Cell Culture Technologies
Prof. Mainak Das
Department of Biological Science & Bioengineering & Design Programme
Indian Institute of Technology, Kanpur

Lecture - 14
Interaction of cell and glass/poly-carbonate surface – I

Welcome back to the lecture series in annual cell culture or cell culture technologies. So, we have into the third week and we have finished the first 3 lectures of the third week. So, today we are entering to the fourth lecture. So, as of now, if you look at it, we have extensively talked about how to lay the foundation stern of a facility specifically at all possibilities, I may have missed out certain things. So, please feel free you know add up on the existing layout of things, but I believe that I have pretty much covered most of it, what is needed in the modern day cell culture facilities till in future things are going to move towards more of a micro fabrication lab on a chip.

So, that is one section, later half of the course, we are going to kind of you know discuss couple of classes; how things are changing, how they are smaller version of incubators and laminar flow hoods and all those kind of things which are you know popping up in the market there are lot of RNDS going on across the world. So, today after giving you this over all out line of how you can setup a lab, what we will do after this virtual lab will enter to that virtual world where we will be talking about the dishes or the culture vessels and the CDs of culture vessels and what you have to keep in mind how you have to keep in mind; what are the pre requisites and what all measures you have to take for swift functioning of your research. So, those of we have done little bit of a cell culture or have worked in any of the bioengineering or biology or chemical engineering labs or material science lab as where the work little bit of biology side.

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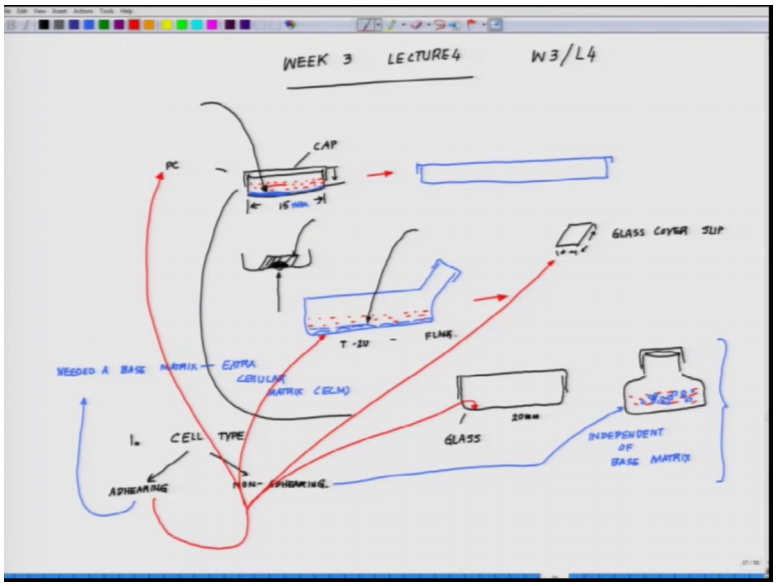

You must have seen some form of vessels they call it T 70; T 20 flux and likewise. So, just to give you an over view what all the different shapes of vessels you must have seen. So, let us start today's class, we are into the week 3 and lecture 4, W 3, L 4, Now some of the dishes, you must have seen something like this 15 mm dishes transparent dishes of polycarbonate very thin polycarbonate you must have seen this you have you have a cap you have the dimension around 15 centimeter and the height will be around you can see for example, I will not be able to tell you the exact something like this something like one digit what will be the height something like this much is the height.

So, you can call it like this much and you have a lit, now what you do is the cells are growing on this base out here the cell growths and you fill it with medium halfway through you cannot fill a lot a medium because you do not want the cells to submerged in such a way that they are deprived of oxygen. So, now, this is one kind of dishes you must have seen then you must have seen 90 mm dishes.

This is 15 mm dishes, then you have 90 mm dishes which are bigger like this with a little like this, then you have something called at T 20 flask, you must have seen this flask which are from the side view of the flask looks like this and the cells grows here and again this is how far, you fill the medium and these flask comes in different sizes there a bigger version of this flask just the same way as this kind of dishes a bigger version smaller version of dishes and flask sometime in a very old text book, if you somewhat

some of you referred, but such things are no more existing or most of the lab does not do it anymore, it was something which was followed in the very early days in National Institute of Health, United States at Maryland.

So, we had this very interesting concept they use to have a instead of this polycarbonate that was very similar stuff of glass you can get them fabricated very thin glass the reason for some of these to be of made of a glass was exactly the same reason what I mentioned in one of the very recently previous classes, what I told you that numerical aperture of the thickness of the material is very critical when you are visualizing with the microscope numerical aperture of the objective. So, earlier when there were not very many objectives which could be used for the polycarbonate, they use to have this glass stuff and then followed by there is something in between which came. So, this was glass and these were all polycarbonate PCM just replace it by PC, in between there was something which is even much more funny.

So, these 2 have this polycarbonate stuff like this and there is to be a small, this to cut a hole on the bottom and top of that these to put a glass cover slip. So, essentially most of the cell growth was happening on top of that glass cover slip and that cut use to help to visualize it using a microscope because that cut was good enough to get of complete fields view or these to cut it likely bigger like you know see for example, this cut and this big to cut. So, these are if you say more or less the kind of vessels which will you come across in any cell culture lab, but what is important here is the part 2 of this the signs where we are going to deal with and one more thing, you will see people can directly grow cells on the base of these dishes on the base of these dishes on the glass covers slip. So, you have these glass cover slips of different dimensions, you can give very thin glass cover slips which are very suitable substrate and you can grow the cells on top of glass cover slips.

And similarly you have the exactly the same thing 15 mm dishes or 20 mm dishes of glass and you have this T 20, T 80 flask. Now first thing, what is important for you to realize what kind of vessel you are going to use. So, in order to address this problem there are 2 different kind of questions, you have to ask question 1 cell type; cell type means are we talking about adhering cells or non adhering cells. So, if we are talking about non adhering cells. So, it means we are talking about these cells, see for example, I have a vessel like this and I have the medium filled with it this is the medium and I have

these cells coming in. So, they are growing on suspension this is called suspension culture if it is a suspension culture, it really does not matter because it does not require any kind of base matrix to grow it is independent of the base matrix these non adhering are independent of base matrix fair enough now at this stage we are not talking about these ones because these are a different cause of cells what we deal with.

IF we talk about the adhering cells which needed a base matrix and this base matrix means extra cellular matrix or ECM. So, if your cell type needs an ECM, then you have to figure a way around this what are the ACM molecules which will be needing because there are 2 options here again, let us before I get into the details either your adhering cells grow directly on polycarbonate or directly on, of course, this is also polycarbonate or directly on glass cover slips or directly on that if these cells of somewhere other. So, the way it works is very simple let me tell you. So, see for example, let us (Refer Time: 12:10) of the color. So, green color we have represent the cell.

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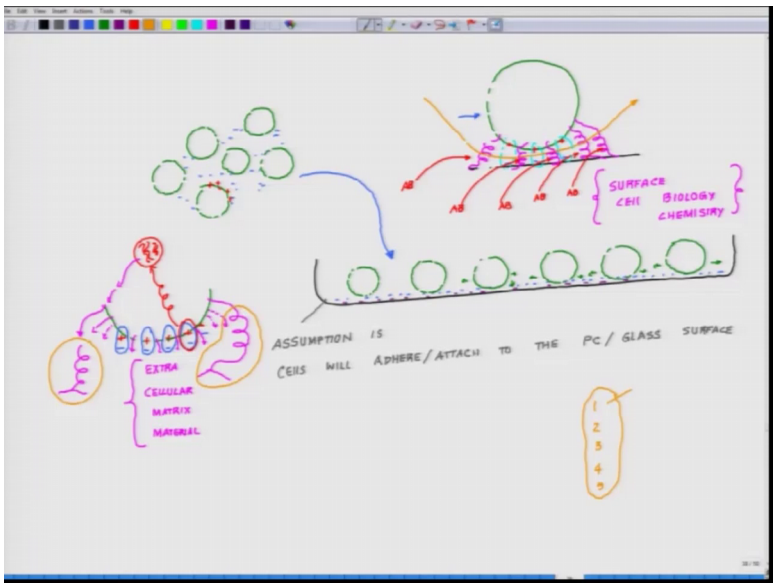

So, these are the cells what you have to play it this now just this if 1000s of cells what you going to play it on a substrate. So, here is your substrate or this is the dish where you are where cell where you are going to play it the cells. So, what will happen? So, what you do with small amount of medium, so very small amount. So, that you know you allow this cells you played this cells on it on this culture dish, on the culture dish at the base you have small that little bit of a medium, right, it will be very little which will kind

of form a layer work of it and this cells will be rolling on it like this and litronic and see another microscope; how wonderfully the cells will be rolling they may many of them may try to see after keep the base parallel to the surface it has to be at 180 degree.

So, that you know on in either side it should not tilled now this is a time T 0 as soon as you have plated the cells look like this and of course, there are lateral mobilities which are happening faster slower depending on how gently or plating them, but there will be certain degree of mobility you know all directions, they will have and eventually you will see its funny to see them one of the microscope eventually they settle down at some point. Now the first thing these cells does now I am introducing a red color and that. So, see for example, assume assumption is so my assumption is cells will adhere or attached to the polycarbonate or slash glass surface that can only happen that can only happen when these cells on the surface.

They have some kind of a charge maybe positive charge negative charge whatever now see for example, on surface they have certain degree of positive charge and the polycarbonate or the glass what you are having is having some form of a negative charge on its surface. So, every material has certain degree of charge on it, surface it is a very static charge, but there will be certain degree of charge on the surface. Now the positive charge of the cell surface the interaction is something like this. So, here you have the cell positively charge on the surface some at this positive you might be wondering from where, it is coming, see of the all these like a proteins server other positively charge irons which are kind of embedded on them. So, just for the timing assume that it has certain area positively charge molecule or you can assume the other way there as a lot of negatively charge.

So, then you have to think the other way. So, at this point, I am assuming there your cells surface is positively charge, see the point is positive negative, it is just we give a some ball, but jus appositively charge which will attract and your surface has say negatively charge. So, there will be electro static interaction between these charge moieties which will ensure the initial how to put it like this the initial week linkage is forming there, they will not be a very strong linkages, there will be you know kind of something like this slightly fluency, but you know they will be all I will not say kind of will adhere very fast, but there will be something which there will be kind of you know at this own; they will be kind of you know rocking like this.

There will be the first set of interactions which will happen now post this interaction something else is going to happen, what is that something else, something else is upon this interactions these cells started to secrete certain protein or certain matrix molecule which I am showing in the pink color, those are called extra, the name is self explanatory; extra cellular matrix material; extra cellular matrix material. These are material which are cumples of different kind of proteins and carbohydrates and CDs of long chain molecules whose rule is something like this, see for example, this is your cell now and here is the substrate and here you have the first set of interaction with the substrate with the positively and negatively charge is where we have the first set of interaction which will held them to kind of you know settle down at a spot for a while, now these extra cellular stuff is something like this something like this.

So, it is kind of anchoring to the surface these are anchoring molecules depending on I am just kind of you know extended for your understanding and very interestingly almost this is one experiment, I did somewhere here on; if you remember, somewhere on 2003 or 2002, I remember in may be 2001 that we did a very funny experiment which is plated the cell like this. So, we plated the cell and by a very brute force method after plating after say 2 hours; after 6 hours, after 10 hours, we remove the cell from the surface, what we observe and after we remove to the cell from the surface.

So, what we are left with essentially if you see this picture; we are then if you could remove the cell from surface without destroying its surrounding environment. So, we have extra cellular matrix which is possibly or probably is deposited on the surface. Now in this situation, if you have antibodies, again this extracellular matrix protein. So, for those who are not aware of; what I am telling about in terms of the antibodies; antibodies are just imagine, I have a specific molecule, there is a binder which is very specific to that molecule. So, those are called as antibiotic for a very lame and understanding for you, if I have an antibody against these is putting at ABS, then what we observed is almost within this is how low we have gone, I think we will manage to went all the way up to 1 hour.

So, soon after plating you see their traces of extracellular matrix deposit it which otherwise a surface whenever had anything except polycarbonate so; that means, this first set of interaction which is happening out here between the positive and negative triggers signal to the nucleus of the cell where the DNA recipes and tells the DNA; dude

I need these extracellular proteins; extra cellular matrix protein to be synthesized and anchor myself because I want to settle down here because we are dating with this surface now.

So, is something like this; so, think of it one small electrostatic interactions could really trigger the DNA to plates role and that starts a very unique way of looking at the whole problem from the perspective of surface biochemistry or surfaced cellular biology surface cell chemistry or cell biology which over way you prefer to use the word and this is something which is very critical might wonder; where this is all going to go this is something critical to figure out why certain cells in our body and before I tell, this it may had one more interesting aspect to this.

Now, this nature of this kind of molecules which are formed; extracellular matrix is unique for cell type to cell type in terms of quantity at quality; that means, see for example, there a set of say; I say that just for your understanding sake, I say there 5 different kind of ECM complexes which have from now for each cell type. It is not essential that they all will be using all the 5 types, some may use 2, some may 1, 3, some may use 5, but now if suppose you have a situation where 2 cells uses all the 5, then 2, you can distinguish because they may use differentially they may they may secrete one larger as compared to 2, 3, 4, 5, they may secrete 2 and 3 larger as compared to 1, 4, 5.

So, they can have in number of combinations, but that brings us to a very interesting point extra cellular matrix are cell specific, depending on the cell type they secrete that unique form of extracellular matrix now you have to ponder, but you think over it before I continue this story in the next class. What does that mean and what is the implication of understanding? This interaction, I will close in here.

Thank you; thanks for your patient listening, will continue this next class.