Cell Culture Technologies
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Lecture - 34
Skeletal Muscle Cell Culture

Welcome back to the lecture series in Cell Culture. So, last class where we ended I talked to you about the phenotype of the myotube. So, while we talk about the phenotype of myotube we talked about that it has multiple nuclei aligned in a line. And this tube has the inherent capability to contract. Sometime it is a spontaneous contraction sometime it needs a neuronal signal will come later into the part to of it. But in the rats it has been observed if you grow from the fetal rat most of these myotubes show a spontaneous contraction.

Now whenever a muscle contracts there are 2 things which happens there is a sliding filament motion of the actin myosin filament on top of each other, this is what contraction happens like this reverse direction motion. So, you can refer to any textbook in animal physiology or you can go through some other lecture series what I have given in there you can really figure that out it is fairly straightforward. But in order to negotiate that sliding filament there is a need of a calcium spike, the calcium has to come into that milieu it is something like when I do in the last class. I will pick it up from here.

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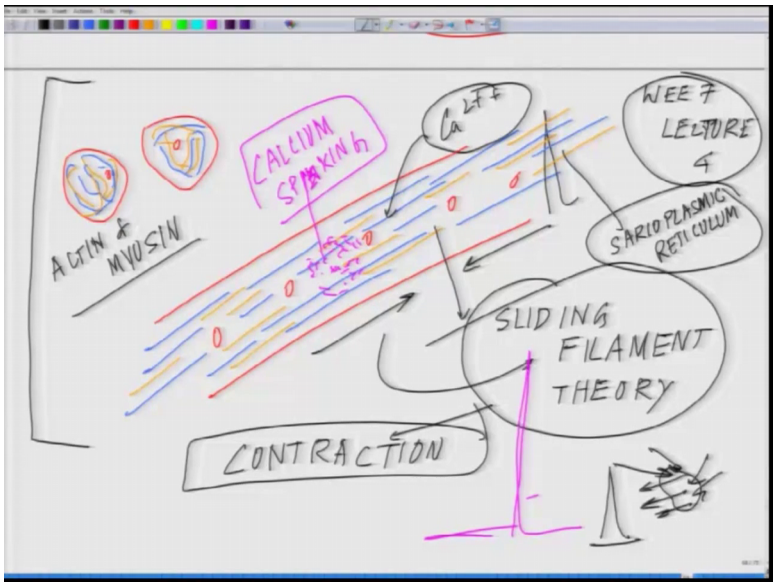

So, this is week 7 lecture 4. So, so there is a slide for the sliding filament to appen what will you we observing is at a particular time there will be a these dots are showing the calcium spike. So, there will be a calcium spiking. And it is a very transient spike it is it happens like this and falls like this, very, very transient spike. But then who controls the transient spike? This a critical question. This transient spike is controlled by organelle which is present in the myotubes.

Now, I am adding complexity whenever you are trying to grow massively (Refer Time: 02:51) kind of keep that in mind. Organelle which is present on the myotubes which is called sarcoplasmic reticulum. And sarcoplasmic reticulum is kind of a sponge, it you squeeze it the calcium will come out and then it will pull it back. So, it is something like if this organelle is present out here. It will throw away the calcium and immediately it will pull it back. And the time window which is there for it you give the calcium out and pull it back is what determine the calcium spike.

So, this negotiation of a calcium spike by sarcoplasmic reticulum is also essential that you retain in the cell culture dish. If you cannot then it is you are not really making the right kind of myotubes, or not fully fullfledged phenotypic features you are unable to observe.

Now sarcoplasmic reticulum is a calcium deliverer, but it gets influenced or it gets activated by 2 kinds of receptor, if this is sarcoplasmic reticulum SR.

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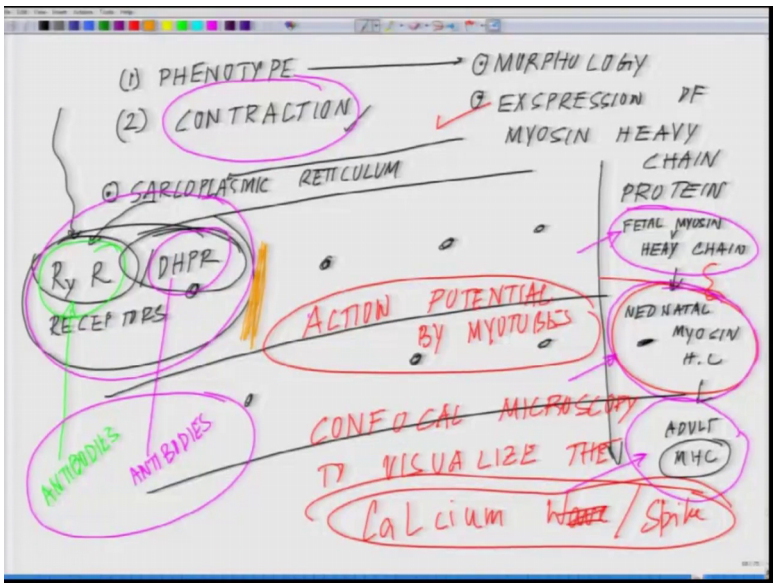

Then This is activated by 2 unique kind of sensors on it is surface, which are calcium sensors and I am putting 2 different colors to indicate. Highlighted the red one and the green one please see here. One is called ryanodine receptor the other in one is called DHPR receptor. These are calcium channels. D stand for di, H stand for hydro, P stands for pyridine, R stands for receptors.

So, a sarcoplasmic reticulum essentially has 2 different sensors in the form of ryanodine RYR in short it is also called RYR. RY stand for ryanodine, R stand the third second R stand for receptors then you have D H P R dihydropyridine receptors right. So, these 2 receptors these 2 calcium sensors helps it to throw out calcium and pull it back. So, this is how the calcium dynamics works. So, this is in tandem with this spike what I have drawn the sarcoplasmic reticulum.

Now when you are developing an in vitro system something like this, now here I have a myotube right series of my tubes which are growing here I have the martin you created myotubes growing in a dish. Now what is expected is, first of all we talked about phenotype. Yes you are lucky that you have a phenotype of myotube sure. Next step you have a multi next step what is expected in functionality is that it should contract either by stimulation or without stimulation contract or contraction step 2. If it contracts.

So, in terms of the phenotype not only the gross morphology, but the expression of expression of myosin heavy chain protein. Interestingly while the muscle develops. So, if you recollect when I showed you this picture. These myosins say for example, I indicated myosin by blue. So, these myosins change their form once they form the myotubes there is a morphological or not morphological is the wrong word. There is a subtype expression.

So, these are mostly much more mature form of myosin what you seen in my tubes, as compared to the myosin which are there in the individual cells. So, this transformation from they call it fetal myosin heavy chain to neonatal myosin heavy chain. HC is heavy chain and then eventually adult myosin heavy chain, MHC stand for myosin heavy chain

So, these are the transformation which are happening. So, if you really are a successful if you claim yourself to a very successful grow at least you should be able to reach up to this point. You should have a neonatal myosin heavy chain expression that transition from fetal myosin heavy chain to neonatal myosin heavy chain. And in should happen in

the cell culture dish if it does not happen it means this expression or in terms of the phenotype is not up to the mark. This is very important that you keep in mind that just making something would not work second thing once is myosin heavy chain expression, we have talked about second thing in order of course, this is a physical parameter that you will be seeing contraction. But then this brings us to the third phenotypic difference which is are we expressing of course, you have to locate for the sarcoplasmic reticulum that circularized in reticulum is well form and that you can only proved by R we seeing ryanodine receptor as well as BHPR receptors. These 2 are critical in this journey, that these cells these tissues should be able to express these particular. And another important thing these 2 unique marker DHPR and RYR should be in close proximity with each other something like this In a very, very close proximity.

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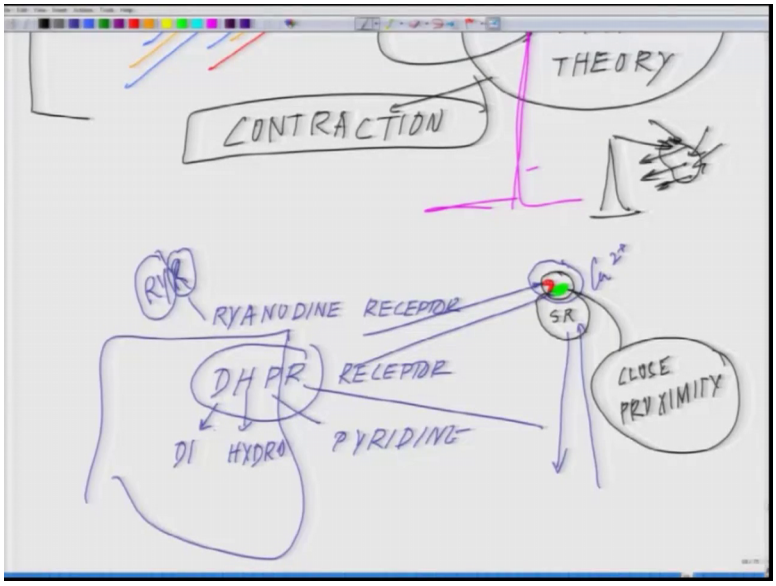

Almost it will look like that they are to their same entity, almost they are something like this it should not be part otherwise this whole calcium spiking is not going to happen.

So, how you do So? You have to have a specific and type antibodies against say DHPR and specific antibody against RYR. So, if you have their specific antibodies, then you can label them separately. So, when you are culturing skeletal muscle. You should have all these different parameters in mind that how I can you know say with conclusiveness or say with certainty that yes I am observing all the features what is there in an mature

tissue in vivo. If I do not have that then I have to you know go back to the basics and figure out.

So, what you need as a tool you need antibody for fetal myosin heavy chain at some point you have to see that, very early phase that there is an expression of fetal myosin heavy chain. Then you should have needed antibodies for neonatal myosin heavy chain. You needed antibodies if you can have a prolonged culture for adult myosin heavy chain. You need your antibodies against DHPR and ryanodine receptors. And you should be able to see the physical contraction, plus using electrophysiology techniques you should be able to see the action potentials generated by the muscle cells.

Action potential by myotubes and next characterization what will be very important, whether are we successful or not will be you should be able to use confocal microscopy to visualize the calcium wave or the spike. Wave is the wrong word here I would say calcium spike. Because it is possible that highly possible and what people does that you see a spike like this. So, at some point you will see a disturbance. So, if you have the right kind of So, when you have to do a calcium measurement then you need it specific dyes which will bind to the calcium.

So, now you can make a wish list that these are the things what I have to procure if I had to kind of say with certainty mine vitro system works like this. So, this needs a lot of planning and a lot of most importantly needs a lot of groundwork and study one has to do literally study that you know this is what I say it is it is not about whether I am successful or I am failed what is important did I do the groundwork right, did I look through every my new detail. That you know this is what am I looking forward to that is very critical that you go through that rigor of going through that whole paradigm that you know these are what is expected from me that these parameters are being taken into account.

So, talking about a defined system for skeletal muscle first thing one has to have a define media first even earlier to that one have to know which each group you are collecting the tissue, as I have already mentioned about the advantage and disadvantage of different ages. So, if you really pick up the tissue from say neonatal or post birth you will have a lot of fibroblast out there.

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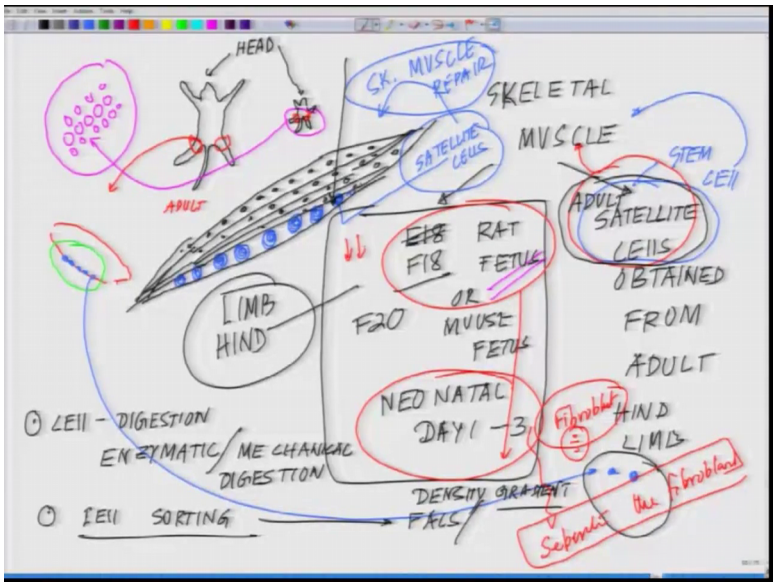

So, they will outsmart your muscle cells because when you are pulling out this tissue. So, if you take a tissue from this zone or this zone you will have lot of fibroblasts. And these fibroblasts divide very fast. And if they divide very fast then they will outsmart your skeletal muscle.

So, you have to have a separation technique involved here by which you can separate the fibroblasts. Once you separate the fibroblasts, you may still have some contamination where you can not help it. Antibodies well I still have because you really cannot separate out there are ways. So, one of the ways how fibroblast could be separated out from muscle cell is, you just put the tissues there put the single cell suspension there some of the cells settle down faster as compared to the other ones. And mostly the fibroblasts settle down faster.

So, if you know the fibroblast settle down, you can always take the suspension and remove it and that will have more of the muscle cells. There are some tricks which you go through the literature will figure out that is not a big deal. So, now, depending on the age your fibroblast contamination is going to increase. So, if you are taking something like E18 you have a very minimal amount of fibroblast contamination. But that has it is advantage as well as disadvantage. Disadvantage is your growth will be slow because fibroblast helps secrete several supporting factors what does skeletal muscles need it. But if you can compensate for those factors using your cell culture medium then you can reap

a very proper to harvest from it. But that that again demands that you understand the basic biology right. So, depending on the age group what you are picking up you will have this fibroblast contamination.

Once you override that by picking up the right age next is you need it to have a define mediums, why mediums? Because a part will be the dividing one you remember I told you a you need 2 2 phases of this. One will be this phase which is this phase second will be this phase dividing plus differentiation plus and only differentiation.

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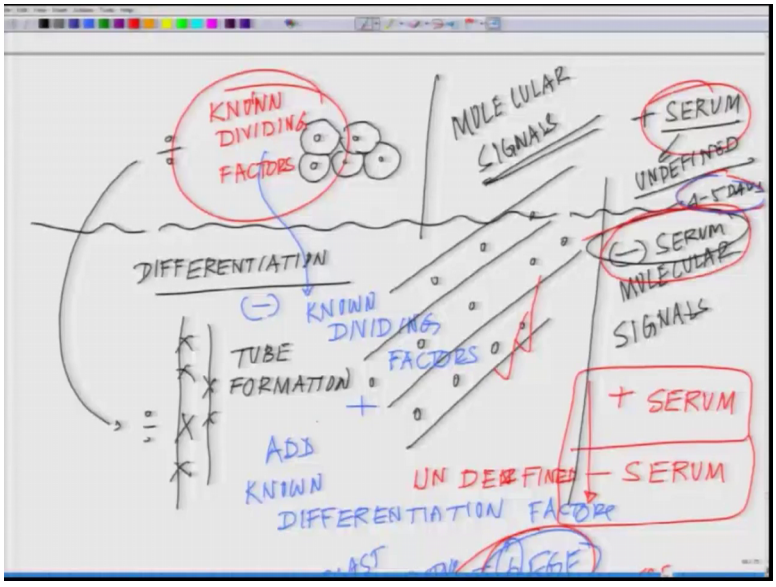

Followed by you needed all these different parameters to be taken into account.

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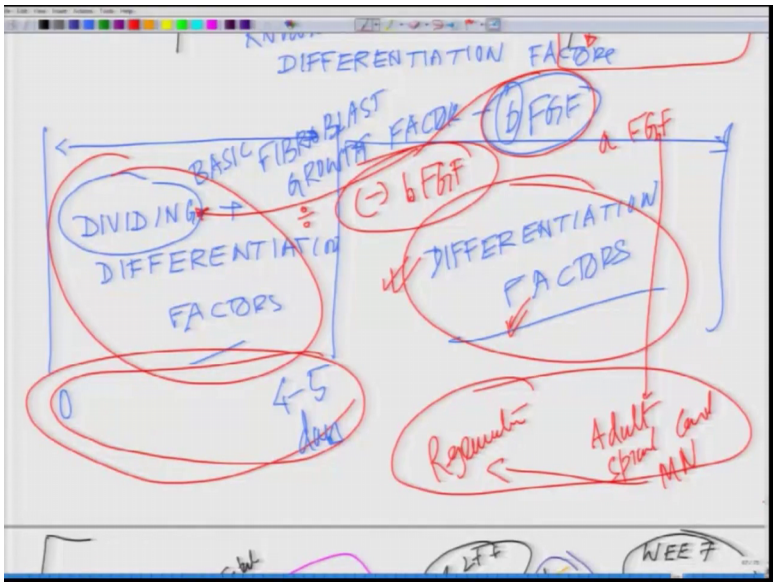

The morphology expression of the myosin heavy chain, the fetal myosin heavy chain, neonatal myosin heavy chain. And if you go further adult myosin heavy chain, presence of sarcoplasmic reticulum ryanodine receptor DHPR receptors, the action potentials minor myotube their contraction and followed by the calcium weight measuring the calcium wave. Now, realize that how intense the process of developing cell culture models are it is not just throwing some cells and growing some cells, it is about systematically figuring out how close you are to the real life.

So, I will close in here next class will explore further.

Thank you.