

BME449 Tissue Engineering



Lecture #10

Cell Culture Techniques in Tissue Engineering-II

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Primary culture

- Cells when surgically or enzymatically removed from an organism and placed in suitable culture environment will attach and grow are called as primary culture
- Primary cells have a finite life span
- Primary culture contains a very heterogeneous population of cells
- Subculturing of primary cells leads to the generation of cell lines
- Cells such as macrophages and neurons do not divide in vitro so can be used as primary cultures

Selective adhesion during *in vitro* culture

- modification of the surface or special coatings.
Ex: Fibronectin coating
- pre-plating method: adopt the time for cell adherence.
- Gradient centrifugation:
 - a) rate zonal and b) isopycnic

Selective adhesion during *in vitro* culture

- Antibody driven separation:

FACS: a fluorescent dye is coupled to an antibody of interest and then, after labeling of the cells with the antibody, the cells can be separated in an electronic fluorescence activated cell sorter

MACS: Magnetic activated cell sorting

Cell Lines

Finite Cell Lines

- A limited number of subcultures, or passages.
- Senescence after approximately thirty cycles of division



Telomere critical minimum length

Continuous Cell Lines

Continuous Cell Lines

- Cell lines which either occur spontaneously or induced virally or chemically transformed into Continuous cell lines
- Characteristics of continuous cell lines
 - smaller, more rounded, less adherent with a higher nucleus /cytoplasm ratio
 - Fast growth
 - reduced serum and anchorage dependence
 - ability to grow up to higher cell density
 - different in phenotypes from donor tissue
 - stop expressing tissue specific genes

Types of cells

On the basis of morphology (shape & appearance) or on their functional characteristics. They are divided into three:

- Epithelial-like cells attach to a substrate and appears flattened and polygonal in shape
- Lymphoblast-like cells attach less and remain with a rounder shape
- Fibroblast-like cells attach to a substrate and appears elongated and bipolar