Cell Culture Technologies

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Lecture – 20
Cell Surface Substrate Patterning

Welcome back, we were talking about how you can process the cover slips. This is something very tricky because most of the cover slips suppliers they will claim that you know a very good quality glass and all those kind of things. But over the years what I have realized that what is over the case you need to do some sort of a treatment you can do a plasma cleaning, you can do a acid cleaning. So, plasma cleaning you needed a plasma setup, but even before you do plasma cleaning it is always a good idea whenever you receive the box get the batch number written somewhere.

So, that you have a track in your register and then the first thing you should do you should put them in soap water small amount of soap just I will leave it to your intelligence or to your judgment and a small little soap solution soak them. And if possible if you can swirl it using a magnetic stirrer that may be a better idea that way the soap will kind of you know will be all over the surface of the glass.

Once you do the soap treatment then you wash it, may be a soap treatment of 5- 6 hours or maybe overnight if you feel like that we you know you reduce your you take a bunch of them the whole box so, that you can use it over a period of time. The next thing is it is a good idea to etch the surface a little bit etching could be done by putting it in a piranha solution (Refer Time: 01:57) concentrate HNO 3 and Hydrogen Fluoride HF these are the ones which kind of make the surface little rough.

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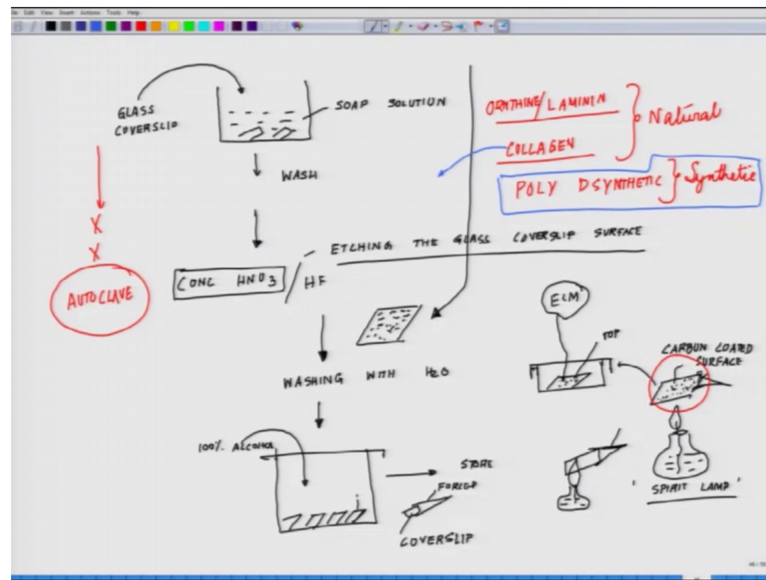

And this roughness of the surface of a cover slip starting from here or here will be very helpful why when will we talk about absorbing the proteins.

Now, once you have etched the surface. So, you just have to drain the water after washing after soap wash and in that put the piranha or the HF solution just be extremely careful because both of these are really splash you will become injured. So, be very careful take proper care, proper gloves, I would necessary mask, and then do these kind of things because in a regular biology lab where you are not doing chemistry on regular basis people are little you know not very careful on these matters. So, just be careful and try to do it very close to the sink. So, that you know even if there is a splash acid splash you can immediately you know put that exposed body part and try not to have exposed body parts.

Once you put the nitric acid or hydrogen fluoride into the system you leave it if you can swirl it is a good idea, but be again be very careful you leave it there for 6 7 8 hours or maybe again overnight or half a day. Then drain the acid or the hydrogen fluoride solution very carefully very very carefully. And be careful about draining it because when you are draining these kind of solutions you should have a setup because you cannot drain such things through the sink. There has to be a jerrycan or something where you can collect it because draining such things in the water pipe can really pollute the surrounding water table if you are doing it in large scale.

So, just be careful follow these basic thumb rules, then again wash it in water in running water and this cleaning is very critical. This cleaning has to be done very thoroughly put on the gloves first of all wash several times and then put on the gloves and then slowly you know kind of you know allow it to really the water to you know roll through all over the place. So, that the last trace of acid is kind of removed. Once you have done this then the best idea is put 100 percent alcohol drain the whole water put 100 percent alcohol in it. So, what all you did. So, you did the etching and after the etching this is the etching of the glass cover surfaces then what you do you etched it after etching.

Now, you are washing with water washing with water a thorough rinsing thoroughly with water followed by now you have the cover slips in a beaker like this, now you pour 100 percent alcohol and in this solution you just put a paraffin thing and you can store it. Generally I used to store them in 4 degrees centigrade somewhere in the corner now suppose today you need 6 covered slips for your work. So, the way I used to do it this is from my experience I am telling you and this is how and most of the old school people use to do it. So, take a bunsen burner or a spirit lamp I am drawing it with any spirit lamp.

Now, pull out say one cover slip on a using a Forceps and here you have the Coverslip and you hold it very gently and this do it very very carefully. Because you may burn your hand and just flame it do not touch the flame really it is kind of you hold it here like this, and just you are doing a mild flaming doing a mild flaming. Just with that mild flaming once you do the flame you observe something very interesting you may find there may be a bit of a deposition of mild slight carbon there is slight very mild black do not get worried that is actually good that slight carbon carbon is actually good.

Because again this is coming from experience this is not the textbooks are going to tell you that mild carbon layer which is formed because of that flaming in the spirit lamp and it is always given good to do it in a spirit lamp it was like kind of very neatly. Once and you are doing everything inside the laminar flow hood so, you have to be again very cautious because there is a air current which is moving be very careful be very very careful.

Once you do the flaming there will be a slight kind of you know blackening and then you can place it in a sterile 15 mm dish. This is now all ready for any kind of coating or

anything and preferred that wherever you have that carbon coated side try to keep that side on the top so that on that side. So, this is carbon coated surface on that surface you are having you expose that surface at the top to have the proteins or whatever extracellular matrix you want to deposit on top of it all the ECM stuff you want to deposit this is how it works.

There are people who will tell you several things and one more thing try to avoid with glass cover slips. This is the do not I am just putting it do not autoclave do not ever autoclave glass cover slips do not because it changes the property because of the high pressure and that moisture it changes the properties of the glass, and at times it changes it is optical properties that visualization becomes a problem and it changes things drastically.

So, please avoid autoclaving the glass cover slips. Once you have these glass cover slips ready, now you are good to go to coat them with different kind of proteins what part of which we have talked about you have Laminin Ornithine actually rather ornithine laminin because you put the ornithine first followed by Laminin, you have Collagen, you have Poly D Lysine which is a Synthetic substrate. So, these 2 are the natural and this is the synthetic substrate.

Now, at this stage I will take a slight detour to talk about suppose your lab does not get a reasonable supply of good quality collagen. There is a very easier way to isolate collagen which has been followed years together by most of the cell culture lab that is called RAT tail methods RAT Tail Collagen.

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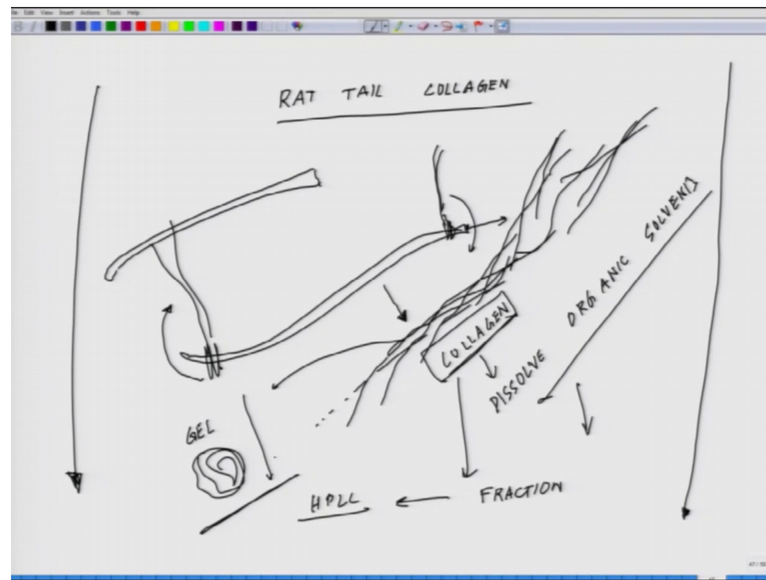

So, the way it is being done is very interesting. There are RATs which are being sacrificed right. What you do you just take that tail of the RAT? So, we do something like this right tail of the RAT.

Now, you take a hemostat kind of things clippers. There are 2 kinds of like clippers you attach the clippers and then what you do is start to twist it in reverse direction. As you will twist it you will realize from the RAT tail you will get this white color threads, which will be coming out and that reasonably thick, but you know and you start collecting those threads something like this white color threads. These threads are high quality collagen, then you have to dissolve it in other Organic Solvents there are different organic solvents and there are different protocols which are given.

And once you dissolve them then you have to run it through a column to get the fraction you are asking for and then of course, you can do a HPLC purification or something depending on how pure you want it, but this is one of the simplest ways where you can obtain collagen if your lab does not have a supply or you are running out of it immediately or want.

So, you just go to your animal facility now ask them do are you sacrificing rats because everybody uses rats or mice or something, but mostly it is good to do with the rat because you get quite a good amount. So, you just take one of them and it something like

this they have a quick. So, you have their and their tail like this. You move one in this direction other one is the reverse direction one if it is one is going clockwise the other one will go to reverse clockwise so, anti clockwise fine.

Once you do like that that is how these threads are going to come out. There are specific protocols which are being followed and if you go online you will find them, but this is how a rat tail collagen is being isolated and that is this could be used you can use this collagen to make Gels you can make 3 d Gels and you can make thin layers depending on at what level you are diluting the solution. So, this is one of the easiest and simplest way how you can obtain collagen.

Now, having said this now let us go back and revisit what all surfaces we have talked about. So, if you look at it.

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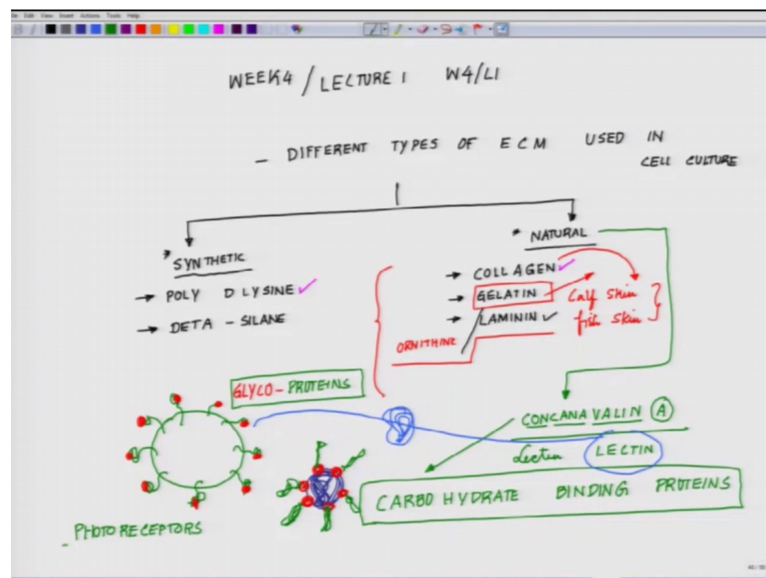

Now we have talked about Laminin let us add the other word in that game, which is Ornithine and Gelatin which I havent talked about which is basically a mix of you know collagen along with partly with laminin and it is kind of a mixture is the same thing, but these are all natural sources. This is gelatin at times could we get from Calf Skin, you can get fish skin gelatin there are several sources from where you can get the gelatin, but it is nothing, but it is a complex collagen matrix.

There is one more natural extracellular matrix which is very rarely used, which is called Concana Valin A. Concana Valin A in a is a lectin what is a lectin. So, lectins are Carbohydrate Binding Proteins; Carbo Hydrate Binding Proteins suppose your cell surface has lot of carbohydrate or glycoproteins remaining theory. There are cells which have those kind of phase if for example, you have a cell like this, which has lot of glycoproteins which are coming out like reporting means protein with a carbohydrate moiety.

Suppose let it join the carbohydrate part of the glycoprotein something like this. So, these are Glycol-Protein. Now these glycoproteins will bind to the lectins which are present a lectin can bind to a carbohydrate because lectin is a protein which could bind to the carbohydrate moiety. What you essentially will have something like this if this is the lectin in a blue color, you will have a glycoprotein part or the carbohydrate part will bind to it something like this.

There are cells like photoreceptor cells especially photoreceptors of fishes and other animals whose structure is very interesting. So, if you remember the structure of the photoreceptors looks like this.

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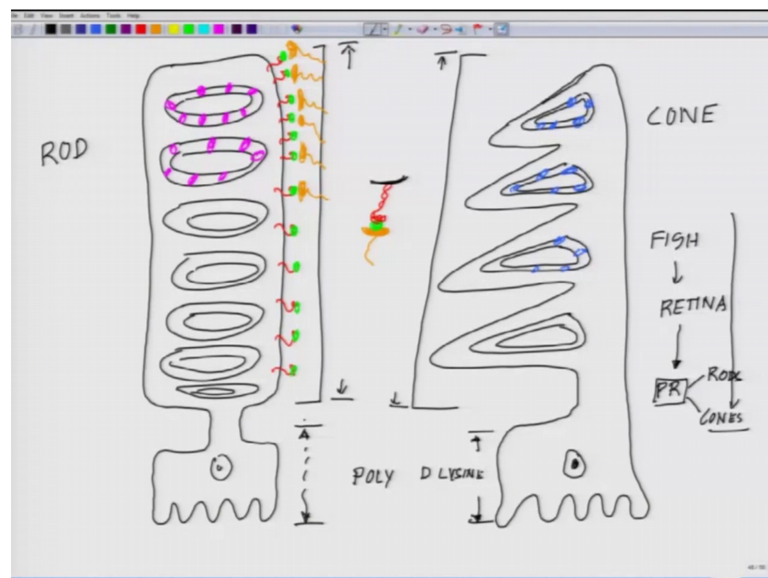

There are 2 kinds of photoreceptors in our body the Rods and the Cones and they look like this. These are the cones they look like cones whereas, here have the rod photoreceptors here these are the rods.

If you look at this structure these are very specialized neuronal structures present in the eyes. So, this is your Cone and this is your Rod. And your rhodopsins molecules are sitting here for the rod which I am showing in pink colors the rhodopsins are sitting here whereas, for the cones depending on which colors they are indicating the collapsing molecules or colour identifiers are sitting here.

Now, if you look at these cells the interesting part of these kind of cells are they have a very extended structure like this, which is the sensory apparatus of these structures very extended I mean think of the length with respect to the rest of the cell body rest of the cell body is very small right. If you just look this is the only part which is this is the attachment part forget about it this is only what is left there. Interestingly we figured out that these cells it is very difficult to attack them in substrate like Poly D Lysine contrary to this these adhere very nicely on concana valin surface why is it so I will distribute this paper for you guys to read apparently this surface out here it has lot of glycoproteins out here it is red I am showing them like oh proteins and on that I am just showing the glycol entity or the carbohydrate entity it is a glycoprotein.

Now say for example, you bring in a concana valin in close proximity to this. Let us indicate the concana valin with orange color I saw yeah orange color here is the concana valin moiety. There will be a very interesting adhesion with this concana valin because there will be any interactions between the concana valin substrate belong with the carbohydrate entity of it and here is the protein part of it and of course, here is the cell membrane.

It is very interesting to note the cell type to cell type you have to really work it out in depth in detail in mine to is details to figure out that which extracellular matrix will do good as compared to the other one. And this only comes via experiments you really do not have any like in a magic wand I do this it is going to work it really never happens like that, like this experiment took us quite a couple of years to figure out that you know is the concana valin maybe a better substrate because now these rod and cone cells what at that time we are isolating this rod and cone cells from the fish retina. Which is easy

and I will kind of explain this one in the next class how you would get one such example of primary culture.

So, isolating the PR PR stands for the photoreceptor cell which is either rods or cones. It took us a while to figure out how you can have a long term ROD and CONE culture and that is where we figure out you can have it if you have a substrate. So, what we did is very interesting we had these cover slips.

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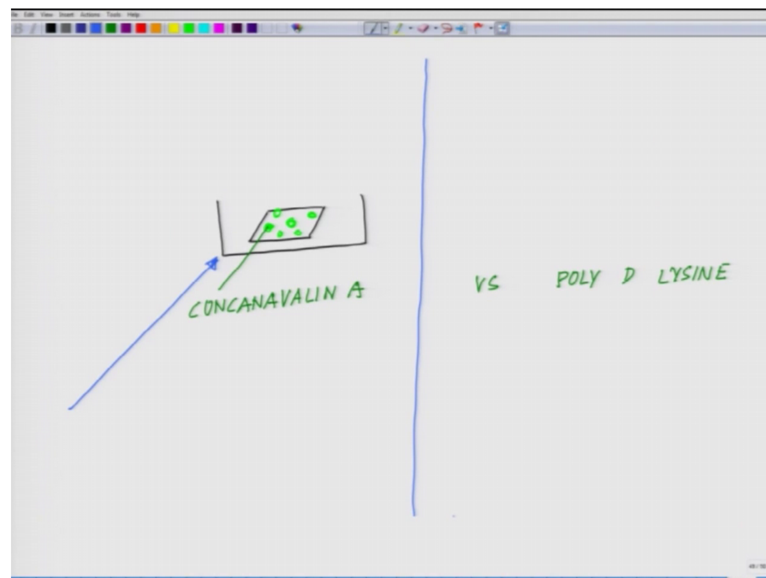

And exactly the way we prepare the cover slips. So, the cover slips were all coated with concana valin and this is concana valin and we made a comparative study concana valin A versus Poly D Lysine. And we observed that most of the rods and cones cells prefer that concana valin substrate, they do not at all adhere on Poly D Lysine absolutely 0 adherence to Poly D Lysine substrate. That was a very stunning revelation for us while doing these kind of experiments on extracellular matrix and I am pretty sure there are many such undiscovered wealth which may open up a totally new dimension about cell culture.

I will close in here with this and next class we will continue little bit more on synthetic substrate and how these could be used for patterning and all other things.

Thank you.