Cell Culture Technologies
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Lecture – 38
Advance Cell Culture Modules – III

Welcome back to the lecture series in Cell Culture. So, for last classes we have been talking about how we can measure using a micro cantilever system, how we can measure the force generated by the myotubes. Now in the last class we talked about that if the cantilever bends and if you have a way to measure the change in the angle of the cantilever then you can do so. So in order to do that there are certain setups which have been currently being used, here is the setup what I personally have the privilege of developing ok.

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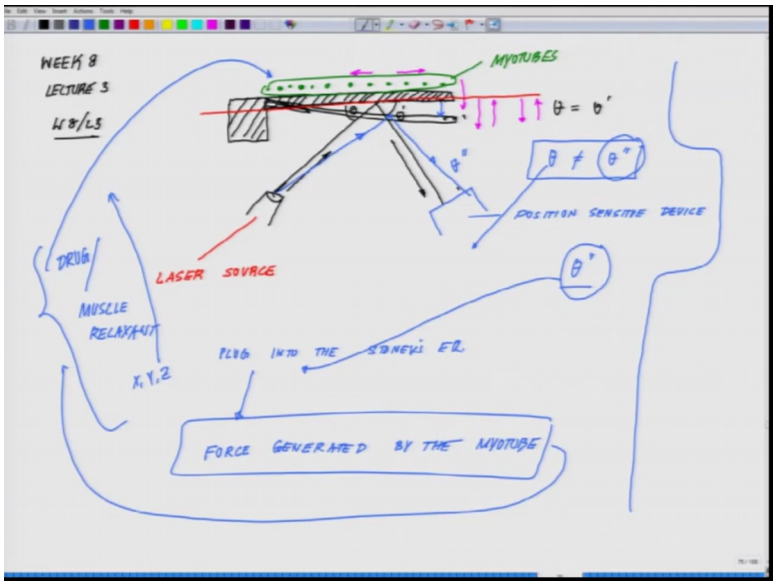

So, this is your single cantilever, what you do? You place a laser source here a very pointed laser source, we and a very benign laser source, what you do you shine a laser like this oh by the way this is our week 8 lecture 3, w 8 L3. You shine a laser on this cantilever and what will happen to this laser this laser will bounce back like this. So, this is how your shining it hits on that, silicon cantilever and it bounces back. So, now, once it bounces back you can track it by having a position sensitive device or some kind of a device here which will see at what angle this laser is bouncing back.

So, say for example, you are sending it an angle of say theta and it is coming back at an angle of theta prime. So, if theta is equal to theta prime, it means this cantilever is not moving or at that particular instant it was at the baseline. So, this is your baseline. So, now, you have a position sensitive device which will tell you what is the bend, and this is your laser source. Now on this setup you grow the cells assuming that they will form structures like this, which are the myotubes. Now these myotubes while will contract will generate a motion like this. So, they will deflect from the baseline and they will go back to the baseline, they will deflect from the baseline they will go back to the baseline.

While doing so, the cantilever out here will bend slightly like this very slight bending which happens in the cantilever structure. So, this is the cantilever line I am trying to draw force bending oh shit. Now during this bending what will happen? As this will bend the laser light will go like this, and it will bounce it will bounce back at a different theta double prime. Now the angle with which it is going is say theta and angle you are getting post bending is say if it is going are theta, you are getting a theta prime which are not equal. Now that stuff will tell you this value. So, if you know of course, you know this value which is 0.

So, this theta prime value which is the value what you have do plug into the Stoneys equation, and this will give you the force generated by the myotube. Now say for example, you add a say a drug or some other molecule like muscle relaxant or something say for example, you have a confusion whether this particular drug acts on neuron or acts on a muscle. So, you add something some that that x y z whatever.

Now, upon adding if you see the change in the force generated by the myotube, then in a very clean system in vitro setup, you will be able to predict how a specific drug is going to behave in a system like this. So, this is one of the most cleanest system we had the privilege of developing, where we really could show a technology how technology can change, the future of in vitro systems where a micro machine device is being used to measure the contractile force generated by the muscle. Having said this I told you that there is an electrical component involved in it.

So, let us talk about the electrical component. So, whenever we talk about myotube or a muscle they are governed by motor neurons.

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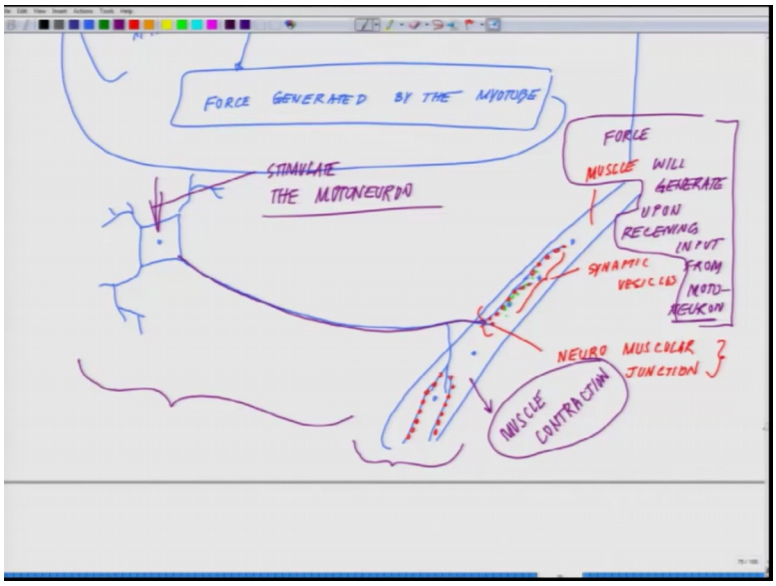

So, the way it is something like this say for example, here you have a muscle sorry here you have a muscle or a myotube growing like this and here your motor neuron which synapse on this muscle and at the synapse what you observe are the synaptic vesicle. So, here you have the synaptic vesicles or this particular part is called neuromuscular junction.

So, the significance of neuromuscular junction lies in the fact that, neuromuscular junction decides how much this muscle will contract. If the muscle is not spontaneously contracting muscle which most of our muscles are not the skeletal muscle, and it is the neurotransmitter which is secreted at this zone kind of decides, the force muscle will generate upon receiving input from moto neuron. So, having seen this now we are opening a little bit more complexity where you needed to have two different cell type in a culture dish or integrate it on top of a micro cantilever, I will come to that.

So, now we know how we can measure the muscle contraction, but what we do not know is if this muscle is not spontaneously contracting, how we can integrate it with a neuron which will make this muscle to contract by sending signal in the form of action potential, or you can even I put this electrode here to tell you can even stimulate the muscle its sorry stimulate the moto neuron.

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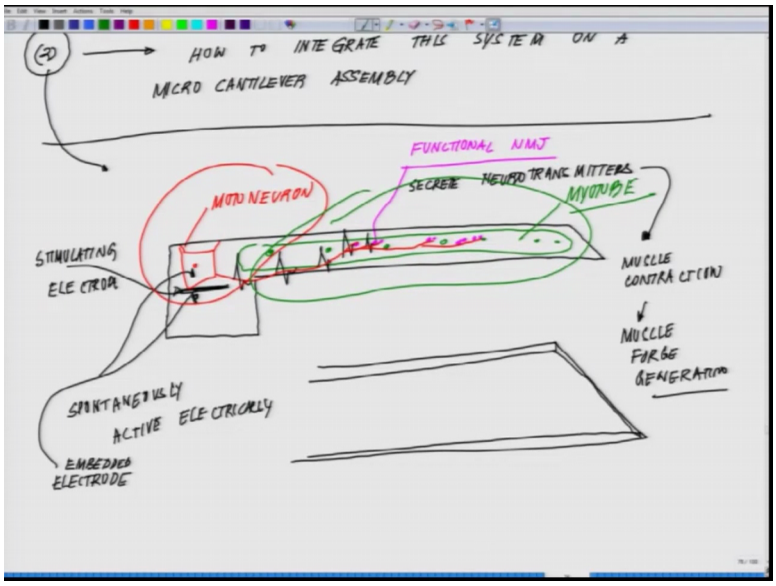

So, there are two problems here and lets write down the problems one, how to co culture a muscle and a neuron here a means a population right. Muscle and a neuron cell types to study neuro muscular junction which in short many peoples call it as NMJ.

So, how to co culture a muscle and a neuron cell types to study neuromuscular junction this is your problem is statement one, the problem is statement two is how to integrate this system on a micro cantilever assembly. So, before I get into it I will first follow this part what exactly is what you wanted to do at some point of time, which is still a dream, but something I personally believe in future will be resolved.

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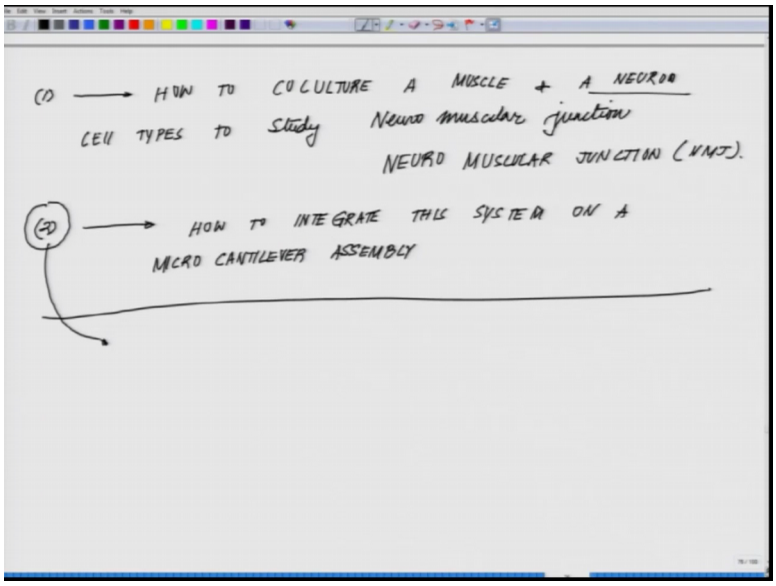

So, the idea was or idea is even till this date is. So, here you have a cantilever right I have already talked about in depth about cantilever sorry not touching upon that part, I am just showing the surface cut of the top, and the this is the this is the thickness what I was trying to tell you ok.

So, now the idea is you have a myotube growing out here a multinucleated tube and somewhere or other you integrate it with a neuron, which will be forming synapses on it which will be of course, a moto neuron and here you have a myotube. Now in order to do so, you are making this whole setup further more complex, then it is already there because not only that this muscle has to interact with the neuron or the neuron has to interact with the muscle, what is important is that they form the neuromuscular junctions here. Without a functional neuromuscular junction or functional NMJ or neuromuscular junction this whole process cannot occur (Refer Time: 16:41). This moto neuron has to be spontaneously active electrically or you have to have a way, by virtue of which you can separately grow them on top of some form electrode which will be in embedded into the system which is falls under the whole idea of embedded system, embedded electrode and your cell should sit on that embedded electrode and you can use this embedded electrode as a stimulating electrode.

So, the idea is you stimulate the neurons. So, neuron the action potentials will travel and will reach the neuromuscular junctions and upon reaching there, they will secrete

neurotransmitters. Those neurotransmitter secretion will lead to muscle contraction further lead to muscle force generation. So, what you need it in such a setup is you needed a common medium which will allow growth of both this moto neuron as well as the myotube. And there was not something which was really easy it is kind of because if you look at your own body the way it is, if you look at this spinal cord. So, here you have your spinal cord and your moto neurons are sitting in the ventral horn, and here you have the muscle.

So, this is part of your CNS, you have a different set of a spinal fluid and everything whereas, here this is outside that is where these junction are being formed. So, one of the challenge what we were facing at that point of time was, how to develop a common medium and. Secondly, once you develop a common medium which has to be again a defined medium serum free, how to plate the cells whom are you going to plate earlier, and whom are you going to plate later. This is another very important thing because you have to realize I told you that the muscle will go through this whole rigor or division like this is the paradigm they are going to follow, where as neurons they own divide they will be sitting there and they will form then your muscular junctions like this.

So, one has to make a call that how we are going to set up the structure so that we can extract the maximum information out of it. So, I will close in here in the next class we will further this is a story of neuromuscular junction and its challenges in the process.