Date: 13 November, 2020

POST GRADUATE (P. G.) COURSE, MONSOON SEMESTER, 2020

VMC 609: TECHNIQUES IN MICROBIOLOGY AND IMMUNOLOGY

Topic: Cryopreservation and reconstitution of preserved cell lines

Dr Manoj Kumar

Assistant Professor, Department of Veterinary Microbiology, Bihar Veterinary College, Patna.

INTRODUCTION

- Cryopreservation: Storage of live material at temperatures so low, that all biological processes are suspended (-196°C) and material does not decompose.
    - Cryopreservation allows virtually indefinite storage of biological material without deterioration over a time scale of at least several thousands of years.
    - Techniques are available for the preservation of microorganisms, isolated tissue cells, small multicellular organisms, and even more complex organisms such as embryos.

CELL LINES

- Finite life, senesce after approximately thirty cycles of division
- Usually diploid and maintain some degree of differentiation.
- It is essential to establish a system to maintain such lines for long periods

ADVANTAGES OF FREEZING CELLS

- Cryopreservation minimizes:
    - genetic change
    - Senescence leading to extinction of cell line
    - Transformation to tumor related properties
    - Contamination
    - Distribution to others
- Saving reagents, time
- Equipment failure such as incubator
- Cross-contamination by other cell lines

CRYOPROTECTANT:

- Cryoprotectants (CPs) are macromolecules added to the freezing medium to protect the cells from the detrimental effects of intracellular ice crystal formation or from the solution effects, during the process of freezing and thawing.

- Solution effects : Forms of injury to cells, cooled slowly enough to prevent damaging intracellular ice crystal formation .

TYPES OF CRYOPROTECTANTS

- Permeating CPs: (INTRACELLULAR)

- Non permeating CPs: (EXTRACELLULAR)

MECHANISM OF CRYOPROTECTANTS

- Cryoprotectants acts by
  - salt buffering action (bind water and less ice crystal formed)
  - gives more time to cell for de-hydrate and interact with cell membrane to make it less brittle during freezing.

PERMEATING CPS :

- Lower the freezing point of the solution, increase the viscosity and thus reduce diffusion in solutions.
- Replace intracellular water to prevent the formation of large ice crystals intracellularly.
- Ability to reduce the amount of water which freezes as ice.
- Reduce exposure of cell to elevated salt and solute concentration.
- Glycerol (GLY), Dimethyl Sulfoxide (DMSO), Propylene glycol (PG), and Ethylene glycol (EG).

NON- PERMEATING CPS: (EXTRACELLULAR)

- LOW MOLECULAR WEIGHT
  - It changes the water balance of cells by shrinking cells before freezing.
  - Sucrose, Maltose, Trehalose, Sorbitol and Acetamide.
- HIGH MOLECULAR WEIGHT
  - Closing the cell membrane defect.
  - Repair damaged cell membrane post thawing.
  - Polyvinyl Pyrorolidone (PVP), Dextran, Serum,BSA

Ethylene glycol is preferred cryoprotectant for cryopreservation.

BACKGROUND OF CELL FREEZING

- **Optimal Cell Freezing Is Characterized By**
  - Maximum number of viable cells upon thawing
  - Minimum intracellular crystal formation
  - Minimum formation of foci of high solute concentration

- Optimal Freezing Is Accomplished By
  - Cooling slowly so water escapes
  - Cooling fast enough to avoid crystal formation
  - Use hydrophylic cryoprotectant
  - Storing at lowest possible temperatures
    - minimize negative effect of solute foci on proteins
  - Thaw rapidly
    - minimize crystal growth and solute gradients

CELL CONCENTRATION AND FREEZING MEDIUM

- High Cell Concentration Seems To Enhance Survival
  - Possibly due to "leakiness" effect from cryogenic damage
  - Centrifugation is avoided since dilution of cryoprotectant is high when reseeding
- Freezing Medium
  - DMSO, Glycerol
  - DMSO used at 5-15%, 10% is common
  - DMSO should be stored in glass or polypropylene

PROCEDURE

- View cultures using an inverted microscope to assess the degree of cell density and confirm the absence of bacterial and fungal contaminants.
- Harvest cells in the log phase of growth.
- For adherent cell lines harvest cells as close to 80 - 90% confluency as possible.
- Bring adherent and semi adherent cells into suspension using trypsin/EDTA as described previously and re-suspend in a volume of fresh medium at least equivalent to the volume of trypsin. Suspension cell lines can be used directly.
- Remove a small aliquot of cells (100-200µl) and perform a cell count.
- Ideally, the cell viability should be in excess of 90% in order to achieve a good recovery after freezing.

- Centrifuge the remaining culture at 150 x g for 5 minutes.
- Re-suspend cells at a concentration of 2-4x106 cells per ml in freeze medium.
- Pipette 1ml aliquots of cells into cryoprotective ampoules that have been labelled with the cell line name, passage number, lot number, cell concentration and date.
- Place ampoules inside a passive freezer e.g. Nalgene Mr. Frosty Freezing Container. Fill freezer with isopropyl alcohol and place at -80°C overnight.
- Frozen ampoules should be transferred to the vapor phase of a liquid nitrogen storage vessel and the locations recorded.

CRYOPRESERVATION OF CELL LINE

Procedure
Assessment of cell
Harvest of cell
Resuspend cell in cell media
Removal and counting of cell
Centrifugation of remaining cells at 150 × g for 5 minutes
Resuspend cell in freeze media
Pipetting of 1ml. of cells
Transfer of ampoules in rate controlled freezer
Transfer to liquid nitrogen storage vessel

Thanks

ACKNOWLEDGEMENT

- Contents and images taken from sources on http://www.google.com/ are for study purpose and non commercial use.