SUCCESSFUL ORGAN TRANSPLANTS : THE METHOD TO LONG-TERM HEART PRESERVATION

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1. Abstract

Organ preservation is necessary to maintain organ stability during storage so that at reperfusion where the organ is surgically placed in the recipient, the organ functions. In modern day heart transplant preservation, hypothermic storage is implemented where, once removed from the donor, hearts are cooled to and kept at temperature a few degrees above the freezing point until they reach the recipient [2]. However, clinical research from Massachusetts General hospital states that about three out of four donated hearts have to be discarded due to short preservation life [1]. We propose a solution that aims to address this issue and extend the preservation life of heart transplants. Our proposal is to implement a preservation solution composed of cryoprotectants of great potency, little toxicity, and high stability, and to rapidly freeze heart transplants to around -196℃ through vitrification.

2. Summary of the significance of the targeted problem

Effective organ preservation is important to successful transplantation and ensures organ viability until the transplant is transferred into the patient. As of now, 60% of donated hearts and lungs are discarded yearly because they can only be kept on ice between 4 and 6 hours [4]. Due to this low preservation efficiency, a lot of patients aren't able to get heart transplants, so by solving this issue, life expectancy rates would increase. With a short heart preservation time, doctors are faced with a time limit of transporting heart from donor to recipient without having much time to have an accurate analysis of immunologic matching, a huge issue that patients face. Even after a successful heart transplant, it is not uncommon for patients to have heart issues years later because of their bodies eventually rejecting the foreign heart. Antibodies, proteins produced by the immune system to protect the body, will not recognize the foreign heart cells and tag them, causing immune cells to attack heart cells, killing it. With improved preservation however, doctors will have more time to match donor hearts to recipients, avoiding this problem of the immune system attacking the heart, reducing the problem of organ rejection and lowering costs of future care. Better preservation would make it so that the heart isn't in a dire need for blood, which allows doctors to have longer times for transplantation, increasing the donor pool of hearts.

3. Descriptions of all state-of-the-art solutions and their insufficiencies

As of now, hypothermic storage at a temperature range of 4-8°C and perfusion with a preservation solution serves as the basis for nearly all methods of heart transplant preservation [4]. Hypothermia decreases metabolism and "the rate at which intracellular enzymes degrade essential cellular components necessary for organ viability" [4], thus preventing heart

deterioration in ischemic conditions where there is no blood supply. Components of preservation solutions include impermeant agents, osmotic molecules, purine nucleotide precursors, antioxidants, enzyme inhibitors, buffers, and electrolytes mimicking intracellular or extracellular fluids [5]. Although the components vary depending on the solution (e.g. Collins Solution, UW Solution), all formulas provide a "physical and biochemical environment that maintains viability of the structural components of the tissue during hypothermic metabolic arrest", and minimize reperfusion injury [4]. However, as previously stated, heart transplants can only last 4-6 hours, so physicians have very little time to find a proper recipient match, transport the organ, and place it in the recipient; thus, the success rate of heart transplants is incredibly low [1].

Long-term preservation must be utilized in order to increase the success rate of heart transplants. It requires "cooling to ultra-low temperatures (below −100 °C) in the process of cryopreservation" [5] in order to prevent further tissue deterioration. However, cooling to sub-zero temperatures leads to uncontrolled extracellular ice formation in heart cells. A growing lattice of ice crystals can pierce and separate tissue, disrupting heart function [5]. In addition, extracellular ice formation osmotically draws water from inside cells, causing cells to shrink and possibly damaging cell membrane and structure [5]. Current cryopreservation methods dissolve cryoprotectants (CPAs), or protective chemicals such as glycerol, propylene glycol, and dimethyl sulfoxide, in the preservation solution to lower the melting point of the solution and prevent intracellular ice from forming. In addition, they carefully control cooling and thawing rates. However, those methods still fail to prevent excessive ice formation and organ injury [5].

A non-ischemic preservation method known as ex vivo perfusion avoids issues of ice formation. This method involves a machine keeping an organ warm by continuously pumping

blood and nutrients (e.g. potassium, sodium, and oxygen) through the organ, and is commonly used for kidney transplants [1]. However, to use ex vivo perfusion when banking organs for months or years is too costly and requires too large of a supply of blood and nutrients for replenishment. Another method that has been explored to attempt to avoid issues of ice formation is vitrification, a form of cryopreservation that transforms a substance into a glass, or a noncrystalline solid, through freezing [7]. Methods to vitrify and preserve sperm, oocytes, and embryos have been developed, but those for tissues and organs have not yet been developed [7]. This is because in order to prevent ice crystallization, current methods for vitrification rely on high concentrations of cryoprotectants "in the region of 60% weight/volume" [5] that are overly toxic levels for human tissue. In addition, such a high concentration of cryoprotectants renders the preservation solution "too viscous to work their way fully into larger tissues and organs" [1]. As such, uncontrolled ice formation in organs remains a critical factor restricting the development of methods for biobanking organs for extended periods of time.

4. **A detailed description explaining your team's solution to the problem and how it overcomes these insufficiencies backed by research**

The heart transplant preservation solution proposed is to permeate organs with a solution of CPAs and then vitrify the organs. The preservation solution will consist of glucose, 3-O-methyl-D-glucose (3-OMG), synthetic ice modulators, and stress tolerance enhancers. Glucose is an essential component in the antifreeze produced by the wood frog, a species that is able to survive in incredibly cold environments by hibernating while frozen. The glucose keeps the frog's cell membranes safe from injury by preventing extracellular ice crystal formation [4]. Molecules of glucose do so by replacing water molecules and preventing them from forming

hydrogen bonds [7]; thus, glucose lowers the freezing point of fluids surrounding cells. With this knowledge, glucose can be used as a cryoprotectant that lowers the freezing point of fluids inside heart transplants to prevent destructive ice crystals from forming during freezing.

Wood frogs have high concentrations of glucose in their antifreeze, but for human tissue, such high concentrations of glucose are far too toxic [3]. As a result, the preservation solution used cannot be solely based on glucose; the glucose concentration must be lower, and other CPAs must be added that aid in protecting the heart transplants during freezing. 3-OMG is a nontoxic glucose derivative that has previously demonstrated colligative resistance to cell shrinking; because it is metabolically inactive, it is able to accumulate in cells and maintain resistance to cell shrinking [2]. In addition, it has been shown to stabilize the phospholipid bilayer, inhibit intracellular ice formation, and decrease metabolic rate by inhibiting glycolysis, all properties necessary to prevent cell death [2]. Reports state that 3-OMG "improves the viability and quality of hepatocytes preserved in vitro" and "enables supercooling of whole livers" [2]. The next components to the preservation solution are synthetic ice modulators, or compounds that influence the ice nucleation (formation of small ice crystal embryos on membrane proteins). The polymers polyvinyl alcohol (X-1000) and/or polyglycerol (Z-1000) are two types that have been been used to preserve a variety of cell types and have shown to "effectively suppress ice nucleation events in aqueous systems even at concentrations as low as 1 ppm, much lower than most other ice control agents, by selectively binding surfaces of molecules that would otherwise promote the formation of ice nuclei" [2]. X-1000 and Z-1000 are "nontoxic, readily permeate cell membranes, and remain active at temperatures ranging from 0 ° C all the way to glass transition temperatures (below −120 ° C)" [9]. Either one may be used in

the preservation solution. The final component of the preservation solution are stress tolerance enhancers, which minimize cryoinjury and maintain cellular integrity during freezing. They do so by decreasing metabolic rates and preserving cellular macromolecules, which is important due to the reduced capacity to replace damaged macromolecules during freezing [9]. Options for stress tolerance enhancers to use in the preservation solution include the antioxidant catalase and the cell-permeable apoptosis inhibitors pifithrin. This preservation solution of glucose, 3-OMG, synthetic ice modulators, and stress tolerance enhancers combines CPAs of great potency, little toxicity, and high stability to create a safer and more effective solution than standard cryopreservation.

The vitrification process will consist of rapidly cooling the heart transplants to temperatures around -196℃. By freezing to such temperatures below hypothermic temperatures, the rates of biochemical reactions and intracellular enzyme activity will be sufficiently decreased. Lysosomes are an especially important group of enzymes that must be slowed, because they play large roles in necrosis, and are capable of digesting cellular components essential for organ viability. All enzymes work best at a certain optimal temperature and pH level depending on their function and location. Thus, when the temperature of organs such as the heart is drastically lowered, lysosome activity will be drastically reduced to very low rates. As a result, cell death and tissue deterioration rates will be low enough to extend the normal heart preservation period of 4-6 hours to weeks, months, or even years.

The cooling rate used must be rapid enough in order to limit time for crystallization and prevent intracellular ice formation, a destructive issue in conventional cryopreservation methods [5]. To accomplish this, a cooling rate of at least 100,000 °C/min will be used that will supercool

the heart cells to amorphous glass devoid of ice crystals. With rates higher than 100,000 °C/min, even pure water, which is very difficult to vitrify due to low viscosity, can be vitrified [9]. Another benefit of using such a high cooling rate is that it lowers the required CPA concentration, so issues of CPA toxicity can be further avoided.

5. **Anticipation of any obstacles that stand in the way of the creation of this technology.**

It may be hard to monitor the toxicity of the preservation solution; the pH, temperature, concentrations, and other such factors may be difficult to control. To address this, the individual CPA components of the solution should be tested on tissue at varying concentrations to discover any toxic effects, which may include damaged cell membranes, impaired enzyme functions, diminished cell or embryo development, and damaged macromolecules such as DNA. With any toxic CPAs, they should be experimented with to find what precise conditions and measurements of the CPA make it non-toxic. Once these optimal conditions and measurements of each CPA are found, the CPAs can be combined to create an optimal preservation solution that will not damage heart cells once utilized.

In order to guard against the possibility that toxicity cannot be avoided or fluctuating toxicity levels of the preservation solution due to fluctuating conditions, caspase inhibitors will be included in the preservation solution. CPA toxicity in the preservation solution may cause caspases, proteases, or kinases, all enzymes that play roles in apoptosis, to be released, and thus cause apoptosis within the heart cells [10]. However, the caspase inhibitors would interfere with the caspase activity and thus prevent apoptosis.

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