Cryopreservation

•This process preserves organelles, cells, tissues, or any other biological constructs by cooling the samples to very low temperatures (typically -80 °C using solid CO2 or −196 °C using liquid Nitrogen).

•It converses water present in the cells from liquid to solid-state.

•The cell water requires a much lower temperature to freeze (up to -68°C) due to the presence of salts and organic molecules in the cells, in comparison to the freezing point of pure water (around 0°C).

- Two commonly used cryoprotective agents(CPA) are dimethyl sulfoxide (DMSO) and glycerol.
- **Glycerol**:- used for cryoprotection of RBC.
- **DMSO**:- used for protection of most other cells and tissues.
- A sugar called **trehalose** is capable of surviving extreme dehydration, so used for the preservation of **sperm, stem cells, and blood cells.**
- First successful cryopreservation of fish sperm was reported in the 1950s.

- **Types:**
- (1) slow freezing
- (2) vitrification (solidification of the aqueous part of the cell or tissue into a noncrystalline glassy phase)
- (3) subzero nonfreezing storage
- (4) preservation in the dry state.

Cryopreservation Steps

1.Harvesting or Selection of material–

volume, density, pH, and morphology should be checked

2. Addition of cryo-protectant –

- glycerol, salts, sugars, and glycol is added to the samples
- It reduces the freezing point of the medium and allows a slower cooling rate, to reduce the risk of crystallization.

3. Freezing –

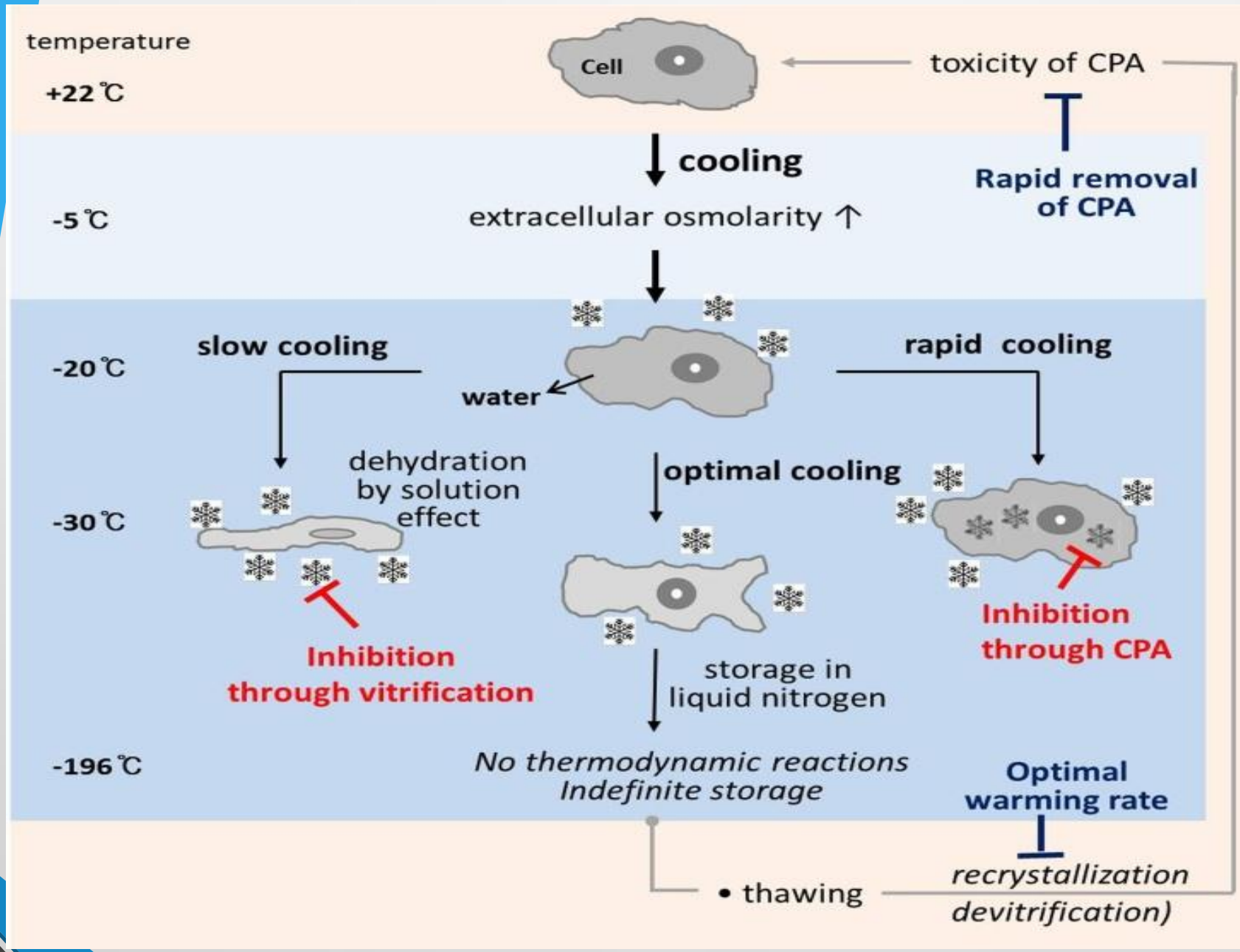
Different methods are applied by exposing the cells to the warm solutions of cryoprotective agents.

4. Storage in liquid nitrogen–

- samples are stored in -80°C for at least 5 to 24 hours.
- Then transferred to the storage vessels.

5. Thawing-

It controls the rate of cooling to prevent cellular damage caused by crystallization.


temperature
+22 ℃
Cell
toxicity of CPA
Rapid removal of CPA
cooling
-5 ℃
extracellular osmolarity ↑
slow cooling
rapid cooling
-20 ℃
water
dehydration by solution effect
optimal cooling
-30 ℃
Inhibition through vitrification
Inhibition through CPA
storage in liquid nitrogen
-196 ℃
No thermodynamic reactions
Indefinite storage
Optimal warming rate
• thawing
recrystallization
devitrification)

Cryoinjury

- Caused by extra- or intracellularly concentrated solutes and intracellular ice formation.

CPAs

- The CPA,:- usually **fluid** that reduces the freezing injury during the cryopreservation process.
- **It reduces** the amount of ice formed irrespective of temperature, cell type, cooling rate, and warming rate.
- Divided into two categories:

(1) **cell membrane-permeating** cryoprotectants”:- dimethyl sulfoxide (DMSO), glycerol,

(2) **nonmembrane-permeating** cryoprotectants:- 2-methyl-2,4-pentanediol and polyvinyl pyrrolidone, hydroxyethyl starch, and various sugars.

Limitations for Cryopreservation:

- Proper technical and theoretical knowledge s required.
- Can cause genetic drift and can alter cellular activity and structure.
- Higher concentrations of CPAs can damage cells like DMSO may alter chromosome stability, and lead to a risk of tumor formation.

Uses:- in cancer treatments

- freeze and store hematopoietic stem cells, (found in the bone marrow and peripheral blood).
- Hematopoietic stem cells are collected from a patient’s bone marrow prior to high-dose chemotherapy treatment since it is toxic to the bone marrow.
- After treatment, the patient’s cryopreserved cells are thawed and infused back into the body.

- Also used to freeze and store human sperm and embryos that are generated by in vitro fertilization (IVF).
- These frozen embryos are used for late pregnancy.
- But it is associated with a significant increase in the risk of cancer among children born from such embryos.

Other applications of Cryopreservation:-

- widely used in cryosurgery, molecular biology, ecology, food science, plant physiology, and in many medical applications. Other applications are:

1.Seed Bank.

2.Gene Bank.

3.Blood transfusion.

4.In vitro fertilization.

5.Organ transplantation.

6.Artificial insemination.

7.Storage of rare germplasm.

8.Freezing of cell cultures.

Benefits of Cryopreservation

- Fertility treatments.
- Minimal space and labor required.
- Safety from genetic contamination.
- Preserve Biological samples for a longer period of time.
- Protects samples from disease and microbial contamination.
- Prevents genetic drift by cryopreservation of gametes, embryos, etc.

Risks associated with cryopreservation

- Cellular damage occurs during the freezing stage, because of :-

  1)solution effects,

  2) extracellular ice formation,

  3)dehydration

  4)intracellular ice formation.

- These effects can be reduced by cryoprotectants.
- Once the frozen stage is reached, it is safe from further damage.

Solution effects

High concentrations of some solutes can be very damaging.

Extracellular ice formation:-

When tissues are cooled slowly, water migrates out of cells, and ice forms in the extracellular space.

- Too much extracellular ice can cause mechanical damage to the cell membrane due to crushing.

Dehydration

- The migration of water can also cause cellular dehydration, which directly causes Cellular damage.

Intracellular ice formation

- Some organisms and tissues can tolerate some extracellular ice, but intracellular ice is almost fatal to cells.

Prevention of risks

- intracellular freezing is lethal, so can be avoided by **slow cooling** to permit sufficient water to leave the cell during the freezing of the extracellular fluid.

- An optimum cooling rate of 1°C/min is maintained for many cells after treating them with glycerol or DMSO.