Cell Culture Technologies
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Lecture – 23
Rules for Mechanical Dissociation of Tissue

Welcome back to the lecture series in Cell Culture Technology. So, in the last lecture we talked about the mechanical dissociation of the hippocampal tissue and this whole mechanical dissociation process is uniform. I am just taking up one simple example and one has to be slightly careful while you are doing mechanical dissociation, while you are kind of you know offering a shear force to the piece of tissue to dissociate it into single cell suspension you ensure that you are not picking up air bubbles those air bubbles damage the tissue big time.

So, you will always have to do it very gently. So, there is some thumb rules out here in mechanical dissociation.

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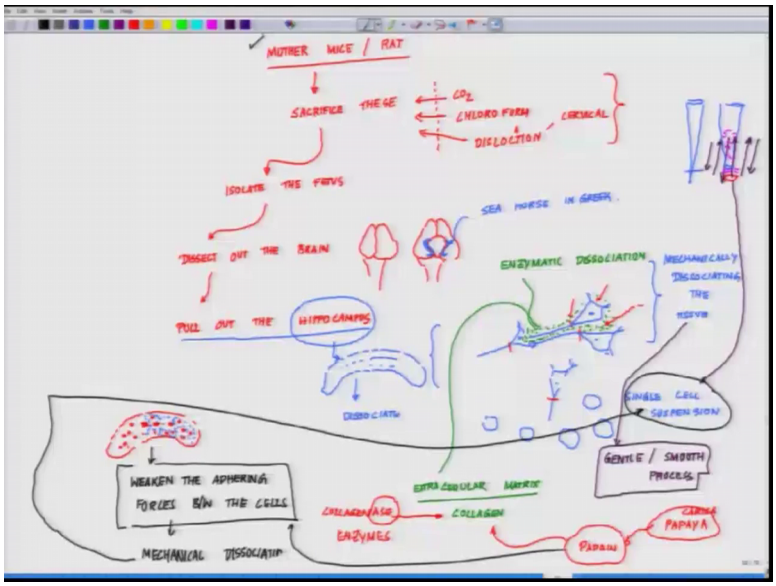

You have to be very gentle and fairly smooth process it has to be done with utmost care, the other method which especially for this particular thing I will not recommend because and I will give you a few references sites, few references you will see what you can do is say for example, in this case you have this is extracellular matrix. So, you follow something called enzymatic dissociation we will come later where you really needed

enzymatic dissociation, enzymatic dissociation essentially means say for example, these extracellular ok.

Extracellular matrix has say collagen; we have already talked about collagen you remember. So, what I will be using to break this collagen, what I will do I will use an enzyme which will break the collagen I will use collagenase a sc whenever means it is an enzyme. So, I use collagen is enzyme at a limited concentration you have to be very careful what is the concentration you are using or I can use another protease which will break these bonds of collagen, I can use papain, what is papain? This is one enzyme which is derived from papaya we all eat this as a vegetable as well as a fruit, but this papaya which is carica papaya and you must have seen many people say, if you are having a stomach upset or something or like you know you should take some papaya. What it does it essentially the whip papain works is that say for example, and this is this will very important in the phase 2 of it you will realize why I am kind of. So, say for example, this is the tissue and these are the cellular elements in the red dots right.

Now, what papain does is, let us represent papain molecule as a grain it kind of infiltrate into these zones and the job it is does is weaken the adhering forces between the cells, this is what it does and in that process followed by this treatment. If you do offer a mechanical shearing or mechanical dissociation what you obtain is again the same thing what we talked about here a single cell suspension, but the single cell suspension in this case goes through 2 processes you first did a papain processing followed by a mechanical dissociation. But when we talk about the fetal tissue to isolate hippocampus from mother mice or rats like for the fetus you will really do not need to go through this whole rigor or go through this whole you really can do it mechanical dissociation.

So, now, what you are having once you are mechanical it dissociated it.

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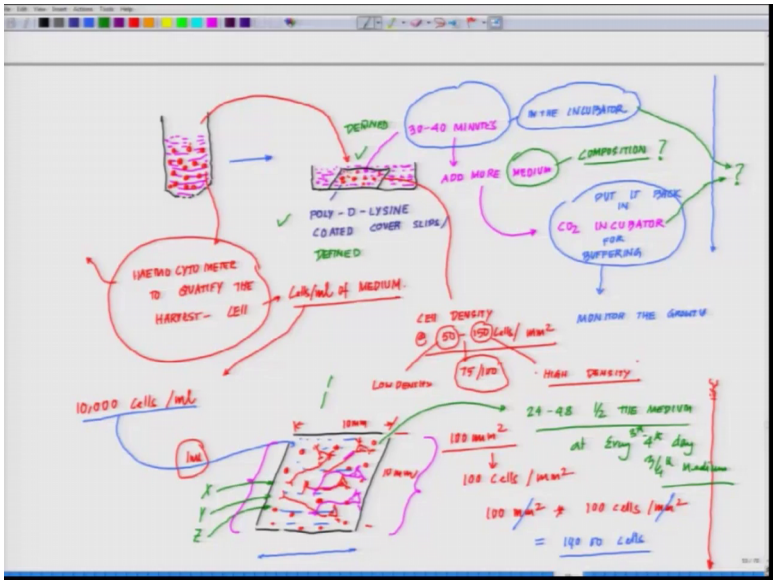

Now, you have this in a tube. So, say for example, this is the tube where you have collected. So, you have a single cell suspension in a medium. So, again as of now we have not talked about medium just assume that we you have a particular composition of medium where these cells will grow or a buffered solution supplemented by different growth factors. So, this is your medium and here in red dots I am showing the single cell suspensions here the air sitting, now what you do you take these and beforehand having said this beforehand you have to prepared your cover slips or substrate. So, in the last class we talked to you about how to prepare substrate you have to prepare the cover slaves, have to coat them with substrate of your choice.

So, in this case for hippocampal neuron one of the favorite choice is poly d lysine I will come to other substrates where it is even much more preferred than poly d lysine, but at this state just keep that in mind. So, you have, here is your cover slip and here you put medium in which they will grow, before putting medium I have said this before putting medium you take a small part of this cell suspension and and of course, I missed out on one point is these are poly d lysine coated cover slips or glass. So, now, on this what you do you take the suspension, a small amount of suspension first and you would try to play played the cells like this you just put them there, they will find out with a little bit of a medium in that, but try to played them and without adding any further medium for next 30 to 40 minutes and then after 30 to 40 minutes add more medium this is where you are submerging it, but do not go very high I told you just good enough because say so that they can derive the oxygen from the environment easily ok.

Add more medium and of course, again put it back in the co 2 incubator will come back to the co 2 incubator and the buffering system. So, this is is the zone you remember I told you there has to be everything defined. So, this is your how we do the buffering I will I will come to this very soon and here is your put it back in co 2 incubator for buffering and here also where you left it in the incubator ok and now you monitor the growth.

So, what I have not told you as of now is this other part composition what is that medium this I have not told you, co 2 incubator fundamental concept in terms of the buffering I have not told you, but I have told you about a substrate and how you are isolating the cells. So, I have defined 2 things, if you look at carefully I have defined 2 things this is all defined and this part is defined. Now once you plated the cells and you monitored them under the microscope. So, this is what you are going to see, these single cell suspension they will these cells will regenerate and they will form a random network depending on how close or how had a distance you are plating them.

So, typically there is certain cell density of plating what you have to do. So, this is another thing which I have not mentioned here cell density, mostly for these such small neurons are typically at the rate of I would say you know anything between 50 to 150 cells per millimeter square typically, depending on because there are 2 things you have to realize here about the cell density and this has to be defined very clearly either you follow 50 or you follow 150 or you follow anything in between say 75 or 100, I personally has always typically worked with 100 cells per millimeter square unless otherwise there is a demand for a very low density culture.

So, when we go for 50 we talk about a very low density and it took 150 these are high density cultures again there are people who may disagree they can go a little higher also, but I personally after working with hippocampal neuron for more than a decade I will not recommend that. So, if you go very low density the problem is for the cells to so cells they grow in colonies and when we grow in a colony we have interaction with our neighbors and you know in the information transfer and all those kind of stuff which happens which is strengthened say for example, your neighbor tells you, you know there is a there is this problem happening. So, be careful or what will not come on this day. So, you know so it is kind of mutually helping each other in a colony, cells are exactly like that. There are colonies there are colonies where they continuously crosstalk with each

other and if you sparsely seed them so one is here another is another one kilometer away another.

So, that interaction is going to be missing and some of these interactions are survival instant concept or survival therapies for them, they survival because they crosstalk they interact. So, that could be one of the problem one could face, when we talk about a very low density culture and we will come there are situations where you have to do a very low density culture well talk about patterning and all that kind of stuff will be coming soon to this, but if you do a very high density culture you can. So, the problem is that then there will be a resource crunch, it is just like you put too many people in a confined location and now you have resources that is not an issue, but how the resource will reach to the cells which are kind of you know covered by other cells and it creates an anaerobic situation and there are series of problems, which because it the way the metabolism will function it will be functioning at that tremendous pace and you do not always get into that kind of situation right.

So, you will always have to optimize what rate you are going to follow, but then having seen this, whatever density you follow once you have to keep on following that it cannot change it. So, how this is being done is once you isolate the cell, then you do a hemo use a hemocytometer, hemo cytometer to quantify the harvest in this case cell. So, once you have quantified the harvest. So, you know how many cells per ml you are having here per ml of medium hum per ml of medium, Now, if you know this many cells are there per ml of medium and you want certain specific number of cells to be plated in a certain area what you have to do say for example, I say I want the let us take a practical situation

So, I say. So, there are say 10000 cells per ml, now I want to play. So, I say I want to play it in a cover slip whatever size I am not defining that at this stage, I say I want to plate say for example, let us take a number now. So, I say I have a cover slip of say 10 millimeter by 10 millimeter. So, that makes it 100 millimeter square accept it. Now I said if I have a 100 millimeter square I want a density of say 100 cells per millimeter square ,if I 100 cells per millimeter square then I will be needing 100 millimeter square multiplied by 100 cells per millimeter square.

So, your millimeter square millimeter square, cancel. So, what you are having cells. So, what you are having is 10000 cells. So, you have one ml now you have to spread this one

ml on these cover on what single cover slip in such a way that it uniformly spreads in the beginning. So, the cells now will spread all over the place in a uniform way. So, you are taking one ml and of course, you may have a stock off like you know 10 ml. So, you can place this 10 cover slips in that situation. So, depending on what will be your density you have to do the back calculation here very carefully and you have to be very meticulously careful you cannot afford to have in 1 culture, I did a 100 cells the other culture I did at 200 cells and a third culture I was somewhere in between every time the data what will be deriving from it will go hey where.

So, please, be very careful these are things which are not being told, but as graduate students as I independent p i you have to be cautious you have to be very careful if you are a peer then you have to ensure that your lab staff follows this or if you are a graduate student that is, I mean much more important because this is your experiment which everybody wants to repeat at some place in the world and they should not come across come on I mean time this is the effect I see the next time I see another effect these small errors can cause very dearly ok.

So, now, once I have told you about how you. So, this hem cytometer calculation this you can really pull out any manual where they do the blood analysis will figure out how much you have to put and how much cells you are seeing. So, this is fair enough, so he plated the cells and once you plated the cells. So, this is called the cell plating.

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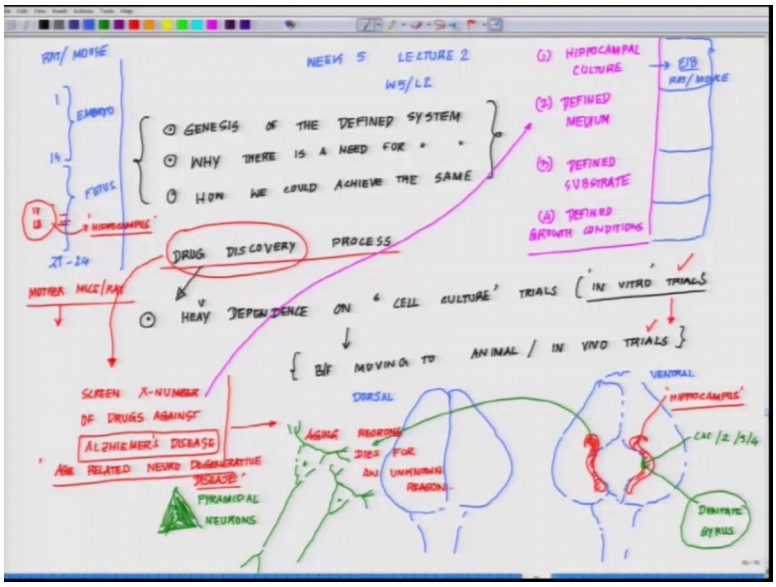

So, if you remember I told you that go back to the master chart what all you have to be careful. So, it is here self plating rules remember told you that this you have to be very very careful, how much cells you are plating. So, when we talk about define condition these are those small small parameters which will come very handy we talked about the isolation rules then we talked about let me add one more in your master slide cell count and followed by now cell plating.

So, making sense how small small details are going to influence the results you are going to interpret now coming back here I was. So, you have done the cell plating now what will you observe with it if it is a hippocampal culture within 24 hours you will start seeing these cells will be sending out processes and within 2 3 days you will see these cells are going very neatly, but they will have a very random connectivity all over the place. Who is forming synapse with which self cannot be you know dictated, now I told you this industry wants to do an Alzheimer's although I testing.

So, it puts the drug which is say represented by x drug or y drug or z drug into this medium and one more thing whenever you are going this part in primary culture the first medium change what they are recommended is what happens after. Generally, it is recommended after 24 to 48 hours it should change half the medium, just have the medium, you warm the medium in a bath and just replace half the medium and followed by that at every fourth depending on what is the density of culture third or fourth day change three forth medium, some people completely change the medium which I not fully recommend for a reason because the cells secrete lot of factors which are helpful for their survival.

So, if you remove everything out of it. So, that cells are devoid they have to again redo the whole exercise. So, I would say three forth of a media may change every fourth day likewise. So, you have these drugs now at your disposal.

So, what I will do, I will close in here in the next lecture we will talk about how these drugs are going to act and how the company is going to interpret and what all next gen problems which will come what they have to define, ok.

Thank you.