**Cell Culture Technologies**
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**Lecture – 32**
**Condition for Regenerated Cells**

Welcome back to the lecture series in Cell Culture. So, in the last class we summarize the 3 techniques of cell separation, where we talked about density gradients centrifugation, we talked about immuno panning and we talked about fluorescent assisted cell sorting. Now coming back one small tail piece which I did not cover while we talked about the immuno panning of the motoneurons which I just wanted to tell you, how powerful these techniques are provided you understand the developed biology of most of these different cell types.

So, in the embryonic motoneurons which are present in the ventral horn if you remember that I showed you that diagram I am just going to go back, yes.

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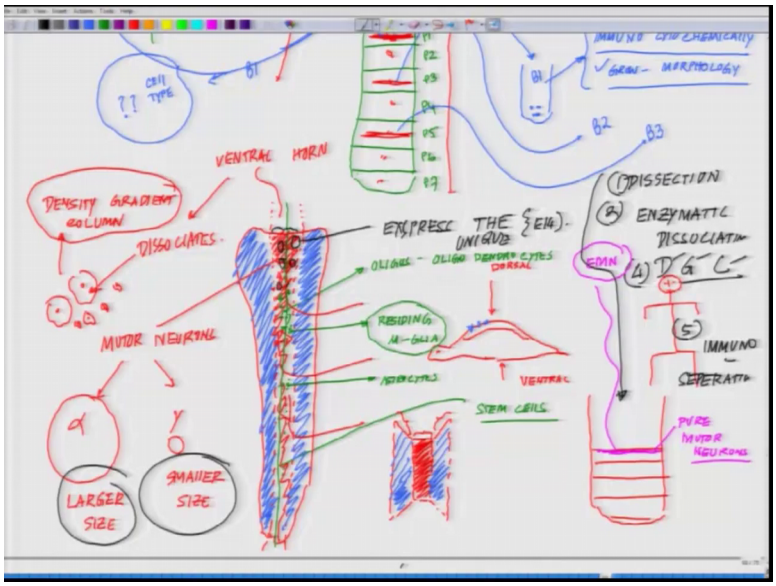


In the ventral horn motoneurons where I told you that you have 2 types like you have the larger size you have the smaller size. But what is important here I wanted to highlight is that, these ventral horn motoneurons these large motoneurons express that unique the unique extracellular or unique antibody at only or at specifically at around E14

embryonic day 14. And after that all the cells here express it is something like them I will show you. So, we are into week 7 lecture 2.

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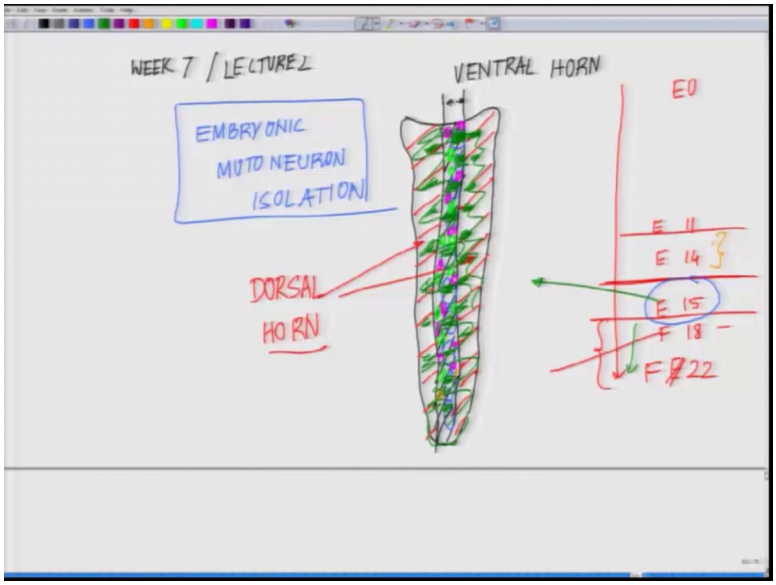


So, say for example, this is the spinal. Cord and this is the ventral horn. The shaded region is the ventral horn, ventral horn. And this is the dorsal horn, dorsal horn. Now interestingly So, the embryonic day starts from say E0 to say E22 when sorry, this is actually not calling run it is called fetal. So, embryonic day last still so, is halfway through. So, around E11 this is also called embryonic. And up to 14 it is call an embryonic day 14 15 after post 15 it is called fetus.

So, around I would say E15 and then F I would say F 18 likewise. Maybe you can consider from 18 they it is called a fetus it is, why it is called a fetus is at that particular time the whole anatomical features comes out perfect. Prior to that the whole anatomical development was kind of not you can cannot really fully make out. It is there you know that this is a small pup or something, but not fully that is why these 2 distinctions are there.

So, coming back when we talk to you about separation of embryonic motoneurons. So, this is what we are kind of revisit visiting this problem in the light of our new techniques, what we have learned cell sorting by immuno panning density gradient and fluorescence activated nested cell sorting embryonic motoneurons isolation. Now so, these motoneurons are sitting here right. So now, within this there are many other cells which

are sitting there. Now what is unique about the motoneuron at E14 or E mostly E14 you can think about is they express a very, very unique marker, which cannot be seen any anyone of them like, it this green is that marker what I am. This is that E14 the story at E14 only these cells are expressing this marker, but as time progresses as we move from F 18 and likewise, 16 17 then this antibody or this marker not antibody this marker is expressed all over.

Why I wanted to highlight this point? This deserves a different highlight because of the reason that unless you exactly know the developmental to paradigm of a particular tissue type, mark my word you should not jump on to cell sorting. First of all you try to understand what you are doing, if the specific antibody is there and you have a or a specific marker is there sorry pardon my, language it is not antibody is the a specific marker is there, on the cell surface and you have a counter antibody to bind to it then only you should go for it. But say for example if somebody uses this paradigm which is supposed to be followed at E15, you try to use it at E 18 it is just not going to work I mean you will get a lot of contaminating other cell types right.

So, these are some of the points what I wish that you people should invest your energy whenever you are doing cell sorting you should read the literature thoroughly that you know how things have developed what others have done. And where you are where you want to take it to the next level, what are those critical question what you are trying to address. So, it is very important that you go through this whole roller coaster ride of understanding that why it is so significant, that you understand that is why I kind of you know revisited this that these profound tools can come very handy provided you know the power of these tools. If you do not understand the power of this tool you can misuse a tool which many a times I see and which saddens me, but that is how it is ok.

So, having said this, now I will switch gears from cell sorting part to a next level. That is in a culture dish when you grow cells. What is our anticipation? Or anticipation is that it should not only maintains it is phenotype the cells should not only maintain it is phenotype it should be able to maintain it is functionality as far as it can afford. So, having said this say for example, we wanted to culture a cell which secretes insulin. And now we have to ensure that those cells in the culture dish indeed secrete insulin may not be with the same intensity with which it does in the body, but maybe fifty percent of that or maybe 10 percent of that maybe even 5 percent of that I will accept it or maybe even 2

percent of that, but it has to continue with that function. Or say for example, I say I wanted to culture neurons great.

Now what is the function of a neuron? It is a electrically active cells. So, electrically active cells means it fires action potentials. Now if I have neurons which does not fire action potential in the culture dish, or if I claim that you know from x y z stem cells I have derived neurons in the culture, with all possible immune markers I show it. For me it would not make any sense unless otherwise it has been shown that s indeed those neurons what I have cultured are electrically active. If they are not then why there is a problem. There is some serious issues out here. And it is very important and whenever we venture into the world of cell culture, one has to keep this basic fundamental ideas in mind that what are you targeting, what is the goal.

So, say for example, whenever I asked this thing. So, so the critical question let us put the critical question first ok.

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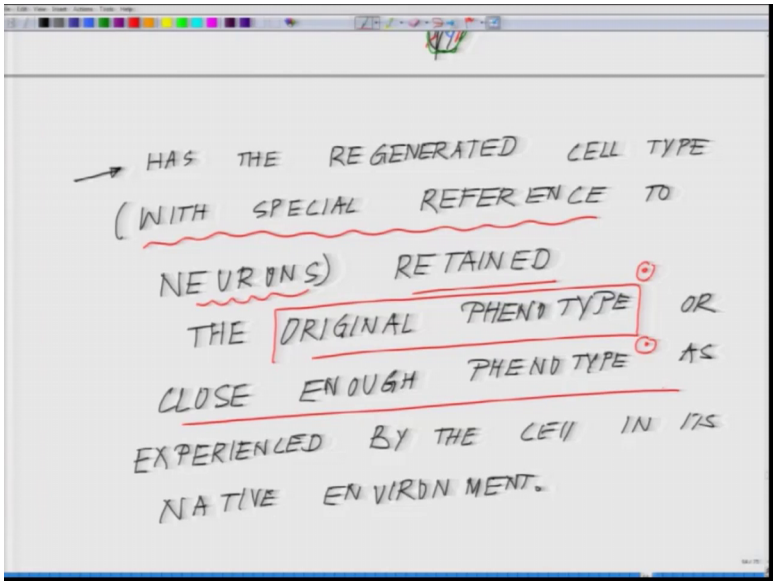


So, the critical question is that has the re generated because you are regenerating the cells in the culture, regenerated cell type with if I say with special reference to neurons. I take the liberty with special reference to neurons retained the original phenotype or close enough phenotype as experienced by the cell in it is native environment. So, when we talk about native environment. So, we are talking about something like this.

So, let us repeat. It has the regenerated cell type with a special of course, we are talking about new neurons in this situation, which could be any other cell type with a special reference to neurons or other cells retained the original phenotype this is part one. Or close enough phenotype experience by the cell in it is native environment. So, this is part one the phenotype.

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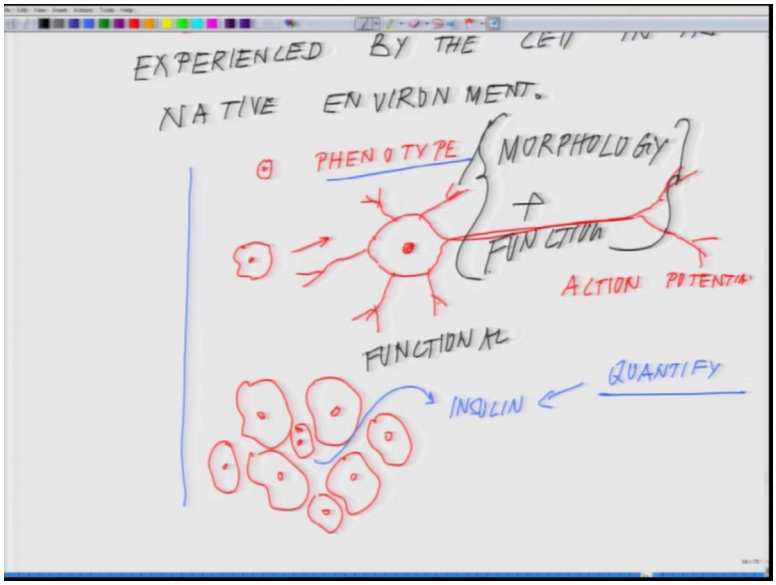


So, what we mean by phenotype here is say for example, I say this cell is this time to become neuron. So, I will expect that this cell will have a processes which will have an axon dendritic tree and it will express all the necessary ion channels to behave like a neuron. So, in other word it should be able to shoot an action potential. Similarly if I say this cell is these community of cells what I am growing is suppose to secrete insulin, then I expect that they will form this kind of clustering and should be able to see they are secreting insulin which of course, I have to quantify by some kind of titration or whatever right.

So, here apart from the phenotype I introduce another aspect which is called functional. So, morphology plus function. Could these 2 parameters being retain in the cell culture dish? Unless this is being retained the purpose will not be achieved of having a model which is close enough. So, having said this and again as I told you that I will be taking mostly the case of neurons here, because they are the most challenging ones. So, for example, again if I take another excitable cell I say I am culturing cardiomyocytes, you

know what will be my first question are the beating? Are those cells in the dish beating? Are the contracting like that? If they are not then I will have doubt that are you really regaining the phenotype as well as a functionality they look like great like phenotypes sure, but then are they functionally active if they are not it means we have to do something. Similarly if you culture our muscle. So, it is skeletal muscle my first question again will be curious stimulate it. So, that it contracts because all our skeletal muscle what we have they have both electrical as well as mechanical function. Are we there? Or I will ask a question that you are growing the muscle say for example, let us take the example of muscles.

So, generally whenever we talk about muscle culture, we talked about there are 2 possible ways and here we will talk about say skeletal muscle.

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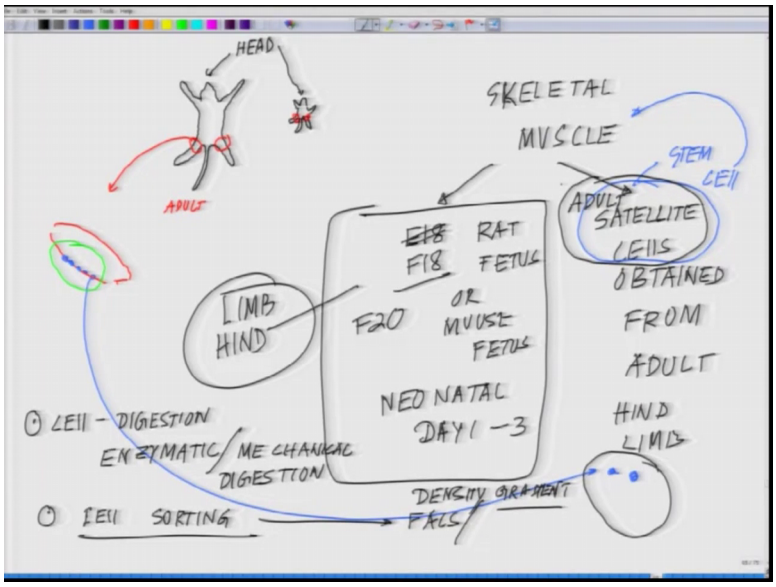


Let us take the example of skeletal muscle first. So, mostly skeletal muscle could be culture from 2 different sources. One source is your E 18 sorry, F 18 rat fetus or MUUSE fetus E 18 it could be F 18 it could be F 20 F 20 or it could be a neonatal, which is day one to day 3. Should not exceed because it is really challenging to an isolate the tissues and dissociate it. All you date for do it from satellite cells obtained from adult hind limb. And the reason why the follow hind limb. So, here also this is all from the hind limb, hind limb. Reason they do it from the hind limb is that you get sufficient amount of tissue. Say for example, it is so it is something like this if ok.

So, basically the tissue is collected and if it is in fetus it will be something like this and if it is an adult. So, this is the head similarly here is the head and here you have the hind limb. So, most of the tissue is collected from here. Now the satellites cell if we talk about this as an adult. So, satellite cells are sitting like this if this is the tissue just making the hind limb underneath out here. And these satellite cells are collected. So, these satellite cells are essentially it is stem cells this time to become a skeletal muscle. And suppose you have to isolate these satellite cells from here you needed to do a lot of the techniques what you have red cell digestion or enzymatic digestion, enzymatic slash mechanical digestion one. Second what you have to do is followed by that after this mechanical and enzymatic digestion.

Next step will be cell sorting once you sort out these cells then you have to may have to use FACS or some other density gradient or FACS or some kind of immune marker to isolate this satellite cells and then you have to grow them. So, this is a long route where you have to grow from the adult satellite cells. And again when you grow it from the adult satellite cells your success rate is not that easy, it is kind of tricky.

Now, you might wonder why we needed to use the satellite cells. There are reasons for it. And that is where I am going to come that why you cannot use the other cell type. And that is precisely is where we will be discussing about skeletal muscle that how they grow and how you have to be careful while we will be doing this kind of cultures.

So, I will close in here in the next class we will follow it up from here that how to isolate these cells and what are the phenotypes we see and what are the functional analogues of it.

Thank you.