Cell Culture Technologies
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Lecture – 30
Cell separation & in Vitro Myelination Cell Culture Mode – V

Let us start the fifth class of sixth week. So, in the last class I introduced you and we talked extensively about the density gradient centrifugation and if we go back to the class you will realize that I told you that most of these work achieved on adult hippocampal neuron around 1995 to 2000. Then between 2000 and 2003 much of these technologies were translated for spinal cord neurons and I told you around 1980s embryonic motor neuron separation followed density gradient centrifugation.

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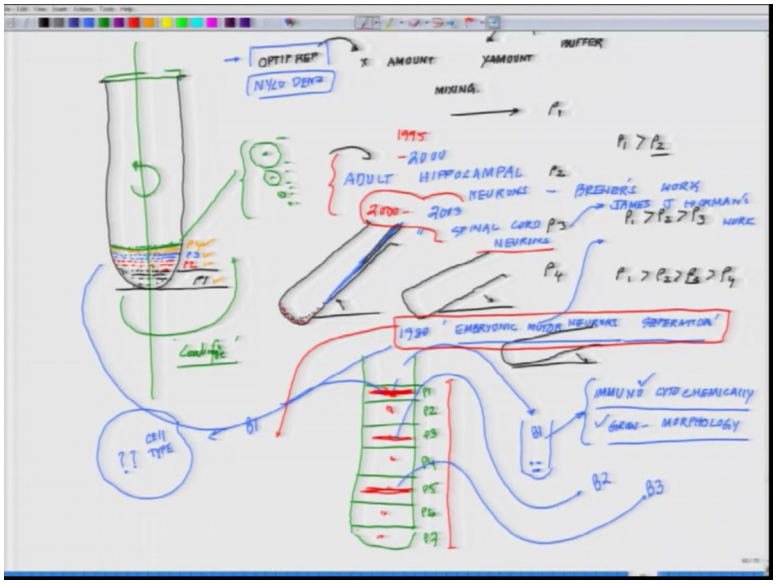

So, there also the logic was same because when you look at this spinal cord of embryonic motor neurons. So, the way the cells are being separated if you look at the spinal cord.

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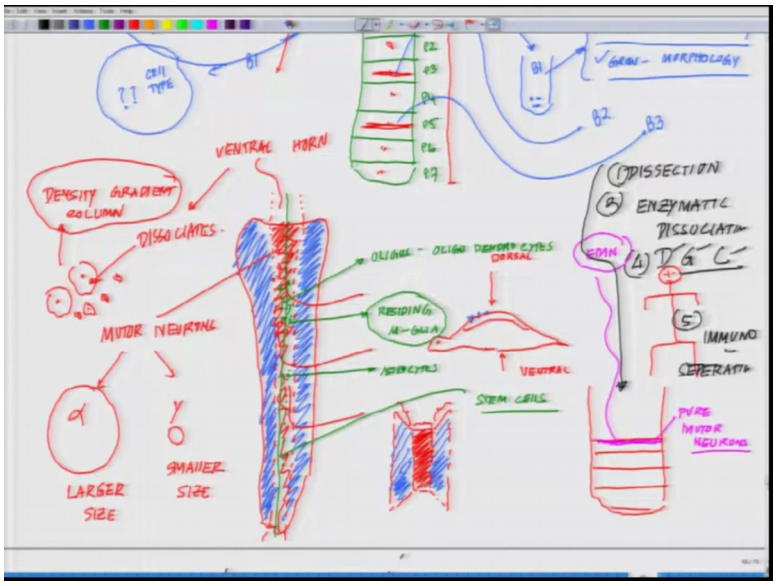

So, the spinal cord is something like this and then embryonic and as well as in addered. So, when you see the animal from the top you get a dorsal view when you see from the bottom see for example, you are seeing the animal from the top, this gives you a dorsal view. You see the bottom this gives you a ventral view now if you look at this spinal cord spinal cord is in this animal it will be like this or say for example, this is human. So, if you look at the spinal cord from this side you open the visera and look at the spinal cord you will be looking at the spinal cord from the ventral side whereas, if you look it from the back you will look it from the dorsal side ok.

So, keep that concept in mind. So, it is very interesting the spinal cord is kind of like this. Just like you have done paper folding you fold this wherever I drew the line you fold it. So, what you get something like this then right after folding we will be getting something like this. So, this is a kind of a central part this is the side and this is the other side.

Now, these two sides fold outward in other word these two sides if this is the ventral horn if this is you are looking at me at ventral side and if I keep it like this it will be folding will be like this. So, you see my arms on both sides you are try to look at like this if you look at my arms on both sides this side and this side. So, those are the green. So, this is the part where both my this is where both my arms are. So, this is the red part. Now, let me just show you out here, let me (Refer Time: 03:41) this is the red part like this and this, this part on the arm this is what you see this is the blue shaded region one

second, this is the blue shaded region and it folds like this. So, it seems that the blue part is as if on the top it is the same structure like this its curves like this and this is the blue part the one which I am shading now.

So, now if you want to culture motor neurons you have to know the location of the motor neuron spinal cord motor neurons. So, the location of the spinal cord motor neurons are here which is called the ventral horn, but now these cells along with the motor neurons they have there are two types there will be alpha or will be gamma. So, the differentiation does not happen. So, early, but in the embryonic phase, but it happens in the adder, but the alpha are the ones which are the biggest one and the gamma are the one which are the smaller ones these are smaller size these are larger size.

Now, apart from it you have a series of if you have the alphas here you have the gamma somewhere out here you have a series of other cell types which are present here which includes your oligos, residing microglia and oligos or oligo dendrocytes residing microglia astrocytes and there are some along the central canal which is essentially a location like this just at the center you have lot of stem cells.

Now, again the same problem if we remember, except now there is no more adult hippocampal neuron ellipse I am talking to you.

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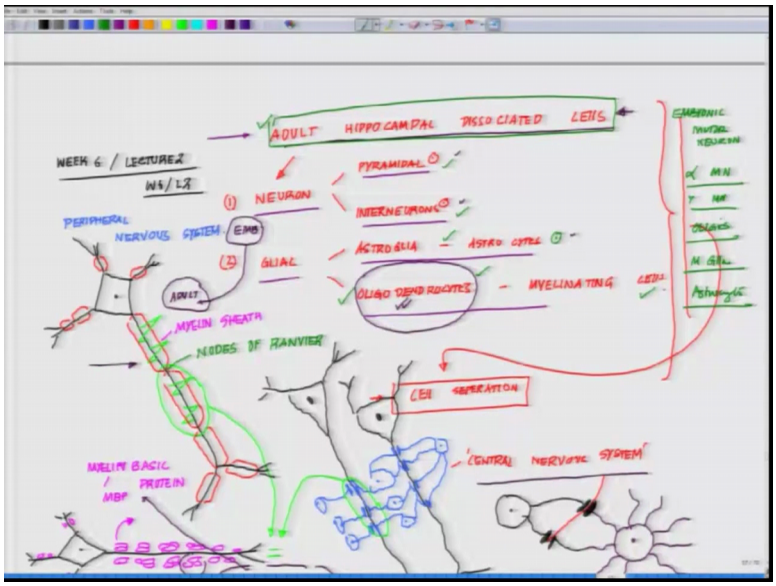

Now, this become embryonic motor neuron culture and what you have alpha motor neuron mn gamma motor neuron possibly very small neurons then you have oligos you have microglia residing microglia, you have astrocytes and series of them and the technique which is followed, which was followed was the same and this was the very very first paper if I could dug out the paper I will definitely circulated they form a density gradient column and on a density gradient column they separate out these cells this was the very, but before you do so, you have to first take the embryo and you have to dissect out only the this part.

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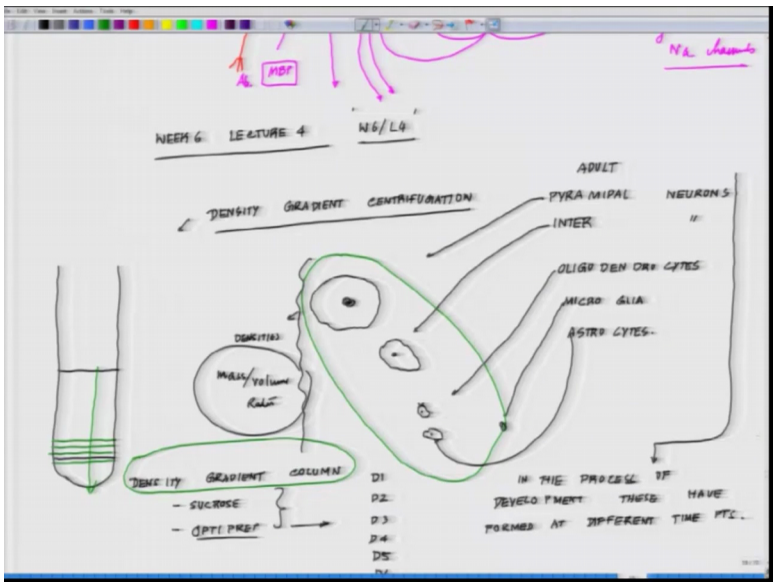

And once you dissect out this part which is called the ventral horn then you dissociate the cells of ventral horn and these cells then are being separated using density gradient column. On a density gradient column you separate out these cells.

Once you separate out these cells what you will be achieving is something like this on a density gradient column the largest cells which are the motor neurons they will form a wonderful layer if you are really good at it a small thin layer out here which will be the pure motor neurons. And if I remember it right this was done with sucrose as well as with nycodenz and there are specific devices which are being used to measure the density I will give you those papers there you can check it out what the kind of devices which are being used to make these kind of density gradients columns.

So, these pure motor neurons which are the embryonic motor neuron EMN embryonic motor neuron are the source of studying motor neuron disorders from embryonic system and again the process is density gradient.

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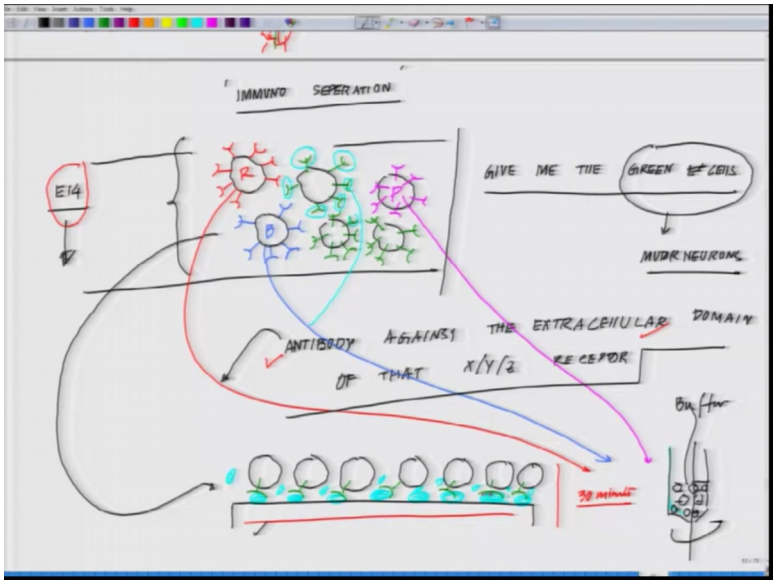

But there is an advancement to that process which happens that is what I wanted to share now which is immuno separation of cells, immuno separation of cells is first of all try to understand the basic fundamental before I can.

So, a cell is a dynamic system it expresses a whole sorts of receptors on its surface and a cell distinguishes itself by the kind of receptor it is expressing now see for example, during the development of the cell if you know that at a specific time point that x or y cell is going to express a specific receptor on its surface then if you have a technology by virtue of which you can grow antibodies against that receptor then in a pool of different cell types you can separate out that cell well.

Let me draw it that will make more sense say for example, I have these different cells same size you know i, but they are of different types I know that. How I know there are of different types I know that these cells have receptors like, this I am just showing the color coding that way it will make more sense to and these are external receptors. So, we see green colored blue colored.

Now, I give you a situation I said you give me the green label or green cells and I believe these green cells are say motor neurons and this process and their development may be a very unique phase. So, I know that say at E 14 motor neuron expresses this receptor, but after by E 16 or E 17 there we gain a mix up mix in match is going to happen, but if I know this is a zone where there is an unique situation that these motor neurons will be exclusively expressing a receptor and this is where I always say.

If you want to become a good cell culturist you should always keep a tab on the progress and development biology this will be always helpful if you understand development or if you can keep a tab on the different development taking place in different biology it will be very helpful to make very judicious judgment. So, at around E 14 there are motor neurons most of the motor neuron expresses are very unique receptors. I am just telling the story how people went into you know separation of neurons motor neurons as a case study.

Now, what they did was very interesting parallelly there was some individual who developed earlier than that an antibody against this part, what I am circling antibody against the extra cellular domain of that receptor domain of that x y z receptor this was already known.

Now, somebody did a very smart thing you will love what they did and I will send you the paper then you will realize what they did they took this antibody. So, they know this antibody and what they did. So, you have a dish on this dish. So, I am labeling the antibody with say this color they coat the dish with this antibody now dishes coated with the antibody.

Now, what you do you take this mixture of cell and you roll the mixture of cell on top of it. So, what will happen the cells if they are having the receptors for this particular thing they will go and bind and other cells which are the red cells blue cells pink cells they all will be moved out because they do not have an attaching antibody to it right.

Now, this is what forms the basis of immuno separation it is a very simple concept, but you just have to get this idea and that is why I am kind of drawing in different colors, but catch you really have to know your cell type or your concern cell type does it express that antibody or not. And in motor neuron biology some very smart moves which happen

and I will give you the papers which you kind of give you those unique papers which really change the way the field has progressed.

And they are very discreet event which took place like E 14 motor neuron expresses a particular extracellular receptor this was discovered by a gentleman called Johnson and centrally Missouri back in 1980s probably or earlier than 80s earlier to that there was one gentleman I have forgotten his name who for a totally different reason absolutely nothing to do with it developed a antibody against the accessory domain of it.

And then there came a third person who had a idea about these two what he did was creating a test bit like that and they could get all the motor neurons separated from all the rest, very smart technique. And then what you do then you need to have a computed by (Refer Time: 17:12) you have to remove this how it there are two ways you can remove you can tap it very hard we tap it then this attachment is not very prominent.

And in order to achieve this you need almost 30 minutes you have to you know keep them on the dish after 30 minutes what you going to tap them and these cells will detach or what you can do you put some excess antibodies there and which will kind of competitively will bind to this to the receptors and will remove thus remove these cells. And you can collect them in a you can one second you can collect them in a different centrifuge to in a buffer medium and you can spin them down and you have a pure population.

So, this forms the basis of immuno separation of motor neurons is not it a cool technique. So, we started with now think of it where we started. So, first what it requires? It requires a proper dissection ability step one then it needs enzymatic dissociation and then it requires density gradient centrifugation D stand for density g for gradient centrifugation.

Then from there you can do a immuno separation you see all these different steps by virtue of which you can get a very very pure population of the cell of your choice. So, this is how you achieve these very interesting field. Now if you go back in the last lecture of fifth week now do you feel more learn at that given your situation I do not know which one of here of your own set of situations.

You should be able to use these different techniques to achieve the goal and here I must mention you something very critical in the last lecture. So, this whole thing is a very very

time dependent one unless this is an E 14 spinal cord you will not see this pattern by E 15 the pattern changes. So, that is what I say that you have to have an understanding of developmental biology or at least you should keep a tab how the development is happening so that you can separate out things you know exactly this antibody or this particular receptor will be there I can utilize I can capitalize on it. So, this is how most of these things happen that they happen it is not there is any plan there is any planning that is how it happened it just happen to be like that.

So, with this I will closing this lecture with the density gradient and immuno separation next week our goal will be to understand the 2 D and the 3 D cultures again with a special reference to neurons which will kind of help you to appreciate how the whole field is moving.

Thank you.