Exercise 7 Observation of Cultured Cells

Purpose of Procedure
Critical examination of cell cultures.

Applications
Checking consistency during routine maintenance; evaluation of status of cultures before feeding, subculture, or cryopreservation; assessment of response to new or experimental conditions; detection of overt contamination or misidentification and cross-contamination.

Training Objectives
- Familiarization with appearance of cell cultures of different types and at different densities;
- Awareness of problems of misidentification and cross-contamination;
- Use of microscope and camera;
- Distinction between microbial contaminated and uncontaminated, and between healthy and unhealthy cultures;
- Assessment of growth phase of culture and need for medium replenishment or subculture.

 Supervision: Continuous during observation, then intermittent during photography.

Time: 30 min.

Background Information
Morphology, photography (see Section 18.5, 18.5). Standard protocols: Microscopy and photography (see Protocols 18.1, 18.5). [Ancillary protocols: Staining (see Protocols 18.2, 18.3); cytocentrifuge (see Protocol 18.4); indirect immunofluorescence (see Protocol 18.6).

Demonstration materials or operations: Photographic examples of cell morphology, phase contrast of living cultures, as well as fixed and stained preparations; archival photos of cells to be used later in exercises; types of culture vessel suitable for morphological studies such as Petri dishes (see Fig. 7.4).

△ Safety Note. No special safety requirements.

Instructions for Exercise 7
Outline
Examine and photograph a range of cell lines at different cell densities.

Equipment and Materials
- Range of flask or Petri dish cell cultures at different densities preferably with normal and transformed variants of the same cell (e.g., 3T3 and SV3T3, or BHK21-C13 and BHK21-PyY) at densities including mid-log phase (~ 50% confluent with evidence of mitoses), confluent (100% of growth area covered and cells packed but not piling up), and postconfluent (cells multilayering and piling up if transformed). If available, include suspension cell cultures at low and high concentrations.
- If possible, include examples of contaminated cultures (preferably not Petri dishes to avoid risk of spread) and unhealthy cultures, such as cultures that have gone too long without feeding.
- Inverted microscope with 4x, 10x, and 20x phase-contrast objectives and condenser.

Procedure
1. Set up microscope [and adjust lighting for Kohler illumination and center phase rings if necessary].
2. Bring cultures from incubator. It is best to examine a few flasks at a time, rather than have too many out of the incubator for a prolonged period. Choose a pair, for example, the same cells at low or high density, or a normal and transformed version of the same cell type.
3. Examine each culture by eye, looking for turbidity of the medium, a fall in pH, granularity, vacuolization, or detached cells. Also try to identify type of cells in monolayer and look for signs of patterning. This can be normal, for example, swirling patterns of fibroblasts at confluence, or transformed, for example, random growth without patterning.
4. Examine at low power (4x objective) by phase-contrast microscopy on inverted microscope, and check cell density and any sign of cell-cell interaction, aggregation, or detachment.
5. Examine at medium (10x objective) and high (20x objective) power and check for the healthy status of the cells, signs of rounding up, contraction of the monolayer, or detachment. Check also for evidence of patterning, indicative of normal fibroblastic growth, or piling up and random overgrowth, evidence of transformation.
6. Check for any sign of microbial contamination.
7. Check homogeneity of culture; evidence of heterogeneity may indicate genetic instability, transformation, or cross-contamination.

Observations and Record
(1) Look for differences in growth pattern, cell density, and morphology in related cultures.
(2) Assess health status of cells.
(3) Is there any sign of contamination?
(4) Are cells ready for feeding (see Section 12.3.2) or passage (see Section 12.4.1)?

Data
Qualitative:
(1) Record your observations on morphology, shape, and patterning for all cultures.
(2) Note any contaminations.
(3) Confirm healthy status or otherwise.