Cell Culture Techniques for In Vivo Grafting

Mouse models for in vivo imaging of cancer established by in vivo grafting of syngeneic or xenogeneic cells in recipient animals are commonly practiced. Success of these studies relies upon solid cell culture technique. This article covers sterile technique, thawing and freezing, subculture and preparation of cells for grafting.

1. Sterile Technique:

**Use Sterile Technique to prevent contamination from bacteria, fungi and mycoplasma and cross contamination with other cell lines.*

- Sanitize the cabinet using 70% ethanol before commencing work.
- Sanitize gloves by washing them in 70% ethanol and allowing to air dry for 30 seconds before commencing work.
- Put all materials and equipment into the cabinet prior to starting work after sanitizing the exterior surfaces with 70% ethanol.
- While working, do not contaminate hands or gloves by touching anything outside the cabinet (especially face and hair). If gloves become contaminated re-sanitize with 70% ethanol as above before proceeding.
- Discard gloves after handling contaminated cultures and at the end of all cell culture procedures.
- Equipment in the cabinet or that which will be taken into the cabinet during cell culture procedure (media bottles, pipette tip boxes, pipette aids) should be wiped with tissue soaked with 70% ethanol prior to use.
- Movement within and immediately outside the cabinet must not be rapid. Slow movement will allow the air within the cabinet to circulate properly.
- Speech, sneezing and coughing must be directed away from the cabinet so as not to disrupt the airflow.
- After completing work disinfect all equipment and material before removing from the cabinet. Spray the work surfaces inside the cabinet with 70% ethanol and wipe dry with tissue. Dispose of tissue by autoclaving.
- Sanitize the cabinet with 10 – 30 min UV light. Warning – plastics will crack and become brittle over time with repeated exposure to UV light. Only some cabinets have timed UV lights. Ensure they are not left on for extended periods.

2.Thaw Frozen Cell Vial:

** Some cryoprotectants, such as DMSO, are toxic above 4^{o}C, therefore it is essential that cultures are thawed quickly and diluted in culture medium to minimize the toxic effects.*

- Remove ampule from liquid nitrogen and thaw cells in a 37^{o}C waterbath. Submerge only the lower half of the ampule. Allow to thaw until a small amount of ice remains in the vial - usually 1-2 minute.
- Transfer to class II safety cabinet.

- Wipe the outside of the ampule with a tissue moistened (not excessively) with 70% alcohol hold tissue over ampule to loosen lid.
- Slowly, dropwise, pipette cells into a 15 ml conical tube with 5ml pre-warmed cell culture medium to dilute out the DMSO.
- Centrifuge at 1000 rpm for 5min.
- Aspirate supernatant and resuspend in 5ml cell culture medium.
- Transfer suspension to T25 Flask, cultivate in incubator at 5% CO2 in air, 37^{o}C.
- Aspirate and change medium the next day (after evaluating plating/adhesion of the cells).

** Expand and freeze down cells immediately to generate back up stock.*
** To prevent genetic drift in culture, start from fresh frozen stock every 10 weeks.*
** For in vivo grafting, use cells from < 15 passages.*

3.Routine Cell SubCulture:

In general, cell lines are split twice a week*. Procedures:

- Unpack flasks, pipettes etc. under the laminar flow hood.
- Assess cell morphology and confluency in a tightly capped flask under an inverted microscope. Split at 70-90% confluency.
- Transfer flask into biosafety cabinet. Handle only a single cell line at a time, preventing cross contamination.
- Label new flasks with the cell line name, date, split ratio and passage number.
- Pipette media into new flasks (10 ml media into T-25 flask, 25 ml media into T-75 flask, or 50 ml media into T-225 flask) and cap flasks.
- Aspirate media from cells.
- Wash cells 3x with PBS without Ca^{2+}/Mg^{2+} (7 ml for T-25, 10 ml for T-75, or 15 ml volume for T-162/T-225 flasks).
- Aspirate PBS.
- Add Trypsin-EDTA solution (0.25% Trypsin*, 1mM EDTA Na_4) (1ml for T25, 3ml for T75 flask) to detach cells from flask for 1 min. Gently rotate flask, confirm under microscope, flick flask to detach cells.
- Add fresh medium with serum to inactivate trypsin (5 ml for T-25 flask, 10 ml for T-75 flask, or 20 ml for T-162/T-225 flask) and pipette up and down to separate cell clumps.
- Dilute appropriately into new flasks (e.g. 1:5 split ratio).
- Cultivate cells as described above.

** Additional Info can be found at: http://www.mnstate.edu/biotech/CellCultureProtocol.pdf* and *the Jove Videos:*
Trypsinizing and Subculturing Mammalian Cells -Richard Ricardo, Katy Phelan, Molecular Pathology Laboratory, Inc.
Counting and Determining the Viability of Cultured CellsRichard Ricardo, Katy PhelanMolecular Pathology Laboratory Network, Inc.

4.Freezing of Cells:

- Freeze cells in 5% DMSO in complete medium (containing 10% FBS) at $1x10^6$/ml.
- Lower temperature at -1°C/ minute, using a freezing container (e.g. CoolCell, Biocision) in a -80°C Freezer.
- Always use dry ice to transfer the cells to permanent storage (LN_2) to avoid temperature rise and cell damage. Cryovial contents can rise from -75°C to over -50°C in less a minute if exposed to room temperature air.
- It is strongly recommended that all frozen cell cultures be checked for viability before the stock culture is terminated.

** Additional information can be seen at the Jove-videos:*

Freezing, Thawing, and Packaging Cells for Transport - Richard Ricardo, Katy Phelan, Molecular Pathology Laboratory Network, Inc.
CoolCell - Controlled Cell Freezing w/o Alcohol or Maintenance Cost - Rolf Ehrhardt, Brian Schryver, Jeff Schryver,Biocision

5.Preparation of Cells for Grafting:

- Tumor cells are trypsinized, and resuspended in DPBS or serum free medium to a concentration of $1\text{-}3x10^6$ cells in 100ul.
- Dilute cells in DPBS and mix well before each injection. Draw the cells into the syringe without a needle to prevent shearing. Attach the needle to the syringe, and before injecting flick or invert the syringe to ensure the cells are in suspension.
- If cell line is labeled with an optical reporter - verify light emission prior to injection. Serially dilute cells in a 96 well plate and measure photon emission to determine photons/cell. This is a great quality control assay for stable transfection and light emission and will predict sensitivity in vivo.

** Tumor cells can be injected subcutaneously (20-100ul), intraperitoneally (200-750ul), intravenously (50-100ul), by an intracardiac route (50-100ul), or orthotopically (20-50ul).*
** Recommendations: Use a 25g 5/8" needle for s.c. and i.p. injections of adult mice, 26 g 1/2" needle for i.v. injections, 26-27g 1/2" needle for i.c. injections, and a 30g 1/2" needle for some orthotopic injections.*