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Cryopreservation of Biological Membrane- A Review Paper

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ABSTRACT:

In the present period, preservation and its effects on biological materials are widely used in a variety of sectors, including biomedicine, organ transplantation, regenerative medicine, and drug development. Because donated organs cannot be kept alive until a suitable receiver is discovered, the scarcity of organs leads to a serious medical issue. Here, we go over the most recent cryopreservation techniques and uses. The key difficulties will be discussed, including scaling up to huge volumes and complicated tissue, avoiding ice formation, reducing cryoprotectant toxicity, and outlining future prospects for the area.

Keywords: cryopreservation, vitrification, thawing, cryoprotective agents,

INTRODUCTION:

The goal of cryopreservation is to preserve the vitality and stability of biological tissue, fluids, and medicinal compounds by storing them at extremely low temperatures. It has long been understood that biological material can be kept longer at low temperatures. Since Boyle commented on the ability of ice to preserve human bodies and made several attempts to freeze and examine live animals, he discovered species of frog and fish that could survive encasing in ice. Supporting archaeological findings show that icehouses were used to store food in Mesopotamia as early as 2000 BC.

At low temperatures, biological metabolism and chemical interaction in living cells are drastically reduced, a characteristic that may enable long-term cell preservation. The problem is that freezing is fatal to the majority of living things because it causes cell damage and restrictions due to the special characteristics of water molecules that are both within and outside of cells when ice crystals form. [1]

The purpose of cryopreservation is to preserve the integrity of cells by carefully preserving biological subjects in a condition of suspended animation at cryogenic temperatures for an extended period of time.[2]

VITRIFICATION

When an aqueous solid is created without crystalline ice through ultrarapid cooling at a temperature below -130°C, this process is known as vitrification. The vitrification process causes the solution to become glassy, which means that chemical and physical processes in the vitrified materials are severely constrained, effectively stopping biological time without the creation of ice crystals. In contrast to gradual equilibrium freezing, this process is also referred to as non-equilibrium vitrification.[3]

Ice does not have enough time to create a crystalline structure during vitrification, instead transitioning to an amorphous state that is a snapshot of the liquid state. Cells must be dehydrated before being exposed to subzero temperatures by being exposed to high concentrations of cryoprotectants in order for vitrification to be successful.

Live cells and tissues are often vitrified by being submerged immediately in liquid nitrogen. Cryoprotectants or their mixes are applied to the cells first to enhance the viscosity of the fluid inside and outside the cells by removing some of the water. [4]

THAWING

The procedure of warming biological samples in order to regulate cooling and prevent crystallization-related cell damage.

A culture that has been frozen finds the thawing process distressing. A significant percentage of the cells will survive the surgery if appropriate technique and haste are used. For the best outcomes while freezing and thawing, we advise that you closely adhere to the instructions included with your cells and other reagents. [5]

- Rapidly defrost frozen cells in a 37°C water bath (1 minute).

- Before incubating the thawed cells, dilute them gradually with pre-warmed growth media.
- High density plate-thawed cells to enhance recovery

Wear personal protective equipment, such as a face mask or goggles, at all times, and practise correct aseptic technique while working in a laminar flow hood. When thawed, cryovials stored in liquid phase pose an explosion risk.

- DMSO, which is known to make it easier for organic molecules to enter tissues, is present in some freezing media. Utilize tools when manipulating solutions containing DMSO.

CRYOPROTECTANTS

In order to lessen or prevent damage to the structures and characteristics of membrane lipids, proteins, and nucleic acids, cryoprotectants are utilised to safeguard the integrity of cell membranes and the intracellular environment. Depending on the permeability of their cell membranes, they are split into two groups.

Nonpermeable cryoprotectants, including polyvinyl pyrrolidone, polyethylene oxide, and sugars (such as trehalose and sucrose), starches (such as hydroxyethyl starch), cannot enter cells and remain extracellular during cryopreservation. They are typically used to safeguard cells during quick cooling. Due to the extracellular presence of cryoprotectants, the extracellular solution will undergo vitrification after cooling has begun. As a result, as extracellular viscosity rises, the water transport between inside and outside of cells will be impeded. [7]

A drop in cell volume occurs when a slow freezing rate is used because it gives intracellular water enough time to leave cells under the influence of osmotic pressure. In order to solve this issue, permeable cryoprotectants that can enter cells, such as dimethyl sulfoxide (DMSO), glycerol, ethylene glycol, and propylene glycol, can be used to stimulate the vitrification of the intracellular environment prior to the formation of ice crystals and thereby prevent an excessive loss of cell volume. [8]

More cryoprotectants made from natural ingredients have recently been created. Bacillus subtilis SB-MYP-1 cell damage caused by freeze-drying stress has been shown to be prevented by a new soybean flour product that contains protein (34.20 g/100 g), carbohydrate (36.00 g/100 g), and fat (21.10 g/100 g). A novel cryoprotectant for bovine spermatozoa has been developed using Rhodiola sachalinensis saccharide, which is derived from the rhizome of Herba Rhodiolae. L-carnitine, a naturally occurring zwitterionic molecule, has also been shown to significantly increase the survival rate of ornamental cichlid fish following exposure to a cold shock9. Cryopreservation of cells, embryos, and organs has also made use of antifreeze proteins from marine creatures.

Cryoprotective agents	**Membrane permeability**	**Possible toxicity**	**Applied in cryopreservation**
Cell Banker series	Yes	Unknown but less than that of DMSO	Adipose tissue-derived stem cells Amniotic fluid Bone marrow Mammalian cells Synovium
Dimethylsulfoxide (DMSO)	Yes	Reduction in heart rate Toxicity to cell membrane	Adipocyte tissue Amniotic fluid and umbilical cord Bone marrow Dental pulp Embryo (combined with EG or propylene glycol) Embryonic stem cells (alone or combined with EG) Hepatocytes Microorganisms Oocyte (combined with EG) Platelet Teeth Testicular cell/tissue
Ethylene glycol (EG)	Yes	Gastrointestinal irritation Pulmonary edema Lung inflammation	Amniotic fluid Dental pulp
Glycerol	Yes	Renal failure	Amniotic fluid Microorganisms Red blood cell Spermatozoa Teeth

Trehalose	No	Relatively less toxic	Adipose-derived stem cells (combined with vitrification) Embryo (combined with vitrification) Ovarian tissue (combined with vitrification) Red blood cell Spermatozoa Stem cells (combined with propylene glycol)
Propylene glycol (1,2-propanediol)	Yes	Impairment in the developmental potential of mouse zygotes	Embryo Hepatocyte

CONCLUSION

With the right methods and techniques chosen for a particular tissue, ideal cryopreservation can be accomplished. The best cryopreservation strategy for each type of tissue can be determined by experiments with different types of tissue. These procedures' synthesis enables the selection of parameters appropriate for the cryopreservation of composite organs, enabling the development of the perfect mixture of cryoprotectors for each organ and its constituent tissues and cells.

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