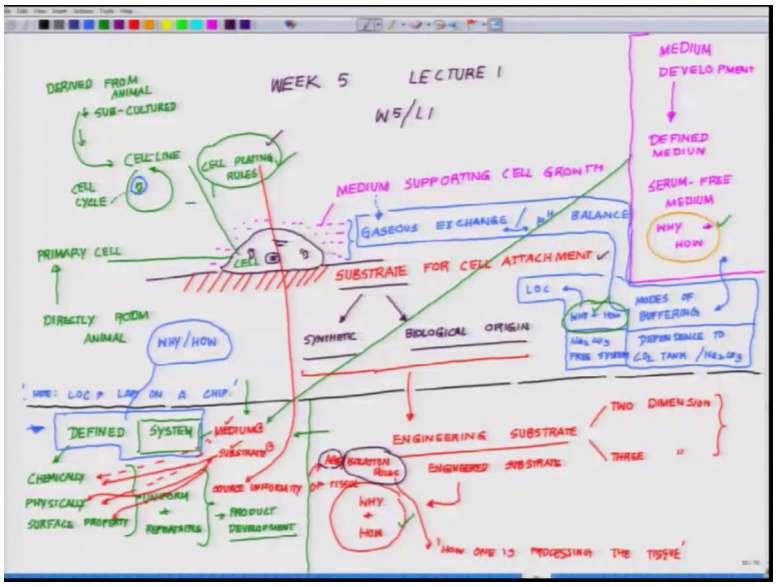
Cell Culture Technologies
Prof. Mainak Das
Department of Biological Sciences & Bioengineering & Design Programme
Indian Institute of Technology, Kanpur

Lecture – 22
Mechanical Dissociation of Hippocampal Tissue

Welcome back to the lecture series on Cell Culture Technology. So, if you remember in the last class we talked about the define system and we decided upon that, this class we will talk about the genesis of the defined system and why and how we could achieve it. So, we are talking about a defined system we have to look into the major stakeholder of cell culture technologies one of the biggest stakeholder in the cell culture technology in the world is the pharmaceutical industries, why is it so? Because any drug discovery goes through different channels different procedures.

So, first of all chemists synthesize say you know x number of molecule for a particular y disease or they isolate x number of molecules from nature or some other sources for some is specific kind of you know pathological situation. So, down they needed to do the testing of course, they cannot directly go to the animal testing, they have to go through a cell culture test cell culture or testing in the cells in vitro. So, the first testing so if you just that mean start putting them down. So, that today we are into week 5, lecture 2, W 5, L 2.

(Refer Slide Time: 02:00)



Now, where we are starting today is genesis of the define system is one, second how of course, of course, why there, which is essentially answering why there is a need for define system and how we could achieve the same. Now, as I told you the, we just switch gears and told you the drug discovery industry drug discovery process.

Heavy dependence on cell culture, cell culture trials before moving to animal or in vivo trial and these are all in vitro trials. So, now, since I have jot down thing how you should think over this it is. So, this needs bit of a thinking process what we really meant by that. So, say for example, we wanted to prove a point, let us talk about let me say that system is working towards a medicine which will say prevent Alzheimer's fine. Now, you have to test this, if you have to test this you need it first of all in vitro set up.

So, let us jot down the points. So, this is how I am going to proceed say for example, our industry. So, we will, we will have a case study which will be a imaginary case study, but that imaginary cases study well or hypothetical cases study will help you to appreciate what we meant by the defined system. So, we will be coming to the defined system from a different angle. So, bear with me. So, say it say for example, drug discovery process, here is the drug discovery process and in that drug discovery process the goal is now to screen some x number of drugs against Alzheimer's disease.

So, lets enumerate what all will be needing. So, of course, we know the processes there has to be in vitro trial, there has to be a in vivo trial and it should follow that sequence you cannot and remember you cannot jump to a in vivo trial without doing the correct in vitro trial. Now, what was existing in the industry something like this when we talk about screening Alzheimer's drugs you will see many many work what they will do I will tell you, they will before, I get into this those who does not know what happens in Alzheimer's.

So, you lose the hippocampal neuron of the brain. So, if you look at the brain. So, here is your brain this is the dorsal view you are looking at the brain from the top and here is the ventral view of the brain where you are looking at the brain from the ventral side, this is a ventral and this is dorsal and out here is this is this position slightly deep inside lies hippocampus you, you really need good surgical skills to isolate the hippocampus you cannot do it. So, easily you really need to go down and pull out this tissue.

Now, what happens in Alzheimer's for those who are not aware of it and for those who knows it, these cells which are part of this which are essentially interesting neurons with a very nice pyramidal geometry, their cell body looks like pyramids and they are large cells. So, this is how most of them looks like if you look at the cell body it will look more like more like a pyramid like this. So, there is a 3 dimensionality into the structure. So, these are called pyramidal neurons.

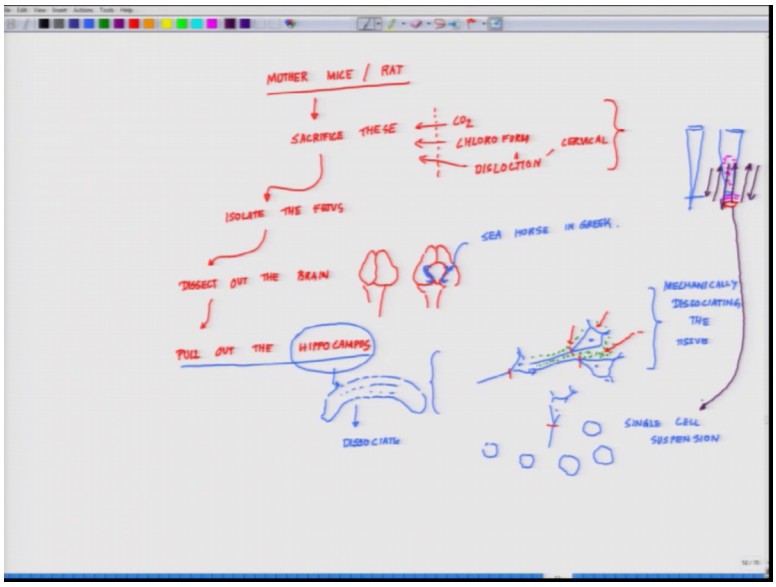
So, these pyramidal neurons form a series of circuits within this structure which are called ca 1 2 3 4 and of course, there is a zone called dented gyrus and these pyramidal neurons starts to die at a certain point in life in Alzheimer's mostly these are age related disorder. So, when we talk about Alzheimer's we talk about age related, mostly again this is mostly because age related neuro degenerative disease and this age related neuro degenerative disease means these aging neurons in other word as you are aging the neurons are also aging, these aging neurons dies for an unknown reason, ok.

So, now you want it to screen a drug for these aging neurons what paradigms followed in many places and some now they are shifting, I will tell you the existing paradigm for. So, this is your case study so now let us see; what are the paradigms which people are following. So, the first thing what you will be needing for this is one you need hippocampal culture. So, which is has to be a primary culture because there is nothing called hippocampal cell line or something because these are completely differentiated cells, hippocampal cultures. Then what you need is defined medium I will come to that why then you need define substrate fourth define growth conditions these are the most optimal things what will make this a story to explore further.

So, what people are doing, now let us see: what is the current status. So, the current status is now I am putting here on this side, now you have the hippocampals culture which is people use embryonic day eighteenth rat or mouse what does that mean. So, a rat or a pregnant rat or a pregnant mice goes through a pregnancy phase of 21 to you know 23 days somewhere in between, on eighteenth day the fetus. So, there are 2, 2 words which are being used. So, when they go through this whole cycle of 1 to 21, 21 to 24 days. So, up to fourteenth day this is called an embryo and from fourteenth day till birth it is called fetus ok.

So, this is the cycle a rat or a mouse goes through, now on day 18 somewhere out here seventeenth, eighteenth the hippocampus is fully developed in the fetus. So, what you do you take the mother, my mother mice or rat you sacrifice it. So, let me go to that next slide that will make more sense ok.

(Refer Slide Time: 15:10)



So, mother mice or slash rat sacrifice the subject, now how you are sacrificing is important are you using co 2 or using chloroform or using cervical dislocation has to be fixed, that methodology has to be you know fixed which methodology you are using then post this you isolate the fetus then you dissect out the brain then you pull out the hippocampus.

So, here you are having the brain and as I told you that, that is the dorsal side and here is the ventral side of it and here is your concern tissue. So, you really needed a very good pair of hands to pull out the hippocampus once you pull out the hippocampus it is more or less it looks like this, this is we are talking about the fetal hippocampus something like this. Though this word hippocampus had come from there is meaning is a sea horse in Greek so it is a shape is like a sea horse so, that is why it got the name.

So, here is your isolated tissue hippocampus, now this hippocampus what you do you would associate this tissue, what I meant by dissociation is that if you imagine this tissue it is something like this it is a network of neurons pyramidal neurons, very small teeny tiny pyramidal neurons its form a huge, huge networks how there. Now, when you are

dissociating it what you are essentially trying to do. So, in between you have a lot of extracellular matrix which is I am just giving 2, 3 neurons here showing you, but there are thousands and thousands of them forming this tissue.

So, what you do using some xyz technique out here interview. So, you kind of you know split the cells into a single suspension. So, what you wanted to achieve is you have the cells separated out like this, but while doing the process most likelihood you are going to break some of these. So, what will be getting as a single cell suspension most of them will break you will be getting like this, these are the individual cells which you are going to get out and this is called single cell suspension. Now, when we talk about this part how you are going to do this you have to realize that your options are to either you mechanically dissociate them, mechanically disassociating the tissue.

So, you can mechanically dissociate the tissue because these are very very soft, what you can do is that you take a, you take a pipette tip and you just cut the tip a little bit, just say for example, you have a pipette tip like this so you cut it. So, what you are left with is something like slightly bo white- bore. So, you, you have your tissue out here in the medium. So, along with one second, you take the tissue in, out, in, out, in, out, in, that process what will happen you will be obtaining what we call as single cell suspension. So, it is a kind of a shear force by virtue of which you are breaking the tissue, it is a very brute force method , but this brute force method does work for tissue which are soft and that is good enough especially for hippocampal neuron that is good enough to do this.

So, what I will do is here I will close in and the next class will continue from here to explain that how this tissue will be plate it because see in this process what we are happening, if you go back the slide before we talked about cell plating rules and here I mention about age isolation rules. So, we are talking about the isolation rules now. So, this is your map which you have to keep in mind as long as you could follow this map you will be able to follow these lectures very smoothly.

So, I will close in here and we will continue with this hippocampal thing and we will talk about what are the challenges what the industries are facing. So, we will just stop here, ok.

Thank you.