Cell Culture Technologies
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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 prerequisite 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 kind 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 it little bit louder on this.

So, we have this non adhering cells 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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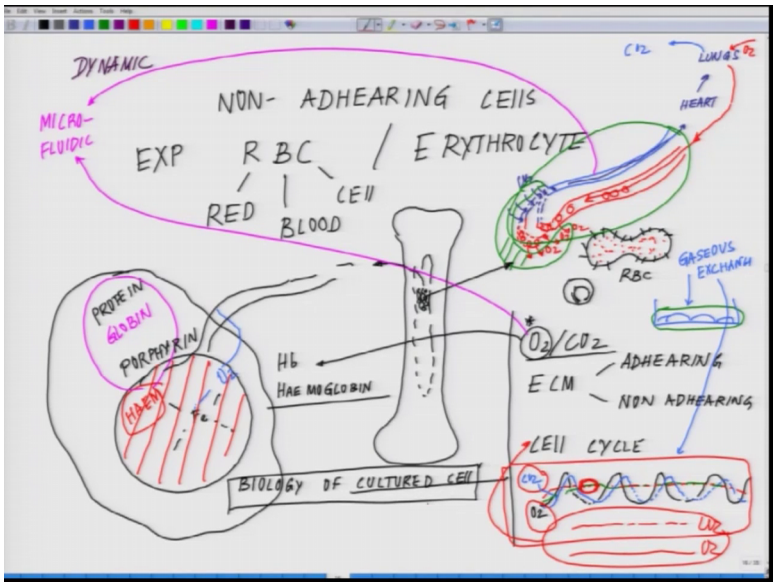

Let us once again, this is our week 1, lecture 4. So, this is a fourth lecture and talking about non adhering cells. So, if you taken for example, if we take the example of RBC or red blood cell which is also called erythrocytes. These erythrocytes have their origin in the bone marrow somewhere out here, 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 get 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 r b c. 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 take 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 h b, 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 r b c's and r b c is responsible for going to. 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 C O 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 c o 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 in a manner like this, realizing because this is the flow which consumes and parallely 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 c o 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 represent 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 kind 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 given us a very different understanding about the dynamics of cellular growth is a continuous process 24 7 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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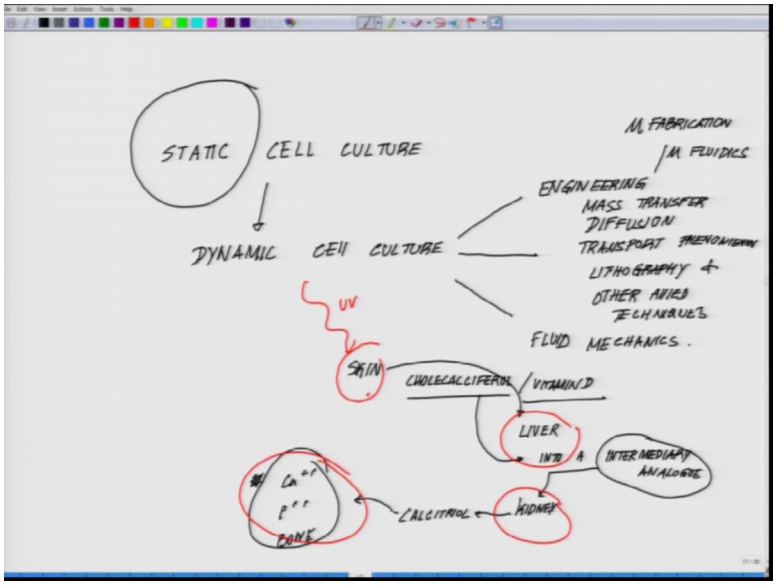

So, you can say that modern cell culture from static cell culture the future will be more of a dynamic cell culture and such dynamic cell culture will be read, needing lot of understanding of engineering and within engineering micro fabrication, micro fluidics, mass transfer diffusion, lot of understanding 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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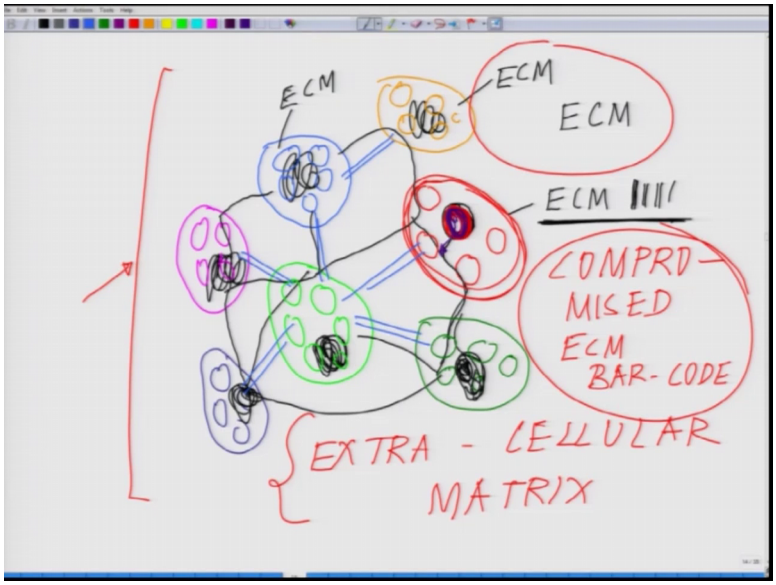

Just when I drew this, so there is always information exchange which is taking place between these multisystem systems, multi system structures. So, say for example, one simple example let me give you, what I mean by that?

So, you know that our, we are being always advised and you know in the morning go out and because there is u v rays which are slightly helpful. So, what we observe the u v 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 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 C O 2. Having said this, this brings us to 2 different situation, such sometime such situation this situation which is currently prevailing this is the constant level of C O 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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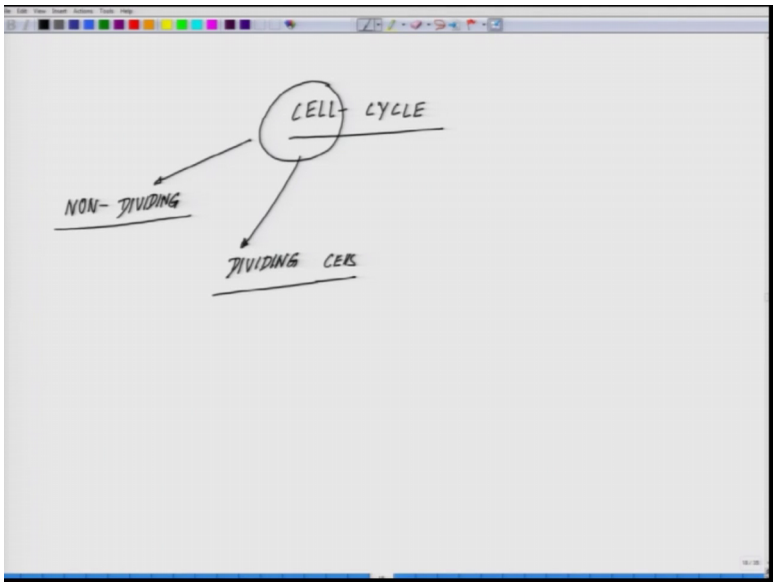

So, let us first of all talk about where we suppose to start is cell cycle. So, here let me highlight that you can, in the previous class I told you that you can distinguish between adhering cell and non adhering cells right. Now I am introducing that we can classify the cells under 2 groups dividing cells and non dividing cells. So, put a 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 neurons 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.