Cell Culture Technologies
Prof. Mainak Das
Department of Biological Sciences & Bioengineering & Design Programme
Indian Institute of Technology, Kanpur

Lecture – 36
Advance Cell Culture Modules – I

Welcome back to the lecture series in Cell Culture Technology. So, today we are into the 8th week which is essentially the final week of it. So, the way I have designed this week is will have multiple a small modules of 20 to 21 minutes. And there will be few extra modules apart from these 5 classes scheduled for the week which will be adding up to it, which will help you to understand the current or I should say the most advanced technologies currently followed all over the world or at least emerging which will definitely change the face of cell culture technology in the next 20 to 25 years. And I wish all of you should be braced up for that, because that is your generation when you really have to know how these technologies can come very handy.

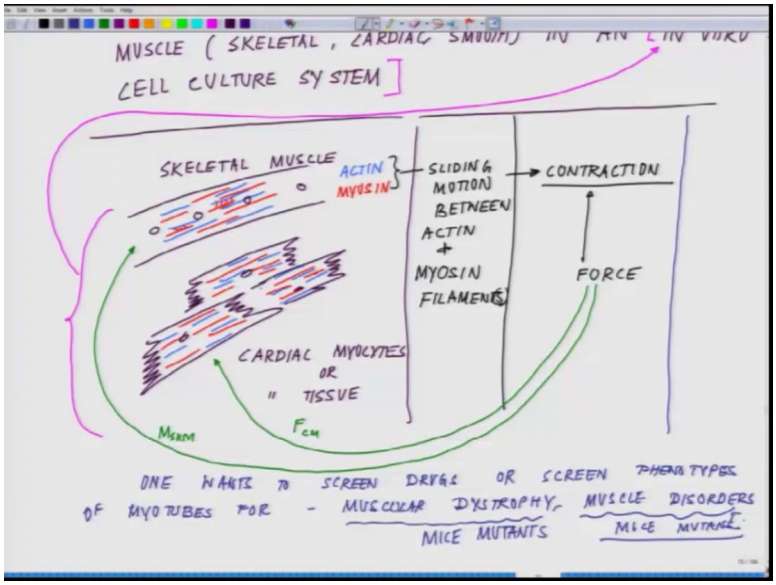
So, the first lecture today we will be follow up on what we finished in the last class. So, if you remember in the last class we talked about growing different kind of muscle cells. Skeletal muscle, we talked about the cardiac muscle, and we talked about how the sliding filament theory works and how the muscle contracts and everything. But what is important for us to understand most of these excitable tissues includes the neurons skeletal muscle, cardiac muscle, smooth muscle some of the endocrine cells, not only they have electrical activity they indeed show mechanical activity. Specially the cardiac myocytes skeletal muscle and I told you about the development of the skeleton muscle where they form small myotubes and myotubes becomes myofibers. Myofibers become what you see multiple myofibers joining together to become a muscle.

So, if they show contractile activity which is mechanical activity, anything will show some form of mechanical activity generates a force. So, based on the force one can essentially figure out what kind of muscle it is, in other word just to take you a little bit to the molecular side of it. While we were discussing the muscle I told you the muscles are made up of 2 key structural proteins, myosin and actin or actin and myosin. So, during sliding filament motion it is the actin and myosin chains are sliding over one another. That I told you that you can look through any textbook you will be able to figure

that out how that works or as a matter if you go to Google and say sliding filament theory, you will be able to figure that out.

So, this sliding motion of actin and myosin over one another is a function of the muscle type. What does that mean? So, the speed with which the sliding is taking place on between the myosin and actin, through the myosin heads is a function of the muscle type. Or in other word that speed determines at what rate it is going to contract. So, when we talk about the contraction we talked about the speed of this movement we are essentially talking about the force being generated by the muscle in that whole process. So, and that force. So, let me formalize it from of the text. So, so we are in to Week 8 and lecture 1 w 8 l 1 ok.

(Refer Slide Time: 04:14)

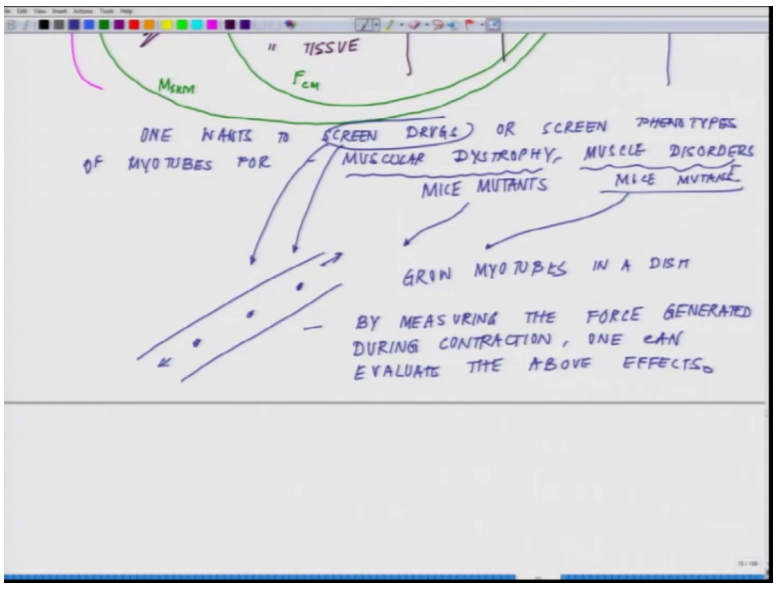


So, what we are trying to do today is measuring the force generated by muscle, it could be skeletal muscle, it could be cardiac muscle skeletal cardiac smooth muscle. Measuring the force generated by muscle in an in vitro cell culture system. This is the topic what we are going to discuss. And what all points I kind of highlighted, I told you that whether it is a skeletal muscle like this, skeletal muscle like this or a cardiac myocyte like this cardiac myocytes or cardiac tissue myocytes or cardiac tissue like this. They are made up of actin and myosin filaments. And these actin and myosin filaments slide over one another ok.

Actin and myosin and there is myosin of the myosin heads which are kind of you know connected like this like this. So, similarly here you have actin and myosin. So now, and these actin and myosin essentially leads to what we call as there is a sliding motion between actin and myosin filaments. These sliding motion between actin and myosin filaments leads to we talked about contraction. And this contraction is nothing but this contraction has a force. Now this force essentially determines this force is different for cardiac tissue, this force is different for this skeletal muscle. So, if this one I call it as fc this is f skm cm for cardiac muscle. Now how our challenging our question is how you can determine these 2 forces in an in vitro cell culture setup.

So now, having saved this why are we doing it? Whenever we try to do something one has to first of all justify. So, see for example, one wants to screen drugs or screen phenotypes of myotubes for muscular dystrophy. Or any kind of muscle related disorders or other muscle disorders. One needed a tool just the way we needed we have the cell culture tools which will tell you the cells are growing or not and how they are growing you needed a very specific kind of tool to do. So, a tool from where you can measure the force, how we can do this? So, say for example, you have this different kind of muscular dystrophy mice different mutant mice mutants muscle disorder you have different mice mutants mice rat whatever you want to use ok.

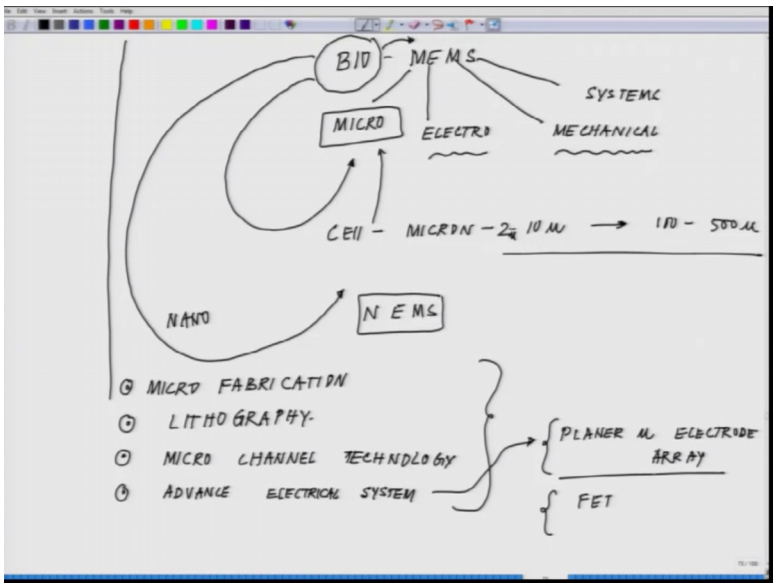
(Refer Slide Time: 10:36)



Now, what you can do is you can use these to Grow myotubes. So, I have already talked to you myotubes are the smallest unit of muscle in the case of skeletal muscle. These are the myotubes grow myotubes in a dish or you can suppose you want to screen drugs. So, you have this culture. So, you are putting different kind of drugs and you know measuring the contraction. So, what you are essentially doing is by measuring the force generated during contraction one can, one can evaluate the above effects. Now globally there are a different kind of techniques which are being used, but today I am going to share one technology which I had the privilege of being an integral part of developing the technology is called micro fabrication of cantilevers and using cantilevers for measuring the force generated by the muscle.

So, this falls under micro electromechanical systems or MEMS based systems which are very frequently nowadays used you all must have heard about this word at some point of other called bio MEMS.

(Refer Slide Time: 12:42)



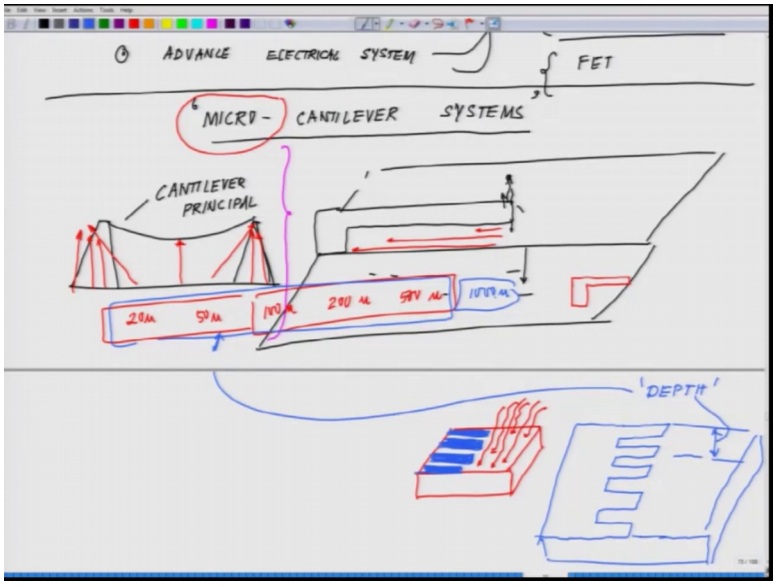
Where M stands for micro, E stands for electro, M stand for meccano, or mechanical, S stand for systems. Micro electromechanical systems bio. And when on these MEMS you have a biological component integrate to it, you have the possibility or you have the technical know how or you have a scope to measure any kind of mechanical as well as electrical phenomena in a micro system, because all your biological samples if you are dealing with. If it is a cell then we are talking about micron dimension anything between

you know 10 micron to it could be less than 10 micron of course, I mean say 2 micron to 10 micron to 100 to 500 micron.

So, these are all micro systems. If you talk about much more smaller system, nano then you have to go to NEMS. Nano electromechanical systems. So, restructures which you are making are very small structures. So, depending on at what range you are developing these devices. So, these are devices on which you are integrating a biological component. So, in other word the next generation cell culture where it is heading and that is what we will be discussing in these last few lectures is more on the area of technologies like what I will be talking mostly here. Micro fabrication lithography micro channel technology we will talk about this regarding hippocampal neuron micro channel technology and advanced electrical systems. Where we will be talking a little bit here and there about planer micro electrode array and a little bit literature I will give you about field effect transistors or f e ts in cell culture.

So, let us start of with micro fabrication. What is really micro fabrication technology and how it comes very handy in micro fabrication?

(Refer Slide Time: 16:06)



So, earlier just few minutes back I told you we used micro cantilever systems, essentially water cantilevers. Cantilevers are all must have heard about AFM right. So, cantilevers are something some structures like this. You must have seen bridges there are no pillars in between it is kind of a hanging bridge and they say they work on cantilever principle.

Those of you have heard about howrah bridge the old howrah bridge. Bridge is something like not a single support system. So, these are those bridges which are based on cantilever principle. In other word where all the force components are kind of know for like this ok.

So, cantilever essentially. So, this is just for your understanding sake I just gave it. Cantilever are essentially a very closest analogy what you can draw is imagine in a swimming pool you must have seen this diving boards right, there are diving boards like you know stand there and it moves like this. Imagine a diving board of a swimming pool. So, if this is the swimming pool you have out here this is the swimming pool underneath. So, this is where So, this is where you are standing. So, you must have seen a person kind of standing here and this moves like back and forth like this. And then this person jumps into the water. So, essentially this suspended plank on which this person moves this is called a cantilever which you can physically see. Now think of it I reduce the size of this cantilever to the level of micron.

And once I reduce the level to a micron level say I make it say 200 micron or I make it 500 micron or I make it 100 micron. Depending on how far I wanted to go I can go up to fifty I can go to 20 depending on again this all depends on what is your needs and requirements. When I develop this kind of micro cantilever. So, then these are called a small mechanical devices or micro cantilever devices. Now on such micro cantilever devices you can use such. So, the way you make it is. So, another question is how you really develop these kind of structures. So, there are multiple ways by which you can do it. One of the ways what we followed is say for example, you take a block structure like this we use silicon.

So, I am just giving an example of silicon and then what you do you have a mask which have a pattern like this. So now, you place the mask and using laser or other etching techniques it started to h out the surface. You are continuously etching out the surface. As you keep on etching out the surface the resultant stuff will be like this. You will have an array of micro cantilever which will be etched out from the surface of course, with a certain amount of depth profile which will be how there ok.

So, there is a depth component associated with it and that could be seen here. This is the depth. So, that depth could vary from say I would say anything between again anything

between these numbers depending on how much you want to do it. It could be thousand micron that you can always vary.

o, with this background I will close in the class in the next class we will integrate some of the tissues into it and we will see what all power the system can offering.

Thank you.