Cell Culture Technologies

Prof. Mainak Das
Department of Biological Science & Bioengineering & Design Programme
Indian Institute of Technology, Kanpur

Lecture – 24
Drug molecule testing

Welcome back to the lecture series in Cell Culture Technology. So, we are on the fifth week and today we are initiating the fourth class of the fifth week. So, if you remember in the last class we talked about our case study where a company who has a set of molecules or drug molecules which it wishes to test for Alzheimer patients. So, in order to do that, the first level of tests will be on the cell culture plates whether it is working or not.

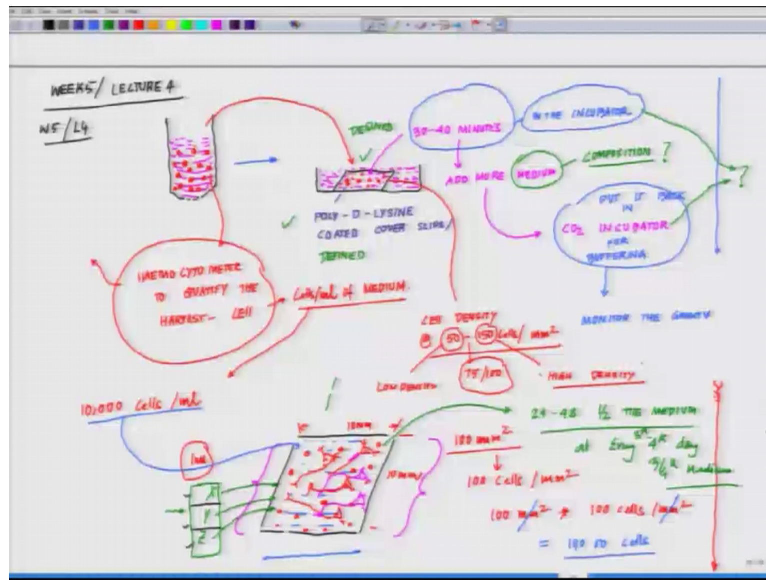
So, we reached up to the point how to set up a cell culture or neural cell culture of hippocampal neuron, since these are the neurons which dies during Alzheimer's disease. So, there is a neurodegenerative it is basically Alzheimer's is a neurodegenerative disorder, which leads to the death of hippocampal neuron over a period of time, and eventually the individual loses his or her own identity and eventually they die.

And it is a kind of a very very sad disease because you are you because you know your name or you could identify yourself in a surrounding, but in an Alzheimer's patient you do not know who you are, because all your memory traces fades because of the death of the hippocampal neurons, and there are a lot of efforts across the world to understand what these kind of disease is and since it picks up at a later age.

So, you need a lot of care and it is especially for such patient, it is very difficult to hold them at home because you know if you are going out for work any other elderly person who is suffering from Alzheimer's there are a lot of issues, because you know they may get lost they may go out of the house and they may get lost because they would not be able to identify their own house. It is a kind of a very sad and a very sorry state of affair.

So, with this background we were kind of you know developing a case study that, what a company can do in terms of testing the drugs. So, we will into this part. So, this is where we where just before I start

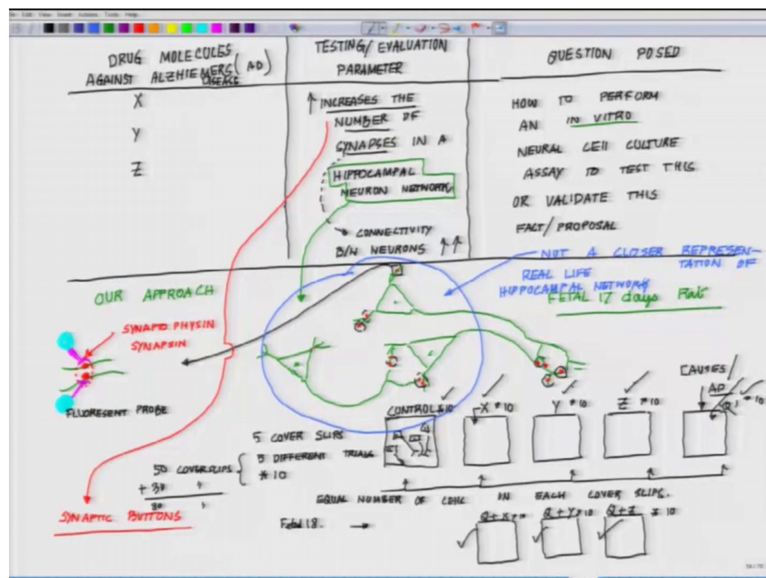
(Refer Slide Time: 02:52)



So, today we are initiating our week 5 and our lecture 4and W 5 L 4 fine. So, now, say for example, to keep it simple you have three different drug molecules x y and z right.

Now, these three molecules the company believes could influence or could help in those Alzheimer patients. So, in order to test that you have to have certain parameters what are the parameters say for example, again this is whole hypothetical situation.

(Refer Slide Time: 03:41)



So, we will be going by which set the parameter we say x y z these are the drug molecules fine drug molecules against Alzheimer’s.

So, what are the testing parameters or evaluation parameter testing or evaluation parameter; here are the evolution parameters. So, it is believed that all these three drugs increases the number of synapses in a network, this is all hypothetical we are talking about number of synapses in a hippocampal neuron network, in other word what will be. So, increase in number of synapses further, what this will lead to connectivity between neurons increases right ok.

So, the question posed question which was posed in front of us as cell culturist is how to perform an in vitro neural cell culture assay, to test this or validate this fact or proposal and what is the proposal? That it will increase a number of synapses.

So, what all we did our approach now comes now change the color our approach I am just now systematically putting it in front of you. So, we needed a hippocampal neuronal network. So, this is all in vitro in vitro. So, we do not have to worry about the animal studies at this time. So, we reach this part that we have a pyramidal neuron culture system, which a random culture system is no as such order or anything. So, we developed a culture system, using fetal 17 day rat and this is our hippocampal neuron culture.

So, whenever we talk about this increases the number of synapses. So, what we are talking about the synapses actually form between or dendrite and an axon or even an axon and axons in other locations which they form synapses. So, let us identify the synapses by say let me use red color. So, these are the zones where synapses will be forming and this may be getting some input from somewhere or ok.

Now, you culture the cells a finite number of cells using on four different cover slips, you have to of course, increase their application number. So, I put 5 there is a reason why I put 5. So, these are five cover slips or in other word you can say 5 different trials. So, one of and on each one of them will be say replicating them say you know 10 times.

So, we will be getting obtaining data from say in this case 50 cover slips fine making sense. So, we have 5 different case studies. So, our three cases studies are already defined x y z and this is your control, and this one this particular one this one could be a something which causes. If suppose you know that it causes that a specific disease which causes or hunch there is a hunch that it causes ad some other molecules Alzheimer’s disease ad stands for Alzheimer’s disease let me put that in case you forget ad ok.

Now, some molecules say Q right just a random molecule this is all hypothetical now on each one of this. So, you have for each you have multiplied by 10, multiplied by 10, multiplied by 10, multiplied by 10. So, you are having some 50 cover slips with five different possible treatment out here you what you do you plate equal number of cells and all of them on each cover slips.

So, you have to ensure couple of things while you are doing that you have to ensure the source animal are of the same age they are all your fetal 18 and you are picking it perfectly under the same conditions you have to grow all of them. Now you have all the cells and you have to decide when are you going to put your drug in the beginning or later or whatever ok.

Now, as a first set of control what you do, you this is control with nothing and you add the drug and you see whether in a without any damage in the cells is it increasing the total number of synapses, how you get validate it? You can validate it by counting the total number of synapses, you can do that by fluorescently labeling the synapses you can. So, whenever we talk about the synapse it is a zone where say this is one neuron this is another neuron and they are forming synapses. So, it will be a something like this.

So, there are fluorescent tags synaptophysin and synapsin, synaptophysin, these are the proteins which are present there synaptophysin synapsin there are several others. These are the proteins if these proteins are present there and if you have a antibody against this protein. So, the pink color is showing the antibody and if this antibody is further labeled with a fluorescent row.

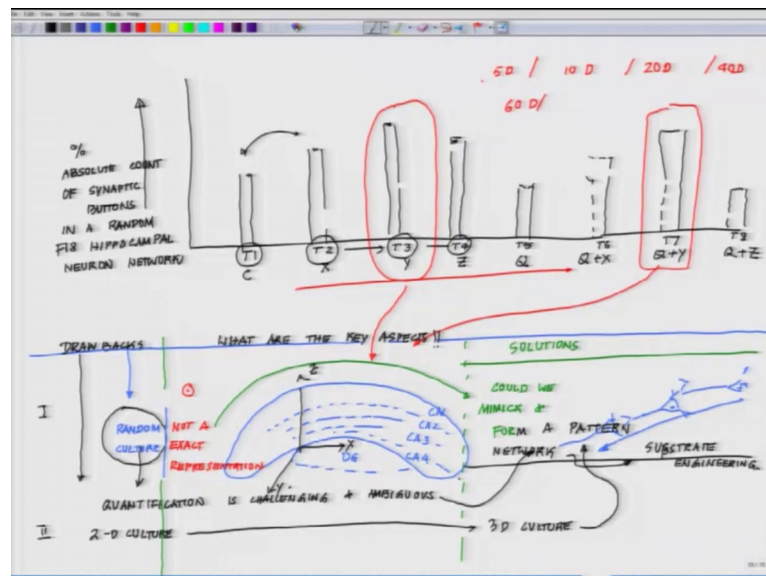
Say for example, I showed the fluorescent row with this color this is the fluorescent row out here this is the fluorescent row. So, what you can do essentially you can count these are called synaptic buttons, you can count the total number of buttons you have to do a bit of a statistics here you can count the total number of buttons and you can say without anything if we add this x or y or z what is the total number of synapses, in per unit area and whatever ok.

That I will leave it up to your which so ever, way you wanted to do that analysis followed by you have this negative control here; obviously, if there is some damaging element you add the damaging element Q, and you see the reduction in number of

synapses synaptic buttons. So, what you are quantifying? If I go to the quantifying what am I quantifying here. So, I am quantifying this in terms of synaptic buttons.

So, now you see try all this three then you have this Q and here you realize you have to increase, three more treatment though three more treatments are. So, which I did not in the beginning told you now I am adding them what do you do? You put the Q plus say x, Q plus y, Q plus z again all the applications are 10. So, you realize we are now talking about you already have 50, now we are talking about 50 plus 30, cover slips you have 8 cover slips to look into this whole thing and it is a very random network.

(Refer Slide Time: 15:13)



Now what you how you have to quantify if you go by the statistics, so you have something like this, you have 8 different treatment with you right if I go back 1, 2, 3, 4, 5, 6, 7, 8, a different treatment. So, T 1, T 2, T 3, T 4, T 5, T 6, T 7, T 8. So, this is your control this is x this is y this is z this is q, this is Q plus x, this is Q plus y, this is Q plus z.

And here you are either you go by percentage or absolute count of synaptic buttons in a random F18 fetal 18 hippocampal neuron network. So, now, you have some percentage. So, you will see certain values coming suppose I assume y performs the best z is somewhere in between. So, you can have the bar graph like this something like this, then say for example, Q really brings it down just hypothetically I am just putting say for

example, Q here you see some sort of an improvement in say why you see a much more improvement and here you see some improvement or something not ok.

Now, what you have to do all these eight treatments you have to do a comparison, is it significantly different like is T 1 significantly different from T 2 vice versa, T 2 significantly different T 3, T 3 to T 4 likewise since T 1 to T 4 and you have to do a whole lot of statistical analysis to come to the point that out of all these things which one stands out or nothing stands out based on that see say for example, we say ok.

Now, we have this promising thing with say this one say for example, then what we will do this molecule will be taken for the next level of trial, but this has to be repeated several several several times, and not only that this has to be done over time window say for example, for how many days this acts say for example, this culture is this culture of say 5 days, when the assay day is being done 10 days 20 days 40 days 2 months 60 days ok.

So, at different time point how this whole thing is happening, this needs tremendous amount of support in terms of the cell culture and expertise in it, but then when we saved this let us see what are the problems you are going to face in this. There are certain problems which are going to emerge and that is what I am going to highlight now, what are those interesting problems what you are going to face.

The first problem you try to realize this is the word I have been using very repeatedly is it is a random culture right. Now your hippocampus if you look at the tissue if you remember when I showed you the tissue. So, you have CA one CA two CA three CA four you have the dentate gyrus sitting here dg CA 1, CA 2, CA 3, CA 4 all these pathways which are rolling this is a very very systemic pathway something like this. So, very very unique systematic pathway.

So, when we talk about a random scenario, what I showed you in the previous slide if you look at it is a very random network. So, this is not the true representation this is not a closer representation to not mark my word, not a closer representation of a real life hippocampal network yes it is part of it, but not close to the real life ok.

Random culture not a exact representation point one. So, challenge could we have could be mimicked could we mimic and form a solutions. So, I think solutions possible

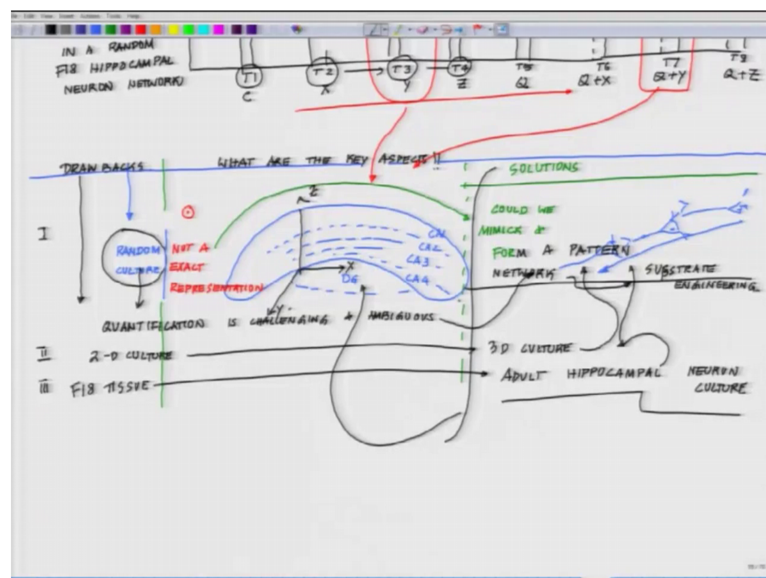
solutions of course, not solutions should be possible solutions. So, this is the we are talking about the drawbacks here, drawbacks what are the key aspects.

Now, solution could be mimicked and form a pattern network point 1. Now that will take us to the domain of substrate engineering. Second what is the second drawback if you look at it is a random culture first second inherent drawback with the system is in a random culture quantification becomes very difficult, quantification is challenging and ambiguous, that can only be resolved if we have a pattern network. So, they are linked.

The second major drawback is what we have used is 2D culture. It is a two dimensional culture, but this whole structure is a it has a z axis right it has a z axis, it has a x axis it has a y axis fair enough. So, where is the z axis now could we make a three d culture and the challenge will be could we make a 3D pattern culture that is where the future is.

You are almost trying to mimic that organ in our dish and that will take us to lab on a chip concept third point now three d and 3D pattern. So, we are taking one step and the most challenging part of the game is something else.

(Refer Slide Time: 24:45)



Now, what you are using is an F18 tissue, but they all close in Alzheimer does not happen to a fetus right it happens to an adult.

Now, you want it to represent a disease which causes to an adult, by using a culture which is of fetus that is something really ponder upon are we representing the right edge

by picking up that model. So, what are our options here is our option, you have to do this in adult. Now adult hippocampal neuron culture and this adult hippocampal neuron culture further if it could be in three dimension as well as a pattern.

So, all the three together that will be the bingo if we could really create a model, which will be almost I am not saying the closes, but definitely will be closer than what we are doing today. So, you realize we took up a simple case will close in here in the next class we will talk about this.

Thank you.