Cell Culture Technologies
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Lecture – 02
Philosophy and Complexity in Cell Culture

Welcome come back to the Cell Culture Technology. So, in the first class this is our second class of the first week, the first class I told you when we take a part of the tissue and dissociate them and grow the cells outside or even the tissue as a matter fact. World biggest challenge is how close are we to the in vivo setup or the conditions what are cell experiences inside the body just to appreciate that. So, let us start with is.

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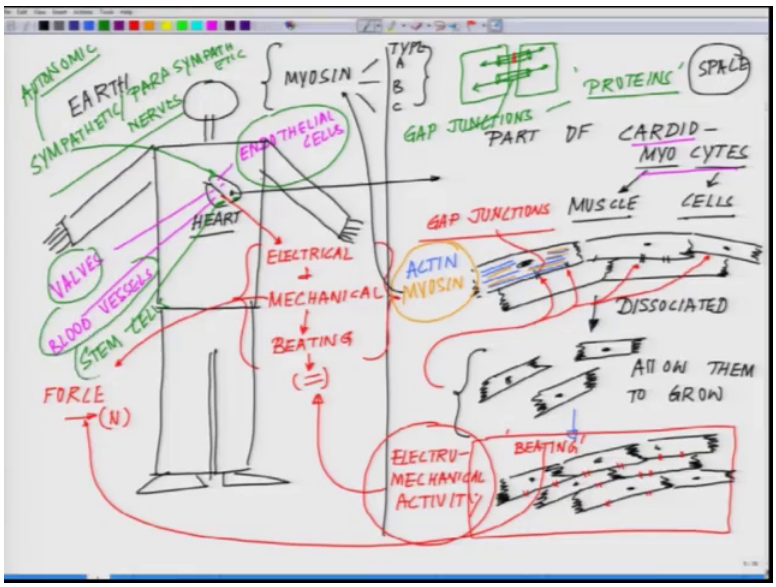

So, we are in to week 1 class 2. So, what we are asking is simple. So, say for example, this is say this is an intact in vivo system and say for example, I took a part of the cardiac tissue outside, this is heart and took part of Cardiomyocyte; cardio means heart myocytes means myo means muscle cardiac muscle and cytes means cell those of you who do not have the wide range of biology background, this is the breakup of that word Cardiomyocyte cardio means taken from the heart.

So, it take part of this and these (Refer Time: 02:33) kind of you know look like this, and there are connectors called gap junctions and I am getting to the detail of it at this point like this. Now I take this tissue cardiac tissue dissociate them into cardiac myocytes and

allow them to grow in a dish. I have dissociated them as I mention you, and allow them to grow. Once I allow them to grow outside the system what do I expect from them the first thing what do I do expect from them is to regain this tissue level site to architecture; when I talk about the tissue level site to architecture they should develop these specialized structure called gap junctions. Gap junctions are small tube like structure those are from biology, aware of those who are from non biology background. So, simple say for example, there are just imagine like there are two rooms, there is a room here is a adjacent room say for example, let me let me draw it is to make it simple this is one room and this is another room ok.

Now, if I put some connector like this. So, these two rooms can cross talk with each other right like this. These connectors are called gap junction in technical term and these are nothing, but these are proteins, these are membrane proteins which are forming these kind of junctions. So, cardiac cells have this classic feature they have series of gap junctions, and there is reason for we will come later in to that why there is those gap junctions are important because it is a structure where the electrical stimulation has to in a spread out in multiple direction like this, and it has its own function. And of course, there is one more thing this gap junction may be sometime gate it or non gate it. They may only open in one direction or they may not they may have both directionality. So, the first thing what I anticipate for these cardiac cells to join with each other coming close and form those gap junctions.

So, essentially what I will be expecting next to happen is this here. I have these cells coming close like this, like this, like this and forming those reforming those gap junctions are they doing that. Now next thing when we talk about cardiac, you touch your heart it is beating. This beating means these cells what you are saying here in your body these cells are essentially they are performing two function; they are forming a electrical function and a mechanical function. When I talk about mechanical function; that means, what I does telling is the beating and this electrical function and the mechanical functions are coupled with each other. If not these are independent thing that you know if I am doing this, I will not do this they are all inter linked with each other if there is an electrical impulse it will lead to the mechanical vibration or beating whatever you call that.

So, for these cells if they have to grow outside, and they should be in a position which should be able to mimic these electromechanical event in a dish. So, these cells I expect now they should be able to do that electro mechanical activity, similar to that of in your body. It should be similar even if it is not similar then we know there is a deviation, and we will have to quantify those deviation, it is not that is a negative thing it is a very positive thing then we know that we have a (Refer Time: 07:43) to improve and I will tell you why these are important, because these are really really important for those people who dream of artificial organs. I mean in futuristic world where they say we can replace the heart yeah its sure, but we do not know the basics we would not be able to achieve that goal or people say that we can replace a kidney, we can replace something I mean there is a whole password all over this tissue culture world are you know re general medicine, but the fact is this all lies in the basics.

The basics are not clear these are fancy dreams, I will (Refer Time: 08:17) one is living is a fools paradise without knowing the basics. So, we have to really know what all you can achieve and I will tell you that why a times people just get (Refer Time: 08:28) without realizing the ground realities, there is certain ground realities. So, it will generate an electro mechanical activity. Now this electro mechanical activity your first evidence you should be able to see on a dish under microscope, that these cells are beating they are not beating it means yeah you grow something, but they are not functional.

Now if they are beating it means they are generating certain force right it is a mechanical event is a four generation. Now whenever we talk about force, we are talking about some Newton value right something. So, what is it could be nano Newton, it could be Pico Newton, it could be whatever. So, there has to be some unit at what rate at what unit this is mechanical activity is, because that will again tell you that how close you are to the real life my heart is beating.

So, part of my tissue which is going outside should exactly behave the same way. Now if we have to correlate this then we have to understand. These beatings are governed by the two major proteins of muscle, which are now let me put them in those are actin and myosin which I am putting in two different color orange and green. Those are actin and myosin filament, and as a matter of fact depending on the quantity of four generation, the myosin filament sub type changes. In other word what I means to say is the myosin present in these muscles of my body, which are the skeletal muscle are entirely different

force dynamics, then the force dynamics of the cardiac cells or cardiac myosin which are present. There not only that within this muscle there are certain structures or muscle spindle, they behave entirely differently their myosin properties are entirely different.

Similarly, there are myosins in your gut the whole smooth muscles which is lining the hole or which is forming in the hole alimentary canal, where you are eating the food and the food is moving through a (Refer Time: 11:13) moment across the digestive system, those myosin's are different, but it is that myosin critically acting does play a role, but it is this myosin sub types, which dictates say type whatever fast type slow type whatever. So, I am just putting a b c just for imaginary sake I am putting it like that. So, these cells which are growing in the dish now, should express those myosin's which are really present here, then only there is a possibility that they will be generating the similar force as generated inside the system. So, you realizing that when you are trying to build something outside this is something, suppose of this is on earth you are trying to grow something and if you are growing this is totally different environment for it. It is any space total different it has no clue why it has no clue think of it for a minute.

Now, this heart which is here, it is surrounded by not only heart has only cardio myocytes, the heart consist of endothelial cells then there are cells which are forming the valves, there are blood vessels, there are sympathetic and parasympathetic nerves or rather autonomic nerves, there are stem cells cardiac step cells which are present there. So, these cardiac myocyte what you have isolated are coming from a (Refer Time: 13:24) not I mean it comes from we more than several surrounding cell. So, it means you can imagine it like this, as a cartoon way you can imagine like that suppose there are houses.

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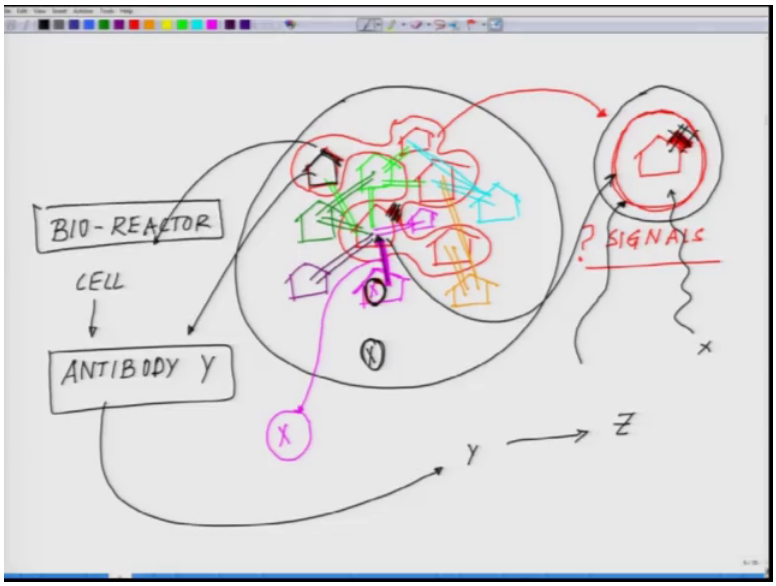

I am just trying few houses for (Refer Time: 13:42) and that will that will help you to appreciate it, you are seeing different color of colors houses what I am drawing through. Now it is a kind of a colony where every house have different color and yet some houses which are of similar color. So, you see red you see light green, you see dark green, you see Prussian blue likewise.

Now what I am asking is that I only wanted to study these houses in isolation and that to not in this system outside the system. Now the things is that one can study that is really no problem, one has to keep in mind that there is always a interaction between this between this between this between this, there is a interaction between these ones, the interaction between these ones, these ones. So, when you are trying to study these red houses in isolation, you will be missing there will be signals which you are missing and you have to accept, this error it is not an error rather it is a acute system you are growing. So, in you may not be able to achieve the exact perfection it is not possible I mean its accepted, because I do not know these many interactions which is happening, but this give me an opportunity when I am going this in isolation i.

So, say for example, just try to take an imagination slightly beyond it say for example, I given hypothesis I say these pink color houses. You see this pink color houses these pink color houses are communicating with this red color houses by a compound called say by some means say x. So, this is my hypothesis I said fine and I say that when x is sent to

these red house the red becomes red develop something say you know develop another roof like this. So, it is fine if this is my hypothesis how I can prove this hypothesis, whether this is really true or not. So, what I do? I have to fist a of all figure out what is this x compound, and I grove these in absolute isolation and then in this I introduce that x, and I wanted to see does the x develop that additional roof or not. If it does so that means, whatever I am seeing in this milieu or whatever is my guess is true, if it is not that it means it is much more complex than I am understanding.

So, you see we open up a problem and this is how we have to understand that cell culture, tissue culture, explants culture whatever you call it, these are acute systems these are not I mean one has to be very cautious and careful that what kind of data are we interpreting out of it. Because once there in isolation say for example, these cells just talk about a reverse situation, these cells are not allowed if this x whatever you talking about this x thing, this x compound is secreted by this pink house to ensure that if this does not form this kind of a structure, is not allowed to from this second roof. Now I say well if this is true, then if I grow this in isolation it should be able to form this roof second roof if this is true.

Then it means that x compound is important and you can do the reverse experiment you put x here, you should not be able to see this growth. So, realizing that water profoundly powerful tool this could be provided you ask the right question, and if you do this whole process like just like a (Refer Time: 19:02) technique taking I just follow it without even putting my brain into place, you will end up with of course, you will get some results I am not denying that, but something which may be very ambiguous and many other people may not able to repeat it.

So, one has to realize that before using a tool one has to understand how close you are to the in vivo condition or you do not care, there are people who use cells there is another group of people we talk about them, who use cell as a simple bio reactor what do they do? Say very simple all of you heard about antibodies right, we get antibodies production of antibody. So, I say for example, these cell type and the I am representing the analog of this these red cell types which are there, they produced antibody say you know y. What I do I take out these things, I understand the condition under which and I produce terms of antibodies for some zee function. I can use the cell as a simple in that situation I am

using the cell as a sample by reactor, and there are many such studies in biotechnology where people use them as bioreactor, we are just producing unit as a matter of fact.

Lot of oil production which is happening the algae we are using algae as the bioreactor, because it is producing algae we have isolating the oil and that is it. So, we are not really bothered about it, our goal is very clear we are using this as a tool; simple tool produce some xyz compound of our own commercial, medical, significance full stop. So, unless you know what you are asking for. Unless you are sure the question you are asking and another thing here, when I talk about when I was telling this, this one I made this complex picture in front of you I told you this is this is the real situation. So, how far is your culture?

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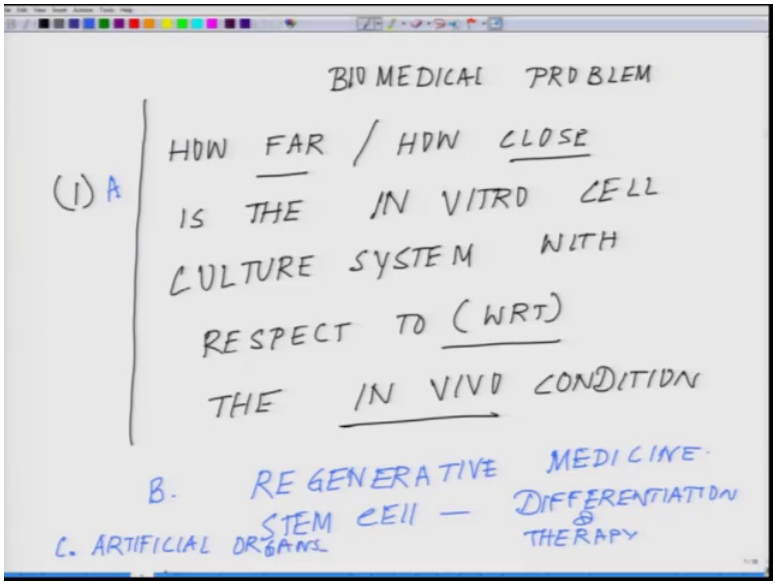

So, one thing I have to realize fundamental question what you would ask how far or how close is the invitro cell culture system with respect to the invivo condition. This is one fundamental question one have to ask, provided if you are working on some biomedical problem ok.

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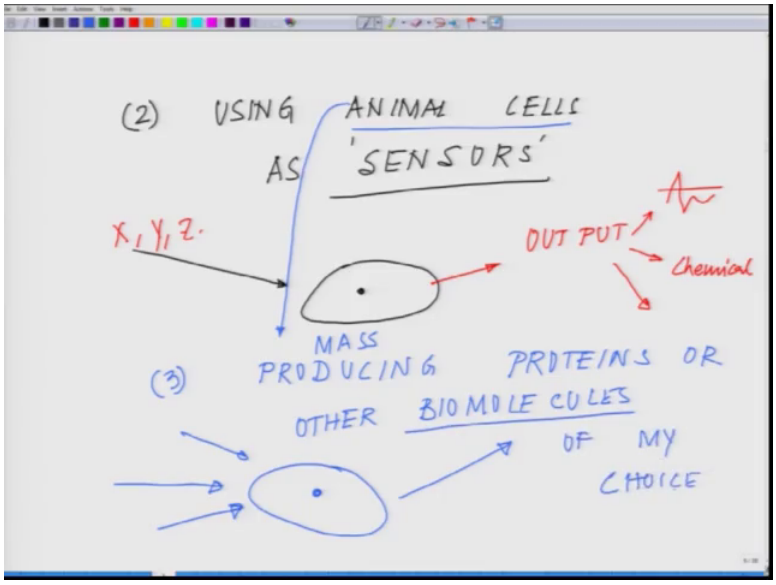

This is one situation the next is two; using animal cells as sensors what do I mean by sensors say for example, you can use most of our cells are very sensitive to different kind of toxins. So, I have this culture dish put that toxins I know how it reacts. So, based on that I am using this as a simple sensor device for testing for a screening or as some condition where I am really I am not sure what this toxin is going to do I use this as a simple sensor. So, I use the cell this is your cell, I use this as a sensor system. So, I have this compound say x y z whatever, and I have an output here to measure; what is the output that could be electrical output, it could be chemical output or it could be some other physical parameters within it, similarly I can use animal cells for reducing or mass producing proteins or other by molecules.

So, in other word I am using cells giving them the right set of conditions of course, you one has to study through cell culture, to produce by molecules of my choice. So, you see technique is one, but one has to ask the right set of questions or I study there is another side which is previous to this one I put this is A as a B side. I use this as a regenerative medicine, where which is one of the very fashionable topic currently and the stem cell differentiation and therapy, and which eventually may lead to artificial organs. So, one technique which most of the people considered technique for me it is a follows a (Refer Time: 25:56) a subject. Unless you philosophy is it properly you will never be able to know the power of this in its tremendous power trust my words it has tremendous power, but one has to know what you are doing. So, the whole solve purpose of introducing a course which otherwise always believing as a simple you know when you go to a lab,

you will learn from this seeing you know this is how yield culture cell, that is it you know that is just a technique.

Let us come out of that mind set absolutely, next embrace this from a different word try to imagine it can create a structures. We can create next generation sensors, we can create the cheapest sensors we can have we can produce our own set of anti bodies in an artificial synthetic systems. Let us think beyond this because let us use cells as a tool, we govern them we tell them what we wanted to do, we tell them then which part of the DNA we want to put you know transcribed. Let us go take it to that level where we can truly see we are doing a cell engineering when here is the platform, where we can do the cell engineering right within these limitations.

So, with this hope we will be proceeding further in the next class, we will talk little bit more about these conditions. We will take over from one of these pictures that what all the forces, what all you have to take into account we are in (Refer Time: 27:29) talk about synthetic systems in vivo systems, when you are translating what all you needed there is a biology behind this whole thing what are those biological parameters, and I will try throughout the course I will try to make it very generic. So, that anybody who is the viewer of this course, what is ever field they are, it should be tangible to them.

So, they are with my using the simple languages instead of using technical jargons, which I am not going to use towards the course and if I use I am going to write it down here thank you.

Thanks for your patience listening.