Cell Culture Technologies
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Lecture - 11
State of the art facility in cell culture Lab – I

Welcome back to the lecture series on Cell Culture Technology. So, this is our third week what we will be initiating. And if you recollect up to a second week or during the second week we have started the layout design. So, you become a pi or you get a job in the industry and you have to set up a cell culture facility. So, how you will proceed? What are those critical steps which will not be really written in a book, but you kind of have to keep in mind how to move. So, if you recollect back. So, one aspect what we have taken into account is the lab is divided into 2 parts. The part one where some of the kind of dissections which may cause infections or which may cause a lot of microbial contamination should be done in one part whereas, the other part is dedicated for doing the real culture work.

So, in that context if you recollect we divided the lab into 2 parts right. So, another interesting thing which many new modern labs do follow is or rather should be very clearly followed that they maintain a specific set of footwears. So, say for example, if you remember I told you that you have a shoe rack and all that stuff. So, you could have very dedicated foot wears which could be used for the cell culture facility itself. And apart from it if you remember where we talked about entering into inside the lab.

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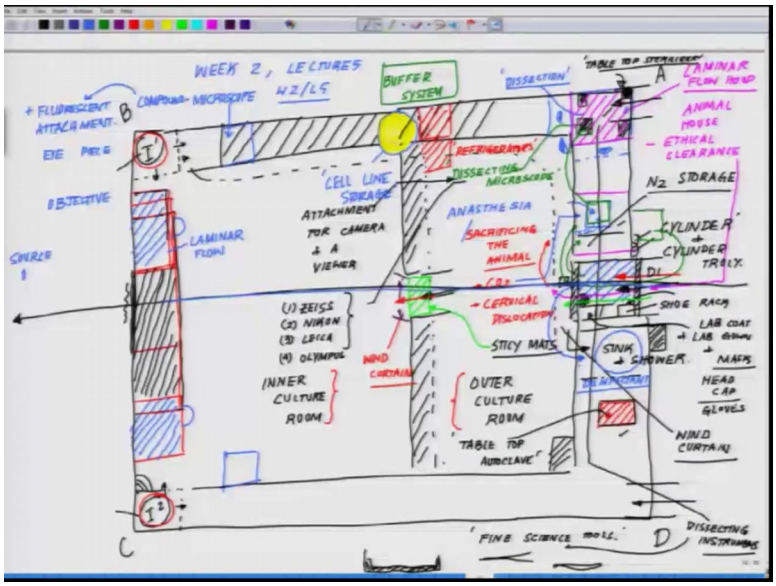

If you think of this zone where you were entering. There are something called sticky mats, the sticky mats. This is sticky mats are basically if you put your feet it is kind of you know. So, they are there to you know to trap the dust, it is kind of a dust reducer into the system. And most of these rooms are being arraigned in a way that the air is being continuously pumped out of these rooms. So, to ensure that the air inside both these rooms are being continuously pumped out and circulated; that means, what you are essentially doing is you are continuously recycling the air and that made demand that the roof what you make you may need to make the roof of false roof. So, on top of the false roof you may have to put different kind of setup you know from the air.

So, these are some of the points what you always have to keep in mind even you have to be concerned from the word where you have to talk to your colleagues in engineering who may help you, or in your respective works department of the institute that how I can have a false roof like say for example, I am standing in this room where I know there is a false roof. So, you all have heard about a false roof it is something like. So, this is the height of the room and you just create another false roof using some kind of a polymer and in between there is a gap, a significant gap there. There you put all the pipe holes and everything you know (Refer Time: 04:27) the air out and you know circulate they are in peculiar way. So, these are some of the interesting details which you always have to keep in mind. So, if we just look through the picture. So, you will see there is an entry port here. Then we talked about the sink and the shower. Then we talked about a tabletop

autoclave here you see the tabletop autoclave. Then you have a sacrificial zone where you sacrifice the animal by using ethel carbon dioxide cervical dislocation whatever animal ethics people recommended you. Then you have a dissection assembly where you do all sorts of dissection and everything. Then from here we will leave the rest of the space, now you are moving into the second room which is the room where you will be performing most of the culture work. And in between I told you that you have a nitrogen storage which has to be replenished time to time.

And apart from it what you can do is out here I am using a light green color from entering from this one to this part, because you have partitioned the room you can put the sticky mats. So, that way that will prevent a further level of checkpoint or we could have kind of a sliding door out here which will ensure or restrict entry and the exit along this point. And there is one more think which could come very handy if you can please refrigerator somewhere out here in the corner. Because you will be needing multiple refrigerators. Because you have to store mediums will factors several things.

So, you may think of having a refrigerator in one of the corners. And try to use only mostly the corners, because that will be a let us say will lot of your space. And whenever you design that you want to place an refrigerator you can have this part the countertop may be cut at that point and you can place the refrigerator in such a way that you are not conceiving that space. So, we will have one refrigerator out here depending on what is a strength off the lag now from here you move to the mainframe out here. You could have if you do not want to put a sliding door you could have a wind curtain through which you entered here. Now here you have to be really careful because this is the zone where most of your work will be happening.

So, I have already told you that this is where you will be placing the incubators formed in the corners. So, you are not in direct hit of the pathway. And you will be needing laminar flow hood you can have maybe one or 2 laminar flow hood like this either. So, if this is your countertop along the side of the countertop out here, but you have to ensure that you are not obstructing the opening of the incubator door depending on which side of the incubator door, it in which side you want to open the incubator door. So, you have laminar flow hood sitting here. So, again if you noticed one interesting aspect. So, if I have to like put one single line like this is your entry and cross.

So, of course, this is a wall here assuming there is a wall, or a glass or something. So, there is a blockage here it is it is not really like in a movie, but I am not putting any of the major piece of instrument which will come on the way of the air current. Try to avoid this as much as you can. Do not prefer to keep anything which is coming on the way. It is kind of very widely advised that minimize that somewhere another minimize it as much as you can. So, you have this laminar flow hoods here you have the counter top of course, which I am shading in black now we are the countertop for you for all other works whatever.

Now, you have needed to have we will what server purpose you have in the lab, but you needed to have a compound microscope decent compound microscope. Now this is something we will be discussing little bit more. So, let us get the purpose. So, you have either say line what you are maintaining. So, you have to have I told you about the nitrogens storage and everything. So, you have to have a storage area of our say for example, a storage area out here for storing the cell line this you will be shading what I am doing. So, you will have cell line storage. And this is a tank which has to be replenished time to time with the with nitrogen as your every time you are opening it.

Now, whether you what doing a cell line culture or you are doing a primary culture. One thing is very critical you have to observe the cells and as your passaging the cell or your plating that cell you need to observe the cell time and again. So, you have to have a microscope and I will recommend you going by the same logic where you want to put it prefer something which is not coming in the direct way, because you are viewing the cell. So, it should not come on this way, your options are on this counter top somewhere here in this side or somewhere on this wall somewhere it is this wall or that wall which one. So, is your requirements like cannot tell that, but I can recommend you what all you have to wide.

You have to place microscope. And this is a microscope which will be or compound microscope. Now whether you wanted to have a fluorescent attachment with it not. So, (Refer Time: 12:17) a microscope plus because these are all addend up, that I will leave it to your requirements and to your wisdom. Because that decision only you can take whether you want a fluorescent attachment or not. But what I will recommend here is something else where you are plating the cells that is important. Why that is important

because if you are working on substrate which are opaque. Say for example, I have a opaque substrate like this, and I played the cells and top of it.

So, it is a tissue engineering it is a some kind of hydro gel or a cryogel or some kind of metal or some kind of a biomaterial something I am testing the bio compatibility and the cells around top of it right. Now in that case you will have to have a microscope which is upright. Or now when we talk about and upright microscope the thing is that those who have used microscope and those who have in used the microscope you have the basic knowledge that in your courier must have right it that it has a objective. So, you have an eyepiece. So, let us put it like this. So, we will have an eyepiece we will have a source of light eyepiece give an objective.

Now, and you have a source of light. These are the things and you have couple of assembly of lens. So, do not worry about that now through the eyepiece of course, you are viewing the object. Now you are options are two. So, options are this. Say for example, this is why your sample is light source may come from top light source may come from side light source may come from bottom. Now if your this is stuff on which the cells are. So, just to visualize it let me just put it A slightly different way.

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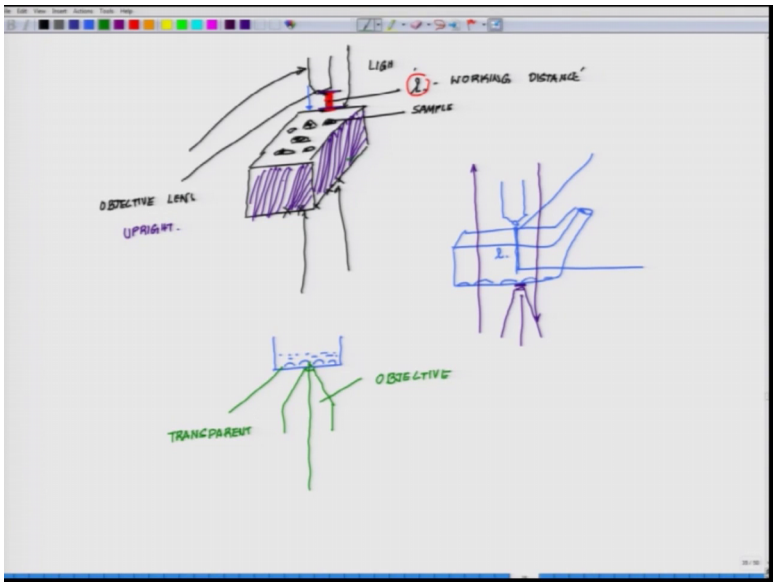

Now say for example, you have this substrate through which I am just purposefully making if that thick the light does not pass.

So, your only we can view it is from the top. And you have to shining the light from the top. So, this is how your shining the light and the cells are growing on top of it. And we your ring the cells and these cells are reach. So, 4 dag you will be needing an upright microscope means, you have this objective which is coming from the top. So, we have again from the top your objective is coming from the top. But the problem with such upright microscopes are the working distance means, this distance between the sample. So, look at this distance between the sample and the micro and the objective tip. So, this is the objective lens. And this is where you have the sample and this distance this distance let us say I just represent by l.

This working distance is critical why this is critical. Because most of the microscope if you see will have a very short working distance what does that mean? That means, that l what you see what I have put there l up to here I am just circling it with this distance what you see, this distance this l. This l this micro microscope in this case if it is a very small l and this microscope objective has to come very close to the sample. Now think of it suppose you have a t 20 flask you have these flasks on which cells are being cultured right. Which are itself are kind of you know 3 dimensional thing. Now you are cells are sitting out here. Realize and you have an upright microscope those objectives are and this is the amount this is the kind of working distance I am talking about this is the l.

So, automatically you want be able to see much visualization will be compromised, but since it is a t 20 flasks I will come to the earth side very soon that you do not need the upright in that case. But since your substrate is opaque it is not allowing any light to come from the bottom your only option is to use a upright objective in that situation. And for such in upright objective you have to ensure it is a long distance objective what does that mean; that means, this distance should be higher. So, if this is your sample you should have the objective this for and yet you will you will be able to see it. That is something which no seller of microscope will tell you they will come and they will tell you n number of info which is to cell there objective. They will not take into account at what are the problems you are going to face.

Either you have to be up front very clear that this is the kind of sample I have to visualize and you convert them. Or you explore it by yourself. Because this is a protein and problem I have seen over the years when we have set up labs, one negligence or not side coasted us very dearly that shit why we did like that it should have dyed other way.

So, if you are confident that your samples will not be like this. Then you have on a slightly easier site then you can go for. So, if you know that your plating surface is transparent light can move back and forth. Then you are go to go, then you can use objective which is coming from the low from underneath it is no more apprised objective here. So, objective is from the bottom. So, even objective can touch the base of it, because touching the base of the sample which is not in contact we want help very simple say for example, this is your dish. And inside your cells are growing right and your use your media. So obviously, the microscope can always touch the base of this vessel, it want microscope objective can touch the base of those vessel because it is plastic right and it is transparent, and you can shine the light through this path and you can visualize it.

So, in that case you have a inverted assembly your objective is underneath you have an inverted assembly, inverted microscope in that case where the objective is below you do not have to worry about the working distance you can really come close to the sample provided this is transparent. Here this was opaque or translucent. So, your options are very clear either and another interesting part I used to heam of, but as of now it is kind of not. So, easy to do you cannot convert a prior to an inverted in microscope. So, if you know that you have 2 kind of samples it people working in the lab one which will be say for example, your work some kind of biomaterial coating which is opaque.

Then you have to have set up with long working distance you will have an upright. Microscope. And you have the other people who will be using on a transparent either you can use the upright one, but the problem there are issues with upright one 2, then in that case you have to another microscope which is inverted. And even with inverted this is another thing which most of these giant coat and code manufacturers own tell you have to ask then this and I myself got like kind of you know, once I do. But definitely was in not being told the right side of information that if to ask then that.

Is this objective for plastic or glass? There is something on the lens objective lens if you open the objective lens you will see there is a number which will be given like n is equal to something. I am now getting the microscope detail what does that mean, but that is the one which will tell you that suppose your samples are in a glass dish. So, glass has a different kind of thickness and different kind of optical properties where is if it is on a

plastic which is basically polycarbonate like you know, this kind of t a t or whatever like you know vessels they are different material with the different optical properties.

So, they have a different kind of thickness. For that you have a specifically different objectives. So, many a times you by a objective and then you realize that objective is not really the objective you are asking for because you are unable to visualize a wonderful microscope, but you are unable to visualize things. So, these are some of the points which will never be part of your standard textbook. But this is what comes from experience. So, I will recommend in terms of the microscope you be very careful because this is a big investment. This is not a easy investment any good quality microscope costs of fortune.

So, I will close in here in the next class we will continue it further and we will explain some of the other integrity details what will be needing in this journey of learning about cell culture and making it a state of art facility.

Thank you.