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January 01, 2006

Standard Operating Procedures: Corneal Culturing

Office of Environmental Health and Safety, Northeastern University

Recommended Citation

Office of Environmental Health and Safety, Northeastern University, "Standard Operating Procedures: Corneal Culturing" (2006). *Office of Environmental Health & Safety Standard Operating Procedures.* Paper 3. http://hdl.handle.net/2047/d20003705

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CORNEAL CULTURING
(ISOLATION OF FIBROBLASTS BY MIGRATION FROM CORNEAL EXPLANTS IN CULTURE)
Extracellular Matrix Engineering Research Laboratory

Date: September 25th, 2006
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References:

1. *Xiaoxing Guo, Schepens Eye Research Institute, Personal communication*
2. *Freshney, Culture of Animal Cells handbook, 4th edition, page 158*

MATERIALS

***Note**: unless otherwise stated, all materials need to be STERILE.*

- Cornea explants (see "Dissection of.." protocol).
- Autoclaved glass petri dish(es)
- Autoclaved 1x PBS with 1% antibiotic (Sigma#A4468) **add antibiotic after autoclaving.
- Autoclaved Tweezers/forceps
- 6-well tissue culture plates (BD 353046).
- Fibroblast culture medium
    - Dulbecco's Modified Eagle Medium (DMEM)
    - 10% fetal bovine serum (FBS)
    - 1% antibiotic/antimycotic SIGMA#A4668
- Trypsin/EDTA Sigma # T4049 (stored at -20°C)
- Lab coat, gloves

PROCEDURE FOR CULTURING CORNEAS

1. Corneas should be handled with clean tweezers and forceps, and gloves should be worn at all times. Tweezers should be autoclaved prior to procedure but can be cleaned during procedure by soaking in alcohol.

2. 10-12 sections are obtained per cornea according to the "Dissection of Bovine Eyes" protocol. The sections corresponding to the 2 corneas of an animal will be plated evenly distributed into the 6-well tissue culture dish.

3. Use a pipette to remove the extra PBS and moisture around the tissue once it is sitting in the wells of the tissue culture dish. This will help the tissue to attach to the bottom of the wells; the tissue should not be completely dry, though.

4. Let the tissue sit for ten minutes in the well (inside the biological hood) before carefully adding 1mL of media. This is in order to favor attachment.

5. Place the plate(s) inside the incubator.

6. 24 hours later, add another 1.0 -2.0mL of media. **Always be extremely careful removing tissue samples from incubator – laminar flow hood and back, as you do not want the samples to detach from the bottom of the wells.**

7. Tissue should be fed every 3-5 days, by removing 50% of media, and replacing with fresh fibroblast culture media. Continue to replace the media until a substantial outgrowth of cells is observed.

PROCEDURE FOR PASSAGING CELLS

1. This should be done when the cells have become confluent; this is when ~80 – 90% of the view through a microscope is cells. It is a gauge, not an exact measurement.

2. Gently remove the medium with a pipette; **do not pour it out** as it may contaminate the mouth of the culture jar.

3. Rinse twice with PBS, in order to remove the presence of FBS (FBS is thought to interact with EDTA, stopping it from loosening the cells as efficiently). To rinse, add 10mL of PBS and shake gently, so that PBS will touch all surfaces. Then remove with a pipette.

4. Add trypsin/EDTA solution ; 1mL for T25 flasks, 3mL for T75 flasks; 500uL per well of 6 well dish. Then incubate flask at 37°C for 3-5 minutes, checking under the microscope to see when all cells have been released. The newer the EDTA, the more quickly it will act.

5. Gently remove the trypsin solution and re-suspend newly detached cells in new media and mix with pipette to generate a new uniform cell suspension.

6. Add new suspension to new flasks, splitting the volume by sight. Complete with fresh medium. Do not add anything on the original culture flask.

7. Be sure to label the new flasks with the new passage number, cell density, and date of passage.