Cell Culture Technologies
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Lecture – 28
Cell Seperation and In Vitro Myelination Cell Culture Mode – III

Let us resume our conversation about the myelination process in an in vitro setup how you can achieve. So, in the last class remember I introduced the peripheral neurons and I allowed them to grow. So, let us pick it up from there, week 6 a lecture 3.

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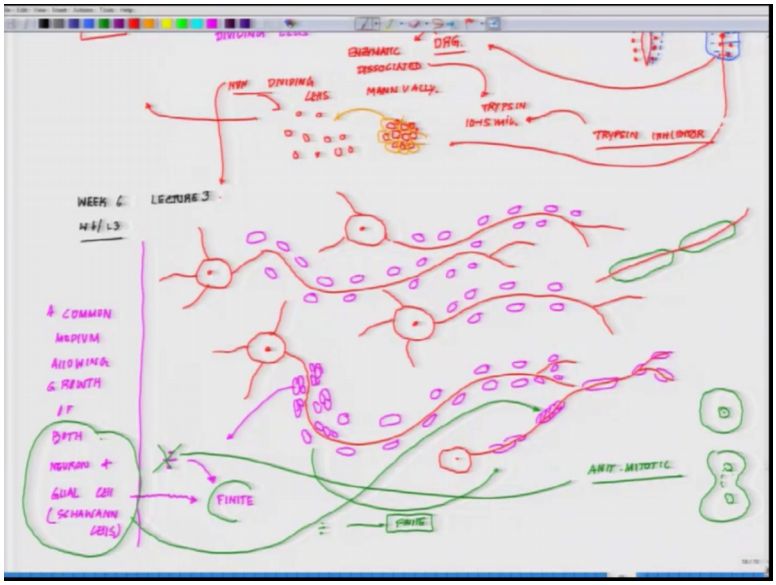

Now at this stage you are introducing I told you that allow them to grow 3 4 days then you introduce the Schwann cells which are in smaller in size. So, once you allow the Schwann cells to get there what are the possibilities. So, one thing which could happen is the Schwann cells will migrate towards the axon and along the axon the Schwann cells may form exactly the way it happens they may slowly divide like this and they may form what I was trying to tell you is something like out here something like this or this may not even ever happen.

So, they go in close in there and they started forming the sheets like this they go and wrap around it this is one thing which could happen. Now when I have said you we could target the problem in a different way before I get into that now think of it. So, once they are getting in, so they will have a finite they have to it has to be ensured that their

division because these cells will be dividing it should be finite if it is more than it then it is going to outnumber. So, in the same culture model you have to ensure that you have first common medium allowing growth of both neuron and gual cells in this case the Schwann cells.

But then one has to ensure these cells which in order to achieve its biological role of forming the myelination where it has to divide this division has to be finite. Why it has to be finite because otherwise this is going to completely cover the dish will not be able to really figure it out how one can do so then possibly at some point or other in order to restrict the division you may have to use something called an anti mitotic. Anti mitotic is something which prevents the mitosis to happen.

Now, one has to be very very careful something like furacy and that there are several such compound cytosine arabinocytes which is one of such compounds which basically what the way they work is very simple. So, when the cell is dividing say for example, this one cell which is dividing. So, you must have seen that there are two level of divisions which takes place one is the nucleus divide and then the cytoplasm divides. But this division is being happening because of all those centrioles and all those formation of mitosis and all those thing we remember.

So, cytosine arabinocytes ensured that they do not separate out which is kind of a very ugly way of doing suppressing the cell division, but these are some of the very oldest techniques which are being used, but there are other techniques where you can deprive them from serum other unknown factors you can stop their division. But one has to do that division stopping that division is essential, but in a very controlled way so that you can achieve your goal of seeing the myelination happening as well as seeing the nodes of ranvier formation and their electrical properties they regain it. So, we have not touched that point yet.

So, now coming back what is the other way you can look at it other way you can look at it is another interesting way where many people have tried is say for example, you form a bed of myelination, myelinating, cells on the bed of myelinating cells you grow them.

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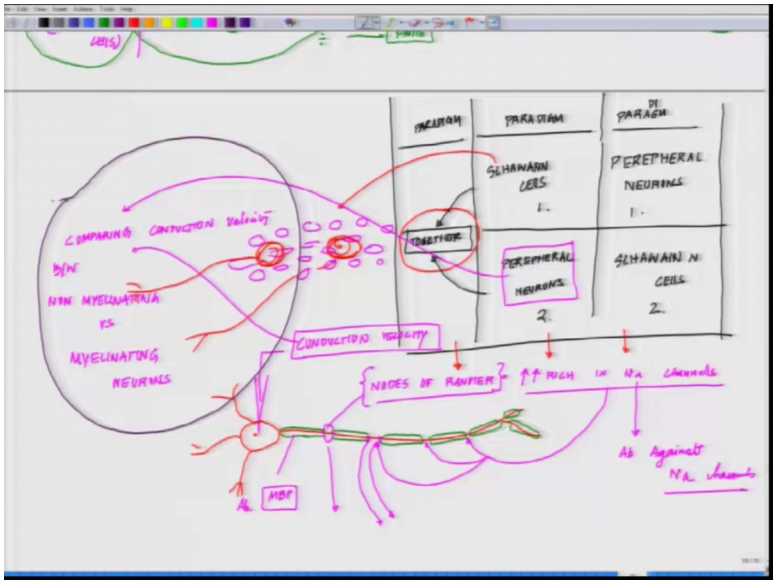

So, here what you did first case is study when I said you put the peripheral neurons first followed by Schwann cells second, you can reverse the order you could have if this is one paradigm you are following there is another paradigm you can follow where you put the Schwann cells first and you put the peripheral neurons second or you could have a third paradigm where you put the Schwann cells and the peripheral neurons together. There only three things you can do.

So, if you break up the problem right then in one of the three experiment of course, your luck has to even your side and your hard work also you will be able to achieve the problem. The second situation is that you put the Schwann cells first out here and top of that you start putting the neurons assuming that as the neuron will be sending out its processes these Schwann cells will come along and form the wrapping or you have a third situation which is out here you put them together.

So, at this stage I will give you the papers what has been achieved I will not really divulge that I will leave it to your judgment how we did it, but what is important is part two of the paper what I am going to discuss with you how we, but we went through the whole rigor the whole idea about telling you these stories from real time is this is how we have to understand cell culture. The modern day cell culture is no more like you know add this add that and something happened no you have to think rationally our body has formed in a systematic way component 1 component 2 component 3 component 4 and slowly it has build it itself and now what you are trying to do is he wanted to see an in vitro development outside the system that is not a easy job. It is something exceptionally

challenging and how you can achieve it, it will be a long drawn process it will be a long whole journey of ours to understand even going even close to it, but it all start with that simple modesty of doing these experiments right.

So, this is the way we thought it out I still remember that what are the possibilities this is one possibility, this is the second possibility, this is the third possibility and we think around it on a dish that which one is going to work. So, these are some of the stuff which I want you guys to think, but then what one has to achieve now here is the interesting part one, one has to achieve. So, now, if you look at the neurons when they are forming a myelination this is the classic part what you will be observing - these are the dendrites and assume that this is where you are seeing the myelination right.

So, now this zone which we call as nodes of ranvier this particular spot is very rich in sodium channels right. So, now, when you achieve if you believe you have achieved it first thing you have to see whether you could make or form a nodes of ranvier or not. So, you have to stain these cells in a culture before you claim that you have done it is with myelin basic protein we have talked about it the antibody against myelin basic protein you have to identify the nodes of ranvier by a specific sodium channel antibodies against sodium channel and at this nodes of ranvier there are different nodal proteins you have to develop or you have to get the antibodies against them to make a claim that you have achieved this.

And on top of that and on top of that you have to have electrical recording from these neurons and you have to measure you have to measure the conduction velocity and you have to compare the conduction velocity of these neurons along with a neuron without myelination, comparing conduction velocity between non myelinating versus myelinating neurons. Then only you will be able to make a claim of whether you have achieved it or not.

Now, we try to see that how are trivia problem called cell culture can become so very interesting and so very fascinating to appreciate that the possibilities the of possibilities is unparalleled there are so many beautiful things you can achieve in a dish and that will give you a joy to see this is how the life is formed. So, now, I mean seen this, this is one part of the problem where we started let us go back and let us do not lose track of it because I have digress a bit. So, this is where we all started right.

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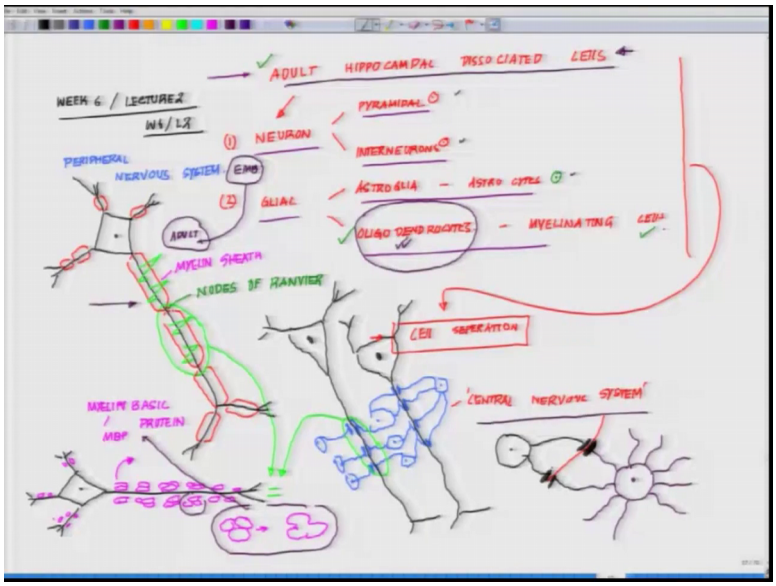

So, this was the problem which I am supposed to target, but I think I will be able to target this problem it will take me another couple of more classes before I kind of hit upon that problem. Now having seen this where we started. So, now, you have a fairly good idea how to have a cell culture model for peripheral neurons now how to achieve for central nervous system neurons we have not talked about it yet right.

Now, let us break the problem now I could come here if you have to break the problem. So, you will be needing oligodendrocytes if you want to achieve a myelination and you will be needing adult neurons. So, just like this problem in the peripheral nervous system we use the embryonic system in order to achieve it before we graduate into adult and to the best of my knowledge as of now there are hardly any groups in the world which has achieved myelination on adult neurons not that I know of, but anyone of you find it out in a culture dish people have a achieved that be a really great thing, but it is exceptionally challenging.

But here also we will talk about the myelination problem with respect to the cell culture context with respect to embryonic neuron because these are some of the models which has been successfully demonstrated that it is possible. As of now technologically it is. So, mark my word it is technologically very very challenging to target the second level of problem where you will be using the adult neuron to myelin get myelinated ok.

So, now in terms of the oligodendrocyte myelination, so here I told you the way the myelination is happening these cells are coming close and eventually losing their identity and secreting myelin basic protein which is this one and this is how the myelination coverage coverage is happening and then you have the nodes of ranvier where you have a significant population of sodium channels which are labeling. But in the case of central nervous system if you want to achieve it this does not work like that. Being the central nervous system the myelination is carried out by oligodendrocyte and the way oligodendrocyte works is that oligodendrocyte cells have they resemble something like this initially they look like this and then they send out processes like this - multiple processes on multiple directions very very multipolar cells and these multipolar cells after coming in contact with any axon they form something like this, that is after coming there and this is the part where they secrete the myelination protein.

So, if there is another, if there is another oligo coming from the other direction. So, it will form a sandwich just imagine in between there is a process going on and from here something like this and the another direction something like this, slowly using multiple oligodendrocytes you will it will myelinate. The central nervous system neuron, so central nervous system neuron does not their processes does not become as thick as the peripheral nervous system neuron where you have a thick coating and covering because central nervous system has a limited space to expand it cannot expand beyond it.

So, now in order to achieve it if you really want to achieve their first target is you needed to have a source of neuron now again let us break up the problem in terms of the way we break it up here now achieving myelination.

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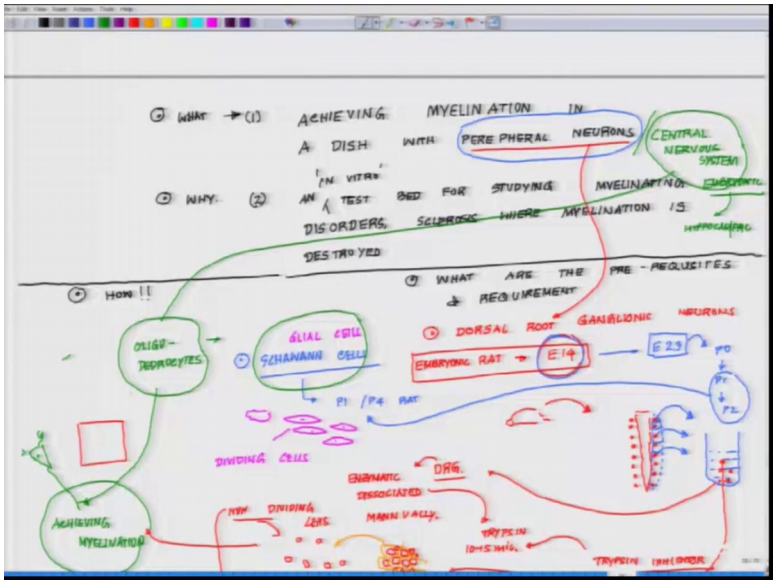

Now I am just changing peripheral, now I make it central nervous system this is central nervous system then its corresponding myelination disorder of course, that remains the same. In order to achieve now instead of Schwann cells this will become oligo dendrocytes.

So, central nervous system and oligodendrocyte and the problem is achieving myelination of course, for neuronal source central nervous system you are using embryonic tissue which depending on what kind of cells you want to achieve for what kind of cells if you are using oligos and of course, your best wet will be hippocampal neurons which I have already talked to you. So, you have hippocampal neurons which are basically the pyramidal neurons of embryonic origin and he wanted them to get myelinated with oligodendrocytes.

So, what is important here for you is to understand the kind of problems what the modern cell culture will be dealt. These are the kind of problem one has to appreciate and what level which kind of tissue you are using it does not matter I am taking and this study in terms of the nervous system because this is the that is the most fastidious one may be you can translate it with any other tissue whether it is a pancreatic tissue liver tissue kidney whatever it does not matter.

What it matters is how you target the problem that is the most critically important things and in a modern day if you really want to make a difference and you have to have a proper paradigm to do achieve this how you are going to do it in terms of paradigm this

is what I am talking about that who will come first what will be their paradigm this paradigm has to be defined one second you need a defined protocol so that everybody can follow then you have to have a defined medium, then you have to have a defined surface. Once you can pull all this together you really can contribute in a big way in developing defining systems to achieve these kind of problems.

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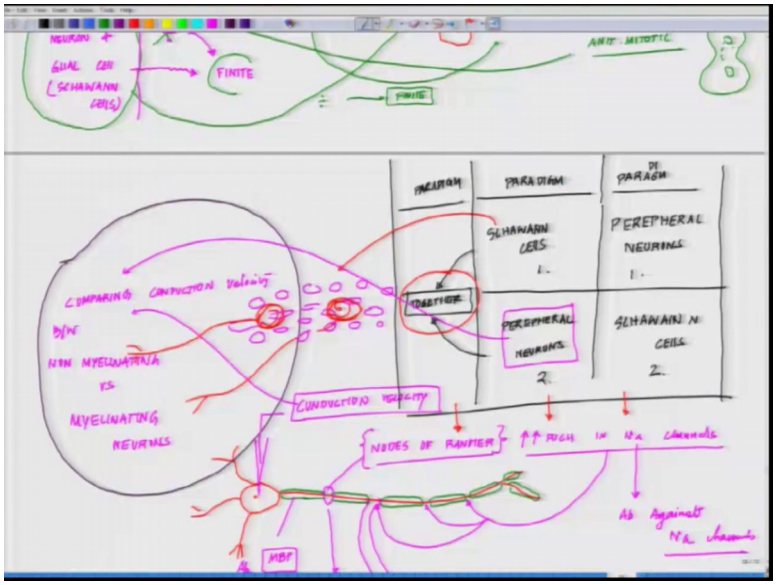

So, with this background with this aspect I will close in this lecture and I will send the handouts which will help you to appreciate these kind of problems and how these could be targeted there are whole sorts of protocols which are involved in it, but the important part is to understand the philosophy of targeting this kind of problem and how we can make a difference in the drug discovery process.

Thank you.