Cell Culture Technologies
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Lecture – 37
Advance Cell Culture Modules – II

Welcome back to the lecture series in Cell Culture Technology. So, in the last class we are into the 8h week and we are starting our week 8 lecture 2.

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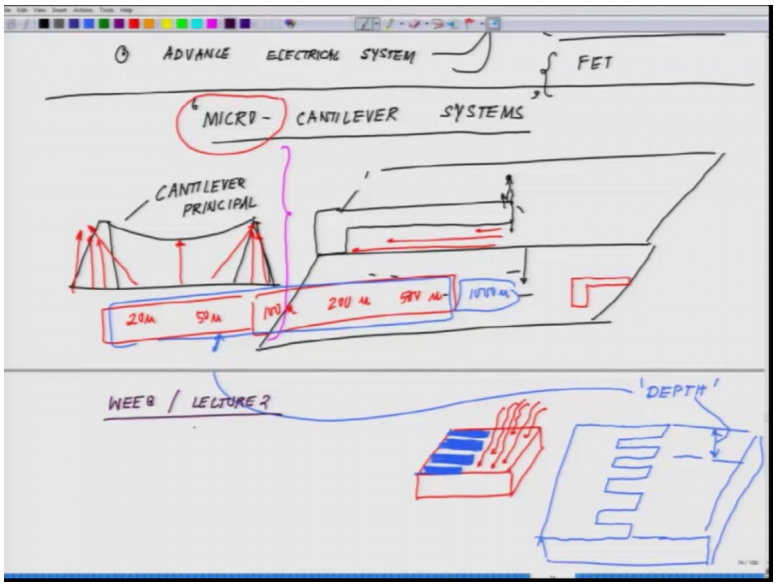

So, continuing with our previous plan We are generating or measuring the force generated by the muscle in an in vitro culture system.

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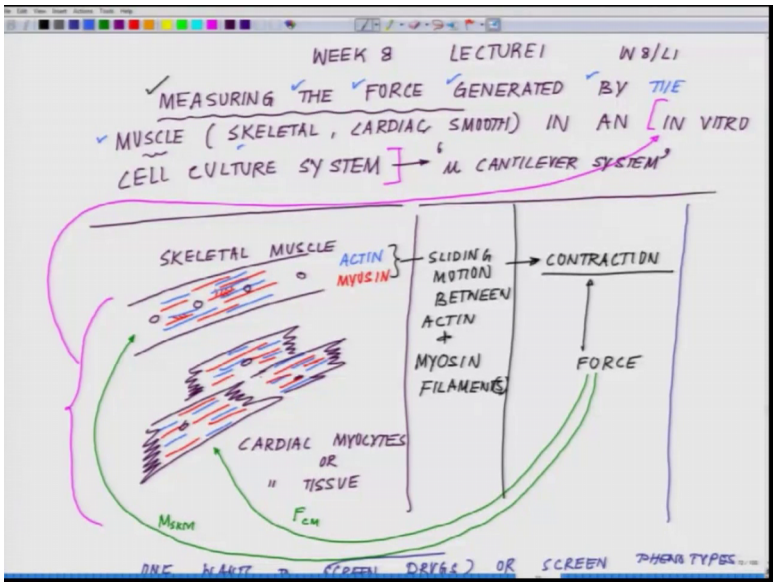

And in vitro culture system out here is micro cantilever system. So, as of now we have talked about the development of micro cantilever, how we do it?

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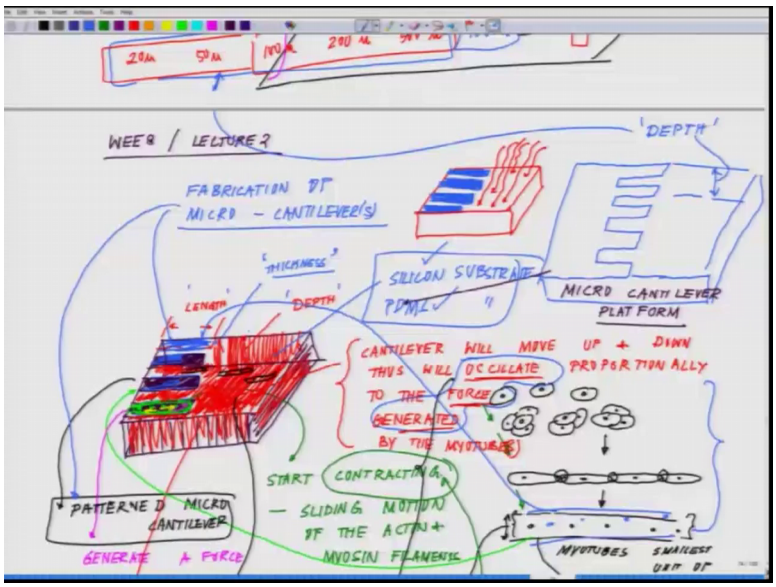

So, this is what we get micro cantilever plat form.

So now, imagine this as swimming pool with multiple diving board boards. Like this I am just putting 3 for understanding sake instead of you know, crowding it imagine maybe you could have a area of 20, 40, 100 like that. A fourth one you really cannot see. So, get a 3D perspective into it. And this is how it looks like. And I told you that there is

a depth into it. So, it is kind of a close thing, and there is length that is in your control. And this has thickness. That thickness is very important, I will come why that thickness is important.

So, this is how a micro cantilever system looks like. Now thickness of the cantilever decides, how much flexing it will show. In other word these cantilever is just like I give you the example of that diving board. Where there is this is kind of oscillating in an oscillation oscillating motion like this. So, this oscillation power at this hinge joint, how much it is going to move back and forth is a function of how rigid the structure is or how thick the structure is or how thinner the structure is. Thinner the structure you will see much more better motion.

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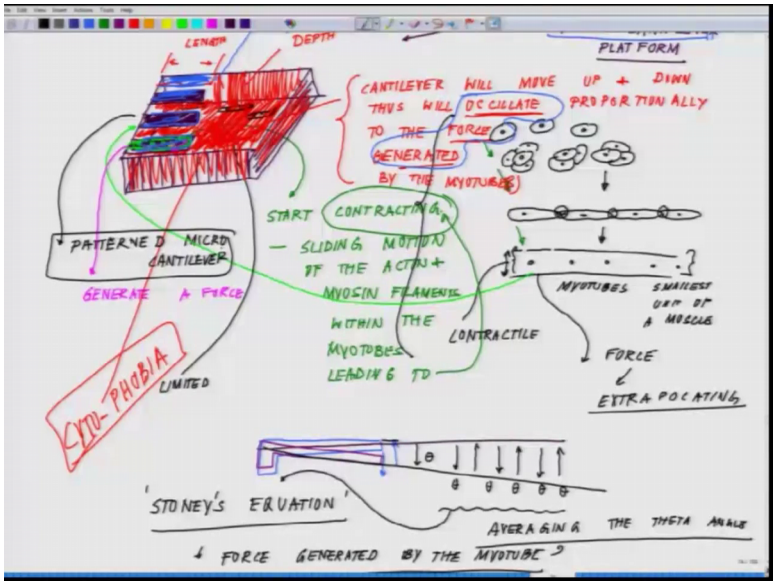

So, that is why this thickness does matter. Now we wanted to say for example, have a case a study, we wanted to measure the force generated by skeletal muscle.

So, again just let us visit the skeletal muscle biology as a backup. So, you have these muscle cells in a culture and that do we want to do in a culture right. You have these muscle cells. They divide spots I told you and upon division, post division they align like this. And post alignment they merge like this they lose their individual identity like this is the part they are missing. This is the part they are merging together and they form a structures like this, which are called myotubes. And these myotubes are essentially the

smallest unit of muscle. So, if you could measure the force or contractile force generated by the muscle, then you could have a fairly good idea about how the muscle will behave.

Now, one of the approaches you allow this whole process of these muscle cells going through this whole saga of forming myotubes on top of the cantilever. So, what we essentially happen is you played the cells on top of it I mean, like this the yellow are showing the cells. And these cells eventually will divide and will be like this and eventually they will form myotubes like structure like. This not myotube like structure they will form the myotubes another example out here. You have the myotubes getting form now these myotubes as we have already mentioned are contractile structures. These myotubes will eventually as their form they will start contracting. In other word there will be a sliding motion of the actin and myosin filaments within the myotubes leading to contraction.

Now this contraction once these myotubes start to contract here. So, the motion will be something like this, and this contraction will generate force. Now while this muscle is contracting on this surface. So, it is the same analogy as I gave you out here. A person is kind of you know thumping is like on top of the diving board. So, once this person start doing it this diving board starts to you know oscillate like this. So, if this analogy if a you have understood here is the second analogy I am giving you that the muscle while contracting will make this cantilever move up and down. Or the cantilever will move up and down. I do not see a side motion happening there, because it is already kind of in a hold that. So, this could have a moment like this only, it will be moving up and down thus will oscillate proportional, proportionally to the force generated by the myotubes ok.

So, depending on the force generated by the myotubes, there will be a certain kind of an oscillation which will be happening out here. Now while this oscillation is taking place. So, you can correlate 2 things. So, this oscillation is directly proportional to the force generated. These 2 words go hand in hand. Now while this event is happening? If you can some way or other measure.

So, say for example, if this is the cantilever you have. If somewhere other you can measure the change in angle in other word while this is oscillating, if this is your base baseline let me draw the baseline. If this is your baseline. So, while it will oscillate your next stage will be something like this.

So, there will be right, So, your baseline from the baseline there will be a shift of an angle out here which is we denote it as by theta hold on and then again it goes back. So, it came down and it comes back to it is ground original position. Then again it bend and again it goes back again it bends again it goes back again it bends again it goes back. And if you repeatedly measure this theta angle and most likely would it is not going to change, but even if there is a slight change in the theta angle you averaged it out all this theta averaging the theta angle.

Once you average out the theta angle you can use an equation which is called stoneys equation. Stoneys equation was developed long time back summer early 90s ninetieth century. I will leave it for you to look into, you can feed in this value of theta into the stoneys equation. And you can essentially measure based on stoneys equation the force generated on or generated by the myotube. And this force you can back calculate you will see it is approximately close to the force generated if I extrapolate the value. Because this is the value you are getting for a single myotube right, out here for a single myotube of a finite dimension.

Now, if you extrapolate the force generated by this, extrapolating you will be able to get a reasonably close estimate of the force which probably will be generated. Or which is being generated by the individual muscle. Now having said this is not a such an easy technology you have to realize. And while you make a well like this there is a very limited amount of medium which can be placed there. And that needs a lot of optimization lot of process which has to take place. And Secondly, there is another challenge to this there is no way that you can prevent the myotubes forming here and the distance is. So, less or the depth is.

So, profile So less that those myotubes may come in the bottom and can occlude the motion of the cantilever motion. How you can avoid it? The way you can avoid it is say for example, you coat this surface or you spray this surface is something which will not allow the growth of the cells. Some form of some kind of surfaces by which you are coating it. While the mask is already there you first of all coat it with such a way in such a way that nothing ever grows on these walls or not on these surfaces.

So, you are kind of ensuring that these surfaces are cyto phobic. They have a phobia the cells of a phobia to grow on these surfaces. And parallely you coat these surfaces with

something which will one second which will allow the growth of the cells. Now you have the cantilevers which will promote the growth and you have the background which will not promote the growth. If you could achieve that then for sure now what you are having is what we call as a pattern micro cantilever. And such pattern micro cantilever are very profoundly powerful tool to address the issue of using micro electro mechanical systems.

So, here you only see the mechanical part of it and I will introduce the electrical part of it soon. So now, based on it you can now let us go back what is our problem is statement. Our problem is statement was or is measuring I am going by word by word the force generated by muscle, whether it is the skeletal you can use cardiac you can use smooth in an in vitro cell culture system, which is a micro cantilever system. So, what we have learned here in these 2 classes is how using the modern micro fabrication technology, you can develop cantilever cantilevers micro cantilevers.

Here it is fabrication of micro cantilever, followed by how you can pattern such cantilevers. And after patterning you have a very finite space which is exclusively reserved on the top surface of the cantilever where you can grow the cells and based on your normal substrate data those cells should form micro cantilever sorry, should form myotubes. And what you do once this myotubes are form you look at them under the microscope of course, this is where you need you will you will be needing an upright microscope you remember the very earlier I told you that what all things you have to use. And most of these kind of cantilevers are made on silicon substrate or they are made on pdml substrate.

So, there are several ways there are other polymers which could be use PDMS polydimethylsiloxane is one such member which could be used for making this cantilever. You can make them on pure silicon substrates I personally have done it on pure silicon substrates. So, you have a mask. So, you take the block of the silicon you have the mask you place the mask and you etch the whole surface up to the level of thickness what you are wanting of the cantilever structure, and you use this structure to plate the cells on top of it post patterning So that it is not the cells will grow randomly all over the place.

Now, you have to place it in such a way that you allow sufficient medium to cover it. So, that these cells get enough nutrients to grow. And once they are form on top of the micro cantilever, you have to a range as setup by virtue of which you can measure the force which are being generated. Now what is that set up and what is that assembly? That still I have not talk. I have told you that if somewhere either you can measure the bending. So, how you measure the bending?

So, what we will do in the next class we will talk about how you develop that setup.

Thanks a lot.