Cell Culture Technologies
Prof. Mainak Das
Department of Biological Science & Bioengineering & Design Programme
Indian Institute of Technology, Kanpur

Lecture – 06
Biology Of The Cultured Cells

Welcome back to the lecture series in animal cell culture or cell culture technologies. So, in the first week, we introduced the subject and then we went on exploring the different aspect of the biology of the cultured cells. So, if we recap, when we talked about culturing the cells, we talked about you know take a part of the tissue make a single suspension and then you culture it on a dish.

So, first thing which has to be considered; what is the demand of the cell of these cells for oxygen. So, we talked about some kind of a condition creating a condition where proper oxygen to carbon dioxide balance is being maintained, then the dish where you are growing the cell should mimic the extracellular matrix of the cell in your condition that is the animal and we talked about the role of extracellular matrix how these cementing materials plays a critical role in determining the fate of the cell.

Post that of course, while talking about the adhering bring of the cell, we talked about classification one has to understand is it adhering cell, we are culturing or we are culturing a non adhering cell then followed by this, we talked about the cell cycle progression, are we dealing with cells with limited time division or we are dealing with fully differentiated cell and we introduce some of the terminologies; cell cycle leading to cell division followed by differentiation and at times differentiation where the cell loses its as per the definition permanently loses its function which it is supposed to do post differentiation, then we talked about de-adaptation where the cell which suppose to produce a specific function de-adapts or does not perform at under a specific conditions, but that could be rectified see in other words de adaptation is a reversible phenomena.

So, these are some of the points and finally, I insisted you to understand that how many passages a cell lines; suppose you are using a cell line which has been immortalized or it can grow n number of cycles, but then to a fundamental question come; what is a practical number, really a cell can be use over possible you know division; what we are ritualizing. So, one really has to know that because otherwise through every passage a

cell goes through a life cycle and the cell loses some of its telomere and eventually lot of its function kind of gets compromised, but then it all depends how you want to treat the cell and for what purpose you are growing the cell.

Are you using it for a specific sensor application or you are using it for understanding certain event happening at the cellular level or you are using it for some kind of hybridoma technique like you know antibody production or you are using it for production of some other x, y, z chemical. So, that particular aspect will determine how much carefully have to be with cell type you are dealing with. So, let us resume the second week by kind of summarizing the aspect what we have already dealt and couple of other aspect which we have not talked about to start off with.

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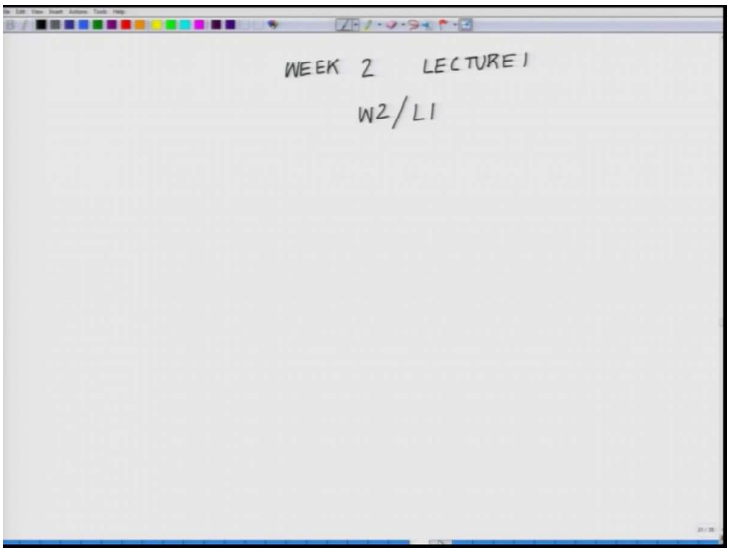

So, we are into week 2, lecture 1; W 2, L 1.

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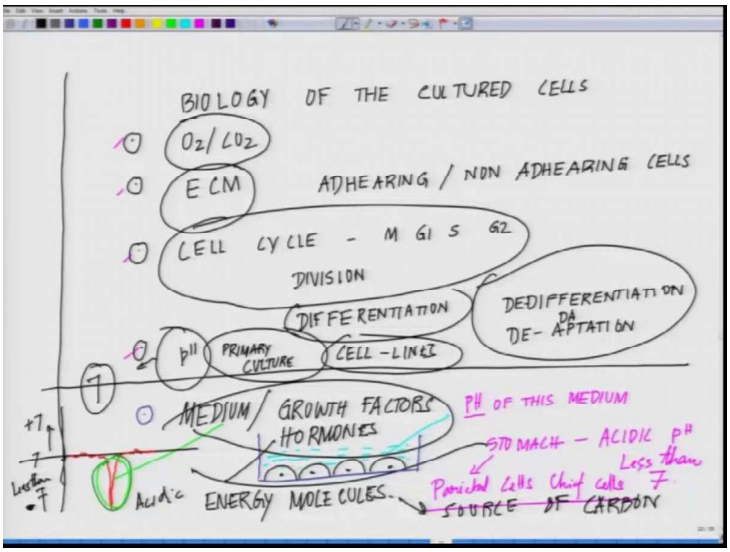

So, talking about biology of the cultured cells, so, the point we have already dealt is oxygen and CO2 environment, whatever we are maintaining, we talked about extra cellular matrix and we talked about adhering and non-adhering cells, then we talked about cell cycle progression. So, we will be having mitosis phase G 1 phase synthesis phase G 2 phase and talked about cell division then we talked about differentiation then dedifferentiation and de-adaptation; this is what we have already covered. This part is there with us, there are few more points in order to completely appreciate the biology of the cultured cell; what we will be dealing with and one of the very critical important things what we will be dealing with is the PH under what PH a cell is an adapted to.

So, generally we know our body PH is at 7, but while you are growing the cell, there are or let us put it other way; while the cells are growing in your body, we have this blood which is slowing through you have these extra cellular matrix proteins; extra cellular matrix solution which is continuously washing out that spot ensuring it to make a PH 7. So, when we talk about cell culture just for your imagination sake, think of it, here we have a dish and, in that dish, we have the cells which are growing this half moon shaped; these are the cells and here you have the medium in which they are growing. Now how one can handle the PH of this medium and how do we know what kind of PHB cells will prefer why I am telling you this.

See for example, we have to culture the cells of the stomach which digest the food at a very acidic PH of course, from our knowledge of physiology, we know that that this acid production by the cells parietal cells chief cells which have present in the just for those taking this example in the stomach which is which functions at a very acidic PH. So, it means less than 7. So, the cells which are involved in this is parietal cells chief cells, all these different cells; these cells can withstand at will a very low PH for a transient period of time because of course, in the stomach it is not that it is a place which is filled with acid these cells are specifically produces the acid by reacting the hydrogen iron and the chloride iron.

But what is important for us to realize that it is not every cell and they utilize this acid to break the food and soon after that this acid is kind of you know again re-absorbed or kind of you know it is destroyed. So, for transient period of time if I have a PH scale out in the stomach, if you think of it, something like PH scale here, so, if I say these are this is 7 and this is plus 7 onward; these are my below 7, see for example, less than 7. So, what we will observe is and this is the of course, the acidic side. So, from the base line value during digestion, thus this environment will go like this for a finite period of time, this thing happens. So, then what we interpret from this is the cells in the stomach can withstand a very low PH for a transient period of time, but in order to regain that kind of activity we have to create such a situation.

So, if we are culturing these kinds of cells, we should know that what is the level they can withstand. Similarly, there are cells if you bring them down to that kind of PH level, they will die, they will not be able to withstand that kind of high proton concentration, right. So, maintaining the PH of a system is extremely critical and knowing the window to which a cell can withstand it because when we are growing things, we are not removing all are the Debris which the cells are producing that kinds of create a situation where the PH may automatically fail there is always a possibility and there is always a danger in the cell culture that your cultured dish goes on an acidic frame. So, this is something which one has to be very careful this is one aspect.

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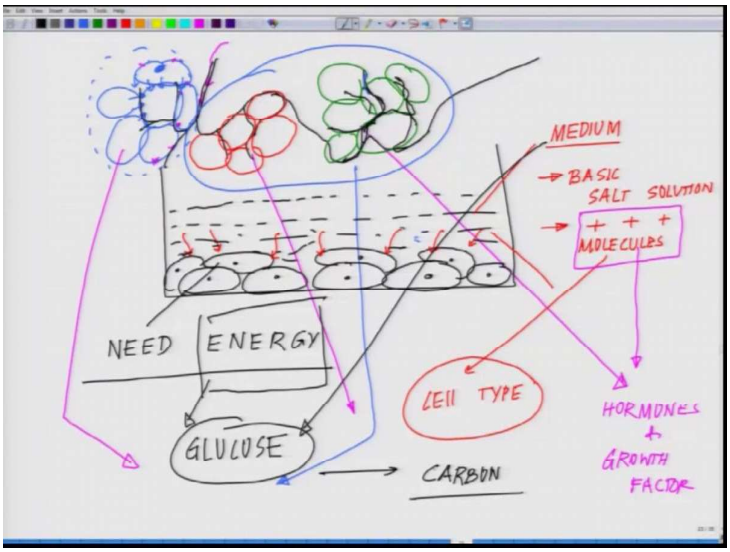

Second aspect in that line is that we have talked about the PH, now to add up what all we have talked about. So, here we have now talked about the PH, the next what will be talking about is see for example, here the cells are growing in a dish adhering cell assuming that these are all adhering cells and these adhering cells are dividing, this is the medium where they are growing. Now on at this condition these cells which are dividing will need energy and it is only source of energy is in medium because none of these cells are synthesizing their own food material they are depending on the medium to supply them with energy.

Now, 2 things; we are introducing one has to know what kind of medium, we are going to use because though the basic salt solution remain more or less same, but at different spots of the body different parts of the body, there are several other added molecules for specific cell types, these are cell type is specific you might wonder, how that is happening. So, such thing happens, see for example, if you consider your body just that old analogy; what I gave you for example, these are colonies of cells in your body some are red some are green some are blue like this and blood vessel is traveling like this which is bringing the necessary nutrients out here, but still every cell type has a specific requirement.

So, many of the factors which see for example, these colony of blue cells will be needing, they are surrounding brothers and sisters secrete those and through blood

vessels or through direct contact, these are being you know transmitted to this cells or blood brings specific say hormones or growth factor whose receptors are very unique on specific cell type. So, in a way again coming back to that fundamental concept that cells in a very dynamic environment extremely dynamic extremely changing, whereas, whenever we do a cultured we essentially follow very static model at least as of now the most of the models which are being followed across the world are very static model.

So, each cell type possibly needs if we are growing a pure culture of any specific cell type, say for example, I grow these blue cell as separately or the red cell as separately or the green cell as separately, I mean it to provide those specific molecules which are cell type is specific, they mostly come in the form of hormones and grow factors the complexity arises if I have to grow, say for example, 2 different cell types together under that situation see green needs a different kind if growth factor and red needs a different kind of growth factor. So, how I ensure that the growth factor received by green which I am providing from outside out here does not interact with the red, there is only one possibility either red does not have a receptor for it then you are very lucky or red will have receptor for it.

And that adds up to the complexity in a real life what happens there are blood vessels which are crawling through and they ensure that these binds there, but outside the system that privilege is lost. So, those adds up the complex situation of growing cell in a very controlled environment further, we talked about when we talked about; we talked about the energy molecule. So, every cell, if you look at it mostly they are readily source energy source is glucose. So, every medium what we develop will have glucose as one of the major sources of energy and more over carbon, but that comes with some set of problems which will be coming later we use glutamine and other sources there are reason and rhyme.

So, with this I will close in on to this part I will add that last tail piece what all we have covered. So, we talked about oxygen and carbon dioxide we talked about extra cellular matrix, we talked about the cell cycle dedifferentiation de adaptation and of course, we talked about the cell lines and in between we have talked about little bit very little bit about primary culture and now we talked about then we talked about the PH acetic or basic PH where the most of the cell groups at 7; PH 7 and then we talked about medium

growth factors hormones and the need for energy molecules or source of carbons source of carbon.

So, these are the very basic fundamental; what one has to keep in mind while one is planning to do cell culture and if you have the basic knowledge physiology, then these things should come very handy fine you know; what all my body needs; there is the same condition or close to the same condition, I will have to provide; now if you remember, when we talked in one of the very early classes that whenever I am growing the cells.

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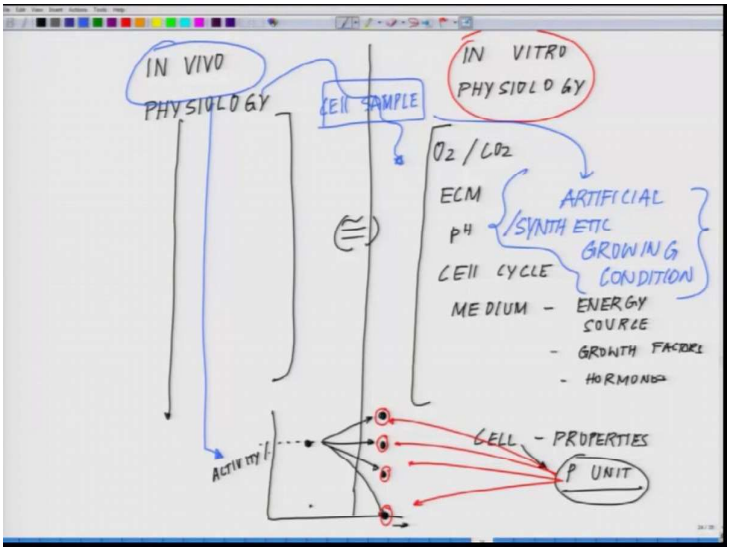

So, see for example, this is my knowledge of in vivo physiology or physiology which is happening inside the cell and here I am with in vitro physiology, what we talked about is in vitro physiology in the form of oxygen CO2 tension extra cellular matrix PH cell cycle medium culture medium of course, and this you have energy source growth factors and hormones.

Now, what is critical for it know is after growing the cells in all these conditions which is we are our goal is to you know come as close as possible almost; similar almost congruent then what we have to do the cells which are growing here their properties have to be evaluated. Say for example, the cell produce certain level of activity an ensign say for example, it has an anti oxidative ensign capacity and it produce a P unit of it, now I have to compare it with in in vivo condition; how this cell is functioning and what is the

quantification parameter of this particular one property or many properties as a matter of fact and if under in vivo condition see for example, if I say activity in the y axis.

So, if the activity of a particular thing is somewhere here seen for example, how far I am in in vitro condition; am I exceeding it am I below it is I seem at it. So, depending on where am I; I have to understand the biology of this cell which is under growing in any in vitro setup or I am not even performing that function I am here 0. So, this sight the one I am circling with red are the one which are the in vitro physiology of the one imaginary aspect the P unit; what I am trying to telling. So, there comes the real challenge how much you have understood the biology of the cell of the cultured cell and how much we have appreciated the in vivo physiology of that particular tissue from where you have derived the cell sample in order to create an artificial condition artificial or synthetic growing condition, fine.

So, understanding of the basic physiology and correlating it with in vitro physiology is very important for a successful execution of an experiment validity of the data in real life and understanding the short falls and short comings what you are under growing while you know performing this task. So, with this; this is our first class and the second week we will move on to the layout and designs.

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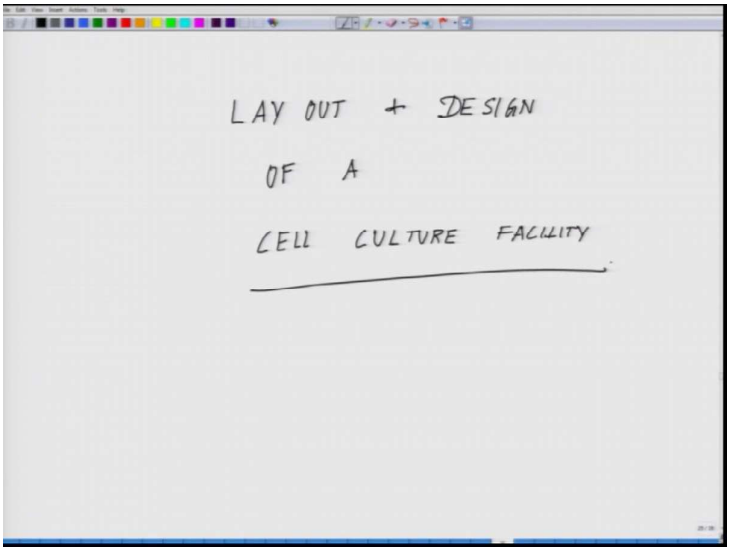


So, next our objective will be move on to with this basic start of I will move on to layout and design of a cell culture facility. So, I will close in here please go through the points and think over it ponder upon it and develop your own philosophy about the subject.

Thank you, thanks for your patience listening.

Module 6 Lecture 1

Cell Culture

Cell culture is the multifaceted process through which cells are isolated from animal or plant and their subsequent growth under controlled artificial conditions, generally outside their natural environment. In this procedure cells are directly isolated from body or disaggregated by enzymatic or mechanical procedure or they may be derived from cell lines or cell strains. The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture. The in vitro propagation of cells has become a common practice in many laboratories for a huge numbers of applications. The ranges of cell types grown are vast. Generally the cells are sensitive to a wide range of compounds and it is therefore necessary to ensure that they come into contact only with those under study and not with extraneous materials. Adherent mammalian cells require a suitable surface for attachment.

Primary cell culture: Primary cell culture is the primary step of cell culturing in which the cell is first isolated from tissue and then proliferated under the appropriate conditions until they consume all available contents for their growth. Now the cell is ready for subculturing by transferring them to new growth medium that furnish more opportunity for continued growth.

Cell lines: A cell line is a permanently established cell culture that will proliferate indefinitely in appropriate fresh medium and space. Cell lines differ from cell strains in that they have absconded the Hayflick limit and become immortalised. The Hayflick limit (or Hayflick Phenomenon) is the number of times a normal cell population will divide before it stops, presumably because the telomeres reach a critical length. A cell line arises from a primary culture at the time of the first successful subculture. The terms finite or continuous are used as prefixes if the status of the culture is known.

Cell Strain: By applying cloning, the positive population of cell lines are selected, therefore this cell lines now becomes a cell strain. A cell strain often acquires additional genetic changes resulting to the initiation of the parent line.

There are two types of cell culture:

Continuous cell culture: A continuous cell culture is one that is apparently capable of an unlimited number of population doublings, often referred to as an immortal cell culture. Such cells may or may not express the characteristics of in vitro neoplastic or malignant transformation. Continuous cell lines are usually aneuploid and often have a chromosome number between the diploid and tetraploid values. There is also considerable variation in chromosome number and constitution among cells in the population (heteroploidy).

Some important properties of Continuous cell lines:

- Reduced serum requirement
- Reduced density limitation of growth
- Growth in semisolid media
- Aneuploidy

Several normal cells do not give rise to continuous cell lines. The classical example are normal human fibroblasts that remain euploid throughout their life span and at crisis (usually around 50 generations) will stop dividing, although they may remain viable for up to 18 months thereafter. Human glia and chick fibroblasts behave similarly. Epidermal cells, on the other hand, have shown gradually increasing life spans with improvements in culture techniques.

Finite cell culture: A finite cell culture is capable of only a limited number of population doublings after which the culture ceases proliferation.

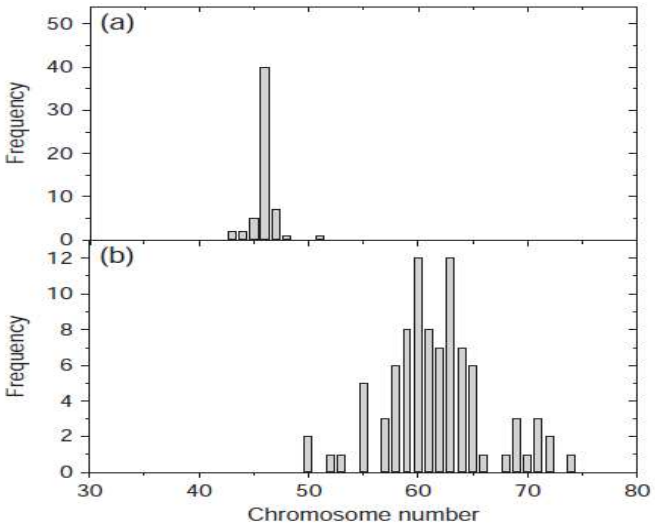


Figure 1: Chromosome Numbers of Finite and Continuous Cell Lines.

(a) A normal human glial cell line. (b) A continuous cell line from human metastatic melanoma.

Table 1: Properties of finite and continuous cell lines

Properties	Finite	Continuous(transformed)
Ploidy	Euploid, Diploid	Aneuploid, Hetroploid
Transformation	Normal	Immortal growth control altered and tumerigenic
Anchorage dependence	Yes	No
Contact inhibition	Yes	No
Density limitation of cell proliferation	Yes	Reduced or lost
Mode of growth	Monolayer	Monolayer or suspension
Maintainance	Cyclic	Steady state possible
Serum Requirement	High	Low
Cloning efficiency	Low	High
Markers	Tissue specific	Chromosomal, enzymatic, antigenic
Special function (e.g. virus susceptibility and differentiation)	May be retained	Often lost
Growth rate	Slow(T_D of 24-96 h)	Rapid(T_D of 12-24 h)
Yield	Low	High
Control parameter	Generation time, tissue specific marker	Stain characteristics

Features of cell line: To use any cell line for the production of biological product, one should have knowledge of following things related to cell lines:

- Age, sex and species of the donor tissue.
- For human cell lines, the donor's medical history and if available, the results of tests performed on the donor for the detection of adventitious agents
- Culture history of the cell line including methods used for the isolation of the tissues from which the line was derived, passage history, media used and history of passage in animals, etc.
- Previous identity testing and the results of all available adventitious agents testing

Characteristics of Cell lines: Each cell line is distinguished by characteristic features which render these cells unique and biomedically or biotechnologically useful.

- The growth pattern and morphological appearance of the cell line should be determined and should be stable from the master cell bank to the end-of-production cells.
- If there are specific markers that may be useful in characterizing the cell line (such as marker chromosomes, specific surface markers), these should be characterized for stability.
- Mostly cultured cell lines are allowed to generate their own ECM (extra cellular matrix), but primary culture and propagation of some specialized cells, exogenous provision of ECM.
- Many transformed cell lines have provided the best model for the induction of differentiation.
- Since normal cells has limited dividing capacity, therefore after a fixed number of population doublings cell lines derived from normal tissue will die out. This is a genetically determined event involving several different genes and this phenomenon is known as senescence. If the cells have an identified finite life expectancy, the total number of population doubling levels through senescence should be determined.

- Some cell lines may avoid senescence and give rise to continuous cell lines. The ability of a cell line to grow continuously probably reflects its capacity for genetic variation, allowing subsequent selection.
- A common feature of many human continuous cell lines is the development of a subtetraploid chromosome number. The alteration in a culture that gives rise to a continuous cell line is commonly called in vitro transformation and may occur spontaneously or be chemically or virally induced.

Table 2: Selection in cell line Development

Stage	Factor influencing Selection	
	Primary explant	Enzymatic disaggregation
Isolation	Mechanical damage	Enzymatic damage
Primary culture	Adhesion of explant, outgrowth(migration), cell proliferation	Cell adhesion and spreading, cell proliferation
First subculture	Trypsine sensitivity, nutrient, hormone, proliferative ability	
Propagation as cell lines	Relative growth rate of different cell, selective growth rate of one lineage, nutrient, hormone and subculture limitation Effect of cell density on predominance of normal or transformed phenotypes	
Senescence, transformation	Normal cell die out, transformed cell grow	

Requirement of cell lines: For the maintenance of Cell line some basic conditions are required. These are described as follows.

1. **pH:** Most cell lines grow well at pH 7.4. Although the optimum pH for cell growth varies relatively little among different cell strains, some normal fibroblast lines perform best at pH 7.4 to pH 7.7, and transformed cells may do better at pH 7.0 to pH 7.4.
2. **Buffering:** Culture media must be buffered under two sets of conditions:
   a) Open dishes, where the evolution of CO_2 causes the pH to rise
   b) Overproduction of CO_2 and lactic acid in transformed cell lines at high cell concentrations, when the pH will fall.
3. **Temperature:** The temperature recommended for most human and warm-blooded animal cell lines is 37°C, closely to body heat, but generally set a little lower for safety, because overheating may become major problem than under heating.

4. **Media:** Although many cell lines are still propagated in medium supplemented with serum, in many instances cultures may now be propagated in serum-free media. Media that have been produced commercially will have been tested for their capability of sustaining the growth of one or more cell lines. However under certain circumstance we can use our own media.
5. **Growth curve:** A growth curve gives three parameters of measurement: (1) the lag phase before cell proliferation is initiated after subculture, indicating whether the cells are having to adapt to different conditions; (2) the doubling time in the middle of the exponential growth phase, indicating the growth promoting capacity of the medium; and (3) the maximum cell concentration attainable indicating whether there are limiting concentrations of certain nutrients. In cell lines whose growth is not sensitive to density (e.g., continuous cell lines), the terminal cell density indicates the total yield possible and usually reflects the total amino acid or glucose concentration.

Generation of cell lines: Stably transfected cell lines are used extensively in drug discovery. Cell lines expresses a target of interest, such as a G-protein coupled receptor (GPCR) or a reporter gene, form the basis for most cell-based compound screening campaigns. In establishing new assays for high throughput screening, creation of the appropriate cell line is a bottleneck. Typically, a stable cell line is created by transfection with a plasmid encoding the target of interest or reporter gene construct, and an additional gene which allows for chemical selection of successfully transfected cells (usually an antibiotic resistance gene). Through a lengthy selection process and subsequent limiting dilution to obtain clones, the desired stable cell line is generated. This process takes approximately 2-3 months, usually yielding 5-10 usable clones and allowing little control over the end result throughout the process.

Technique for cell line generation:

LEAP (Laser-Enabled Analysis and Processing) has been developed for high-throughput laser-mediated cell elimination for cell purification. It is a precise laser-based cell ablation enables cell purification based on fluorescent and morphological criteria. It has whole well imaging system i.e all cells in the well can be analysed. It has F-theta scanning optics i.e Image up to 40X faster than typical HCS (High-content screening) systems. Image magnifications of 3X, 5X, 10X, or 20X can be obtained. Combinations of

8 excitation and 8 emission wavelengths is possible. LEAP images all cells within a well, selects a specific population of cells by gating, and eliminates selected cells at $>10^3$ per second. LEAP can select cells of interest based on fluorescent properties, morphological properties, or a combination of both. By replacing the antibiotic resistance gene used for chemical selection with a gene encoding a fluorescent protein, transfected cells can be selected based on fluorescence. These cells can then be purified using LEAP by specifically eliminating non-fluorescent cells using laser elimination. By selecting cells that remain fluorescent and proliferate over a period of time, stable cell lines are isolated. In addition, fluorescence level may be used to identify cell lines with a specific desired expression level of the transfected construct. The fluorescent reporter gene may also be replaced by a variety of fluorescent cell physiology read outs, enabling the selection of cells based on functional responses.

Applications:

The generation of stably-transfected cell lines is essential for a wide range of applications:

- Cell line can be used for gene function studies
- Drug discovery assays or the production of recombinant proteins can be carried out by cell lines.
- In contrast to transient expression, stable expression of cell line allows long term, as well as defined and reproducible expression of the gene of interest.

Table 3: Types of culture system for cell lines

Culture System	Advantage	Application
Batch culture - Polyclonal	Fast, useful for cells which do not grow in single cell culture	Over expressions, protein expression system (e.g. for Basic research)
Limiting Dilution-monoclonal	Defined cell clone	Study of gene function, protein production(e.g. for therapeutic applications)

In a batch culture system, a mixed population of drug resistant cells is selected on plates or in flasks and can be used directly for experimental analysis. During a limiting dilution procedure, cells are usually diluted and selected e.g., in a 96-well plate for outgrowth of cell clones or single colony growth. Subsequently, colonies can be picked and used to generate monoclonal cell lines.

Culture conditions for generation of stable cell lines:

As for transient transfection experiments, culture conditions (passage number, split rhythm, etc.) of selected cell type are very important for the generation of stably-transfected cell lines. The American Type Culture Collection (ATCC®; www.atcc.org) is a reliable source for various cell types. Generally, the cell line should be passaged two days before the experiment to promote good proliferation and cell physiology. Cell passage should not be higher than 30. Interference of higher passage numbers with integration efficiency is possible and may be cell-type dependent. Depending on the scope of experiment, cells can be cultivated as polyclonal batches or monoclonal single cell clones post transfection.

Transfection Method: Stable expression can be influenced by the transfection method used. The choice of transfection method determines which cell type can be targeted for stable integration. While biochemical transfection reagents can be used to transfer DNA into standard cell lines, efficient delivery of DNA into difficult-to-transfect suspension cell lines or even primary cells is only possible with viral methods or Nucleofection. Unfortunately, viral methods suffer from several limitations, such as time consuming production of vectors and safety concerns.

Table 4: Experimental outlines for the Generation of cell lines

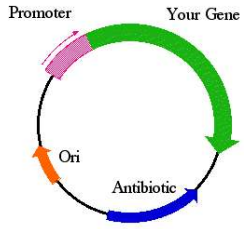
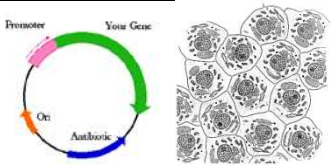
	Procedure Outline	Important Information
 Expression Plasmid	Design experiment and choose cell type, expression vector and transaction method	Make sure that transfection method and expression vector are suitable for the cell type
	Determine the appropriate cell number per plate (only for limiting dilution) and G418 concentration	Cells differ in their susceptibility to G418. The activated concentration of stock G418 can vary from batch to batch
 Expression plasmid + cells	Transfect expression vector into cells.	Amount of expression vector per expression is dependent on transfection method and cell type.
	Plant transfect cells and cultivate cells into medium without G418	Do not add G418 to culture medium immediately after transfection as this may drastically increase mortallity
	Dilute cell into culture plate and start selection 24-48 hour post transfection. Feed every 2-3 days (for batch culture) or 10 days (for limiting dilution)	Choose culture condition [batch culture limiting dilution] depending upon the experimental design. Refresh selection medium is important to avoid false positive cells.
	Analyze stably transfected cell	Make sure the chosen cell is suitable for your application.

Table 5: Commonly used cell lines

Type of cell line	Cell lines	Morphology	Origin	Species	Age	Ploidy	Characteristics
Finite from normal tissue	IMR-90	Fibroblast	Lung	Human	Embryonic	Diploid	Susceptible to human viral infection contact inhibited
	MRC-5	Fibroblast	Lung	Human	Embryonic	Diploid	Susceptible to human viral infection, contact inhibited
	MRC-9	Fibroblast	Lung	Human	Embryonic	Diploid	Susceptible to human viral infection, contact inhibited
	WI-38	Fibroblast	Lung	Human	Embryonic	Diploid	Susceptible to human viral infection, contact inhibited
Continuous from normal tissue	3T3-A31	Fibroblast		Mouse BALB/c	Embryonic	Aneuploid	contact inhibited, radily transformed
	BEAS-2B	Epithelial	Lung	Human	Adult		
	BHK21-C13	Fibroblast	Kidney	Syrian Hamster	Adult	Aneuploid	Transformable by polyoma
	BRL3A	Epithelial	Liver	Rat	New born		Produce IGF-2
	C2	Fibroblastoid	Skeletal muscle	Mouse	Embryonic		Myotubes
	C7	Epitheloid	Hypothalamous	Mouse			Neurophysin, Vasopressin
	COS-1, COS-7	Epitheloid	Kidney	Pig	Adult		Good hosts for DNA transfection
	CHO-K1	Fibroblast	Overy	Chinise Hamster	Adult	Diploid	FactorVIII, Angiotensin II converting enzyme

	HaCaT	Epithelial	Keratinocytes	Human	Adult	Diploid	Comification
	LLC-PKI	Epithelial	Kidney	Pig	Adult	Diploid	Na^+ dependent glucose uptake
	NRK49F	Fibroblast	Kidney	Rat	Adult	Aneuploid	Induction of suspension growth by TGF-α, β
Continuous from neuroplastic tissues	A2780	Epithelial	Overy	Human	Adult	Aneuploid	Chemosensitive with resistant variant
	A549	Epithelial	Lung	Human	Adult	Aneuploid	Synthesize surfactant
	B16	Fibroblast	Melanoma	Mouse	Adult	Aneuploid	Melanin
	HeLa	Epithelial	Cervix	Human	Adult	Aneuploid	G6PD Type A
	HeLa-S3	Epithelial	Cervix	Human	Adult	Aneuploid	High plating efficiency, will grow well in suspension
	HEP-G2	Epitheloid	Heptoma	Human	Adult	Aneuploid	Retain some microsomal metabolizing enzyme
	K-562	Suspention	Myeloid leukemia	Human	Adult	Aneuploid	Hemoglobin
	SK-HEP-1	Endothelial	Hepatoma, Endothelium	Human	Adult	Aneuploid	Factor VIII
	MCF-10	Epithelial	Fibrocytic mammary tissue	Human	Adult	Near Diploid	Dome formation
	HT-29	Epithelial	Colon	Human	Adult	Aneuploid	Differentiation inducible with NaBt

Interesting facts:

- There are several different types of cell lines that can be finite or continuous, prepared from normal tissue as well as neuroplastic tissues.
- Cell bank system is generally used for maintenance of cell line with constant supply of starting material as well as it perform cell lines characterization and detection of cell line cross contamination.
- In drug discovery, stably transfected cell lines are generally used.

Questions:

1. What are the basic types of cell lines and which type of tissue involve in formation of cell line?
2. What are the conditions required for cell line growth?
3. What are the criteria for choosing cell lines?
4. Which type of precaution one should take during cell line maintenance?
5. Give some example of human cell lines and what are the characteristic of those cell lines?

References:

1. Fresheny, I. "Culture of animal cell – A manual of basic technique and specialized application" by; chaptor-2: Biology of cultured cells; page-23
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3. http://atcc.custhelp.com/app/answers/detail/a_id/355/~/definition-of-%22cell-line%22
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M6 L2 Characterization of Cells

Characterization of a cell line is vital for determining its functionality and in proving its authenticity as pure cell line. Special attention must be paid to the possibility that the cell line has become cross-contaminated with an existing continuous cell line or misidentified because of mislabeling or confusion in handling DNA profiling. This has now become the major standard procedure for cell line identification, and a standard procedure with universal application.

The various important factors for cell line characterization are:

(1) It leads to authentication or confirmation that the cell line is not cross-contaminated or misidentified

(2) It is confirmation of the species of origin

(3) It is used for correlation with the tissue of origin, which comprises the following characteristics:

a) Identification of the lineage to which the cell belongs
b) Position of the cells within that lineage (i.e., the stem, precursor, or differentiated status)

(4) For determination whether the cell line is transformed or not:

a) Whether the cell line is finite or continuous?
b) Whether the cell line expresses properties associated with malignancy?

(5) It indicates whether the cell line is prone to genetic instability and phenotypic variation

(6) Identification of specific cell lines within a group from the same origin, selected cell strains, or hybrid cell lines, all of which require demonstration of features unique to that cell line or cell strain

Table 1: Decisive factors for characterization of cell lines and corresponding methods

Decisive factor	**Method**
DNA profile	PCR of microsatellite repeats
Karyotype	Chromosome spread with banding
Isoenzyme analysis	Agar gel electrophoresis
Genome analysis	Microarray
Gene expression analysis	Microarray
Proteomics	Microarray
Cell surface antigen	Immunohistochemistry
Cytoskeleton	Immunocytochemistry with antibodies specific cytokeratins

Parameters of Characterization

The nature of the technique used for characterization depends on the type of work being carried out. Some of the parameters are:

1. In case molecular technology, DNA profiling or analysis of gene expression are most useful.
2. A cytology laboratory may prefer to use chromosome analysis coupled with FISH (fluorescence in situ hybridization) and chromosome painting. Chromosomal analysis also known as karyotyping, is one of the best traditional methods for distinguishing among species. Chromosome banding patterns can be used to distinguish individual chromosomes. Chromosome painting, explicitly using combinations of specific molecular probes that hybridize to individual chromosomes, adds further resolution and specificity to this technique. These probes identify individual chromosome pairs and are species specific. Chromosome painting is a good method for distinguishing between human and mouse chromosomes in potential cross-contaminations.

3. A laboratory with immunological capability may prefer to use MHC (Major Histo compatibility complex) analysis (e.g., HLA typing) coupled with lineage specific markers.

   Combined with a functional assay related to our own interests, these procedures should provide sufficient data to authenticate a cell line as well as confirm that it is suited to the concerned.

4. **Lineage or Tissue markers:** The progression of cells down a particular differentiation pathway towards a specific differentiated cell type and can be considered as a lineage, and as cells progress down this path they acquire lineage markers specific to the lineage and distinct from markers expressed by the stem cells. These markers often reflect the embryological origin of the cells from a particular germ layer. Lineage markers are helpful in establishing the relationship of a particular cell line to its tissue of origin. There are some lineage markers which are described as follows:

a) **Cell surface antigen:** These markers are particularly useful in sorting hematopoietic cells and have also been effective in discriminating epithelium from mesenchymally derived stroma with antibodies such as anti- and anti-HMFG 1 and, distinguishing among epithelial lineages, and identifying neuroectodermally derived cells (e.g., with anti-A2B5).

b) **Intermediate filament proteins**: These are among the most widely used lineage or tissue markers. Glial fibrillary acidic protein (GFAP) for astrocytes and desmin for muscle are the most specific, whereas cytokeratin marks epithelial cells and mesothelium.

c) **Differentiated products and functions**: Haemoglobin for erythroid cells, myosin or tropomyosin for muscle, melanin for melanocytes, and serum albumin for hepatocytes are examples of specific cell type markers, but like all differentiation markers, they depend on the complete expression of the differentiated phenotype.

Transport of inorganic ions, and the resultant transfer of water, is characteristic of absorptive and secretary epithelia. Polarized transport can also be demonstrated in epithelial and endothelial cells using Boyden chambers or filter well inserts. Other tissue-specific functions that can be expressed in vitro include muscle contraction and depolarization of nerve cell membrane.

d) **Enzymes:** Three parameters are available in enzymatic characterization:

- The constitutive level (in the absence of inducers or repressors)
- The induced or adaptive level (the response to inducers and repressors)
- Isoenzyme polymorphisms

Table 1: Enzymatic markers used for cell line

Enzyme	Cell types	Inducer	Repressor
Alkaline ephosphatase	Type II pneumocyte (in lung alveolus)	Dexamethasone, Oncostain, IL-6	TGF-β
Alkaline Phosphatase	Enterocytes	Dexamethanose, NaBt collagen, Matrigel	-
Angiotensin-converting enzyme	Endothelium	Collagen, Matrigel	-
Creatine Kinase BB	Neurons, neuroendocrine cells, SCLC	-	-
Creatine Kinase MM	Muscle cells	IGF-II	FGF-1,2,7
DOPA-decarboxylase	Neuron, SCLC	-	-
Glutamyl synthetase	Astroglia (brain)	Hydrocortisone	Glutamine
Neuron specific enolase	Neuron, neuroendocrine cell	-	-
Non-specific esterase	Macrophage	PMA, Vitamin D3	-
Proline hydrolase	Fibroblasts	Vitamin C	-
Sucrase	Enterocytes	NaBt	-

e) **Regulation:** The level of expression of many differentiated products is under the regulatory control of environmental influences, such as nutrients, hormones, the matrix, and adjacent cell. Hence the measurement of specific lineage markers may require preincubation of the cells in, for example, a hormone such as hydrocortisone, specific growth factors, or growth of the cells on extracellular matrix of the correct type.

f) Lineage fidelity: Lineage markers are more properly regarded as tissue or cell type markers, as they are often more characteristic of the function of the cell than its embryonic origin.

5.**Unique Markers:** Unique markers include specific chromosomal aberrations (e.g., deletions, translocations, polysomy), major histocompatibility (MHC) group antigens (e.g., HLA in humans), which are highly polymorphic, and DNA fingerprinting or SLTR DNA profiling. Enzymic deficiencies, such as thymidine kinase deficiency (TK−) and drug resistance such as vinblastine resistance (usually coupled to the expression of the P-glycoprotein by one of the *mdr* genes that code for the efflux protein) are not truly unique, but they may be used to distinguish among cell lines from the same tissues but different donors.

6. Transformation: The transformation status forms a major element in cell line characterization and is dealt with separately.

a) **Cell Morphology:** Observation of morphology is the simplest and most direct technique used to identify cells. Most of these are related to the plasticity of cellular morphology in response to different culture conditions. For example, epithelial cells growing in the center of a confluent sheet are usually regular, polygonal, and with a clearly defined birefringent edge, whereas the same cells growing at the edge of a patch may be more irregular and distended and, if transformed, may break away from the patch and become fibroblast-like in shape.

b) **Microscopy:** The inverted microscope is one of the most important tools in the tissue culture laboratory, but it is often used incorrectly. As the thickness of the closed culture vessel makes observation difficult from above, because of the long working distance, the culture vessel is placed on the stage, illuminated from above, and observed from below. As the thickness of the wall of the culture vessel still limits the

working distance, the maximum objective magnification is usually limited to 40X. The use of phase-contrast optics, where an annular light path is masked by a corresponding dark ring in the objective and only diffracted light is visible, enables unstained cells to be viewed with higher contrast than is available by normal illumination. Because this means that the intensity of the light is increased, an infrared filter should be incorporated for prolonged observation of cells.

It is useful to keep a set of photographs at different cell densities for each cell line, prepared shortly after acquisition and at intervals thereafter, as a record in case a morphological change is subsequently suspected. Photographs of cell lines in regular use should be displayed above the inverted microscope. Photographic records can be supplemented with photographs of stained preparations and digital output from DNA profiling and stored with the cell line record in a database or stored separately and linked to the cell line database.

c) **Staining:** A polychromatic blood stain, such as Giemsa, provides a convenient method of preparing a stained culture. Giemsa stain is usually combined with May–Grunwald stain when staining blood, but not when staining cultured cells. Alone, it stains the nucleus pink or magenta, the nucleoli dark blue, and the cytoplasm pale gray-blue. It stains cells fixed in alcohol or formaldehyde but will not work correctly unless the preparation is completely anhydrous.

Chromosome Content: Chromosome content or karyotype is one of the most characteristic and best-defined criteria for identifying cell lines and relating them to the species and sex from which they were derived. Chromosome analysis can also distinguish between normal and transformed cells because the chromosome number is more stable in normal cells (except in mice, where the chromosome complement of normal cells can change quite rapidly after explantation into culture).

Chromosome Banding: This group of techniques was devised to enable individual chromosome pairs to be identified when there is little morphological difference between them. For Giemsa banding, the chromosomal proteins are partially digested by crude trypsin, producing a banded appearance on subsequent staining. Trypsinization is not required for quinacrine banding. The banding pattern is characteristic for each chromosome pair.

Other methods for banding are:

a) Using trypsin and EDTA rather than trypsin alone
b) Q-banding, which stains the cells in 5% (w/v) quinacrine dihydrochloride in 45% acetic acid, followed by rinsing Giemsa banding the slide, and mounting it in deionized water at pH 4.5
c) C-banding, which emphasizes the centromeric regions

Techniques have been developed for discriminating between human and mouse chromosomes, principally to aid the karyotypic analysis of human-mouse hybrids. These methods include fluorescent staining with Hoechst 33258, which causes mouse centromeres to fluoresce more brightly than human centromeres.

Chromosome painting: Chromosome paints are available commercially from a number of sources. The hybridization and detection protocols vary with each commercial source, but a general scheme is available. Karyotypic analysis is carried out classically by chromosome banding, using dyes that differentially stain the chromosomes. Thus each chromosome is identified by its banding pattern. However, traditional banding techniques cannot characterize many complex chromosomal aberrations. New karyotyping methods based on chromosome painting techniques—namely spectral karyotyping (SKY) and multicolour fluorescence in situ hybridization (M-FISH)—have been developed. These techniques allow the simultaneous visualization of all 23 human chromosomes in different colours.

Chromosome Analysis

The following are methods by which the chromosome complement may be analyzed:

(1) Chromosome count: Count the chromosome number per spread for between 50 and 100 spreads. (The chromosomes need not be banded.)

(2) Karyotype: Digitally photograph about 10 or 20 good spreads of banded chromosomes. Image analysis can be used to sort chromosome images automatically to generate karyotypes.

Chromosome counting and karyotyping allow species identification of the cells and, when banding is used, distinguish individual cell line variations and marker chromosomes. However, karyotyping is time-consuming, and chromosome counting with a quick check on gross chromosome morphology may be sufficient to confirm or exclude a suspected cross-contamination.

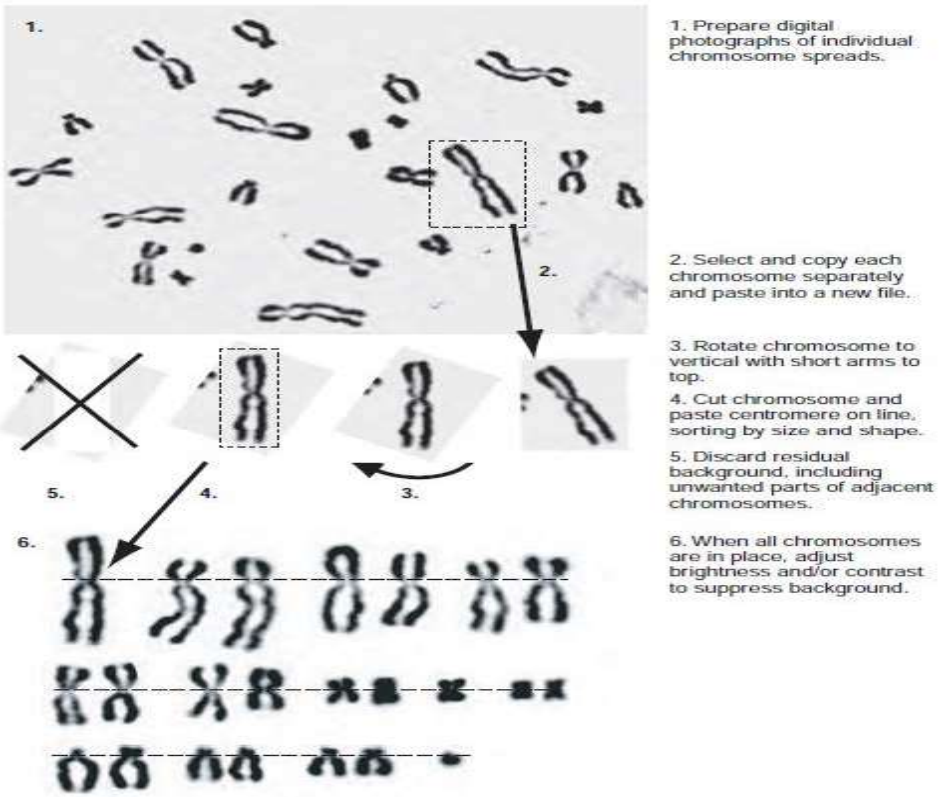

Figure 1: Karyotype Preparation Steps in the preparation of a karyotype from digital microphotographs of metaphase spread. Chinese hamster cells recloned from the Y-5 strain.

DNA Analysis: DNA content can be measured by propidium iodide fluorescence with a CCD camera or flow cytometry, although the generation of the necessary single-cell suspension will, of course, destroy the topography of the specimen. DNA can be estimated in homogenates with Hoechst 33258 and other DNA fluorochromes such as DAPI, propidium iodide, or Pico Green (Molecular Probes). Analysis of DNA content is particularly useful in the characterization of transformed cells that are often aneuploid and heteroploid.

DNA Hybridization: Hybridization of specific molecular probes to unique DNA sequences (Southern blotting) can provide information about species specific regions, amplified regions of the DNA, or altered base sequences that are characteristic to that cell line. Thus strain-specific gene amplifications, such as amplification of the dihydrofolate reductase (DHFR) gene, may be detected in cell lines selected for resistance to methotrexate; amplification of the MDR gene in vinblastine-resistant cells overexpression of a specific oncogene, or oncogenes in transformed cell lines or deletion, or loss, of heterozygosity in suppressor genes. Although DNA aberrations can be detected in restriction digests of extracts of whole DNA, this is limited by the amount of DNA required.

It is more common to use the polymerase chain reaction (PCR) with a primer specific to the sequence of interest, enabling detection in relatively small numbers of cells. Alternatively, specific probes can be used to detect specific DNA sequences by in situ hybridization having the advantage of displaying topographical differences and heterogeneity within a cell population.

DNA fingerprinting: DNA fingerprints appear to be quite stable in culture, and cell lines from the same origin, but maintained separately in different laboratories for many years, still retain the same or very similar DNA fingerprints. DNA fingerprinting is a very powerful tool in determining the origin of a cell line, if the original cell line, or DNA from it or from the donor individual, has been retained. This emphasizes the need to retain a blood, tissue, or DNA sample when tissue is isolated for primary culture. Furthermore, if a cross-contamination or misidentification is suspected, this can be investigated by fingerprinting the cells and all potential contaminant.

Antigenic Markers: Immunostaining and ELISA assays are among the most useful techniques available for cell line characterization facilitated by the abundance of antibodies and kits which is commercially available. Antibody is essential to be certain of its specificity by using appropriate control material. This is true for monoclonal antibodies and polyclonal antisera alike; a monoclonal antibody is highly specific for a particular epitope.

Immunostaining: Antibody localization is determined by fluorescence, wherein the antibody is conjugated to a fluorochrome, such as fluorescein or rhodamine, or by the deposition of a precipitated product from the activity of horseradish peroxidase or alkaline phosphatase conjugated to the antibody. Various methods have been used to enhance the sensitivity of detection of these methods, particularly the peroxidase linked methods. In the peroxidase–anti-peroxidase (PAP) technique, a further amplifying tier is added by reaction with peroxidase conjugated to anti-peroxidase antibody from the same species as the primary antibody. Even greater sensitivity has been obtained by using a biotin-conjugated second antibody with streptavidin conjugated to peroxidase or alkaline phosphatase or gold-conjugated second antibody with subsequent silver intensification.

Differentiation: Many of the characteristics described under antigenic markers or enzyme activities may also be regarded as markers of differentiation, and as such they can help to correlate cell lines with their tissue of origin as well as define their phenotypic status. Although sometimes constitutively expressed (e.g., melanin in B16 melanoma or Factor VIII in endothelial cells), expression of differentiated lineage markers may need to be induced before detection is possible.

Interesting facts:

- EDTA, a chelator of divalent cations, is added to trypsin solutions to enhance activity.
- The calcium and magnesium in the extracellular matrix, which aids in cell-cell adhesion, also obliterates the peptide bonds that trypsin acts on.
- The EDTA is added to remove the calcium and magnesium from the cell surface which allows trypsin to hydrolyze specific peptide bonds. This activity can be arrested by adding a serum media mixture or a trypsin inhibitor (from soybean, for example) in serum-free systems.

Questions:

1. Why characterization of cell line is necessary?
2. What are the parameters on which characterization depends?
3. What is the role of trypsin and EDTA in cell culture? How EDTA help trypsin in cell detachment?

References:

1. Fresheny, I.; "Culture of animal cell – A manual of basic technique and specialized application" by; chaptor-15: Characterization

M6 L3 Contamination in cell culture

Contamination: Contamination is the presence of a minor and unwanted constituent (contaminant) in material, physical body, natural environment, at a workplace, etc. In biological sciences accidental introduction of foreign material (contamination) can seriously distort the results of experiments where small samples are used. In cases where the contaminant is a living microorganism, it can often multiply and take over the experiment, especially cultures, and render them useless.

Source of Contamination: Maintaining asepsis is one of the most difficult challenges to work with living cells. There are several potential routes to contamination including failure in the sterilization procedures for solutions, glassware and pipettes, turbulence and particulates (dust and spores) in the air in the room, poorly maintained incubators and refrigerators, faulty laminar-flow hoods, the importation of contaminated cell lines or biopsies, and lapses in sterile technique.

Table 1: Route of Contamination

Technique	Route or cause	Prevention
(Manipulations, pipetting, dispensing, etc.)	Nonsterile surfaces and equipment	Work area of items not in immediate use should be clear.
	Spillage on necks and outside of bottles and on work surface	Swab regularly with 70% alcohol. Do not pour liquids. Dispense or transfer by pipette, auto dispenser or transfer device. If pouring is unavoidable: (1) do so in one smooth movement, (2) discard the bottle that you pour from, and (3) wipe up any spillage.
	Touching or holding pipettes too low down, touching necks of bottles, inside screw caps.	Hold pipettes above graduations.
	Splash-back from waste beaker	Discard waste into a beaker with a funnel or, preferably, by drawing off the waste into a reservoir by means of a vacuum pump.
	Sedimentary dust or particles of skin settling on the culture or bottle; hands or apparatus held over an open dish or bottle	Do not work over (vertical laminar flow and open bench) or behind and over (horizontal laminar flow) an open bottle or dish.
Work surface	Dust and spillage	Swab the surface with 70% alcohol before during, and after work. Mop up spillage immediately.

Operator hair, hands, breath, clothing	Dust from skin, hair, or clothing dropped or blown into the culture	Wash hands thoroughly or wear gloves. Wear a lint-free lab coat with tight cuffs and gloves overlapping them.
	Aerosols from talking, coughing, sneezing, etc.	Keep talking to be minimum, Avoid working with a cold or throat infection, or wear a mask. Long hair should be tie back or wear a cap. Generally wear a lab coat different from the one which wear in the general lab area or animal house.
Materials and reagents		
Solutions	Non-sterile reagents and media	Filter or autoclave solutions before using them
	Dirty storage conditions	Clean up storage areas and disinfect regularly.
	Inadequate sterilization procedures	Monitor the performance of the autoclave with a recording thermometer or sterility indicator. Check the integrity of filters with a bubble-point or microbial assay after using them. Test all solutions after sterilization.
	Poor commercial supplier	Test solutions; change suppliers.
Glassware and screw caps	Dust and spores from storage	Shroud caps with foil. Wipe bottles with 70% alcohol before taking them into the hood. Replace stocks from the back of the shelf. Do not store anything unsealed for more than 24 h.
	Ineffective sterilization (e.g., an overfilled oven or sealed bottles, preventing the ingress of steam)	Check the temperature of the load throughout the cycle. In the autoclave; keep caps slack on empty bottles. Stack oven and autoclave correctly.
Instruments, pipettes	Ineffective sterilization	Sterilize items by dry heat before using them. Monitor the performance of the oven.
	Contact with a nonsterile surface or some other material	Do not grasp any part of an instrument or pipette that will pass into a culture vessel.

Culture flasks and media bottles in use	Dust and spores from incubator or refrigerator	Use screw caps instead of stoppers. Swab bottles before placing in hood. Box plates and dishes.
	Dirty storage or incubation conditions.	Cover caps and necks of bottles with aluminum foil during storage or incubation. Wipe flasks and bottles with 70% alcohol before using them. Clean out stores and incubators regularly.
	Media under the cap and spreading to the outside of the bottle.	Discard all bottles that show spillage on the outside of the neck. Do not pour.
Equipment and facilities	Room air Drafts, eddies, turbulence, dust, aerosols	Clean filtered air. Reduce traffic and extraneous activity. Wipe the floor and work surfaces regularly.
Laminar-flow hoods	Perforated filter	Check filters regularly for holes and leaks.
	Change of filter needed	Check the pressure drop across the filter.
	Spillages, particularly in crevices or below a work surface	Keep around and below the work surface clear regularly. Let alcohol run into crevices.
Equipment and facilities		
Room air	Drafts, eddies, turbulence, dust, aerosols	Clean filtered air. Reduce traffic and extraneous activity. Wipe the floor and work surfaces regularly.
Laminar-flow hoods	Perforated filter	Check filters regularly for holes and leaks.
	Change of filter needed	Check the pressure drop across the filter.
	Spillages, particularly in crevices or below a work surface	Clear around and below the work surface regularly. Let alcohol run into crevices.
Dry incubators	Growth of molds and bacteria on spillages	Wipe up any spillage with 70% alcohol on a swab. Clean out incubators regularly.
CO_2, humidified incubators	Growth of molds and bacteria on walls and shelves in a humid atmosphere.	Clean out with detergent followed by 70% alcohol

	Spores, etc., carried on forced-air circulation	Enclose open dishes in plastic boxes with close-fitting lids (but do not seal the lids). Swab incubators with 70% alcohol before opening them. Put a fungicide or bacteriocide in humidifying water (but check first for toxicity).
Other equipment	Dust on cylinders, pumps, etc	Wipe with 70% alcohol before bring in
Mites, insects, and other infestations in wooden furniture, or benches, in incubators, and on mice, etc., taken from the animal house	Entry of mites, etc., into sterile packages	Seal all sterile packs. Avoid wooden furniture if possible; use plastic laminate, one-piece, or stainless steel bench tops. If wooden furniture is used, seal it with polyurethane varnish or wax polish and wash it regularly with disinfectant. Keep animals out of the tissue culture lab.
Importation of biological materials		
Tissue samples	Infected at source or during dissection	Do not bring animals into the tissue culture lab. Incorporate antibiotics into the dissection fluid. Dip all potentially infected large-tissue samples in 70% alcohol for 30 s.
Incoming cell lines	Contaminated at the source or during transit	Handle these cell lines alone, preferably in quarantine, after all other sterile work is finished. Swab down the bench or hood after use with 2% phenolic disinfectant in 70% alcohol, and do not use it until the next morning. Check for contamination by growing a culture for two weeks without antibiotics. (Keep a duplicate culture in antibiotics at the first subculture.) Check for contamination visually, by phase-contrast microscopy and Hoechst stain for mycoplasma. Using indicator cells allows screening before first subculture.

Monitoring For Contamination:

Potential sources of contamination are enumerated along with the precautions that should be taken to avoid them. Even in the best laboratories contaminations do arise, so the following procedure is generally recommended:

(1) Contamination by eye and with a microscope at each handling of a culture should be checked properly.

(2) If it is suspected that a culture is contaminated and the fact cannot be confirmed in situ, the hood or bench should be kept clear except suspected culture and Pasteur pipettes. Because of the potential risk to other cultures, this should be better to do after all your other culture work is finished. A sample should be removed from the culture and placed on a microscope slide. Slide should be checked with a microscope, preferably by phase contrast. If it is confirmed that the culture is contaminated, pipettes should be discarded, hood or bench should be swabbed with 70% alcohol containing a phenolic disinfectant. The hood or bench should not be used until the next day.

(3) Nature of the contamination should be recorded.

(4) If the contamination is new and is not widespread, the culture, the medium bottle used to feed it, and any other reagent (e.g., trypsin) that has been used in conjunction with the culture should be discarded properly into disinfectant, preferably in a fume hood and outside the tissue culture area.

(5) If the contamination is new and widespread all media, stock solutions, trypsin, and so forth in current use should be discarded immediately.

(6) If the same kind of contamination has occurred before check stock solutions for contamination (a) by incubation alone or in nutrient broth (b) by plating out the solution on nutrient agar. If (a) and (b) prove negative, but contamination is still suspected, 100 mL of solution should be incubated, filtered it through a 0.2-μm filter, and plated out filter on nutrient agar with an uninoculated control.

(7) If the contamination is widespread, multispecific, and repeated then one should check (a) the laboratory's sterilization procedures (e.g., the temperatures of ovens and autoclaves, particularly in the center of the load, the duration of the sterilization cycle), (b) the packaging and storage practices, (e.g., unsealed glassware should be resterilized every 24 h), and (c) the integrity of the aseptic room and laminar-flow hood filters.

(8) One should not be attempting to decontaminate cultures unless they are irreplaceable.

Visible Microbial Contamination: Characteristic features of microbial contamination are as follows:

(1) A sudden change in pH, usually a decrease with most bacterial infections, very little change with yeast until the contamination is heavy, and sometimes an increase in pH with fungal contamination.

(2) Cloudiness in the medium, sometimes with a slight film or scum on the surface or spots on the growth surface that dissipate when the flask is moved

(3) Under a 10X objective, spaces between cells will appear granular and may shimmer with bacterial contamination. Yeasts appear as separate round or ovoid particles that may bud off smaller particles. Fungi produce thin filamentous mycelia and, sometimes, denser clumps of spores which may be blue or green. With toxic infection, some deterioration of the cells will be apparent.

(4) Under a 100X objective, it may be possible to resolve individual bacteria and distinguish between rods and cocci. At this magnification, the shimmering that is visible in some infections will be seen to be caused by mobility of bacteria. Some bacteria form clumps or associate with the cultured cells.

(5) With a slide preparation, the morphology of the bacteria can be resolved with a 100× objective, but this is not usually necessary. Microbial infection may be confused with precipitates of media constituents (particularly protein) or with cell debris, but can be distinguished by their regular morphology. Precipitates may be crystalline or globular and irregular and are not usually as uniform in size. Clumps of bacteria may be confused with precipitated protein, but, particularly if shaken, many single or strings of bacteria will be seen. If you are in doubt, plate out a sample of medium on nutrient agar.

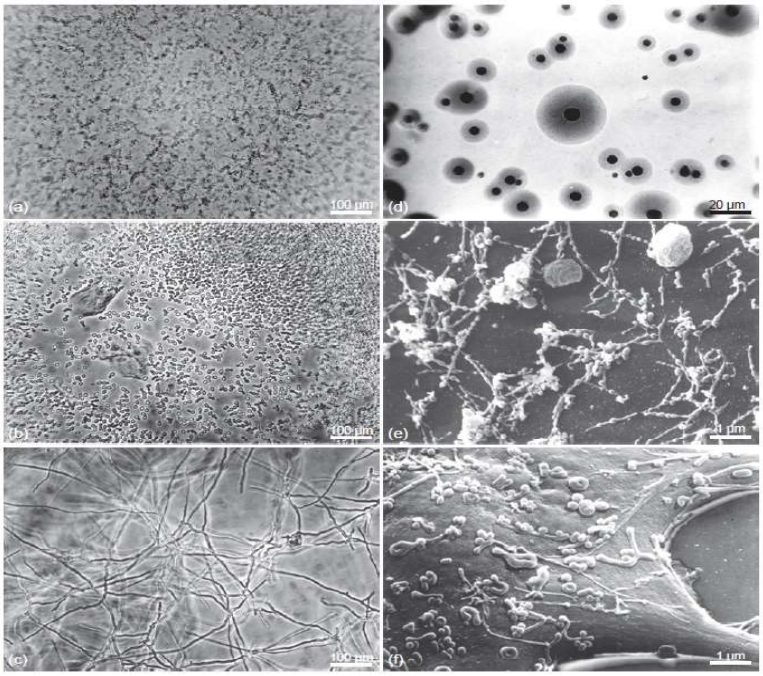

Figure-1: Types of Contamination. Examples of microorganisms found to contaminate cell cultures. (a) Bacteria. (b) Yeast. (c) Mold. (d) Mycoplasma colonies growing on special nutrient agar (e, f). Scanning electron micrograph of mycoplasma growing on the surface of cultured cells. From where has this figure been taken

Ways of Disposal of Contaminated Cultures: The following procedures are generally used for disposal of contaminated culture:

- It is important to ensure that all contaminated material is disposed of correctly. Culture vessels should be removed from the culture area, unopened if possible, and autoclaved.
- Open items, such as Petri dishes with the lids in place, and pipettes or other items that have come in contact with a contaminated culture should be immersed in hypochlorite disinfectant (Petri dishes can be opened while submerged).
- If only one of a series of similar cultures is contaminated, it is necessary to discard the bottle of medium that was used with it, but if the contamination is widespread, then all medium as well as all other stock solutions and reagents, used with these cells, should be discarded into hypochlorite.

Eradication of Contamination: Eradication of Contamination in cell culture is a challenging job during safe culturing. There are different way for different organism, some example are given as follows:

Case-I: Bacteria, Fungi, and Yeasts: The most reliable method of eliminating a microbial contamination is to discard the culture and the medium and reagents used with it as treating a culture may be unsuccessful or lead to the development of an antibiotic-resistant microorganism. This procedure is optimal; however, the majority of cell lines do not form spheroids in this manner. Alternatively, aggregates may be formed from cell suspensions in stationary flasks, previously base-coated with agar. Aggregates may be left in the original flasks or transferred individually (by pipette) to multi well plates, where continued growth over weeks will yield spheroids of maximum size, about 1000 μm.

Decontamination should be attempted only in extreme situations, under quarantine, and with expert supervision. If unsuccessful, the culture and associated reagents should be autoclaved as soon as failure becomes obvious. The general rule remains that contaminated cultures are discarded and that decontamination is not attempted unless it is absolutely vital to retain the cell strain. In any event, complete decontamination is difficult to achieve, particularly with yeast, and attempts to do so may produce hardier, antibiotic-resistant strains.

Case-II: Eradication of Viral Contamination: There are no reliable methods for eliminating viruses from a culture at present; disposal or tolerances are the only options.

Cross Contamination: During the development of tissue culture, a number of cell strains have evolved with very short doubling times and high plating efficiencies. Although these properties make such cell lines valuable experimental material, they also make them potentially hazardous for cross-infecting other cell lines.

Interesting facts:

1. Once cell lines are infected, they may undergo spontaneous differentiation or altered function due to adaptation, which may have a profound impact on experimental results.

Questions:

1. What are the possible routes of contamination in cell culture?
2. What are possible methods for monitoring contamination?
3. What are the general procedures for disposal of contaminated culture?

References:

1. Fresheny**,** I.; "Culture of animal cell – A manual of basic technique and specialized application"
2. http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Stem-Cell-Research/Stem-Cell-Analysis/Cell-Line-Contamination.html.

M6 L4 Cell line differentiation

Differentiation: Differentiation in cell line is the process which leads to the expression of phenotypic properties and characteristic of the functionally mature cell in vivo. It is the phenomenon in which less specialized cell develops or matures to become more distinct in form and function. This may be irreversible when there is cessation of DNA synthesis in the erythroblast nucleus, neuron, or mature keratinocyte. The process may be reversible, when the dedifferentiation of mature hepatocytes into precursors happens during liver regeneration. Some of the properties of the differentiated cells are adaptive, such as albumin synthesis in differentiated hepatocytes, which is often lost in culture but can be reinduced. Differentiation is the combination of constitutive (stably expressed without induction) and adaptive (subject to positive and negative regulation of expression) properties found in the mature cell.

Terminal differentiation: Terminal differentiation is another type of differentiation in which a cell has progressed down a particular lineage to a point at which the mature phenotype is fully expressed and beyond which the cell cannot progress. This stage may be reversible in some cells, such as fibrocytes, that can revert to a less differentiated phenotype, or even a stem cell, and resume proliferation. It may be irreversible in cells like erythrocytes, neurons, skeletal muscle, or keratinized squamous cells. The growth of cells on floating collagen has been used to improve the survival of epithelial cells and promote terminal differentiation

Pluripotent cell: A cell that is able to differentiate into all cell types of the adult organism is known as pluripotent. Such cells are called embryonic stem cells in animals and meristematic cells in higher plants.

Totipotent cell: A cell that is able to differentiate into all cell types, including the placental tissue, is known as totipotent. In mammals, only the zygote and subsequent blastomeres are totipotent.

Control of Differentiation: Differentiation is controlled by various parameters. There are five major parameters that control differentiation. Figure 1 shows the diagrammatic representation of the figures.

1. Cell–cell interaction
2. Cell–matrix interaction
3. Cell shape and polarity
4. Oxygen tension
5. Soluble systemic factors

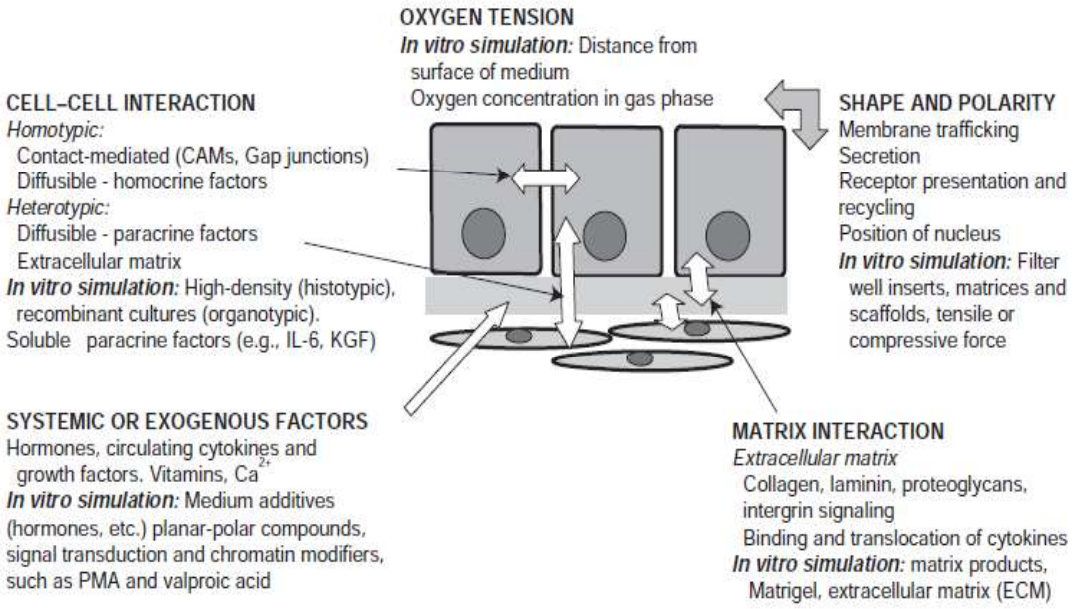

Figure 1: Parameter factors for cell differentiation.

1. Cell- cell Interaction: Cell-cell interactions are of two types, Homotypic and heterotypic. They can be detailed as follows:

Homotypic cell-cell interaction: Homologous cell interaction occurs at high cell density. It may involve gap junctional communication in which metabolites, second messengers such as cyclic AMP, diacylglycerol (DAG), Ca^{2+}, or electrical charge may be involved. This interaction harmonizes the expression of differentiation within a population of similar cells, rather than initiating its expression. Homotypic cell–cell adhesion molecules, (CAMs) or cadherins, which are calcium-dependent, provide another mechanism by which contacting cells may interact. These adhesion molecules promote interaction primarily between like cells via identical, reciprocally acting,

extracellular domains, and they appear to have signal transduction potential via phosphorylation of the intracellular domains.

Heterotypic cell-cell interaction: Heterologous cell interaction such as between mesodermally and endodermally or ectodermally derived cells is responsible for initiating and promoting differentiation. During and immediately after gastrulation in the embryo, and later during organogenesis, mutual interaction between cells originating in different germ layers promotes differentiation.

2. Cell–Matrix Interactions

Animal cells are not surrounded by cell walls. Animal cells are surrounded by a plasma membrane which is complex mixture of glycoproteins and proteoglycans surface that is highly specific for each tissue, and even for parts of a tissue. Recreation of this complex microenvironment, involving cell–cell and cell–matrix interactions has been shown to be vital in the expression of the mature keratinocyte phenotype in the reconstruction of skin equivalents and the maintenance of the stem cell compartment. Collagen has been found to be essential for the functional expression of many epithelial cells and for endothelium to mature into capillaries. Small polypeptides containing this sequence effectively block matrix-induced differentiation, implying that the intact matrix molecule is required. Defined matrices are required; although fibronectin, laminin, collagen, and numerous other matrix constituents are commercially available, the specificity probably lies largely in the proteoglycan moiety, within which there is the potential for wide variability, particularly in the number, type, and distribution of the sulfated glycosaminoglycan, such as heparan sulfate. The extracellular matrix may also play important role in the modulation of growth factor activity. One type of extracellular matrix is exemplified by the thin, sheet-like basal laminae, previously called basement membranes, upon which layers of epithelial cells rest. In addition to supporting sheets of epithelial cells, basal laminae surround muscle cells, adipose cells, and peripheral nerves. Extracellular matrix is most abundant in connective tissues.

3. Polarity and cell shape: Various studies shows that growth of the cells on collagen gel and the subsequent release of the gel from the bottom of the dish with a spatula or bent Pasteur pipette are required for full maturation of cell. This process allows shrinkage of the gel and modification in the shape of the cell from flattened to cuboidal or even columnar shape. Following the shape change and also possibly due to contact to medium through the gel, the cells develop polarity which is visible by electron microscopy. When the nucleus becomes asymmetrically distributed nearer to the bottom of the cell an active Golgi complex is formed and secretion is observed towards the apical surface.

4. Oxygen Tension: Gas exchange enhances when positioning the cells at the air–liquid interface, particularly facilitating oxygen uptake without raising the partial pressure and risking free radical toxicity. It is also possible that the thin film above mimics the physicochemical conditions in vivo (surface tension, lack of nutrients) as well as oxygenation.

Table 1: Common Physiological Inducers of Differentiation

Type of Inducer	Common Inducers	Cell type
Steroid and related	Hydrocorticosone	Glia, glioma Lung alveolar typeII cells Hepatocytes Mammary epithelium Myloid leukemia
	Retinoid	Tracheobranchial epithelium Endothelium Enterocytes (Caco-2) Embryonal carcinoma Malanoma Myeloid leukemia Neuroblastoma
Peptide Hormones	Melanotropin	Melanocytes
	Thyotropin	Thyroid
	Erythropoietin	Erythroblasts
	Prolactin	Mammary epithelium
	Insulin	Mammary epithelium
Cytokines	Nerve growth factor	Neuron
	Glia maturation factor, CNTF, PDGF, BMP2	Glial cell
	Epimorphin	Kidney epithelium
	Fibrocyte-pneumocyte factor	Type II pneumocyte
	Interferon-α, β	A549 cells

		HL60, myeloid leukemia
	Interferon-γ	Neuroblastoma
	CNTF	Type 2 astrocytes
	IL-6, OSM	AS49
	BMP	1 OT 1/2
	KGF	Keratinocytes Prostatic epithelium
	HGF	Kidney (MDCK) Hepatocyte
	TGF-β	Bronchial epithelium Melanocyte
	Endothelium	Melanocyte
Vitamins	Vitamin E	Neuroblastoma
	Vitamin D3	Monocytes (U937) Myeloma Enterocyte (IEC-6)
	Vitamin K	Hepatoma Kidney epithelium
Minerals	Ca++	Keranocytes

Table 2: Common Non-Physiological Inducers of Differentiation

Type of Inducer	Common Inducers	Cell type	Fate
Planar- polar compound	DMSO	Murin erytholeukemia	Immature erythrocytes
		Myeloma	Granulocyte
		Neuroblastoma	Neurons
		Mammary epithelium	Secretory epithelium
		Hepatocyte precursors, HepaRG hepatoma	Hepatocyte
	Sodium butyrate	Erythroleukemia	Immature erythrocytes
		Colon cancer	Absorptive epithelium
	N- methyl acetamide	Glioma	Astrocyte
	N- methyl formamide, Dimethyl formamide	Colon cancer	Absorptive epithelium
	HMBA	Erythroleukemia	Immature erythrocytes
	Butylated hydroxyanisole	Adipose derived stem cells	Neuron
	Benzodiazepines	Erythroleukemia	Immature erythrocytes
Cytotoxic drugs	Genistein	Erythroleukemia	Immature erythrocytes
	Cytosin arabinoside	Myeloid leukemia	Granulocyte
	Mitocin C, anthracyclines	Melanoma	Melanocyte
	Metotrexate	Colorectal carcinoma	Adsorptive & mucin-secreting epithelium
Chromatin modifiers	Valproic acid	Adipose derived stem cells	Neuron Neurite extension

		PC12 cells	
	Azacytidine	NG108-15 neuronal cells	Cholinergic neuron
Signal transduction modifiers	Isobutylmethyl xanthine	Adipose derived stem cells	Adipocytes
	Forkoline	Adipose derived stem cells	Neuron
	PMA	Bronchial epithelium	Squamous epithelium
		Mammary epithelium	
		Colon(HT29, Caco-2)	Ductal morphogenesis
		Monocyte leukemia(U937)	Monocytes
		Erythroleukemia(K562)	Immature erythrocytes
		Neuroblastoma	Neurite growth

Relationship between Differentiation and Malignancy:

With increasing progression of cancer, histology of a tumor indicates poorer differentiation, and from a prognostic standpoint, patients with poorly differentiated tumors generally have a lower survival rate than patients with differentiated tumors. Cancer is principally a malfunction of cells to differentiate normally. It is therefore surprising to find that many tumors grown in tissue culture can be induced to differentiate. Indeed much of the fundamental data on cellular differentiation has been derived from murine leukemia, mouse and human myeloma, hepatoma, and neuroblastoma. Nevertheless, there appears to be an inverse relationship between the expression of differentiated properties and the expression of malignancy associated properties, even to the extent that the induction of differentiation has often been proposed as a mode of therapy. In correct environmental conditions, and assuming that the appropriate cells are present, partial, or even complete, differentiation is achievable in cell culture. As a general approach to promoting differentiation, as opposed to cell proliferation and propagation, the following aspects need to be taken care.

i. Selection of the correct cell type by use of appropriate isolation conditions and a selective medium.
ii. Growing the cells to a high cell density (>1 × 10^5 cells/cm^2) on the appropriate matrix. The matrix may be collagen of a type that is appropriate to the site of origin of the cells, with or without fibronectin or laminin, or it may be more complex, tissue derived or cell derived, such as Matrigel or a synthetic matrix (e.g., poly-D-lysine for neurons).
iii. Changing the cells to a differentiating medium rather than a propagation medium for other cell types this step may require defining the growth factors appropriate to maintaining cell proliferation and those responsible for inducing differentiation.
iv. Addition of differentiation-inducing agents, such as glucocorticoids, retinoids, vitamin D_3, DMSO, HMBA, prostaglandins and cytokines, such as bFGF, EGF, KGF, HGF, IL-6, OSM, TGF-β, interferons, NGF, and melanocyte-stimulating hormone (MSH), as appropriate for the type of cell.
v. Addition of the interacting cell type during the growth phase, the induction phase or both phases. Selection of the correct cell type is not always clear, but lung fibroblasts for lung epithelial maturation, glial cells for neuronal maturation, and bone marrow stromal cells for hematopoietic cells are some of the better-characterized examples.

Equilibrium between cell proliferation and differentiation:

Cells in culture can be thought to be in a state of equilibrium between cell proliferation and differentiation. Normal culture conditions i.e. in low cell density, mitogens in the medium will favor cell proliferation, whereas high cell density and addition of differentiation factors will induce differentiation. The position of the equilibrium will depend on culture conditions. Dedifferentiation of the culture may be due to the effect of growth factors or cytokines inducing a more proliferative phenotype, reprogramming of gene expression, or overgrowth of a precursor cell type. The relationship between differentiation and cell proliferation may become relaxed but it is not lost. For example, B16 melanoma cells still produce more pigment at a high cell density and at a low rate of cell proliferation than at a low cell density and a high rate of cell proliferation.

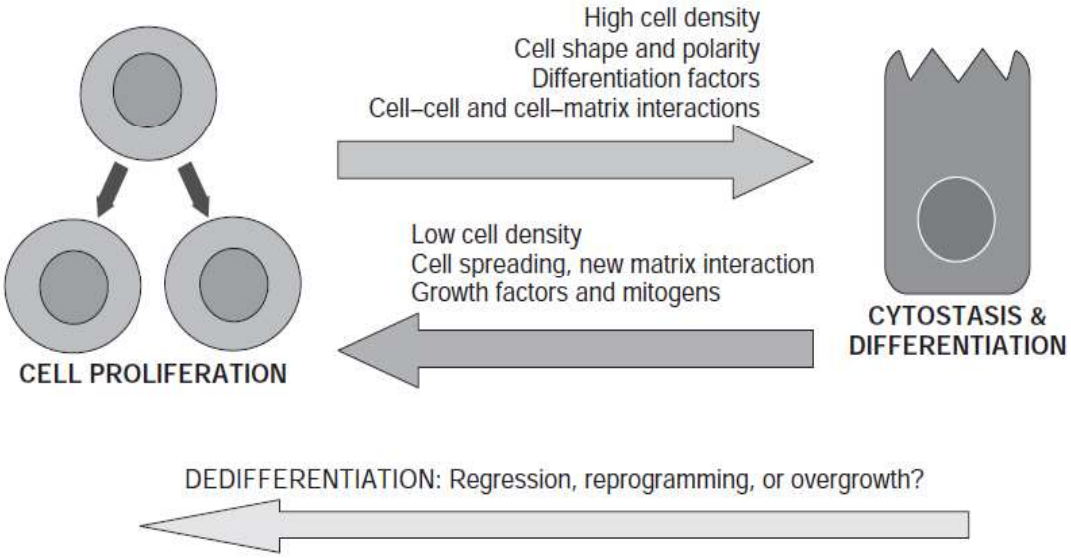

Figure 2: Differentiation and Proliferation

As differentiation progresses, cell division is reduced and ultimately ceases. In most cell systems, cell proliferation is incompatible with the expression of differentiated properties. Tumor cells can sometimes break this restriction, and in melanoma, for example, melanin continues to be synthesized while the cells are proliferating. Even in these situation, synthesis of the differentiated product increases when division stops.

Differentiation from stem cells:

It may be useful to think of a cell culture as being an equilibrium between stem cells, undifferentiated precursor cells, and mature differentiated cells and to suppose that the equilibrium may shift according to the environmental conditions.

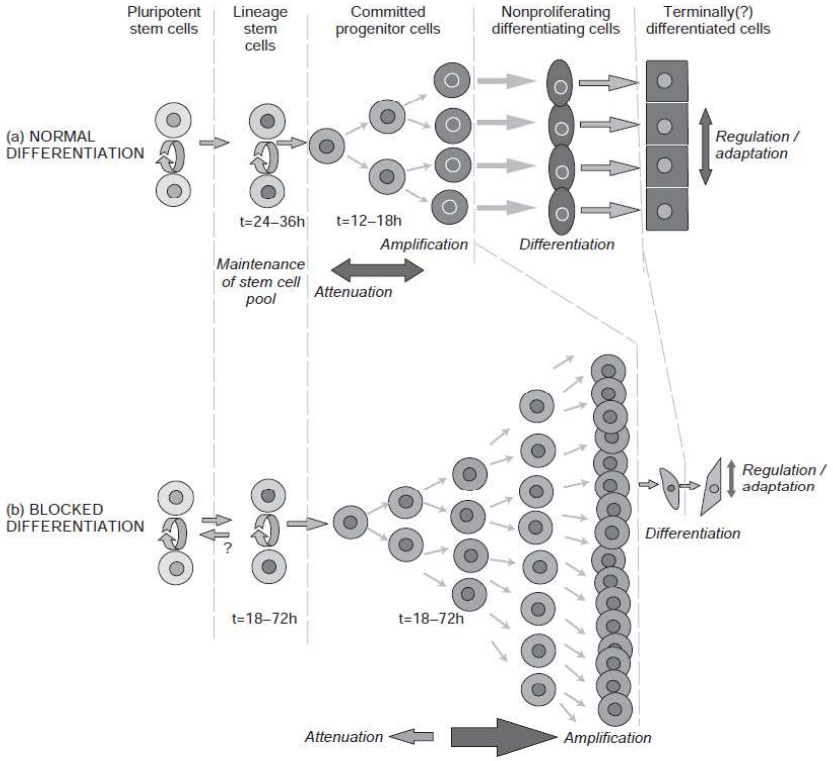

Figure 3: (a) In vivo, a small stem cell pool gives rise to a proliferating progenitor compartment that produces the differentiated cell pool. (b) In vitro, differentiation is limited proliferation, and the population becomes predominantly of progenitor cells, though stem cells may also be present.

Diamond nanoparticles have also been used to modify the substrate for the proliferation and differentiation of neural stem cells and the configuration of the growth surface can also be altered by photoetching. Treatment of the substrate with denatured collagen improves the attachment of many types of cells, such as epithelial cells, and the nondenatured gel may be necessary for the expression of differentiated functions.

Interesting facts:

1. Cells in culture can be thought to be in a state of equilibrium between cell proliferation and differentiation. Normal culture conditions i.e. in low cell density, mitogens in the medium will favor cell proliferation, whereas high cell density and addition of differentiation factors will induce differentiation.

2. Patients with poorly differentiated tumors generally have a lower survival rate than patients with differentiated tumors.

Questions:

1. What is differentiation? What are the major pathways of differentiation?
2. What are the parameters that are required for control of differentiations?
3. What are the relationship between differentiation and malignancy?

References:

1. Fresheny, I.; "Culture of animal cell – A manual of basic technique and specialized application.

M6 L5 Three dimensional cell culture

Three Dimentional Culture: Three-Dimensional (3-D) cell cultures have been widely used in biomedical research since the early decades of this century. The potential of 3-D cell cultures is currently being exploited in various areas of biomedical research. One reason for the recent progress in research on multi cell systems may be the increasing interaction between researchers working in different fields of biomedical science and using similar 3-D culture techniques. Such a research effort mirrors the common need for improved and more refined in vitro models as a link between cell-free systems or single cells and organs or whole organisms in vivo. One major advantage of 3-D cell cultures is their well-defined geometry, which makes it possible to directly relate structure to function and which enables theoretical analyses such as diffusion fields. Subsequently, the most promising data on these cultures may be obtained by using techniques allowing for spatial resolution. Combining such approaches with molecular analysis has clearly confirmed that, in comparison with conventional cultures, cells in 3-D cultures more closely resemble the in vivo situation with regard to cell shape and cellular environment. These parameters (shape and environment) can determine gene expression and the biological behaviour of the cells. In contrast to 2D monolayer, 3D cell culture models are modular, adaptable biomedical systems consisting range in complexity from a single cell type (monotypic), representing the minimum unit of the differentiated tissue *in vivo* to complex co-culture models that recapitulate both the 3D architecture and the multicellular complexity of the parental tissue. There will always be a number of questions that can only be answered by investigations using single cells or cell-free systems. At the same time, 3-D cultures cannot completely replace the testing of biological mechanisms for their relevance in vivo, e.g., in knockout animals

Effect of Cell Density:

Cell–cell interaction is manifested at the simplest level when a cell culture reaches confluence and the constituent cells begin to interact more strongly with each other because of contact mediated signaling, formation of junctional complexes and increased potential for exchange of homocrine factors. The first noticeable effect is cessation of cell motility (contact inhibition) and withdrawal from cell cycle (density limitation of cell proliferation) in normal cells and reduced cell proliferation and increased apoptosis in transformed cells.

Reciprocal Interaction:

When different cells interact in their population, they have tendency to show reciprocal effect on their respective phenotypes, and the resultant phenotypic changes lead to new interactions. Cell interaction is therefore not just a single event, but a continuing cascade of events. Similarly exogenous signals do not initiate a single event, as may be the case with homogeneous populations, but initiate a new cascade, as a result of the exogenously modified phenotype of one or both partners.

Choice of Model for Three Dimensional Cultures:

There are two major way to approach these goals.

- One is to accept the cellular distribution within the tissue, explant it and maintain it as an organ culture.
- The second is to purify and propagate individual cell lineages, study them alone under conditions of homologous cell interaction, recombine them, and study their mutual interactions.

Types of Three Dimensional cultures: There are three main types of three dimensional cultures:

- Organ culture
- Histotypic culture
- Organotypic culture

1) **Organ culture:** Organ culture in which whole organs or representative parts are maintained as small fragments in culture and retain their intrinsic distribution, numerical and spatial, of participating cells. Organ culture seeks to retain the original structural relationship of cells of the same or different types, and hence their interactive function in order to study the effect of exogenous stimuli on further development. Organ culture seeks to retain the original structural relationship of cells of the same or different types and hence their interactive function, in order to study the effect of exogenous stimuli on further development.
2) **Histotypic culture:** Histotypic culture in which propagated cells are grown alone to high density in a three-dimensional matrix or are allowed to form three-dimensional aggregates in suspension
3) **Organotypic culture:** Organotypic culture in which cells of different lineages are recombined in experimentally determined ratios and spatial relationships to recreate a component of the organ under study

Organ Culture:

Gas and Nutrient Exchange: A major deficiency in tissue architecture in organ culture is the absence of a vascular system, limiting the size (by diffusion) and potentially the polarity of the cells within the organ culture. When cells are cultured as a solid mass of tissue, gaseous diffusion and the exchange of nutrients and metabolites is from the periphery, and the rate of this diffusion limits the size of the tissue. The dimensions of individual cells cultured in suspension or as a monolayer are such that diffusion is not limiting, but survival of cells in aggregates beyond about 250 μm in diameter (~5000 cell diameters) starts to become limited by diffusion, and at or above 1.0 mm in diameter (~2.5×10^5 cell diameters) central necrosis is often apparent. To alleviate this problem, organ cultures are usually placed at the interface between the liquid and gaseous phases, to facilitate gas exchange while retaining access to nutrients. This is achieved by most

system by positioning the explant in a filter well insert on a raft or gel exposed to the air, but explants anchored to a solid substrate can also be aerated by rocking the culture, exposing it alternately to a liquid medium and a gas phase or by using a roller bottle or rotating tube rack. Anchorage to a solid substrate can lead to the development of an outgrowth of cells from the explant and resultant alterations in geometry even though this effect can be minimized by using a hydrophobic surface. One of the advantages of culture at the gas–liquid interface is that the explant retains a spherical geometry if the liquid is maintained at the correct level. If the liquid is too deep, gas exchange is impaired whereas if it is too shallow, surface tension will tend to flatten the explants and promote outgrowth. Permeation of oxygen increases by using increasing O_2 concentrations up to pure oxygen or by using hyperbaric oxygen. As increasing the O_2 tension will not facilitate CO_2 release or nutrient metabolite exchange, the benefits of increased oxygen may be overridden by other limiting factors.

Structural Integrity: The maintenance of structural integrity is the main reason for adopting organ culture as an in vitro technique in preference to cell culture. Whereas cell culture utilizes cells dissociated by mechanical or enzymatic techniques or spontaneous migration, organ culture deliberately maintains the cellular associations found in the tissue.

Growth and Differentiation: There is a relationship between growth and differentiation such that differentiated cells no longer proliferate. It is also possible that cessation of growth, regardless of cell density, may contribute to the induction of differentiation, if only by providing a permissive phenotypic state that is receptive to exogenous inducers of differentiation. Because of density limitation of cell proliferation and the physical restrictions imposed by organ culture geometry, most organ cultures do not grow or if they do proliferation is limited to the outer cell layers. Hence the status of the culture is permissive to differentiation and the appropriate cellular interactions and soluble inducers are provided as an ideal environment for differentiation to occur.

Limitations of Organ Culture:

- Organ cultures depend largely on histological techniques and they do not impart themselves readily into biochemical and molecular analyses.
- Biochemical monitoring requires reproducibility between samples, which is less easily achieved in organ culture than in propagated cell lines, because of sampling variation in preparing an organ culture, minor differences in handling and geometry, and variations in the ratios of cell types among cultures.
- Organ cultures are also more difficult to prepare than replicate cultures from a propagated cell line and do not have the advantage of a characterized reference stock to which they may be related.
- Organ culture is essentially a technique for studying the behaviour of integrated tissues rather than isolated cells.
- Organ culture has contributed a great deal to our understanding of developmental biology and tissue interactions and that it will continue to do so in the absence of adequate synthetic systems.

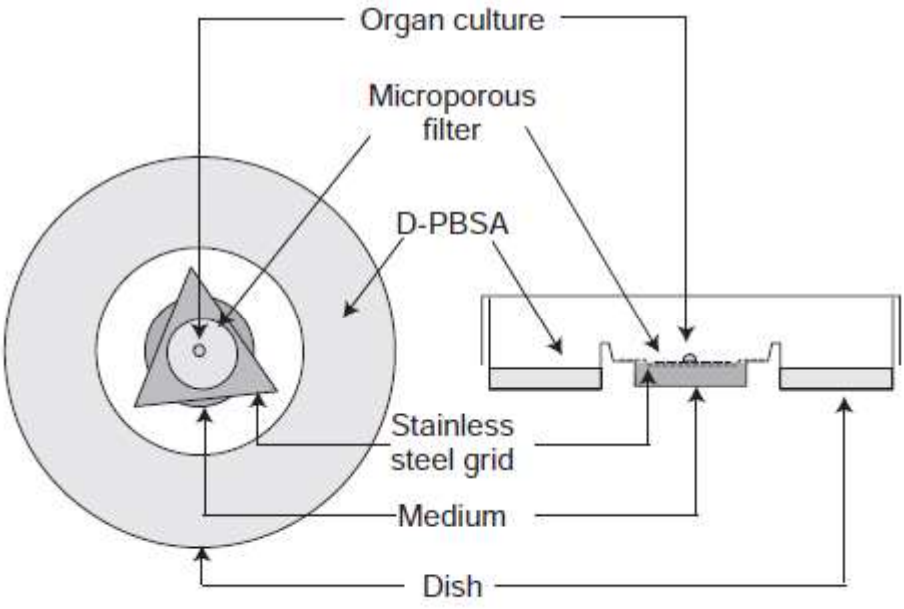

Figure 1: Organ Culture Small fragment of tissue on a filter laid on top of a stainless steel grid over the central well of an organ culture dish

Histotypic culture:

Histotypic culture is defined as high-density cell culture with the cell density approaching that of the tissue in vivo. Various attempts have been made to regenerate tissue-like architecture from dispersed monolayer cultures. As cells reach a high density, medium nutrients will become limiting. To avoid this, the ratio of medium volume to cell number should remain approximately as it was in low density culture. This can be achieved by seeding cells on a small coverslip in the center of a large non–tissue-culture grade dish or by use of filter well inserts, which give the opportunity for the formation of both high-density polarized cultures and heterotypic combinations of cell types to create organotypic cultures. A high medium-to-cell ratio can also be maintained by perfusion.

Gel and Sponge Techniques: Use of three-dimensional sponges and gels has increased extensively with the development of tissue engineering. Two commonly used gel in this technique.

- **Collagen gel:** Collagen gel (native collagen, as distinct from denatured collagen coating) provides a matrix for the morphogenesis of primitive epithelial structures. Many different types of cell can be shown to penetrate such matrices and establish a tissue-like histology.
- **Matrigel:** Matrigel is a commercial product derived from the extracellular matrix of the Engelbreth–Holm–Swarm (EHS) mouse sarcoma which has been used for coating plastic but can also be used in gel form. It is composed of laminin, collagen, fibronectin, and proteoglycans with a number of bound growth factors, although it can be obtained in a growth factor-depleted form. It has been used as a substrate for epithelial morphogenesis formation of capillaries from endothelial cells and in the study of malignant invasion.

Organotypic Culture:

High density three-dimensional culture involving the recombination of different cell lineages may be referred to as organotypic culture, a term that used to distinguish these reconstruction techniques from organ culture where the original cells are not dissociated. The key event that distinguishes these constructs from histotypic culture is the introduction of heterotypic cell interaction including diffusible paracrine effects and signaling implicating the extracellular matrix. The relationship of the cells allows the

generation of a structured microenvironment, cell polarity and enhanced differentiation. Creation of organotypic culture by mixing cells randomly and allowing them to interact and sort, as can happen spontaneously particularly with embryonic cells or the construct may be design to keep the interacting cells separate so that their interactions may be studied.

Tissue Equivalents: The advent of filter well technology boosted by its commercial availability, has produced a rapid expansion in the study of organotypic culture methods.

Tissue Engineering: Just as organotypic culture needs cell interaction, constructs for tissue engineering often require similar interactions, as in the interaction between endothelium and smooth muscle in blood vessel reconstruction. In addition to biological interactions, some constructs require physical forces; skeletal muscle needs tensile stress, bone and cartilage needs compressive stress, and vascular endothelium in a blood vessel construct needs pulsatile flow.

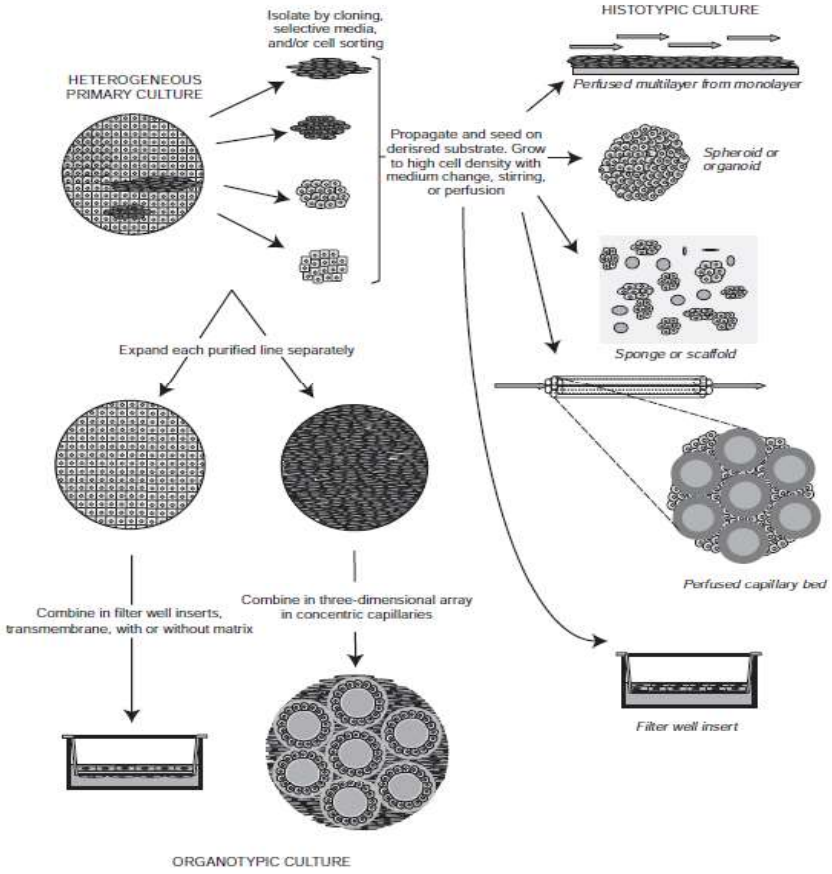

Figure 2: Histotypic and Organotypic Culture

Imaging Cells in 3-D Constructs: As we know that microscopic observation becomes difficult when cells are incorporated into a scaffold in a three-dimensional organotypic construct, alternative methods must be used to visualize the status of the cells within the construct. This can be done by NMR if the bioreactor housing the constructs is placed within an NMR detector and the output displayed as an MRI, and the emission spectrum being analysed.

Interesting facts:

1. Organ culture is a technique for studying the behaviour of integrated tissues.
2. Organ culture has contributed a great deal to our understanding of developmental biology and tissue interactions.

Questions:

1. What are the types of three dimensional cultures?
2. What are the advantages of three dimensional cultures?
3. What are Gel and Sponge Techniques and how its use increase with increasing genetic engineering?

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1. Mueller-Klieser, W.; Three-dimensional cell cultures: from molecular mechanisms to clinical applications. *The American Physiological Society,* 1997, C1109-C1123
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3. Barrila, J., Radtke, A.L., Crabbé, A., Sarker, S. F., Herbst-Kralovetz, M. M., Ott, C. M., Nickerson, C.A. Organotypic 3D cell culture models: using the rotating wall vessel to study host–pathogen interactions

M6 L6 Role of matrix in cell growth

Role of matrix in cell growth: Matrix is an insoluble, dynamic gel in the cytoplasm, believed to be involved in cell shape determination and locomotive mechanism, across a solid substrate. It consists of polymeric microtubules, actin microfilaments and intermediate filaments interacting with a number of other proteins.

Extracellular matrix (ECM): The extracellular matrix (ECM) is a part of three connective tissue layers (endomysium, perimysium, and epimysium) surrounding muscle fibres. Extracellular matrix is composed of proteins including collagens and proteoglycans.

Component of extra cellular matrix: ECM is comprised variously of collagen, laminin, fibronectin, hyaluronan and proteoglycans such as beta glycan, decorin, perlecan, and syndecan-1, some of which bind to growth factors or cytokines.

- Proteoglycans in extracellular matrix form a cross-linked meshwork with fibrous proteins
- Some proteins bind multiple other proteins and glycosaminoglycans (fibronectin).
- Integrin is a family of proteins that mediate signalling between cell interior and extracellular matrix
- Mass of interactions between cells and matrix not only anchors cells to matrix but also provides paths that direct migration of cells in developing tissue and (through integrin) conveys information in both directions across plasma membrane. Figure 1 depicts structure of cell matrix.

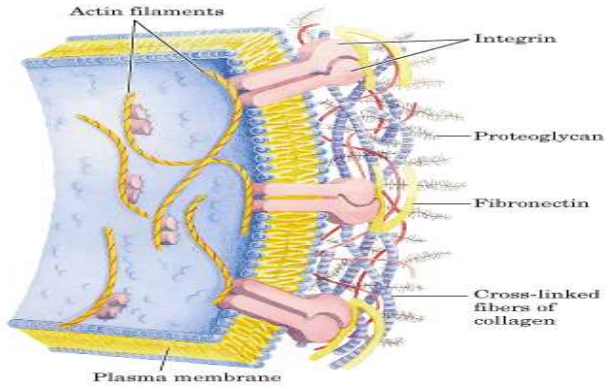

Figure 1: Cell matrix

ECMs are composed of proteins such as collagen and elastin that serve as scaffolds for cells as well as networks of various adhesion ligands and growth factors, which promote cell signalling. ECM is complex in both structure and composition.

Role of matrix: There are some important roles that matrix play in biological system which is described as follows.

- Matrixes are generally used for providing support
- It involve in segregating tissues from one another
- It takes part in regulation of intercellular communication
- Extracellular Matrix cells have been found to cause regrowth and healing of tissue.
- Some time it acts as fibrosis
- The use of ECM constituents can be highly beneficial in enhancing cell survival, proliferation, or differentiation, but unless recombinant molecules are used
- In human foetuses, the extracellular matrix works with stem cells to grow and regrow all parts of the human body and foetus can regrow anything that gets damaged in the womb
- In case of injury repair and tissue engineering, the extracellular matrix serves two main functions

a) It prevents the immune system by triggering from the injury and responding with inflammation and scar tissue

b) It facilitates the surrounding cells to repair the tissue instead of forming scar tissue

Molecular components: Components of the ECM are produced intracellularly by resident cells and secreted into the ECM through exocytosis. Once secreted, they then aggregate with the existing matrix. The ECM is composed of an interlocking mesh of fibrous proteins and glycosaminoglycans (GAGs).

Proteoglycans: Since we know that, GAGs are carbohydrate polymers and are usually attached to extracellular matrix proteins to form proteoglycans (exception-hyaluronic acid). Proteoglycans have a net negative charge that attracts positively charged sodium ions (Na^+) which attracts water molecules via osmosis, keeping the ECM and resident cells hydrated. Proteoglycans may also help to trap and store growth factors within the ECM. There are the different types of proteoglycan found within the extracellular matrix.

1. Heparin sulphate: Heparin sulphate (HS) is a linear polysaccharide found in all animal tissues. It occurs as a proteoglycan (PG) in which two or three HS chains are attached in close proximity to cell surface or extracellular matrix proteins. HS binds to a variety of protein ligands and involve in regulation of a wide variety of biological activities, including developmental processes, angiogenesis, blood coagulation and tumour metastasis. In the extracellular matrix, particularly basement membranes, the multi-domain proteins perlecan, agrin and collagen XVIII are the main proteins to which heparin sulphate is attached.

2. Chondroitin sulphate: Chondroitin sulfates help to provide the tensile strength of cartilage, tendons, ligaments and walls of the aorta. They have also been known to affect neuroplasticity.

3. Keratan sulphate: Keratan sulfates have variable sulfate content and unlike many other GAGs, do not contain uronic acid. They are present in the cornea, cartilage, bones and the horns of animals.

Non-proteoglycan polysaccharide: There are various non-proteoglycan polysaccharides.

1. **Hyaluronic acid:** Hyaluronic acid (or hyaluronan at physiological pH) is a polysaccharide containing alternating residues of D-glucuronic acid and *N*-acetyl glucosamine. Unlike other glycosaminoglycan (GAGs) it is not found as a proteoglycan. Like cellulose and chitin, it is synthesized at the plasma membrane by a transmembrane hyaluronan synthase. Hyaluronan is the only GAG that occurs as a single long polysaccharide chain. Hyaluronate is also an essential component of the extracellular matrix of cartilage and tendons, to which it contributes tensile strength and elasticity as a result of its strong interactions with other components of the matrix. A number of proteoglycans interact with hyaluronan to form large complexes in the extracellular

matrix. A well-characterized example is aggrecan, the major proteoglycan of cartilage. Hyaluronic acid acts as an environmental sign that regulates cell behaviour during embryonic development, healing processes, inflammation and tumour development. It interacts with a specific trans-membrane receptor, CD44.

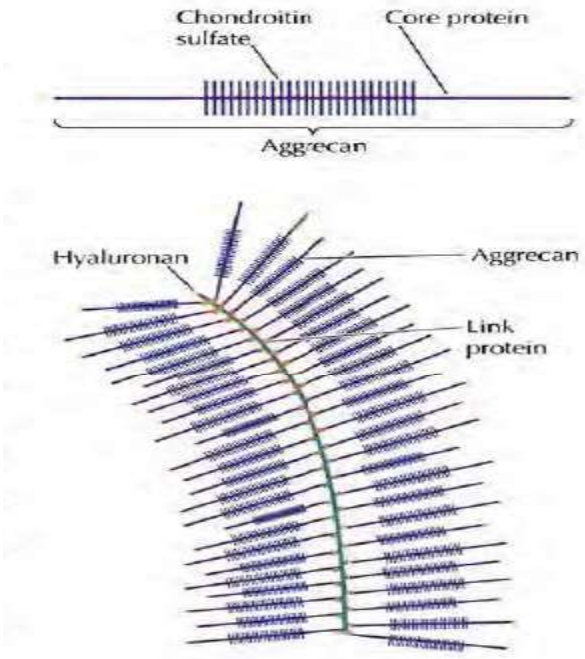

Figure 2: Structure of hyaluronan and aggrecan

2. Matrix Structural Proteins: Extracellular matrices are composed of tough fibrous proteins embedded in a gel-like polysaccharide ground substance-a design basically similar to that of plant cell walls.

Collagen: In ECM of most animals, collagens are the abundantly found structural protein. In fact, collagen is the most abundant protein in the human body and accounts for 90% of bone matrix protein content. Collagens are present in the ECM as fibrillar proteins and give structural support to resident cells. Collagens are a large family of proteins containing at least 27 different members. They are characterized by the formation of triple helices in which three polypeptide chains are wound tightly around one another in a rope-like structure. The different collagen polypeptides can assemble into 42 different trimers. The triple helix domains of the collagens consist of repeats of the amino acid sequence Gly-X-Y. A glycine (the smallest amino acid, with a side chain consisting only of hydrogen) is required in every third position, so that the polypeptide chains can pack together close enough to form the collagen triple helix. Proline is

frequently found in the X position and hydroxyproline in the γ position; because of their ring structure these amino acids stabilize the helical conformations of the polypeptide chains. The unusual amino acid hydroxyproline is formed within the endoplasmic reticulum by modification of proline residues that have already been incorporated into collagen polypeptide chains. Lysine residues in collagen are also frequently converted to hydroxylysines. The hydroxyl groups of these modified amino acids are thought to stabilize the collagen triple helix by forming hydrogen bonds between polypeptide chains. These amino acids are rarely found in other proteins although hydroxyproline is also common in some of the glycoproteins of plant cell walls.

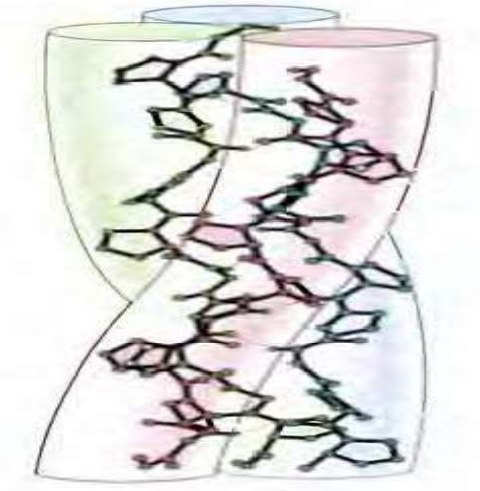

Figure 3: Structure of triple helix collagen

The collagen can be divided into several families according to the types of structure they form:

Table 1: Classification of collagens

Collagen class	Type	Tissue Distribution
Fibril- forming	I	Most connective tissue
	II	Cartilage and vitreous humors (e.g. skin and lung)
	III	Tissue containing collagen I
	V	Cartilage
	XI	Bone and cornea
	XXIV	Eye, ear and lung
	XXVII	Cartilage
Fibril-associated	IX	Cartilase
	XII	Tissue containing collagen I
	XIV	Tissue containing collagen I
	XVI	Many tissue
	XIX	Many tissue
	XX	Cornea
	XXI	Many tissue
	XXII	Cell junctions
	XXVI	Testis and overy
Network forming	IV	Basal laminae
	VIII	Many tissues
	X	Cartilase
Anchoring fibrils	VII	Attachement of basal laminae to underlying connective tissue
Transmembrane	XVII	Skin hemidesmosomes
	XXV	Nerve cells

The most abundant type of collagen (type I collagen) is one of the fibril forming collagens that are the basic structural components of connective tissues. The polypeptide chains of these collagens consist of approximately a thousand amino acids or 330 Gly-X-Y repeats. After being secreted from the cell these collagens assemble into collagen fibrils in which the triple helical molecules are associated in regular staggered arrays. These fibrils do not form within the cell because the fibril forming collagens are synthesized as soluble precursors (procollagens) that contain nonhelical segments at both ends of the polypeptide chain. Procollagen is cleaved to collagen after its secretion, so the assembly of collagen into fibrils take place only outside the cell. The association of collagen molecules in fibrils is further strengthened by the formation of covalent

crosslinks between the side chains of lysine and hydroxylysine residues. Frequently, the fibrils further associate with one another to form collagen fibers, which can be several micrometers in diameter.

Elastin: In contrast to collagens, Elastins give elasticity to tissues, allowing them to stretch when needed and then return to their original state. This is useful in blood vessels, the lungs, in skin, and the ligaments. Elastins are synthesized by fibroblasts and smooth muscle cells. Elastins are highly insoluble, and tropoelastins are secreted inside a chaperone molecule, which releases the precursor molecule upon contact with a fiber of mature elastin. Tropoelastins are then deaminated to become incorporated into the elastin strand. Diseases such as cutis laxa and Williams syndrome are associated with deficient or absence of elastin fibers in the ECM.

Matrix adhesion proteins: Matrix adhesion proteins, the final class of extracellular matrix constituents are responsible for linking the components of the matrix to one another and to the surfaces of cells. They interact with collagen and proteoglycans to specify matrix organization and are the major binding sites for integrins.

1. Fibronectin: Fibronectin is the principal adhesion protein of connective tissues. Fibronectin is a dimeric glycoprotein consisting of two polypeptide chains, each containing nearly 2500 amino acids. Fibronectin are proteins that connect cells with collagen fibers in the ECM, allowing cells to move through the ECM. Within the extracellular matrix, fibronectin is often cross-linked into fibrils. Fibronectin has binding sites for both collagen and GAGs so it cross-links these matrix components. Fibronectins bind collagen and cell surface integrins, causing a reorganization of the cell's cytoskeleton and facilitating cell movement. Fibronectin are secreted by cells in an unfolded, inactive form. Binding to integrins unfolds fibronectin molecules, allowing them to form dimers so that they can function properly. Fibronectins also help at the site of tissue injury by binding to platelets during blood clotting and facilitating cell movement to the affected areas during wound healing.

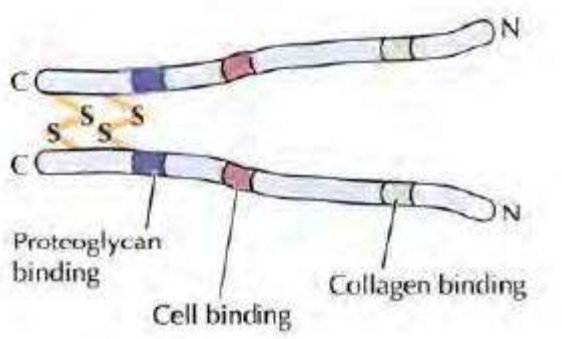

Figure 4: Structure of Fibronectin

2. Laminin: In almost all animals, Basal laminae contain distinct adhesion proteins of the laminin family. Laminins are heterotrimers of α, β and γ subunits which are the products of five α genes, four β genes, and three γ genes. Like type IV collagen, laminins can self-assemble into meshlike polymers. Such laminin networks are the major structural components of the basal laminae synthesized in very early embryos, which do not contain collagen. The laminins also have binding sites for cell surface receptors such as integrins, type IV collagen, and the heparan sulfate proteoglycan, perlecan. In addition, laminins are tightly associated with another adhesion protein, called entactin, which also binds to type IV collagen. As a result of these multiple interactions, laminin, entactin, type IV collagen, and perlecan form cross-linked networks in the basal lamina. They also support in cell adhesion.

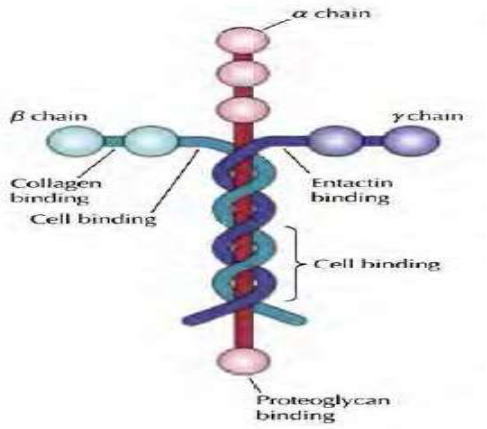

Figure 5: Structure of laminin

Cell adhesion to the ECM: Many cells bind to components of the extracellular matrix. Cell adhesion can occur in two ways by focal adhesions, connecting the ECM to actin filaments of the cell, and hemi-desmosomes, connecting the ECM to intermediate filaments such as keratin. This cell-to-ECM adhesion is regulated by specific cell surface cellular adhesion molecules (CAM) known as integrins. The integrins are a family of transmembrane proteins consisting of two subunits, designated α and β. Integrins are cell surface proteins that bind cells to ECM structures such as fibronectin and laminin, and also to integrin proteins on the surface of other cells.

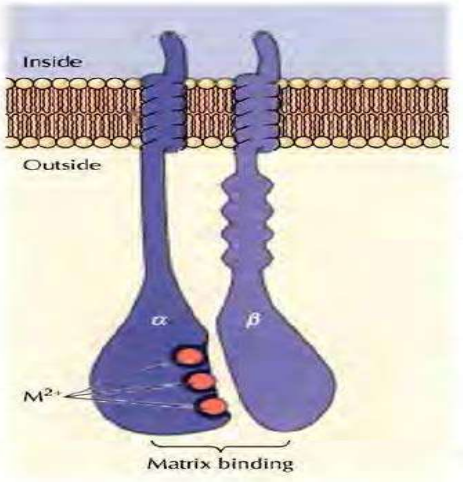

Figure 6: Structure of Integrin

Fibronectins bind to ECM macromolecules and facilitate their binding to transmembrane integrins. The attachment of fibronectin to the extracellular domain initiates intracellular signaling pathways as well as association with the cellular cytoskeleton via a set of adaptor molecules such as actin.

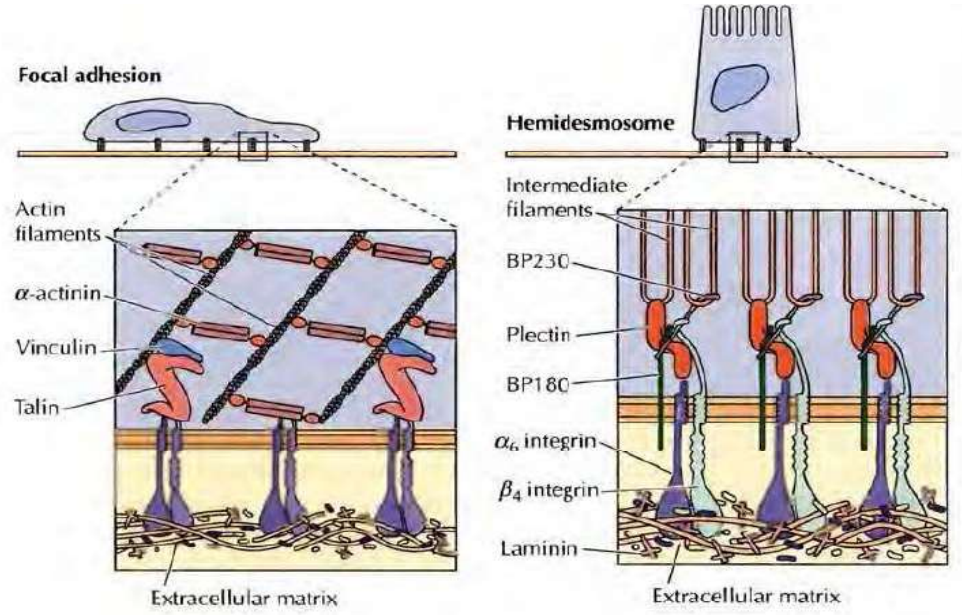

Figure 7: Focal adhesion and Hemidesmosome

Commercially available matrices:

Commercially available matrices such as Matrigel™ (Becton Dickinson) from the Engel breth–Holm–Swarm (EHS) sarcoma, contain laminin, fibronectin, and proteoglycans, with laminin predominating. Other matrix products include Pronectin F (Protein Polymer Technologies), laminin, fibronectin, vitronectin entactin (UBI), heparan sulfate, EHS Natrix (BD Biosciences), ECL (US Biological), and Cell-tak (BD Biosciences). Some of these products are purified, whereas others are a mixture of matrix products that have been poorly characterized and may also contain bound growth factors. If cell adhesion for survival is the main objective, and defined substrates are inadequate, the use of these matrices is acceptable, but if mechanistic studies are being carried out, they can only be an intermediate stage on the road to a completely defined substrate.

Table 2: Examples of some matrix

Material	Composition	Source
Matrigel	Laminin, fibronectin, collagen IV, proteoglycans, growth factors (growth factor depleted available)	EHS sarcoma
EHS Natrix	Laminin, fibronectin, collagen IV, proteoglycan, growth factors	Cell line from EHS sarcoma
Cell-Tak	Polyphenolic protein	Mytilus edulis
Collagens(various)	Collagen I, II, III, IV	Human, bovine, rat tail
ProNectin F	Protein polymer with multiple copies of RGD containing epitops	Recombinant
Laminin	Attachement protein from basement membrane	Natural
Laminin	Attachement protein from from basement membrane	Recombinant
Fibronectin	Attachement protein from from extracellular matrix	Natural
Fibronectin	Attachement protein from from extracellular matrix	Recombinant
Heparan sulfate	Matrix proteoglycan	Natural
ECL	Enactin-collagen IV-laminin	Natural
Vitronectin	Attachement protein from extracellular matrix	Natural
ECM	Extracellular matrix	natural

Interesting facts:

1. Collagens are abundantly found structural protein in the human body and accounts for 90% of bone matrix protein content.

2. Elastins give elasticity to tissues, allowing them to stretch when needed and then return to their original state.

3. Fibronectins bind to ECM macromolecules and facilitate their binding to transmembrane integrins.

4. Basal laminae contain distinct adhesion proteins of the laminin family in almost all animals.

Questions:

1. Explain the role of matrix in cell culture?
2. Give the detail about matrix material that are generally used?
3. What is the difference between proteoglycan and non-proteoglycan polysaccharide and how can they involve in formation of matrix?

References:

1. http://www.biology-online.org/dictionary/Cell_matrix
2. Cooper GM The Cell: A Molecular Approach. 4th edition; chaptor-14: Cell walls, the extracellular matrix, and cell interactions
3. Fresheny, I. "Culture of Animal Cells- A Manual of Basic Technique and Specialized Applications"
4. What are the types of matrixes? What are the advantages of matrix adhetion protein?

Cell Culture Technologies
Prof. Mainak Das
Department of Biological Sciences & Bioengineering & Design Programme
Indian Institute of Technology, Kanpur

Lecture – 04
Cell Cycle Concept

Welcome back to the lecture series in Cell Culture Technology. So, during the first 3 class we have dealt with the myriad of opportunities which cell culture technology has offered and will be offering in future, were a glance of it using cell culture as a tool for sensing as a sensor element as a bioreactor to produce different kind of compounds of biomedical as well as other significance.

Apart from it we highlighted the fact that understanding the fundamentals of cell culture technology and the basic philosophies will be one of the very basic prerequisites for stem cell biology and regenerative medicine. Apart from it the dream of man kind of having artificial organs and when I talk about artificial organ here, we are not I am not talking about extracorporeal devices where we have dialysis bags or all those other kinds of things or you now heart lung machine. I am talking about kidney developed from biological elements very similar to our existing kidney or a heart developed using cardio myocytes endothelial cells so and so forth, that is where the future is.

Now, while talking about the biology of the cells we talked about the oxygen tension, oxygen and carbon dioxide very briefly of course, we will come back to this thing. Then we talked about extracellular matrix protein and there we discuss the opportunity of using extracellular matrix system to discover newer and newer extra cellular matrix which may be even synthetic analogues. And how a cancer cell cheats by moving out from its actual extracellular matrix profile and kind of you know like a rogue cell colonizes at different places, while talking about this we also talked about the concept of adhering cell or non-adhering cell. So, we have exclusively talked about the adhering cells, but just if you think of its little bit louder on this.

So, we have this non-adhering cell which those start their journey from even adhering environment say for example, if you take blood if you think of the blood cells to be something like this ok.

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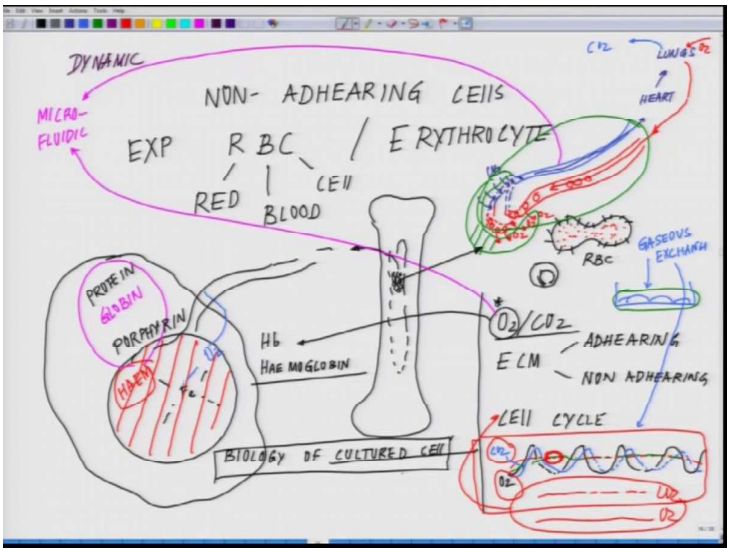

Let us once again, this is our week 1, lecture 4. So, this is a fourth lecture and talking about non-adhering cells. So, if you taken for example, if we take the example of RBC or red blood cell which is also called erythrocytes. These erythrocytes have their origin in the bone marrow somewhere out here, from here these erythrocytes initially they divide from hematopoietic stem cells and they migrate and they migrate through the blood vessels and they have a limited life is span afterward it gets destroyed.

So, one interesting feature about these cells are that, they travel all over the body yet really, they anchor at any point. So, apparently it looks like there, if this is a red blood cell which is devoid of it is a like kind of you know hollow stuff with something like the side view is concave disk, by concave disk if you look at RBC. So, it looks like the extracellular composition in such that that it does not get adhere at any site which is remarkable the different from an adhering cell, where the extracellular perturbation which are coming out from the cells have the ability to adhere. So, if one has to develop a culture system for non-adhering cells, adhering we have talked about you know they has taken to a surface and then we have to have a different kind of paradigm realize and this paradigm has to be totally different and talking about this while sighting this example of a bone marrow.

Let us talk about the third aspect of, we have talked about oxygen and CO_2, we have talked about e c m and we have briefly talked about adhering and non-adhering cells.

Now let us talk about the concept of cell cycle, this is we are talking about and the broad heading of biology of cultured cells. So, before I move on to the cell cycle part I wish to highlight one point which I missed out while I was talking to you about oxygen and carbon dioxide. So, if you realize in our body the oxygen supply is met by the molecule called hemoglobin Hb, hemoglobin which is complex it is a globin protein with a haem complex haem complex is nothing, but iron and it is its found coordination complex and it is entrapped in a porphyrin ring and this whole thing is kind of you know place inside a protein call globin.

So, this is essentially is the color imparting one call the haem fragment and this part is the globin protein. So, that is why it makes its hemoglobin and hemoglobin is responsible for attaching to the oxygen and transporting its. So, hemoglobin is the key part of the RBC's. So, this is how in a real-life scenario happens, like these are the vessels or the veins and these are the arteries and of course, there is a connecting zone something like this, but the capillaries and they exchange then there is narrowing down which is happening like this similarly simplistic way I am just putting it together something like that.

So, now concern tissues are sitting out here, the oxygenated blood or the red blood cells travel here and unload their oxygen, is oxygen is taken up by the tissues and the veins out here takes up the CO_2 which is given away by these cells this is brought back via heart goes to the lungs and from the lungs any picks up the oxygen throw away the carbon dioxide picks up the O_2, throw away the CO_2 and it oxygen further comes back after attaching the hemoglobin.

Now, if you realize each one of these tissues which are there in our body, they are not continuously in an oxygenated environment. So, the oxygen profile if you just try to imagine in any part of your body is kind of fluctuating in a manner like this, realizing because this is the flow which consumes and parallelly with a small delay you will see if this black what I drew is of oxygen and there will be a carbon dioxide peaking which will be something like this. So, what essentially, I wish to highlight which I forgot in the previous class is oxygen and CO_2 is dynamically changing in a real-life scenario, but if you grow cells in a dish like this say for example, this is the dish K where you are growing the cell. So, what we essentially do is that we keep the gaseous exchange, because there is no other way for us, gaseous exchange something like this we have to

draw it will be something like this for both the gases or with us little bit of a say for example, if one color represents one gas the other one will be something like this.

Which is absolutely different from what happens in the real life, but then how we could get around this problem that will introduce us to the world of the next gen cell culture technologies which will be just like these kinds of vessels, what you see the arteries and the veins. The next gen cell culture technology will be using micro fluidics systems to mimic the conditions which are there in real life and maybe such micro fluidic structures will be given us a very different understanding about the dynamics of cellular growth is a continuous process 24X7 multiplied by x as long as those cells or that individual is alive.

It is a continuous process which is happening, heart is pumping the blood is going loaded with oxygen downloading the oxygen at a specific at all tissues picks up, upload the carbon dioxide comes back again do the same thing in a side sinusoidal manner it is continuously wearing. But could a mimic those conditions, food for thought will be coming later, once we will talk about this micro fluidic channel and use of micro fluidics how to do all the kind of things or people are progressing what are the next level of technologies and what are the technological blockages.

There are some any blockages also it is not so easy, but such systems are unlike last 100 years these are more dynamic system.

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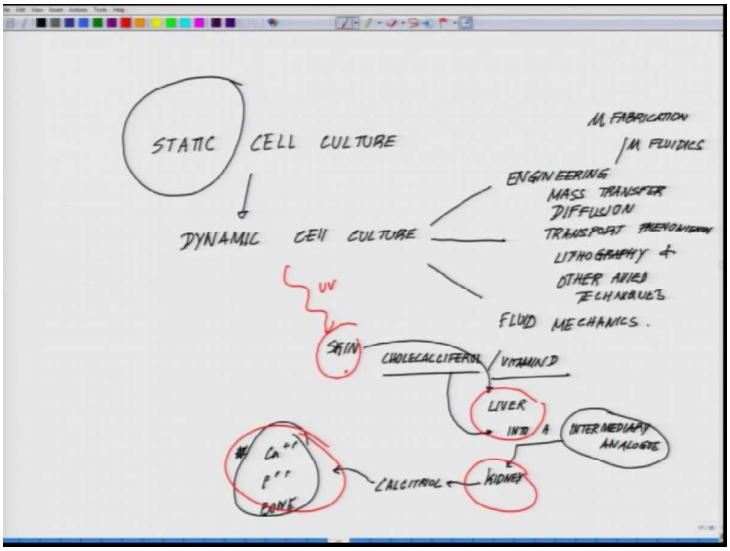

So, you can say that modern cell culture from static cell culture the future will be more of a dynamic cell culture and such dynamic cell culture will be read, needing lot of understanding of engineering and within engineering micro fabrication, micro fluidics, mass transfer diffusion, lot of understanding of transport phenomena, lithography and other and other allied techniques and fluid mechanics.

So, 100 years round the line probably the way today's text books are written those will change because, those will change because we are assuring into a very in different time, but slowly we are realizing that we cannot study a system of this magnitude where every second or every minute the milieu is getting fresh and up or getting perturbed by static systems, not only that.

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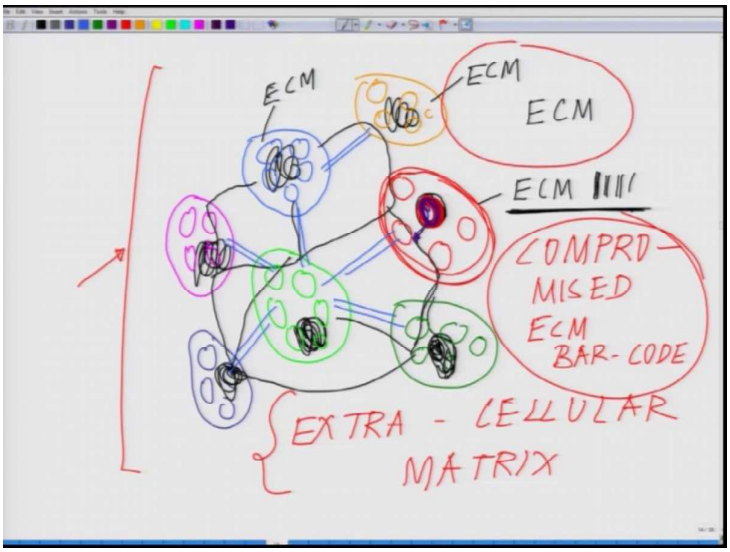

Just when I drew this, so there is always information exchange which is taking place between these multisystem systems, multi system structures. So, say for example, one simple example let me give you, what I mean by that?

So, you know that our, we are being always advised and you know in the morning go out and because there are u v rays which are slightly helpful. So, what we observe the UV what happened steroids derived from cholesterol which are present in our skin, underneath the skin they get converted into vitamin d and for this reaction to happen you need ultraviolet rays. Now once that happens this is remaining d which is also called cholecalciferol, cholecalciferol from a, from your skin just think of it, from your skin is

transported to the liver. This is cholecalciferol or vitamin d it is transported to the liver in the lever this cholecalciferol is converted into intermediary analogue, this analogue is this intermediary analogue is transported to the kidney, where this is transformed into and hormone called calcitriol and calcitriol is the hormone which is secreted by the kidney which is responsible for absorbing calcium and phosphorus by the bone.

So, what you see here is there is a cross talk happening between skin, liver, kidney, bone that is the complexity or that is the integrity of such systems are. So, future cell culture technology will be very dynamic. So, that is where we (Refer time: 20:41) rest on we I told you that you know, whenever we will have to talk about these things we have to talk by pulling everything together we cannot talk in isolation that you know I see this I observe this in, but then you have to have what is the implication of it? Why these particular cell types behave since such a way? Where we maintain the conditions, what is needed and again one has to realize if we maintain a higher oxygen tension, this may lead to oxidative like oxidative damage because higher oxygen tension always leads to the free radical formation.

That is the penalty we pay for being an oxygenated environment. So, we have to ensure that the oxygen tension is not maintained at a higher level for a prolonged period of time, similarly that brings us that to the fact that how very systematically we can keep the oxygen tension low and ensure there is not much accumulation of CO_2. Having said this, this brings us to 2 different situations, such sometime such situation this situation which is currently prevailing this is the constant level of CO_2 and oxygen which is mostly supplied by the air unlike what is happening in real life can lead to certain cell cycle issues.

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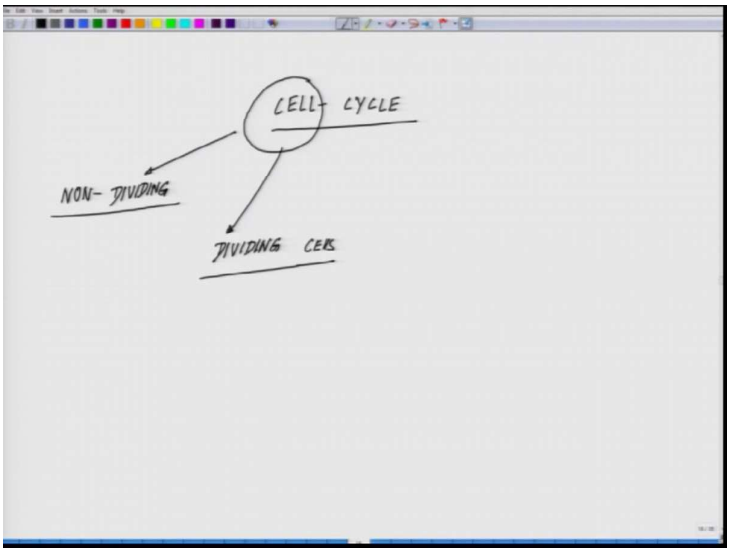

So, let us first of all talk about where we suppose to start is cell cycle. So, here let me highlight that you can, in the previous class I told you that you can distinguish between adhering cell and non-adhering cells right. Now I am introducing that we can classify the cells under 2 groups dividing cells and non-dividing cells. So, I am classifying the cells now as non-dividing and dividing. So, if we talk about dividing and non-dividing what are the cells which comes in your mind, one of the cells which you must all be aware of are neurons, they do not divide, at least the terminally differentiate a neuron do not divide. So, I used 2 terms here terminally differentiated what does that mean, I will come to that.

Similarly, there are cardio myocytes which do not divide yet you have your skin whole surface is a continuous rejuvenation, there is a continuous division which is taking place at the epidermal layer from all the way if you remember all the 5 layers there is continuously I rejuvenate in process which is happening, now a time that can we come on control as we have discussed in the previous class which may lead to cancer is like situation or tumor. So, what will do next is we will talk about the different stages of division and how that influences the way we are conducting the culture.

So, I will close in here in the next class we will start off with the cell cycle.

Thank you.

Cell Culture Technologies
Prof. Mainak Das
Department of Biological Sciences & Bioengineering & Design Programme
Indian Institute of Technology, Kanpur

Lecture - 05
Dividing Cells

Welcome back to the lecture series in Cell Culture Technology.

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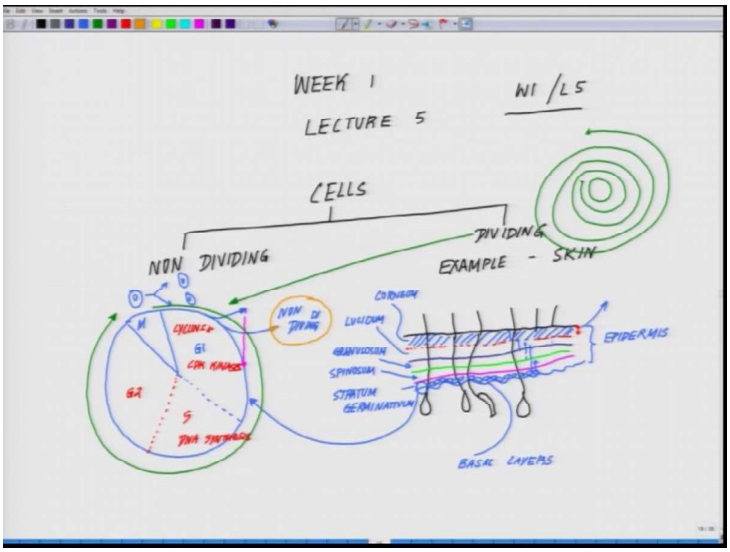

Today we are in the 5th lecture of the 1st week it is put that down otherwise I will miss it out it is Week 1 lecture 5 W1/L 5.

We closed on in the previous lecture in the fourth lecture on cell cycle, where is the fag end of the lecture I introduce the 2 cell types broadly speaking; the Dividing and the Non-dividing cell. And in the non-dividing cell I give the example of a neuronal cell, cardiac cell and in the dividing type I talked about the skin cells I am just taking example there are so many of this. Whenever you talk about dividing cell think of it say if I take the let us start it. So, talking about the cells Non-dividing and this side and Dividing on other side.

So, my example was skin now if you look at the skin itself skin consists of 2 layers epidermal layer and the dermal layer. If you look at your own skin to the something like this is. The skin the hairs are coming out like this and this here cell bodies are kind of

sitting like this and underneath it we will see first layer like this, second layer like this I am just broadening it up, but is not that thick of layer third layer like this, fourth layer like this and fifth layer which is something like this. So, I am just kind of you know this is the epidermis. And within an epidermis the layers are this is stratum Germinativum stratum this one is Stratum Granulosum in between is Stratum Spinosum, Stratum Lucidum, and Stratum Corneum.

The interesting part is this layer this layer contains some apart from other cells like mortal cells and melanocytes this layer contains something called basal cells. These cells you can considered them as the stem cells or the germ cells for you know the germ cells is wrong word sorry let me take back the skin stem cells which divide to form the skin layer. So, whatever divides here they move like this layer by layer them move and then they come to the uppermost layer. And this whole process this migration of the cell takes around 15 to 20 days and at the top layer these cells survive for 2 to 3 weeks before they are sluft off and the previous layer moves to the upper layer.

It is always like if the lowermost layer is 5 next is 4 then 3 2 1. So, from 5 something germ to germs to 4 from 4 that 1 germ to 3 from 3 to 2 2 to 1 and then form 1 0 it gets you know sluft of from the skin similarly this whole process continuous now coming said this now start let us talk about this cell cycle process. This individual cell has can go through something called a process of mitosis M phase what we call as the mitosis is the M phase after the mitosis where the chromosome separates out and the 2 daughter cells are formed. This is the cell now and here the nucleus it is chromatic condensers and the 2 daughter cells are formed.

At this stage next stage pose mitosis this cell has a choice this is called the gas phase G 1 phase it has a choice and it is a decision to make whether it is going to terminally go out of dividing and it becomes non-dividing or non-dividing or it temporarily goes out of division and then in due course it again comes back to do the division cycle. There are several chip points here as this several cyclins and cdk kinases which are involved I am not getting in into those. So, there are several such molecules which are dealing with its cyclins and cdk kinases and there are several check points, which ensures whether they are going to go further or not. Then comes a phase called S phase if they decide to you know go further and this is the S phase where DNA synthesis happens. And followed by

a G 2 phase where the again have a choice again have a choice and there are lot of checkpoints out here.

So, cell essentially goes through this cycle. Now that brings us to a very interesting question that how many times a dividing cell will be going through this cycle is this number finite or is this number infinite say for example, a particular cell like this how many cycles 1 2 3 4 likewise one and so forth how many cycles it can do. Because with every cycle there is something in it is chromosome called telomere the telomere length reduces and as a matter of fact the length of the telemeter can tell you which is part of the chromosome can tell you that what is the edge of it? Because as more and more telomere lengths are lost eventually the cell loses it is viability loses it is original characteristics. If cell at this stage say for example, at G 1 phase out here decides to finally, land up that it will become non-dividing it will permanently go to the non-dividing phase, then it has to decide what final function it will attain what does that mean.

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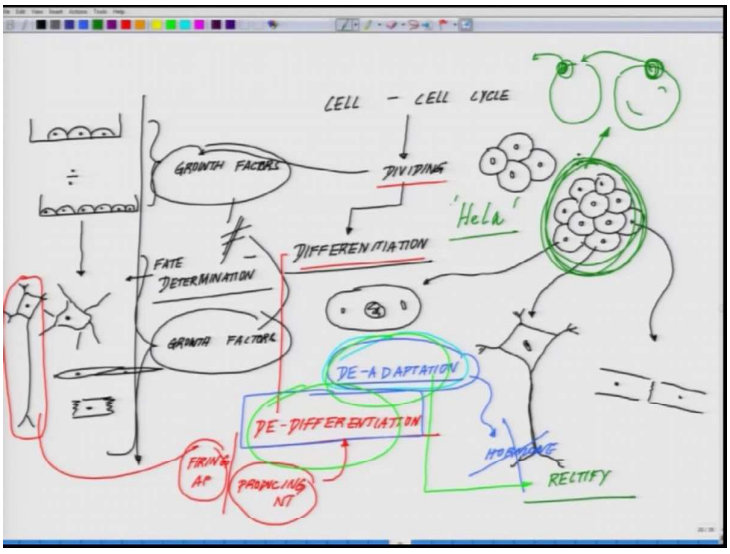

So, there are 2 3 phases apart from the cycle it goes through broadly speaking cell of course, it goes to cell cycle. Now while it is cell cycle it is doing 2 options some point or other it has it has been dividing maybe for few days or few hours whatever it has must have dividing, and after dividing if it decides at a certain point of it is division that it is not going to go any further division then it has to take it is final identity what I meant by it is final identity means whether it will be a cell which will be producing a particular

kind of hormone, or it will be a cell which will be producing a particular kind of hormone, or it will be a cell which will be present in the alveoli, or it will be a cell present in the heart as cardiomyocytes, or it will be a cell producing insulin, or it will be a cell produce saying a some other hormones like you know gonadotropins. So, that paid determination of a cell post is division is called differentiation.

So, this is a mass of cell sitting out here which is dividing now it divide form a bigger mass this. Now from this mass some of the cells decided that you know they will decide their own fate. Some of them say becomes like fully differentiate itself they will not divide any further they may secrete something some from specific population may decide that they become neuron and they want you know divide any further there is some from in population a specific population decided that they will become muscle cells or some other cell time. This fate determination process weight determination is called differentiation and the conditions for dividing cell is different from a differentiation.

So, since that brings us to a point while we are culturing cells in a dish we have to make a call are we holding the cell type on a dividing phase or they will be differentiated. So, say for example, I have a cultured dish here where I have these cells which are sitting and suppose they are dividing and spreading further. Now at this the conditions which will be there the dividing conditions the growth factors and the surrounding milieu will be entirely different for these cells when they decide they are fate determination whether they will be come from here they will be come and neuron or they become you know some real cell something like these astrocytes or they will become schwann cells or they become cardiomyocytes that is it. So, the conditions out here if you talk about the growth factors here they are totally different these 2 conditions are very unequal.

That is brings us to another point that whenever we try to emulate a biological system outside, we have to emulator dynamic system and that was the reason why in the previous class before I teach this aspect or highlight these aspects to you. I talked about the concept of introducing micro fluidic systems, where you can change milieu and a very nano molar picomolar concentration by you know flowing certain specific growth factors certain point of time and then secret then sending another set of growth factor which you know will may promote differentiation or some other aspect. In that whole process there is another word which will be coming very handy and that I am just putting

in grade now this is the word called between division and differentiation there is another word called De-Differentiation, what is de differentiation?

De-differentiation is a situation, when a cell forgets or loses it is ability of it is differentiated behavior what does the mean say for example, this becomes a neuron. Now this loses it is characteristics of say Firing action potentials or Producing certain kind of neurotransmitters it loses it is ability; it means this cell has reached a de differentiation it has forgot10 or it has lost permanently well this part we will kind of keep we will take it with a bench of salt because we do not know the future of cell biology may tell a totally different story that we can again get back de differentiated cell back into all it is definition statistics. So, I will be I will be little cautious on it.

So, but for the time being a de-differentiated cell loses it is that unique capability of is differentiated state. And there is another one another word which comes pretty handy here called De Adaptation say for example, a cell is adapted to produce say a particular hormone. Now this particular hormone production for some reason or other it is adapted in that particular milieu you keep this cell out in this milieu it will behave differently say for example, let me pick up that previous class example previous say for example, here you have a milieu.

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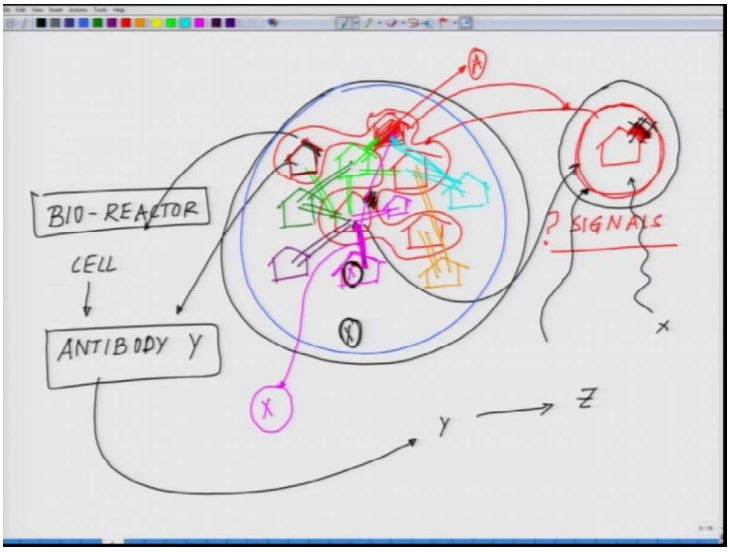

Now if you keep this red I consider each house as a cell in this milieu it will do certain XYZ stuff say for example, it produces compound A.

But if you remove it and keep grow it in isolation it may not be able to produce at compound a because in order for this red one to produce compound a may need influence from this pink one, from these green ones, or from this blue one. And if these influences are missing then what will happen is this cell will be de-adapting to that situation.

This process is called the de adaptation. These words are very important that we understand that is it a de differentiated cell we will come later when all these things we will be dealing more and more or is it de adapted could this de adaptation be rectified could you rectify the de adaptation. Similarly, those who are using cell lines. So, I am just using a word which I have been introduced. So, cell lines are cells which are continuously dividing and people isolate such colonies of cell and store it in liquid nitrogen and take them out and again culture them again subculture them we will come to these words do not worry about it what is culturing what is sub culturing and all those things.

But just for the time being think of it key I am a mass of cell, unique mass of cell, and I can grow them, I can divide them, I can take a part of it like this, and I can store it, and I go this once and do my experiment I pulled out this part and take again growth and, again store a part of it; likewise every time whenever I am dividing them I am taking a part and store it in liquid nitrogen when after that again I am using it and I can make it in number of aliquots of it and I take aliquot I grow it and I again a take small part of it. These processes called sub culturing your continuously growing them, but I told you something and one such say line which is very commonly used call ‘Hela’ cell. Again, will after the name of henrietta lack we will come to this story of this hela cell, but here what I wish to highlight is something much more I should say interesting and precautionary.

Every time a cell is going through this cycle it loses I told you part of it is telomere because telemeter is activity changes. Now, there are several labs in the world who use hela cells somewhere in 19 50 these whole cells line has developed. We have no clue that how many cycles these cells have gone through and still we are using it, and there are several reports I will dug out I will try to dug out those reports some of them for you to read those cells behave now very deserved several places this is happening.

Cell lines over period of time growing them in an synthetic artificial conditions time and again time and again time and again time and again gives us results which are fairly (Refer Time: 22:12) and why is it I told you that every cell is this division process, what I tried to tell you is this division process what I am telling is it never ending you can divide it forever possibly no it has a threshold limit and many a times we do push it beyond that limit.

Whenever you are sub culturing or you know this process this process for passaging the cells over generations after generations one has to keep track that how many time this is divided at least because of course, you do not even know that when you receive the stock how many times it has already divided before this, but at least you can keep a tab or at an what you one can do is something called a carrier typing process, where you can analyze the chromosomes time to time if you are a person who are using those kind of you know cells cell lines you should carrier type it an see, how close or it is resembling to the original stock or the description the original stock some 40 50 years back how close you are too that or has it reached to a point that in a it has to be you know thrown away it is no more really worth using .

One has to always go through that exercise time and again time and again to figure out, that hope I am not using something which is which will give me ambiguous results. And in order to appreciate that one has to understand this basic fundamental the this process is a chemical process, it goes through that cycle and it has it is checkpoints check and balance in the form of cyclins and cdk kinases which are playing tremendous role what I am I interrupting what is the question I am having does this question matters about it is genetic integrity of that cell maybe it does not matter may be you are using it is a sensor it does not matter as long as that sensing a protein is being synthesized by it is perfectly fine.

But maybe it matters you were doing some other kind of experiments with it. These are the basic questions as a cell culturist one has to ask without asking these questions I mean it will be a blind walk in (Refer Time: 24:44) we will get some results and you will of course, in a paper also, but are you sure what you are talking, or what you are documenting.

That is why understanding of this fundamental concept is very important we will come back. So, do not worry for those who are unable to track what is this cell line concept I am introducing we will come back to this, but for the time being understand that the cell either will divide an or some after division they will reach a point where they would not divide any further there will be differentiated. Yet there will be certain cells who will lose their permanently as of now what we know from the literature their differentiation ability and they are called de-differentiated cells. And yet there are certain cells which does not lose it permanently that differentiated potential their de adaptive cells. There will be several such things which will happen and then there are terms called transformations and all which will come which will be coming later once we will talk about the cell line cultures.

As of now what all things we have talked about is the Oxygen and Carbon dioxide milieu we have talked about the extracellular matrix, we have talked about the dynamic nature of in vivo system and how we can emulate that in vivo system using the modern technology is a micro fluidic system and we will be talking later about all those end up detail and the most recent studies in that area till 20 20 20 20 17.

And now we are talking about the cell cycle very briefly to give you a feel about the challenges what you will be coming across while we will be talking about the cell lines and primary culture again this is another new term, we will come back later into it. I will close in here in the next class we will talk little bit more about the biology of the cultured cell before we move on to the other aspect of cell culture technology.

Thanks for your patience listening.

Cell Culture Technologies
Prof. Mainak Das
Department of Biological Science & Bioengineering & Design Programme
Indian Institute of Technology, Kanpur

Lecture – 01
Introduction of Cell Culture Technology

Good evening, and welcome you all to this new course which will be starting today on cell culture technology. So, the name cell culture, whenever it comes gross people have the first feeling that well, it is a lab training or a kind of a technique which is used by most of the labs, who worked at the interface area biology chemistry environmental sciences and all other allied fields might metabolic sciences say for example. So, it is just a simple technique what most of the kind of understand that you know cells which are cultured in a synthetic or artificial environment at least that is what the commoner or you ask any graduated student or under graduate student that is what they will answer why we really want to offer a course on this area.

So, and as a matter of fact when I floated this course there are people has been that that is a practical training how really want to you know put a cross a course which is mostly a practical training people have cell culture labs. So, for me to put it across is though the name is just cell culture, but there is a philosophy behind this, whole thing, there are some basic rules and moreover, there is tremendous amount of science and art involved in it and if somebody who wants to do cell culture or wants to learn cell culture.

Appreciate these basic philosophies or the paradigm or the mile stones, then it will be easy for that individual to design the problems in better and most importantly to appreciate; how far they can go. Say for example, in mathematics there are several techniques, right or in physics there are several techniques, similarly in chemistry, there are several techniques say spectroscopic technique, but we have course; the basic logics behind the spectroscopy; whether it is a Raman spectroscopy, whether it is a FTIR, whether it is a NMR, if you know those; at least the basics, then you know with your problem, what all you can derive using this spectroscopy or say for example, crystallography.

Similarly, several mathematical tools similarly like statics there are these are tools, but if you know the tools and the origin of the tools and the power of the tools, it will help you

to become much wiser in designing our problem. Similarly cell culture is a tool, but tool whose journey if you look back is now more than 100 years old and there are handful of books, you will come across and some of them; I have already mentioned like (Refer Time: 04:11) tissue cultured book or few other books like gorsline bankers, neural cell culture book and there are manuals published by companies like Gibco Invitrogenor; currently which is known as life science technologies and they are really nice guide.

But one thing which I personally have felt is that somewhere these books are more or less like a protocol book, but cell culture has such as not just a mere protocol is definitely, there is a significant part of it which is protocol driven just like an molecular biology in a (Refer Time: 05:01) manual, even you can go through it, but a still molecular biology has a subject has tremendous amount of chemical logics and unless you understand that you will be working like a blind person; that is precisely what happens in cell culture that my young student or young enthusiast take under graduate or a post graduate; just embraces that this is technically just let follow it.

Without putting logics and place that why am I doing it what is the basic reason behind it. So, in this course, what we will do; try to do of course, we will talk about the techniques, but mostly we will be talking about the philosophy and what drives us and what are the possible options some explored some unexplored, as I mentioned, just a couple of minutes back; the history of cell culture is now more than 100 years old and if you look back like if you are an good historian or if you are interested in scientific history, if you look back the very first paper which at least known to us which were published in this field were around 1908; 1912; it is that back even much earlier than the first world war; first world war started in nineteen fourteen right.

So, this was the paper from Harrison; one of the pioneers and what he did for the first time, he grew an explant; explant means part of some animal systems body or a matrix and a matrix was very interesting thing it was a spider net he did not know where to grow. So, if we break up this problem what he was trying to do, but before what is trying to do let us go back a little? So, if this field which has its first set of publication coming almost 117 years back, then for most what we will try to do? We will try to appreciate this whole history of 100 years; what is happened, how things are progressed and where this can take us.

So, there were points and it is not that in just 1 or 2 classes, we are going to finish this off as we will be proceeding through the course time to time, we will try to go back and see you know where it all started that will kind of give me an idea that where to go, if you do not know the history; if you do not know the time; how it is it has been progressing it is very tough to build up a story what will be the next landmark thing which is going to come up there.

So, on one hand as I; when I started the lecture today, I told you that this is used as a technique for certain people, yet there are a lot many people who have spend their life time in or devoted their life time in discovering or making this field to march ahead. So, keeping this mind, let us go little back to 18th century, when the cellular theory was which was given. So, one of the theories was (Refer Time: 09:07) means cell existing form preexisting cells.

Here, I will just take a slight (Refer Time: 09:16) for you course title is cell culture technology and in this particular next series of 40 lectures, we will be exclusively concentrating on animal cells, this is just for your note; we are not taking plant cells into consideration here because that will become way too diverse we will talk only about animal cells. So, cell culture technology; you can put it within bracket animal cells and of course, we will be talking about all sorts of animals, we starting from myelin cells, even human cell culture to mammalian cells like rats' mice all the way to amphibians like lower order including fishes.

This is the range the spectrum; what will be covering coming back to the basic fundamentals cell arises from preexisting cells so; that means, each one each of our cells in our body arises from the previous one, if this is true, then could we do this thing outside the system; what is that mean; that means, say for example, at these you know epidermal cells growing all over the skin I take a small part of it. So, what I will be taking out will be a small tissue?

So, all of you are aware about the organization; you have cells cluster of cells performing a common function. It is called a tissue; I take a tissue. So, each cell is adhering to the other cell with a cementing matrix I dissolve the cementing matrix. So, what I have are individual cells and if I take this cell outside the system. So, no more in part of body now

and plate it somewhere will it behave the same way as it happens in my body is it making sense let me just put it now graphically for you to appreciate it.

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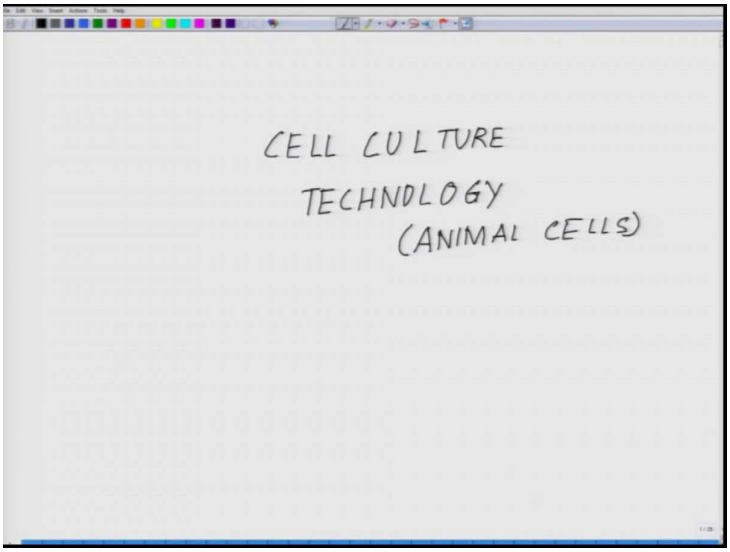

So, we start off with; so, our cell culture technology and we will be dealing as I mentioned exclusively with animal cells and what I am trying to tell you is that say for example, from an animal.

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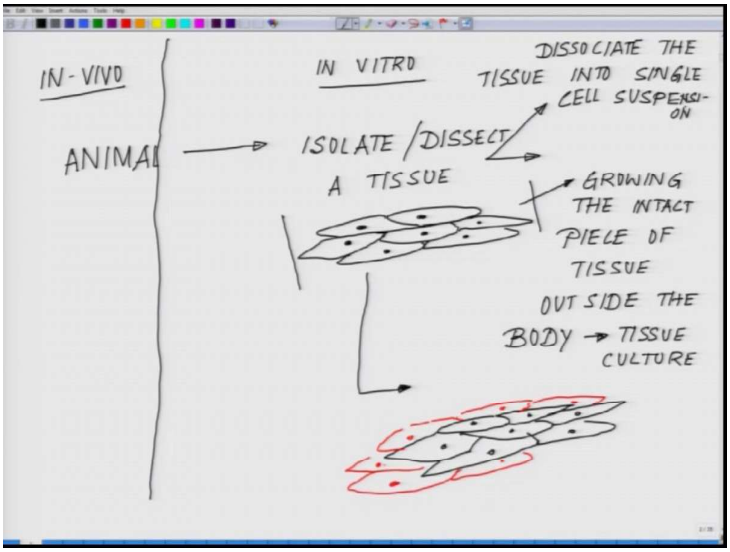

I isolate or dissect a tissue. Now once I dissect the tissue. So, it will be something like this. Now for example, it is a mass of cells which I have isolated. Now once I isolate this

I have 2 options, either I just grow them as it is which we will call as outside the system now up to this, this was all inside which is in vivo. So, there is a term which is used for this if you directly studying within the animal is called in vivo. Now I have in an in vitro condition which is outside the system. Now I directly grow this piece of tissue; growing the intact piece of tissue outside the body.

In that situation, I will call it a tissue culture. Of course, if this is a dividing cell then I will be expecting my expectation with this in a tissue cultured dish will be these are the old cells which are present then going by the theory of omni cellule cells existing arriving from preexisting cells, I should be able to see the development of the new cells, the one which I have shown in red. So, there will be dividing and there will be forming a mass kind of a structure, right, these red ones are the ones which are growing outside the system all what I do I dissect this tissue and I follow another route where I dissociate the tissue into single cell suspension.

What does; that means, let me suspension; that means, so, I have this tissue, here the original tissue which I took out from the animal.

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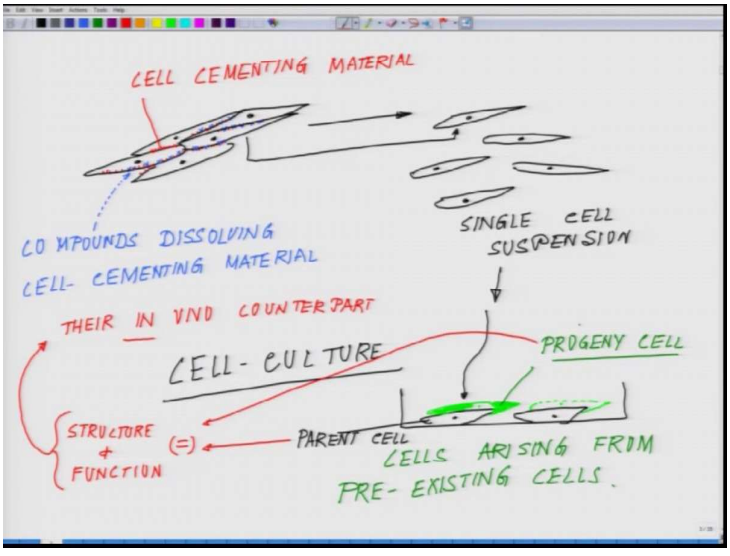

So, this tissue as multiple cells and each one of the cells are anchor to each other where I am putting these small-small red stumps, these are the extra cellular matrix or the cementing material which are making these cells to adhere to each other. Now what I do

I take some kind of an enzyme or some kind of a system by which I break down or chew away those cementing materials? So, let me introduce that.

So, I introduce some form of a compound which will start to nullify them. So, once this cementing material is dissolved, what I will be leaving behind will be after this; let me put this is the cell cementing material and I am not introduce any technical terminology at this point, we will come later on to all those thing cell cementing material and so these are compounds dissolving cell cementing material. What we will be getting after that will be suspension single cells like this which are no more adhere to each other. They are suspension and this is called the word which I used in the previous slide; single cell suspension. So, this is called single cell suspension.

Now, if this single cell suspension is given a right environment to grow; say for example, in a day short somewhere and let me introduce this complexity, let me finish this then I will introduce this complexity what is that and where I stopped, if I allow them to grow on a synthetic environment and they should be able to exactly behave like they should be able to divide like this. So, this green is showing the dividing which is proving the point omni cellule cell arising from pre- existing cells. Now second situation what I told you is called cell culture; the first one is tissue culture second one is called cell culture.

So, there will be these 2 words have been used very oppositely in the literature because when Harrison, I told you that 1903; the first paper by Harrison when Harrison did actually, what Harrison did was something like a tissue culture he took an explant, this is that is why it is called an explant. He took a explants or part of the tissue and grew it on a spider net. So, it was; I think it was a neural tissue and it shows the extension of the neural cell bar; neural cell process is moving along the threads of the spider net. So, essentially the term which is coined was tissue culture, but over period of time; what we will be talking mostly about cell culture because the reason is there are different terms for all this things when you start with single cell you allow it to grow to form a mass or form a tissue; there is a next level, if you allow the tissue to in a three dimension that is called a plant sometime people directly get the explants. So, here we will be dealing at deferent level.

So, to start off with in order to since course title is cell culture, I told you; this is purely what is the cell culture, but we will be covering the whole spectrum from cell culture to

tissue culture to its plant culture, all those things we will be covering, but for the basics, it should be very clear to you; what you are doing and now we are into 21st century. So, you should not use the wrong because it is not the wrong; it is just the confused. So, it is better to use the right terminology; what I am doing? I am doing cell culture; am I doing tissue culture, am I doing explants culture, it should be very clear to you there should not be any room for any ambiguity.

Now, coming back if it is omni cellule cellule; cell arises from the pre-existing cell by Rudolf Virtue, when he proposed this, it just part of the basic principles of cell theory. Now the question is yes indeed what here when I am drawing that yes from the pre-existing cell, I am getting another cell sure, sorry, I mean out here; out here, yeah definitely, from cell this green one, this is the new cell. Now the question is how much closely this new cell which arose from the pre-existing cell is similar to its parent.

So, if this one is the parent, this is the parent cell, this is the parent cell and this is the progeny cell, yes, they look similar, but are they truly similar in terms of structure. So, if I have to compare between these 2, what I will be comparing is structure and function are they same not only that are their structure function same as their in vivo counterpart, what is the in vivo counterpart, here are these cells say for example, if I just add little bit more here, like you know if I talk about the cell culture here, if these individual cells which are growing instead of the tissue and if these are there next generation forming are these cells properties of these cells, these cells are they equal to the properties of these cells.

If it is; so, there are 2 options hypothetically one, they all behave exactly the same behave the same way means all their expression profile of different proteins all other parameters are same or being outside the system because when they are inside the system, any cell which is growing here, it is exposed to blood vessels, it is exposed to other tissue, it is exposed to n number of things, but the very moment I take this part or any part of the body and growing it outside the system, it is in a totally different environment, it is in totally synthetic totally different thing it may do many blizzard things which inside the body it cannot do.

Say for example, a cell which is growing here, it is under normal control of several parameters, it can their own be any out growth or anything, but same cell, there is a

possibility if I take it and grow it outside, it may behave like a cancer cell, it may behave like something else which we have no clue because it does not have any restriction boundaries it can grow. So, how close when we are growing something outside the system, how close we are to the in vivo set up because that will determine a lot of things that how what you are interpreting you experimental interpretation of using a technique based on this and you wanted to extra polite and claim that this is what is happening in an animal will depend whole lot on how close these 2 systems are, if they are not close enough how far they are. So, say for example, now let us draw the lines. So, what will be doing in the next class? We will start to draw the lines that how close are these cells to their in vivo counter parts.

So, let us close in here for the first class and we will go to the next where we will be discussing this similarity and this similarity and we will conclude that where all the real challenges lie.

Thank you for a patience listening.

Cell Culture Technologies
Prof. Mainak Das
Department of Biological Sciences & Bioengineering & Design Programme
Indian Institute of Technology, Kanpur

Lecture – 07
Layout(s) & Design(s) of Cell Culture Facility

Welcome back to the lecture series in Cell Culture Technology. So, we are into the second week and today we are starting the second lecture of the second week. So, the thrust area of this lecture will be mostly layout and design of a cell culture facility. So, as you go through the text books about different kind of layouts and designs there are certain salient features which will be highlighted. So, todays lecture I will be talking exclusively from my personal experience of setting up cell culture facilities in different parts of the world under different financial constraints and for different kind of purpose.

So, the first and foremost important thing which occurs to me should be taken into account that what level of facility are we looking forward to, what is the objective and I am not talking about like you know how big, how small what is the objective? Say for example, to tell you this if we or if you are setting up a facility in a lab which is working on say cell biology or you know some like the velum biology or something and you want to have a small cell culture facility, the requirements will be different as compared to say for example, if a biosensor lab you just use the cell as just to maintain some kind of a cell to put it on some kind of sensor device cell based biosensors of course, I am talking about.

Then your requirements will be kind of different, similarly if it is a tissue engineering lab where cell tissue interaction with the materials are being tested, again the requirements will be of different type yet if you are a chemistry department which wants to a nurse cream different kind of fluorescent molecule, different kind of anti cancer drugs, a different kind of newly formulated compounds your requirements will be different.

So, you see or say for example, so let me jot down these points ok.

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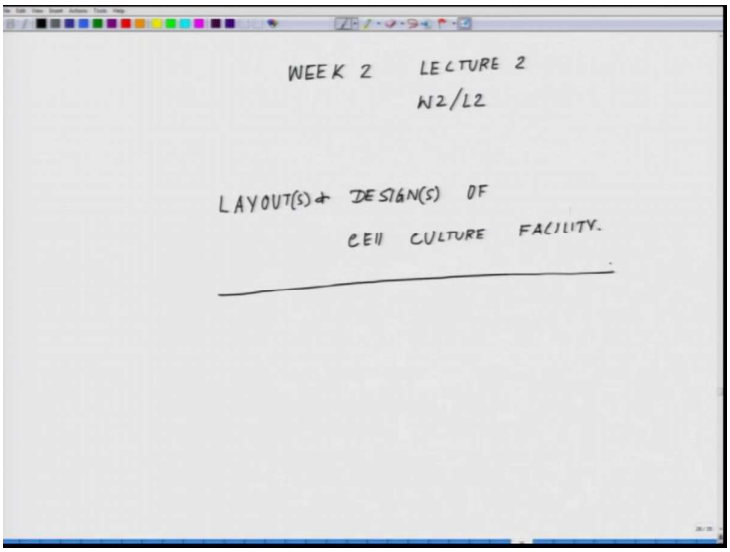

So, we are into week 2 lecture 2. So, W 2, L 2 and so what we are dealing today is layout and designs, layout and design of cell culture facility, facility. So, now, I told you that there are different kinds of you know facility say for example, what are the, what are the major kind of objectives?

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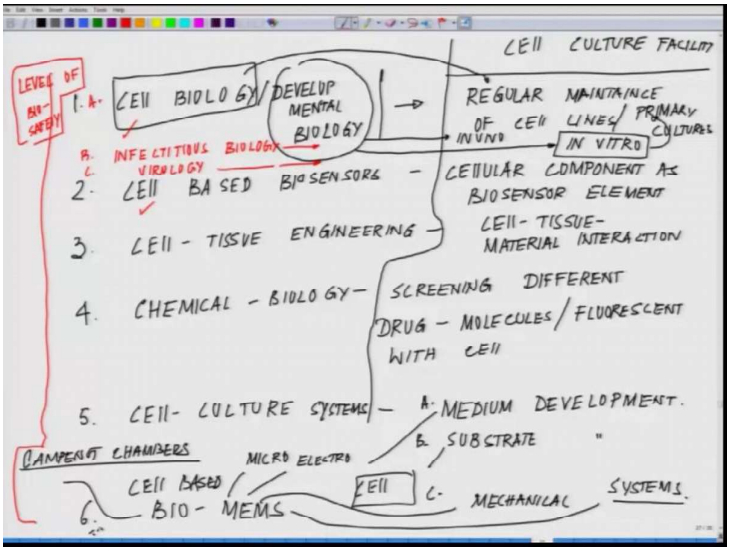

So, one we talked about if it is a cell biology lab and it wants to have a facility whose major work are cell biology or in our development biology lab and I will come later, what are the specification, say for example, a lab working on cell based biosensors or a

lab working on cell tissue engineering or chemical biology lab where, essentially the idea is the screening different drug molecules, the interaction with cells with cell or not only drug molecule different say fluorescent molecules. Similarly cell tissue engineering the major thrust area is cell tissue material interaction, cell based biosensors is mostly cellular component as biosensor element.

Similarly, cell and development biology, if they want to have a cell culture facility. So, they have something you know regular maintenance of cell line and again it depends on what kind of diploma biology you are doing, you could be could have in vivo where you are exclusively dealing with animals and histology or you have an in vitro development biology where you are doing everything outside the system with isolated cells then definitely cell lines and primary cultures.

I will come later what does that mean and of course, in a point of cell biology there you are doing with different kind of cell lines and all the stuff. So, I mean this list can go on what all you can do similarly there is another fifth group which exclusively work on developing cell culture systems itself, these are the labs which exclusively work on medium development, medium for cell culture of course, they work on substrate development, substrate for culturing the cells these are all for the. Then they do a different kind of or let us put it like this, there is another emerging area where there is a area of bio mems, cell based bio mems structure mems here stands for micro electro mechanical S stand for systems ok.

So, there are cell based bio mems structures which are being developed for say for example, history goes back to like things like campaign or chambers will come later, what are those different aspects are something like you know modified, one second. Campaign or chambers and several other things people work on bio robotics and all those areas. So, first and foremost thing what has to be understood is what is the objective? because there are constraints what are the constraints what one comes across. So, first what is the objective and what is the purpose.

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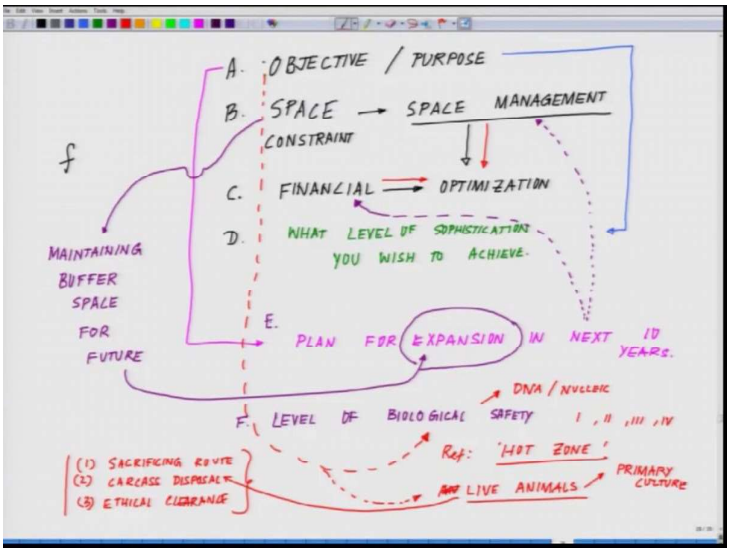

So, let us get all the functions right, what are the functions what are important to understand.

So, the major is objectives or the purpose slash purpose. So, we have already talked about the purpose in the previous slide. You see, these are the different kind of purpose we are telling. So, you should know what is your purpose what kind of lab you really wanted it to be after the purpose comes the constraints, what are the constraints? the first constraint which we see all over the world a common constraint respective of the fact whether you have money, where you do not have money, how much money?

Is space, space is a huge constraints all over the world, like if you go to places like a national institute of health at Maryland, better stuff you will be surprised like you know you have seen the office cubicles most of you have seen some time or other small office cubicles, it is almost that is the size of the facility you get for cell culture. And there people will be crammed in like you know three or four groups sharing such facilities. So, all over the world this is very critical because getting a space getting designated space with all the, like you know the vacuum systems and all those things it is not easy because you need certain special things if you want to make in more and more sophisticated.

So, space is a big constraint and the next constraint come, if your space is a constraint related to it is space management and that is where the whole design layout comes very handy space management. This is very critical because first of all you are constrained by

space. So, let me put it like that space constraint and then you have to manage the space and if you talk about the manage the space, then we have to talk about what will be the kind of equipments or the size of the equipments you wanted to get for such constrained space point 2, we will again come back to this space constraint point 3 is your financial constraint because wherever you set up a lab you will always have a financial constraints and optimization.

So, this arrow now I am again redoing that arrow a space and a finance these has to be optimized very neatly and then come based on your objectives, what are the objectives you have set for yourself? You have to decide what level of sophistication you wish to achieve, what does that mean? say for example, your lab regularly maintains a particular cell line I have already talked about cell line right at the first week and you just your job on these to maintain the cell line and you just passage them every week after week, subculture them and grow them in and then you just take them and do your experiment.

You probably may not need a very high end microscope for that purpose and then you just have to observe the cells they are doing fine, if that is the objective then you are not going to spend on a very high end microscope right, on the other hand if your lab does a lot of cell biology work and that completely relies on your cell culture and probably you may need a very high end microscope facility and you may prefer to have a microscopic facility then and there adjacent to your working bench.

So, these decisions are very critical provided you are very sure what is the purpose and what is the objective and adding to the purpose and the objective is there is another thing which comes is plan for expansion in next 10 years. Because, unless you have a very clear cut plan this is how you want to explore or say for example, you are not very sure suppose your new faculty starting something, you are not very sure here I may do this, I mean that happens with all of us right. You know when I set up my lab I still remember and as a matter of fact when I set up blast for my for my employers I always told them this simple thing like at this point I do not know like I may love to you know explore this areas.

So, what do you do you do not consume the whole area, you kind of cover the idea by somewhere or other it should not look like that you are not utilizing that area, but it should have a buffer space. So, tomorrow say 5 years down the line you wanted to

explore that site and I wanted to do this, but where I am having a space because I have got all this big giant stuff and have covered up everything, that should not happen that farsightedness is very critical that we all grow, our desire changes, our scientific problem demands us to explore areas how you do it. So, that brings you to that critical point what I was mentioning earlier is both this is a direct function of your space management as well as your, at that point financial management, these 2 are very critical and I used another one word.

I am just before we forget and I forget maintaining and this is I believe each one of us should you know keep in mind, maintaining buffer space for future expansion this is very critical. So, unless you have that buffer space you will kind of you know realize that like, I should have you know save that space I could have utilized it later that comes the next. What level of sophistication you want to achieve and what are the, what are your plans for expansion. Next thing if I put this as E next important thing is that what is the level of biosafety are we talking about say and this of course, comes in the objective in the beginning level of once again biological safety, what does that mean?

So, before I get into that, so this is of course, a very direct aspect. So, say for example, I wanted to work with a very deadly virus called Ebola which is a bioterrorist agent or in order to work with Marburg virus, hell I mean this is like something if I am not wrong there are handful of labs, maybe across the world there will be 3 or 4 labs who can handle that level or you want to work with a very deadly strain of TB tuberculosis which is common in India are we kicked to do so? Because the person, the user suppose I am using this. So, first of all I am putting myself into a threat I may you know get the infection the other person who are working they may get an infection and not only that and if we cannot contain it then the whole area the whole community will get an infection and these are deadly stuff.

So, first and foremost the power of the objective, when I talk about the purpose if we remember the purpose out here what are you working on. So, now, I add a few more points are you working on say, now I am adding few other points out here say one a is this then, are you working on infectious biology, are you working on virology, infectious biology virology. So, that brings us to the point of level of biosafety, the country if you are using a kind of regular cell lines it really does not matter, I know I mean nobody really bothers, I mean it its perfectly fine or using some kind of you know some

straightforward materials directly derived from biology your system should not be a problem. Then comes another interesting aspect, since we are living in an era of, so add up to that in cell biology section develop biology section, are we working on transgenics does it involves d n a transfer are you working on knockouts are you working on R N A I.

So, that demands different level of precautions which has to be taken, because the very moment you are handling with the DNA material or any kind of genetic material there are certain rules and regulation or the law of the land wherever you go, whether you were in India whether you are abroad any country, every chronic country has very stringent rules extremely stringent. So, one has to know those rules and regulations a priori or much beforehand for your design. So, you know I want to do this and then you realize that that in that particular building you really do not have the proper kind of exhaust or you do not have a proper way to you know contain the building you do not have a proper run away or escape route and you wanted to open up facility which kind of put others in hazard.

So, these are very critical, exceptionally critical and you have to be very clear that what are my because you are dealing with systems which not only is hazardous to you, could be hazardous to others unless you are sure unless you are very clear in your mind you should not lay down, first of all think over it that is why I am jotting down this point, what is the objective and these are the points which textbooks will not say, but when you will go to the day to day life (Refer Time:23:51) take any textbook I mean they will kind of you know brush aside this things. Where the practical reality is this, these are you come to know once you go to job, once you have to set up a lab then oh no you cannot do this, oh no this is not allowed or in this building we cannot do this and I completely understand their point.

Because certain buildings are not even made for that purpose as a matter of fact so these things you should keep very clear in your mind what level because based on that we have these levels called biosafety levels 1, biosafety level 2, biosafety level 3, biosafety level 4 and for those who are little inquisitive to learn about much of these dangers should read a very nice book the hot zone. If you find it online or I will try to you know someone of if cant contact me if I, if I have a e copy I will forward this, you should read this book this is a very interesting book about virus deadly viruses escaping out and how

that could influence our life big way ok.

So, next is biosafety level, what you have to be very critical and in that of course, I have already mentioned you have to very clear are you working with DNA are you working with, you know other forms of nucleic acids and what is so on and so ever. Then you have to realize in that objective line, am I going to use live animals? in other word say for example, I wanted to work on primary cultures. So, primary cultures are essentially when for culturing you directly derive the tissue from live animal, you have to kill animal or if the tissue is just take a small part of the skin then you can you know give the animal necessary first aid and you know and exercise the animal, take the tissue and you know give the animal necessary first aid and you know good to go or you want to sacrifice the animal.

Now, if you want to sacrifice the animal and you want to pull the tissue out of that animal that demands different kind of facility support, because when we are talking about sacrificing the animal what we are essentially talking about. If you have animal sacrificing facility right not only that the biological waste which are coming out, first of all any animal killing has to be done in a humane manner, in an ethical manner, it is one underlying prerequisite. So, first how you are killing the animal, will come in depth here are different ways to do. So, then how many you wanted to kill, then once you take out your desired tissue how you dispose the animal? Where you bury it or where you know put it to rest? These are very ethical and very human aspects which one has to be very clear and how really want to do it because then there are every institute across the world, recognized institutes have animal ethics committees then you have to take ethical clearances, you should know what all ethical clearances we needed to know.

Barring aside some animals like you know, it can work with fishes without any ethical clearances and there are few lower animals you can work with possibly drosophila and all those small systems where you do not need a ethical clearances. But apart from it the very moment you move to the rodent's guinea pigs they all need ethical clearances. So, what sort of ethical clearances you will be needing? So, whenever we are talking about live animal or you know primary culture from right live animal. So, all these points will come to play that, sacrificing method, sacrificing root calchas or the cadaver disposal and most important they all fall under do you have to have an ethical clearance.

So, you realize even much before I hit upon the topic of the how the layout of a lab will be, there are other things which one has to take into account. So, close in here we will continue, we will resume our journey of understanding what all basic prerequisites one has to take before one think of developing one of the state of the art facility so bonded upon it think over it.

And thanks for your patience.

Cell Culture Technologies
Prof. Mainak Das
Department of Biological Science & Bioengineering & Design Programme
Indian Institute of Technology, Kanpur

Lecture – 02
Philosophy and Complexity in Cell Culture

Welcome come back to the Cell Culture Technology. So, in the first class this is our second class of the first week, the first class I told you when we take a part of the tissue and dissociate them and grow the cells outside or even the tissue as a matter fact. World biggest challenge is how close are we to the in vivo setup or the conditions what are cell experiences inside the body just to appreciate that. So, let us start with is.

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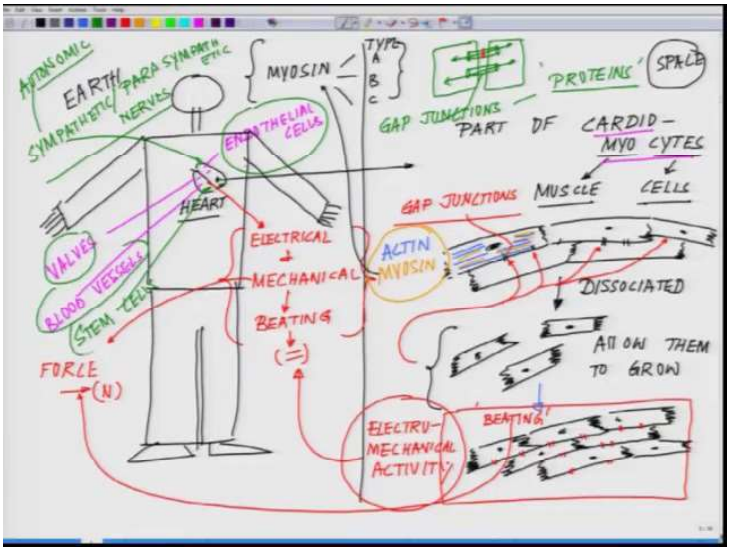

So, we are in to week 1 class 2. So, what we are asking is simple. So, say for example, this is said this is an intact in vivo system and say for example, I took a part of the cardiac tissue outside, this is heart and took part of Cardiomyocyte; cardio means heart myocytes mean myo means muscle cardiac muscle and cytes means cell those of you who do not have the wide range of biology background, this is the breakup of that word Cardiomyocyte cardio means taken from the heart.

So, it takes part of this and these (Refer Time: 02:33) kind of you know look like this, and there are connectors called gap junctions and I am getting to the detail of it at this point like this. Now I take this tissue cardiac tissue dissociate them into cardiac myocytes

and allow them to grow in a dish. I have dissociated them as I mention you, and allow them to grow. Once I allow them to grow outside the system what do I expect from them the first thing what do I do expect from them is to regain this tissue level site to architecture; when I talk about the tissue level site to architecture they should develop these specialized structures called gap junctions. Gap junctions are small tube-like structure those are from biology, aware of those who are from non-biology background. So, simply say for example, there are just imagine like there are two rooms, there is a room here is an adjacent room say for example, let me let me draw it is to make it simple this is one room and this is another room.

Now, if I put some connector like this. So, these two rooms can cross talk with each other right like this. These connectors are called gap junction in technical term and these are nothing, but these are proteins, these are membrane proteins which are forming these kinds of junctions. So, cardiac cells have this classic feature they have series of gap junctions, and there is reason for we will come later in to that why there is those gap junctions are important because it is a structure where the electrical stimulation has to in a spread out in multiple direction like this, and it has its own function. And of course, there is one more thing this gap junction may be sometime gate it or non-gate it. They may only open in one direction or they may not they may have both directionalities. So, the first thing what I anticipate for these cardiac cells to join with each other coming close and form those gap junctions.

So, essentially what I will be expecting next to happen is this here. I have these cells coming close like this, like this, like this and forming those reforming those gap junctions are they doing that. Now next thing when we talk about cardiac, you touch your heart it is beating. This beating means these cells what you are saying here in your body these cells are essentially they are performing two function; they are forming an electrical function and a mechanical function. When I talk about mechanical function; that means, what I does telling is the beating and this electrical function and the mechanical functions are coupled with each other. If not, these are independent thing that you know if I am doing this, I will not do this they are all inter linked with each other if there is an electrical impulse it will lead to the mechanical vibration or beating whatever you call that.

So, for these cells if they have to grow outside, and they should be in a position which should be able to mimic these electromechanical events in a dish. So, these cells I expect now they should be able to do that electro mechanical activity, similar to that of in your body. It should be similar even if it is not similar then we know there is a deviation, and we will have to quantify those deviation, it is not that is a negative thing it is a very positive thing then we know that we have a (Refer Time: 07:43) to improve and I will tell you why these are important, because these are really important for those people who dream of artificial organs. I mean in futuristic world where they say we can replace the heart yeah its sure, but we do not know the basics we would not be able to achieve that goal or people say that we can replace a kidney, we can replace something I mean there is a whole password all over this tissue culture world are you know re general medicine, but the fact is this all lies in the basics.

The basics are not clear these are fancy dreams, I will (Refer Time: 08:17) one is living is a fool's paradise without knowing the basics. So, we have to really know what all you can achieve and I will tell you that why a times people just get (Refer Time: 08:28) without realizing the ground realities, there is certain ground realities. So, it will generate an electro mechanical activity. Now this electro mechanical activity your first evidence you should be able to see on a dish under microscope, that these cells are beating they are not beating it means yeah you grow something, but they are not functional.

Now if they are beating it means they are generating certain force right it is a mechanical event is a four generation. Now whenever we talk about force, we are talking about some Newton value right something. So, what is it could be nano Newton, it could be Pico Newton, it could be whatever. So, there has to be some unit at what rate at what unit this is mechanical activity is, because that will again tell you that how close you are to the real life my heart is beating.

So, part of my tissue which is going outside should exactly behave the same way. Now if we have to correlate this then we have to understand. These beatings are governed by the two major proteins of muscle, which are now let me put them in those are actin and myosin which I am putting in two different color orange and green. Those are actin and myosin filament, and as a matter of fact depending on the quantity of four generation, the myosin filament sub type changes. In other word what I mean to say is the myosin present in these muscles of my body, which are the skeletal muscle are entirely different

force dynamics, then the force dynamics of the cardiac cells or cardiac myosin which are present. There not only that within this muscle there are certain structures or muscle spindle, they behave entirely differently their myosin properties are entirely different.

Similarly, there are myosin's in your gut the whole smooth muscles which is lining the hole or which is forming in the hole alimentary canal, where you are eating the food and the food is moving through a (Refer Time: 11:13) moment across the digestive system, those myosin's are different, but it is that myosin critically acting does play a role, but it is this myosin sub types, which dictates say type whatever fast type slow type whatever. So, I am just putting a b c just for imaginary sake I am putting it like that. So, these cells which are growing in the dish now, should express those myosin's which are really present here, then only there is a possibility that they will be generating the similar force as generated inside the system. So, you realizing that when you are trying to build something outside this is something, suppose of this is on earth you are trying to grow something and if you are growing this is totally different environment for it. It is any space totally different it has no clue why it has no clue think of it for a minute.

Now, this heart which is here, it is surrounded by not only heart has only cardio myocytes, the heart consists of endothelial cells then there are cells which are forming the valves, there are blood vessels, there are sympathetic and parasympathetic nerves or rather autonomic nerves, there are stem cells cardiac step cells which are present there. So, these cardiac myocytes what you have isolated are coming from a (Refer Time: 13:24) not I mean it comes from we more than several surrounding cell. So, it means you can imagine it like this, as a cartoon way you can imagine like that suppose there are houses.

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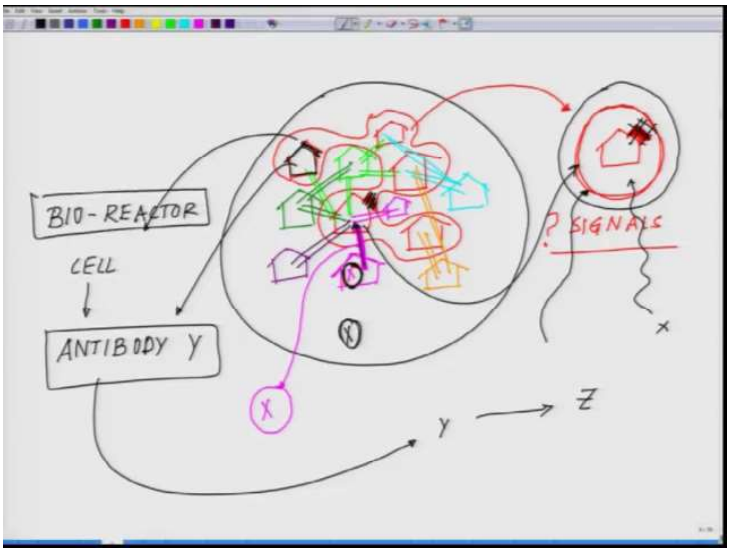

I am just trying few houses for (Refer Time: 13:42) and that will that will help you to appreciate it, you are seeing different color of colors houses what I am drawing through. Now it is a kind of a colony where every house has different color and yet some houses which are of similar color. So, you see red you see light green, you see dark green, you see Prussian blue likewise.

Now what I am asking is that I only wanted to study these houses in isolation and that to not in this system outside the system. Now the things are that one can study that is really no problem, one has to keep in mind that there is always an interaction between this between this between this between this, there is an interaction between these ones, the interaction between these ones, these ones. So, when you are trying to study these red houses in isolation, you will be missing there will be signals which you are missing and you have to accept, this error it is not an error rather it is an acute system you are growing. So, in you may not be able to achieve the exact perfection it is not possible I mean its accepted, because I do not know these many interactions which is happening, but this give me an opportunity when I am going this in isolation.

So, say for example, just try to take an imagination slightly beyond it say for example, I given hypothesis I say these pink color houses. You see this pink color houses these pink color houses are communicating with this red color houses by a compound called say by some means say x. So, this is my hypothesis I said fine and I say that when x is sent to

these red houses the red becomes red develop something say you know develop another roof like this. So, it is fine if this is my hypothesis how I can prove this hypothesis, whether this is really true or not. So, what I do? I have to fist a of all figure out what is this x compound, and I grove these in absolute isolation and then in this I introduce that x, and I wanted to see does the x develop that additional roof or not. If it does so that means, whatever I am seeing in this milieu or whatever is my guess is true, if it is not that it means it is much more complex than I am understanding.

So, you see we open up a problem and this is how we have to understand that cell culture, tissue culture, explants culture whatever you call it, these are acute systems these are not I mean one has to be very cautious and careful that what kind of data are we interpreting out of it. Because once there in isolation say for example, these cells just talk about a reverse situation, these cells are not allowed if this x whatever you talking about this x thing, this x compound is secreted by this pink house to ensure that if this does not form this kind of a structure, is not allowed to from this second roof. Now I say well if this is true, then if I grow this in isolation it should be able to form this roof second roof if this is true.

Then it means that x compound is important and you can do the reverse experiment you put x here, you should not be able to see this growth. So, realizing that water profoundly powerful tool this could be provided you ask the right question, and if you do this whole process like just like a (Refer Time: 19:02) technique taking I just follow it without even putting my brain into place, you will end up with of course, you will get some results I am not denying that, but something which may be very ambiguous and many other people may not able to repeat it.

So, one has to realize that before using a tool one has to understand how close you are to the in vivo condition or you do not care, there are people who use cells there is another group of people we talk about them, who use cell as a simple bio reactor what do they do? Say very simple all of you heard about antibodies right, we get antibodies production of antibody. So, I say for example, these cell type and the I am representing the analog of this these red cell types which are there, they produced antibody say you know y. What I do I take out these things, I understand the condition under which and I produce terms of antibodies for some z function. I can use the cell as a simple in that situation I am using

the cell as a sample by reactor, and there are many such studies in biotechnology where people use them as bioreactor, we are just producing unit as a matter of fact.

Lot of oil production which is happening the algae we are using algae as the bioreactor, because it is producing algae we have isolating the oil and that is it. So, we are not really bothered about it, our goal is very clear we are using this as a tool; simple tool produces some xyz compound of our own commercial, medical, significance full stop. So, unless you know what you are asking for. Unless you are sure the question you are asking and another thing here, when I talk about when I was telling this, this one I made this complex picture in front of you I told you this is this is the real situation. So, how far is your culture?

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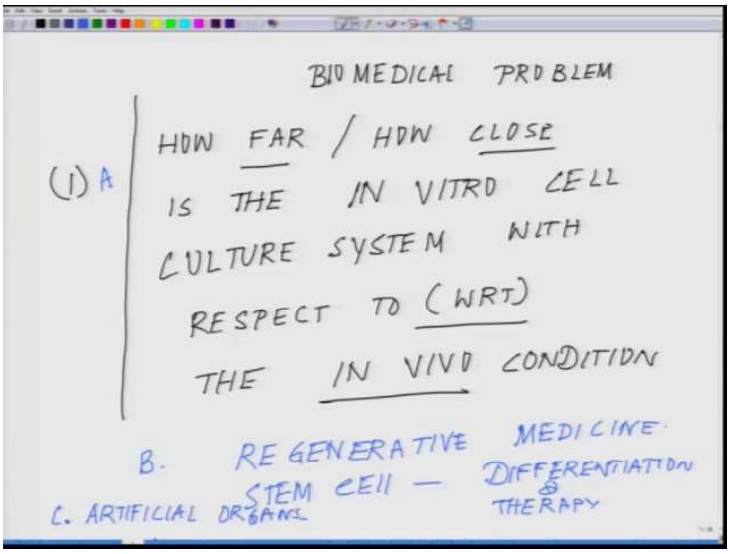

So, one thing I have to realize fundamental question what you would ask how far or how close is the invitro cell culture system with respect to the in vivo condition. This is one fundamental question one has to ask, provided if you are working on some biomedical problem ok.

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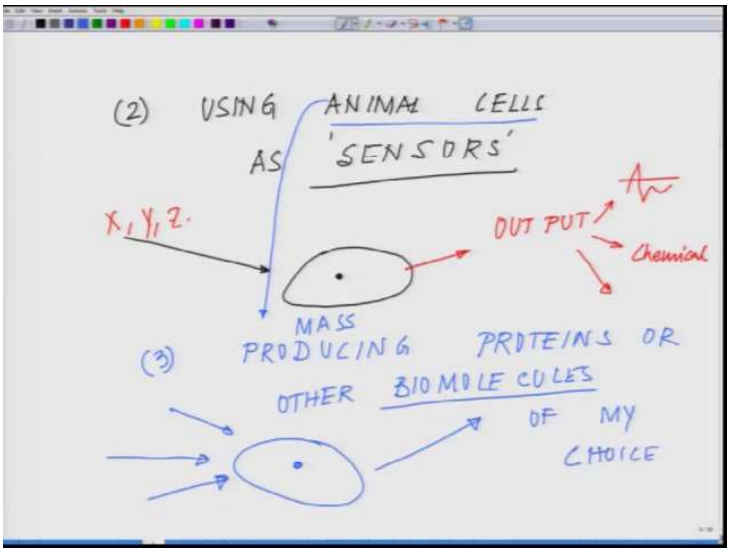

This is one situation the next is two; using animal cells as sensors what do I mean by sensors say for example, you can use most of our cells are very sensitive to different kind of toxins. So, I have this culture dish put that toxins I know how it reacts. So, based on that I am using this as a simple sensor device for testing for a screening or as some condition where I am really I am not sure what this toxin is going to do I use this as a simple sensor. So, I use the cell this is your cell, I use this as a sensor system. So, I have this compound say x y z whatever, and I have an output here to measure; what is the output that could be electrical output, it could be chemical output or it could be some other physical parameters within it, similarly I can use animal cells for reducing or mass-producing proteins or other by molecules.

So, in other word I am using cells giving them the right set of conditions of course, you one has to study through cell culture, to produce by molecules of my choice. So, you see technique is one, but one has to ask the right set of questions or I study there is another side which is previous to this one I put this is A as a B side. I use this as a regenerative medicine, where which is one of the very fashionable topics currently and the stem cell differentiation and therapy, and which eventually may lead to artificial organs. So, one technique which most of the people considered technique for me it is a follows a (Refer Time: 25:56) a subject. Unless your philosophy is it properly you will never be able to know the power of this in its tremendous power trust my words it has tremendous power, but one has to know what you are doing. So, the whole solve purpose of introducing a course which otherwise always believing as a simple you know when you go to a lab,

you will learn from this seeing you know this is how yield culture cell, that is it you know that is just a technique.

Let us come out of that mind set absolutely, next embrace this from a different word try to imagine it can create a structure. We can create next generation sensors, we can create the cheapest sensors we can have we can produce our own set of anti-bodies in an artificial synthetic system. Let us think beyond this because let us use cells as a tool, we govern them we tell them what we wanted to do, we tell them then which part of the DNA we want to put you know transcribed. Let us go take it to that level where we can truly see we are doing a cell engineering when here is the platform, where we can do the cell engineering right within these limitations.

So, with this hope we will be proceeding further in the next class, we will talk little bit more about these conditions. We will take over from one of these pictures that what all the forces, what all you have to take into account we are in (Refer Time: 27:29) talk about synthetic systems in vivo systems, when you are translating what all you needed there is a biology behind this whole thing what are those biological parameters, and I will try throughout the course I will try to make it very generic. So, that anybody who is the viewer of this course, what is ever field they are, it should be tangible to them.

So, they are with my using the simple languages instead of using technical jargons, which I am not going to use towards the course and if I use I am going to write it down here thank you.

Thanks for your patience listening.

Cell Culture Technologies
Prof. Mainak Das
Department of Biological Sciences & Bioengineering & Design Programme
Indian Institute of Technology, Kanpur

Lecture - 03
To Grow the Cell Outside the Body

Welcome back to the course on Cell Culture technology today we are into the 3rd class of the 1st week. The first class I made a humble attempt to kind of request you to think beyond cell culture as just a culture technique. And I told you that at every point of it there is a philosophy, there is a philosophizing subject instead of blindly following certain things and there is simple chemistry, physics, and biology involved in it.

Today next 2 classes what 2 to 3 whatever you know whatever you take us, we will talk about the biology of the cells visible the in vivo and the in vitro conditions. Because what is I understand the biology of the cells it will help us to recreate a situation which is similar to that of inside the body.

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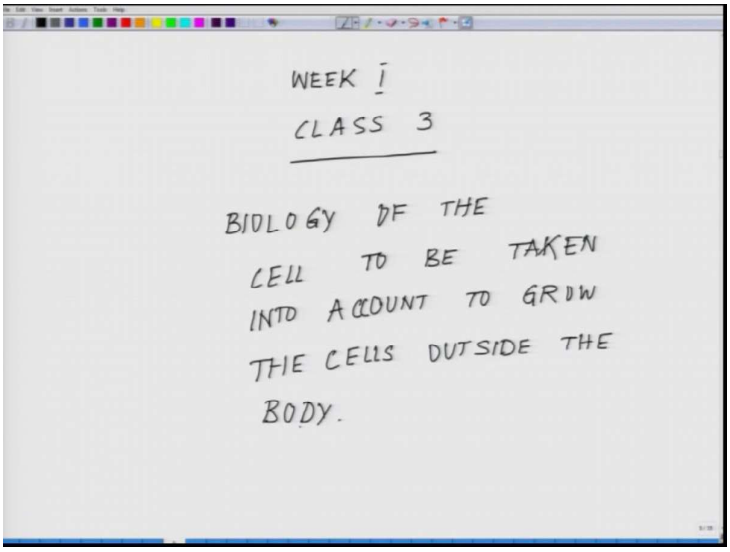

So, we are into Week 1 and today is our class 3. Today we will talk about the Biology of the cell to be considered to grow the cells outside the body; this is the key point what will be dealing next few classes. In order to address this point first of all we have to understand under what conditions of cells grow inside the body. So, again let us take the example of one of the previous examples where you dealt with.

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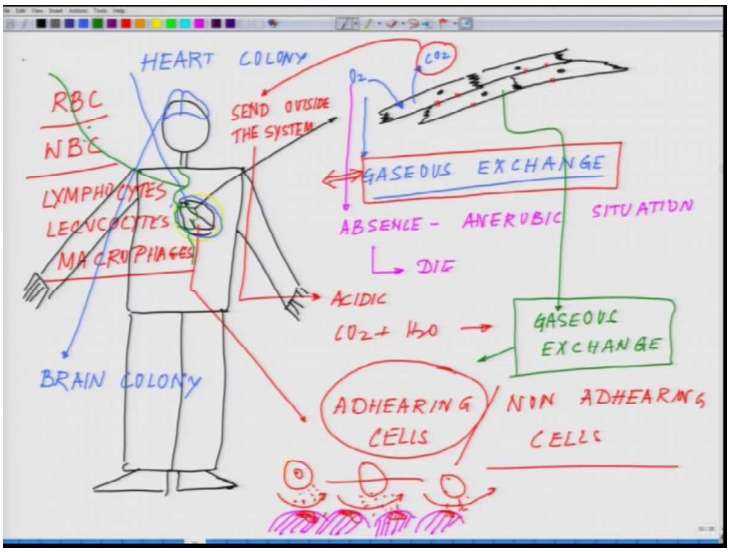

So, say for example, if to take the same explain again and just take the example of the cardiac cell or (Refer Time: 03:44) So, these cells which are growing out here a cardiac monoxide which I have already explained earlier and here are the gap junctions.

These cells out here have the first parameter they need Oxygen and they give out Carbon dioxide. There is something called they have a mechanism of gaseous exchange inside the system very first thing you needed. Because in the absence of oxygen in it is absence which is an Anaerobic situation these cells will die. They need sufficient oxygen and not only that this carbon dioxide which is produced here has to be sent outside the system. Otherwise this will make the environment acidic because CO_2 plus H_2O we know that it will perform carbonic acid.

That is something one has to keep in mind. There has to be a proper Gaseous exchange which should take place, without the Gaseous exchange these cells are not going to grow. So, first thing what one has to keep in mind if you are taking out this tissue out here and you should be able to provide right milieu of gaseous exchange. And we will come to that how the gaseous exchange how this milieu is being maintained in a system outside the system parameter one.

Next parameter is this we have to figure if you remember that in one of the lectures is give a pause I say if these cells adhered to the surface. There are 2 kind of cells; Cells

which are not here of course they are other parts of what they have, they are Adhering cell and Non-Adhering cells which cells are we culturing.

So, flocking about Non-adhering cells; adhering cells you could see these are all adhered to each other right in our hand. Now talking about the Non-adhering cells, blood cells they are circulating in your body and Non-adhering right, they do not adhere they adhere than will collapse right because they are to carry oxygen.

So, are we talking about RBC red blood cells, are we talking about WBC wide blood cells or we talking about of course RBC in this all the Lymphocytes, Leukocytes Macrophages are we trying to culture this, and if we trying to culture them do you want them to adhere or we want them to you know in a suspension to move around what are we culturing this is the very critical point. Think of it the hard tissue which is growing out here it is exposed to blood and that blood contains lot of Non-adhering cells moving through and those Non-adhering cells while they are moving through say for example, this is the Non-adhering cell once again let me change the color Non-adhering cells it is rolling you know it is moving through, but what we while it is moving through it my secret out certain things right.

In a real life we do not know what it is secreting and that what it is secreting to it is surrounding main influences the another cell which is an adhering cell sitting underneath; this pink colors are there adhering cells which are sitting underneath and top of that this red cells is moving through by secreting certain xyz compound xyz chemical and this is the influencing some of it is biology we really not know that.

So, first we have to decide our we culturing Adhering or Non-adhering at this point I will stick to the adhering cells, we talk about the adhering we are not taking account the non-adhering cell that is why give a pause in somewhere in my first or second lecture that you know are we talking about what I did not introduced that complexity.

Here I thought that I bring it back. If you are talking about adhering cells, the cells the way it works is something like this say for example, I have a cell like this.

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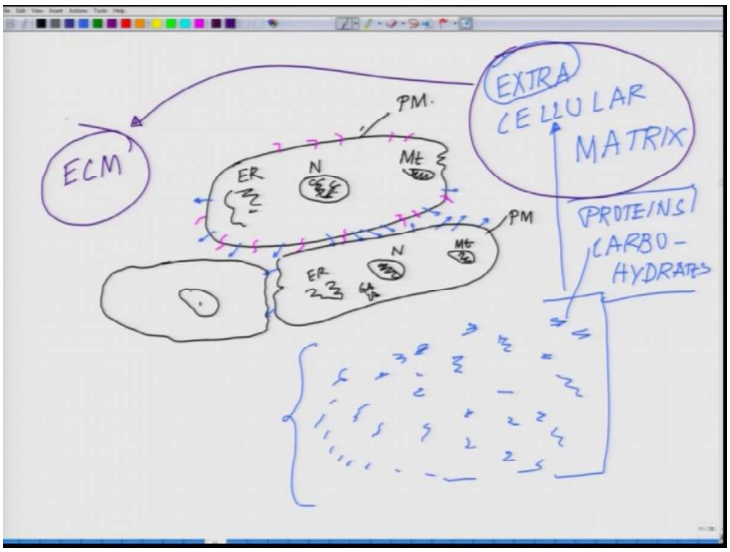

Here I have a nucleolus with the DNA sitting there we have all sorts of orgonal mitochondria endoplasmic reticulum ER nucleus Mt stand for mitochondria likewise we have the plasma membrane PM. These cells have lot of you know membrane proteins which are setting there, now if these cells have to adhere to a substrate or adhere to each other they secrete specific cementing materials and by virtue of which these blue arrows coming out are telling or telling about the cementing materials. These cementing materials helps it to adhere to another cell and it is vicinity of it is type or another type of or whatever GS s the golgi apparatus likewise.

Again these 2 cells then form the colony say for example, they these 2 secrete another set of then it invites the third one to form. They are slowly form in a colony. So, in order for them to adhere these compounds which are shown in blue out here this forms a matrix. If you could remove all the cells what you will be seeing behind there will be left behind will be something like this. Those blue color arrows what I am trying to show and this matrix is called in technical terms it is called extra cellular, because it is extra it is not inside the cell right it is an extra outside the cell extra this is not part of the cell extra cellular matrix it forms a matrix like this there is a matrix. And this extra cellular matrix is mostly proteins and part of carbohydrates and there are metal ions and several things which are involved in it.

This extra cellular matrix is one critical feature in our body which ensures the cells remain adhered to their specific locations because this particular aspect is very critical when will be talking later about cancer itself. This extra cellular matrix is kind of we can call it is a signature of the colony of cells what do I mean by the colony of cells or let me come back to the previous diagram. This is the I name it has the heart colony, now out here where I have the brain this is the brain colony.

Now, I have this Stomach and everything this is the say Stomach colony then the series of things, each one of them are colonies and within colonies there are sub colonies how is it. So, say for example, if I recope in the heart within it say the structure of the heart is something like this.

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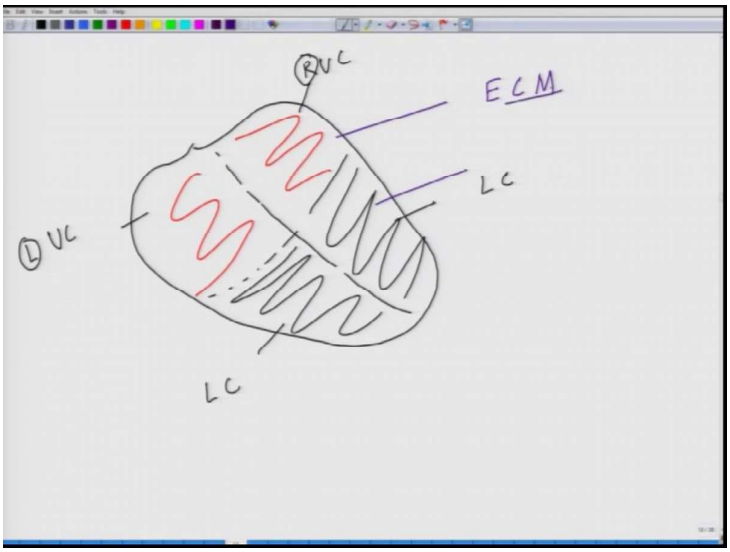

Where it has 4 chambers like this you know, now within it these are the 2 Upper chambers beginning on which angle you are looking at it from which side L stand for left R stand for right. UC stand for Upper chamber; LC stand for Lower chamber.

Within this colony there are multiple colonies and each one of these have different cells types which are involved in its ventricular cells this is the lower once are the ventricular cells, which are covering this part ventricles, the lower 2 chambers are called ventricles these are called auricles and they have different cells types, mild variations there are different colonies. And different colonies lead to different extra cellular matrix the term which I introduce in the. So, in short this is called and biological jargon they call it ECM

extra cellular matrix is nothing, but identification of that location these cells grows here under these conditions.

It is a very classic signature of extra cellular matrix. Now if I have to coming back here if I have to grow these cells outside. So, you take out these cells first thing what have to mimic is if I these cells out here.

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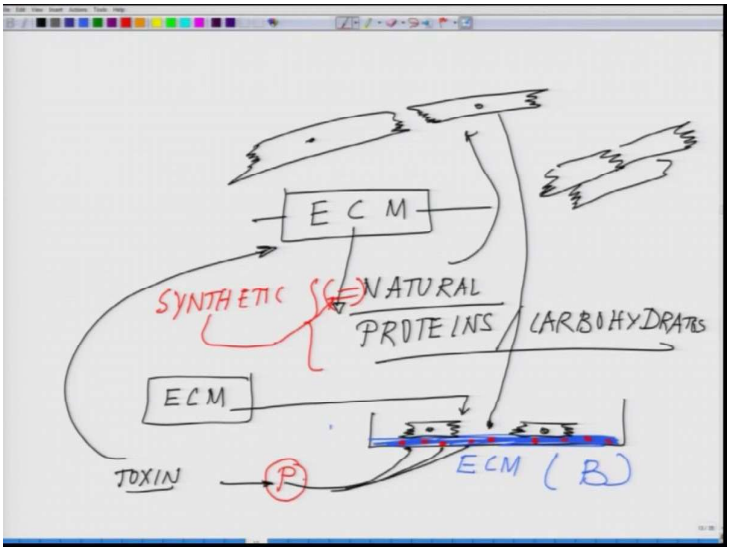

I should have the right set of extra cellular matrix (ECM). I have tried to grow the cells outside on a dish, then it is the prime prerequisite is if I have a dish like this the dish should have the right set of extra cellular matrix. So, I should we able to could this I am just putting blue because since I showed you the extracellular matrix in blue I am just putting it with blue should have the right set of extracellular matrix protein out here.

Now, I am talking about extra cellular matrix protein. If one has a hypothesis saying that the see for example, I mark this extra cellular matrix as say I call this as blue; this blue is showing the extra cellular matrix and I called it has B. Now if I give you hypothetical situation if I say if in the extra cellular matrix B, you add compound P, which I am representing by say compound P something like this. And if you grow these cardiac myocytes out here on top of this then it will change the properties of cardiac myocyte they will not adhered to each other.

In that situation you will only see the cells sitting there they will not move, they will not migrate and form the structure what I showed you then if they have to form I should show something like this like if you remember when I showed you this is structure they should show the electromechanical activity and all this thing they should come close to each other remember this is structure which you showed you in probably in the first or second lecture.

They will not be coming close to each other. Now here I am telling you that you can actually test such things if you say this P will actually prevent their migration. And say for example; P is some form of a Toxin which binds to extra cellular matrix and prevent the cardiac myocyte to come close to each other. So, how to test it here is an example.

So, you see if you know the basics then you can answer infinite number of questions, but you have to understand the basics very right because as I will go through you will see you know you just culture the station many people as a matter of fact because of lot of you know, but in graduate students you know they do not even understand the significance the by coated dishes to culture things without even realizing that there is a tremendous chemistry and biology involved in it is not something like you know just mix something mix match something is going to happen it does not work that this is a logic. And one has to because it is said a very tough process I mean one has to go through that whole grill to understand that what is the significance of having these kinds of understanding or sat for example, we start predicting that.

These are ECM which are natural these are natural ECM which consists of as I have mentioned of proteins and flush carbohydrates. Now I have a synthetic molecule a synthetic analogue which exactly mimics a natural analogue with discovery. And we will talk about it they are a synthetic analogue which exactly behave the same way as they are biological counterpart how to discover it if you do not know the basics and within scientific within this one can have synthetic; synthetic things which maybe I in organic counterpart I be an inorganic molecule, but in order to desperate one has to understand the biology and chemistry behind it. Because I told you there is another very interesting thing which is related to this extracellular matrix protein there is coming back to this picture.

Now, when you talk about carcinoma or cancer cells; what are really cancer cells are cancer cells are originating from your own body these cells say for example, we talk about a colony let me draw it something say for example, you consider your body as colony.

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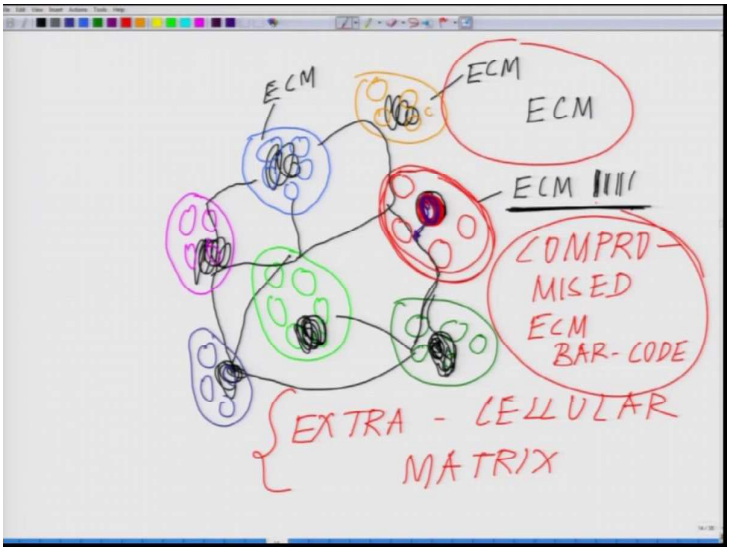

As such say a blue colony, red colony, green colony, ta green colony, orange colony, pink colony, fade blue colony this is good enough, and each one of these colonies I have different you know cells which governs them right these are the different cells. And this whole system lives in harmony without you know creating recues one minute, wherever right and they are connected with each other with blood vessels and you know them cross talk with each other which is system which is functioning in complete harmony with it with itself.

And they are happy go lucky family now say for example, in this colony say for example, one of the kids become rogue this is that rogue kit. And this rogue kit decided that I am going to create problem, what this will do, it will travel it will come out of it.

Now, think of it these colonies identification of this colonies are they are ECM. There is a different ECM here extra cellular matrix there is a different ECM here there is a different ECM here. ECM is their barcode you remember the barcode whenever you buy a material, you see the barcode it is the barcode of that cell we decide which part of the body it is going grow. But here this rogue fellow decided that it will deceive the barcode

and it will go anywhere and it will is going to grow it will invade may it may invade this part and you know it will grow like this because it is now more of a suspended cell it can grow anywhere and said it does not have the anymore the barcode to grow it has you know kicked out it is barcode, it goes here and you know you need create trouble it may go here and create trouble wherever it decides.

It is a rogue fellow. So, all of us sudden harmonious system goes for a different swing and that is precisely what happens in a cancer cell; a cell which decides that hey I do not want to be in part of this colony how I cannot become part of the colony I shut off my bar coding and I cheat this colony the red colony if I start with the red colony I cheat the red colony if people I moved out from that colony, I want to the green colony or the light green colony, or to the pink colony and I spread myself. Because I do not have any barcode now I can go anywhere and as much as I can grow, this is precisely what happens and that is why I am telling you understanding this part of the biology of understanding this extra-cellular matrix extra again I am reiterating this point because try to understand the science behind its extra-cellular matrix is so, so very important.

So, please as the next generation upcoming scientist do not follow things blindly please thing this is hollow is a chemistry behind it without that biology does not exist, there has to be a basic fundamental chemistry behind it. So, please think over it I have seen people buying dishes coated with some extra is are extra cellular matrix and they grow cells surely the something will grow I mean whose stopping them from growing like explicit itself there is a thumb rule python. That is not the way to do the science one has to understand the basic concepts what is happening? If I change this. So, it should be able to play with the cells you know if I change this extra cellular matrix this is what is what is going to happen if I use this is the what is going to happen how the cell behavior changes as the matter fact within the same extra-cellular matrix with slight difference their physical parameter changes.

This is that part where when a cell becomes rogue, when the cell becomes cancerous because this extracellular matrix signature or barcode is compromised. So, for those who are from outside biology you can call it as the compromised ECM barcode this is how you should remember it the barcode has been compromised. When the barcode is compromised they can cheat anybody and they do cheat they go to some other part of the body and then create rockers. So, this is how it works. This is second aspect what one

has to understand that what the ECM does. In the next class we will follow further with the biology of B cells which you help you because based on this.

We have talked today about the gaseous exchange in how we mimic it we have not talk to the mimic it, but how we have to mimic that condition second, we talked about the ECM right. We will follow it up in the next class.

Thank you, and thanks for your patience.