Cell Culture Technologies
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Lecture – 31
Florescent Assisted Cell Sorting

Welcome back to the lecture series in a Cell Culture. So, we are in to the 7th week. And as of now we have a using a case study of neurons. We have talked about how to separate different kind of neurons and 2 process processes what we have discussed in depth, which are kind of you know can be followed by any lab at any point of time is a density gradient centrifugation. And this density gradient centrifugation, this process using sucrose or nycodenz or optic prep as the material to make the density gradients.

This technique can be used for cell separation of pancreatic cells this, could be used for the liver cells, this could be used for any cell type, provided there are few prerequisite to that provided you obtain a single cell suspension. What does that mean? That means so, whenever we take out a chunk of a tissue. So, it consists of millions of cells.

So now you have to have a way by virtue of which you use some form of enzymatic or mechanical or some treatment, where individual cell dissociate out from the tissue. So, it no more maintains the tissue architecture instead it becomes a single cell suspension, something like this I mean just start off with.

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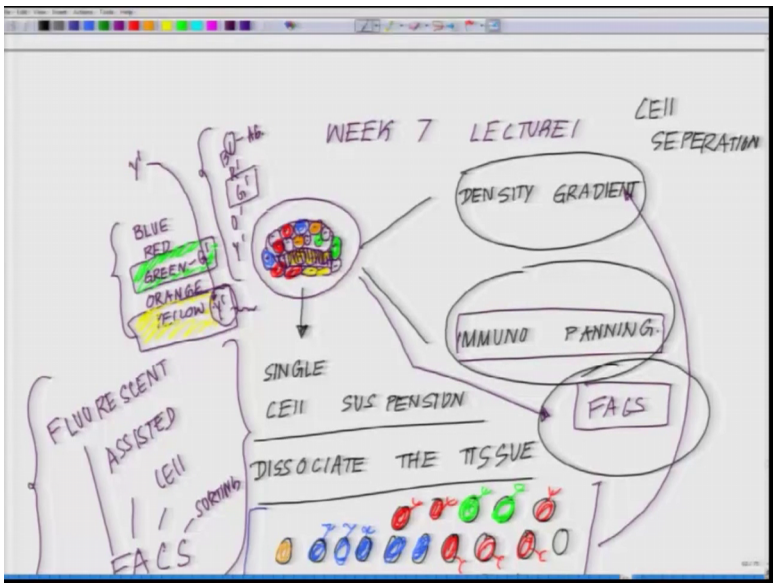

So, this is your week 7 lecture 1.

So, say for example, you have this piece of tissue what you have to dissociate and these are the individual cells say for example, something like a 3 dimensional tissue. Now if I have to use this let us say for example, I see I have certain cells which are in orange, certain cells which are green these are different cell types based on their different functionality some cells which are yellow like this.

This is how the and you want to separate all these different cell types. So, the in order to use whether you use. So, we have talked about 2 techniques right. We have talked about density gradient and we have talked about immuno panning where you are using an immune marker.

Now, for both these techniques what is very essential is that from this mass you get single cell suspension. And the single cell suspension means essentially if you dissociate this tissue if you dissociate the tissue what you will be getting is something like this. One second something like this and (Refer Time: 04:56) have.

So now if you get this kind of mix and the individual cells have different size, then by virtue of which you can follow the density gradient. Provided each one of these population have different densities to follow. Now say for example, they do not have different densities to follow, then if they have different kind of immune markers, something like this and you have an idea that these are the immune markers which are present there.

So, then what you can do? Then you can follow something called an immuno panning what we have discussed in the last class, further a extension of the technique is cell sorting. You can use FACS, which is fluorescent assisted cell sorting. FACS which is essential if F stands for fluorescent, A stand for assisted, C stand for cell and S stand for sorting. This is another modern technique.

So, what is really FACS? So, we have talked about two. So now, I am adding a third frame to this which is your FACS, what really facts is all about? So, we will discuss a little bit about in this class about what is FACS. So, even before getting into any kind of study on facts try to understand the basic fundamental logic what is happening. So, say for example, you are going to a stadium to see a match or something.

So, there is a thousand people and each one of you or each one of there are. So, say for example, I divide that vip gallery, say my vip gallery then I say this is only exclusively reserved for families of some big it is, and then I reserved section for some sportsmen or something. Then I said this is a general. So, I have 5 different categories. Now when I am entering I am entering through one common gate right.

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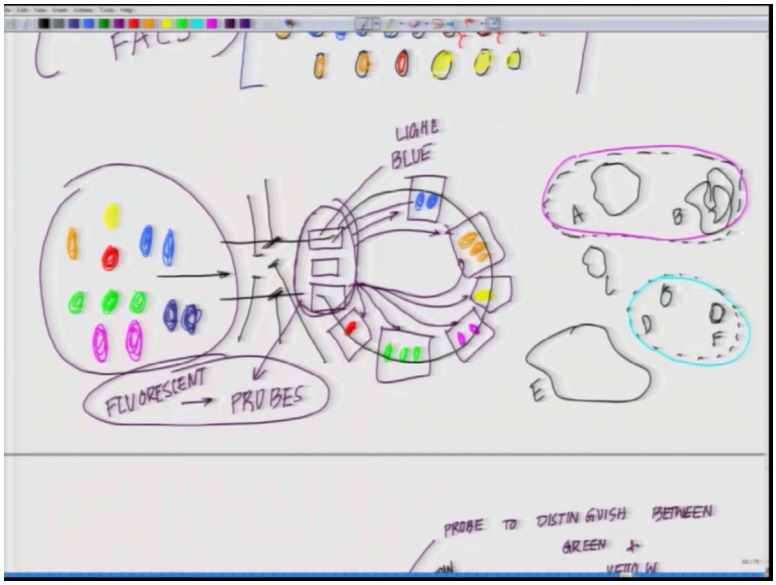

But the difference So, say for example, this is the common gate through which I am entering. And this is the arena where we are supposed to go, and here people are coming of different types. And these are the different kind of people coming to an view the match. Now see for example, out here out at this place, I have a sensor out. So, each one of these people are coming. So, the sensor will tell this is blue light blue.

So, these light blue should be asked to you know move to this spot this is the arena. Now this is red, this red should be asked to this is reserved for the red should move here. These are greens the green should be here. And these are say pink, pink should be here. These are yellow should be sitting here. These are light say orange; orange should be sitting here ok.

So, what I wanted to say is say for example, on the cell surface you have specific markers. If you consider these as different cells, instead of these are individuals who are holding different color tag of tickets. So, they have this ticket for it. So, I have this color. So, for you this place is reserved. So, for light blue this place is for light green this is

reserved, for pink this is reserved, for yellow this part is reserved, for red this part is reserved.

So, there are locations which are reserved now, but you have to location is not an issue is how you separate them out. That is the most critical point. So, in order to separate them out the first thing what one has to do if you get these specific cell type say for example, I get this, I get this, I get this, I get this, I get this. So, there are 5 cell types say for example, I am getting A B C D E.

So, I told you one options I have is that I separate them out by density gradient which is possible actually, but if they are of the same size then the density gradient would not work out. Because the density of the 2 particles say for example, I just introduce another one say f. Now between d and f you hardly will see a density difference or similarly between A and B there will be very little option that there will be a huge density difference, you understand?

So, in this situation say for So now, what you have you have? These 2 population this 2 population which will behave differently as compared to this population. Now how to separate them out? Now one option is that you label them just like you are entering the arena to see a match. You have a specific kind of color coded tickets. You may have a orange one you may have a green one, you may have a blue one, you have a red one.

Now, based on that code here there are sentries who are standing out here they will tell you that you are sitting spot is here or your sitting spot is here or yours is here or yours is here or yours is here or here or here whatever. Now at the microscopic level these sentries will be replaced by specific probes. And the kind of probe are we talking about is called fluorescent probes.

The probe which will distinguish oneself from another based on the fluorescence signature which, either the cell has an inherent fluorescence signature or you introduce a fluorescent level on the cell. The say for example, you know you have a mixture like this a mixture of cells like this now go back to the previous diagram where we started. So, you know this is your tissue has this kind of it may be a pancreatic tissue it could be a liver tissue it could be a spinal cord it does not matter that is irrespective ok.

I am taking examples of neurons, because I have done intense work on that area. So, I know exactly how, but the logics it is not that from the neuronal book I picked up the logic logics were standard cell culture logic, but then you have to take a cell type to tell you and based on your situation you will have to pick up the conditions and, but you have to understand what all be sick tools you have.

So, this whole purpose of this exercise is you know what all scientific or cell culture tools you are having at your disposal in order to crack a problem right. So, coming back. So, if you look at it. So, say for example, how many colors? We have we have say we have blue and these colors are very symbolic here it is it is nothing to do with the real life colors red, you have green and you have a orange and you have yellow.

Now, suppose you know these you have these 5 different kind of cells. And you know there are unique antibodies which could be bound on their surface, which say for example, I take say now I label the different antibodies, antibody B antibody R antibody G just sign. That this sign is saying that this is an antibody G o prime Y prime.

Now, if I bring Y prime into the game, I know only yellow cells it will adhere to the yellow cells right. No issues now I bring. So now, these cells will have a tag Y prime, now what I have to do? I mean if this is a primary antibody I have to have a fluorescent tag which will distinguish these cells. So, say for example, I want to separate from all these 5 different cell type I wanted to separate 2 cell type just a hypothetical sake. The green and the yellow ones. So, what I do I put a G prime.

So now I have 2 cells which are now labeled with 2 fluorescent probes, yellow and the green, right.

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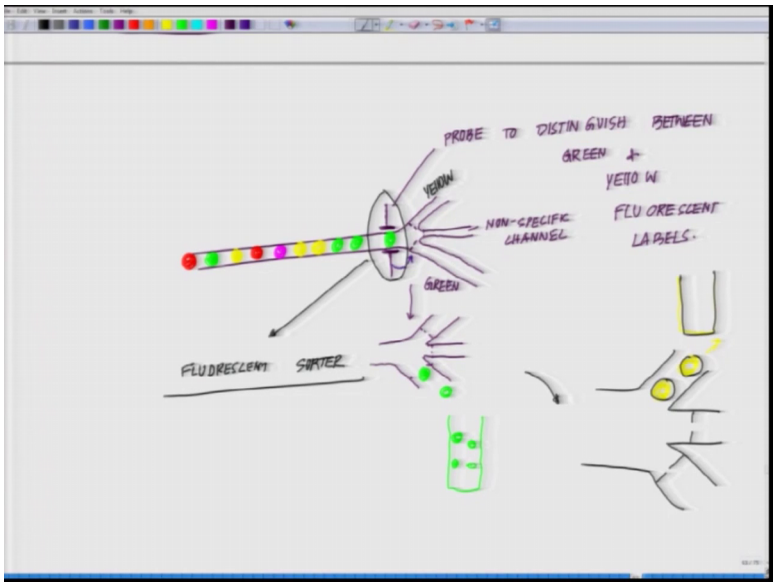

Now, I have a sensor say for example, I allow the cells to flow. So, I have something like this a very narrow tube through which only one cell at a time can flow. And here I have a sensor and here I have a gate.

Now, I have multiple cells which are coming through right and yet I have actually this drawing was a slight mistake and I am just putting it. Now putting it now I am getting cells of these kinds. Maybe yellow right and at a time only one cell is coming through red pink you can yellow any other green likewise right.

Now, here you are having the probe. Probe to distinguish in this case study. Probe to distinguish between green and yellow fluorescent labels. So, so this channel. So, we are having 2 channels now right. So, this is one channel which I call this as a yellow channel and there is another channel, which we call this as a green channel. Because this is what I am going to separate out. And this is a non specific channel right.

Now, what will happen once the cell will approach here say for example, a green cell approaches here. Now as soon as this sensor senses this it will send a command signal which will remove this. So, o k by the way there is a third gate here. So, it will immediately open this gate and the next phase what you will see, this is this is this will remain close, but this green gate is now open and this cell would not move.

So now you have a collection vessel where you are collecting all the green ones similarly if there is a yellow one coming through. So, what will happen real life? Now yellow cell coming this gate will remain closed this will open and the yellow cell will move here and you can collect all the yellow cells in your bucket.

So now from 5 cell types now you are slowly narrowing down to 2 different cell types. So, you see this is how. So, this fluorescent sorted. So, depending on how powerful is your sorter. So, this is your fluorescent sorter. So, it is the same analogy what I told you in when you enter a stadium. So, here you have the sentries standing there who are ensuring which part of the stadium you should go.

So, these fluorescent cell sorter fluorescent assets assisted cell sorter are one of the very potential tools in the modern cell culture labs. And this comes very handy when you have a complex situation like this where you have multiple cell types to be separated out, but the fact is still the same.

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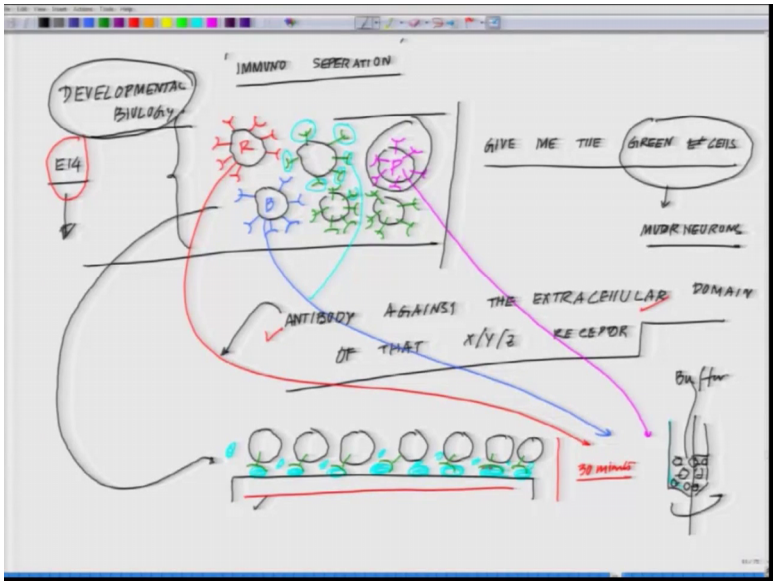

What I showed you in the last class, that you have to have those unique markers. Without this basic fundamentals still the same, you have to have those basic markers, without that really to sort them out is would not would not be easy job.

So now in the light of this. So, you have density gradient centrifugation, you have immuno panning you have fluorescent assisted cell sorter. So, we have 3 different techniques by virtue of which one can do a cell separation.

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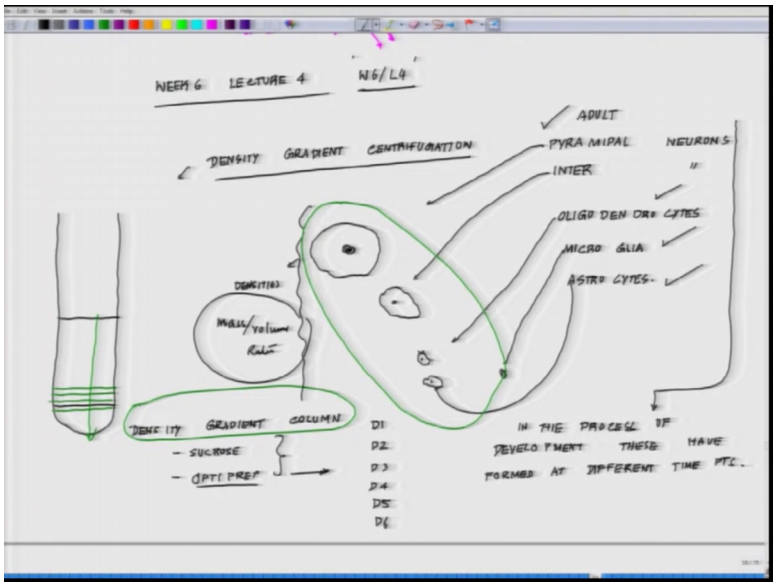

And now in the light of this if you go back and think what all we covered think over it. You have different kind of neurons different all oligos microglia astrocytes and not only you can separate them out using density gradient, you can separate them out further separation can be achieved by virtue of which they are immuno panning.

As a matter of FACS it will be surprised to know regarding this immuno panning if you really know at what point of time these specific cell express unique markers on their surface. All these kind of fluorescent cell sorting can be really very formidable technique by virtue of each you can isolate them.

So, I will close in here for these classes in the next class well go further and discuss about some of the other aspects which will help to have a much more real life model of cell culture.

Thank you.