Cytological techniques and It's Applications

Course Code: ZOOL 5002 (Instrumentation and techniques for research)

PhD Course Work

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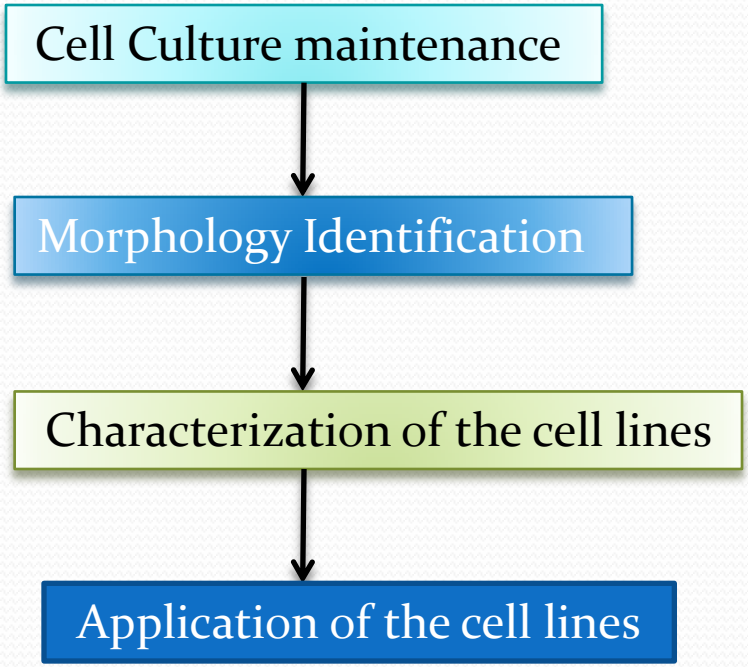
Cytological techniques

These techniques Includes:



Cell Culture maintenance

1.Introduction

• Cell culture can be defined as process of growing cell *in* -vitro in artificial environment/ medium.

• The cell culture was fist time successfully undertaken by Ross Harrison in 1907.

•A **cell line** is a permanently established cell culture, that will proliferate indefinitely in appropriate fresh medium and space. Also they have acquired homogenous genotypic and phenotypic characteristics.

•Normal cell line: Divides a limited number of times.

•Cell line having the capacity for infinite survival is called Immortal.

• The immortal cell lines follows the Hayflick limit (number of times a cell population will divide before it stops).

2. Basic requirement of cell lines: For the maintenance of cell line some basic conditions are required. These are described as follows:

pH: Most cell lines grow well at pH 7.4. Although the optimum pH for cell growth varies relatively little among different cell type. For Example normal fibroblast lines grows best at pH 7.4 to pH 7.7, and transformed cells may do better at pH 7.0 to pH 7.4.

Buffering: Culture media must be buffered under two sets of conditions:

a) The pH of the medium can be maintained by CO_2/HCO_3 ion buffer system and using CO_2 Incubator.

b) Overproduction of CO_2 and lactic acid in transformed cell lines at high cell concentrations, when the pH will fall.

Temperature: The temperature recommended for most human and warm-blooded animal cell lines is 37°C.

Media: Specific growth media supplemented with serum, in many instances cultures may now be propagated in serum-free media. Medium types depend on cell type, some example of media; RPMI1640, DMEM etc.

3. Detection of Contamination in cell culture:

• In generals the contamination can be observed in culture medium in form of turbidity, pH changes, color change of the media and multinucleated cells.

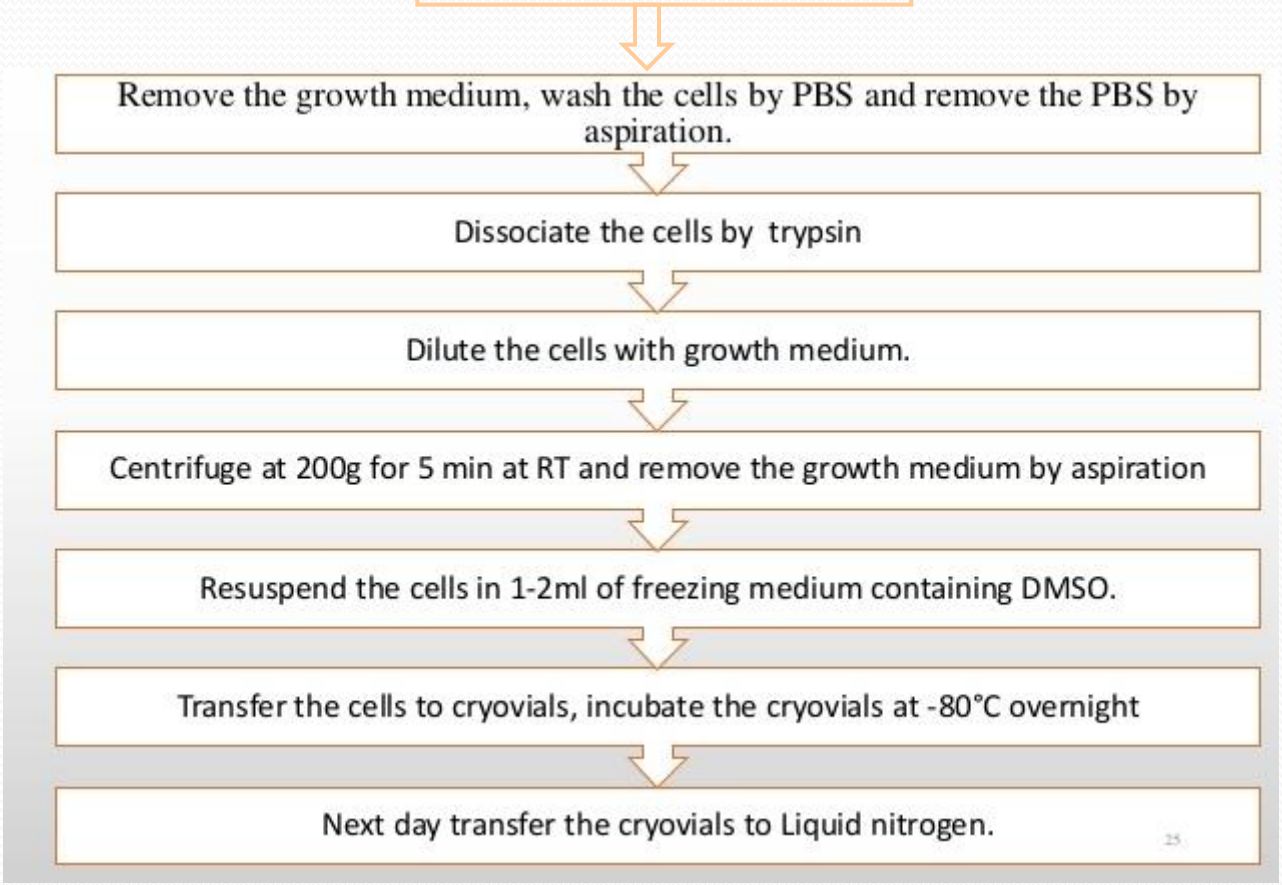
• Yeast, Bacteria and fungi usually shows some characteristics feature like changes in turbidity and pH of the medium.

• Mycoplasma contamination is very serious problem of the cell culture and can't be decontaminate very easily.

•. Mycoplasma can be detected by staining with DNA staining dye (Hoechst 33258) and PCR amplification of mycoplasmal RNA.

•The best way to eliminate the contamination is to discard the infected cell lines.

4. Cryopreservation



5. Some basics cell culture Instruments:

Laminar Air Flow Cabinet

Autoclave

Co2 Incubators

Refrigerator

Culture Flask

Inverted Microscope

Water Purification System

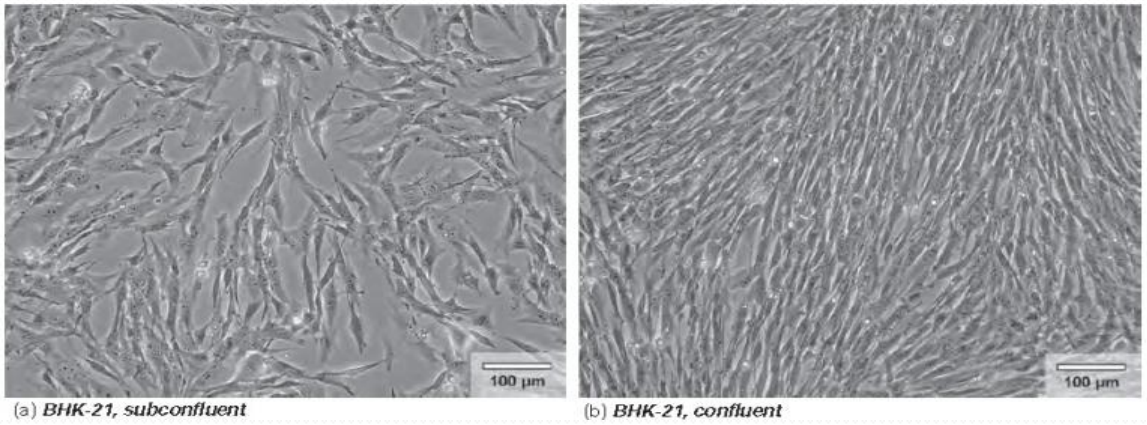
Syringe Filter

Centrifuge Machine

Morphology Identification

•The morphological identification of cell can be done under the Microscope, we can indentify the cells based on cell shape and size.

• Also the growth pattern of the cell can be predicted whether semi-confluent or confluent.



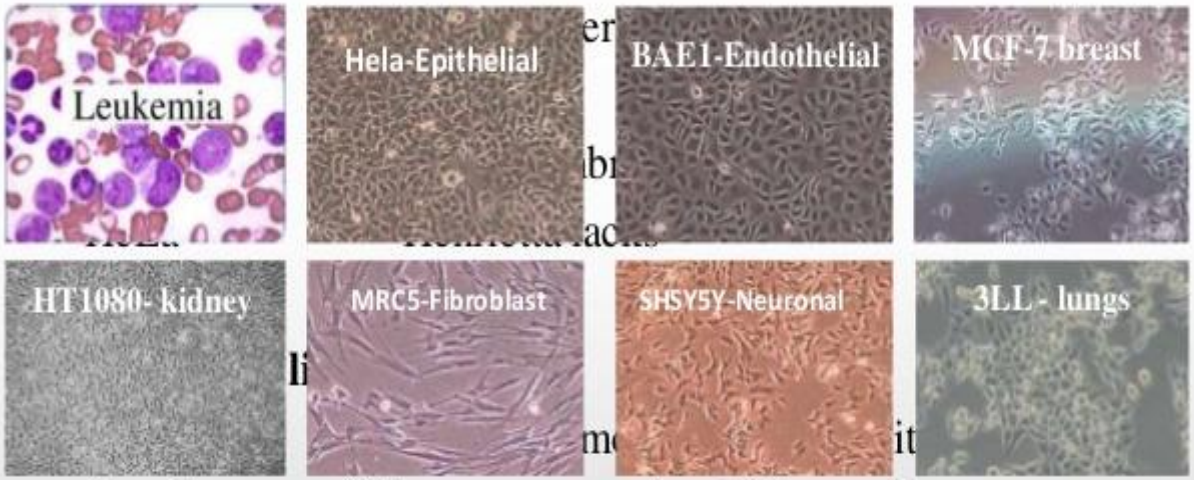
(a) *BHK-21, subconfluent*

(b) *BHK-21, confluent*

(e) *Vero, subconfluent*

(f) *Vero, confluent*

Some most common Human cell line system



- Cos-7 African green monkey kidney cells
- And others such as CHO from hamster, sf9 & sf21 from insect cells

Characterization of the cell lines

TABLE 15.1. Characterization of Cell Lines and Cell Strains

Criterion	Method
DNA profile[a]	PCR of microsatellite repeats
Karyotype[a]	Chromosome spread with banding
Isoenzyme analysis	Agar gel electrophoresis
Genome analysis	Microarray
Gene expression analysis	Microarray
Proteomics	Microarray
Cell surface antigens	Immunohistochemistry
Cytoskeleton	Immunocytochemistry with antibodies to specific cytokeratins

Most Suitable Authentication

Freshney, 2010

Different Cell Bank agencies worldwide provides characterized cells for study:

Cell Bank	Web Site
NCCS (National centre for cell science), Pune	https://www.atcc.org
ATCC (American Type Culture Collection)	https://www.atcc.org
Coriell Cell repository	https://www.coriell.org
ECACC (he European Collection of Authenticated Cell Cultures)	https://www.phe-culturecollections.org.uk/collections/ecacc.aspxhttps
DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen)	https://www.dsmz.de
JCRB (apanese Collection of Research Bioresources Cell Bank)	https://cellbank.nibiohn.go.jp/english
RIKEN gene bank	https://en.brc.riken.jp

T-47D (ATCC® HTB-133™)

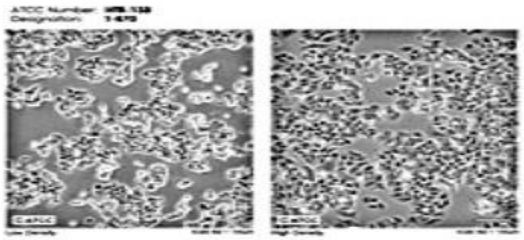
Organism: ***Homo sapiens*, human** / Tissue: **mammary gland; pleural effusion** / Cell T

GENERAL INFORMATION | CHARACTERISTICS | CULTURE METHOD | SPECIFICATIONS | HISTORY

Karyotype

This is a hypotriploid human cell line. The modal chromosome number is 65 occurring at 50% and polyploidy at 0.8%. 18 marker chromosomes are common to most cells, of which 7 are paired and 11 are single-copied. The t(8q14q), t(9q17q), t(10q17p) are among 7 paired markers common to most cells. N7, N9, and N10 are absent and N11 is generally present in 4 copies. DM's occurred, but infrequently. Q-band examination did not show the presence of a Y chromosome.

Images



Derivation

The T-47 line was isolated by I. Keydar from a pleural effusion obtained from a 54 year old female patient with an infiltrating ductal carcinoma of the breast.

Clinical Data

female
The T-47 line was isolated by I. Keydar from a pleural effusion obtained from a 54 year old female patient with an infiltrating ductal carcinoma of the breast.

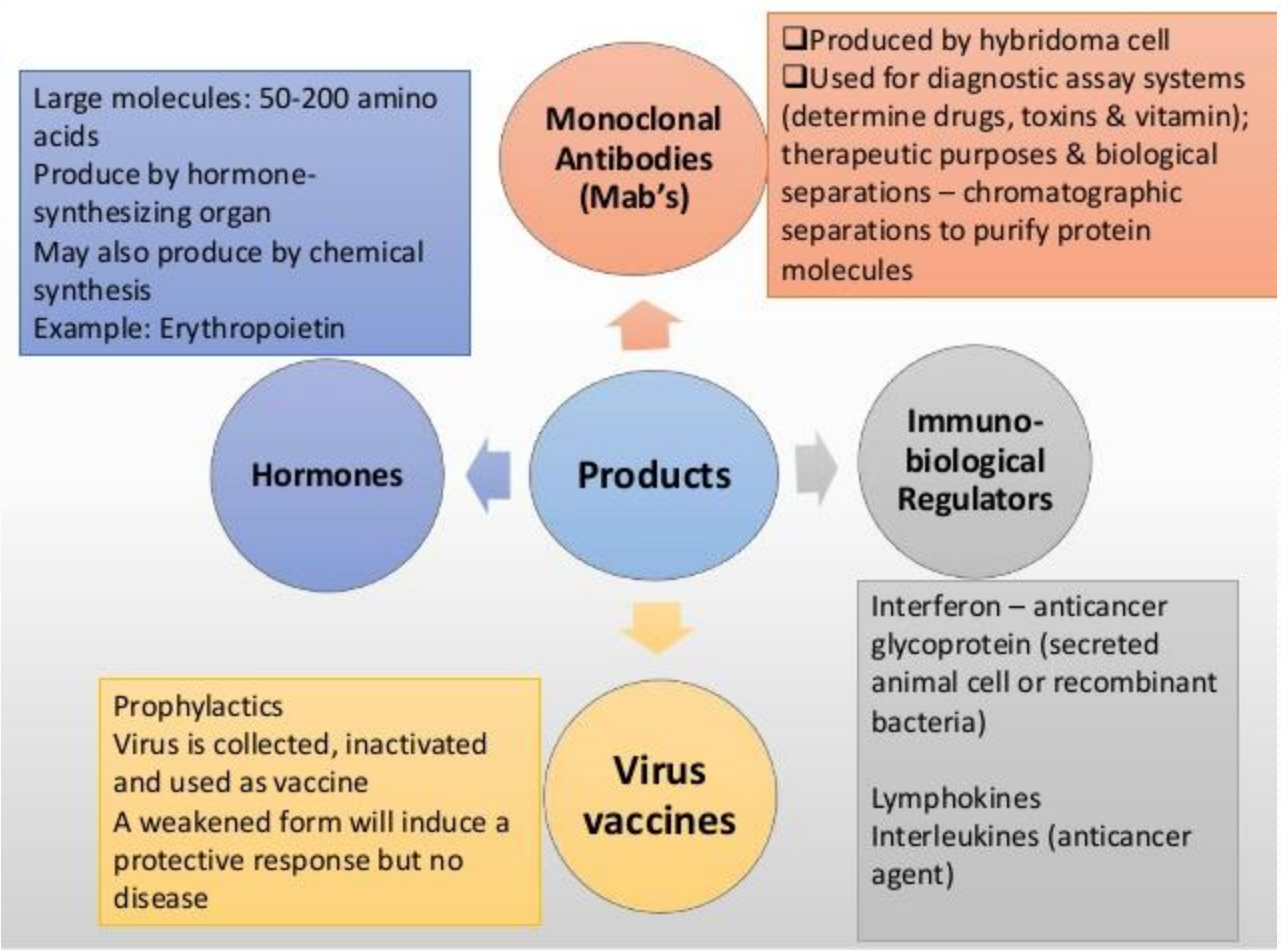
Markers

calcitonin, expressed
androgen receptor, expressed
estrogen receptor, expressed
progesterone receptor, expressed

Application of the cell lines

•Cell culture based products have lots of therapeutics applications.

There are following products are available in the markets:



References:

1. Fresheny, I. “Culture of Animal Cells- A Manual of Basic Technique and Specialized Applications”.

2. http://www.biology-online.org/dictionary/Cell_matrix

Thank You