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Standard Operating Procedures: Isolation of Fibroblasts by Migration From Corneal Explants in Culture - No Collagenase

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ISOLATION OF FIBROBLASTS BY MIGRATION FROM CORNEAL EXPLANTS IN CULTURE – NO COLLAGENASE
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
- 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.