Cell Culture Technologies
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Lecture – 19
Cell Surface Interface

Welcome back.

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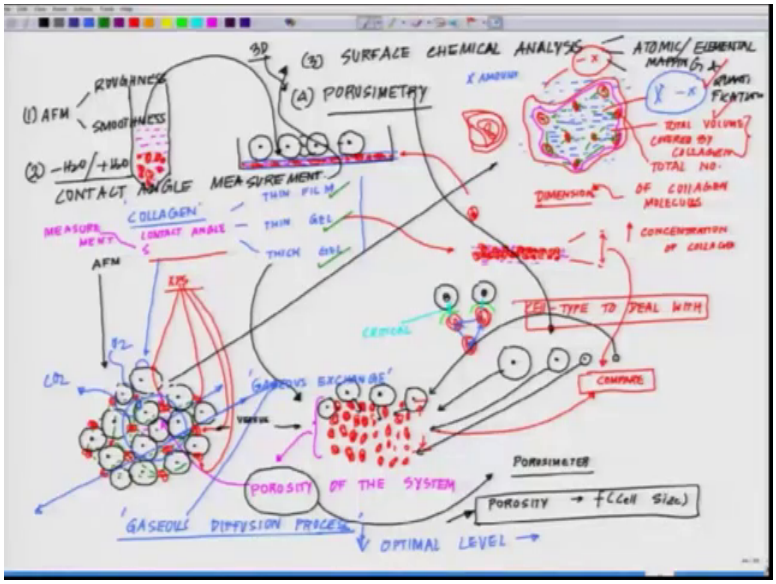

So, what we are telling you is if you increase the number of molecules out here of collagen and you wanted to make a gel. So, this is what it will be it will be more like a 3 dimensional assembly now and you wanted to grow the cells in it.

Now what are your options? Your options are you have the cells exclusively on the surface like this, which is kind of if you think of it will be a wastage of space. So, you may want that the cells to penetrate and have that 3 dimensionality because whenever you are growing a cell on top of like this. So, you are realizing that cells are only interacting at one point of contact or maybe a little bit between each other being close to each other like this ok.

But it is not getting that whole 3 dimensional feature or 3 dimensional signaling I wanted to have a 3 dimensional signature to this. If you wanted to do a 3 dimensional signature then your best way is now you have to control the porosity of the system, porosity of the

system and talking about the porosity of the system. Now if you look at this now you need something because these cells just physically look at it these black cells cannot really penetrate into these spaces, these spaces which are there put another colour that will make more sense they cannot penetrate into this surface. Now what you can do is you can change the porosity of these molecules.

Now, in order to change the porosity, now realizing these molecules are moving a little far away, you are realizing I am now stretching the molecules far away from each other. Now how I can ensure that there is an interaction between them, you realize the problem it is not that of a trivia in order to do that then you need modulatory molecular handles, I am standing here I want to in a touch you. So, here is my arm is stretch. So, another arm comes and I couple like this. So, if this is one molecule standing here another one molecule standing here I have couplers.

So, then you have to use some kind of couplers which will create kind of you know a ne2rk, a mass, a mesh like this. So, you have to introduce another new component, a handle which will create this kind of mesh now you look at it. Now you are having those cells which you are placing here some of them will be getting inside like this, like this, like this, like this, like this, like this, this, like this, this like this.

Now, could you compare this picture versus this picture. So, now, when you talk of the porosity of the system; that means, now I am adding one more dimension to this you have to have porosimeter when you want you to make gel out of it. So, now, you see how the system is slowly evolving in your brain. So, first of all we talked about surface analysis with an afm here also that will apply, we will be using atomic force microscopy to analyze the surface. Here also you will have to use contact angle measurements to see how this gel is behaving on the surface because otherwise if the surface does not allow and then the medium will not you know move into these spaces fine.

So, then comes contact angle measurements, followed by once you do a contact angle measurements you should do an xps that will give you the surface characteristics sorry the surface characteristics of the native gel. So, I have just added the cells, but do not get confused your native gel was something like just to redraw this thing once again redraw, that will make that will not confused you.

So, this is with the different kind of porosity. So, you have the surface like this and here are the interactions right. How you do the porosity measurements that is very important now for the. So, the third thing comes is the porosity. So, how do you do porosity very simply we try to understand these things do not mug up anything, just put your goddamn logic in place. So, what do you do you take a finite amount of fluid, this fluid could be anything people use mercury, porosimetry per people use cds of them do not get another try to pick up a simple thing, you take a simple fluid you know that x amount of fluid I am taking take.

Now, once you do this you allow the fluid to get soaked again into these spaces fine. So, based on that if you took it, took at total amount of fluid some fluid we will not get into this and some fluid will get into the system. Now, you can back calculate and figure out how much fluid has gone into the system. So, you can back calculate it so this extra stuff so, let me put a pin. So, this whole extra stuff is useless this is not going anywhere. So, you can remove it. So, this is minus x amount coming out this is whatever you know, if this blue is the original x minus x is the amount which is getting there.

Now, in this molecule you know the dimension of the collagen and the total number of collagen molecules, total number of collagen molecules and if you know the total number of collagen molecules that you have of course, back calculate using (Refer Time: 07:56) number about, what is the concentration you started with and the dimension of it that you can see in the literature. So, if you know that the dimension of collagen molecule in terms of like, say what is the dimension of it. So, if you know the dimension of it and if you know the total number then you can back calculate the total volume covered by collagen.

Now, you have this value, you have this value you know how much is the pore space this is the simplest way how one calculate do not fall into the complexity of it these are very simple techniques, but you just have to put your brain in place to figure them out. So, now, you can vary the porosity of the 3 dimensional matrix and by varying the porosity of the 3 dimensional matrix depending on the. So, you have to take everything into account you may have a cell this big you may have cell this small you are in a smaller cells you know in a smaller cell. So, depending on the size of the cell what porosity you are going to use will be porosity will be, will be a function of cell size ok.

So, porosity is a function of your cell size. So, if you know the porosity so let us summarize again because there are too many things I am dealing. So, first for surface analysis or analysis of your material you use the a f m, atomic force microscopy that will kind of give you the roughness or smoothness of the surface whichever way you want to explain it. One next followed by that what you can do is you can do a, I am just putting it symbolically contact angle measurements, contact angle measurement 3 followed by that what you can do is surface chemical analysis right.

So, where you have the atomic or slash elemental mapping and quantification, then you can go for if it is a gel or if it is 3 d material what you are handling you can go for porosimetry. So, you see at your disposal you have so many different techniques and you can vary the porosity depending on which cell type you wanted to deal with, not only that this 3 dimensional matrix are to be created in such a way that the gaseous exchange happens properly because, in such micro environment if you see the interior of the micro environment there are metabolites which are being given out form of c o 2 and the cells will be needing oxygen. So, the gaseous diffusion process gaseous exchange or gaseous diffusion process happens at an optimal level right.

So, by using simple physical techniques you really can build up your own story, you can target your, your own problem it is what because see each one off will be will be working on different aspects unless you know that tools at your disposal you will not be able to answer many of the questions which you wish to answer you will love to answer, but then how I am going to do this, is there a technique yes there are techniques, but one has to know the techniques.

Now, coming back so, we talked about the collagen. So, you can make. So, where we digress you can make a thin film of collagen you can make a thin gel of collagen or you can make a thick gel of collagen and when you make a thick gel or even a thin gel you can make a, you can vary the porosity of that material and by varying the porosity there are several other things you can think of in this same line say for example, you wanted to have your cells exposed to a slow releasing material slow releasing drug. So, you can encapsulate your material or that a specific drug in these kind of gel and you can grow the cells on top of it say for example, one of the techniques could be say just imagine like this

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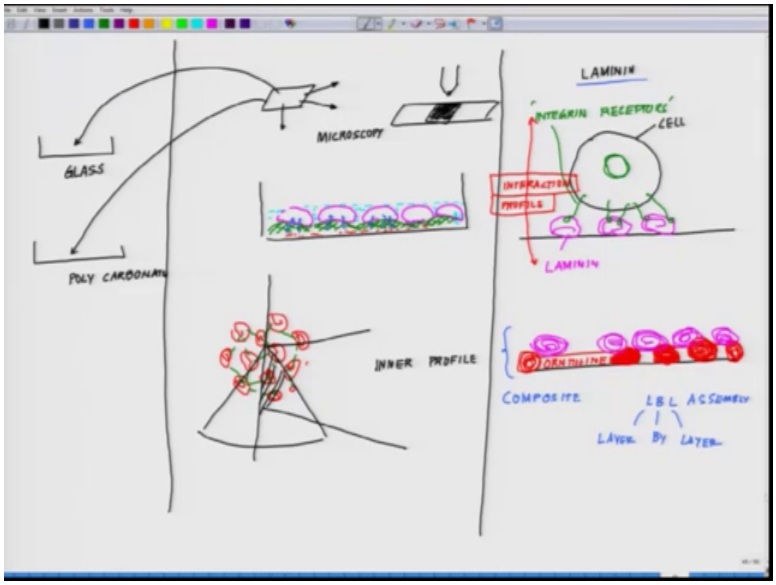

So, you have the drug molecules which are here and on top of that you have this gel like matrix, which are there and we had a very thin very small porosity very slowly releasing situation you can control say the porosity out here. So, on top of this you put the cells, now these cells will be exposed to this particular drug in a slow fashion, because the porosity is less. So, the porosity is less then automatically the release of the drug will be slow.

So, based on the porosity you really can develop different kind of gels thin gel, thick gels different of the structure and not only that the interesting part is there is one more technique what you can use you can even take this like I showed you this picture. So, say for example, you form a porous structure like this say for example, like this a different kind of interaction say for example, this I call it a surface structure and you have these handler molecules which are in anchoring them there are several of them. So, I am not getting to that part there, I will leave it to your you can even using different kind of electron beam using different kind of etching electron beam lithography you really can cut across it in 3 dimension and you can really see the interior profile of it, of that gel I did using different kind of electronic beam lithography.

So, these are very advanced techniques where people really try to see the interactions between the cell and the substrate I am not recommending at this stage, but at least have an idea how you can use these different kind of tools. So, next in the line once we talk

about collagen next in that line is laminin, but before I get into laminin when we were talking about (Refer Time: 18:01) we talked about the first interaction is this interaction.

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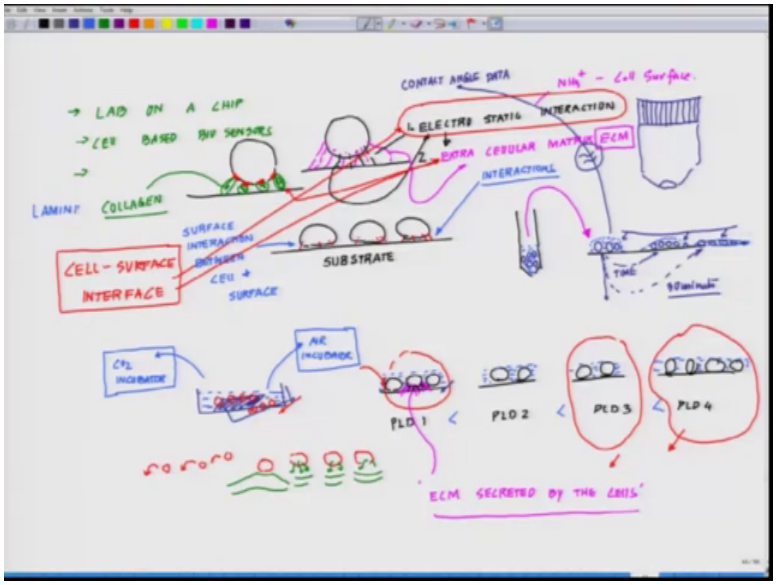

The electrostatic interaction which happens right, followed by a extracellular matrix secreted by the cell and leading to the attachment.

Further strengthening the attachment, now I in this slide I introduce here side by side and will help you to understand a situation like this, where you have the collagen sitting there. So, the collagen itself is a so, the green one is showing the collagen. So, the collagen itself is a extracellular matrix floating, the cellular interaction collagen has its own receptor on the cell surface and they bind like this. So, here you are not really relying on this part for the attachment you realize it, you are only relying on this part.

So; that means, the force dynamics are going to change of the attachment and this is critical that you could distinguish it should be able to distinguish between different interactions which are happening at the cell surface interface. It is extremely essential that you people understand this interaction at what all forces are governing this, this force, this force which all the forces without having a complete idea about this you will not be able to appreciate the subject to its core.

Now, in the same line we were about to introduce the laminin, laminin is much more simpler in that respect the way the interaction happens. So, laminin has its, if if you look

at the cell this is the cell and you have a substrate glass surface on which you are coating it with laminin molecules. So, laminin has its respective integral receptors on the cell surface. So, there is kind of an interaction like this, here is your cell. So, here is the cell, here you have laminin and here you have the integrin receptors present on the cell surface and the interaction between happens between integrin and laminin. Here is the interaction, here is the interaction profile this interaction helps the cells to attach to the laminate surface.

But it is the interesting, laminin by itself is a very tricky molecule for it to adhere to the surface of the glass. So, many a times before you coat the substrate with laminin if you want to use laminin as the extracellular matrix, it may be a very fair idea that you take the glass and by the way we have to talk a little bit about the glass also. You take the glass substrate or the polycarbonate substrate whatever you want you to use you coat it with another protein called ornithine, ornithine. So, you have a coating of ornithine something like this, coating of ornithine like this, on top of ornithine you code the laminin do not be so confident that laminin will directly adhere to the substrate of the glass. From experience I am telling you I have tried this experiment several times and most of the time I have failed because laminin has this peculiar and these interactions are not so easy that you can predict sitting on a computer and oh you know this is going to interact it really does not happen like that because unless you are at the ground reality.

So, if you see the old books, old manuals you will see the older guys from the old school they have already mentioned this very clearly you need it ornithine some positively charged there on which the laminin will interact better. So, in other word whenever you are doing something like this, you are making the substrate further complex it is more of a composite now adhering composite or layer by layer assembly as we call that, layer by layer assembly lbl assembly layer by layer.

So, for ornithine this is pretty critical that you ensure that it attached to the substrate, now coming to one aspect which I have not touched is are you growing in 2 dimension on a glass or on polycarbonate your options are mostly fall under these 2 glass or polycarbonate if you are using polycarbonate which is a sterile polycarbonate 15 mm or 90 mm or you know 60 mm dishes which comes. You do not have a much of an option on that you just have to coat it with your xyz protein if you are using on a thin laminin side and most of the time the way cell cultures are being done, we use we try to grow the

cells on glass cover slips and we place the glass cover slips in it or we place the glass cover in we directly the reason being if we have to do a immuno saito chemistry if you have to do an assay. So, all these things are easy to visualize or see from a glass cover slip especially microscopy becomes much more easy when it is on a grass cover slip ok.

So, you just take the glass you make a slide take the whole glass thing and you prepare a slide like this. So, here is your like you know sitting there and you visualize it. So, this is how most of these things you know kind of work. So now, coming back where I was, so now whenever you are using glass so these glasses arrives at your disposal in your lab in bulk we really never test a glass. So, few problems what you will face in real life will working with glass, glass cover slips is that most of the glass comes with significant amount of oil or grease which you cannot see.

So, in that situation the first step, there are some step wise procedure which has to be followed in order to ensure that the glass is in a good shape to be used for cell culture and it follows a certain specific protocols. The first step what one should do upon receiving the glass is you put it in a soap solution overnight step one, take the glass cover slips and put them in then take a beaker you take the glass cover slips raise the glass cover slips like this in a soap solution.

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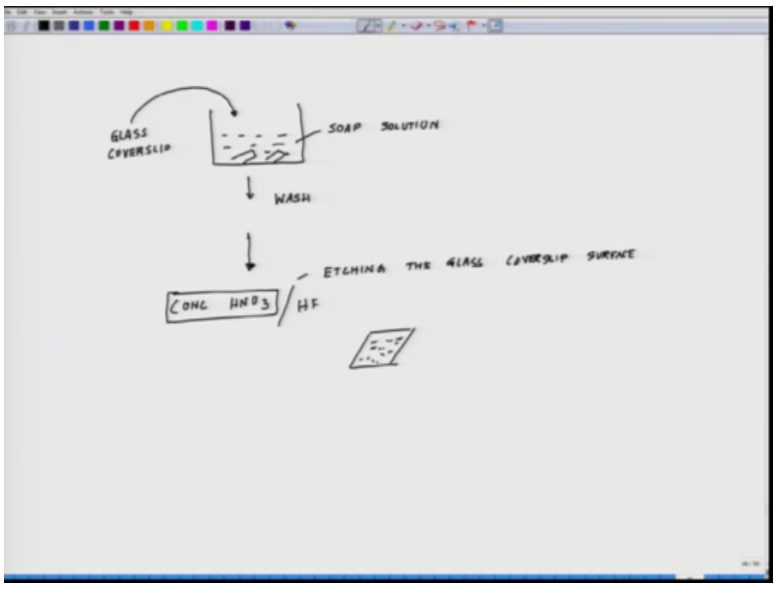

Next thing what you do you wash it completely, next day and then treat the glass these glass exactly in the same beaker on concentrated hno 3, this is where you are really really etching the glass cover slip surface. So, you actually make it slightly more rough than normal. So, what essentially hno 3 does over glass like this it will etch out some of you can even there are people from old school they use hydrogen fluoride which is even much more stronger, but you really can you can actually tweak the time and kind of an you know make it good enough for your purpose.

So, what I will do I will close in here and we will continue about this glass treatment from this point on, ok.

Thank you.