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Change History

Revision	Description
12/12/2019	New Document for PDMR Public Website

RELATED DOCUMENTS

Available on the PDMR website: https://pdmr.cancer.gov/sops

SOP50102: PDX Implantation, Expansion and Cryopreservation (Subcutaneous)
SOP50103: Histopathological Assessment of Patient-Derived Xenografts
SOP30103: Initial Culture, Sub-culture, and Cryopreservation of Early-Passage Adherent Patient-Derived Tumor Cultures (PDCs)
SOP30104: Initial Culture, Sub-culture, and Cryopreservation of Early-Passage Suspension Patient-Derived Tumor Cultures (PDCs)
SOP40103: Passaging and Sub-culture of Patient-Derived Organoid (PDOrg) Cultures

1.0 PURPOSE/SCOPE

This Standing Operating Procedure (SOP) describes the procedures for preparation and subcutaneous implantation of patient-derived cell (PDCs) or organoid (PDOrg) cultures to generate Cell Line Xenografts (CLXs) under BSL-2 safety criteria. This SOP is used/performed by the Biological Testing Branch (BTB) at NCI-Frederick, Frederick National Laboratory for Cancer Research.

2.0 SAFETY

BTB treats all patient tissue and mice carrying patient tumors as a possible health threat as the human tissue could retain human pathogenic agents. Mice are housed in barrier facilities using full micro-isolator technique with all work, including husbandry, provided by experienced technical staff. The primary mouse strain used is the NOD.*Cg-Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) which is highly susceptible to infection due to their profound immunodeficiency. All materials coming into the barrier facilities are decontaminated by autoclaving, or chemical means including the use of chlorine dioxide gas, Clorox Healthcare Hydrogen Peroxide spray/wipes and Virkon® dip tanks for non-autoclavable items.

3.0 MATERIALS & EQUIPMENT

3.1 Implant Materials & Equipment

3.1.1 1000 µL pipettor and sterile tips (pre-chilled at -20°C)

3.1.2 Sterile scissors and forceps

3.1.3 Matrigel® (BD BioSciences, Bedford, MA.) or Cultrex Basement Membrane Extract Type 3 (BME3), Pathclear, (R&D Systems, Cat#: 3632-001-02)

- **IMPORTANT:** All basement membrane extract purchases should be submitted specifying mouse rodent pathogen screened, LDEV-Negative material. If not, there is a possibility of LDEV contamination which can result in LDEV+ tumors.

3.1.4 RPMI-1640 media (Thermo Fisher Scientific, Cat#: 11875093)

3.1.5 1-cc low dead space syringe with luer-lock hub, sterile (pre-chilled at -20°C) (Exel Cat#: 26048-1)

3.1.6 Sterile 20- and/or 18-gauge needles (pre-chilled at -20°C)

3.1.7 NOD.*Cg-Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) mice, sex-matched to human patient

3.1.8 Isoflurane and anesthesia machine for delivery (optional)

3.1.9 Clorox Healthcare® Hydrogen Peroxide Spray, Bleach (5.25% Hypochlorite) diluted 1:10, 2% Virkon®, or similar disinfectant

4.0 CELL HARVEST AND IMPLANTATION

4.1 General Notes

- Patient-derived cell (PDCs) or organoid (PDOrg) cultures should be in log phase at the time of harvest.
- Use previous experience with cultures to determine optimal number of flasks/wells to harvest for implant.
- All sample preparation should be performed in a Biological Safety Cabinet (BSC) using sterile instruments and technique. Maintain sterility at all times.
- Single-cell preparations/suspensions **must** be kept at **4°C** to maintain viability and reduce cell clumping.
- Mice should be ready for implantation **prior to harvesting cell and/or organoid cultures.** This includes shaving of the implantation site and identifying all individual animals (e.g., ear tag, tattoo, chip).
- If the operator is more comfortable anesthetizing the mouse for implantation then isoflurane can be used. All anesthesia guidelines must be observed.

4.2 PDC Culture Harvest and Implantation

4.2.1 Harvest cells for implantation.

4.2.1.1 For adherent cultures, follow SOP Step 8.2.1-8.2.6 in the PDMR SOP30103.

4.2.1.2 For suspension cultures, follow SOP Step 8.2.1-8.2.4 in the PDMR SOP30104.

4.2.2 Determine the viable cell count for the harvested cells, pellet the cells (200xg for 5 mins), and resuspend them to a final concentration of $2x10^8$ cells/mL in RPMI-1640 media without serum (at 4ºC). Keep cells on ice to maintain viability.

4.2.3 Transfer cells to the in vivo laboratory according to your institution's safety regulations. Cells should be implanted as soon as practical. The cells should be kept on ice until implantation.

4.2.4 Resuspend the cells by gentle agitation or pipetting.

4.2.5 Add an equal volume of Matrigel®/BME (stored per Manufacturer's instructions) using a pre-chilled pipet or 1-cc syringe. Mix the cells and Matrigel®/BME3 by gentle trituration without introduction of air bubbles. This provides a final cell concentration of $1x10^8$ cells/mL. Keep on ice.

4.2.6 A total of 100 µL is subcutaneously injected into each NSG host to provide a final implant inoculum of $1x10^7$ cells/mouse.

4.2.6.1 Draw adequate air (0.1 – 0.2 mL) into the pre-chilled 1-cc syringe with 20-ga needle to allow full discharge of the cell inoculum.

4.2.6.2 Draw 100 µL of the cell inoculum into each pre-chilled syringe/needle and keep the needle/syringe cold until it is injected. It is recommended that multiple doses not be drawn into a single syringe as the Matrigel®/BME3 will begin to gel as it warms. Rather, use a single syringe/needle for each mouse being inoculated.

4.2.6.3 Inject the inoculum subcutaneously at the desired location (e.g., post-axillary; flank; near mammary fat pad). To avoid leakage of the inoculum through the needle penetration site it is recommended the needle be passed at least 1.5 cm from the entry point to the injection site.

4.2.6.4 Slowly withdraw the needle and return the mouse to its cage.

4.2.7 Additional details on subcutaneous implantation methods can be found in the PDMR SOP50102.

4.3 PDOrg (organoid) Culture Harvest and Implantation

4.3.1 Harvest organoids for implantation following the PDMR SOP40103 steps 5.3.1-5.3.8 for passaging organoid cultures. The Dispase II step is used to break-down the BME2 domes. Do not triturate or attempt to dissociate the organoids.

4.3.1.1 Harvest all wells into 50-mL tubes, combining as needed to minimize the number of tubes.

4.3.1.2 If more than one 50-mL tube is needed for initial collection, after the first centrifugation resuspend the organoids in a minimal amount of Wash media and combine them into a single 50-mL tube.

4.3.2 Adjust final volume to 51 mL with Wash Media

4.3.3 Invert to mix and remove 1 mL of the organoid suspension. Allow remaining 50 mL to sit on ice.

4.3.3.1 Determine viable cell count of the 1-mL aliquot by digesting organoids to a homogeneous single cell suspension and counting as detailed in SOP40104 Step 5.3.8

4.3.4 Calculate the total cell count in the remaining 50-mL sample of organoids based upon the cell count in the 1-ml aliquot.

4.3.4.1 Calculate the volume needed to prepare a final cell density of $1x10^6$ cells/mL.

4.3.4.2 Pellet the organoids by centrifugation at 200xg for 5 min.

4.3.4.3 Remove the supernatant and gently resuspend the organoids in the calculated volume of RPMI-1640 media without serum (at 4ºC).

4.3.4.4 Aliquot the organoid suspension into 1.5 ml tubes at 1 mL/tube ($1x10^6$ cells/tube) ensuring the organoids remain homogenously suspended during this process.

4.3.5 Transfer cells to the in vivo laboratory according to your institution's safety regulations. Cells should be implanted as soon as practical. The cells should be maintained at 4°C until implantation.

4.3.6 Prepare organoids for implantation:

4.3.6.1 Allow organoid aliquots to sit undisturbed so the organoids settle to the bottom of the conical tubes then remove as much media as possible with a pipettor.

4.3.6.2 Add 100 µL of cold Matrigel®/BME3 to each tube and mix by gentle trituration without introduction of air bubbles to provide a final implant inoculum of $1x10^6$ cells/mouse.

4.3.6.3 Draw adequate air (0.1 – 0.2 mL) into the pre-chilled 1-cc syringe with 20-ga needle to allow full discharge of the inoculum.

- If the organoids are large and there is concern a 20-ga needle may not properly accommodate them, then an 18-ga needle can be used for administration provided local IACUC needle size guidelines are followed.

4.3.6.4 Draw the full cell inoculum in a single tube into each syringe/needle and keep the needle/syringe cold until it is injected. Use a single syringe/needle for each mouse being inoculated so the organoid density given to each mouse is consistent.

4.3.6.5 Inject the entire inoculum from a single tube subcutaneously (SC) at the desired location (e.g., post-axillary; flank; near mammary fat pad). To avoid leakage of the inoculum through the needle penetration site it is recommended the needle be passed at least 1.5 cm from the entry point to the injection site.

4.3.6.6 Slowly withdraw the needle and return the mouse to its cage.

5.0 GROWTH ASSESSMENT

5.1 Details on Growth Assessment outlined in the PDMR SOP50102, Section 7.0 and on collection of tumor material for Quality Control in Section 8.0

5.2 Notes on CLXs that differ from PDXs:

5.2.1 It is recommended to harvest CLX tumors in the 500-1000 mm^3 size range as there is typically less necrotic tissue present. (Individual institutional guidelines for maximum allowable tumor burden should be followed)

5.3 Static and No-Growth Monitoring

5.3.1 Animals with no signs of tumor growth are euthanized at 180 days post-implant. While CLXs frequently grow faster than the parental PDX, some models can still grow slowly as compared to traditional cell line xenograft experiments.

6.0 CLEAN-UP

6.1.1 All materials coming into contact with patient derived cultures as well as the mice carrying patient derived samples are treated as a potential health threat (BSL-2 precautions) since the human tissues could retain human pathogenic agents even if they do not replicate in mouse cells (e.g., HIV, HPV, etc).

6.1.2 Flush/soak any items (e.g., tubes, syringes, petri dishes, lab mats, etc) that were in contact with human tissue with disinfectant (e.g., 10% bleach, commercial hydrogen peroxide disinfectant, 2% Virkon®) for a minimum of 10 minutes before disposal in biohazard waste or sharps containers (follow institutional guidelines and manufacturer's recommendations).

6.1.3 For items that can't be rinsed (e.g., micropipettors), wipe down thoroughly with Clorox Healthcare® Hydrogen Peroxide wipes or other appropriate disinfectants.