Cell Handling and Maintenance – Proficiency in Culturing, Passaging, and Maintaining Mammalian Cells

Introduction:

As researchers working with mammalian cells, it is crucial to have an in-depth understanding of how to handle and maintain them properly. The success of your experiments often depends on how well your cells are cultured and maintained. This lecture will guide you through essential techniques, best practices, and tips to become proficient in handling these cells.

1. Understanding Mammalian Cells:

Let's briefly understand the two main types of mammalian cell cultures:

- **Adherent Cells:**
  - These cells **adhere to the surface** of the culture vessel (e.g., flasks, dishes, or plates).
  - Common examples include fibroblasts, epithelial cells, and various cancer cell lines.
  - **Culture environment**: Typically require a surface-coated vessel and a medium that supports attachment.
- **Suspension Cells:**
  - These cells **float freely** in the culture medium.
  - They do not require a surface for attachment, making them easier to handle in larger volumes.
  - Common examples include hematopoietic cells, lymphocytes, and some transformed cell lines.
  - **Culture environment**: Cultured in suspension media in flasks or bioreactors.

Both types of cells require different handling, but the basic principles of good culture practice apply to both.

2. Culturing Mammalian Cells:

Culturing mammalian cells involves creating a suitable environment for cell growth. This means providing the right temperature, pH, nutrients, and space for cells to grow and divide.

A. Preparing the Culture Medium:

- **Basic Components**: A typical mammalian culture medium contains amino acids, vitamins, salts, glucose, and serum (e.g., fetal bovine serum or FBS).

- **Serum-free Medium**: Some applications require serum-free mediums, but these can be more complex as they often include growth factors and other supplements.
- **pH and Osmolarity**: Check that the medium is properly buffered and that the osmolarity matches that of mammalian cells (~300 mOsm/L).

B. Incubator Settings:

- **Temperature**: 37°C is the standard for most mammalian cells, but specialized cells may require different conditions (e.g., 33°C for certain hybridomas).
- **CO_2**: A 5% CO_2 incubator ensures that the pH of the culture medium remains stable, mimicking the physiological environment of mammalian cells.

C. Sterility:

- **Aseptic Technique**: This is crucial for preventing contamination. Always work in a biosafety cabinet (laminar flow hood) and wear gloves, a lab coat, and, if needed, a face mask. Disinfect all surfaces with 70% ethanol.
- **Handling of Reagents**: Always use sterile techniques when handling media, pipettes, and cell suspensions.

3. Passaging Mammalian Cells:

Passaging, or subculturing, is the process of transferring cells from an old culture vessel to a fresh one to allow continued growth.

A. Adherent Cells:

1. **Check Confluence**: Before passaging, check the confluence of the culture. For most cell lines, around 70-80% confluence is ideal.
2. **Remove Medium**: Aspirate the old culture medium from the flask or plate.
3. **Wash with PBS**: Gently rinse the cells with phosphate-buffered saline (PBS) to remove any residual medium or serum.
4. **Detachment**: For adherent cells, use a **trypsin-EDTA solution** (trypsin is an enzyme that breaks down the proteins holding the cells to the surface). Add just enough solution to cover the cells and incubate for a few minutes at 37°C.
5. **Neutralize Trypsin**: Add fresh culture medium containing serum to neutralize the trypsin action.
6. **Resuspension**: Gently pipette to dislodge the cells and resuspend them in fresh medium.
7. **Transfer to New Flask**: Seed the cells at the desired density into a new culture vessel.

B. Suspension Cells:

1. **Check Cell Density**: For suspension cells, ensure the cell density is within the optimal range for growth (usually 1–10 million cells per mL).
2. **Transfer and Dilute**: Suspension cells are easier to passage because they don't require detachment. Simply transfer the cells to a new culture vessel and dilute with fresh medium.
3. **Mixing**: Gently mix the cells to ensure a uniform suspension.

Note: When passaging, it's important to be gentle to minimize stress and damage to cells. Harsh pipetting or over-trypsinization can lead to poor recovery rates.

4. Maintaining Mammalian Cells:

To keep cells healthy and proliferating, maintenance goes beyond just feeding and passaging them.

A. Regular Medium Changes:

- For adherent cells, change the medium **every 2-3 days**. For suspension cells, this may be every day depending on the cell density.
- Always check the pH and appearance of the medium. If it turns yellow or cloudy, it's time for a change.

B. Temperature and Incubator Conditions:

- Ensure that your incubator is functioning properly and maintains a stable environment. Fluctuations in temperature or CO_2 concentration can stress cells and affect their growth.

C. Monitoring Growth:

- **Microscopy**: Regularly examine cells under a microscope to check for morphology, confluence, and overall health.
- **Cell Counting**: For suspension cells, count the cells using a hemocytometer or automated cell counter to determine growth rates and whether passaging is required.

D. Cryopreservation:

- **Freezing**: Mammalian cells can be preserved by freezing them in **cryopreservation medium** (typically containing 10% DMSO and 90% serum). Freeze at -80°C first before transferring to liquid nitrogen for long-term storage.
- **Thawing**: Rapidly thaw frozen cells by placing the vial in a 37°C water bath, and then carefully resuspend them in fresh medium.

5. Troubleshooting Common Problems:

Despite best practices, sometimes problems can arise when culturing mammalian cells. Here are a few common issues and solutions:

A. Contamination:

- **Bacterial/Fungal**: Contamination is a significant risk, especially with serum-based media. Regularly inspect cultures under the microscope for signs of contamination (e.g., cloudy media, irregular growth patterns).
- **Cross-Contamination**: Always label and keep cell lines separate to avoid mixing different cell types.

B. Low Cell Viability:

- **Passage Too Early/Too Late**: Passaging too early (before cells are fully attached or grown) or too late (causing overcrowding) can lead to stress and reduced viability.
- **Improper Medium**: Ensure the medium is appropriate for the specific cell type.

C. Poor Growth:

- **Incorrect Culture Conditions**: Double-check the incubator settings, and ensure that cells are not exposed to temperature fluctuations or CO_2 imbalances.
- **Over-confluency**: Avoid allowing adherent cells to reach too high a confluence. Overcrowded cells may experience nutrient depletion and oxygen shortage.

6. Conclusion:

Mastering the techniques of **culturing, passaging, and maintaining mammalian cells** is essential for any cell-based experiment. The key takeaways are:

- Regular monitoring and care of your cells will ensure their health and reproducibility in experiments.
- Always work aseptically to prevent contamination.
- Understand the specific requirements of the cell lines you are working with, whether they are adherent or in suspension.
- Proper passaging and medium maintenance will keep cells at optimal growth conditions.

By following these techniques and guidelines, you'll set yourself up for success in your cell culture work.