Cryopreservation and Thawing of Mammalian Cells: Freezing and Storing Cells for Long-Term Use

Introduction

If you've worked in cell culture or are interested in cell biology, you've probably come across the terms "cryopreservation" and "thawing" in research. We're going to break down these processes and understand why they are vital in maintaining the viability and integrity of mammalian cells.

Mammalian cells are delicate and can be affected by a variety of environmental factors, making it important to preserve them for future use in experiments. Cryopreservation allows us to freeze and store cells for long-term use, while proper thawing techniques ensure the cells retain their functionality and vitality.

1. What is Cryopreservation?

Cryopreservation is the process of freezing biological samples, such as mammalian cells, to sub-zero temperatures for long-term storage. This method preserves the cells by halting all metabolic and biological processes, including cell division, effectively putting the cells in a state of suspended animation.

But why is cryopreservation so crucial?

- **Preserving cell lines**: Cells, especially rare or genetically modified ones, are invaluable. Freezing allows researchers to store these cells for future experiments.
- **Reproducibility in experiments**: Cryopreservation ensures that you can use the same batch of cells in multiple experiments, eliminating the variation that might arise from starting cultures from scratch each time.
- **Resource efficiency**: Cryopreserving cells helps avoid the need for constant propagation, saving time and resources.

2. The Cryopreservation Process

Let's break down the key steps involved in cryopreserving mammalian cells.

Step 1: Preparing the Cells

Before you freeze the cells, there are several critical preparation steps to ensure their health is maintained:

- **Cell harvesting**: First, you need to collect the cells you plan to cryopreserve. This is typically done by trypsinizing adherent cells or harvesting suspension cells.

- **Centrifugation**: After harvesting, the cells are typically centrifuged to remove excess media and concentrated into a small volume of culture medium.
- **Cryoprotectant addition**: Cryoprotectants are essential for protecting cells from freezing damage. The most commonly used cryoprotectant for mammalian cells is **dimethyl sulfoxide (DMSO)**, but others, such as **glycerol**, are also used depending on the cell type.

Cryoprotectants work by preventing the formation of large ice crystals inside cells. These ice crystals can puncture and rupture cell membranes. Cryoprotectants lower the freezing point of water inside the cells and reduce the formation of harmful ice.

Step 2: Freezing the Cells

Once the cells are prepared and suspended in cryoprotectant, the next step is to freeze them. It is important to freeze cells gradually to avoid damaging them.

- **Controlled-rate freezing**: The freezing process should be slow to prevent the formation of large ice crystals. Typically, cells are cooled at a rate of about **1°C per minute**. This can be done using a **controlled-rate freezer** or even a simple alcohol-based freezing container.
- **Optimal freezing temperature**: The temperature of the freezing process should drop to approximately **-80°C** before the cells are transferred to liquid nitrogen storage. Liquid nitrogen, which has a temperature of **-196°C**, is used for long-term storage because it is cold enough to preserve biological material indefinitely without causing damage.
- **Storage in liquid nitrogen**: Once the cells are frozen, they are stored in cryovials and placed in liquid nitrogen tanks, where they can remain stable for months or even years.

3. Why Cryopreservation Works

To understand why cryopreservation is effective, let's look at the science behind it. At low temperatures, the metabolic activities of cells slow down or stop entirely. The cryoprotectants help minimize the formation of ice crystals inside the cells, which is critical because these ice crystals can puncture cell membranes and cause irreparable damage.

- **Vitrification**: At sufficiently low temperatures, the cryoprotectants also help the cells enter a **vitrified state**, meaning they form a glass-like structure instead of crystalline ice. This is ideal for cell survival.

The key to success lies in balancing the **cooling rate**, the **cryoprotectant concentration**, and ensuring the proper **storage conditions** to preserve the cells' viability and functionality.

4. Thawing Cells – Bringing Them Back to Life

Now, let's shift gears and talk about the second critical part of cryopreservation: **thawing**. Once cells are frozen and stored, the time will come to use them. The process of thawing is just as crucial as freezing.

1. Quick Thawing: The Key to Cell Viability

When it's time to thaw the cells, the process must be rapid. This is because thawing cells slowly can lead to the formation of ice crystals again, which could damage the cells.

- **Water bath method**: Typically, cryovials are placed directly into a **37°C water bath** for rapid thawing. It's essential to ensure that the vial is not submerged fully in water to avoid contamination. Gently swirling the vial in the bath will ensure uniform thawing.
- **Thawing time**: The cells should only be in the water bath long enough to fully thaw (usually around 1-2 minutes). It's essential to monitor this closely, as prolonged thawing can lead to the breakdown of cell membranes.

2. Post-Thaw Recovery: Diluting the Cryoprotectant

Once the cells are thawed, the cryoprotectant must be removed because it can be toxic to cells if left in contact for too long.

- **Dilution**: Cells are usually transferred into a fresh culture medium to dilute out the cryoprotectant. This process helps to protect the cells from the toxic effects of the cryoprotectant, which is used in high concentrations during freezing but should be washed away post-thaw.
- **Gentle handling**: It's crucial to handle the cells gently during the thawing process, as they can be fragile right after thawing. Avoid harsh pipetting or centrifugation that could damage the cells.

3. Incubation and Monitoring

Once thawed, the cells are placed into culture dishes and incubated at optimal conditions. Monitoring cell health and viability in the first 24 to 48 hours is important to ensure successful recovery.

- **Cell viability assays**: Using assays like **trypan blue exclusion** or **live/dead staining** can help you assess how many cells survived the freezing and thawing process. Ideally, over 70-80% of cells should be viable for them to be considered healthy and ready for use in experiments.

5. Challenges in Cryopreservation and Thawing

While cryopreservation and thawing are highly effective, there are challenges:

- **Cell death**: Not all cells survive the freezing and thawing process. For example, some cell types, like primary cells or some stem cells, are more sensitive to cryopreservation.
- **Cryoprotectant toxicity**: High concentrations of cryoprotectants can be toxic to cells, so balancing the concentration and exposure time is critical.
- **Contamination risk**: When thawing cells, contamination can occur if sterile techniques are not followed. Always work in a sterile environment, especially when transferring cells after thawing.

To mitigate these challenges, it's important to optimize the cryopreservation protocol for the specific cell type you are working with, as different cells require different conditions for optimal survival.

6. Best Practices for Cryopreservation and Thawing

- **Optimize the freezing rate**: Use a controlled-rate freezer or a controlled environment for slow freezing.
- **Use the right cryoprotectant**: For mammalian cells, DMSO is commonly used. However, for some cell types, other cryoprotectants may be required.
- **Monitor thawing carefully**: Use a 37°C water bath for rapid thawing and ensure cells are transferred to fresh medium immediately afterward to dilute out the cryoprotectant.
- **Handle cells gently**: Both during freezing and thawing, be sure to avoid any harsh physical handling of cells to prevent mechanical damage.
- **Assess viability**: Always check the viability of your cells after thawing to ensure they are healthy and ready for use in experiments.

Conclusion

In conclusion, **cryopreservation** is a powerful technique that allows for the long-term storage and preservation of mammalian cells. By carefully following the steps for freezing and thawing, you can ensure that cells maintain their viability and function, allowing them to be used in future experiments without compromising your results. Whether you are storing cell lines for research or creating a cell bank for clinical applications, mastering cryopreservation and thawing is essential for ensuring reproducible and reliable results.