Cell Culture Technologies
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Lecture – 39
Advance Cell Culture Modules – IV

Welcome back to the lecture series in Cell Culture Technology. So, today we end of the 4th lecture. So, in the last lecture I talked to you about the challenges of developing a neuromuscular junction. There are 2 challenges what we put forward, week 8 lecture 4.

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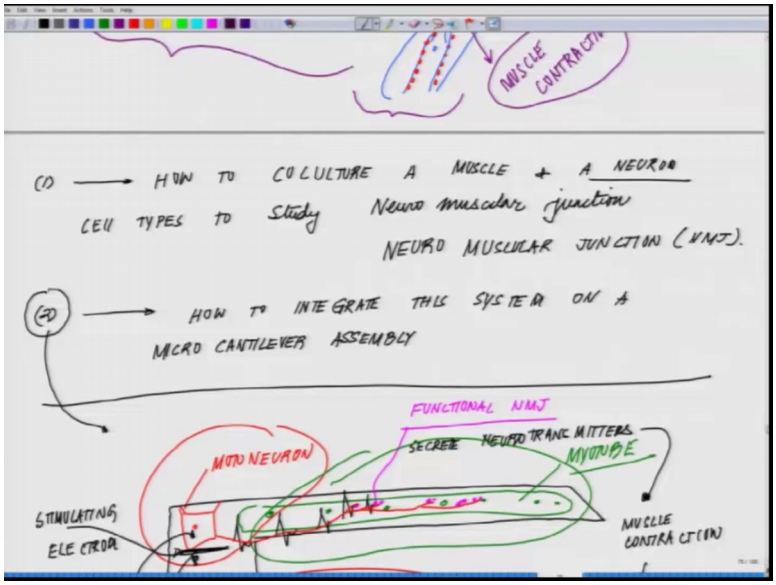

So, these are the 2 challenges. How to integrate the system on a micro cantilever assembly? So, one and the other challenge was how to co culture a muscle and a neuron cell type to study neuromuscular junction. So, these 2 problem comes with another additional issue that you wanted to have a defined medium which is a serum free and it should be a common medium.

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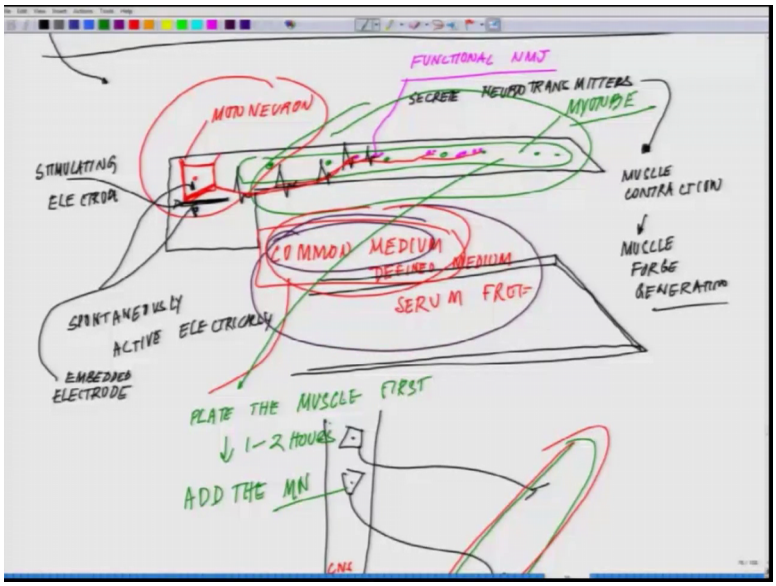

This was very challenging from the word go. Because when you have to grow the muscle try to realize you know how the in vitro systems can become so very daunting for to work with. And try to appreciate that how you can bypass all these things so neatly.

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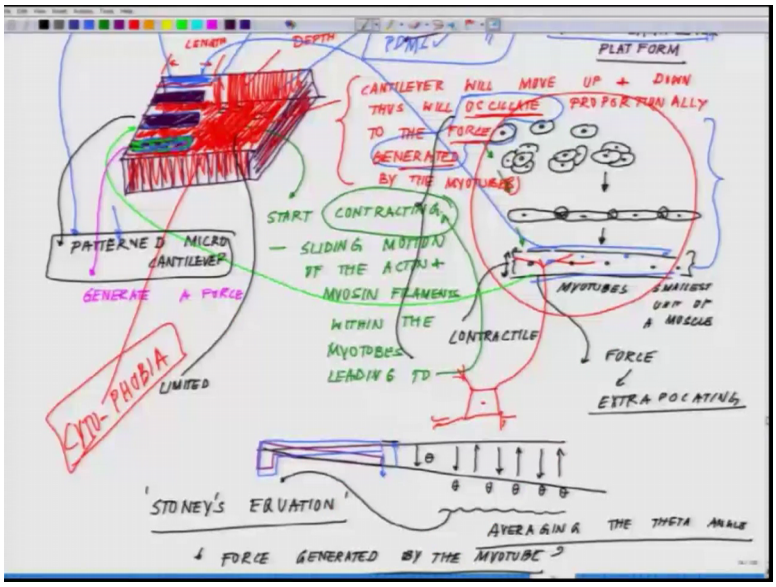

So, when you talk about muscle if you remember the first thing what we told you is this is the picture right out here. So, these are the muscle cells which divide pose division they form these kind of alignment. And then of course so, this is the phase of I told you about the differentiation.

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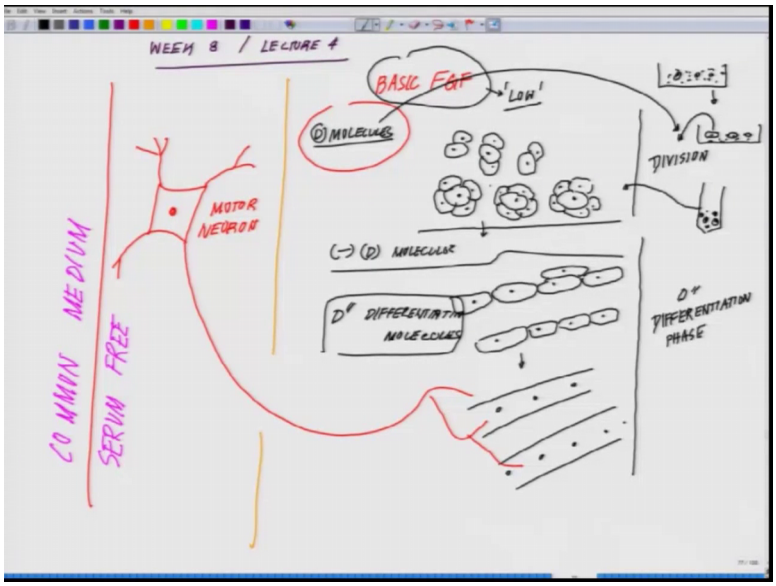

So, so these are your muscle cells you are plating in a dish. Your initial plating these are muscle cells, post plating you have medium which will promote their division initially for 3 4 days. You are allowing them to divide pose division they will align like this. They will be elongated they will be aligning like this. And soon after that they will lose their individual identity and they become a tube like this.

Now, in this process this is which is the division phase, this is which is the differentiation phase, differentiation phase. Now in order to divide. So, you have to use molecules which allows the division. And then you have to, so I am just putting that dividing molecules. Then you have to withdraw the minus sign showing the D molecules and allow the cells to grow in D prime D double prime which is in the milli of differentiation molecules ok.

So, this journey becomes complicated when you are adding into the system. Another component which is a neuron which never divides. So, here you have the in this case it is a motor neuron which did not divide. Now whenever you are using anything which these kind of molecules which are mostly basic fibroblast growth factors, which are used and this media this whole thing has to be a common medium. This has to be a common medium and it should be serum free.

So, the way we achieved it took us a while to achieve this interesting feat. But we eventually did achieve it. So, what we did first is that, we took the muscle as I have

mentioned in the previous class. And we did a first level where the muscles are contaminated with fibroblasts. And all we just allow the muscle to sit there and the fibroblasts kind of settle down and the muscle cells were in the top. What you do that you take out this suspension and you take out the suspension. So, you have the more or less the pure myocytes sitting out there, then what you do? You played these myocytes on a dish. So, once we played this myocytes. You tweak around with the basic fibroblast growth factor concentration you use very, very low concentration of course, that has it is own problem because then the cells own divide in sufficient number. But that is one degree of compromise you have to do.

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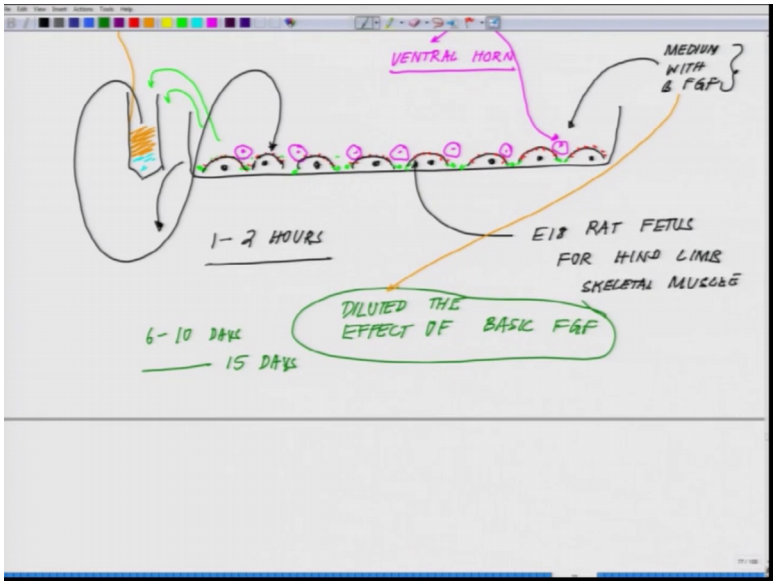

So, you played the cells and on top of it you put the medium. So, what you have in a dish is something like this. So, here you have the cell culture dish where you are growing them. So, you have these muscle cells which are kind of you know settled on the surface after panning, I told you how you are removing the fibroblasts because the fibroblasts will be settling down faster. So, you have the muscle cells are there. Then you add the medium with FGF. And there are other components in the handout, I will give you can have a look at it what are the other components which are being used.

Now, you leave this in a dish for approximately 1 to 2 hours. It is during this time most of these cells will first of all of course, most of this different growth components will bind on the surface of the cells. And will initiate the necessary reaction what they have to

do. This is this red color showing all the different kind of binding of FGF and other growth factors which are being used. And simultaneously these cells will start to secrete their own extracellular matrix.

Now, after one hour while still the culture is 1 to 2 hours, what you do? You remove a small part of the medium very small part of the medium, good enough that it just mildly covered the top. And then you introduce your motor neurons, they will be sitting something like this. And these motor neuron mostly comes from case of rat in case of fetal culture they come from E14 rat embryo. They are coming from ventral horn of spinal cord. Whereas, your skeletal muscle for the cultures are coming from E18 rat fetus for hind limb skeletal muscle. So, that is where you are getting myotubes.

So, after 1 to 2 hours you add these motor neurons. Then of course, the medium what you have removed initially you replace it back because you have to realize in the very early phase the cells secretes so many important growth factors, so many, it is a plethora of growth factors they secrete which are extremely essential. Extremely, extremely essential and while you are putting the medium back you add some fresh medium on top of it, which is without basic FGF. So, what you essentially did? You actually in this whole process you diluted the effect of basic FGF. And if you can hold this culture for I would say anything between 6 to 10 days and slightly longer say 15 days, you will see wonderful neuromuscular junctions getting formed like this.

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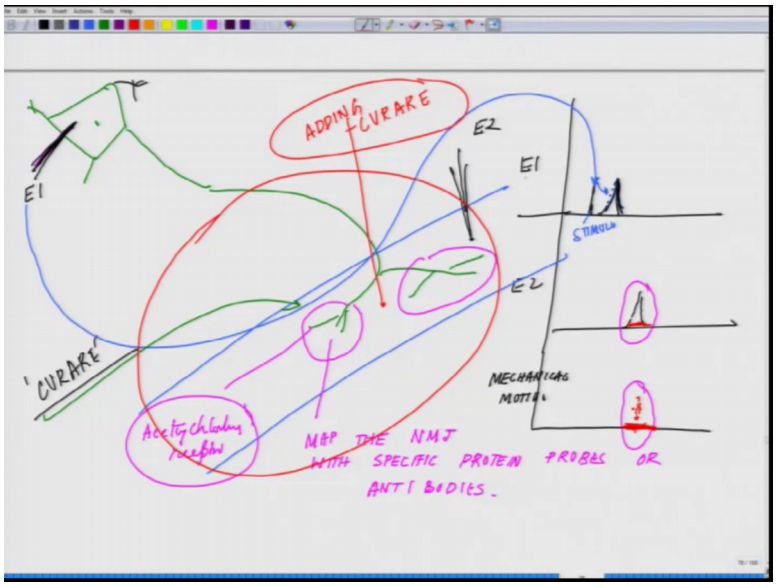

And one of the most beautiful test you can do is there a couple of tests you can do. One test is say for example, you have you know, you see the motor neuron out there. You stimulate the motor neuron with an electrode you should be able to see with a time delay, you should be able to see a contraction taking place in the muscle. Or you should be also able to see parallely and if this is E1 this is electrode 1 and what you will see is to scale this is showing for E1 and this is E8.

So, here you are giving the impulse at E1. And you will see there is an electrical activity generated out here. And then with a delay you will see another electrical activity happening in the muscle out here. And simultaneously you will see a mechanical motion or a contraction happening at the zone. So, this is your first impulse coming through. So, this is the stimulus say for example, you needed a stimulus. This is the first impulse this is the an electrical activity of the neuron followed by out here the electrical activity of the muscle. And simultaneously the mechanical activity of the muscle. You really can physically see it, apart from it there is another way you can evaluate it, is that out here out here you can map the NMJ or neuromuscular junction with specific protein probes or antibodies probes or antibodies.

Simultaneously the antibodies could be you know, you can probe them for I would say against acetylcholine receptor. Because there are a lot of acetylcholine receptors which are present on the muscle. You can probe them with bungarotoxin. So, bungarotoxin is a toxin which will block the transmission in the neuromuscular junction. So, you can parallely do one more thing, you suppose given a stimulation here. And you add something there is a drug called curare, which is used by the tribes in amazon basin to (Refer Time: 14:36) deers or other animals. What it do? What it does is curare upon binding it paralyze the neuromuscular junction.

So, if you add curare and if you given stimulus you can have any stimulus, but you would not see any signal out here. Your signal will be missing out here. And there would not be any emotion out here. This is the effect of curare. Curare can block the neuromuscular junction in a very unique way. It is a by the way it is a reversible blocker after a while curare's blockage stopped. So, you can actually use these kind of things and not only that if you are seeing that this whole setup is spontaneously contracting. You can stop the contraction by adding curare. You will see upon addition of curare there will be shaking like this and then the muscle will stop.

So, all these things can be seen in vitro and these are proven trackers I will hand over all the papers to you, these can be seen very neatly and they could be evaluated, but our problem was even much more challenging. We wanted to do this on top of a cantilever this is what we are kind of looking forward to. So, in order to do that, what we needed was a common medium and a different plating rules. So, the plating rule was or E is you plate the muscle first and wait for 1 to 2 hours and then add the motor neuron. And I told you the source of motor neuron which is from the E14 ventral horn of the rat embryo and skeletal muscle you are collecting from E18 rat fetus.

So, if you can really pull it through as of now to the best of my knowledge, as of now no one has been successful in achieving this kind of such a neat system muscle has been done. But really integrating it in the neuron and still we will take a little bit more time, because this is not a easy system to work with, but these are some of the cleanest systems which in years to come it is going to change the face of cell culture or traditional cell cultures what people have been following for years together like these are the modern day technology where we are assuring into where cell culture is no more a territorial dominance of the biologist, it is now a very integrated journey of engineers mechanical engineers, chemical engineers, electrical engineers and designers biomedical scientists, biologists, physicists, chemists.

So, in a way you have to have a fairly good idea about cell patterning you should be able to know the different kind of a thin layer technology a thin layer technology another area integrating electrode into such structures.

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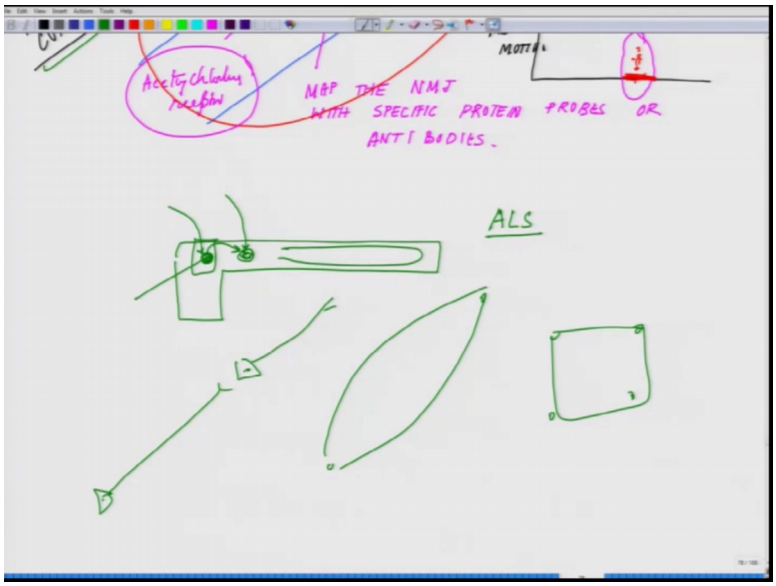

So, as of now this approach also like on a cantilever say for example, here you have a cantilever having embedded electrode or embedded electrode systems, these are not easy job. We you are doing at a micron level and these are still it will take a little bit more time. Technology is there, but really to you know make it happen the way it is it is not something so easy ok.

So, in this frog segment what we are trying to do and is kind of exposing you to those technologies which are still not part of textbook. These are futuristic technologies, but these are the technologies which we will drive, the future say for example, I wanted to test a system for ALS amyotrophic lateral sclerosis. That is a situation when martin neuron is unable to communicate with the muscle, because they die out to create a system how you can do it? These are the system you want to study Parkinson. And you want to have 3 layers say for example, a motor neuron coming from substantia nigra, a motor neuron in the spinal cord and here is the muscle.

So, the signal which is missing is here substantia nigra neuron is not sending a signal, how one can really study make a Parkinson model on a dish. Or how you could have a cell patterning technology to make circuit is like this, circuit is like this, where you can study Alzheimer's. And in one of the small modules end of it I will talk about the micro channel technology, where we will talk about how this micro channel technology could come very handy to study single neuron to single neuron communication with an

hippocampus which could throw a lot of light into our understanding or the deep understanding of the signals generated by the hippocampal neuron while cross talking with each other.

So, I will close in here. So, in the last class in the series will talk the electrical excitability and how we achieve it ok.

Thank you.