Chapter 3
Industrial
Cell Biology

Outline:

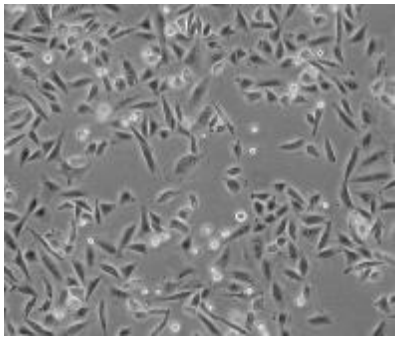
- 3.1 Mammalian cell culture
- 3.2 Applications of mammalian cell
- 3.3 Types of cell cultured *in vitro*
- 3.4 Morphology of cell in culture
- 3.5 Culturing mammalian cell in the laboratory

Learning outcomes:

- Define mammalian cell culture.
- Describe the important of mammalian cell culture in the production of recombinant protein.

3.1 Introduction

- **Cells, previously growing in a human or animal modified to grow in plastic or glass**
  - **In the body = *in vivo***
  - **On plastic or glass = *in vitro***
- **Mamalian Cell culture: the removal of cells from an animal and their subsequent growth in a favorable artificial environment.**

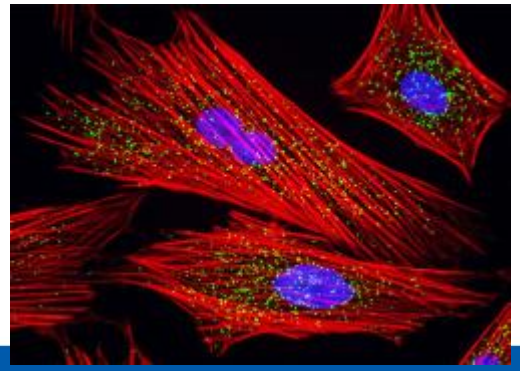


3.1 Introduction

- **Host of choice for production of post translational modifications (PTM) recombinant proteins.**
- **Bacteria – no PTM**
- **Yeast – PTM is not complex**

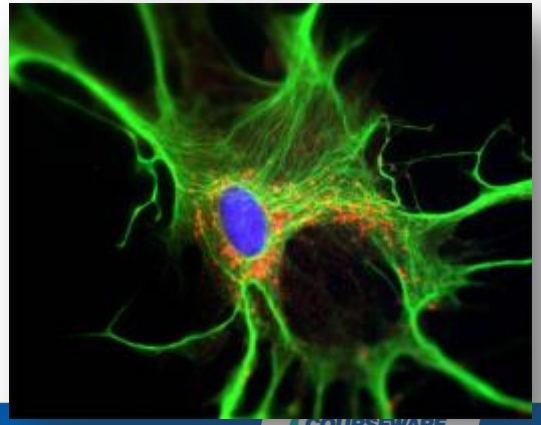
3.2 Applications of mammalian cell

- **Genetic Engineering - Production of commercial recombinant proteins (PTM), large scale production of viruses for use in vaccine production e.g. polio, rabies, chicken pox, hepatitis B & measles**
- **Virology - Cultivation of virus for vaccine production, also used to study there infectious cycle.**



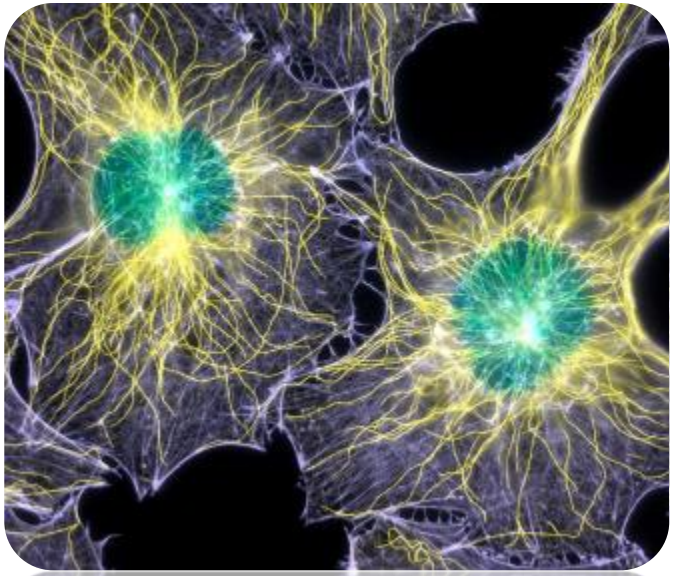
3.2 Applications of mammalian cell

- **Gene therapy** - Cells having a functional gene can be replaced to cells which are having non-functional gene
- **Model systems** - Studying basic cell biology, interactions between disease causing agents and cells, effects of drugs on cells, process and triggering of aging & nutritional studies



3.2 Applications of mammalian cell

- **Toxicity testing - Study the effects of new drugs**
- **Cancer research - Study the function of various chemicals, virus & radiation to convert normal cultured cells to cancerous cells**



3.3 Types of cell cultured *in vitro*

Isolation of cell lines for *in vitro* culture

Resected Tissue

↓

Cell or tissue culture *in vitro*

↓

Primary culture

↓ Sub-culture

Secondary culture

↓ Sub-culture

Cell Line

↓

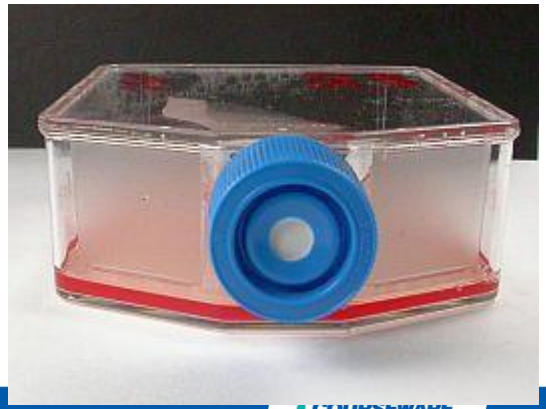
Immortalization

3.3 Types of cell cultured *in vitro*

- **Primary culture**
- **Cells when surgically or enzymatically removed from an organism and placed in suitable culture environment will attach and grow**
- **have a finite life span**
- **Retain differentiated phenotype**
- **contains a very heterogeneous population of cells**

3.3 Types of cell cultured *in vitro*

- **Secondary cultures**
- **Derived from a primary cell culture**
- **Isolated by selection or cloning**
- **Becoming a more homogeneous cell population**
- **Finite life span *in vitro***
- **Retain differentiated phenotype**
- **Mainly anchorage dependant**

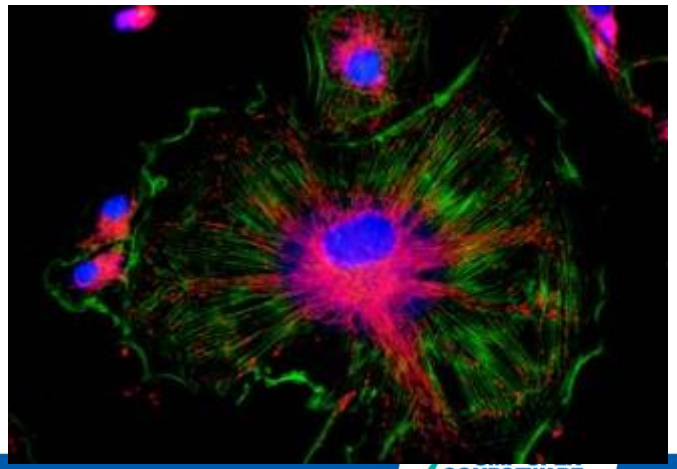


3.3 Types of cell cultured *in vitro*

- **Continuous cultures**
- **Derived from a primary or secondary culture**
- **Immortalised:**
  - **Spontaneously (e.g.: spontaneous genetic mutation)**
  - **By transformation vectors (e.g.: viruses &/or plasmids)**

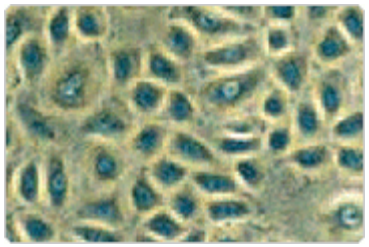
3.3 Types of cell cultured *in vitro*

- **Continuous cultures**
- **Serially propagated in culture showing an increased growth rate**
- **Homogeneous cell population**
- **Loss of anchorage dependency**
- **Infinite life span *in vitro***

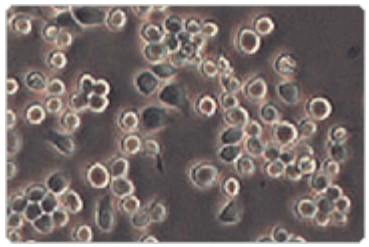


3.4 Morphology of cell in culture

Three categories:



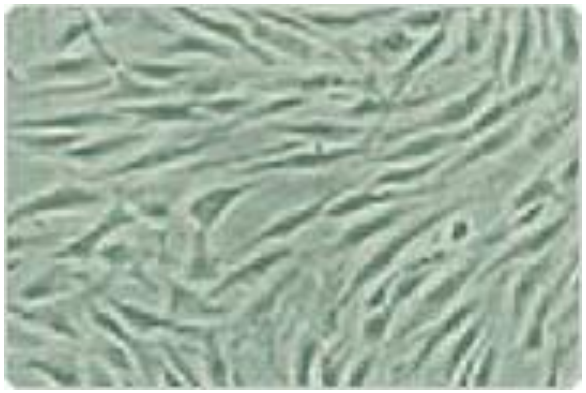
- **Epithelial** like-attached to a substrate and appears flattened and polygonal in shape



Lymphoblast like- cells do not attach remain in suspension with a spherical shape

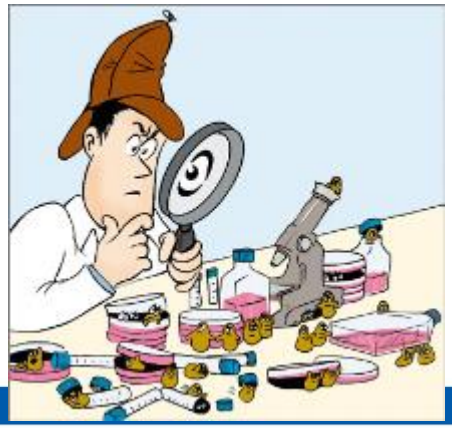
3.4 Morphology of cell in culture

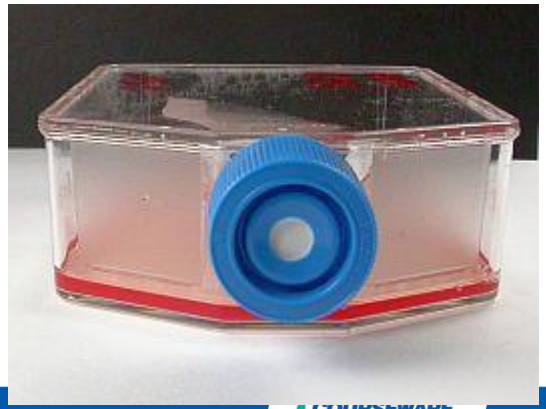
- **Fibroblast like- cells attached to an substrate appears elongated and bipolar**



3.5 Culturing mammalian cell in the laboratory

- **Substrate or liquid (cell culture flask)**
- **Nutrients (culture media)**
- **Growth factor**
- **Hormone**
- **Environment (CO_2, temperature 37°C, humidity)**
- **Sterility (aseptic technique, antibiotics and antimycotics)**





3.5 Culturing mammalian cell in the laboratory

- **Most cells are anchorage-dependent and must be cultured while attached to a solid or semi-solid substrate (adherent or monolayer culture), while others can be grown floating in the culture medium (suspension culture).**

3.5 Culturing mammalian cell in the laboratory

Revive frozen cell population
Isolate from tissue

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Maintain in culture (aseptic technique)

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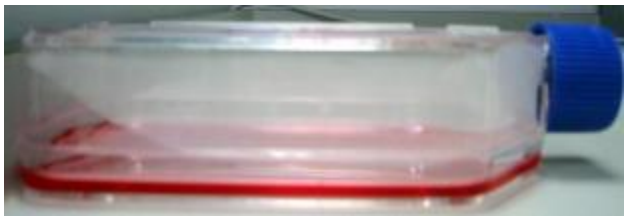
Sub-culture (passaging)

↓ **Count cells**

Cryopreservation

Containment level 2 cell culture laboratory



Typical cell culture flask

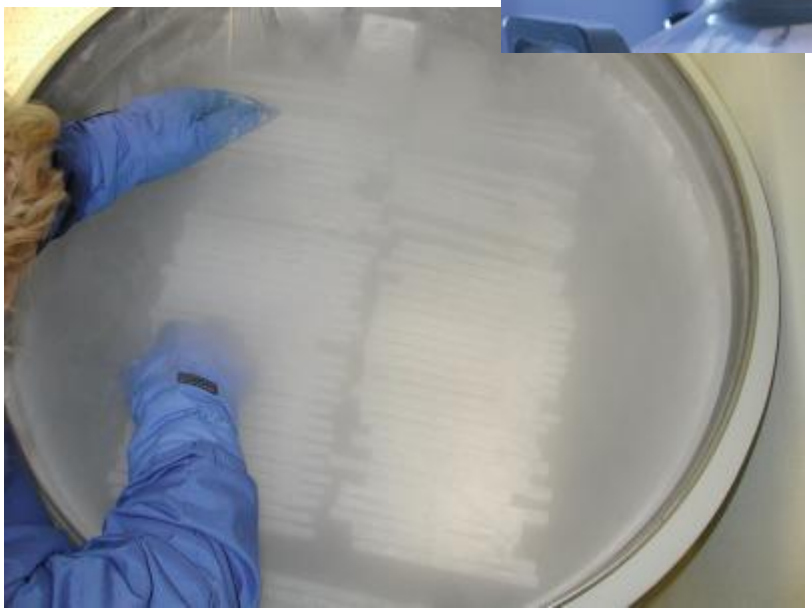
'Mr Frosty' Used to freeze cells

3.5 Culturing mammalian cell in the laboratory

- Cryopreservation
- If a surplus of cells are available from subculturing, they should be treated with the appropriate protective agent/ cryoprotectant (e.g., DMSO or glycerol) and stored at temperatures below –130° C (cryopreservation) until they are needed.

3.5 Culturing mammalian cell in the laboratory

- Cryopreservation

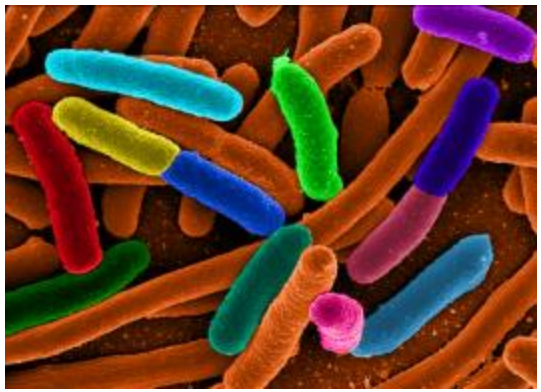


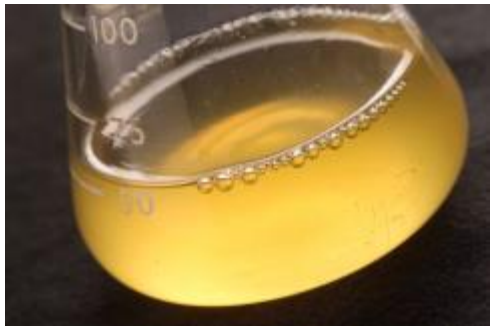
Discussion

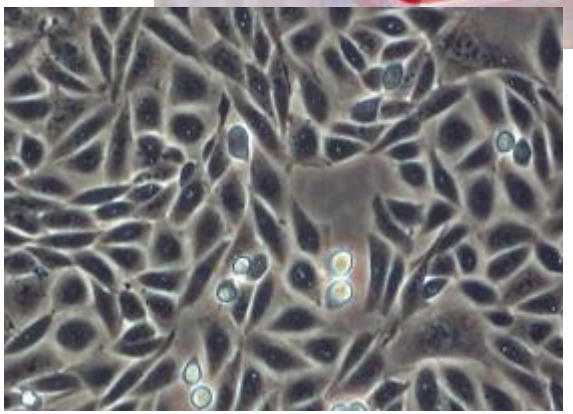
- Imagine that you are a technopreneur and own a company, you would like to produce a recombinant growth hormone that needs PTM, how would you produce the product?

Discussion

- Compare and contrast bacterial culture system and mammalian culture system.







Extra reading

- http://www.biology.arizona.edu/cell_bio/tutorials/cells/cells.html
- http://userpages.umbc.edu/~jwolf/method5.htm
- http://www.youtube.com/watch?v=ZBDSok3SMRY&feature=BFa&list=PLE1088CA918E254A1
- http://media.invitrogen.com.edgesuite.net/Cell-Culture/videos/CellCultureBasics.html?CID=ccbvid1

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