**Cell Culture Technologies**
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**Lecture – 26**
**Cell Seperation & In Vitro Myelination Cell Culture Mode – I**

Welcome back to the lecture series in Animal Cell Culture. So, last week if you remember we talked about the first level how we can create a system for adult neuronal culture. So, on the week 6 we will continue with that and the different issues different challenges and what all modern techniques what we can use and as a matter of fact, this week as well as the next week we will concentrate on some of these current technologies which are being followed. The idea is here we are taking examples of neuronal cells because these are the most fastidious one the most tedious one, but if you can get a hang on these cells then the basics remains the same, for any other cell type you really can translate it that is the whole reason why I am kind of seeking care because there are several problems what you will be facing while will be culturing neuronal cells.

First problem is they are extremely challenging regenerative ability in a culture dish even if you can regenerate them how good neuronal morphology will be regenerated is the second level of challenge, third level of challenges these cells have unique connectivity by virtue of which they cross talk with their surrounding how you can regain that connectivity. As if you remember in one of the slides we mention that how we can pattern them followed by even if you can go this part you have to ensure that these cells are electrically active.

So, how you ensured that these cells are electrically active in the culture. So, there are several problems. So, while we are developing in vitro systems one has to keep in mind that these factors are being taken into account so that you get a robust model to explore and emulate the adult or the embryonic or the fetal type of conditions. So, in the last class of the week 5 that there is a flip actually if you remember where we ended was let us have a recap.

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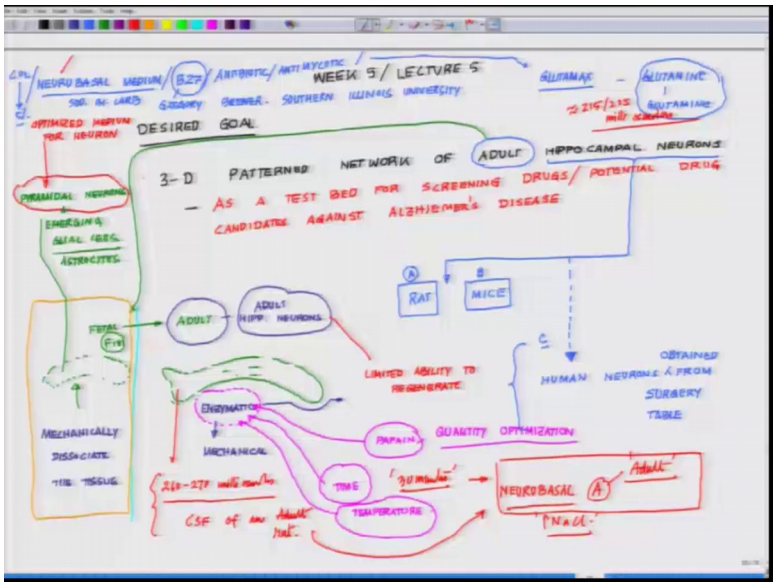


So, this is what we are targeting 3D pattern network of adult hippocampal neurons as a testbed for screening drugs potential drug candidate against alzheimers disease. This is alzheimers disease here is a test case that is it there is nothing I mean you can make it parkinson in that case your hippocampal neuron culture will become substantial niagra or you can take an example of say liver cell culture the disease will change or you can say pancreatic cell culture the conditions are going to change, but end of the day what one has to understand is most of these things are self assembled pattern 3 D structure.

The two three things - self assembled because nature has its own roots of scaffolding and forming a three dimensional structure second it is three dimensional third it is a three dimensional geometry which is dynamic it grows if there is an uncontrolled growth then it is called tumor or cancer or and this three dimensional geometry is cross talking with all its part, so there is an internal communication channel which is happening irrespective of which cell it is further this three dimensional assembly whichever form it is whether it is in the brain whether it is in pancreas whether it is in liver whether, it is in lungs it is interacting with other systems like endothelial system which is supplying the blood and it is interacting with the nervous system which is giving its necessary impulse how to act how to react it is interacting with the endocrine system it is interacting with its own other types in an around.

So, in nutshell what the future if you ask me that where really all these is a small bits and pieces of informations are taking us and all the studies and I am going to shares one of you have requested me I have not send that, but I am going to upload them very soon. One of the critical part of all these things that we are trying to rebuilt a system in a dish a small prototype of it maybe in a two dimension and in future in a three dimension. So, in a way we should have a miniaturized testbed which carries mark my word which carries the minimum signature bare minimum signature of the whole system it is like representative signature that say xl or x neuron does these these these things in its whole total context, but here I have a small prototype which can perform these rate limiting bare minimum functions.

So, in case of neuron one such rate limiting bare minimum function is these neuron should be electrically active and should fire if they fail to fire then these cultures are of no use. So, in other word they should be able to do their job of synthesizing electro transmitters and discharging the transmitters and thereby allowing the electrical impulse to carry from one neuron to the next neuron and if we could miniaturized in such a way that it follows a path. Apart from it the problem becomes way more complex and these are the points which I haven in really discussed is say for example neurons are lying in layers layer 1, layer 2, layer 3, layer 4.

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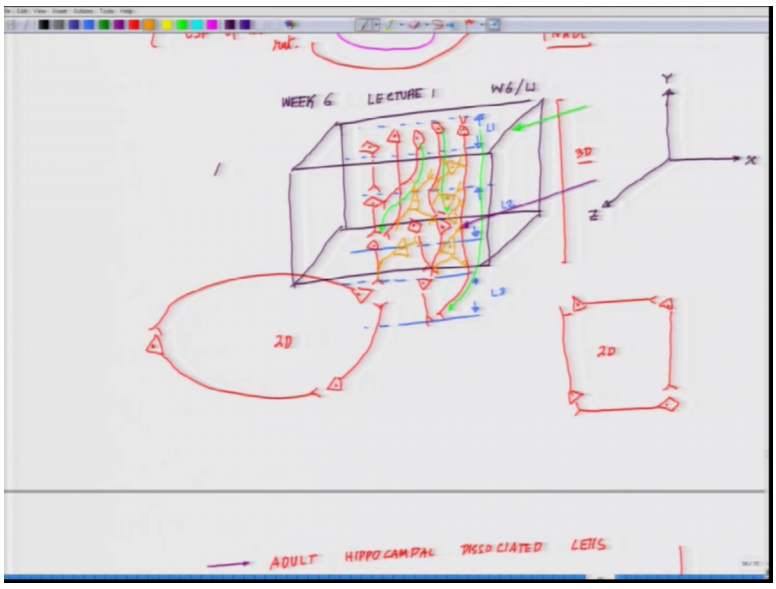

So, it is something like just in just add a point that we are into we are into week 6 and lecture 1; W 6 L 1. So, what I wanted to say, say for example. So, these neurons are kind of sitting like this let me draw a box to give you that feel exactly what I am trying to tell you. So, this is a three dimensional structure that is how brain is this is your x axis. this is your y axis and this is your height or the z axis. Now the way they are arranged is something like this. So, for example, in one layer one neuron. So, this is forming a contact here, this one is forming further a contact here, maybe another layer in the nearby me directly go and form a contact here, another neuron sitting in this vicinity make criss cross and form a contact here we just look at the complexity and just in a small section I am putting up the complexity is now imagine that how really a 3 D network will really look like. So, you see that if we try to demarcate them as different layers say for example, this is layer 1, this is layer 2, this is a layer 3.

So, now, we do not have any control when if we talk about a network that whether layer 1 is interacting with say layer one is interacting with in this case with layer 3 or layer 1 is interacting with layer 2 as well as layer one is interacting with both layer 2 and layer 3. So, there are different kind of layering and connectivities which are happening in a three dimensional network. So, when we talk about a three dimensional network it is unlike when we talk about two d network where it is very easy say for example, I want to make a pattern like this.

So, I can make a oval shape pattern like that we can make a pattern like this say for example, squarish pattern we will come into these kind of pattern geometries soon and I will provide you not this week and in the next week I will provide you some of the videos. So, you can make a squarish pattern you can make a lot of such patterns and those pattern making is comparatively marquai out comparatively easier in a two dimensional part two dimensional structure as compared to making a something like a 3D pattern and why these 3 D patterns are. So, very important is that from one layer how it is cross talking with another layer is still a tricky problem for us to challenge in vitro setup and these things does matter why does matter you may have a series of say neurons sitting here as a particular neurotransmitter type you may have another set sitting somewhere out here of another neurotransmitter types or some mix.

So, these kind of interactions eventually determine much of the physiological highlights of the system and if you really want to understand the physiology of the system. One

really really has to recreate these models in an in vitro setup. So, keeping these some of these basic paradigms in mind let me pick up where we left in the last class.

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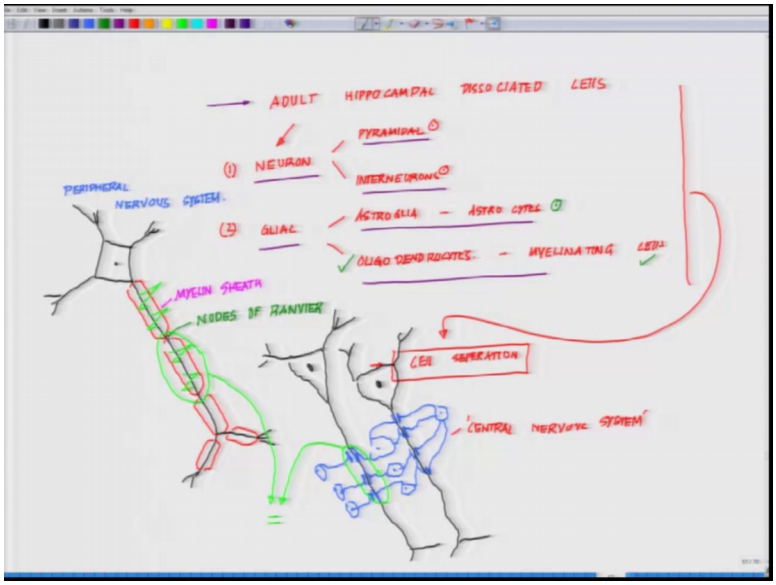


So, in the last class if you remember this is where we were we have the adult hippocampal dissociated cells which consists of neurons and the glial cells within the neurons you have the pyramidal cells you have the interneurons you have astroglia or astrocytes or oligodendrocytes.

Now, when you have to make a culture in real life these cells are all sitting together, so you have if you see the network, network is something like this. So, within this network now I am introducing. So, if you imagine this is the three d network within this network you will have oligos which are sitting like this, which will be myelinating this, now try to understand the complexity here and then we will talk about how we are going to separate out these cells these are the oligos these oligos are involved in the myelination process.

So, those of you who are not aware of the myelination process in the nervous system just to give you a brief idea. So, here you have a classic neuron and whenever we draw the neurons we drew it like this right. So, you must have seen in the textbook they have this kind of picture right and both side as if along the axon you have a sleeve like a structure. This sleeve like a structure is what it will be written as myelination or myelin sheath and in between out here you will see written nodes of ranvier on the contrary, so this is a classic neuron which is in the peripheral nervous system. On the contrary if you see a

neuron in the central nervous system if you draw say if I draw a pyramidal neuron in the central nervous system of hippocampus which has pyramidal like cell body here the processes here the nucleus yes let me draw another one that I do not make more sense to show the myelination pattern.

So, in between in a cross section if you ever take a brain slice cut the brain slice or take the hippocampus you will see a series of cells which I am now showing in blue they will be sitting like this a multi polar cell bodies very small this is I am kind of drawing it very big multi polar cell bodies and they will be arrange like this. I am just taking example of two cells to show you, but this could be way more complex. You are seeing that what it is doing as if these blue color cells are forming a similar structure as a very similar kind of covering as you see here. So, this is a form of myelination what you see in the central nervous system neuron.

What is myelination? So, we are aware of the fact that neuronal cells are carrying electrical impulses right, now when they are carrying electrical impulses just like any other cord which is carrying electrical or anywhere you always put an insulation across it why - because otherwise if you touch a necked where you will get shock and get short because the current will start passing through your body and god forbids it may cause irreparable damage. So, similarly when the neurons are carrying those current impulses, they may influence the surrounding neuron which is by its side.

So, as for them not to influence the surrounding neuron such that the signal which is being generated the signal fidelity remains intact and it travels through these cells have our insulated covering and that insulation is called myelin sheath. It is a protein secreted by two different kind of cells at two different places of the body. So, I have to give you this idea because that way all these words we will make sense and you will be able to appreciate that how this whole thing is orchestrated when we talk about a defined system right.

So, what I will do in this class after giving you this brief idea because now we are dealing with this oligodendrocyte astroglia and then once you know the function of all these things I will be defining how one can separate them out and how can one reintroduce all these things into the system.

So, the first thing what we will be doing not in this class in the next class we will talk about how in real life a cell get myelinated and how if you have to create a cell culture model how you can regain it or how you can reintroduce the cell to see whether such myelination happens or not. Because a neuronal cell without its myelination will not be able to carry over the electrical signal to a long distance it will lost because there is no covering on top of it. And what server model you form will be incomplete without or inconsequential without that kind of myelination cover.

So, I am closing it here in the next class we are starting with the myelination problem and the in vitro setup before we really target this adult hippocampal dissociated cell and how to separate them out because this will slowly take us to the cell separation parameters and how we can really target this problem.

Thank you.