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 taken into account; 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 this cementing materials plays a critical role in determining the fate of the cell.

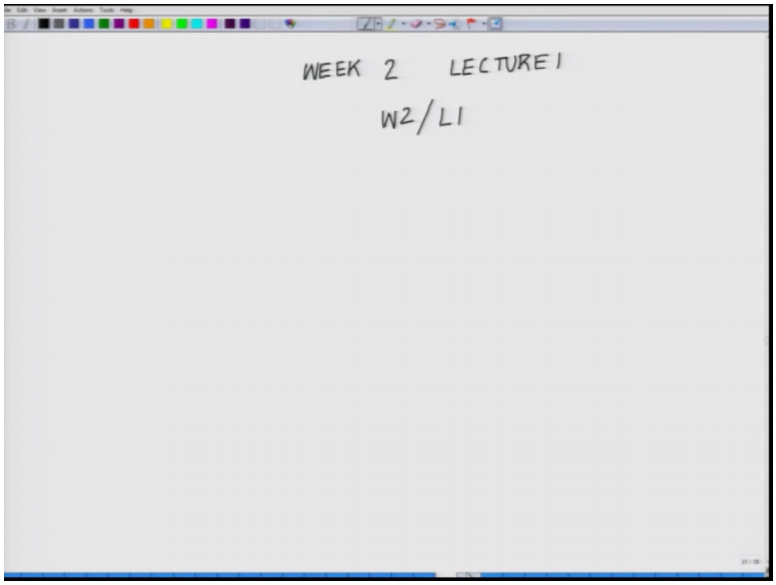
Post that of course, while talking about the adhering bring of the cell, we talked about classification one has to understand is it adhering cell, we are culturing or we are culturing a non adhering cell then followed by this, we talked about the cell cycle progression, are we dealing with cells with 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 passage a cell lines; suppose you are using a cell line which has been immortalized or it can grow n number of cycle, 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.

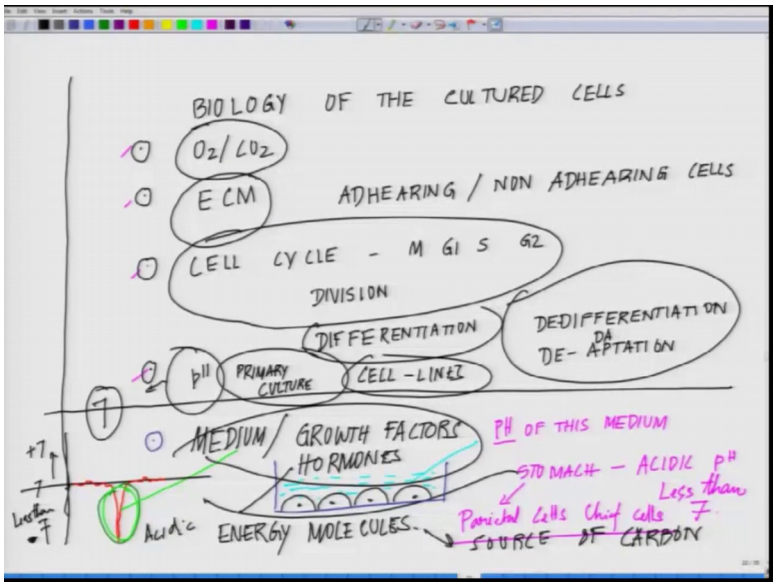
Are you using it for a specific sensor application or you are using it for understanding certain event happening at the cellular level or you are using it for some kind of hybridoma technique like you know antibody production or you are using it for production of some other x, y, z chemical. So, that 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.

(Refer Slide Time: 05:09)



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

(Refer Slide Time: 05:32)



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 have 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 thing what we will be dealing with is the PH under what PH a cell is a adapted to.

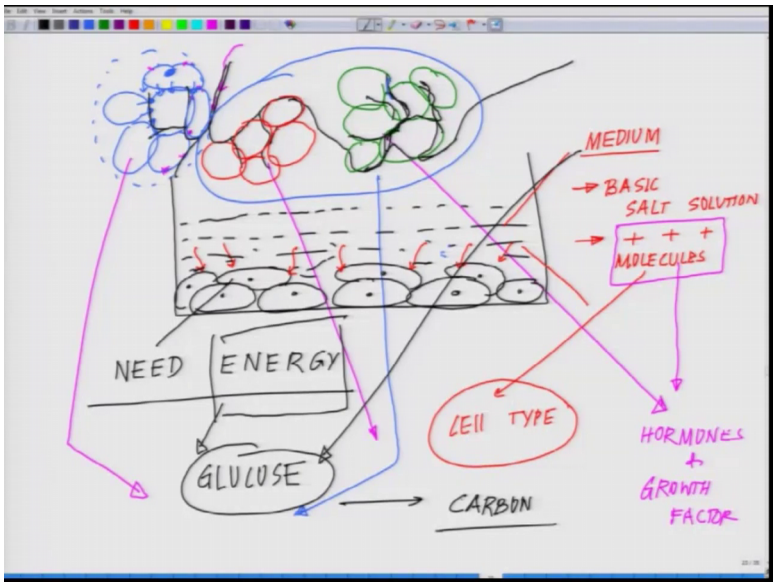
So, generally we know our body PH is at 7, but while you are growing the cell, there are or let us put it other way; while the cells are growing in your body, we have this blood which is slowing through you have these extra cellular matrix proteins; extra cellular matrix solution which is continuously 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 is 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 goes like this for a finite period of time, this thing happens. So, than 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 kind 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 Debri 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.

(Refer Slide Time: 13:24)



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 requirements.

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.

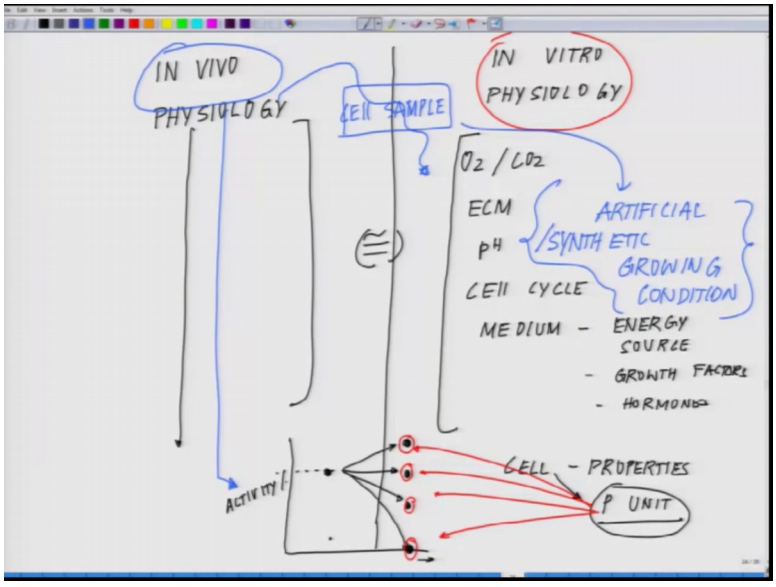
And that adds up to the complexity in a real life what happens there are blood vessels which are crawling through and they ensure that these binds there, but outside the system that privilege is lost. So, those adds up the complex situation of growing cell in a very very controlled environment further, 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 source 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 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.

(Refer Slide Time: 21:38)



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.

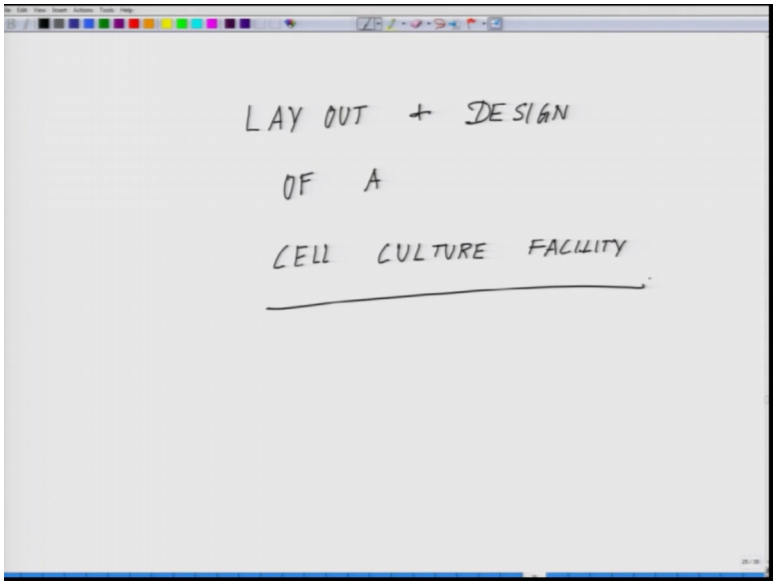
Now, what is critical for it know is after growing the cells in all these conditions which is we are our goal is to you know come as close as possible almost; similar almost congruent then what we have to do the cells which are growing here their properties have to be evaluated. Say for example, the 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 see for example, how far I am in in vitro condition; am I exceeding it am I below it am 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.

(Refer Slide Time: 26:18)



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.