Cell Culture Technologies
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Lecture – 27
Cell Seperation and In Vitro Myelination Cell Culture Mode – II

Welcome back to the lecture series in Cell Culture. So, in the last class where we just ended was about myelination. So, today the first thing we will do we will talk about how the myelination happens in the peripheral system as well as in the central system and they have a very different pattern of myelination. So, if you are culturing peripheral neurons and you have to follow a different paradigm as compared to if you are following a central nervous system neuron.

So, in the peripheral neurons when I drew this, we are into week 6 lecture 2 W 6 L 2.

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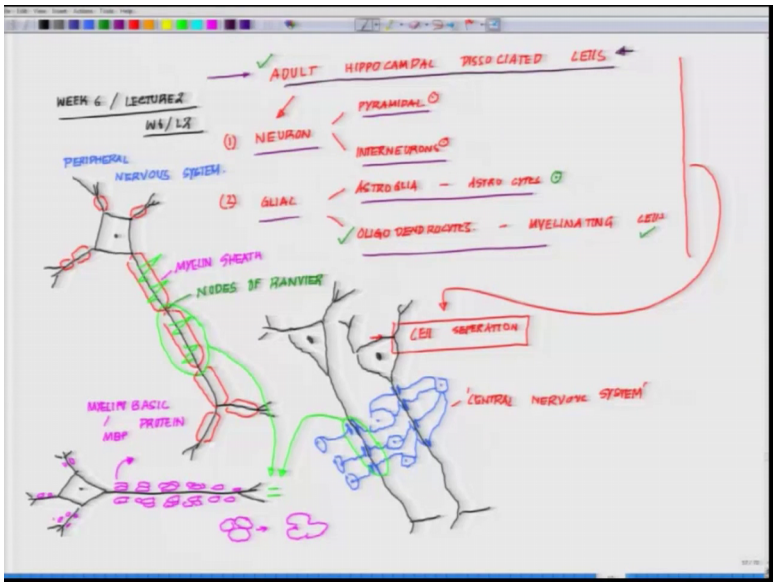

Now, when you talk about the peripheral neurons, this is a neuron the way the myelination happens when these neurons are formed in the body is there are these Schwann cells and this is all about pianos these Schwann cells arrive at the axon and as well as the dendrite because this myelination you will see here to right. So, these Schwann cells which are involved in myelination these are type of glial cells they will arrive along the cell body or along the processes. Now out here what they will do they will divide after reaching something like this.

Upon division these cells kind of go underneath the axon and tries to encircle it or tries to form a cover like this say for example, if this is the axon which is sitting first of all these cells divide and some of them goes down and slowly takes it like this as if it is forming sticking it in a grip like this something like this. And then what happens these cells individual cells which has formed a complete covering like that starts to secrete a myelin basic protein which is called MBP or myelin basic protein and is this myelin protein which is secreted by these cells which form the myelin sheath around them and most of these cells loses part of their identity.

So, the fate of these cells are like this initially they are like this and eventually they become something like this. It seems as if the boundary of cell membrane kind of merges the cell membrane merges it looks like a complete sheet kind of structure that is why it is also called myelin sheep and the sheep kind of anchors on the axon by virtue of these secreted myelin basic protein.

Now, having said this having give you a very small layer of the biology here now I say that you want to culture neurons sure you want to culture peripheral neurons, but then you have to know could you achieve this how you are going to do this think of it. Now I will give you two cases studies before again remember that do not lose the sight of this fact adult hippocampal dissociate cell this is where we are heading, but before we head into this there are several other things what I expect you I should think that why these kind of cell culture models are so very important.

So, this is one problem which boggled us for a while and very fortunate to work with a very close friend of mine who has pioneered this technique of developing myelination sheet on a peripheral neuron in an in vitro culture I will share this his name is John Owen Samsay was one of the gentlemen in University of Cellular Florida where I was working with him on his thesis problem and this is the problem what he solved in his thesis one of the problems he solve many other problems, but this is one of the problem.

So, how he did it in a dish, so I will share the real time experience of how he actually did it. So, the way John approached the problem was very interesting. So, the first thing what he did, so for peripheral neurons what are the sources and please do not lose track that we have to come back I am just diverting from the main course in order to come back there with a much more concrete viewpoints about you people that why we have to

answer these kind of peripheral question before we target on to some of the very very challenging problems of the future regeneration of the adult neurons and in a culture dish how we especially in a culture dish how we can minimize the financial burden of drug discovery.

So, now getting back how this problem was targeted.

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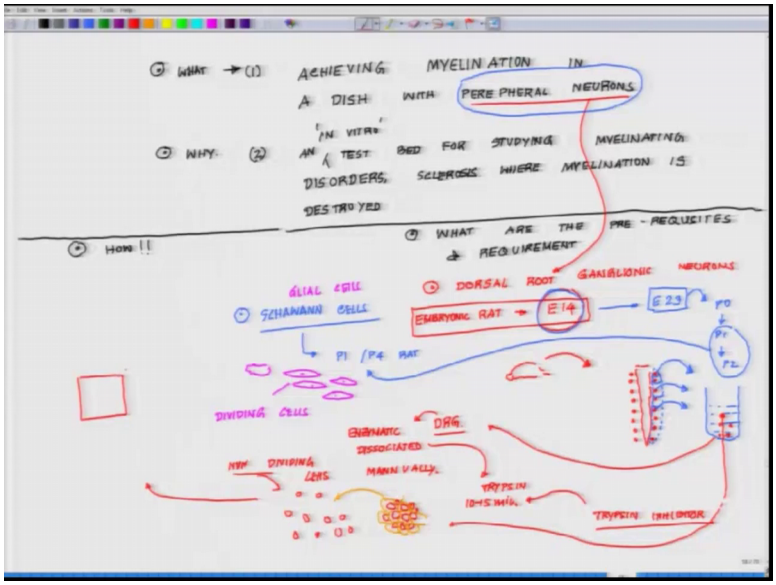

So, the first and foremost idea let us break the problem achieving, the title is achieving myelination in dish with peripheral neuron. So, second this what you want to achieve why test bed for studying myelinating disorders sclerosis where myelination is destroyed. So, you have why what and let me in vitro set up or in vitro test bed. So, this is your what this is your why.

Now, comes how, how you achieve it and this is what we are going to discuss now for next fifteen minutes how you can create such a test bed. Before we see how let us try to figure out what are the requirements what are the prerequisite or requirement. So, we are trying peripheral neurons your easiest source of peripheral neuron will be dorsal root ganglionic neurons which are sensory neurons this is one of the prerequisite you need and how you can obtain dorsal root from where one of the easiest model at least what we have worked for the years is you get it from rat they were fairly good especially from embryonic rat which is E 14. So, generally rats have this 20 to 23 days of pregnancy, so

you can get one from E 14 dorsal root ganglion. So, we are not trying to do it on adults because this was the very very first cut one has to try it out embryonic neuron E 14.

So, from E 14, if you look at how you are going to isolate so there is a dissection challenge here. Dissection challenge is you are handling an embryo which will be very teeny tiny something like something like this. Now first thing what you have to do is you have to isolate the spinal cord and this has to be a micro surgery and around the spinal cord you will see this dorsal root ganglion sitting and very carefully extremely carefully you have to using your surgical acumen you have to isolate these dorsal root ganglion separately these are individual ganglions.

So, now what you have your the collection buffers something like hibernate or something you have these dorsal root ganglions DRGs. Now these DRGs needed to be enzymatically dissociated so that so you can do an enzymatic dissociation by using trypsin or if you are really good you can do it manually also, but you know you have to really careful while doing it manually and most of the anxiety enzymatic dissociation are done with trypsin you can try out for pain, but it is worth trying out trypsin. And if you use trypsin then you have to stop this trypsin reaction within after 10 to 15 minutes using something called trypsin inhibitor because trypsin is going to damage the cell. So, you have to ensure that reaction happens of you know when we talk about these kind of ganglions these ganglions have something like this it is a lot of cells which are sitting there. So, what you are essentially doing while you are putting trypsin you are trying to you know separate out the cells by penetrating in between like this.

So, what you are getting our single cell suspension. So, and these are your DRG neurons which are there at E 14 because if you go further that the population of the Schwann cells are going to go up. So, here one interesting thing one has to realize that the first neuron we first cells on the DRG is are form are the sensory neurons followed by once the sensory neurons are formed simultaneously the Schwann cells are formed and they form the wrapping. It is not that what these wrapping as well as these wrapping as well as this division of the Schwann cells to form that covering is happening parallely first of all they hit their target and simultaneously this process starts.

So, once you get at this kind of single suspension you have your first set of prerequisite ready that is you have the peripheral neuron at your disposal, but now you want to

achieve another thing. So, this is if this is the first prerequisite there is a second prerequisite. Second prerequisite is that you have to you will be needing the Schwann cells because you want to mile in it and this are you are doing a peripheral by relation. So, in order to do that you need a source of Schwann cells, but I just now mentioned you then when you use a E 14 at that time the Schwann cell has informed properly. So, it means in order to obtain a Schwann cells your source animal source has to be a matured animal. So, your option is that you get a Schwann from adult which, but it does not work what you can do for Schwann cells you can take a p 1 or p 4 rat what is p 1 and p 4. So, I told you that the pregnancy goes up to say E 23, 23 days. So, as soon as they are born this is p 0 this is the day will born and p 1 p 2 postnatal. So, around something around p 1 to p 2 or p 4 postnatal after birth those teeny tiny rats those are the source of Schwann cells they work the best.

Now, if you ask me that how all these things are known it is 100 years or last 56 years people are being working. So, it is kind of a long haul journey. So, today we have some ideas; however, you could get those cells which are going to myelinate and likewise one ends forth. So, it all comes through some of the very critical studies in develen biology in vitro development biology. So, we have an idea that at what point these cells will appear and how that information of developmental biology could be exploited to develop an in vitro setup. So, these all think that very interlink.

So, this whole area of cell culture especially any kind of cell culture one has to have a kind of idea about the development problems too without that it will be very tricky it will be something like yes I know and yet I do not know understand my point. So, it is very very kind of I would say that essential that you keep your eyes open that you know what all the basic developments are happening now think of it like I still remember we used to think that if we could get the Schwann cells off from that age, but unfortunately the development does not allow us to do so, so we have to depend on another animal or another each group animal to get the Schwann cells. So, now, that is your second source to isolate the Schwann cells.

So, now once you get the Schwann cells which are like kind of this kind of shape. Now we realize a very interesting thing that when you talk about the Schwann cells they will be dividing these are dividing cells whereas, this is form of glial cells whereas, neurons what will be dealing with are non dividing. Now if you put a non dividing cell with a

dividing cell in a culture and without ensuring that the dividing cells a rest is division the dividing cell will outnumber the non dividing cells and so much so that you lose tab on the problem that you know you will not be able to even locate the non dividing cell.

So, how to target the problem? One of the ways one can target the problem is you first of all plate these cells which sells the non dividing cells. So, you have a dish out here and this is how we achieve the problem or kind of target is the problem.

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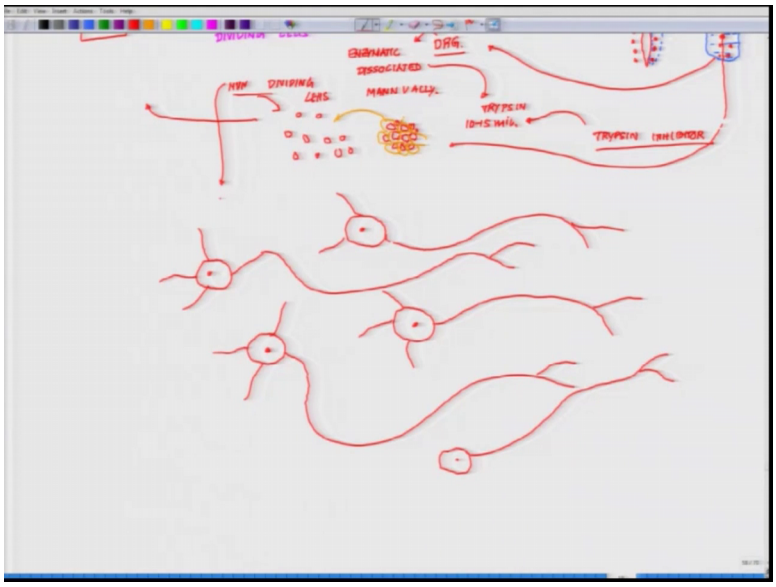

So, you played these, peripheral dorsal root ganglionic neurons in a dish, you will see within 24 hours or so they will start sending out the processes like this and then one of the processes will become larger and will form an axon you allow that part to happen. So, you have the process in front of you now and if you can pattern them to follow a geometry that will be even better if you can really do that.

Now, as they are growing after 2 3 days this is the right time to introduce the Schwann cells. So, these ones you are achieving from the E 14 right non dividing cells. So, now, they have formed their processes something like this, now at this stage you are about to introduce the Schwann cells.

So, closing here we will continue this story after that what will happen and how we can translate it to the adult hippocampal neuron because there are some missing wonderful links.

Thank you.