

UNIT 3.3

Cell Biology

Lab Exercise

Lab Activity

- **Performance Objectives**

To familiarize oneself with mammalian cell culture laboratory procedures and sterile techniques plus microscopic visualization of cells.

- o Learn basic cell culture technique of plating cells.
- o Observe effects of cell substrate on cell adhesion.
- o Analyze cell cytoskeleton and nucleus changes as a result of different substrates and hypergravity stimulation.

- **Materials**

- o Osteoblast cultures
- o Tissue culture hoods
- o Tissue culture incubators
- o Tissue culture plasticware
- o Tissue culture media
- o FITC Phalloidin (actin stain)
- o Rhodamine 123 (mitochondrial stain)
- o Hoechst 3367 (nuclear stain)
- o Fluorescence microscope
- o Confocal microscope

- **Procedure**

The groups will subculture mammalian cells into microscopic observation flasks and allow for cell adhesion and spreading.

- Group 1, allow 45 minutes for cell adhesion
- Group 2, allow 10 minutes for cell adhesion.
- Each group prepares duplicate samples.

1. Fix cells with paraformaldehyde (instructor).
2. Permeabilize fixed cells with cold acetone.
3. Add Rhodamine 123, Phalloidin and Hoechst stains. Stain for 45min.
4. Wash with PBS.
5. Mount with coverslip.
6. Observe in the microscope and record.

- **Wrap-Up Discussion and Evaluation**

Observe and analyze the microscopic effects of adhesion time on cell structure.