My iSCORE Experience:Growing Cells

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The Project

- GROWING/CULTURING EPITHELIAL CELLS
  - Cells used were from human eye corneas
- DONE TO INCREASE CELL NUMBERS FOR FURTHER EXPERIMENTS
- USED FETAL BOVINE MEDIA TO PROMOTE CELL GROWTH
- TOTAL TIME TOOK ABOUT 1 HOUR
- DONE TWICE
  - Separated by a few weeks time
- USED AS A WAY TO GAIN EXPERIENCE IN CELL CULTURING AND TO FURTHER UNDERSTAND IT

The Steps

1. The biosafety cabinet used was first disinfected with a UV light to create a sterile environment
   - Time: Approx. 23 minutes

2. 70% alcohol was used to further disinfect the cabinet
   - 100% alcohol would evaporate too fast

3. 10 mL of fetal bovine and serum (FBS) was mixed with 100 mL of media containing were combined to create complete growth media for cell growth

The Steps (cont.)

4. The cellular mixture was incubated and monitored
   - Microscopes were used to determine if cell samples were ready for the next steps
   - If there was significant growth with little space (~ 70% confluence), the cells were ready
   - Incubator was at 37°C with 5% CO_2 to maintain the buffer, and water was kept inside the incubator walls to maintain cellular growth

5. Trypsin was used to subculture the cells
   - Trypsin breaks the lysine and arginine residues of the cells to dissociate the cells

6. Cells were then transferred into a tube and centrifuged for 5 minutes.
   - Done to separate the cells from the mixture

The Steps (cont.)

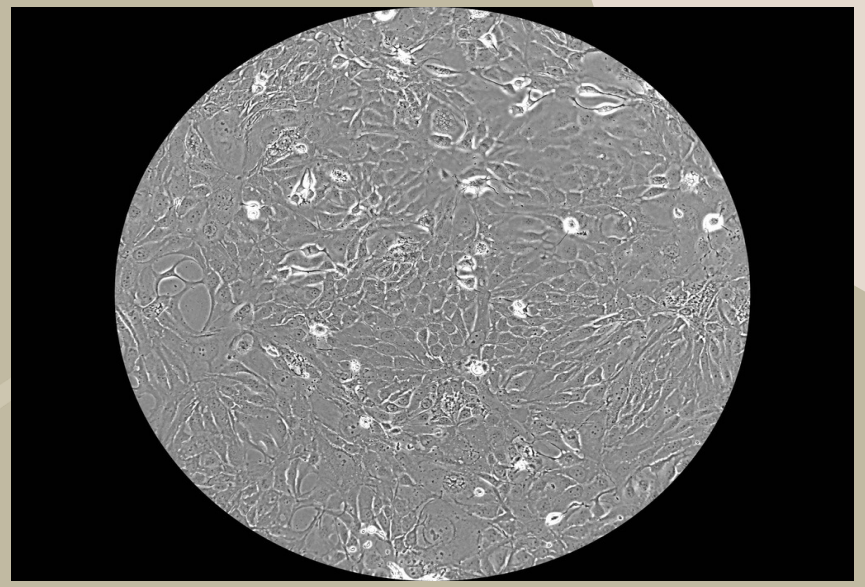
7. Using a vacuum, the media was removed from the tube without sucking up the cells.
   - The cells were on one side of the media

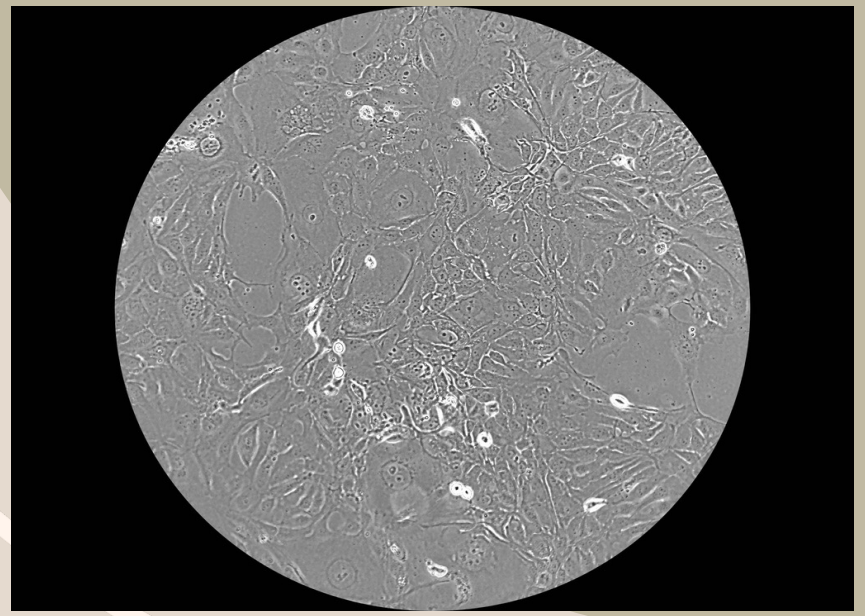
8. New media without trypsin was pipetted into the tube with the cells and mixed in with said cells by repeated pipetting (sucking up and releasing the liquid) into the tube with the pipette

9. The cell mixture was pipetted into a labelled dish and left to incubate for cell growth over the span of 48 hours

The Results

- After 48 hours, the cells were examined under and echo rebel light microscope to check for growth and and possible contaminations
- No contaminants were found in either experiment and cell growth appeared normal
- Overall, both experiments were successful for cell culturing and area was kept clean enough to prevent any sort of contamination to the cells



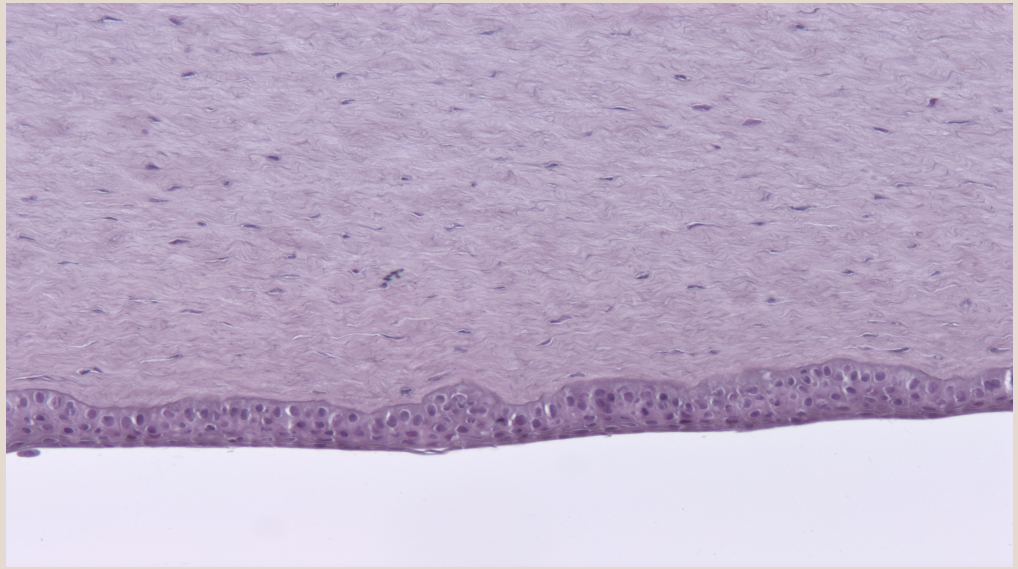


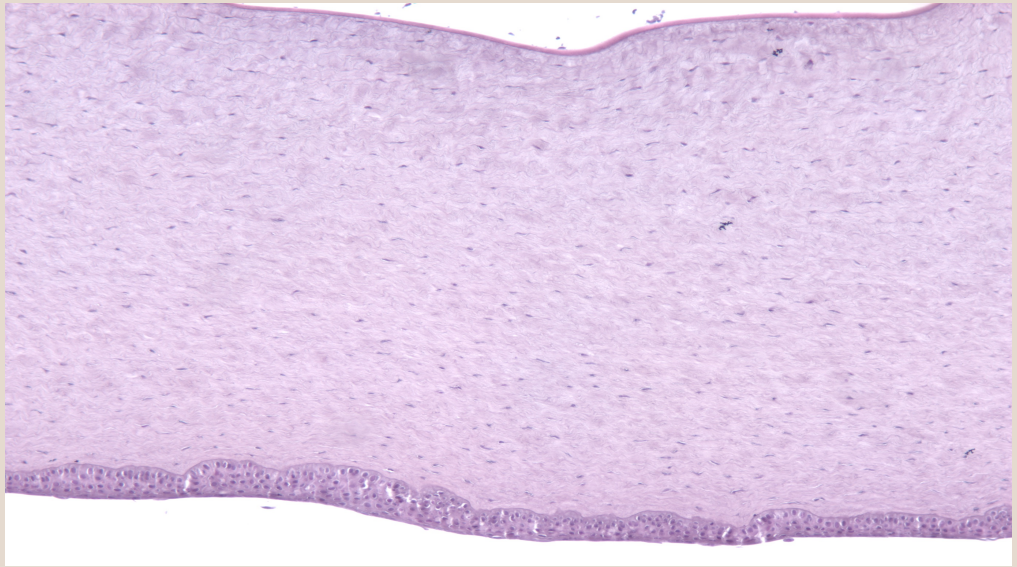
The BCA Method of Protein Estimation

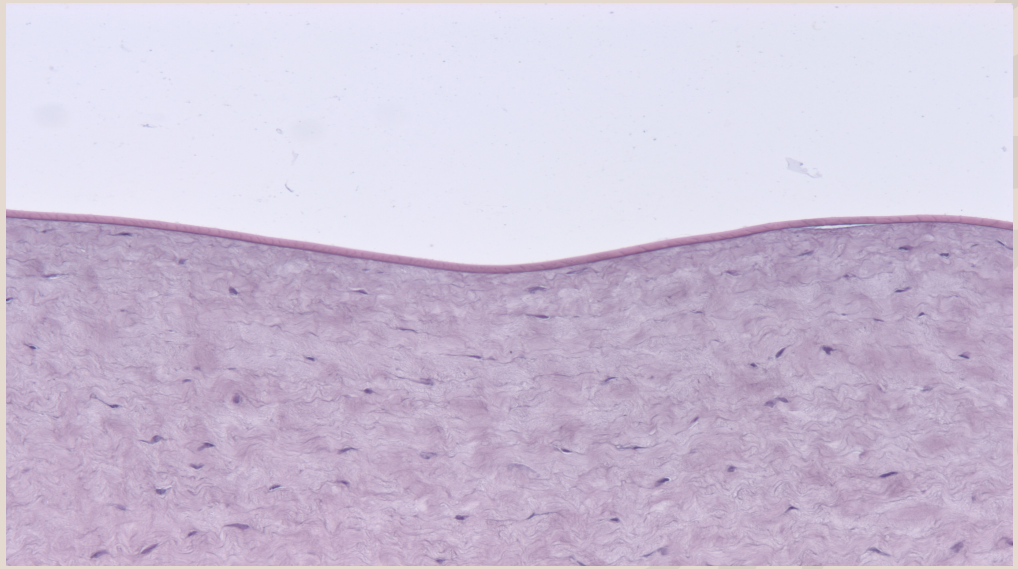
- Proteins extracted from the cells were quantified using the BCA method with a kit from Bio-Rad
- This is done to estimate the protein concentration in a cell culture so that the equal amounts can be loaded for the western blotting experiment
- Absorbance based plate reading method in which the OD is taken at 618nm

Morphology of Rabbit Cornea

Hematoxylin stained slides were viewed under the microscope and the morphology of rabbit culture tissue was discussed where I learned about its components







Cultural Aspects and Differences

- Medical school and PhD programs were different between India the United States
- Movies were different in content between the two countries.
- Country infrastructure differences
  - Ease of living differences
  - Cost of living differences
- Family structure differences

Thank You!!!!

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