Cell Culture Technologies
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Lecture – 29
Cell Separation and In Vitro Myelination Cell Culture Mode – IV

Welcome back to the lecture series in Animal Cell Culture. So, we are into the week 6 and we are starting our fourth lecture. So, if you remember in the week 5 the last lecture this is where we stopped and I told you do not lose sight of it because we will be coming back soon after a bit of a digression of understanding the myelination. So, you have adult hippocampal dissociated cells you have pyramidal cells interneurons you have astroglia you have oligodendrocyte.

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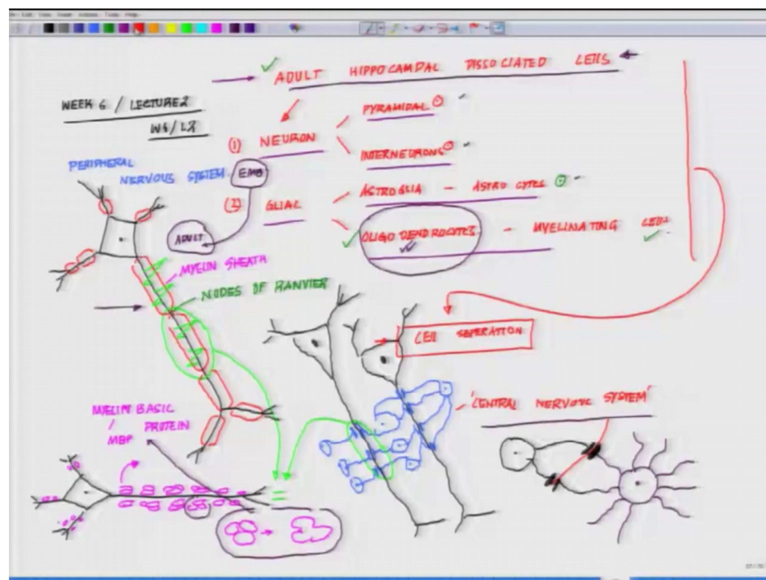

So, now, how one can separate these cells out when you get an adult tissue this is where we start it right. So, this is what you are going to get, piece of tissue which is the hippocampal neuron.

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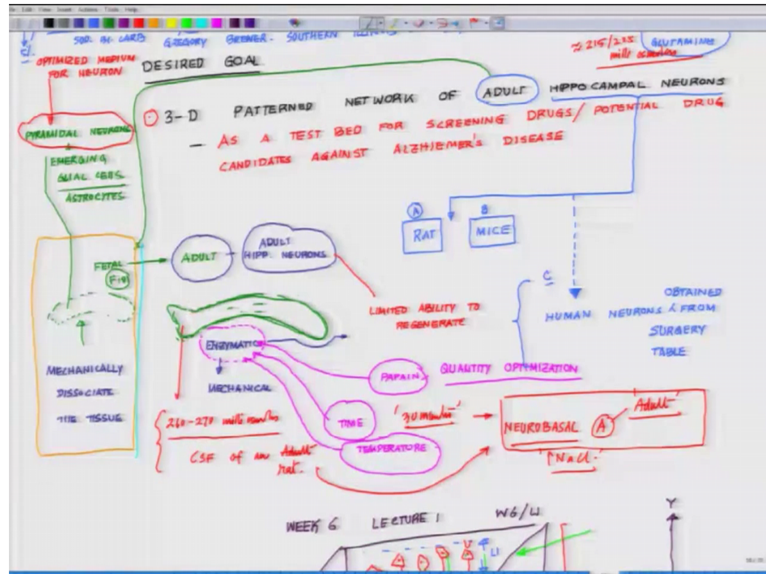

So, the way you are achieving it is that first of all you have to isolate the individual cells and you have to reestablish the contact. So, this is the population what you are getting pyramidal cells interneurons astroglia oligodendrocyte first of all you dissociate these cells and we have talked about the dissociation protocol. Next once you have dissociated these cells how you can separate out in the cells.

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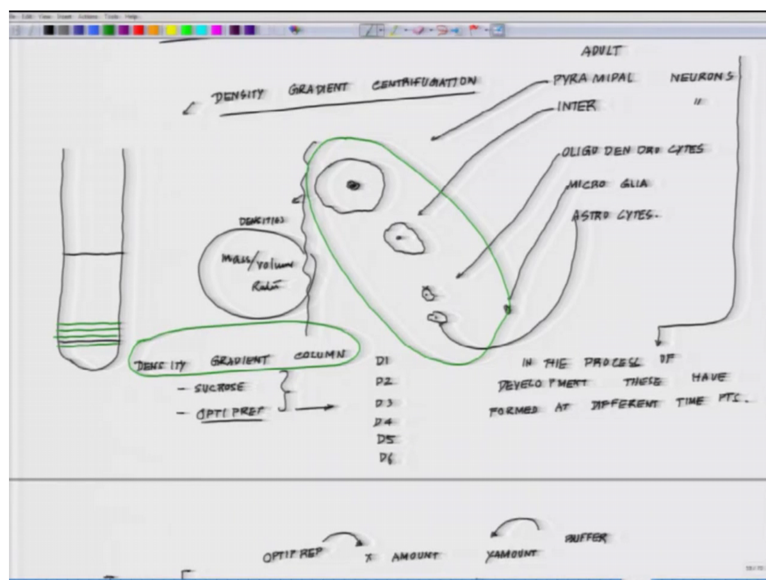

So, one of the ways now I will come to that now we are into week 6 lecture 4 W 6 L 4 this is where we are now when you talk about getting the adult hippocampal neuron and how we are going to separate. So, again just let me enlist pyramidal neurons inter neurons oligo dendrocytes you will have micro glia will have astrocytes. So, one of the

simplest way of cell separation is called density gradient centrifugation density gradient centrifugation what really is density gradient centrifugation.

So, each one of these cells first of all when you isolate the cells try to see when you dissociate them what are the size difference in the population. So, say for example, when you talk about the pyramidal neurons these will be after dissociation when it has lost all if it is an adult we talking about the adult it has lost all its processes is this would be something like this. You see an interneuron contrary you will see slightly smaller whereas, the oligos if you look at them they will be far more smaller microglia will not be able to see it really this will be so small and then you have the astrocytes which are kind of similar to oligodendrocyte and interestingly in the process of development these have formed at different time. So, in other word in the process of development these have formed at different time points.

So, their requirements are also different now one of the easiest way to go for it is. So, each one of these cells will have different densities based on their density which is essentially masses to volume ratio you can separate these cells what one has to one has to do is one has to create a density gradient column. So, what is the density gradient column? So, you have some neutral liquid something it could be sucrose it could be one of the new ones which are being used for last 10 years is opti prep.

So, there are several such molecules which will not sucrose has certain issues with osmolarity though as compared to opti prep which is much more easy to deal with you form a density gradient density gradient means. So, you layer the. So, you can make these solutions of different densities say for example, you take opti prep density 1 density 2 density 3 by mixing it with some suitable buffer D 5 D 6.

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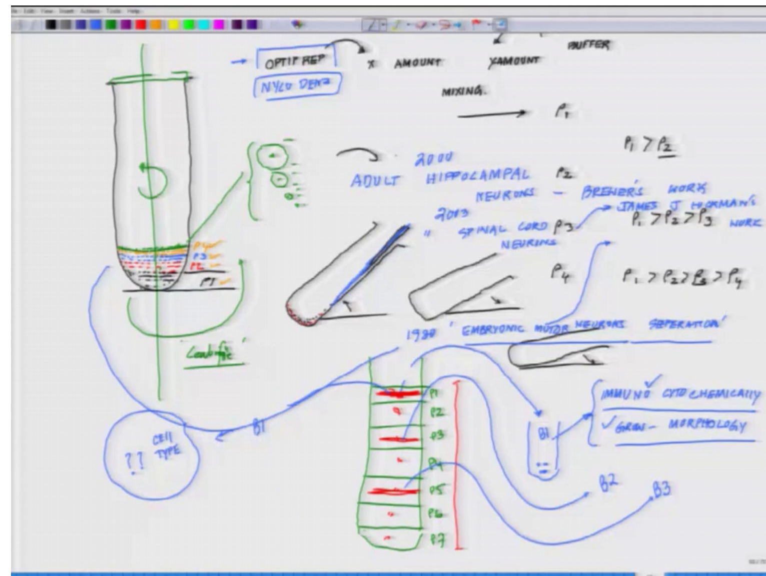

So, let us take this simple example I make 4 density 6 different densities of I take opti prep I am not taking sucrose for a reason there are issues and I do not want to get into that it I take x amount of opti prep and I use a buffer where the neuronal cells will survive and y amount of buffer. So, I mix them at a particular ratio and I achieve particular density say I said density 1. Now I mix them in another ratio I achieve a density two and assuming density 1 is more than density 2. So, it means density 2 what I am getting is lesser density then I make something density three now density 1 density 2 density 3 then I get another one density 4 and I am just mixing them small amount maybe one ml thick just 1 ml, 1.

So, then I get density one density two density 3 and density 4. So, let us just stick to 4. So, now, I take a pipette and on a pipette I layer the highest density 1 ml layering and this layering has to be done very very gently, so density 1. On top and why do you do this layering this took me a long time to learn it it is absolutely trial and error. So, whenever you are pipetting it generally the way we do it you hold the pipette and you just you know dumb the fluid out there, but actually this density gradient should not be done like that. The way one should do this make this density gradient is something like this you till the whole thing like this almost at an very very acute angle that the more the angle is kind of closing to zero like if you can really hold it and this comes by practice there is no other there is no science in it or almost like this that will be the best, The more the acute angle is you cannot make it horizontal reserves it is going to flow out and using a very

narrow bore pipette here is very gently allow the stop to form that layer something like this.

So, this is where your pipette tip is you can further go down actually if you have that margin and slowly you layer it. So, what you achieve is that this is the layer one and you have to always keep it tilt slightly slowly allow it to inner settle down then put the next layer this is your this is density 1 this is density 2, then you put on top of that density 3 and it has to be done very very gently trust me this is not something very easy. Then on top of that you achieve what you, once again density 3 then you have density 4.

So, you are layering different densities where the highest density is lying here second highest here third highest here forth. So, on the top you have the lighters lightest of all. Now, on top of that you have these dissociated cells right you have dissociated these different kind of cells in a particular buffer, and I told you make these layers of. So, for example, layer 1 layer 2 layer 3 layer 4.

So, you make these layers on top of this layer you take say if you make this layer of say 4 ml or 6 ml whatever that I will leave it up to you because it depends on cell type two you are using if you are using the pancreatic cells you have to separate the you know islets of langerhans and all those things you needed a different kind of layering, but logic is the same or you are using the layer cells or you want to separate out the cells of the lungs or kidney you need different concentrations, but the logic of density gradient centrifugation remains the same.

And the reason for me to pick up the neuronal case because those are the last one in the history of this kind of density gradient centrifugation where they are being used the earlier density gradient centrifugation has been used for other cell types. So, on these density gradient columns, so what do you make what I asked you that you make a density gradient column and what is the density gradient column this is the density gradient column what we created now on top of the density gradient column you have this dissociated cell you make a very very very very thin layer on top of it. So, these are the cells which you want to separate out and it has a mix of bigger cells smaller cells even a smaller cell, small cells.

Now one more thing like I will not ask you really to go and figure out what is densities, but people over the years almost last 56 years since this technique is theory in the market

people have a fairly good idea about respective densities of the cells where they will fit in. Now, you take this you seal the lead of it and you spin it in a centrifuge. Now while you spin it in a centrifuge what will happen is and please be cautious of having this topmost layer of the cellular this thing has to be exceptionally exceptionally narrow band. There are people who recommends thicker band, but over the years I have realized that that really does not work and you spin it once you spin it what will happen depending on the density of the cell they will settle down at different density zones. So, in other words if I have a density column like this of say d 1 d 2 d 3 d 4 d 5 d 6 d 7, d 1 d 2. So, I am not following that pattern I am just kind of for your understanding I am d 5 d 6 d 7.

Now the cells of different densities are going to settle down where they find their matching density. In other word if you really really master this technique of density gradient centrifugation depending on the size or the volume of the cell size includes here of course, there you can separate out the cells. This is one of the simplest cheapest and easiest, but that needs a whole lot of optimization protocol you really have to go through the rigor of optimization, but the basic basic basic fundamental of density gradient centrifugation is this. Now if you have a hag on this technique if you really understood the technique the next part is now you understand the basic physics behind it ok.

Now there is a practical side of the story which I have not talked about now we have separated the cells great. Now once you separate the cells now you have to pull them out. So, what we will be seeing is at every level, so you may see a band forming out here now how to pull out that band this is not easy. So, for example, I see three bands three different zones now we need something like a very similar to Hamilton syringe which is used in biochemistry labs it is not really Hamilton, but something of that sort where you have to you know bring it close in and you have to pull out that band in a separate test tube similarly then you have to pull this out at a separate test tube to pull this out at a separate test tube.

Once you see these cells out. So, you have these cells of band see this is the band one this is band two this is band three now when you are doing it for the first time you have no idea which cells are which. Now what you have to do you have to characterize this cell by two methods immuno cyto chemically is one method and you have to grow them to see their morphology how they grow over period of time based on that you will be

able to figure out which bands represent what cell type. So, these are some of the technologies which has been achieved over the years like the very first technology I will I will come of the hippocampal neuron I will come above this panocord neuron where you have to separate out the oligodendrocyte and several other cell types there I will come to that.

So, what I wanted to highlight here. So, these are the basic technologies and the follow up technologies what one has to master before you achieve the goal of developing the defined systems. Earlier for embryonic neurons, so this particular technique, there was another such thing which is called nycodenz you will not find that anymore because they have discontinued this product it is very similar to optipreap nycodenz optipreap sucrose you can use sucrose also, but the sucrose the problem is what I was trying to tell you because most of these cells had a certain degree of osmolarity and sucrose kind of you know because of this molecule it kinds of ruptures the cell at times because it creates osmotic disbalance, but if you are really clever very careful you can achieve it, but I will not recommend unless you have mastered the technique beyond doubt.

So, I would rather request you try something like optipreap or nycodenz if you get it, but most likelihood you are not going to get nycodenz it nycodenz has been discontinued if I remember almost 17 years back (Refer Time: 18:54) and all those the groups who is to produce nycodenz. But these are the different substrate you can call them to achieve a density gradient centrifugation. There is another way you can do this which are becoming in my next class that how you can separate cells. So, as of now in terms of the optiprep separation what all has been achieved.

So, these are some of the literatures I will be giving you, you can separate out the adult hippocampal neurons, now you remember where we left last week and I told you I will come back to that adult hippocampal neurons and this is where we will be referring to Brewer’s work Gregory, Brewer’s work this has been achieved for adult spinal cord neurons. There I will be referring to James J Hickman’s work his group has pioneered the pioneered these techniques of; earlier to that there was a very nice paper very early back in 1982 or 83 off we talk about the adult they are a people who separate out embryonic motor neurons. I have not talked about that I will I will talk about very soon in the next class on it.

Embryonic motor neuron separation I will handle this if I could get a copy of this paper because this is a very old paper where all these density gradient centrifugation has been used. So, this is 19 I would say 80s, this is the early 2000 and this is around 2003. So, this is how this, this area of cell separation in terms of the neuronal cell has progressed.

So, I will close in here before I move on to the next class where we will talk about the other couple of techniques of cell separation.

Thank you.