Electronic Systems for Cancer Diagnosis
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Lecture – 02
Tissue and Cell Culture Techniques: Methods

Hi, welcome to this particular module and as we have seen the last module about cells; let us see in this model what is Cell Culture alright.

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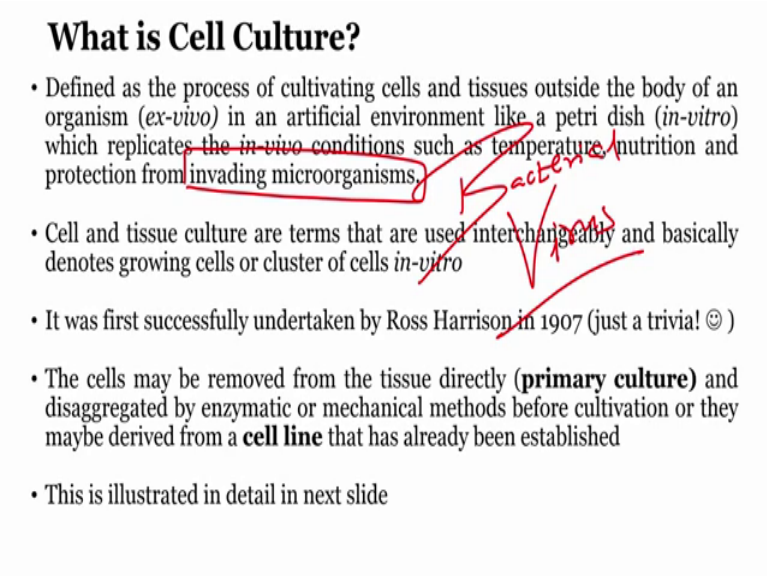

So, if you talk about cell culture it is defined as a process of cultivating cells and tissues outside the body of an organism and when you do that is called ex-vivo. So, if I take the lets say a tissue from an animal and study the tissue is called ex-vivo study or ex-vivo study alright. And, if we are studying the cells or we are growing the cells on a petri dish or we are studying we are growing organoid on a petri dish and we are then loading it in a device or studying that particular organoid or cell in an artificial environment it is called in-vitro ok. In-vivo is where in the we are studying the tissues or we are studying these cells within the body alright within the body, outside the body, in the laboratory.

So in-vivo, ex-vivo, in-vitro this is easy way of understanding, but if you want to define it the body cell culture then we have to understand it is a process of cultivating cells and tissues outside the body of an organism in an artificial environment like a petri dish, which replicates the in-vivo conditions. Such as what are the conditions? Temperature,

nutrition and protecting from invading microorganisms and when you are talking about invading microorganisms, the invading microorganisms for the cells are bacteria which we call bacterial infection, virus which we call viral infection.

So, the enemy to the cells are bacteria and viruses ok. So, then we have to create an environment which will replicate the in-vivo situation, the situation within the body. Such as temperature, nutrition, the CO 2 concentration, the humidity, the oxygen concentration right. And, to protect those cells when we are growing in the laboratory against microorganisms such as bacteria you got it. So, that is the definition of a cell culture. So, let us go back to the slide where, to be see is cells and tissue cultures are terms that are used interchangeably.

And, basically denotes the cells or cluster of cells in-vitro; that means, that I can say cell culture or I can say tissue culture that are used interchangeably. Because, group of cells will form a tissue, group of cells can form organoid, group of cells can form spheroid. Group of cells that is why we can say a tissue culture or we can say a cell culture, cool. So, let us see who discovered first time cell culture. It was first successfully undertaken by Ross Harrison in 1907 and this is just a trivia, just for information.

And, the cells may be removed from the tissue directly called primary culture and this aggregated by enzymatic or mechanical methods before cultivation. Or, they may be derived from a cell line that has already been established by three methods. How? First is if I take the tissue and if I do the enzymatic reaction then the cells will disaggregate right, it will separate, aggregation coming together disaggregation separating out. So, if I use enzymatic reaction I can disaggregate those cells. Second is I can use a mechanical way of disaggregating those cells. Third thing is I can derive further cells from the existing cell line, it is three ways of doing it and all three ways are given or illustrated in the next slide which is right over here.

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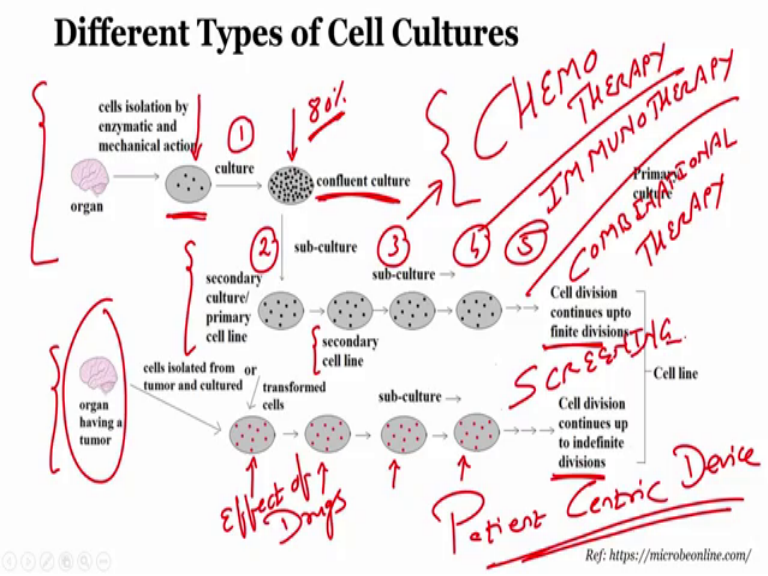

And, you can see here the first one; let us let us understand this particular flow. So, you have a organ, cells can be isolated or you can say the disaggregated by enzymatic and mechanical actions. So, you can see cells here. Now, you had to grow the cells right and the growing of cell is because, of the cell division that we have seen in the last module. And, when the cell reaches to a state which is about 80 percent of its maximum capacity; we say it is a confluent culture confluent culture, this value changes from a lab to lab.

But, but in general it is close to 80 percent ok. So now, we have confluence cells then we have to do a secondary culture or a primary cell line; that means, that we will subculture it when we subculture it and we against card growing the things, it is called secondary cell line. We keep on sub culturing it until the cell division continues up to finite division; that means, that one time we have done the cell culture right; see here you have taken the cells you have culture it. This is first time then you have confluent. So, you can do a second subculture. So, you can say second time then you are again doing it we say third time, again doing it forth time, in it fifth time.

But at some point you will see that further the cells would not divide. So, it is only limited to finite divisions ok. This is one way of doing it and we are talking about primary culture. The second way of doing is the we have organ or a tumor and cells are isolated from tumor and there further cultured here and we keep on culturing further you see. So, again the cells division continues up to invite definite divisions in this case

alright, but at some point we are losing the property of the original tissues. So, I we had to restrict ourselves for few of the sub cultures and then we had to change the cell line the primary cell line right.

Or, we can generate another group of cells from the tissue and we will see by how can we understand the effect of effect of drugs. Whereas, the effect of drugs since we are talking about cancer we say chemotherapy drug c h e m o chemo therapy drugs. Or, we say immunotherapy immuno therapy drugs or we say combinational therapy combinational therapy alright. So, we want to understand from a group of drugs for chemotherapy which group we had to use. Or, I want to understand from the group of drugs existing for immunotherapy which one I have to used or combination therapy which one I had to use.

Then I had to understand the cell culture, I had to use the different drugs onto the cell to understand which drug is more efficient or which has a better efficacy for a particular tumor of ours. And, we can also design a patient centric, what is that patient centric patient centric device for testing or for screening patient centric device for screening s c r e e screening n i n g right screening different drugs. So, the idea is that if you have a three approved drugs by a drug authority right and if a patient comes with a problem or its diagnosed with let us say breast cancer.

So, out of the approved drug which drug we have to use for that particular patient right. We need to know which drug, I can use for a given patient and for that do we have a patient centric platform. Or, we are just relying for the last patient showed a better response to a drug number 3 right and that is why for the next patient, I will continue with that number 3 out of three drugs available. So, how we can come up with a engineering solution and; that means, that can we design a patient centric platform alright. So, that is an idea that what we you had to understand and we want to discuss ok, I will move to the next slide right.

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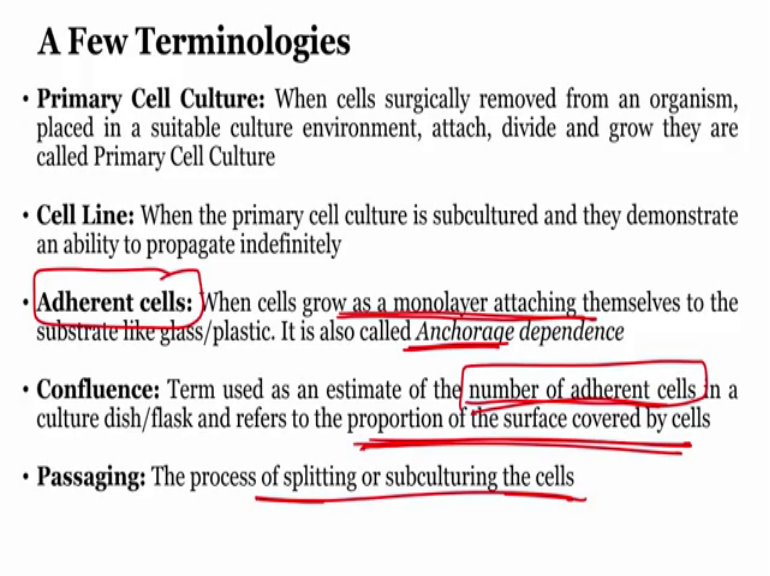

And we need to understand few terminologies. So, what I said is that we will be discussing that patients and take platforms and those designs at some point in these lectures alright series of lectures. So, when you talk about terminologies, we have first term called primary cell culture and that term is when cells surgically removed from an organism placed in a; this is wrong sentence. When cells are surgically removed from an organ not organism from an organ placed in a suitable cell culture environment, attach, divide and grow they are called primary cell culture ok.

So, when you take out the cells from an organ and then; so, when you say organ, organ being said finally, organized like tissues forms organ. So, you take out the cells from the tissue and when take out the sets of the tissue and you place in a suitable culture environment, what will happen? You will attach to each other, it will start dividing and will start growing right and that is called your primary cell culture. What is second definition? Second definition is cell line. So, what is cell line? When the primary cell culture is subculture and they demonstrate then ability to propagate indefinitely right. You can keep on growing a next subsets of cells.

Next one is adherent cells, an adherent cells are when cell grows as a mono layer attaching them self to the substrate like glass or plastic, it is also called anchorage dependence alright. So, this is the mono layer understand this very clearly. Adherent cells are when cells grow as mono layer attaching themselves to a substrate which is glass or

plastic. And, it is also called anchorage dependence. Next word or next term is confluence. The term confluence is used as an estimate of the number of adherent cells in a culture disk, flask and refers to the proportion of the surface covered by cells.

I told you right that in the earlier slide, you see a term called confluent, you see a confluent culture right that is what we are discussing here. Confluence a term used as an estimate of number of adherent cells. What is adherent cells? Yeah, just seen here right in a culture and refers to a proportion of the surface cover cells that is why I said 80 percentage, 85 percentage of the surface covered we have to again subculture the cells. Next is passaging or passaging and the process of splitting of sub culturing of the cells is called passaging. Easy right terms are very easy to understand, very easy to remember.

So, if I want to understand now the tissue culture process flow. What are the tissue culture process flow, let us see in the slide.

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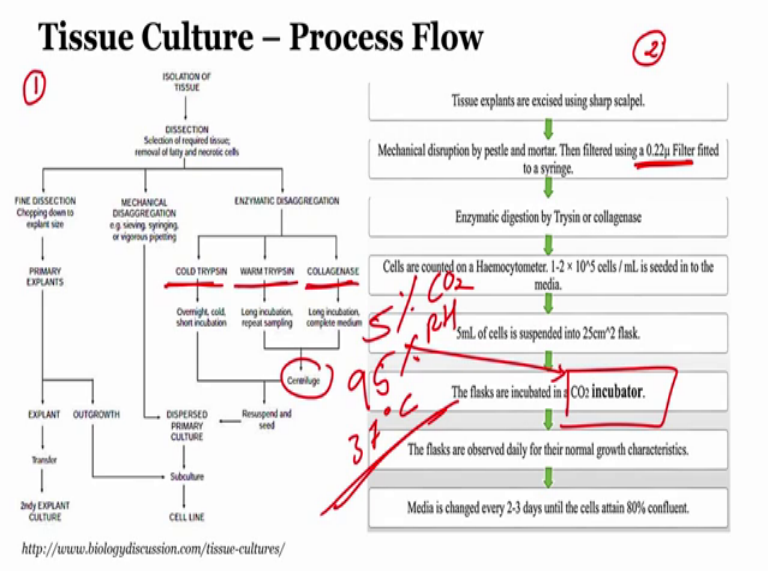

So, let us see let me term like this 1 and this is 2; let us first see first one which is number 1. So, what will do we will isolate a tissue we will dissect right. So, selection of required tissue removal of fatty and necrotic cells and then what will do three things: one is fine dissection by chopping down explant size. That means, when you by chopping other tissue further or we will take out the cells using mechanical d disaggregation or we will take out the cells using enzymatic disaggregation right.

Then if I use the fine dissection, dissection then I have primary implants. Further if I go down I can have explant or I can have outer growth. If I have explant I can transfer it and then I can do the secondary explant culture. If I use for the outgrow then you can do subculture and it forms a cell line. What it we have seen this part and same thing goes for this part right. Now, let us see the mechanical way the mechanical way of disaggregating cells will lead us to dispersed primary culture which will further subculture and you can have a cell line simple right.

When you talk about enzymatic disaggregation, then there are three things: one is called cold trypsin, second is called warm trypsin third is called collagenase and the process is called trypsinization alright. Either you do with cold trypsin or use warm trypsin and or you use collagenase. If I want to use cold trypsin then I have to overnight cold short incubation. If I want to use warm trypsin I have to use long incubation and repeat sampling while, if I use collagenase then long incubation and complete medium.

After that I will centrifuge the cells so that I can get the cells at the bottom of the centrifuge device. I will resuspend and seed the cells to form primary culture subculture and I will have a cell line. This is the process flow for tissue culture based on three techniques: one is trypsinization, second is mechanical disaggregation, third-one is fine dissection or dissections. Let us see the second one process flow very simple. Here first is tissue explants are excised using sharp scalpel. Then mechanical deception by pestle and mortar, then filtering using 0.22 micrometer filter.

Third next one is enzymatic digestion or trypsin or collagenase by not trysin, it is trypsin trypsin t r y p s i n trypsin. Next one is cells are counted on hemocytometer and is seeded into the media. Then we had to take a flask and we had to load 5 ml of cells into 25 centimeter square flasks. Next one we had to incubate this flask in a CO 2 incubator. And, when you do that what will happen the cells will start dividing, attaching to other and then we will reach a confluence state in about 2 to 3 days which is about 80 percentage of confluency right.

I already discussed this thing last time that we have to attain 80 percentage confluency, when we are going to incubate the cells in a CO 2 incubator. What is incubator? And what is CO 2 incubator? This incubator has a properties like it we had to maintain 5 percentage CO 2. There is a relativity of 95 percent relative humidity and then now the

temperature is 37 degree centigrade. This is more like the in-vivo situation, the situation within the body; easy this is what is the tissue culture.

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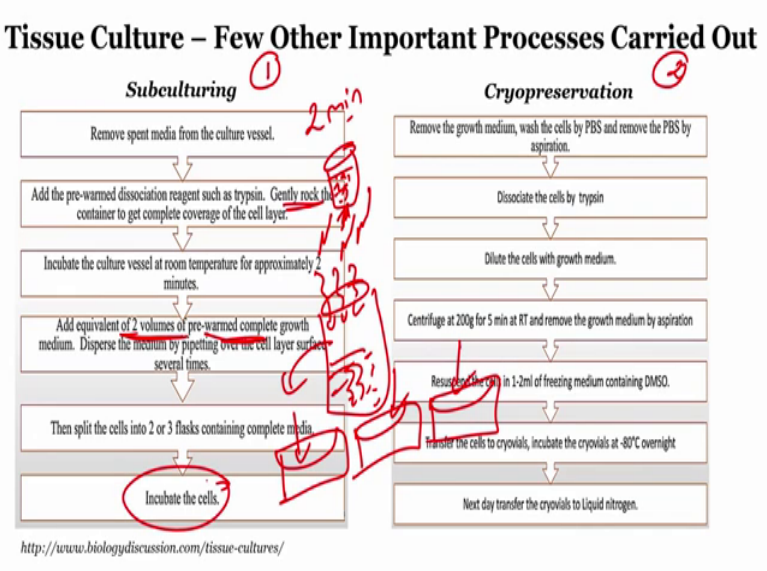

And, if I further want to go and understand the sub culturing and cryopreservation; what can I do. So, let us see the sub culture again, I will name sub culturing as 1 and cryopreservation as 2. So, if I want to do sub culturing I had to process this I had to follow this particular process alright. First is a remove spent media from the cultural vessel, add the pre-warmed dissociation reach a reagent such as trypsin, gently rock the container. Next is incubate the cell culture at room temperature. Then add equivalent well 2 volumes of pre-warmed complete growth medium. Then speed the cells into 2 to 3 flasks and incubate the cells.

So, what exactly; that means, you had to take the cell. So, I will take the cells like this right or in a like this right. Then what I will do and then I will what I will do? I will rock the container little bit rock the container then right then incubate the cell culture vessel at room temperature. So, then I will keep this for 2 minutes in room temperature, after that add equivalent of 2 volumes of pre-warmed growth medium. So, I will take this one right, this was the cells and then in that I will add the 2 volumes of pre-warmed growth media. Then I will take the cells and load into three different flasks ok.

So, I load the cells here by taking from here, I load here and I load here; after loading I will incubate the cells. Incubation is done in a CO 2 incubator at 37 degree centigrade, 5

percent CO 2, 95 percent relative humidity ok. Now, if I want to see cryopreservation what does that mean that, we had to preserve the cells for a long time. For that first is remove the growth media and wash the cells by PBS and remove PBS by aspiration you remove PBS. So, now what we have? You only have cells, then dissociate the cells by trypsin; we will add trypsin. Then dilute the cells with growth medium, we let the growth medium and then centrifuge at 200 g for 5 minutes at room temperature and remove the growth medium by aspirations.

Now, here the cell then we written as resuspend the cells in 1 to 2 ml of freezing medium containing DMSO. And finally, transfer the cell to cryovials; cryovials are the vials that can hold the cells for a longer time in a in a cryopreservation unit. And, then we have to incubate cryovials at minus 80 degree centigrade before we transfer next day the cryovials into liquid nitrogen. This is how we cryopreserve the cells. Now, this is just the information ok. We have to just understand that there are this kind of techniques available by which we use the sub-culturing and cryopreservation alright.

So, this end of this particular module where, we understood how this tissue culture process flow is all about. And, what exactly tissue culture means and then there are subdivisions of the or some of the terms or terminologies that are used in tissue culture.

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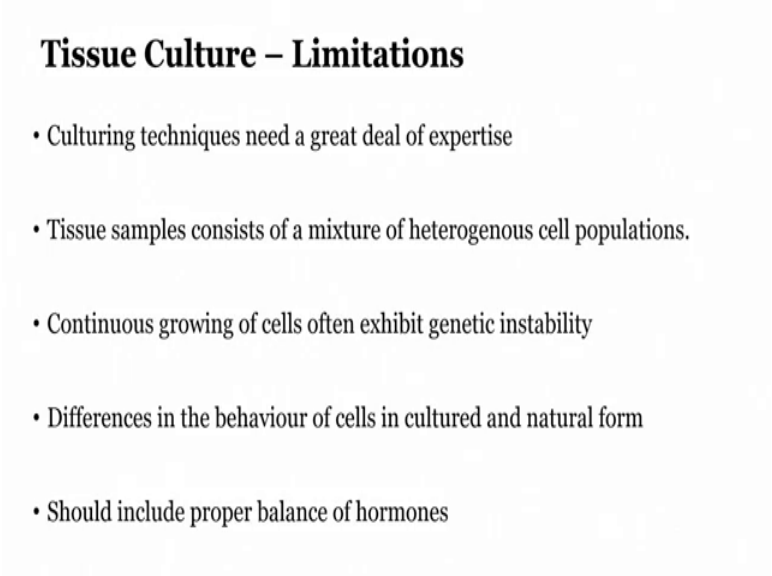

In the next module we will see the advantage, limitation of tissue culture, application of tissue culture.

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Tissue Culture – Applications

- A Tissue culture system is an excellent model system for studying normal physiology, cell biology and biochemistry of cells. For a bioengineering lab, it provides flexibility in experimenting with varying engineering parameters that are used to design the sensors which will finally use primary biological tissues
- It can be used to study the effect of drugs, radiation and toxic components on the cells and tissues. These can be done either through conventional biological protocol based assays or through micro-engineered devices like microfluidics, MEMS, NEMS etc
- Studying mutagenesis and carcinogenesis
- Tissue culture systems are also widely employed in industry for large scale manufacturing of compounds that have biological origins like vaccines, insulin, interferon, and other therapeutic proteins

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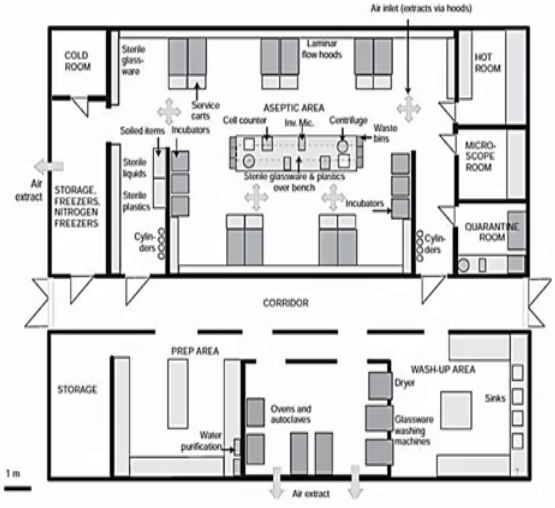
How a Tissue Culture Lab Setup Looks Like?



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And then we will see how a culture lab setup looks like and so on and so forth alright. So, till then you just read once again understand this lecture once again and then I will see you in the next module. Have fun right, just do not keep on reading, have something for yourself. Live your life, enjoy your life, go out, play something. Do extracurricular activities, do not be a bookworm right. Very important to keep your physical health intact along with our mental health alright. So learn these things, have fun I will see you in the next module. Take care.