [2].

2.2. Cooling

Cooling rate is an important factor in both conventional preservation and Vitrification. Cooling rate refers to temperature decreases with respect to time. Cooling is one of the most significant parts of Cryonics, and it is where a significant amount of damage has been done to the body.

For conventional preservation, there are two approaches to reduce cell damage through cooling. The first method involves a gentler cellular dehydration process, which becomes more effective as the ambient temperature increases. This approach shortens the time needed to mitigate damage caused by metabolic alterations, provided that heat removal exceeds the rate of change in extracellular and intracellular ion levels. The second approach suggests that delaying the cooling process may help minimize the formation of harmful intracellular crystals [5]. Extracellular freezing leads to higher solute concentrations in the unfrozen water portion when cooling occurs slowly. This causes the water inside cells to move towards the ice formation outside the cells. Consequently, due to the increasing solute content inside the cells, they lose water and become damaged. However, when cooling happens rapidly, cells don't have enough time to dehydrate in response to the higher solute concentration in the external environment. In such cases, the presence of water inside the cell increases the risk of harmful ice formation within the cell. According to the two-factor hypothesis, there is an optimal cooling rate for each type of cell that results in the highest survival after thawing and the least damage from both solution-related effects and the accumulation of ice inside the cell [6].

2.3. Vitrification

Farrant achieved successful vitrification of whole organs in 1965. In 1985, Rall and Fahy first achieved vitrification preservation of mouse embryos [3]. In 1999, Kuleshova successfully vitrified and preserved mature human oocytes [1]. Vitrification is a transformation in which a liquid transforms into a non-crystalline, glass-like state. Unlike the typical transition from a liquid to a solid crystal or the freezing of certain crystalline materials, the transition to a vitrified or glassy state doesn't entail significant alterations in molecular structure. This distinction is what makes vitrification the preferred approach for cryopreservation, as it preserves the original structure more effectively than freezing [5]. Unlike conventional cooling which emphasizes an optimum cooling rate, vitrification does not. For every interested type of cell, the optimal cooling rate can only be determined experimentally, which is inconvenient, especially for multicellular tissues that may contain different cell types as well as cells in various relationships to one another and to the extracellular environment, all of which affect the optimal cooling rate. Determining the ideal cooling rate for multicellular tissues can be challenging because these tissues consist of different cell types and varying cellular interactions with both each other and the surrounding environment, all of which influence the optimal cooling rate. To find the best cooling rate, experimental testing is necessary for each specific cell type under consideration [3]. Therefore, vitrification is time efficient.

Reducing the toxicity of the cryoprotective agents used in vitrification is crucial in the cryopreservation process because high-concentration vitrification solutions on organs are highly

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harmful to biological materials [5]. It may be necessary to investigate the specific CPA concentration and duration of exposure required to ensure successful vitrification and the prevention of ice formation during the warming process, as these factors may not always be straightforwardly apparent.

3. Stasis

After the preparation process is done, patients are commonly immersed in liquid nitrogen. In traditional medical practices, this method is used for cryopreserving sperm, eggs, embryos, and certain tissue samples. The first cryonics patient, Dr. James Bedford, underwent cryopreservation using this method back in 1967. In contemporary cryonics, submersion in liquid nitrogen remains the predominant approach for temperature maintenance. Using nitrogen offers many advantages, such as being highly reliable, not needing an external power source, and being odorless and silent. Nitrogen can safely interact with patients since it is typically non-reactive. For the hard compartment, a cryostat is a vessel designed to maintain a consistently low cryogenic temperature (below -100 degrees Celsius) internally. A Dewar is a type of cryostat equipped with internal thermal radiation reflectors and excellent vacuum insulation. The Cryonics Institute employs fiberglass cryostats that utilize soft vacuum and perlite for insulation [2].

4. Revival

4.1. Warming

Rewarming is crucial for waking up. However, warming could also do significant damage to the human body.

Similar to the cooling rate, the warming rate also has a connection to internal freezing. Slow warming allows crystals to recrystallize, and this causes damage to the cell. In contrast, rapid warming prevents this from happening since it does not give enough time for it to happen. If the heating rate is not fast enough, harmless small ice crystals formed during the cooling process are likely to continue growing, undergo changes in shape and orientation, or merge into larger ice crystals during the warming process. This phenomenon is referred to as recrystallization [1]. The process of reheating vitrified organs can also be unsuccessful due to ice recrystallization when the rewarming occurs too slowly, or it can result in cracking caused by uneven thermal stress if the rewarming process is not uniform [7].

The danger temperature zone for recrystallization is typically defined as the temperature range between the equilibrium freezing point of a solution and the glass transition temperature. Within this range, the formation (heterogeneous nucleation, homogeneous nucleation) and growth of ice crystals can cause significant damage to cells, potentially leading to cell death [1]. Despite the help of CPAs, achieving the necessary critical warming rates (CWR) to avoid ice crystal formation during the rewarming process is often much more challenging, typically ranging from 10 to 1000 seconds per degree Celsius per minute, compared to the critical cooling rates (CCR) which fall within the range of 1 to 100 seconds per degree Celsius per minute. Moreover, the variability in temperature during the rewarming phase can lead to thermal stress, potentially causing cracks. The primary problem of vitrification lies in the speed and uniformity of the rewarming process [7].

In the process of rewarming, thermal stress damage, and ice crystal damage are often fatal, especially in large-scale sample preservation. Significant progress has been made in the inhibition of ice crystal growth and the elimination of thermal stress during rewarming through external physical field-based techniques such as photothermal rewarming and electromagnetic rewarming [1].

4.2. Nanowarming

Nanoparticles with excellent photothermal conversion properties, combined with near-infrared light, can provide a platform for ultra-fast heating, effectively suppressing recrystallization and devitrification during millimeter-scale cryopreservation and achieving efficient preservation of cells and zebrafish embryos. Magnetic nanoparticles such as Fe3O4 can rapidly convert external magnetic energy into thermal energy [8].

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In nanowarming, CPA solutions and iron oxide nanoparticles (IONPs) are perfused throughout the organ vasculature. By enclosing the organ in a radio-frequency (RF) coil, which generates alternating magnetic fields as a result of electric current flowing through it, the organ is vitrified and then rewarmed [7].

4.3. Cell repair

Partial cellular damage at the tissue and organ level appears hard to avoid. Therefore, successful wake up needs the advancement of cell repair technologies. The brain cells are particularly significant because they store the personalities of the patient [9]. Aside from addressing any harm caused during the cryopreservation process, it is critical to consider the repair of ischemic damage that results from the time lapse between the declaration of legal death and the initiation of cryonics stabilization procedures.

Cell repair is one of the hard parts of Cryonics, especially in the brain cells. Nano repair has been proposed as a theoretical solution to cell damage. The central thesis of nanotechnology is that almost any chemically stable structure that can be specified can be built. Nano repair essentially uses "nanobot" to rearrange biological materials at the molecular level. The molecular constructor consists of two main components: a miniature robotic arm and a well-defined set of chemical reactions that take place at the arm's tip. This assembly system can build precise structures by manipulating reactive materials and operating a series of atomic-level reactions. In essence, it resembles the capabilities demonstrated by ribosomes, but in a more advanced form [9].

The essential information that would prove valuable regarding a frozen brain comprises the precise coordinates and atom types present within it. To provide a comprehensive description of each atom, theoretically, it would describe the states of all their electrons, although this can largely be inferred once we have the coordinates and atom types. With this knowledge and access to advanced technology adhering to the principles of chemistry and physics, we could potentially restore the frozen structure to its initial state after thawing, allowing us to make various atomic-level modifications [9].

5. Further discussion

5.1. Cell volume

CPAs are required to be easily penetrated to the cell membrane, The Cell loses volume undergoing cooling, and regains volume upon warming, rate of cooling causes water to move out of the cell. Therefore, understanding the movement of substances across the membrane in particular water is crucial to Cryonics.

Heating and cooling both cause significant cell volume changes. Although cell and tissue death primarily occur due to intracellular and extracellular ice crystal formation, osmotic stress, and electrolyte disturbance, the exact nature of the primary damage experienced by cells and tissues during the volume-changing process remains uncertain.

Cooling and freezing processes expose cells to significant osmotic stress, cell damage, and a drop in cellular function. The primary reason for this phenomenon is the alteration of the membrane's condition and structure due to temperature fluctuations. When cells cool down, changes in the membrane's fluid-to-gel phase result in the leakage of intracellular solutes. Freezing exacerbates this issue by causing even more shifts from fluid to gel phases in membranes, affecting their permeability to water and external solutes. As a result, this leads to imbalances in osmotic pressure [6].

Four factors that cause cell shrinkage have been identified as osmotic volume decrease (OVD), cooling volume decrease (CVD), freezing volume decrease (FVD), and apoptotic volume decrease (AVD). OVD is the phenomenon of cell shrinkage or dehydration that occurs during the freezing process. When cells are frozen, water flows out of the cells due to increased chemical potential or activity of water within the cells compared to the external medium containing ice. CVD occurs during the cooling process. As the temperature decreases, water flows out of the cells due to changes in the chemical potential of water. OVD, CVD, and FVD are passive volume decreases that are caused by the process of cooling. AVD however is an active volume decrease that is caused by the flow of K^+ and Cl^- ions

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across the membrane. Cell needs to regain the volume when temperature increases, cell triggers regulatory volume increase (RVI), and it is caused by water inflow driven by Na+ influx through the 'hypertonicity-induced cation channels' (HICCs) vasopressin (AVP) is a hormone that plays a role in cell volume regulation and cryo-protection. It has been found to enhance the activity of a specific type of ion channel called HICC, which is involved in the process of cell volume regulation. This hormone can improve cell survival after cryo-preservation and its signaling mechanism is mediated through specific receptors and intracellular signaling pathways. and it would potentially be a cryoprotectant [10].

5.2. Nantechnology

In recent years, research has discovered several advantages of using nanoparticles of different types, sizes, concentrations, volume fractions, and distributions in low-temperature preservation:

Regulating Cell Membrane Permeability: Nanoparticles can help regulate cell membrane permeability, optimizing the addition and removal of cryoprotectants. This assists in protecting cells during freezing and thawing processes.

Improving Heat Transfer and Thermodynamic Properties: Nanoparticles effectively enhance the heat transfer properties of cryoprotectant solutions and modify their thermodynamic properties. This can increase solution viscosity, thereby improving the glass transition of the solution, which is crucial for low-temperature preservation.

Enhancing Cooling and Rewarming Efficiency: Nanoparticles can enhance the efficiency of the cooling and rewarming processes during low-temperature preservation.

Therefore, nanotechnology holds the promise of becoming a key technology for overcoming current bottlenecks in low-temperature preservation [4].

6. Conclusion

To provide a structural explanation, this article divides cryonics into three stages: Preparation, Stasis, and Revival. The preparation phase covers everything from legal death to the patient's placement into the cryostat. The stasis is the maintenance of cryo-stasis. The revival process involves rewarming and cell repair. In the preparation phase, vitrification with the addition of cryoprotective agents (CPA) is a better method compared to the conventional. Vitrification effectively reduces the damage caused by ice crystallization and is less dependent on achieving the optimal cooling rate. Nonetheless, it is crucial to consider the issue of solution toxicity during the vitrification process. Together with liquid nitrogen Cryostats and Dewars ensure the maintenance of cryonics temperatures, offering a silent and odorless environment for the suspended individuals. The warming process during the revival phase is equally important to the cooling procedure, as it emphasizes the speed of heating and the uniformity of heating. To achieve success in Cryonics, future technological advancements may be made in several key areas: nanotechnology, the development of improved CPAs, precise control of cooling and warming rates, and a deeper comprehension of cellular mechanisms, particularly about cell volume.

The technology of Cryonics pushes the boundaries of life, even beyond the limit of death. As cryogenic research continues to advance, it is important that we engage with it from a scientific perspective while staying hopeful.

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