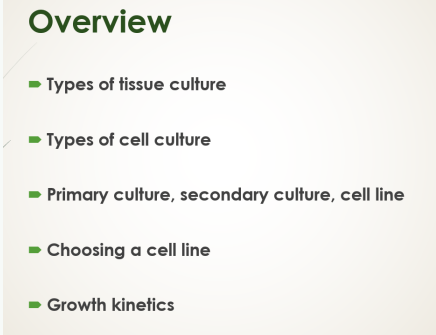
PTC - C7 (Basic Animal Cell Culture) Cheat Sheet

by woozing via cheatography.com/146689/cs/31839/

Overview



Application

1. The study of basic cell biology, cell cycle mechanisms, specialized cell function, cell–cell and cell–matrix interactions.

2. Toxicity testing to study the effects of new drugs.

3. Gene therapy for replacing nonfunctional genes with functional gene-carrying cells.

4. The characterization of cancer cells, the role of various chemicals, viruses, and radiation in cancer cells.

5. Production of vaccines, mABs, and pharmaceutical drugs.

6. Production of viruses for use in vaccine production (e.g., chicken pox, polio, rabies, hepatitis B, and measles).

Types of Tissue Culture

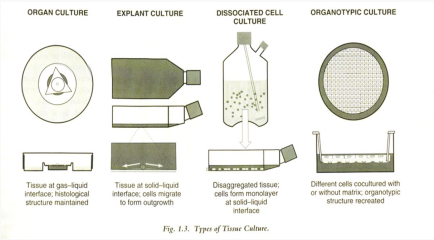


Fig. 1.3. Types of Tissue Culture.

Explant Culture Procedure

1. Obtaining the Explant	-obtained surgically using sterile equipment from mammals, rodents or avian organs or tissues
	-ex 1: a piece of gingival tissue following tooth extraction can be removed as an explant to establish human gingival fibroblasts
	-ex 2: a piece of adipose tissue can be used to establish mesenchymal stem cells

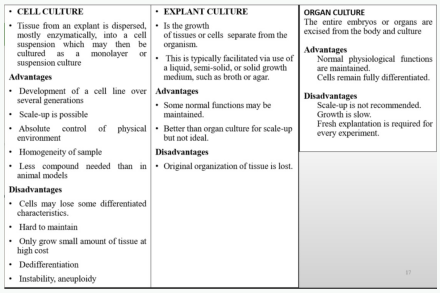
Explant Culture Procedure (cont)

2. Cut and Clean the Explant	-place the explant in a petri dish containing around 1-2 mL of incomplete medium (medium without serum)
	-using a sharp surgical blade, you can cut it (usually around 1×1 mm pieces)
	-collect the pieces of explant using a sterile forceps and wash gently
	-washing can be done by transferring pieces into a centrifuge tube containing around 0.5 mL of incomplete medium
	-gently mix by pipetting the medium 4 to 5 times, and allow the pieces to settle down and remove the upper medium
	-can be repeated 2 or 3 times
3. Culturing the Explants	-obtained explants are aseptically placed on a coated surface and allowed to attach to the surface in the presence of a rich culture medium
	- medium ex: basal minimal media, Dulbecco's Modified Eagle Medium (DMEM) or Minimum Essential Medium Eagle (MEM) supplemented with 10-15% serum
	-cultured in standard tissue culture conditions (pH 7.2-7.4, temperature 37°C, 5% CO2 and humidity) to allow for cell migration and proliferation

Explant Culture Procedure (cont)

	-change the media every 3 days without disturbing the explants
	-depending upon the health and age of the tissue, cells emerge out of the explant within 15-30 days
	-once outgrowth of cells starts from the explant, add 5 mL of medium to the flask in subsequent days
4. Once outgrowth of cells starts from the explant, add 5 mL of medium to the flask in subsequent days	-after the explants are completely surrounded by the cells, you can trypsinise the cells and subculture.
	-it is better to use a lower concentration of trypsin (e.g. <0.25% of trypsin for 5 min)
	-choose an appropriate size of flask for seeding, depending on the total number of cells obtained

Pros and Cons of Types of Tissue Culture



CELL CULTURE

- Tissue from an explant is dispersed, mostly enzymatically, into a cell suspension which may then be cultured as a monolayer or suspension culture

Advantages

- Development of a cell line over several generations
- Scale-up is possible
- Absolute control of physical environment
- Homogeneity of sample
- Less compound needed than in animal models

Disadvantages

- Cells may lose some differentiated characteristics.
- Hard to maintain
- Only grow small amount of tissue at high cost
- Dedifferentiation
- Instability, aneuploidy

EXPLANT CULTURE

- Is the growth of tissues or cells separate from the organism.
- This is typically facilitated via use of a liquid, semi-solid, or solid growth medium, such as broth or agar.

Advantages

- Some normal functions may be maintained.
- Better than organ culture for scale-up but not ideal.

Disadvantages

- Original organization of tissue is lost.

ORGAN CULTURE

The entire embryos or organs are excised from the body and culture

Advantages

- Normal physiological functions are maintained.
- Cells remain fully differentiated.

Disadvantages

- Scale-up is not recommended.
- Growth is slow.
- Fresh explantation is required for every experiment.

Primary Culture

-cultures prepared from tissues taken directly from animals

1. Organ Culture	(google)-organ culture is able to accurately model functions of an organ in various states and conditions by the use of the actual in vitro organ itself
	-maintenance of a piece of tissue, a part of organ or a whole organ in vitro

Primary Culture (cont)

2. Primary Cell Culture	-when taken tissue is dissociated, mechanically or enzymatically, into single cells which could be plated on a coated surface
3. Slice Tissue Culture	-referred to as explant or organotypic cultures
	-small pieces of tissue of interest are simply allowed to attach to an appropriate substrate and are cultured in enriched media
4. Re-aggregate Culture	-dissociated cells is kept in suspension rather than allowed to settle on and attach to solid substrate
	-cells tend to re-aggregate into small balls
	-allowed cells cells to develop in three dimensions
5. Histotypic or histoculture	-culture of intact tissues

(Google) - Histotypic culture is defined as three-dimensional culture of one cell type, while the term organotypic implies the interaction of two or more cell types from a complex tissue or organ.

Types of Cells

1. Epithelial-Like	-cells that are attached to a substrate and appear flattened and polygonal in shape
2. Lymphoblast-Like	-cells that do not attach normally to a substrate but remain in suspension with a spherical shape
3. Fibroblast-Like	-cells that are attached to a substrate and appear elongated and bipolar, frequently forming swirls in heavy cultures

It is important to remember that the culture conditions play an important role in determining shape and that many cell cultures are capable of exhibiting multiple morphologies.

Types of Cell Culture

1. Primary Cell Culture	-Adherent Cell Culture
	-Suspension Cell Culture
2. Secondary Cell Culture	-
3. Cell Line	-Finite Cell Line
	-Continuous Cell Line

1. Primary Cell Culture

-maintenance of growth of cells in culture medium using suitable glass or plastic containers

-using the mechanical or enzymatic methods

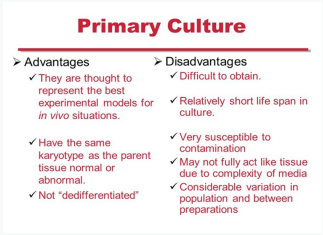
-dissociated directly from the parental tissue (such as kidney, liver)

-they will attach, divide and grow

2 types of primary cell culture depending upon the kind of cells in culture

a) Anchorage Dependent /Adherent cells	-require attachment for cell growth
	-monolayer culture system
	-usually derived from tissues of organs such as kidney where they are immobile and embedded in connective tissue
	(google)-have to be detached from surface before being subcultured
	(google)-growth limited to surface area
b) Suspension Culture/Anch-orage Indepe-ndent cells	-do not require attachment for cell growth/do not attach to the surface of the culture vessels
	-all suspension cultures are derived from cells of the blood system because these cells are also suspended in plasma in vitro e.g. lymphocytes

Pros and Cons of Primary Cell Culture



2. Secondary Cell Cultures

-When a primary culture is sub-cultured, it becomes known as secondary culture or cell line.

3. Cell Line

-cell population derived from a primary culture at the first subculture

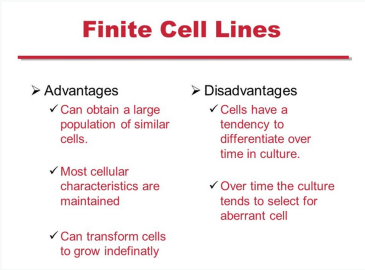
(google)-usually clonal, meaning that the entire population originated from a single common ancestor cell

-the term does not imply homogeneity or the degree to which a culture has been characterized

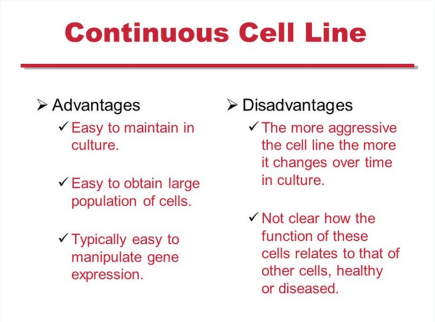
may be finite or continuous depending upon whether it has limited culture life span or it is immortal in culture

a) Finite Cell Lines	-cell lines which have a limited life span and go through a limited number of cell generations
	-growth rate is slow and doubling time is around 24-96 hours
b) Continuous Cell Lines	-grow indefinitely
	-cell lines transformed under laboratory conditions or in vitro culture conditions give rise to continuous cell lines
	-growth rate is rapid and doubling time is 12-24 hours
c) Transf-ormed Cell Line	-cell lines obtained from tumor cells
d) Clonal Cell Line	-cells could be cloned in continuous cell lines to obtain genetically homogenous population

Pros and Cons of Finite Cell Lines



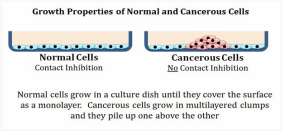
Pros and Cons of Continuous Cell Line



Difference of Normal and Transformed Cells

Normal Cells	Transformed Cells
1. Anchorage-dependent (except blood cells)	1. Nonanchorage-dependent
2. Density-dependent inhibition of proliferation	2. No density-dependent inhibition of proliferation
3. Mortal; Finite Cell Line	3. Immortal; Continuous Cell Line
4. Contact Inhibition; Monolayer Culture	4. No Contact Inhibition; Multilayer Culture
5. Dependent on external growth factor signals for proliferation	5. May not need an external source of growth factors
6. Greater retention of different-iated cellular function	6. Typically loss of differentiated cellular function
	-shorter population doubling time
	-reduced substrate adhesion
	-genetic instability (e.g. show heteroploidy and aneuploidy)

Contact-Inhibition of Growth



Density-dependent Inhibition of Proliferation

-reduction in proliferative activity that correlates with the attainment of confluency, that is，occupancy of all available attachment surface

-can occur before confluence is reached, and reflects diminished nutrient supply and the release of cell-derived factors (including waste products) into the medium

Saturation Density	-population density (cells/cm2) at the point when it reaches density-dependent inhibition of growth
	-population density (cells/cm2) at the point when it reaches density-dependent inhibition of growth

Cell Ageing in Culture

-also known as In vitro cell senescence

-involve progressive alterations in a number of cell characteristics

Normal cell lines commonly have a finite lifespan, that is, they do not grow beyond a finite number of cell generations (population doubli-ngs).

-Eg, the lifespan of normal diploid fibroblasts is in the range of 50-70 population doubling.

Transformed Cells

-cancerous cells

-possess all six hallmarks of cancerous cells :

1. Growth factor independency
2. No response to growth inhibitors
3. Evasion of apoptosis (Natural cell death)
4. Can promote angiogenesis (the development of new blood vessels)
5. Unlimited proliferation - rapid increase
6. Invasive - tending to spread very quickly and undesirably or harmfully

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Immortalization

-Cell lines that have unlimited lifespan arc termed immortal or, preferably, continuous

the term immortalized and transformed are not synonymous

Although infinite lifespan is generally considered to be a characteristic of transformed cells, not all continuous cell lines exhibit alterations in growth control attributed to cellular transformation.

Immortalized Cells

-not yet cancerous, but have sufficient mutations to be able to be passaged forever, unlike non-transformed, non-immortalized cells, which all have a finite passage number

-population of cells from a multicellular organism due to mutation, which can escape normal cellular senescence and keep undergoing division

-this kind of cells can grow in vitro for prolonged periods

Cell Strain

-describe a subcultured population selected on the basis of its expression of specific properties, functional characteristics, or markers

Clonal Culture / Clonal Selection

-clone

-establishment of a cultured cell population derived from a single cell

Sub-culturing (or passage)

-Transfer or transplant cells of an ongoing culture to a new culture vessel so as to propagate the cell population or set up replicate cultures for study.

-Subculturing or splitting cells is required to periodically provide fresh nutrients and growing space for continuously growing cell lines.

-Such cultures may be called secondary cultures (first subculture from primary culture)

Criteria for Subculturing

1. Cell concentration: should not exceed 1 x 10^6 cells/mL for most suspension-growing cells

2. pH: which is linked to cell concentration, and declines as the cell concentration rises

3. Time since last subculture: should fit a regular schedule

4. Cell production requirements: for experimental or production purposes

Pros and Cons of Animal Cell Culture

Advantage	Disadvantage
1. Controlled physiochemical environment (pH, temperature, osmotic pressure, O2, osmolarity etc.)	1. Expertise is needed, so that behavior of cells in culture can be interpreted and regulated.
2. Controlled and defined physiological conditions - nutrient concentration, cell to cell interactions, hormonal control.	2. Need of expertise and technical skill for the development, and regular use of tissue culture.
3. Homogeneity of cell types (achieved through serial passages)/ Homogenous genetic population	3. Ten times more expensive for same quantity of animal tissue; therefore, reasons for its use should be compelling.
4. Economical, since smaller quantities of reagents are needed than in vivo.	4. Unstable aneuploid chromosome constitution.
5. Legal, moral and ethical questions of animal experimentation are avoided.	5. Cost factor is a major limitation.
6. Cost effective screening assays	-Establishment of infrastructure, equipment and other facilities are expensive.
7. Easy production of biopharmaceuticals	-It is estimated that the cost of production of cells is about 10 times higher than direct use of animal tissues.
8. Available in adequate numbers to do chemical study	6. Control of the environmental factors (pH, temperature, dissolved gases, disposal of biohazards) is not easy.
9. Easy to add genes (transfection) or regulate protein levels (RNAi)	7. The native in vivo cells exist in a three- dimensional geometry while in in vitro tissue culture, the propagation of cells occurs on a two dimensional substrate.

Pros and Cons of Animal Cell Culture (cont)

-Due to this, the cell to cell interactive characters are lost.

8. The cell lines may represent one or two types of cells from the native tissue while others may go unrepresented.

9. Tissue culture techniques are associated with the differentiation i.e. loss of the characters of the tissue cells from which they were originally isolated.

-This happens due to adaptation and selection processes while culturing.

10. Continuous cell lines may result in genetic instability of the cells.

-This may ultimately lead to heterogeneity of cells.

Growth Measuring Methods

1. Direct Methods	
2. Cells	-Packed Cell Volume
	-Cell count and viability
	-Colony forming unit
	-Optical density (OD)
3. Tissues	-Fresh weight and dry weight
4. Indirect Method	-Mostly used for large-scale cultures

Growth Observing

1. Increase in turbidity of cells	
2. Increase in size of tissues/ explants	-swelling
	-curling
	-proliferation
3. Decrease in turbidity and size	-death

Growth Observing (cont)

	-apoptosis and necrosis
4. Microscopic observation	-Stereoscope
	-Inverted microscope

Necrosis is caused by factors external to the cell or tissue, such as infection.

Characterization of Cell Lines

a) growth rate

b) karyotyping (C11)

Growth Curve

-established taking into consideration the population doubling time, a lag time, and a saturation density of a particular cell line.

1. Lag Phase	The time the cell population takes to recover from such sub culture, attach to the culture vessel and spread.
2. Log Phase	In this phase the cell number begins to increase exponentially.
3. Plateau Phase	During this phase, the growth rate slows or stops due to exhaustion of growth medium or confluency.

Bacterial Growth Curve

-Unicellular organisms divide by binary fission

-Each cell grows to full size, replicates its genetic material then divides into two identical daughter cells.

-By identical means, two cells divide into four, four into eight and so on, leading to an exponential increase in cell numbers: 1 → 2 → 4 → 8 →2^n

-If we were to plot the number of cells in a population against time, we would get an exponential curve

-Growth usually slows down due to:	a) supply of nutrients becoming exhausted
	b) because metabolism leads to an accumulation of harmful waste substances

Bacterial Growth Curve (cont)

Lag Phase	-When an inoculum of bacteria is first introduced into some growth medium, it will probably require a period to adapt to its new surroundings
	-When an inoculum of bacteria is first introduced into some growth medium, it will probably require a period to adapt to its new surroundings
	-Eg, the carbon source in the medium is unfamiliar, the cells will need time to synthesise the necessary enzymes for its metabolism.
	-Synthesize molecules needed for protein synthesis and enzymes required for cell division
	-no net increase in bacterial numbers, however the cells are metabolically active.
Length of the lag phase depend on:	a) age and general health of the cells in the inoculum
	b) conditions of bacteria before transfer into growth medium
	c) content of the growth medium
Log (expon-ential) Phase	-When the bacteria have acclimatized to their new environment and synthesized the enzymes needed to utilize the available substrates, they are able to start regular division by binary fission.
	-leads to the exponential increase in numbers

Bacterial Growth Curve (cont)

	-under optimal conditions, the population of cells will double in a constant and predictable length of time, known as the generation (doubling) time.
	-Cells are dividing at maximal rate
	-Cells are most susceptible to the action of antibiotics and other deleterious agents
Stationary phase	-exponential phase is limited by environmental factors, and as the rate of growth slows down, the culture enters the next phase
	-The levelling out of the growth curve does not mean that cell division has ceased completely, but rather that the increase due to newly formed cells is cancelled out by a similar number of cell deaths.
	-Occurs when the number of viable cells stops increasing
	-Due to nutrients being used up and/or toxic products accumulating from cell's metabolism
	-as the death rate increases, the overall numbers fall and we enter the final phase of growth.
Death (or de-cline) phase	-As cells die off and the culture is unable to replace them, the total population of viable cells falls.
	-Exponential decrease in the number of viable cells