Cell Culture Technologies
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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 an 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 takes us, we will talk about the biology of the cells visibles 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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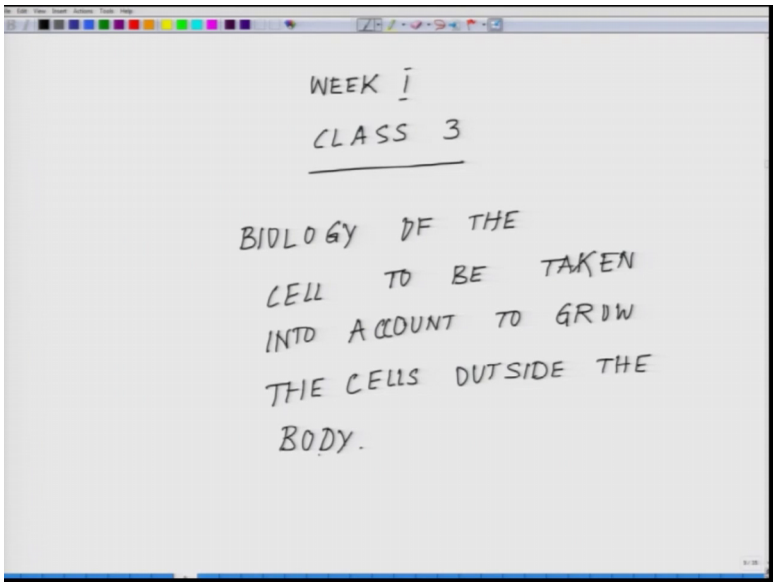

So, we are into Week 1 and today is our class 3. Today we will talk about the Biology of the cell to be taken into account to grow the cells outside the body; this is the key point what will be dealing next few classes. In order to address this point first of all we have to understand under what conditions 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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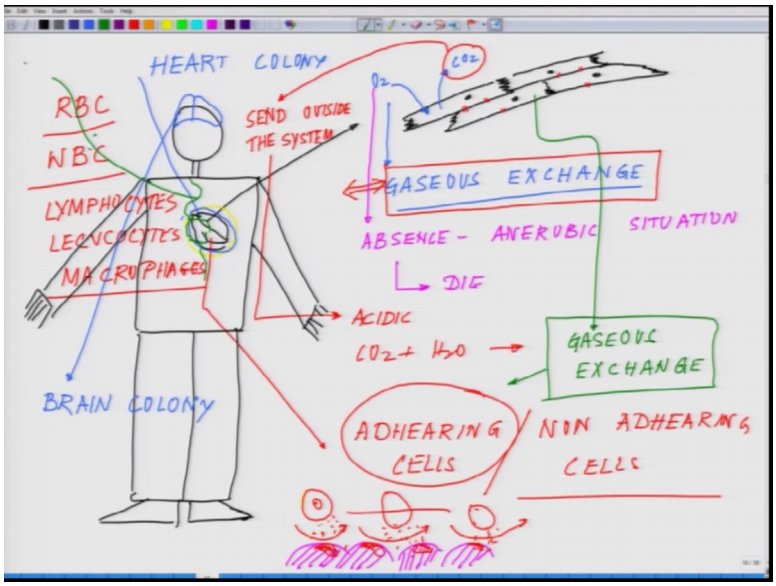

So, say for example, if to take the same explain again and just take the example of the cardiac cell or (Refer Time: 03:44) So, these cells which are growing out here a cardiac monoxides which I have already explained earlier and here are the gap junctions.

These cells out here have the first parameter they need Oxygen and they give out Carbon dioxide and the any oxygen. 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 CO2 plus H2 O 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 lecture 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 x y z compound x y z 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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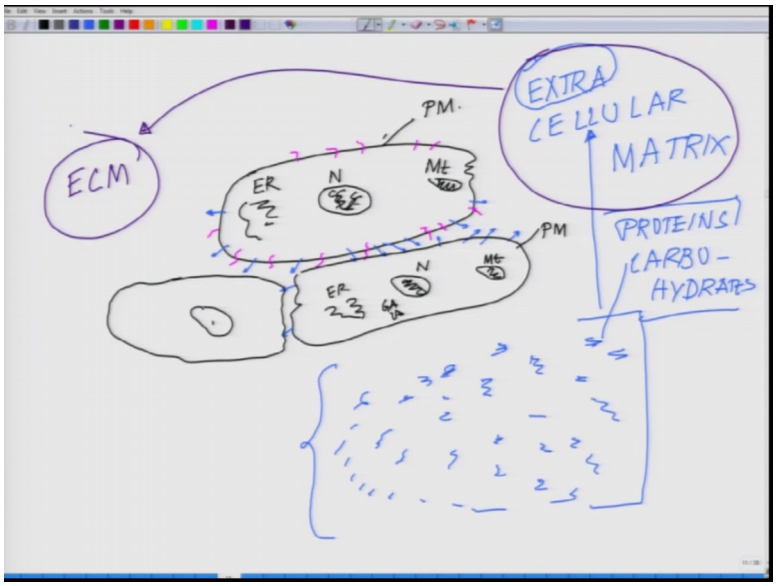

Here I have a nucleolus with the d n a sitting there we have all sorts of orgonal mitochondria endoplasmic reticulum ER nucleus M t stand for mitochondria likewise we have the plasma membrane PM. These cells have lot of you know membrane proteins which are setting there, now if these cells have to adhere 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 invite 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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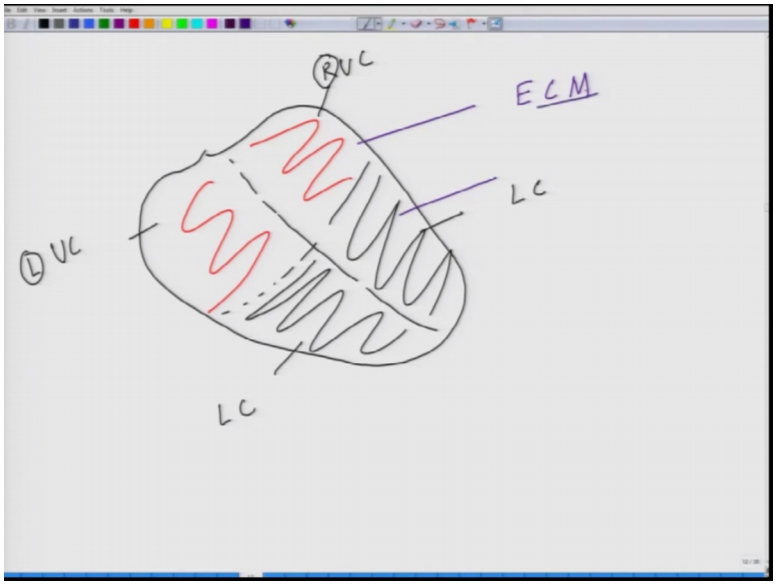

Where it has 4 chambers like this you know, now within it these are the 2 Upper chambers beginning on which angle you are looking at it from which side L stand for left R stand for right. UC stand for Upper chamber; LC stand for Lower chamber.

Within this colony there are multiple colonies and each one of these have different cells types which are involved in it 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 leads 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

ectra 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 this cells first thing what have to mimic is if I these cells out here.

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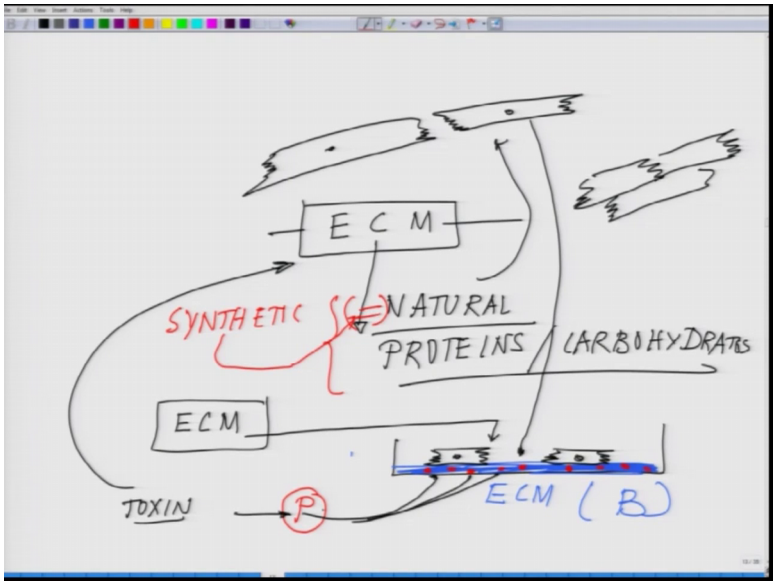

I should have the right set of e c m extra cellular matrix. I have try to grow the cells outside on a dish, then it is the prime prerequisite is if I have a dish like this the dish should have the right set of extra cellular matrix. So, I should we able to could this I am just putting blue because since 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, one can since 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 statiom 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 say it is say a very tough process I mean one has to go through that whole grill to understand that what is the significance of having these kind 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 analogues 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 may be 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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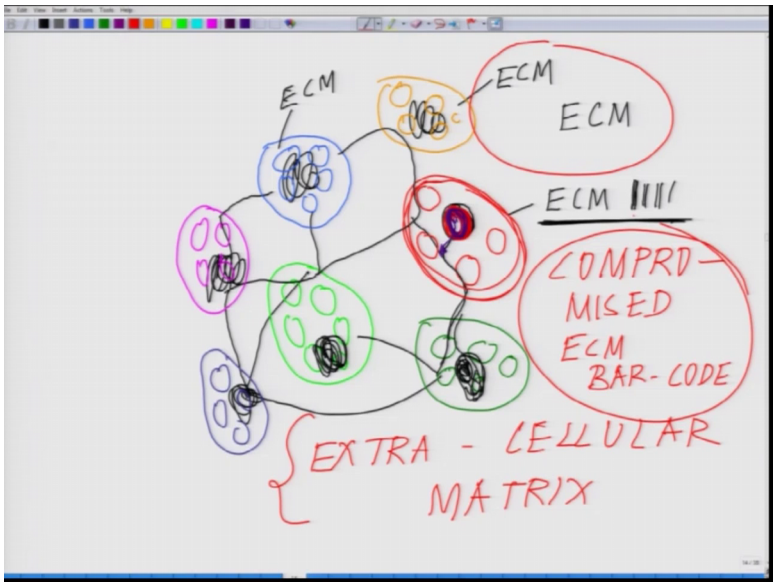

As such say a blue colony, red colony, green colony, ta green colony, orange colony, pink colony, fade blue colony this is good enough, and each one of these colonies I have different you know cells which governs them right these are the different cells. And this whole system lives in harmony without 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 kid 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 buys a material, you see the barcode it is the barcode of that cell we decides 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 ta 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 it extra-cellular matrix is so, 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.