Cell Culture Technologies
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Lecture – 16
Poly D Lysine Deposition

Welcome back to the lecture series in Cell Culture Technology. We are starting the 4th week. This week in the initial part we will be covering about the different kind of extracellular matrix. If you recollect in the last week, while we were discussing about the role of extracellular matrix we talked about the kind of developmental aspects and the way the extracellular matrix anchor the cells on the substrate.

Today what we will do; we will first of all enumerate the different kind of synthetic and natural extracellular matrix which are currently being used in the cell culture technology. And we will talk about the possible interactions what is extracellular matrix materials are conferring on the attaching cell.

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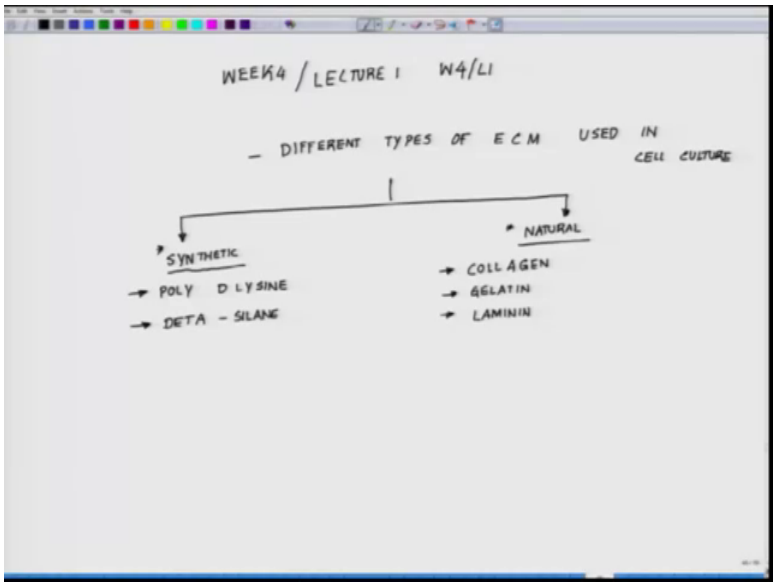

So, to start off with Lecture 1 and we are into week 4 W 4 slash L 1. To start off with we will be talking about different types of extracellular matrix used in cell culture. Let us classify them as we are mentioning into 2 broad categories; Synthetic and Natural. When we talk about Natural we will talk about the one which are known to show this effect either this could be obtained from natural sources directly or using biotechnological

approach they could be produced in mass by you know transferring their jeans into some other secretary proteins salines where you can produce them.

So, talking about the natural ones to start off with we are aware about Collagen matrix protein extracellular matrix protein which is very dominant in ensuring the anchorage between cell to cell and cell to other cell type and collagen has several types. So, we are not getting into the types, but another broad umbrella of collagen. Then we have Laminin the cells are intergreen receptors and this laminins bind to the integral we will come to that. Then in the same family we have a a mix kind of stuff of collagen laminin called Gelatin.

Whereas in the natural or the synthetic side we have Poly D Lysine we have this one I will giving you some literature on this one we will talk about this is basically a Silane dts silane these are teramean groups which are present in them come later into that. Let us start our discussion with this different synthetic and natural extracellular matrix. So, talking about the synthetic matrix it is easy to talk about the synthetic matrix because most of them do not have any specific interaction such. So, the way say Poly D Lysine works it is something like this.

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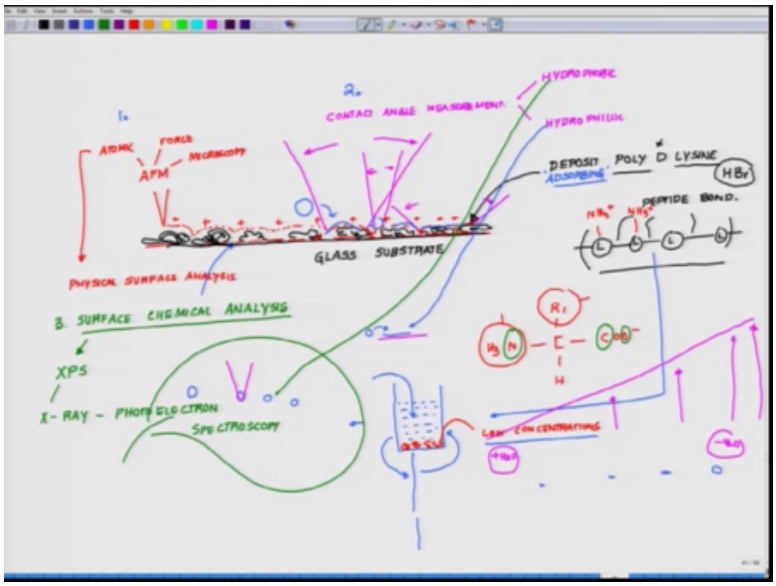

Say for example, here you have a substrate a glass substrate, on a glass substrate you deposit Poly D Lysine; deposit Poly D Lysine. So, talking about Poly D Lysine D is the isomo type and lysine as you know is an amino acid. So, we have a chain of lysine if this

is the lysine residue attached to it (Refer Time: 06:08) bonds out here and if you buy this Poly D Lysine from the market. So, it comes as Poly D Lysine hydrobromide that is to stabilized it will like HBR, but do not worry that thing does not remain in the culture it kind of you know removes, but this is essentially the actual molecule is and while you put it on the surface the molecule becomes something like this, forms a fairly uneven surface because if you take an AFM image I will share an AFM image of this as an additional information.

It something become like this and if you take the AFM tip to do a surface analysis then what you will see something like this, it is a very kind of you know rough surface, upon surface analysis and they are exposed. This has bunch of NH 3 groups positive NH 3 groups which are popping out these NH 3 groups are like this they are popping out like this from the (Refer Time: 07:42) terminal. So, if you remember this is how your amino acids will look like it has coo terminal on one side it is amino groups one other side and it has a R group here and it has a H here right. It has 3 of these amino groups which are present here also few of these amino groups present here also.

And these ones kind of pops out what you see essentially this surface by some kind of a surface interaction on the glass surface they attach, but before you can put them on the substrate. What you have to do is you have to really clean the glass substrate very thoroughly whenever you do this kind because these kind of it is precisely a process of absorption depositing essentially means you are absorbing on the surface you are not doing any chemical coupling of the Poly D Lysine molecule on the substrate.

So, what you do is you take Poly D Lysine in a while you mix deionized water into it and you dissolve it thoroughly and in that process you get a solution. So, the concentration is generally used for Poly D Lysine is fairly low concentration and the exact concentration I will provide through few publish literature it has to be on a lower side. So, always remember one catch about Poly D Lysine is that Poly D Lysine at a higher concentration does not promote cell growth.

Poly D Lysine at a fairly low concentration does promote cell growth, but good enough to make a almost like the surface coverage should be full. So, you have to keep that in mind the surface coverage should be completely full leaving a side of course, there may be little space that you cannot really judge unless otherwise you do atomic force

microscopy on it. Second thing what you can do to analyze the surface. So, this is how we will be proceeding first you should do a AFM atomic force microscopy to analyze the surface.

When you have to really learn and once you exactly learn with that particular composition the surface will look like this then you can repeat it, but time to time you have to cross check. First you should do a Physical surface analysis; second thing what is essential is put a drop of water on this surface and see how the drop of water upon falling on the substrate kind of you know spread out does it remain like this or does it spread out like this or does it spread out like this. So, based on that you measure the scientific term what we call as the contact angle maybe like this it maybe like this or it may be very broad like this. So, that is called contact angle measurements.

These contact angle measurements will give you a fairly good idea about the hydrophobic and hydrophilic nature of the substrate; hydrophobic or hydrophilic how even without getting into the details you can see if. So, all of you have seen this picture you must have all seen suppose there is a leaf and there is a water droplet on it so, water droplet are like this. Whenever we see a water droplet like what is our common sense it says that the water molecule is not really flattening out it means this substrate or this surface of the leaf is hydrophobic right, it does not allow the water molecules the contact point between the water molecule and the leaf is very (Refer Time: 13:24) or minimum.

So, you can really measure the angle just by looking at this picture you can say angle will be something like this very little contact are there narrow contact. On the contrary say for example, there are substrate where you put the water molecule suppose this is a substrate and you put the water molecule the water molecule almost in no time it will become like this from here to here we call them fairly hydrophilic substrate the water can really spread out very fast.

Now, based on this I can suggest you experiments if you try if you increase the concentration of Poly D Lysine on the substrate say for example, here I say about low concentration say for example, you increase this concentration if it keeps on increasing like this. Now there will be a point you will observe you will see that surface becomes water loving to become water hating. So, it means eventually the droplet us of will be

more like this on the substrate, but then that will only happen when you very carefully monitor the surface angle on Poly D Lysine surface by giving concentration.

The reason I am covering this topic in this way because most of the cell culture, which is done currently across the world baring aside few labs very few means you can pretty much count them in the fingers. Most of them do not take these aspects into account and that is where comes a conflicting results between different groups because there maybe glass substrate, where there be certain kind of attachment of these molecules there can be other glass surface, which has not been really a kind of processed properly they may have a different kind of attachment and based on those attachment the contact angle will change.

And since the contact angle changes the cell attachment properties also changes because you have to realize the medium what you are putting out here is a salt solution filled with you know salt and other molecules, but the base is aqueous all the mediums which are been used for cell culture are from are on a water base right. If it is on a water base the problem is this the properties of the water upon putting on the substrate will be similar to the properties which will be experienced by the medium along with the cells.

For the cells to attach you have to have reasonable amount of adhering side and a thin film of water around it. So, that is why I am kind of covering this topic in a slightly different way the things which are not being done, but at the industry level or at the level where you really have to have a perfect quality control there you have to ensure that these things are being followed. What all we talked about we talked about that you have to do an atomic force microscopy as first technique to know the surface, second you have to do a contact angle measurement at the second level of test.

The third thing what you have to do is now when you are putting any compound on the substrate whether it is getting absorbed, or whether it is forming a thin film, or it is forming some kind of chemical coupling. It is always a good idea to do a surface chemical analysis. What I meant by that is you have to know that what all atoms are exposed on the surface of that material or what all elements are on the surface of it. If you look at Poly D Lysine from top you can see you will have carbon you will have nitrogen of course, you have hydrogen these are mostly what will be (Refer Time: 18:36) and of course, you will have oxygen right.

Now, doing a surface analysis and one of the easiest and most thorough surface analysis what you can do is XPS XPS stands for x-RAY-Photo Electron Spectroscopy with few slight changes we will continue from here.

Thank you.