Cell Culture Technologies
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Lecture - 18
Cell Growth Process

Welcome back, what we are telling you is now coming back where we left the problem.

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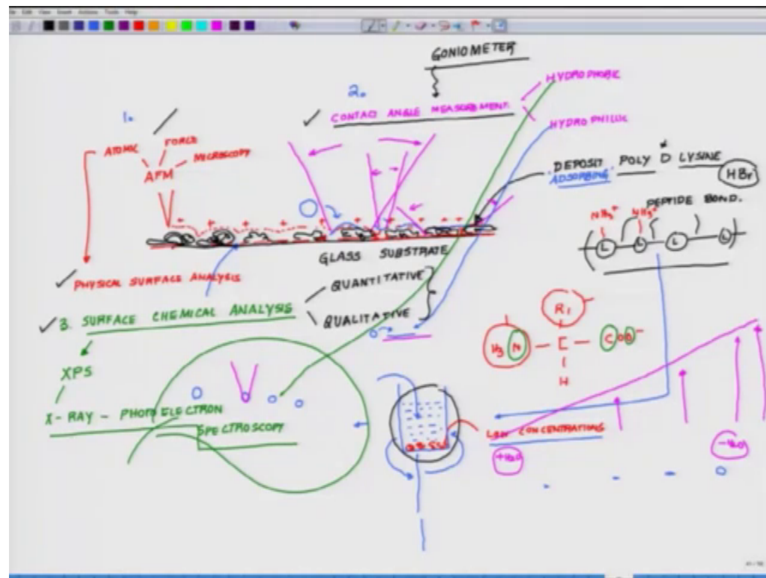

So, we left the problem here With Poly D Lysine and I told you that you can really check it out that, how it is surface properties are changing, how it is charged behaviour will change. You can do an extra photoelectron spectroscopy you can do an AFM you can do a contact angle measurement analysis.

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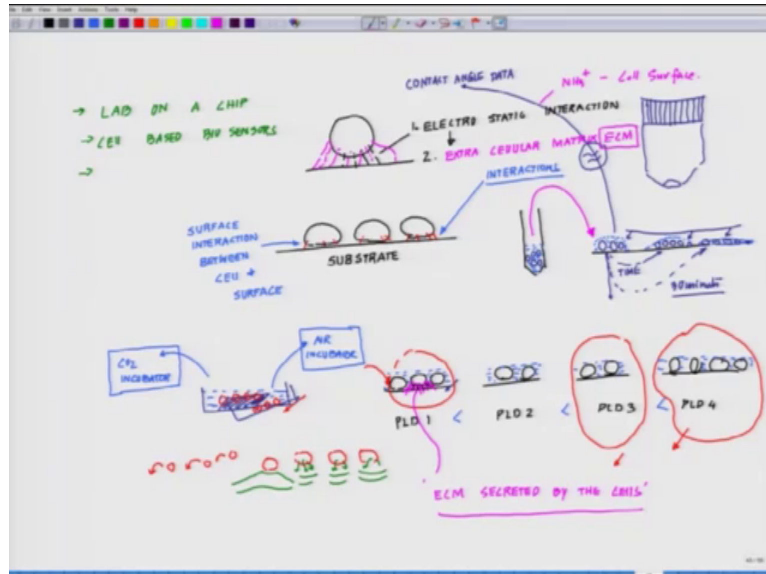

Once you have done this with one substrate the next thing comes you have to now really take this say for example, I have 4 different concentration of Poly D Lysine PLD1 PLD2 PLD3 PLD4. Now comes the next set of experiments where the real life. So, you have to put the cells on them along with the medium. Now seeing their attachment properties and you can do very simple things to see the attachment. So, say for example, you have a cell suspension like this and this is this blue is showing your in the medium ware it is all the cells are suspended.

Now, once you plead the cells you have small thin layer of medium in and around the cells you have this cover slip you take the cell suspension just stop it there with it first of all look at it whether it spreads or not first catch. So, say for example, here you have the substrate and here you have in your small test tube you have the cells what you have isolated and I am showing the (Refer Time: 02:31). So, here you have the cells and cells are suspended in a medium like this.

Once you put this suspension out there, either it will remain like this initially or may remain it for a while or the other option is that it may spread out like this cells are shown in dark blue or it may spread even faster like this, where these are the possibilities and you can map it with respect to time and does this one becomes like this, or this one remains like this, or this one becomes like this you really can see it under the microscopy

So, what you needed is that you needed a microscope over there which will give you an idea that how this molecule is interacting. Now based on this interaction you can see how

the surface is behaving or this does this data matches with your contact angle data which was done without the cell.

How much similarity they have or how much dissimilarity they have how much close in their second thing what you can do is a very easy ways you take this dish you wait for say now thirty minutes and then you just slightly tilt the dish. So, this is on a dish and you just change the angle to something like this just of course, you are going to still fill the medium if these cells are not attaching you will see the cells will be rolling on the surface after half an hour. But if and this is I am talking all from my experience if the cells had to attach by 15 to 30 minutes they will attach and still some of the cells will not attach, that you have to give it as a error margin will all those be there, but if they have to attach and if you tilt this dish little bit.

So, what I am suggesting is see for example, here is the dish where you are growing and see for example, you have a cover slip which is coated with your XYZ substrate and you have these cells which are being here and now you just tilt the dish little bit like this little bit very small maybe a this much like you know just slight tilt if you do a slight tilt if these cells have attached on that substrate and you would not see you will still see some cells which will be rolling down they will be it is a very nice sight to see.

They will be you know hopping down rolling down, but still you will see cells which are more or less looks to you as if there are almost kind of sticking to that surface and at this stage it is very interesting at this stage if you if one can really figure out some way or other one will able to see that since these cells have started secreting extracellular matrix on the surface. So, coming back based on these small queues you can you will be able to figure out whether the cells are properly adhering on that substrate or not.

And the thumb rule is after 30 minutes or sometime even I have gone upto one hour you then fill the whole dish with rest of the medium and put it in the CO 2 incubator for growth. If it is a CO 2 dependent CO 2 incubator is needed or you can also grow it in incube simple here in cubitor depending upon what kind of medium you are using I mean what kind of buffering we will come back come later into that whole concept of buffering and incubator kind of incubator what you can use, but at this point just accept the point you just take it and put it in the incubator and (Refer Time: 08:00) the environment to grow.

Based on the amount of Poly D Lysine you can predict whether the Poly D Lysine is helping the cells to adhere or not helping the cells to adhere and as much if I assume that this is how the dose is changing. Then this particular zone what I am telling you is where you may not see an attachment at least from my experience with neural cell I have seen these are the zones even if they attach eventual growth is kind of very very funky kind of growth. So, with Poly D Lysine a very low concentration does work and the way it works it is believed to be these positive charges which are on the surface of Poly D Lysine these positively positive charge is from NH 2 groups which are present there interact with the surface negative charge of the cell possibly this is what is believed.

It is a purely in the initial phases, it is purely a electrostatic interaction and this electrostatic interaction helps the cell to settle down at a substrate why I am telling you this part it helps them to you know kind of build that first level of. This is electrostatic interaction which is kind of you know keeping the (Refer Time: 09:51) substrate and followed by that it gets that time window to settle down, it is like you know get little of a time and like there is a slope or there is something you are moving, but you just get little time to hold on to a point and then in the meantime what you do, you tell your genetic machinery you start producing extracellular matrix at the substrate to get a better anchorage on that.

These pink what you are seeing are the ACM secreted by the cell. What you see here is one can monitor this whole process in a very systematic way, which otherwise if you look at most of the cell culture were currently happening across the world are not being done. But these becomes more and more important as we talk about using cell based biosensors we talked about using microfluidic devices cell based microfluidic devices we talked about you know lab on a chip. These are some of the emerging technologies like you know lab on a chip cell based Biosensors.

All these things will require a very very thorough surface analysis before one can really translate these technologies to the industry grade level of you know drug screening and other things. We will come later into that part why I am highlighting that because there are issues which are being faced by people while trying to repeat some of these results in different parts of the world and then they realize that it is kind of what I should say it is kind of very incohisive incoherent results which people meet.

Now we talked about the interaction which is possibly happening between Poly D Lysine and collagen a Poly D Lysine and the cell which are present there and there are 2 level. First again just to enumerate the first is a cell out here substrate out here and first is the Electro Static Interaction step one followed by 2 which will have where is the cell upon that stabilization starts to secrete out it is own Extra Cellular Matrix or ECM. So, keeping this paradigm in mind we will move on to and of course, we talked about the interaction between this is the kind of interactions which are possibly happening.

Next in that line now having said this I will take a synthetic situation sorry a natural situation. I have talked about these 2 now let us talk about a natural molecule.

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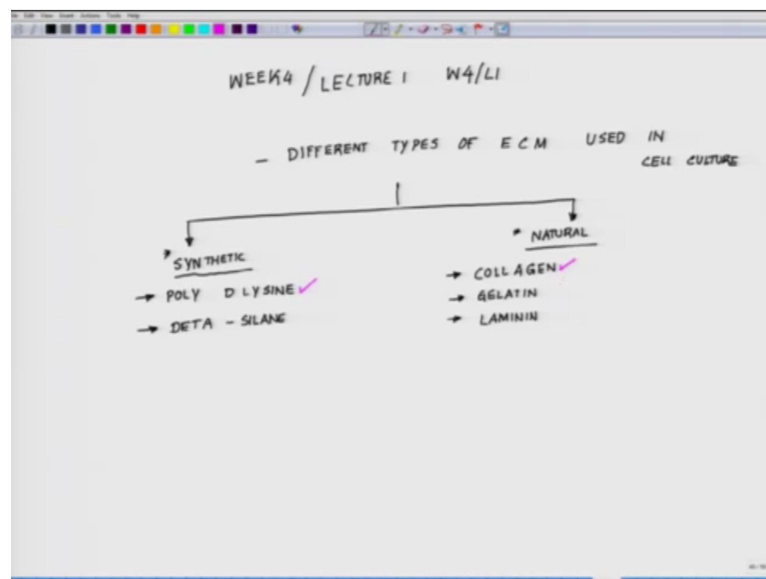

If we talk about if we go back to our list, we talked about this. Now let us talk about Collagen in the case of collagen again you are doing the same thing you take a substrate and you have a collagen protein dissolve in a buffer.

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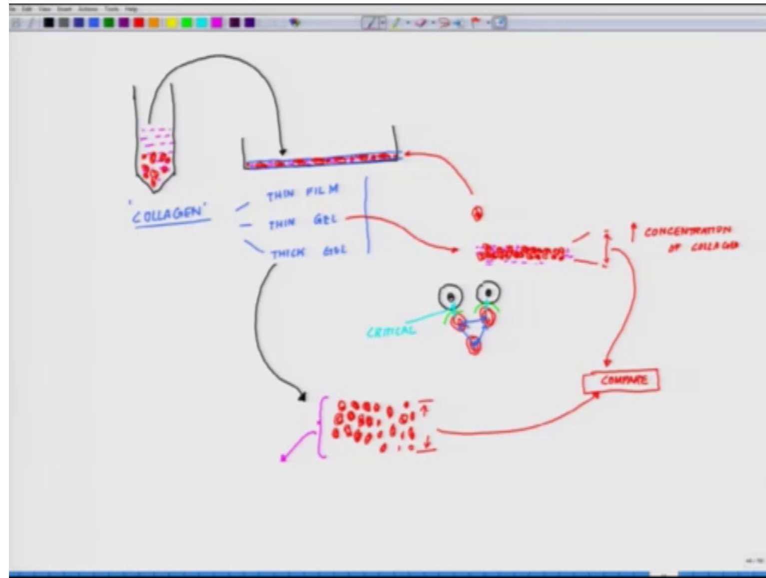

So, here you are not using any more water you may have to use the buffer here is the collagen protein and here you have the buffer in which this is dissolved.

And now again you follow the same thing you create add different concentration you have the collagen already getting there and along with this small amount of buffer. Now what you do is once you coat the substrate, in terms of collagen could be used at different concentrations you can make a one second; you can make a Thin Film of Collagen, you can make a Thin Gel of Collagen and you can make a Thick Gel of Collagen. You have lot of possibilities what you can play around with collagen how you play with this the way you play is out here.

For example, you have this molecule out here. Now if you have to play it as a thin film then you have to have minimalistic number of molecules or minimum number of molecules and you should be able to spread it out all over the substrate the way you see here. It forms a thin film out here, now you wanted to make a gel out of it very simple if you want to make a gel out of it what you do is this thin gel you are making a thin gel. So, increasing the concentration, in another word what you are doing you are increasing the you are introducing a depth feature by increasing the concentration of collagen and thereby you are bringing more collagen molecule closen.

There will be 2 level of interaction; one interaction you have to make made happen is between the individual collagen molecules there is one set of interaction which will be

happening that will bring them close whereas, there is a second level of interaction which is the surface which is exposed which I am showing like this is the surface which is very important for you because this is where the cells are going to interact. So, the black what I am drawing is the cells. This interaction what will be happening here is very critical.

Second thing followed when you wanted to make a thick gel say for example, you want it the whole matrix to be a gel matrix. Which will be something like much more complex interaction which will be coming through something like this, now whenever you are trying you are trying to do this kind of things you are giving much more. So, what you if you compare these 2 this picture and this picture compare if you compare these 2 pictures. What will you observe is the thickness has gone up, the thickness has gone up then there are 2 aspects which will come into play; the first aspect which will be coming to play is what will be the space between these individual molecules of collagen.

So, here you have no more growing the cells not on a glass substrate with thin film or an thin film or a thin gel you are having the whole gel and you want to grow the cells on the gel. In other word you wanted to make a 3 dimensional culture and if you wanted to make a 3 dimensional culture then there are few other parameters which has to be taken into account and we will just continue in another with few slight changes we will continue from here.

Thank you.