Cell Culture Technologies
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Lecture – 33
Introduction of Skeletal Muscle Cell Culture

Welcome back to the lecture series in cell culture. So, in the last class where I stopped is how to isolate their stem cells and I told you that especially the satellite cells of the skeletal muscle. So, similarly you have such stem cells present in the adult cardiac, adult heart and if you know the techniques you can isolate them.

Same way you have such stem cell population sitting in the spinal cord, and there are several efforts which are happening across the world if you isolate those cell types and you know let them divide and make them form neurons and put it back and there are several studies which are or several attempts which are happening all over the world, do you know develop these kind of techniques, but what is the important is the basic fundamentals. You have to understand the basic fundamentals still lies in the cell culture, how you really do it how you can isolate these different cell types, how you can separate them apart, how you can grow them in a control condition, how you can grow them in a defined condition, how do you know that what you are doing in this particular lab will be repeatable in another part of the world.

So, when I started this cultural muscle with you, this was one of the objective I want to tell you that skeletal muscle growth is very interesting. First of all let us talk about the development biology of skeletal muscle growth, and then will come to the challenge of in vitro development biology when we have to talk about how to culture these cells in a dish. So, the way skeletal muscle grows is, why I told you that why you cannot take the direct tissue you only have to rely on the satellite cells to grow them. So, this is start of it.

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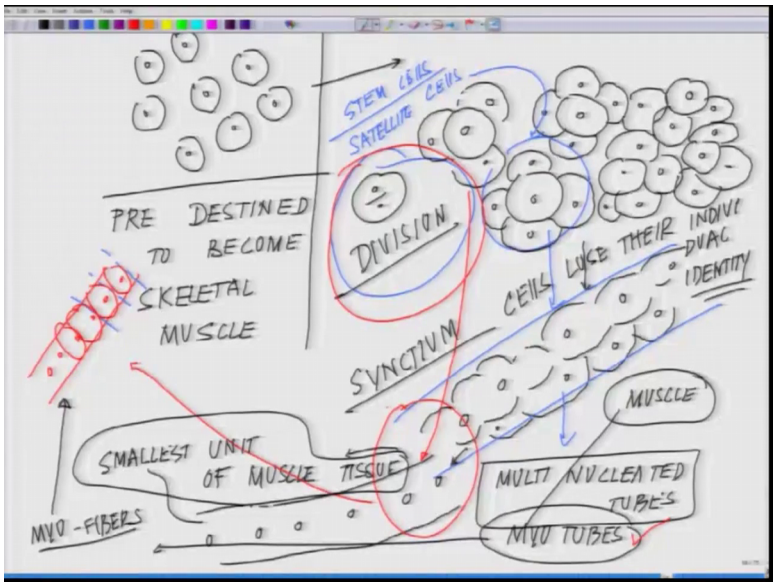

So, this is our lecture 3 and this is week 7.

Now, in the mother's womb the way is skeletal muscle is formed something like this. So, there are certain cells which are predestined to become skeletal muscle right, this population what I am drawing now is that population. These are pre destined to become skeletal muscle. Now these cells reach the specific site may be it may be a limb or some other part wherever the skeletal muscles are needed. So, what they do one of these cells reach their specific spot they align themselves and they divide. So, they divide like this any you really can see. So, this is the division happening. Post division once they have aligned they have to make a particular tissue skeletal tissue, this is the step one.

Next thing what happens is these cells slurry they have a multiple alignment, which happens these cells slowly lose their identity and they become what we call as syncytium. They become a continuous structure like this, and still their nucleus maintains their identity. So, eventually what you see start from starting from there, what you get are they form very interesting tube like structure like this multinucleated tubes, multi nucleated tubes and these multinucleated tubes are called Myo tubes. Myo means muscle and tubes means tubes of muscle and these individual tubes of muscles. So, this is the smallest unit of muscle tissue smallest of all this is the smallest of all unit.

So, these tubes align with each other to forms what is called Myo fibers. And these myo fibers align with each other to form complex muscle tissue. So, it is a complex process

which happens in your body once we are formed. So, first step these are predestined and they reach there. So, there is a division, this huge amount of division. After division these cells lose their individual identity. Followed by that they form tube like structures and these tubes are called multinucleated tubes or Myo tubes.

Now, from a Myo tube you cannot again as of to the best of my knowledge about biology, you cannot again regain a single cell. So, because it us a continuous tube with multiple nucleus. So, there is as of now again as of now there is no way that I separate them and they repair and they give me single cells like this. So, once a Myo tube is formed again with a word of caution, this is what I know. From a Myo tube you cannot regain the cell, but from a cell you can make a Myo tube why I am telling you this story I am telling you this is a story.

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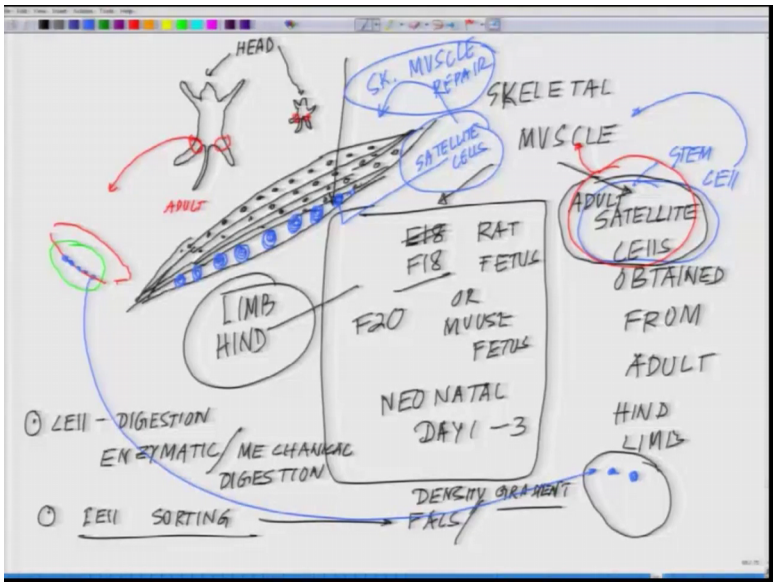

Because in the last class when I told you why you needed adult satellite cells to make a sculptural muscle is the reason. Because out here if you look at this part of the hind limb. So, this hind limb is something like you know what will you see is this kind of wonderful fibers which are making this hind limbs beautiful fiber like structure and these fibers are multinucleated fibers like this, you can stain them you can see wonderful staining and which is maintaining this nice cyto architecture, but when will you try to isolate you would not be able to isolate single cells from there. Your only option underneath it out here somewhere you will have some very interesting individual cells, big individual cells

sitting and apparently this is believed of this is partly proven that these cells whom we called satellite cells are involved in skeletal muscle repair. Obviously, how they will repair? they will follow the same paradigm as has been followed out here. So, you will have these I can just replacing it, you have stem cells or satellite cells in case of skeletal muscle satellite cells. These satellite cells come they divide and then they form these kind of non identifying tubes and eventually they from multiple multinucleated tubes.

So, this is the fundamental developmental paradigm. So, there are two processes they are coming, dividing this is critical for you to understand and will come why is the cell culture analog to this and then they form wonderful tubes. So, in order to culture in a dish a sculptural mussel, your options is now coming back to the previous class, where we left at this stage you are picking up from an embryo or a fetus if 18 fetus. So, at this stage there are a lot of cells in the hind limb of that fetus, which are destined to become muscle is they do not become really and so, the cells are there they are sitting, but they have a aligned completely or they have an aligned really a huge chunk of the population has an aligned completely to form muscle.

So, this is the time when you should isolate, he should the sect out this is.

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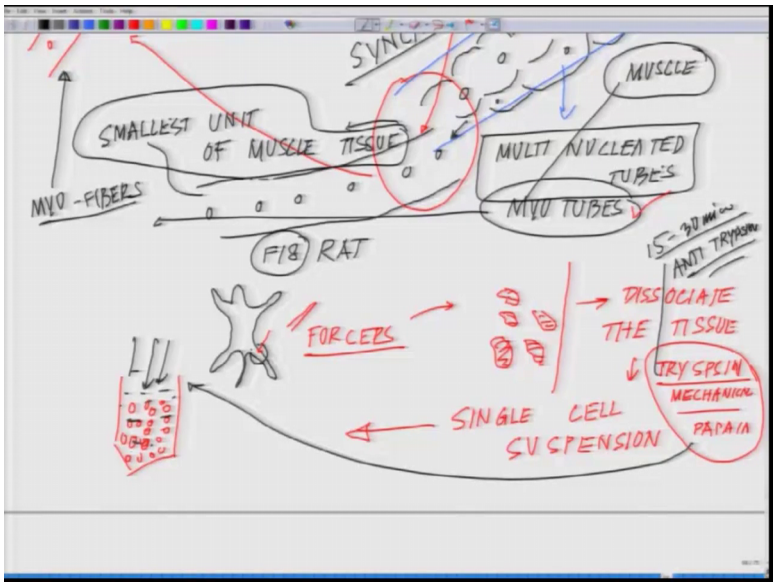

You can take it from the forelimb also. So, it is not that you have to take it from hind limb all the time, but if you do so, do mention in your paper. So, this is say for example, F 18 fetus of rat what you do you from this location out here you open it and using very

nice micro forceps you pull out the chunk of tissue from there. Then what you do? You dissociated; dissociate the tissue into single cell suspension, from these single cell suspensions what you have is single cell suspension something like this.

So, this dissociation mostly people use Trypsin as a dissociating agent sometime Trypsin in plus mechanical dissociation and you can use Papain also, but it is advisable that you use Trypsin because these are very hardy tissues and it is not easy to dissociate them into single cell suspension, but if you use Trypsin you to ensure you use it for a small period of time say 15 minutes to a half an hour, and 15 minutes to half an hour and then you use an anti Trypsin to stop that reaction otherwise it will damage the cells beyond recovery. So, you have to stop it. So, then what you get is a single cell suspension in some form of media where you want you to grow them.

Now, you realize that there are two steps into this reaction, there is a division step there is a differentiation step. Now I am introducing two more terms now one I have already introduced division.

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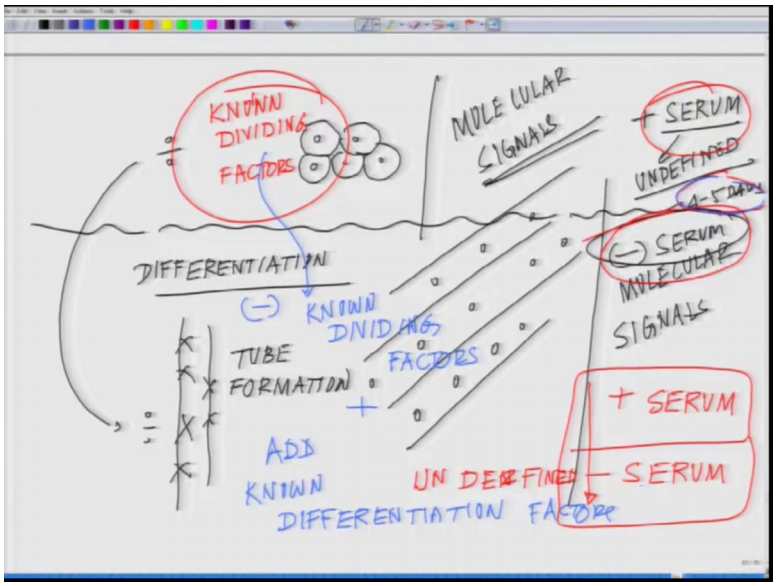

So, division is the zone, where these individual cells are dividing these muscle cells and then there is a differentiation phase. And this differentiation phase is when they are forming these tubes, and it is very interesting to note that this two formation would not happen if still the division signals are coming. These are inhibitory to it if division signals are coming then you cannot allow these cells they will never form a tube. So, it

means the signals which are needed for the differentiation or the molecular signals to be precise, are not same as the molecular signals you needed for division. That brings us a complex equation to do a cell culture. So, it means you have to divide your cultural milieu into two different phases or division phase a differentiation phase and this is one problem you will always face when you are trying to differentiate stem cells this is a common problem, how you get around it, what are the factors.

So, most of the people who were using cell culture prior to I would say two 2004 or 2003 the most of them have used the way they have done it. They culture the first phase of these skeletal muscle cells in serum, which is an undefined condition where these cells divide. So, add serum and the very moment you use serum this is an undefined parameter you really do not have any control on it and then what you do they did. After these cells are divided significantly say 4 to 5 days, then you remove serum your depriving them from that unknown factor or undefined unknown is the wrong word problem undefined followed it and then you remove it. So, you have plus serum and minus serum face, this is how most of the people is to do it which are undefined way of doing things.

So, then came few studies which actually developed the defined system, how they develop the defined system. They define the develop defined system by introducing known dividing factors. All the non dividing factors and after 4-5 days as I mentioned here they remove those known dividing by just removing the medium dividing factors and simultaneously add known differentiation factor or is another roof which has been followed that you start the journey with first 4 days do it like this you add both dividing plus differentiation factors.

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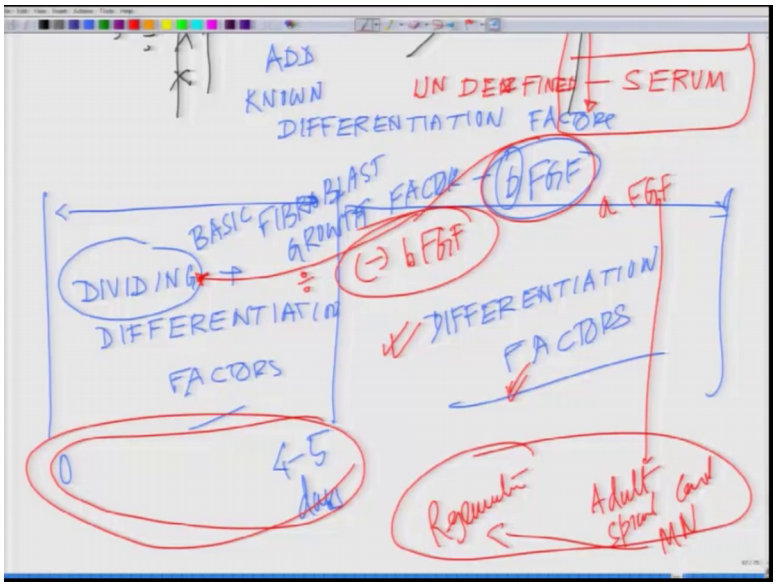

So, you are parallely giving them both the signal say for 0 to 5 days or for 4 to 5 days and then you only give them differentiation factors and these are all known factors ok.

So, what develom valley gist have already proven that these, these, these x y z factors can help in the differentiation or this like one of the dividing factor is called basic fibroblast growth factor which commonly is called basic FGF I mentioned basic because there is an acidic part also there are acidic FGF, which I am not mentioning here they are needed acidic or just for your node. Acidic FGF is needed for adult spinal cord motor neuron regeneration just for your information.

So, here we are talking about basic fibroblast growth factor which is a known dividing signal. It is known in the presence of basic fibroblast growth factor cells will divide, once they divide and sufficiently divide in those first 3-4 days, then what you do from your medium or millio you just remove it. Basic FGF is now removed once the basic FGF fgf is removed then what you are left with they are only the differentiation signals, and these differentiation signals then will give these cells this second fate, and the development of these kind of cell culture models where all the parameters are known all the steps are known, how you do it are term as the defined systems.

But then just by developing a defined system does not prove anything till you see in your dish, that these skeletal muscle what have what you have developed or what you have differentiation difference divided and differentiated in a dish started to contract. If they

do not contract then you have failed in your duty, why now coming back what happened during this phase.

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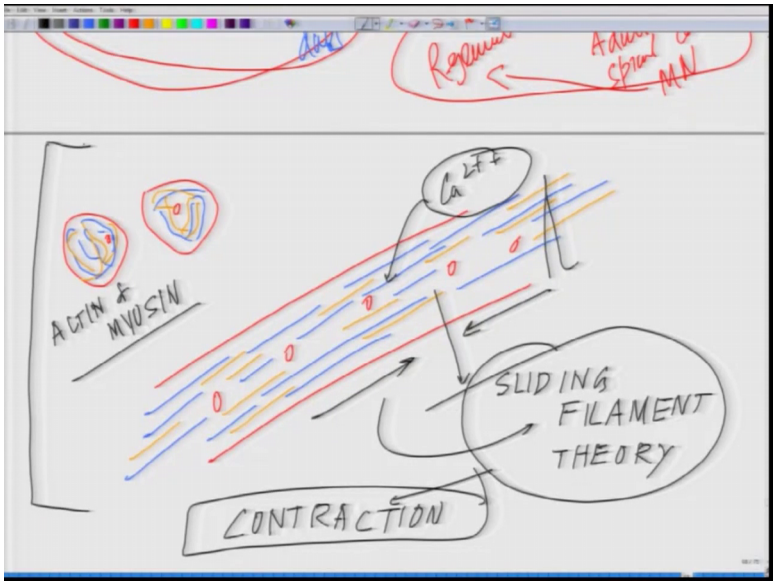

So, when these tubes are formed. So, initially what you have you have these cells and these cells have lot of filamentous protein on their cell membrane, and those filaments mostly are actin and filament actin and myosin which rules most of the muscle cells.

So, when these kind of structures are formed what happened is these actin and myosin filaments align themselves like this and this alignment helps them to follow and this is you have to really refer to animal physiology, what is what we call as sliding filament theory, where the contraction of the muscle takes place because of the sliding filament and this sliding filament is being brought out by a calcium spiking or calcium rush and that spike of calcium brings the sliding filament where this leads to what we call as contraction, this is the whole process.

So, I will close in here in the next class will go little further in depth about it, that how you really can figure out is phenotype supported by the functionality.

Thank you.