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

Lecture – 25
Adult hippocampal Neuron Dissociation

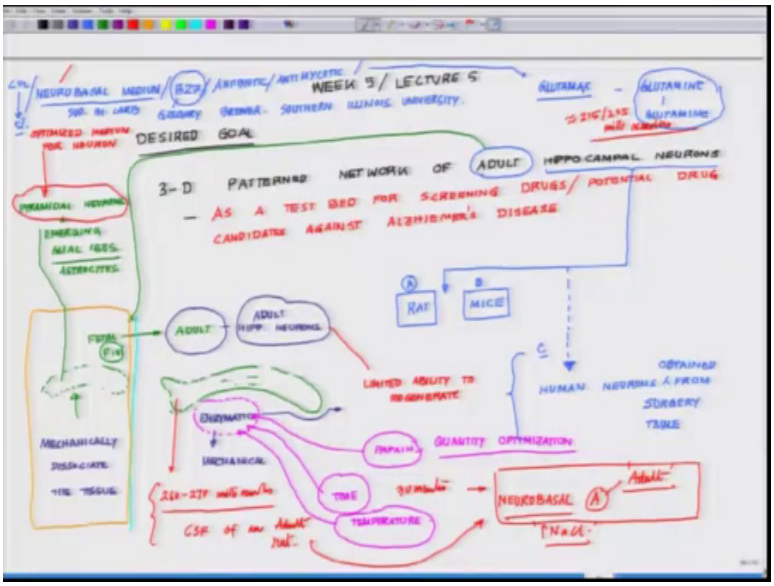
Welcome back into the lecture series on Cell Culture Technology. So, today we will be doing the fifth lecture of the fifth week. So, if you guys recollect in the last lecture we talked about. So, we took a case study that a company has to screen few drugs which are believed to help Alzheimer's patients. And hypothetically in a virtual or we said the quantification parameters as by the application of the drug the number of synapses will increase. Or in other word synaptic button could be quantified in that way.

And while going through it we kind of realized that there are few aspects which demands development of a much more advanced model system that to at the in vitro level. So, the point just if you recollect we came to the point that we needed one point which came out very clearly in front of us is we needed a pattern network. A network which follows a information transfer in a direction.

Second we may off for developing network in 3 dimension. From 2 dimension to 3 dimension. And third emerging concept which kind of made things to think or revisit is it a wise idea to use a fetal hippocampal culture which is fetal day 18 or of the 22 23 days of pregnant mother from where we derive the fetus e 18 fetus is it a good model system to evaluate Alzheimer's system.

So, that brings us to some of the futuristic search for solutions. So, the futuristic search for solution starts with the core goal is we wish to have a 3 dimensional pattern network of adult hippocampal neuron as a test bed. So, let me just put it together.

(Refer Slide Time: 03:16)



So, we are into week 5 lecture 5. So, desired goal is 3 dimensional 3D pattern network of adult hippocampal neurons.

3D pattern network of adult hippocampal neuron as test bed for screening drugs or you can say potential drug candidates against Alzheimer's disease. And of course, in this case we will be using the possible model system animal system could be to 3 possibilities to start off with rat, mouse and if possible if situation permits then going for human neurons from obtain from surgery table ok.

So, your first cut will be A or B and of course, the third one will be c and we will talk about it how you can obtain the human neuron especially this can happen, if say for example, there is a brain tumor surgery which is going on. So, when they remove the tumor from human brain one of the thing which happens is that a part of the good brain tissue kind of is being also lost.

So, if you have this skill set and if you have the technical know how to collect that tissue from the operation theater and process it and of course, use that particular part of the live neurons which are present there you actually can have a human neuron culture on a dish. So, we will come later into that. So, let us think for or let us explore how from. So, the first thing is you have to now your source is going to change. So, your source is changing all the way from fetal to an adult.

So, if you remember when we talked about a fetal hippocampus, it is more like this. It is a kind of a very soft F18 tissue where is in the adult it will be much more bigger something like this. Now this tissue has compared to the fetal one cannot be dissociated by so, mechanical root. So, in the case of fetal you can mechanically dissociate the tissue.

On the other hand if you want to develop an adult cell culture of adult hippocampal neuron culture you cannot do so by mechanically. You will be needing first step will be prior to this will be enzymatic followed by course mechanical. Now when you talk about enzymatic step what enzyme will suit because it is an adult tissue? So, the enzyme has to be I would say it has to be mild enough not to damage these cells, because the regenerating potential these adult hippocampal neuron regenerating potential is very, very limited ability to regenerate ok.

So, if you know it has a very limited ability to regenerate limited ability to regenerate. So, if you know it has a limited ability to regenerate you do not want to damage this tissue extensively. So, for enzymatic treatment it has been observed the enzyme which is reasonably better than other enzyme is called papain. At a fairly low dose and I will give you an additional reference where you can look that how papain or other what dose of a papain will help you.

So, the papain act as an enzymatic dissociate. Now what you do in this case? You have to optimize the time not only you have to optimize. So, your first optimization comes here quantity optimization. Next thing which comes is time. Second thing in the same line temperature sometimes I may need to raise the temperature, but you have to be careful whether you can do it in regular you know or you want to do it in a water bath of say like you know 4 degree or you want to do it at 20 degree that decision you have to figure out over a period of time of optimization.

So, once you optimize this these parameters then you have to realize while you are mechanically dissociating a fetal tissue out here in this situation. In that situation most of the prominent cells which were present there where pyramidal neurons and emerging glial cells which are mostly astrocytes.

Now, one of the thing which over the years cell culturist have done especially those people who are good at neural cell culture. They have optimized the medium for neurons,

optimized medium for neuron what does that mean? Now that means that the medium which has been developed selects or preferentially allows the neurons to grow better as compared to the glial cells.

So, that essentially brings us to a point of course, we know in the brain you have both the glial cells and the neurons and glial cell out numbers the neurons. And glial cells have multiple functions if it if it is an oligodendrocyte, then it supports the myelination. If it is a Schwann cell it supports a myelination outside the central nervous system if it is astrocyte which is present there they will support the homeostasis it will ensure that excess neurotransmitters and neurotransmitter related toxicity are being avoided.

So, when we talk about developing this medium one has to make a call that do you want a mixed culture really absolutely close in to real life culture, but then if you have to do this there is one catch is this that neurons may not divide, but the glial cells will divide at a faster rate. And if you allow the culture for a prolonged period of time then the glial cells will out number the neurons. In a big way out number in the sense will be competing because they will be dividing they will be consuming lot more nutrients and the culture will eventually become overpopulated and you would not be able to keep the culture for a prolonged period of time.

So, you have to think of an optimized way if you want to do a mixed culture that how you can ensure that the population of glial cells are kept at check, that they do not proliferate So much. So, that you know you cannot really handle the culture, but having said this let me tell you if you could really mimic the exact condition, where you have small amount of glial cells attached to it then you are much more closer to the real life situation. And it also influences the electrical activity of these hippocampal neurons.

So, the mediums which are commonly used for fetal hippocampal neuron are called neuro basal medium you can go online and check it out neuro basal medium. And neuro basal medium is supplemented with a composition called B27 this composition was developed primarily by a gentleman called Gregory brewer. At that time he was in southern Illinois University at Springfield Illinois one second yeah, Gregory river southern Illinois University.

Initially he developed something called B10 or 6 something like a medium. And he further took it make it B27. So, B27 stands for 27 different components which are added

to it which primarily supports central nervous system and other neuron growth. Of course, later it has been proved that it also supports the growth of other excitable cells like muscle cells as well as cardiac cells and that was much later it was proved, but initially it was developed to support the growth of especially the hippocampal neuron and other brain neurons. And you have 2 supplement.

So, neuro basal is being buffered using sodium bicarbonate sodium bicarbo buffering. So, you have to use the co 2 incubator. And most of the co 2 incubator prefer 5 percent co 2 for embryonic hippocampal or any of the hippocampal neuron or culture. This is supplemented by antibiotic and anti mycotic. And there is another component which is added which is called glutamax. Glutamax is nothing it is basically a dipeptide of glutamine you needed to add glutamine because this is one component which goes bad very fast.

So, what at some one point like science technology is what you know of initially it was in vitro gene they developed a product where 2 glutamine it is an amino acids you coupled 2 glutamine using a peptide bond you make a dipeptide and that is called glutamax. Apparently it has been observed that glutamax is a much more potent stable compound as compared to using glutamine which otherwise is not really very supportive I mean, it supports it is just it is shelf life is very less once we mix it in the medium.

So, this is the medium which was developed mostly in this field you will see most of the work very initial pioneering work were done by Professor Gregory brewer. So, and his whole idea was to have I mean till this day the kind of work he publishes you will see more of a in vitro analogues for testing different kind of Alzheimer drugs and all those kind of things. So, this is the medium which is used for embryonic or sorry, fetal hippocampal culture.

Now, the modification what has been made in this medium is pretty much all the components remain the same except it is osmolarity has been varied. Because the osmolarity of this medium is approximately 215 to 225 milliosmole. Where is the osmolarity which you needed for adult hippocampal culture is osmolarity of the medium is around 260 to 270 milliosmoles.

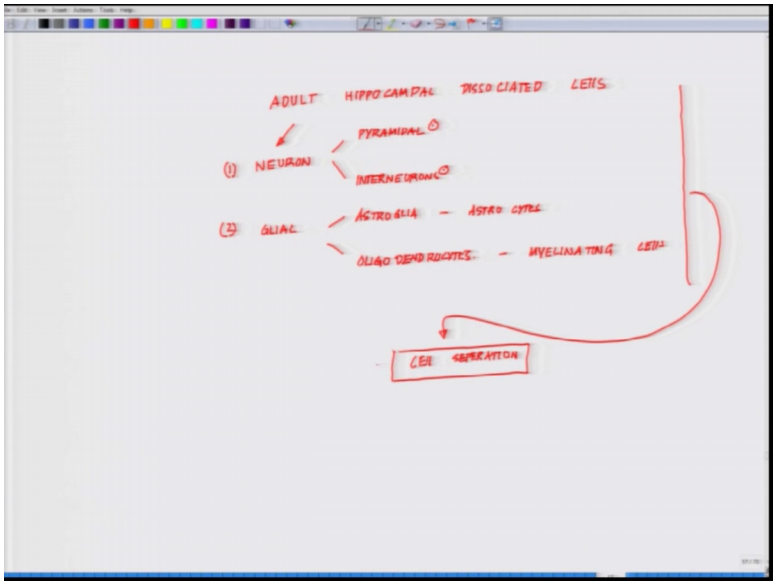
So, this value has come from cerebrospinal fluid of an adult rat. So, the next advancement in this medium which was used for fetus is using neuro basal, but it comes

in the market it comes with A. Where a adult stand for adult. So, neuro basal A and neuro basal regular, the difference is in osmolarity. If I exactly recollect there is a small if there is a higher concentration of sodium chloride in neuro basal A.

So, essentially you can have a neuro basal regular, and you add that pre requisite amount of NaCl to make it neuro basal A. So, this is how from neuro basal you convert it into neuro basal A. So, this is as a matter fact in that course this is the first time I am introducing a medium a specific medium. So, medium remains more or less the same except that now you have to introduce another step which are which is called enzymatic dissociation which is the papain ok.

Now, this dissociation which again the word has to be referred to Gregory brewer, he optimized the condition he found that this dissociation more or less takes around 30 minutes. Now at 30 minutes we dissociated all the cells of hippocampus.

(Refer Slide Time: 23:16)



And this composition consists of your adult hippocampal dissociated cells, what all it consists of?

One, neuron within neuron pyramidal neuron one, inter neuron 2 glial cell astroglia making astrocytes oligo dendrocytes. Which most of them die dies out, but you know you will still have contaminants which are functioned as myelinating cells.

So, what you are getting here think of it? You are getting a mixture of different cells. So, your next challenge how to separate cell separation. So, you see now slowly we are moving with one example we are moving to the complexity of cell culture.

So, I will close in here. So, next class next week we will be starting to some of the basic things again following up on this case study how one can separate different cells. And this paradigm will remain the same only the cell type will vary.

Thank you.