A Review on Cryoprotectant and it's Applications in Cryopreservation

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***Abstract*: Cryoprotectant are usually chemical compounds that prevents tissues and cell from freezing damage. There are different types of cryoprotective agent used for pharmaceutical preservation. (Glycerol, DMSO, Sucrose etc.), During the process of cryopreservation of cell &tissues maintaining of specific storage condition is very important. Cryoprotectant are utilized for various types of preservations. The origin of the cryopreservation at as early 2000BC.There are two types of cryopreservation, very low temperature is used for the process of cryopreservation. The main mechanism of cryopreservation is to protect the tissues &organs for longer period of time. Mainly two types of technique use for cryopreservation (Vitrification &Thawing). Various types of tissues and organs are preserved by using cryoprotectant but it is difficult than the preservations of cells. In addition, some applications and limitation are mentioned.**

***Keywords*: Cryoprotectant, Cryopreservation, glycerol, DMSO, Cryoprotective agen**t.

Introduction:

It is difficult to preserve and store living organisms, Plant tissues, Yeasts, bacteria, fungi, animal tissues, algae, Amino acids, fungi, lipid, based formulation such as solid lipid nanoparticles and other biological material for long period of time. With this challenge, Polge and his team did an excellent discovery.[1]

A cryoprotectant is a substance used to protect biological tissues from freezing damage (i.e., damage caused by ice formation). Cryopreservation is a process in which cells or tissues are preserved by cooling them to sub-zero temperatures, such as 77 K or -196 °C (boiling point of liquid nitrogen). The preservation of structurally intact living cells and tissues through cryopreservation involves using extremely low temperatures. Cryoprotectants that simply increase the total concentration of all solutes in the system, Reduce the amount of ice that forms at a given temperature; but to be biologically acceptable, they must be cell permeable and have low toxicity. At these low temperatures, biochemical reactions that would otherwise lead to tissue damage or cell death are effectively stopped. However, if cryoprotective solutions are not used, preserved cells will be damaged by freezing or thawing when approaching low temperatures or warming to room temperature. [2] In order to prevent cell death during preservation, the temperature of tissues and organs used to be lowered to stop cell death. But at the same time, the cooling rate also affects cell survival. Rapidly cooled conserving cells are prone to cell death due to rapid intracellular ice formation. Sometimes cells can die due to dehydration. Otherwise, cell shrinkage and cell death may also occur due to the presence of hypotonic solutions surrounding the cells.[3]

Cryopreservation enables the storage of a large number of cells and tissues that can be used for scientific research and medical applications, including pancreatic islet and hepatocyte transplantation, blood transfusion, bone marrow transplantation, artificial insemination and in vitro fertilization.[14] Cryopreservation methods can set the stage for indefinite preservation of biological materials by reducing metabolic rates.[16]

Except use of proper cryoprotectant cell will rupture due to "solution effect" injuries, in which the cells' leftover solution alters their shape and mechanical strength before oscillating as they freeze-dry. Sometimes these cryoprotectants are referred to as antifreeze. Although there are various cryoprotectants, glycerol, propylene glycol, and dimethyl sulfoxide (DMSO) are the ones that are most frequently used. It became important to preserve tissues with an appropriate cryoprotectant (5-15%), which aids in the formation of big unfreeze Pockets, in order to ensure cell survival after freezing and thawing at liquid nitrogen temperatures. During the freeze-drying process, these pockets aid in preventing crystal formation and mechanical damage. However, at a certain point, the formation of spherical crystals can destroy cells' chronobiology. Slow freezing is generally preferred for preserving cells because cells are frizzed slowly below their freezing point. These crystals are also known as unfrozen fraction.[3] Low temperatures drastically slow down biological and chemical processes in living cells, a feature that may enable the long-term preservation of cells and tissues. However, freezing is lethal to the majority of living organisms because it results in the formation of intracellular and extracellular ice crystals as well as changes to the chemical environment of cells that result in mechanical stress and harm to the cells. The shift from the water to ice phase is the main challenge that cells must overcome at low temperatures. Slow cooling causes osmotic changes as a result of exposure to highly concentrated intra- and extracellular solutions, or as a result of mechanical interactions between cells and the extracellular ice. Cell damage at rapid cooling rates is attributed to intracellular ice formation. In order to preserve the fine structure of cells, a procedure called cryopreservation keeps biological samples in a condition of suspended animation at a low temperature for a long time. In the presence of a cryoprotective agent (CPA; also known as a cryoprotectant), which changes the rates of water transport, nucleation, and ice crystal formation, the freezing behaviour of the cells can be changed. The underlying physical and biological elements determining the survival of cells at low temperatures during the cooling and warming processes have been examined in many research publications on cryopreservation. Bulk tissues, in contrast to single cell suspensions, experience different heat and mass transfer effects during cryopreservation. As a result, it is more challenging to achieve rapid cooling and warming rates as well as an even distribution of CPAs. For both current and upcoming therapeutic applications as well as basic research, cryopreserved cells or tissues have some benefits. Since cryopreserved cells and tissues are

always available, extensive quality testing can be done on them to evaluate whether they are suitable for transplantation without the need for fresh samples. With the use of CPAs and temperature control technology, the successful cryopreservation of cells and tissues has been gradually rising in recent years. [4]

The current method for tissue cryopreservation uses high CPA concentrations and rapid cooling to prevent ice formation. This process is known as vitrification or ice-free cryopreservation. Simple tissues have been vitrified successfully, but larger tissues have proven more difficult to vitrify since the cooling rate that can be applied drops as sample size increases. Larger tissues can be vitrified with vitrification solutions containing exceptionally high concentrations of CPAs because they enable the use of slower cooling rates3-5. Heart valves have been preserved using vitrification solution VS83, which combines the penetrating agents DMSO, formamide, and propylene glycol at a combined total volume fraction of 83%(v/v). However, excessive CPA concentrations are poisonous to the tissue's resident cells, causing cell death. While not a problem and perhaps even desired in the case of heart valves, many other applications demand that tissues' cells remain viable after thawing. Diffusion kinetics must be studied in order to logically construct techniques for CPA loading tissues for vitrification. [5]

Lazaro Spallanzani (1776) sought to preserve spermatozoa by cooling it in snow, which is when the first instance of semen cryopreservation was documented (Royere et al., 1996). With Polge's discovery of glycerol's cryoprotectant capabilities, science advanced significantly more after that (Polge et al., 1949). [24] Cryopreservation of human sperm is a beneficial treatment strategy to maintain fertility in a variety of potential disorders. Patients receiving chemotherapy, radiation, or other surgical procedures that could result in male reproductive failure must have their sperm cryopreserved. [11]

There are now three main methods for preserving organs: cryopreservation, continuous hypothermic perfusion, and cold storage. Although above-zero storage has several benefits for the preservation of organs over a short period of time, such as a few weeks or months, it is not suitable for this purpose. Continuous hypothermic perfusion (CHP), which simulates physiological blood flow, over time invariably leads to epithelial damage and metabolic alterations, ultimately impairing organ function. Because of this, the period required for organ preservation with CHP often lasts no longer than 5-7 days, which is considerably too short for long-term preservation. The successful cryopreservation of cells, sperm, embryos, and ovarian tissue has led to the belief that cryopreservation is the best method for the long-term preservation of organs. The Cryoprotectant, Cryopreservation, and its types, advancements, applications, and limitations were discussed in this article. [6]

3. Cryoprotective Agent:

Cryoprotective agents fall into one of two general categories: i) permeating DMSO, which could shield against radiation damage and certain biological damage incurred during cryopreservation ii) non-permeating cryoprotectants and Cryoprotectants that can pass through cell membranes, such as glycerol, DMSO, methanol, propanediol, and ethylene glycol. Cryoprotectants that are non-permeating do not pass-through cell membranes. For instance, dextran, sucrose, glucose, and skim milk. Cryoprotective drugs function by raising extracellular osmolality, encouraging dehydration during cooling, and binding leftover water to prevent the harmful consequences of ice formation (IIF). [20] In order to safeguard intracellular structures and biomolecules during cryopreservation, protective chemicals must be able to pass through the cellular membrane. Small, non-ionic molecules are often what penetrate cryoprotective compounds.

The most commonly used membrane permeable cryoprotective agents are dimethyl sulfoxide (DMSO) and glycerol.[12] The CPA, which is usually a fluid, reduces the freezing injury from the cryopreservation process CPAs Should be biologically acceptable, be able to penetrate the cells, and have low toxicity. Various CPAs have Been developed and used to reduce the amount of ice formed at any given temperature, depending on the cell type, cooling rate, and warming rate.[4]

Fig. 1.

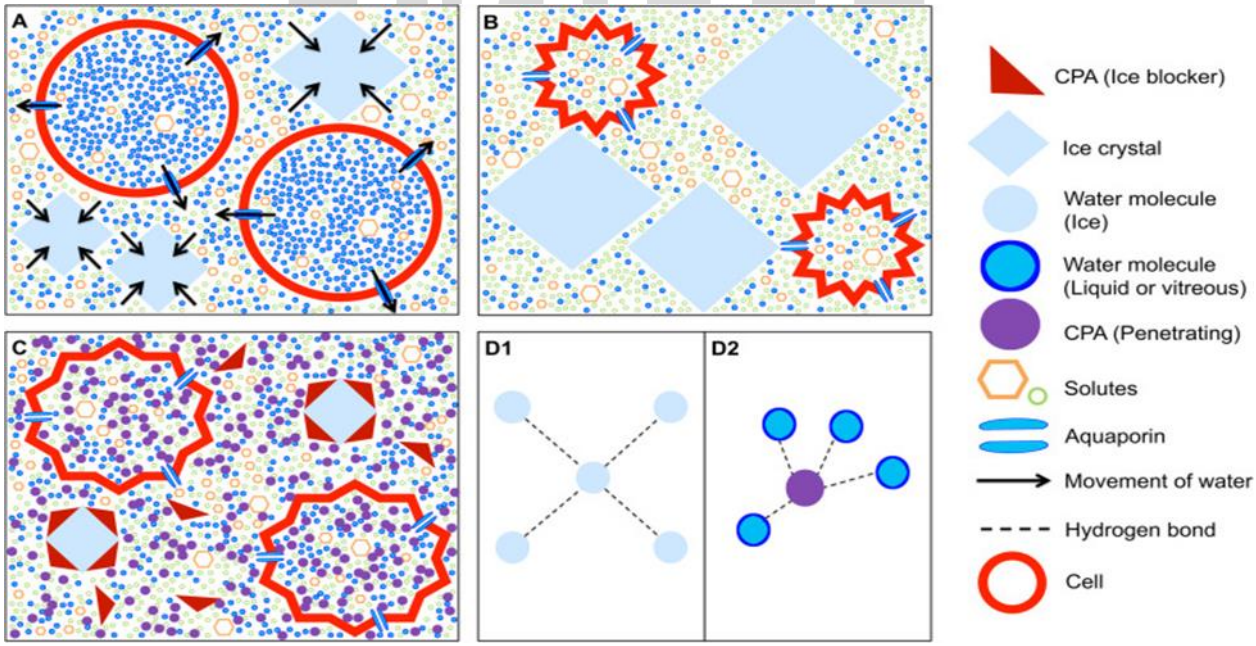

Fig. 1. The operation of cryoprotective agents (CPAs). An Ice first appears in the extracellular environment when a sample is chilled. Since ice keeps solutes out, the concentration of extracellular solutes rises as extracellular water is incorporated into the ice crystals. The cells are then osmotically drained of intracellular water. B The intracellular solute concentration rises and harms unprotected cells. The cell membrane is penetrated by CPAs that enhance intracellular solute concentration, preventing water loss and dilution of other intracellular solutes that, in high quantities, can harm cells. A substance called an ice blocker binds to ice crystals to stop them from developing or to nucleators to stop heterogeneous ice nucleation. D1, D2 Penetrating CPAs cause colligative interference, which lowers the freezing point of the solution by interfering with homogeneous ice nucleation. D1 Ice lattices made of water are regular. D2 A CPA molecule breaks down the hydrogen bonds that hold water molecules together.[9]

Some common cryoprotectant used in pharmaceutical preservation

Glycerol:

Glycerol was first shown to have a cryoprotective effect by Polge et al in 1949, and it remained the most effective addition until Lovelock and Bishop showed that DMSO also had a cryoprotective effect in 1959. [4] The simple polyol (sugar alcohol) molecule known as glycerol or glycerine is a clear, odourless liquid. With water molecules, glycerol creates hydrogen bonds, which gives it good osmotrophic characteristics. Unless and until the temperature is very low, such as 37.8°C, this circumstance makes it difficult to form ice crystals by a mixture (70% glycerol and 30% water). Glycerol is less harmful when present in high concentrations as compared to another cryoprotectant. [1]

Dimethyl sulfoxide:

DMSO was first synthesized in 1866 by Russian scientist Alexander Zaitsev and was widely used for cryopreservation of cultured mammalian cells due to its low cost and relatively low cytotoxicity. Like glycerol, DMSO acts by reducing the concentration of electrolytes in the residual thawed solution in and around a cell at any given temperature. However, reduced survival rate and induction of cell differentiation caused by DNA methylation and histone Alteration have been reported. These negative effects of DMSO in cryopreservation create some difficulties for its use in routine clinics. DMSO is basically an organosulfur derivative. The molecular formula is (CH3)2SO. This colourless solution can dissolve polar and non-polar compounds with typical properties; freezes within 18.5°C. This means that DMSO turns into solids below room temperature, and this property makes it more suitable for cryoprotectants.[4] DMSO can protect against biological damage suffered during cryopreservation and against some aspects of radiation damage. [22]

Polymers:

Another way for regulating cell locations is the encapsulation of CPAs inside a capsule during the resuspension of cells in an encapsulating substance. [1] non-diffusible synthetic polymers can offer the highest cryoprotections of the living cells inside among all encapsulating materials. For instance, polyvinyl alcohol, PEG, & hydroxyethyl starch all have a good tendency to reduce ice crystal size.[3]

2-Methyl-2, 4-pentanediol (MPD):

Although MPD is frequently employed as a precipitant, it can also be utilised as a cryoprotectant in protein crystallography. With both polar and non-polar solvents, it is broadly applicable. It may lead in protein precipitation.

Propylene glycol:

The fluid is frequently used in aircraft. RV or marine antifreeze are trade names for propylene glycol. It also possesses the antifreeze-like properties of an automotive.

Sucrose:

Sucrose is actually a naturally occurring carbohydrate; at low temperatures (45°C), it tends to give preserved cells the nutrients they need; and when combined with DMSO, it maintains good cryoprotective qualities. Extenders, co-cryoprotectants, and other generally used cryoprotectants are employed in cryopreservation. [1]

Cell Banker series:

A recently developed Cell Banker series (Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan) enables rapid cryopreservation of cells at -80°C and has been shown to achieve better survival rates after freezing and thawing.[4] The Cell Banker series of cryoprotective media contains 10% DMSO, a specific polymer, pH modifiers, glucose, bovine serum albumin. These combined cryopreservatives are used to preserve mammalian cells. This technique allows rapid cryopreservation of cells at -80°C and the denote cell survival rate after freezing and thawing was excellent.[3]

List of various cryopreservatives (Table No. 1):[1]

Sr. No.	Name of Cryoprotectant	Sr. No	Name of Cryoprotectant
1.	Acetate	21.	Glycerol
2.	Acetamide	22.	Glucose
3.	Ammonium acetate	23.	Proline
4.	Choline magnesium	24.	Propylene glycol
5.	Chloride sodium bromide	25.	Sodium chloride
6.	Diethyl glycol	26.	Sodium bromide
7.	Dimethylacetamide	27.	Sodium iodide
8.	Ethanol	28.	Triethylene glycol
9.	Erythritol	29.	Valine
10.	Formamide	30	Xylose

Storage

Ideally, cryopreserved cells and tissues should be stored below the glass transition temperature of the solution in which they are stored. This is usually accomplished by storage in liquid nitrogen or nitrogen vapour immediately above the liquid. Commercially available vacuum-insulated tanks are usually used as storage containers. Cell suspensions are usually stored in appropriately sized vials, but there are several specialized supports used for specific cell types, most notably for vitrification. For example, open straws, open straws, and cryoloops have been used for sperm vitrification and storage. There are several key aspects related to storage that must be considered to ensure successful cryopreservation. A critical issue is the potential risk of contamination, particularly when immersed in liquid nitrogen. When liquid nitrogen is produced, it generally has a very low bacterial count. However, contamination can occur during storage and distribution, and any part of the supply chain that is regularly heated, particularly transfer dewars, can introduce significant levels of microbial contamination. Contamination can also occur when filling Dewar flasks if non-sterile liquid nitrogen is sprayed directly onto the samples. This can be aggravated by liquid condensate collecting in the lines between freeze cycles. To alleviate this problem, technology has recently been developed that can provide sterile liquid nitrogen on demand, thereby avoiding contamination [NIGEL G; PERSONCOM.]. The risk of contamination is significantly lower with vapor phase storage. In contrast to liquid nitrogen, however, there can be very large temperature gradients within the vapor phase, which ensure a stable storage temperature. These gradients are exacerbated by the opening of storage containers for sample transfer, content testing, or other reasons. The relatively high temperatures experienced by samples stored in the vapor phase, combined with temperature cycling, can result in reduced viability.[8]

Utilization of cryoprotectant

During cryopreservation, almost 10% cryoprotectant was initially added in a single step. This causes the classic cryobiology shrink-swell reaction, in which cells first shrink due to osmosis and then swell as cryoprotectants enter the cell. Unless and until the volumes of cryoprotectants are equal between the intracellular and extracellular fluids of the swelling cells. Within 10 minutes after setting the concentration and volume, the cell or tissue is ready for freezing. The cooling process is Very slow, mostly less than 1°C/min. The addition of vitrification solutions during cryopreservation should not be 50% in one step due to the high response of osmatic contraction. The material to be vitrified is exposed to various cryoprotective solutions such as 1/8×, ¼×, ½×, 1× full concentration vitrification solution, especially for 20 minutes at each step. Rapid cooling and reheating occur during vitrification. During this process, cryoprotective solutions are perfused through the cells while the Blood flows into the blood vessels. After vitrification, the cryoprotectant can be removed by reversing the steps outlined above.[3]

Cryopreservation:

Origin of cryopreservation

Some of the earliest concepts of cryoprotection were developed by biologists in the 19th and early 20th centuries studying freezing, cold hardiness, and frost resistance in the environment, most commonly in plants. [13] Cryopreservation is the storage of biological material at low temperatures. It has been known since ancient times that biological material has a longer shelf life at low temperatures. In fact, archaeological finds indicate that as early as 2000 BC. Ice houses were used to store food throughout Mesopotamia. The preservative effect of cold was also an interesting topic for early 17th-century experimenters, notably Boyle, who commented on ice's ability to preserve human bodies and made several attempts to freeze and revive live animals, discovering species of frogs. And fish that could survive trapped in the ice. While Boyle could only speculate on the nature of cold, centuries past have shown that its preservative effect is by depriving biological systems of the thermal energy required for normal molecular movement and metabolism, which in turn, cellular processes and decomposition slows down. The ability to reliably generate the extremely low temperatures required for long-term preservation, typically below -100 °C, came with the development of cryogenic technologies in the early 20th century. Modern cryopreservation of living systems in the biotechnological sense dates back to the discovery of the first potent cryoprotective agents (CPAs), also known as
"cryoprotectants", in the 1940s. Lovelock in particular provided crucial early insights into the origins of cryolesion and the action of CPAs Mazur's later work pioneered the use of quantitative models to describe cellular changes during cooling and paved the way for theoretical approaches to the study of cryopreservation. The discipline of cryopreservation Is now well established as a practical means of preserving living cells and tissues, and has increasing applications in biology and medicine. As this report will highlight, cryopreservation now has the potential to bring great benefits to multiple areas of medicine by facilitating the storage of therapeutic cells, tissues and organs.[9]

Procedure

Cryopreservation is the process of preserving living cells and tissues for a long time at extremely low temperatures. There is a lot of variation in the cryobiological response and cryosurvival throughout the freezing and thawing cycle among various mammalian species depending on the cell types or specific cells. The following types of cryopreservation procedures can typically be categorised: a) gradual freezing Vitrification, in which the aqueous environment of the cell or tissue solidifies into a non-crystalline glassy phase; c) sub-zero non-freezing storage; and d) dry state preservation.Storage of mammalian cells in a dry state is generally not possible due to the difficulty of introducing trehalose disaccharide (glucose disaccharide, 342 Da) and amino acids (used as a preservative in plants) into the intracellular space.

The main steps in cryopreservation are as follows:

1) mixing CPA with cells or tissues before cooling,
2) cooling cells or tissues to a low temperature and storing them,
3) heating cells or tissues, and
4) 4) removal of CPAs from the cells or tissues after thawing.

The appropriate use of CPAs is therefore Important to improve the viability of the sample to be cryopreserved.[4]

Mechanism

After properly adding with the appropriate cryoprotectant, water reduces some of its melting point. The various anti-freezing cryoprotectants include propylene glycol and dimethyl sulfoxide (DMSO). For best results, a cryoprotectant concentration of typically 5–15% must be maintained during freezing and thawing of isolated cells. Cryoprotectant causes the formation of relatively large vesicles to press ahead more slowly when cells are frozen in liquid nitrogen. It also causes fewer Salt crystals to form, which protects cells from mechanical harm. Evidence suggests that cryoprotectants rarely form hydrates and that they are relatively less harmful to cells at high concentrations. Within 50 years, the extremely expensive usage of glycerol as cryoprotectants becomes a step toward the discovery of over a hundred more cryoprotectants. Because they penetrate inside of cells and maintain moisture during freeze drying, penetrating cryoprotectants, which have molecular masses of less than 100 Da, are now widely used in the pharmaceutical business. [3]

Fig. 2.

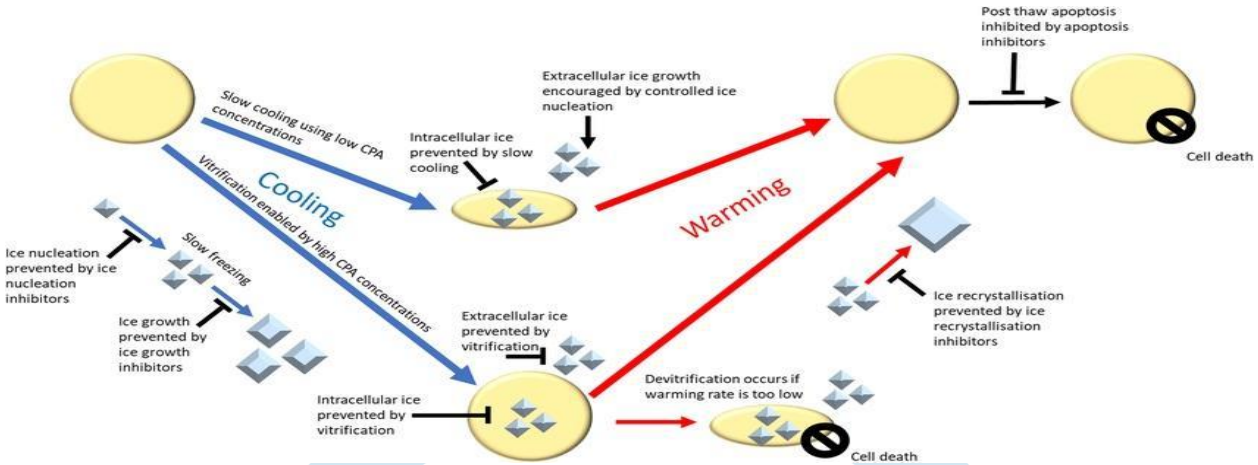

Fig.2 An overview of CPA and cryopreservation mechanisms. Cryopreservation uses slow cooling, where the sample is frozen at a controlled rate to allow water to flow out of the cell and prevent intracellular ice formation, or vitrification, where a high freezing rate and/or high CPA concentration prevent this ice formation in the sample. Ice growth can be further controlled with Ice Nucleation Inhibitors, Controlled Ice Nucleation, Ice Growth Inhibitors, or Ice Recrystallization Inhibitor Devitrification occurs when a vitrified sample is heated too slowly, causing ice to form. Apoptosis inhibitors can also be used to prevent cells from dying after cryopreservation due to stress-induced apoptosis. [9]

Type

Following are the types of Cryopreservation.

Isochoric cryopreservation:

Cryopreservation is largely based on an isobaric (constant pressure) process, where freezing occurs at a pressure of 1 ATM. However, it has its own disadvantages, in this method the intercellular ion concentration increases because the chemical integrity inside the cells changes during freezing, leading to cell damage. However, in the isochoric (constant volume) process, frizz's metabolic rate is constantly changing with every 10-degree drop in temperature. In this isochoric Process, storage at absolute zero temperature is possible and helps maintain cell integrity during slow freezing.

Isobaric cryopreservation

The isobaric cryopreservation technique deals with the preservation of cells at 1 ATM osphiric pressure. This procedure is common, but some limitation of cytocell damage makes it more uncommon.

Hyperbaric cryopreservation: By increasing the elevated pressure, the temperature is lowered below 0 degrees Celsius. This condition results in rapid freezing with intact biological tissue. Therefore, many tissues can be stored by this process, i.e.p. e.g. kidney (10,000 ATM), cells (200 ATM) and liver (70 ATM).[1]

Technique Used for Cryopreservation

Thawing Technique

During rapid tissue cooling, a large number of tiny ice crystals develop in the cells. As the temperature rises, the tiny ice crystals recrystallize. Tissues containing unstable ice crystals recrystallize when the temperature rises from -196 °C to -40 °C or -15 °C. If the temperature falls back to -196°C, the recrystallization stops, but the change that has occurred is not reversed. Any recrystallization Can lead to cell rupture. Studies have shown that tiny intracellular ice crystals are sometimes harmless and cells survive as long as they are relatively small. Let it survive Although rapid thawing is essential for tissue survival, it is impractical.[6]

Vitrification

Vitrification is a process of converting a supercooled liquid into an amorphous, glass-like solid that prevents the formation of ice crystals.[23] Organs are most venerable for frostbite-related damage; They are largely organized and less prone to secreting ice pocket formation compared to tissue suspensions. In order to functionalize organs after freezing, almost all small blood vessel parenchyma cells must survive in large numbers after freezing. Simple cryopreservation is not good enough to address this problem. A famous cryobiologist, Dr. Gregory Fahy, in 1984, proposed vitrification as an alternative to prevent tissue or organs from being damaged by freezing. In cryobiology, vitrification means rapid freezing or conversion to A-glass, loading large organs with a high concentration of cryoprotectant before cooling. After cooling, the organs would be preserved intact in the glass bag. By avoiding the mechanical hindrance caused by ice and maintaining the right ratios of salts and other molecules, vitrification is slowly becoming a clear process in cryopreservation.[1]

Cryopreservation of tissues and Organs

Kidneys
Because the kidneys tolerate ischemia and other extracorporeal treatments well, many researchers have used kidneys to study organ cryopreservation. Macklis et al. washed the kidneys with equilibration fluid to remove blood and then stored the kidney in a sealed perfusion container to cool the organ to 5°C-10°C. DMSO was mixed with perfusion at 100 mL/min to a final concentration of 10% to 15%. Under a pressure of 5.33 kPa (40 mmHg), the kidney was placed in a liquid silicon bath to which Solid CO2 was added at a cooling rate of 0.05-1 °C/min to freeze the organ at -79 °C. After cryopreservation for one week, microwave radiation (intermittent high-power irradiation at 10-s intervals) was used for thawing. As the core temperature rose above 0°C, the highly concentrated DMSO in the wells was eluted with serially diluted DMSO (gradually to zero concentration). After transplantation into the cervical region, half of the kidneys survived and the blood supply was maintained for about 1 month. No significant differences were found Between kidneys stored for 1 hour and 1 week, and histological examination showed that renal arteries, veins and ureters were well preserved.

Hearts Back in the early 1950s of the last centuries, studies on the cryopreservation of hearts concentrated mostly on the ex vivo storage of animal hearts. The currently employed method keeps cardiocytes in an inhibited state by perfusing cryoprotectants into cardiac arteries and cryopreserving at -15 to -80°C, or in liquid nitrogen. Two rabbit hearts were frozen to -21°C using 10% GL by Connaughton and Lewis et al., and one heart started beating again after being warmed up. Robertson et al demonstrated that the damage to the heart produced by freezing was gradual by perfusing hearts with 15% DMSO, cooling them to -12°C to -14°C, and storing the organs for 2 hours. Barner and co. Similar results were obtained by freezing murine hearts in 15%–20% DMSO and storing them for 70 minutes. This resulted in heart damage. According to Karow et al., the heart could retain some functions after being perfused with 15% DMSO (Mg2+) or 6% dextran and stored in a cryogenic environment at -20°C for 20 minutes, but after further time had passed, the function could not be restored. Additionally, their research demonstrated that Mg2+ had potent cardioprotective properties. Also, they stored rabbit atrial slices in a solution that contained 6% dextran and normal saline (2.l mol/LDMSO, 66 mmol/L MgCl2, 44 mmol/L MgSO4) and froze them to -20°C using acetone for cryopreservation. 93%, 86%-87%, and 80% of the hearts survived the various solutions, demonstrating that DMSO was less harmful.

Livers
The cryopreservation of livers is challenging, and no successful cryopreservation of livers has been achieved to yet. Only a few scientists have attempted to keep liver in extremely cold temperatures. Glycerol was injected into the liver by Moss et al., who then kept the organs at -20°C to -60°C for 1–14 days (s). The livers failed to function after being heterotopically transplanted into the pelvis, and all animals died 6 hours later. Wishnies et al. attempted to vitrify human liver slices for cryopreservation and discovered that the cellular enzyme activity in vitrified liver slices were well preserved and were unaffected by the length of storage. [6]

Cryopreservation of human Sperm
Techniques for sperm selection can be applied both before and after cryopreservation to greatly enhance sperm quality. The two methods for sperm selection that are most frequently employed are density gradient and swim-up. [19]
Human sperm can be kept in cryostorage for a considerable amount of time, so the process must be handled carefully and safely. A consent form that specifies the duration (often 5 years) and expense of storage must be signed. Additionally, the consent form must clearly state what will happen to the gametes when the guy passes away. Before sperm is cryopreserved, men should be tested for infectious disorders such hepatitis A, B, C, and HIV. In liquid nitrogen, viruses can potentially spread and behave, as has been described (Tedder et al., 1995). Hepatitis B virus transmission through a polluted liquid nitrogen tank was documented by Tedder et al. in 1995.Because of this, a 6-month quarantine period must be followed before final storage in a tank with other samples. The safety of the storage depends on the kind of straws used as well as the kind and manner of filling. The work by Benifla et al. (2000), which showed that HIV can seep into other freezing straws, underlines the significance of material selection when freezing human sperm. When PVC straws are irradiated to sterilise them, they can no longer be used since they are poisonous. When cooled or heated up, glass vials and PVC straws have the potential to crack or explode, causing leaks into the liquid nitrogen tanks. Ionomeric resin straws are flexible and heat-sealable, guaranteeing a secure, leak-proof solution. Since standard marker labels are temporary, the labelling system on the straws should be durable to low temperatures and preferably located inside the straw. Regular monitoring of the sample storage is necessary, including human or automatic measurement of the liquid nitrogen level, regular cleaning, and annual inventory of the straws and vials present in the container. To ensure the secure and controlled preservation of human sperm, these stringent processes are required. [10] **For** these species, a cryoprotectant with a concentration range of 5 to 30% was utilised for sperm cryopreservation. The cryopreserved aquatic invertebrate sperm has been observed to have motility levels ranging from 5% to 95%. [15]

Recent Advances in Cryopreservation & application
1.Cryopreservation of Red Blood Cells and Platelet
One popular field of application for low-temperature biology is blood cells. Red blood cells (RBCs) (erythrocytes), platelets (thrombocytes), mononuclear cells (lymphocytes, monocytes), and hematopoietic progenitor cells have all been cryopreserved using various techniques.

2.Cryopreservation of Mammalian Oocytes
There are two ways to cryopreserve mammalian oocytes, which are described. One method calls for controlled rate cooling equipment to produce a slow chilling rate and uses a relatively low concentration of the cryoprotectant propanediol combined with

sucrose. From cryopreserved human oocytes, live births have been achieved. The second approach is detailed and uses a low concentration of polyethylene glycol along with a high concentration of the cryoprotectant dimethyl sulfoxide. This approach uses standard drinking straws to cool, which eliminates the need for specialised equipment but necessitates technical proficiency to quickly manoeuvre the oocytes in the highly concentrated cryoprotectant Solutions. Live births from vitrified murine oocytes have been documented. [2]

3.In medical science:

Since ancient times, low temperatures have been employed in healing and to keep food from spoiling. The transit of human organs and the long-term storage of biological specimens, either for the future or just as a record of biodiversity, are being used in fertility treatment.

4.Cryopreservation of sperm:

Human sperm cryopreservation, also known as sperm-banking, is a practise that preserves sperm cells and is now commonly used to store donor and partner spermatozoa before assisted reproductive treatments, to preserve spermatozoa before therapy for cancer, vasectomy, or surgical infertility treatments, and to ensure the recovery of a small number of spermatozoa in several male factor infertility cases. The longest period of successful preservation for human sperm is 21 years.

5.Preservation of micro-biology culture:

Although cell division and metabolism are partially delayed when bacteria and fungi are stored in the refrigerator for a short period of time, this method is not ideal for long-term storage, culture genetic or phenotypic preservation, as cell divisions might result in mutations. [20]

6.Fertility preservation

The preservation of endangered animal species and the restoration of animal and human fertility are both possible through cryopreservation. [21] The use of it in paediatric dentistry It is recognised that exfoliated deciduous teeth can serve as a source of stem cells for a variety of dental tissues. Deciduous teeth that have been scraped off or extracted can be cryopreserved, and the stem cells can be separated and applied in various ways. [25]

Limitations of Cryopreservation.

1.Owing to the fact that the cryopreservation technique has many applications in both basic and clinical research, there are still some limitations. At low temperatures, such as -196 °C (i.e., in liquid nitrogen), cells metabolise absolutely nothing, which inevitably has negative repercussions. [4]

2.It is important for a person to have solid technical and theoretical knowledge of living plant cells as well as the cryopreservation process. [18]

3. The thermal transport limitations associated with the extension of cryopreservation methods to tissue dimensions are broadly analogous to the mass transport problems encountered in tissue cryopreservation.[7]

4.Cryopreservation of tissues/organs is much more complicated and difficult than cryopreservation of single cells. To date, cryopreservation (either in the frozen or vitrified state) of large tissues and organs has generally been unsuccessful. [17]

Conclusion

Cryoprotectant is used for storage and preservation of living organisms, Plant tissues, Yeasts, bacteria, fungi, animal tissues. Etc. There are number of advantages of cryoprotectant in the cryopreservation in the transplantation of organs and tissues (heart, liver, Kidney etc.). Low temperature technology is used for preservation of tissues and organs. Tissues and Organs Preservation are difficult than the preservation of cell. Many Resechars tried cryopreservation technique for organs trasplatation and on it done several experiment on different types of animals but the success rate of survival is very low but Modern emerging cryobank is a promising approach toward Organ transplantation.Organ's transplantation is a most effective way for treatment of end stage organ failure. Nowadays, the donor organs used for clinical transplantation are all preserved at above-zero temperatures. These preservation methods are secure and simple but the storage time is less (4-12 h). Most of the researchers tried to extend the organ storage time by improving protectant and HLA matching to raise the use of stored organs and prolong the long-term survival of organs.

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