Cell Culture Technologies
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Lecture - 35
Cardiac Muscle Cell Culture

Welcome back to the lecture series in Cell Culture. So, in the last class I talked to you about last couple of classes I talked to you about how to grow the skeletal muscle. In the same line today we will talk about how to grow the cardiac muscle. Again talking about cardiac muscle, what is the first criteria of a cardiac muscle will be? Definitely whether the cells contract or not. And do they do the contraction and mind it cardiac cells are spontaneously contracting muscle. So, there are 3 muscle types. So, that is the reason why I would not touch this.

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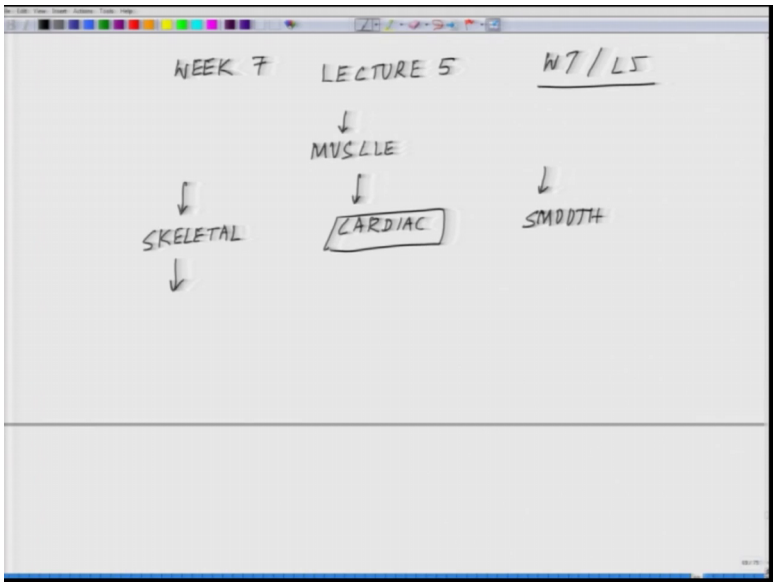

So, let me just pull it down. So, this is a our lecture 5 week 7 w 7 L 5. Now cardiac cells. So, let us talk about the classification of the muscle. Muscle could be classified into 3 groups. Skeletal that we have already talked in depth, cardiac and smooth. So, today we will talk a little bit about the cardiac cells. Because there are several people who are interested in growing cardiac tissue and yet they are not really aware that you know how really to where to start and which is the genuine reason.

So, there are several cell lines, there are actually not several there are there is one cell line similarly there are cell lines in the skeletal muscle to c 2 c 12 this is one of the skeletal muscle cell lines similarly there is a cardiac cell line. But I would recommend that if possible go for a primary culture take the tissue directly from the animal. And one of the best source over the years what I have realized if you have access to a pregnant rats I would see E 14 E 13 to E 14 hearts it just it is it is a very. So, let us start from the very basics.

So, I have already explained you about E 13 E 14 and all that and up to by the time you are reaching E 18 it is called fetal F 18 when we isolate the hippocampus. So, over the years what I realized again with the cardiac tissue also, the more it mature tissue want to isolate the more will be the fibroblast and other cell contaminating your culture. And you will have a very limited control on it because you will have to really manipulate that some of them will grow faster than the others and it is kind of tricky.

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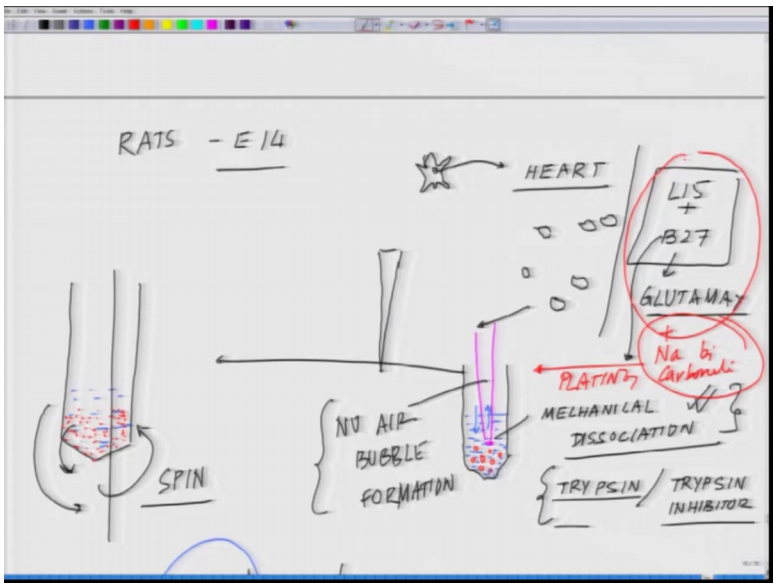

So, what I realize one of the easiest way and this is I am talking in terms of the rats. So, in terms of the rat you take a E 14 small pups. And they look really really cute another microscope if you look at them. What you do you just take out the heart tissue the complete heart. So, in this you would not be able to distinguish. So, easily unless you have a very high end dissecting microscope to isolate whether it is a ventricular or atrium that distinction would not happen, but instead what you can have is that you take out this

small partition and once you will isolate you will observe. So, you can you will still see the blood vessels.

So, the best way is to isolate them in a medium called L 15 L 15 you could supplement L 15 with a something called B 27 and glutamax I will give you the papers we you can look at it which is basically a dipeptide of glutamine which is far more stable compound here. So, you can isolate them in L 15 medium and this works an B 27 is a neuronal factor, but we showed at one point that this supports cardiac growth crazily. Though it was made for neuron, but it does some very interesting stuff.

So, you have these isolated heart from as many you can get. So, then what you do you just kind of you know using it is it is very teeny tiny using your pipette tip you just kind of squeeze it a little bit. So, that the blood which is already there the those bloods kind of you know falls up out from it. So, you have a much more cleaner tissue. Then you have to dissociate. So, you have the complete heart with you a small heart. Easiest way to dissociate will be mechanically. This is I believe the easiest way. Because these hearts are very fragile very, very easy to work with you just in a while you collect this heart like this.

So, say for example, if you could manage to pull like you know 10 from 10 feet or a 10 embryos here is your medium. Now you take your pipette tip out here. And you just pull it back and forth. So, you are essentially what you are doing you are doing a following a mechanical dissociation here. And you will observe that this is good enough good enough to you know dissociate the cells there are people who use a at this stage trypsin and trypsin inhibitor. But if you are a novice and if you are not very keen to use a any kind of enzymatic treatment and anti enzymatic treatment to stop that reaction, I will not recommend you this. I would rather recommend you go for this, one the mechanical dissociation this mechanical dissociation does work and it is a very proven technique for this kind of fragile heart that works fantastic.

So, you slowly and you have to ensure that there is no air bubble formation. There is no air bubble formation taking place here, because such air bubble formation damages the tissue. So, from here what you are going to get is something like this. So, here you have the medium and you have some chunk some single suspension something like this. Depending on how good you are at this is heating the tissue. Now what you do? You

have partly single cell partly small some adhere tissues do not worry about it is perfectly fine. From here what you do? You take this for a spin it down.

So, when once you spin it down what you are going to obtain is something like this. You have your medium and this is spinning is fairly robust you can go for 500 rpm for 5 minutes or 10 minutes there is more than enough.

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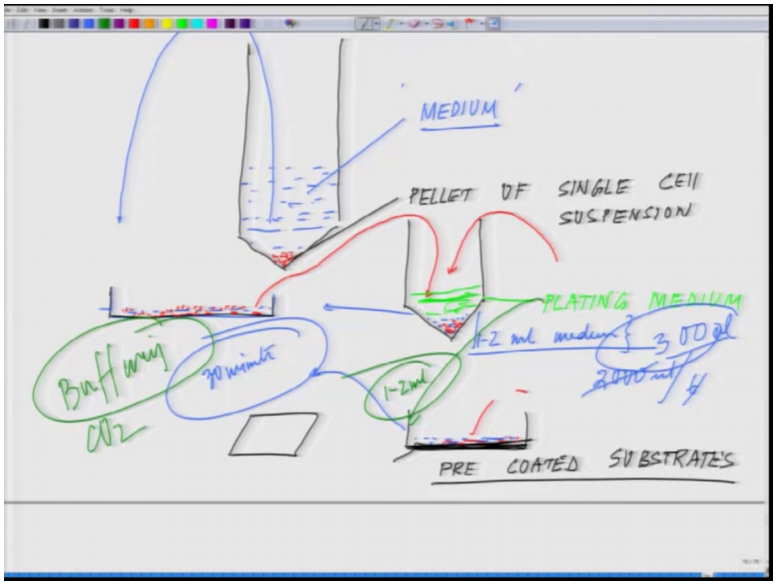

You just have to pellet it. So, here you have the pellet, pellet of single cell suspension. And here you have the medium. Now what you do? In the next step is you aspire it out the medium from here. So, what you are left with is of course, keep some little bit of a medium very little bit good enough not that it does not get dry.

Now, you have a single cell suspension here. I would not say a there will be some which will be chunks, you can get rid of the chunks there is a easier way to do that one shortcut is that at this stage what you do you keep some medium and you know drop it in a dish. Or a dish you know you what you do you have the cells sitting. So, those which are the big chunks they will settle down on the bottom just keep it for 4 5 minutes that is good enough and you can aspire it out the single cell back. So, those cells which are heavier kind of chunks you know they will be kind of you know they will fall down at the base. And you can tilt the dish very gently and you can aspire it out the single cell suspension and put it back in the test tube.

Once I put it back in the test tube now you will be needing the medium where you are going to resuspend it to grow. So, one of the easiest medium what I have developed over the years is you take for this E 14 glutamax plus if you wanted to use a carbon dioxide incubator to grow them, what do you then you do? Sodium bicarbonate I have forgotten the percentage that you can cross check or I will let you know, you add sodium bicarbonate. Now from your dissecting medium to the plating medium the only difference is now you are adding sodium bicarbonate. A once you added the sodium bicarb medium now you are add that.

So, I am just putting a different color for your now here you have the plating medium. Now take these cells and you should have already prepared the cover slips where you want to plate them or the dish or wherever you want to plate them coated with depending on the kind of substrate you are using pre coated. And by the way this pre coated substrate has to be done it is preferable to do 24 hours in advance or at least 12 hours in advance. Do not leave it for the last minute, oh I am dripping the substrate and all that that does not work you have to have a very clear cut plans how we are trying to do it. Pre coated substrates on those pre coated substrate what you do? You put your first a small amount of cell suspension very small amount. It distributed say for example, you have to coat say fifty cover slips or you have to coat say 12 cover slips or 6 cover slips you divide.

So, it is preferable that you resuspend it say 1 to 2 ml of medium plating medium and if it is one ml and you wanted to split it up into say or if it is it you want to split it up into say 2 ml is the easier to work with say 2 ml you want to split it up into 6 cover slips. So, what you do? 2 ml means 2000 micro liter they divide 2000 micro liter by 6. So, essentially around 300 little more than 300 micro liter you are trying to you know plate in every dish or every well or every cover slips what you have.

Now you plate them and spread them out you do not increase the volume. So, this is one thing which I will recommend you, just spread it. Spread it as much as you can and wait for and keep it inside the co 2 incubator. And wait for say you know outside 30 minutes to be very safe. After 30 minutes very gently add a rest of the medium which is around one to 2 ml, that is all you needed to do. So, you isolate the cells isolate the tissue or isolate the organ in this case, mechanically dissociated it, made a single cell suspension.

After you made the single cell suspension you spin it down, pull out the suspension resuspend it in a plating medium and now you plate them.

So, after once you have plated these on the pre coated substrate you wait for 30 minutes, after 30 minutes you fill them with the plating medium, the job is done. And put it back into the co 2 incubator. And your plating medium has sodium bicarbonate which is used for the buffering. And it has been observed apart from the buffering co 2, does have some positive impact on the growth of the cell. It is still not clear why is it so, but such things does happen ok.

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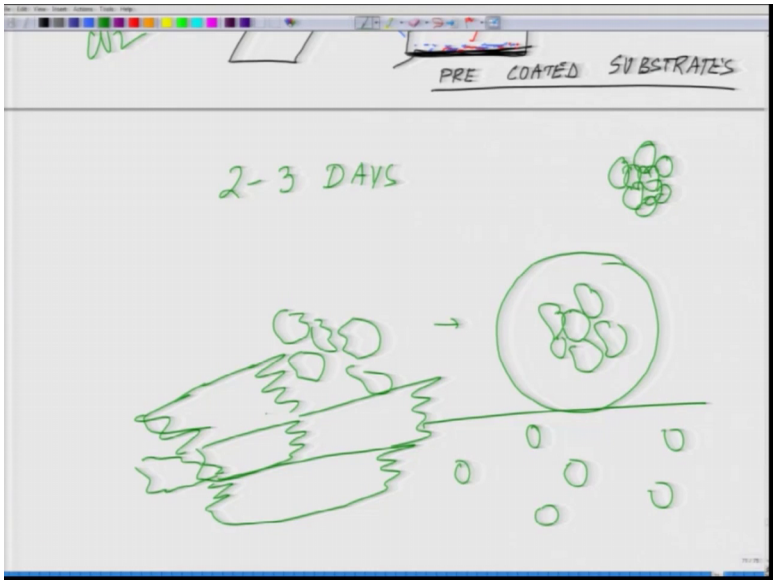

So, now you allow these cells to grow for I would say 2 to 3 days. What will you observe? These individual cells again the same thing, they will start to come close and you have to maintain a certain density So that they could migrate close to each other. If it is a very sparsely populated culture something like this, you may not see the formation of islands. And if it is a very dense culture then also you will see necrosis. So, you have to have an optimal density where you should play your game, where the cells will form almost something like a mono layer.

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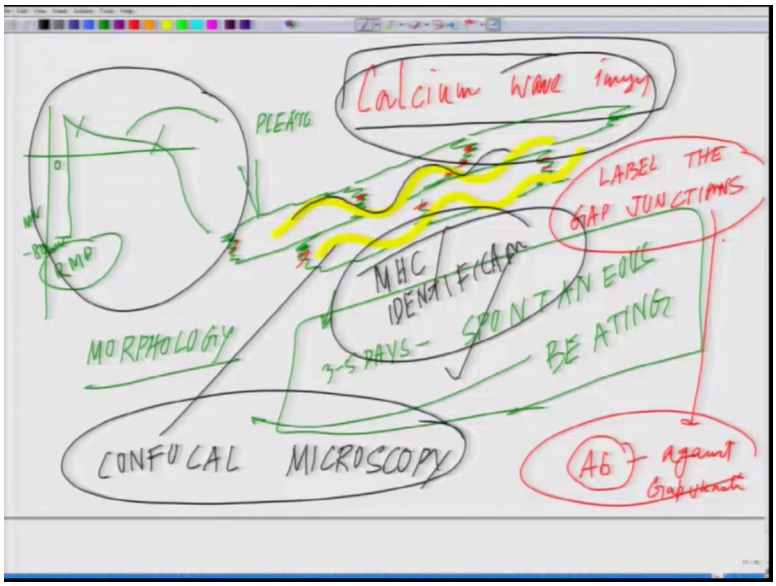

And these cells form something like this. This is the classic morphological aspects what you see with the cardiac myocytes, something like this ok.

Now, these cells after 3 to 5 days will spontaneously beat spontaneous beating, it does not need any additional features into it. Now when they are spontaneously beating. So, first of all as I mentioned in the previous class, you have to the morphology. It should match then spontaneous beating. Next what are the features you have to keep in mind they should be you should do an electrophysiology to see whether they should the classic action potential like that or not. You remember that their cardiac myocytes have this beautiful plateau like action potential and here is of course, overshooting the 0, in terms of the millivolt sitting on minus 80 millivolt resting membrane potential rmp stand for resting membrane potential.

Next you have to label the gap junctions protein, cardiac myocytes have lot of gap junctions which are sitting at different spot, you remember that? So, they have these gap junctions labeling the gap junctions using antibodies a B stand for antibodies against gap junctions. Then what you have to do is the most critical part of cardiac myocyte culture comes needs that is the calcium wave imaging. Because this So, what happens out in these cells are there is a calcium wave which travels along them, something like this.

And the these kind of wave could be easily measured using confocal microscopy, to measuring calcium wave. These calcium implants. There are specific dyes which are

available, because whenever we talk about any kind of culture as I mentioned in the last couple of classes I am continuously hinting out on that. Just growing them throwing them in a dish something grows something happens does not make any sense in the modern day. You have to have everything defined how close you are because other than that you would not be able to compete or the industry will never be interested to take your model systems. Otherwise cell culture will remain that you know you throw something grows that is it and you make some interpretation that is not important.

Important is the technology what you are offering. So, there is a calcium wave which has to be measured. So, if we look into it you have to have the morphology right you should be able to label the gap junctions right. You should be able to see the spontaneous beatings right. You should be able to measure the action potential using patch clamp electrophysiology. And you should have the calcium imaging done using confocal fluorescence microscopy. Once you do this then comes you have to have the myosin heavy chain cardiac has a very different kind of myosin heavy chain, unlike the skeletal muscle because these cells continuously contract.

So, myosin heavy chain identification. What kind of myosin heavy chain is being formed, another important part. So, there are several parts. So, you model system should be able to be as close as possible to the replica of what is happening in the real life. Unless it matches it would not make any sense understand. So, it is very, very essential that you go through this rigor and anyhow be handing over the bunch of reading materials for these different models which will help you to get a better understanding of it.

But keep these in mind it is not just a model it is how close you are to the natural habitat of the cell which is within your body. I will close in here, thank you.

Thank you for your patience listening.