Fabrication Techniques for Mems-based Sensors: Clinical Perspective

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Lecture – 34
Clean Room: Equipments Required

Hi, welcome to this module and in the last module what we have seen, we have seen some cell and tissue culture techniques. So, that is a title of this particular lecture and we have seen what are kind of clean rooms and how you can classify those clean rooms according to ISO standard, right. We have also seen that if it will be nice to have a laboratory in which we have the same kind of clean room environment the reason being that, suppose it is a pharma research, right, you want to test some drug and if you go to a pharmacy where the drugs are tested the class of the clean room is class 10000.

So, if I want to test the same drug or a new drug in my laboratory and give an engineered data and show the data to the pharma industry, they cannot replicate the same data, they cannot take the same data and immediately start production. They need to perform the same test again in their environment. See, the environment is kept constant like class 100 or class 1000 or class 10000 where both the experiments are performed; it is good for the validation. Having said that for the, once we have the fabricated sensors, once we have fabricated microchips microfluidic devices, we want to test it for clinical research.

So, when you talk about clinical research, we talk about biology. And when you talk about biology we talk about cell culture and tissue culture that is why this, this whole lecture is extremely important. Now, once you have the cells, once you have the tissues, once you have the devices how can you use this device or where should you use this device, right. Are there any equipment or are there any chemicals that are required for performing cell culture or tissue culture?

And if the cell is not infectious or it is infectious what kind of stages or what kind of level of equipment should be there for the research purpose.

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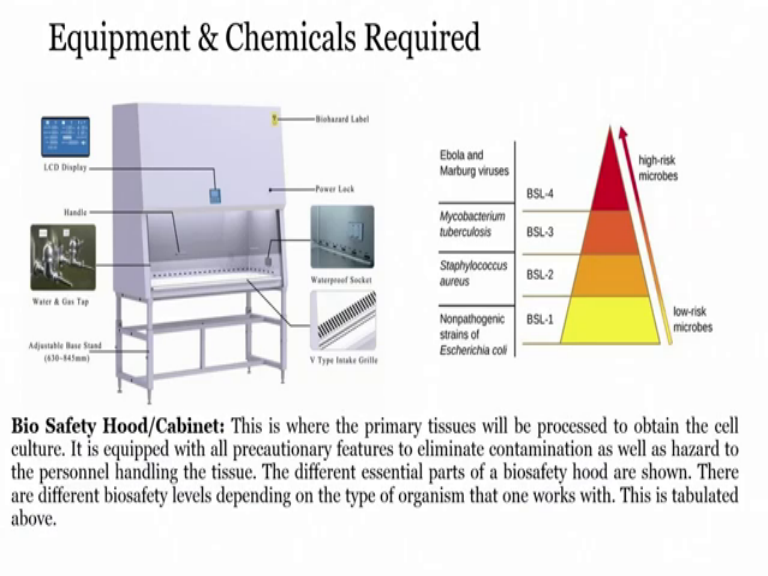

So, let us see in today's module the equipment and chemicals required. Now you can see on the left side is a bio safety hood. And it consists of bio it; it should have this biohazard level, right. Since, we are using if we are using material which is hazardous, there are handles for water and for guests, then the new version of the BSL bio safety hood comes up with a LCD display where you can monitor the environment within the hood, there is a power lock. You can see clearly from this till this there is a complete glass right. So, you should only put the hands from this side that is it. It should be extremely, it is extremely important to take a precaution of not to put your head within the hood, right. This equipment are meant for just pushing your hand and performing your experiment.

There is a waterproof socket and then there is a V type intake grill which is right over here, V type, you can see it here integral. Now from user to user to make the, research comfortable and to for the convenience of the user we have adjustable base stand you can change the height of this base stand. So, that a researcher feels a convenient to work in this particular environment.

Now, when we talk about the class right all level of the bio safety, we start from BSL 1 which is the base of this triangle and this BSL 1 are used for non-pathogenic strains of E. coli. So, this is our low risk microbes and as you go towards high risk microbes, the glass or the safety changes. So, BSL-1 for non-pathogenic strains of E coli, BSL 2 for staph, BSL-3 for tuberculosis, BSL-4 for Ebola and Marburg viruses.

Now, bio safety hood or cabinet this is where the primary tissues will be processed right here. To obtain the cell culture it is equipped with all precautionary features to eliminate contamination as well as hazard to the personnel handling the tissue. The different essential parts of a bio safety hood are shown here, we just talked about it, right. And, the bio safety levels are different depending on the type of organism we are hindering.

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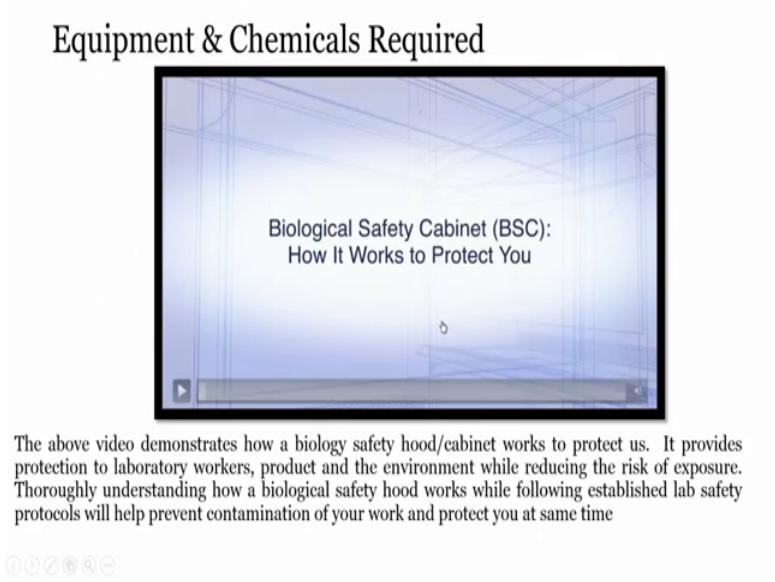

Now, this is the video, this is a video of how the biological safety cabinet protects you and, how it also provides the protection to the laboratory workers, product the product and the environment while reducing the risk of exposure, right. So, thoroughly understanding how a bio safety hood works while following established lab safety protocol will help or prevent contamination of your work. And very important protect you at the same time, right. Precaution is extremely important while performing experiments related to the pathogenic and then non pathogenic, even this is non pathogenic the precaution is extremely important.

So, let us play this video and let us see.

This video demonstrates how biological safety cabinets work to protect you providing protection to laboratory workers product and the environment while reducing the risk of exposure. Thoroughly, understanding how a biological safety cabinet works while following established lab safety protocols will help prevent contamination of your work and protect you at the same time. When used correctly a properly installed and certified

biological safety cabinet provides personnel environmental and product protection for work with biological materials including infectious agents and the combinant DNA. This video depicts a free standing, class 2 type A 2 biological safety cabinet or BSC, it includes Hepa filters for exhaust and supply air the work surface, the opening to the work surface, the airfoil front and rear air intake grilles, the plenum. The BSCs air filtration system works to keep potentially contaminated air from sweeping back onto the worker.

Air flows through the window opening into the front grill, sear the plenum then through the Hepa filters, 70 percent of the filtered air is exhausted, the remaining 70 percent which is now Hepa filtered is recycled back into the workspace. To ensure maximum protection in using a BSC, here are some essential reminders, 1. If the cabinet has been turned off, you must turn it on and wait at least 15 minutes before beginning your work. 2, set up the interior workspace to work from clean to dirty and work consistently from one direction toward the other to prevent cross contamination. 3, place your chair at a comfortable height and in the middle of your workspace to ensure you can reach everything you need inside the cabinet without discomfort.

Please keep in mind that you must work at least 10 centimetres inside the BSC. To guarantee uninterrupted airflow cabinets should never be overcrowded. Overcrowding the BSC can block air grills, airflow can also be disrupted by sudden or sweeping movements, slow direct movements work best, too much foot traffic can cause problems as well and should be kept at a minimum. If pedestrians are unavoidable, keep people at least 1 metre from your BSC and remember to check nearby doors or supply vents to determine if they disrupt the cabinets airflow. When you have completed your work any reusable items should be wiped down with disinfectant before removing them from the BSC.

Next the interior surfaces of the BSC should be decontaminated using the appropriate disinfectant for a contact time recommended for the agent used to be sure a second decontamination is advisable. In summary, there are a small number of best practices to follow in using a biological safety cabinet. Let us go over them one last time work at least 10 centimeters inside of the BSC do not block front and rear grills, too many objects in the BSC can disrupt the airflow. Setup work space in a direction from clean to dirty, use slow direct movements minimizes foot traffic within one meter of the BSC.

Placements of the BSC away from doors and room air supply vents pops maintain air flow. Thank you for watching and stay safe.

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Now, let us see equipment and chemicals acquired we are still in the same topic. And you can see here important thing which is your CO 2 incubator. So, if you see the human body, what is the temperature of our body about 37 degree centigrade, what is humidity? 95 percent RH, relative humidity and how much co two concentration about 5 percent CO 2 right this is how our body is.

So, if I want to use the cells or if I want to grow the cells outside the body that is, in vitro right inside the body in vivo, outside the body in vitro right in vitro, I am growing the cells, ok. I am not using anything from the body, I mean yes if I am using a tissue from the body is ex vivo we, we know this very well, right. So, I have to grow cells in the laboratory and that is why it is a in vitro, in vitro platforms. So, to grow the cells in a controlled environment, we require CO 2 incubator. And parameters of CO 2 incubators are similar to our body temp body parameters like 37 degree centigrade, 95 percent RH, 5 percent CO 2 concentration.

This is a core equipment of any tissue culture lab. Any tissue culture lab in the lab in the in the world you will see a CO 2 incubator. It is a control over contamination which is a major issue in tissue culture methods. See, if there is a bacterial contamination in the cells, cells will start dying. So, to have a controlled environment, we load the cells either

in petri dish or at T 75 flask or a T 20, you have a flask along with the media you can see here T, T 75 flask with media, you can see here petri dish with media and of course, there are cells, right.

So, cells media and it is a T 75 plus, this is a, these are petri dish, these are loaded into the incubator and this incubator has 37 degree centigrade, 5 percent CO 2 and 95 percent relative humidity. You can see we are handling the cells, right we by wearing the gloves, right to avoid any contamination. Since, it is class 10000 the, the gowning requirement the gowning requirement is different than class 1000 is different than class 100, but the prediction is very important.

So, still we have to wear glass we had to wear the lab gown and then we have to use gloves. So, the incubator size varies from table tops to those that can fill an entire small room. Some incubators can even be programmed to cycle through different temperature and humidity levels. So, based on the type of research, you will find different kind of incubators, but in general incubator is equipment to which will help yourselves to sustain and grow in a control environment.

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Now, let us see a next slide. Next slide would be a Refrigerator. What is refrigerator for loading liquid media and growth factors which can be maintained at 4 degree centigrade. And you will see this equipment as a part of the experiment that will be a laboratory

training you will be shown, how the refrigerator for media and growth factors looks like, how the incubator looks like, ok?

So, just not just looking at these slides here, but to actually see how the system looks like, ok. So, you will be shown this refrigerator and liquid media and growth factors maintained are maintained at 4 degree centigrade, enzymes such as trypsin and media components like glutamine serum etcetera are at minus 20 degree centigrade.

Now, the one that you will be shown is at 4 degree centigrade, but there are refrigerators for minus 20 degree centigrade as well. Now, tissue culture plastics are flask 96 well plate, you can see here this is 96 well plate, 48 well plate, 24 well plate, 12 well plate, 6 well plate, these are, these are actually 6 well plate, you can see 6 well 12 well 24 is 96.

So, and then there are petri dishes etcetera, right. This will be treated with a polystyrene. So, all these things are treated with polystyrene. And then you have flask, you can see here T 75 flask, T 25 flask, ok. So, these are the flasks for handling your cells and growing your cells in a controlled environment.

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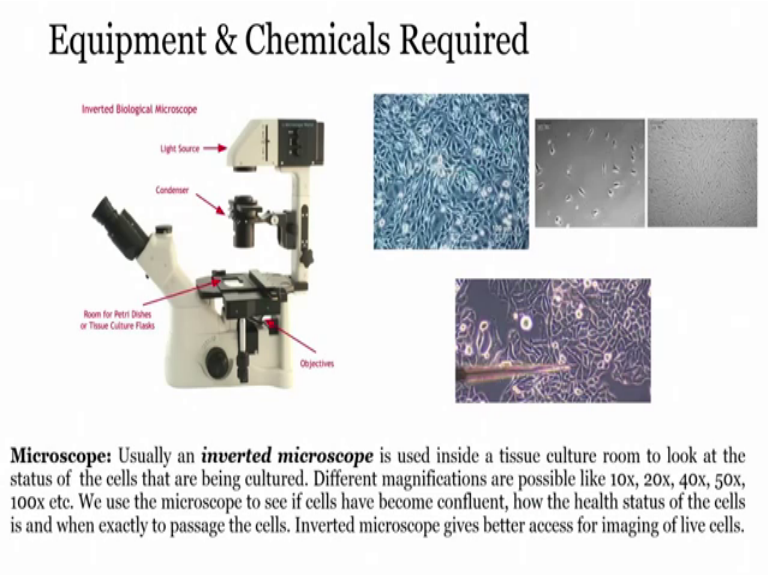

Now, when you talk about, talk about equipment you cannot miss microscope. Generally, in, clinical research setting, you will find microscope which are inverted microscope. And you can see here that there is a room for petri dish or tissue culture flask which you

can keep here. The objectives are at the bottom, the condenser is at the top and of course, the light source is at the top of the condenser.

Now, the inverted microscope is used inside tissue culture room to look at the status of the cell that are been cultured. And different magnifications are possible of course, using the objective lens from 10x to 20x, 40 x to 100 x, etcetera. We use the microscope to see if the cells have become confluent, we have seen the word confluent in the earlier module, right. And how the health status of the cells is and when exactly to passage the cells, right, we have seen what is passaging, correct. Inverted microscope gives a better access for imaging of the license, you can see here the some of the images taken using the inverted microscope, you can clearly see a cells.

In some cases there are fibers in some cases, there are cells depending on what you are focusing on, right. So, inverted microscope is extremely important for cell culture and tissue culture application in a controlled environment; that is about a biology lab. Also a tissue culture lab, also in any clinics, in a pathology. So, see the when we talk about pathology or clinical applications or medicine or pharma, you see the base is still a biology and chemistry, right. When you talk about any engineering the base still remains physics.

So, it is very important physics and math, right. So, it is very important that we, we have our base clear, the fundamentals clear. If I talk about a sensor that is a strain gauge, you should know what is strain gauge, what is strain, what is stress, what is gauge factor, then you come to fabricating a device. So, fundamentals are very important, same thing when you want to learn application of medical electronics or how can you, how can you design a biomedical tool for a clinical research? First thing you should learn is a basic biology, right, chemistry. If that is good, if that is strong it is easy to apply your engineering knowledge and merge it with biology for some clinical research, right. So, let us go to the next slide.

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And next slide is of course, extremely important equipment and that is your autoclave, why it is so important? Because it is used to sterilize the equipment, kill the infectious bacteria kill the pathogens which are left over after our experiment. We cannot just discard these slides, we cannot just throw the slide in a garbage can, No, even is biohazard bags we require a biohazard bags to discard the in vitro platforms which on which we have performed the experiments, right.

So, we can just not discard this platform directly into biohazard bags. For sure we cannot discard in a in a, in a garbage can which is not which does not have any biohazard bags so, but before discarding in biohazard we should go for autoclaving, because autoclaving will kill the remaining pathogens, remaining bacteria; if there are any. And also will help to sterilize the equipment, if you want to reuse the equipment, you have to first sterilize the equipment.

Now, if you have if you know that generally it, it was advisable and still it is advisable to boil the water before we drink. Why to boil the water? So, that the contaminants will be burned will die, if there is any contaminant in the water will die, boil the water, filter the water and then drink, right; same way boiling; that means, heating something at higher temperature, right. In terms of boiling some water, in here we are we are using again a water and we are loading our equipment or utensils that are used for tissue culture or for

or cell culture, even tissue culture and cell culture are interrelated. And thus, we are sterilizing it because we are removing any contaminants any leftover contaminants, right.

So, if you see an autoclave is used to sterilize the equipment utensils, etcetera that are used for tissue culturing both for reuse and safe disposal. Even if you want to dispose it you have to autoclave it. It is like a high tech pressure cooker, it removes microorganisms like virus bacteria, fungus, spores, etcetera using high pressure and high pressure high temperature steam sterilization, right.

So, again autoclave, comes as an important equipment in the biology setting. You will see an autoclave in the laboratory an actual autoclave, how it looks like. Now, let us go to the next slide.

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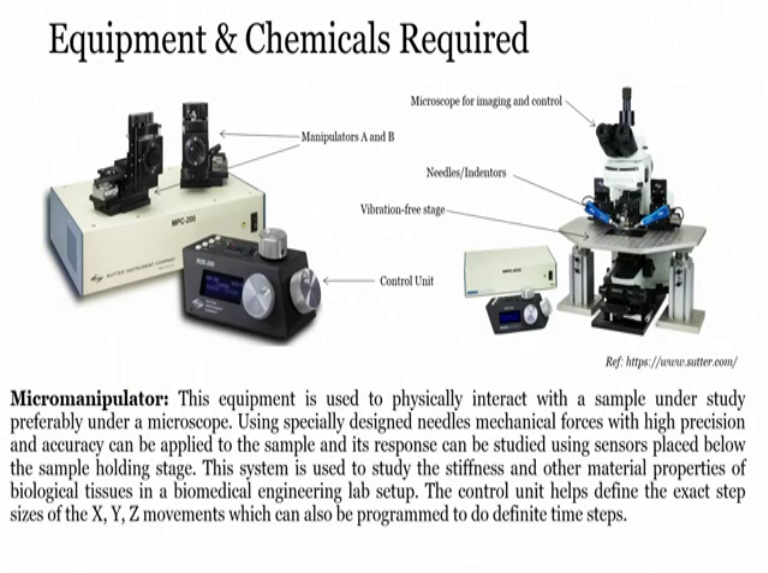

Next slide is micro manipulator; now see this you may not see in all the bio settings ok, but this is extremely important equipment since it can be used for indenting tissues. It can be used to understand indenting cells even, right. Since, the indenter the, the micro manipulator can have can be operated in a step as small as a micron, right it is an extremely important equipment to have in a laboratory for understanding the tissue property.

For example, the, the lecture in which we have studied the electrical thermal and mechanical properties of tissue, how did we study? We had, indenter loaded connected to

a micro manipulator and the micro manipulator used to go down in a z direction and press the tissue and it was pressing the tissue right with a micron force by micron dimensions, right or micron steps. Force, we can know only when we have a load cell to connect at to the indenter, but we were intending the tissue, we are pressing the tissue with a micron dimensions, micron size, micron steps.

There are many other applications. Now, you can see the same thing we were using when we were using a flexible 4 sensor, do you remember flexible 4 sensor with a strain gauge, that strain gauge was made, made out of made out of P dot PSS, right, conducting polymer. So, we had this conducting polymer strain gauge or which we had a insulator on which we had SU 8 pillars and we made it conducting, we loaded it with the 3 printed cone and that 3 D printed cone was connected to the micromanipulator again.

So, micromanipulator comes as a hand equipment for understanding the tissue property in, in the in this particular area where you want to use your engineering devices to understand how the properties of our tissue changes S cancer progresses. Now still we are focusing on a particular disease that is cancer. So, if the cancer is at if, if the tissue is normal and if there is a progress in this issue for example, ductal carcinoma in situ, invasive ductal carcinoma or lobular carcinoma in situ invasive lobular carcinoma.

So, normal benign lobular carcinoma in situ, lobular invasive lobular carcinoma, this is a one stage, right or we can say of normal benign doctor carcinoma in situ and invasive ductal carcinoma this is another line, right where the cancer is in duct cancer is in lobes.

So, these are stages. So, if I want to know the stages of the cancer, if I know the stages or progressor of the disease, if I can correlate this progression with the parameters that I can obtain such as electrical mechanical thermal, it can be other parameters like PH, right. It can be other parameters as well. So, the point is to obtain this parameter, we require micro manipulator. One way, one way to test this tissue is using the micro millimeter; that can be n number of ways, it depends on how the researcher want to use his research for understanding the properties of the tissue.

So, this is from the shutter equipment, you can see here this we have bought in our laboratory. And you will be able to see how the micromanipulator looks like and, the equipment like, it is written here is physically used to physically interact with the sample under study preferably under a microscope using specially designed needles, mechanical

forces with high precision accuracy can be applied, you can see here needles and indenters to the sample. And this response can be studied using sensors placed below the system is used to study the stiffness and material properties of a biological tissue in a engineering lab setup which is biomedical engineering the control unit helps to find the exact step size X, Y, Z.

So, it is not only used for Z movements, it can also be used for X and Y movements and of course, it can be programmable, it can be programmable for definite time steps, right. So, micro manipulator is an, is an equipment that can be used for studying the tissue.

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Let us go to the next one and next one is the microtome. What is microtome? Microtome is a tool used to cut extremely thin slices of material called sections, ok. So, let us say that we have the tissue obtained from biopsy, right and then we want to test IX, this is immunohistochemistry. We need to have a thin slice of tissue which you will stain. And we will understand whether a particular biomarker is present or not, right. So, we have to slice these tissue, to slice these tissue there is an equipment called microtome, ok. And so, this, this tissues are sliced and what is the use of it. So, once the tissues are sliced, it allows the preparation of samples which can be used for observation under light or electron microscopy and also for tissue culturing the, the method itself, is called microtomy, ok.

The, the process the method that is used to slice, the tissue is called microtomy. The section thickness can go as low as 50 nano meter, ok and with an average section being of 100 micron in thickness, ok. So, generally tissue the slice at 100 microns, we can go as low as 50 nano meter microtome or tissue slicing it is also called grossing, we can gross the device; we can gross the tissue, right.

So, if you see the video, this is from the oxy lab and here we will see how the microtome can be used. So, let us see first video and then let us go to the next slide.

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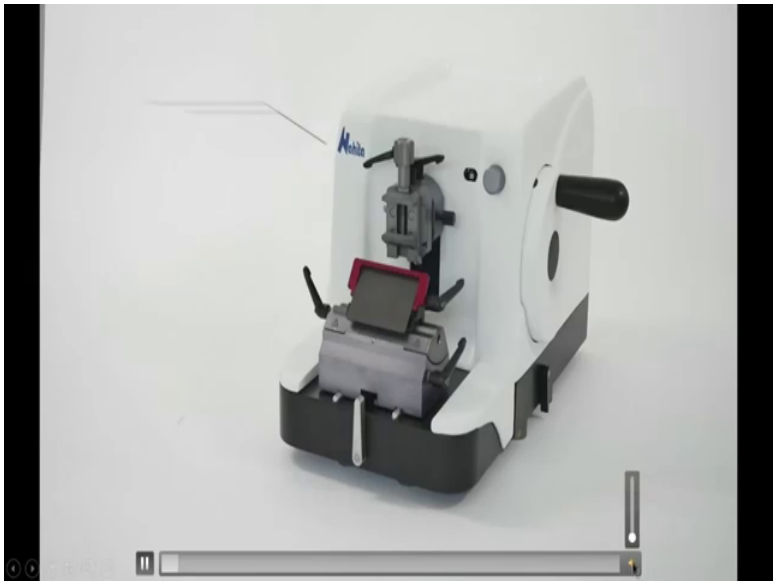

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Centrifuge: what is this equipment? Centrifuge is a equipment used to concentrate in the cell suspensions and remove the supernatant the equipment has controls to set required RPM and time of rotation. We can insert multiple falcon tubes; you can see here there are falcon tubes, right inserted at the same time. And here is a sample video of how it is operated is shown.

Now, in this particular section, in this particular image, right the role of centrifuges, suppose I load in a falcon tube of a sample let us say a blood, blood, ok. So, what will happen? If I centrifuge it the, the blood will be the bottom, there will be RBCS in a center, there will be WBCS on the top, there will be plasma, you can separate the heavy material from the lighter material with the help of centrifuge. In another case if I have cells in PBS and if I want to wash the cells multiple times, I will load the cells with PBS in a falcon tube centrifuge it.

So, the cells will go down and I can throw the supernatant which is the liquid PBS that is on the top and throw it, load another PBS and again centrifuge it, again throw it. So, as you can do washing steps, this is one example of how to use centrifuge.

You can separate a blood like I said, if you lower the blood and if you centrifuge it at a certain RPM then what will happen, the heavier cells will come at the bottom the lighter cells will come at the top and such the layers are formed.

So, we have RBCS, WBCS and plasma you can separate these three things out this is whether the other way of using the centrifuge. So, if you see the video, you will understand how the centrifuge is used, right.

The executive series interviews is the newest addition to the elite product line of insight global biologics and taking advantage of all aspects of superior separation the executive series centrifuge comes fully equipped. A high performing machine with an enhanced look and smaller footprint is now here. When looking at the executive series centrifuge you will first notice it’s large multifunction buttons and clear display to the left is the time button where you will turn the dial to set the time and press start and stop centrifugation. To the right, you will find the speed button which also can be turned to set the RPM speed and press to toggle between VR CCF and RPM display.

These buttons are clear and easy to operate, centrifuge comes with the control deceleration (Refer Time: 35:00) soft braking feature This minimizes cell agitation (Refer Time:35:04) during processing. Up to my separation is achieved with activation providing the best achievable cell concentration for the procedure. To activate this feature, press and hold the short button until you see the beep are on indicated (Refer Time: 35:23). This machine has an automatic lock and release function for lid operation. To open the lid, power up the machine and press the open button. You can manually open the lid without AC power by releasing the pull string at the base of the machine where you can pull to open the lid automatically locks when closed. Looking inside, you will find that the machine comes with the proprietary rotor and two customizable smoothing buckets.

These buckets are designed to swing horizontally and smoothly present to a full vertical position when completed. This prevents the buffy coat from (Refer Time: 36:19) into the plasma and prevents the platelet clip phenomenon which degrades the quality of the final product.

With these new concepts in platelet and bone marrow separation, the executive series centrifuge now makes its mark as it enhances the quality of these products.

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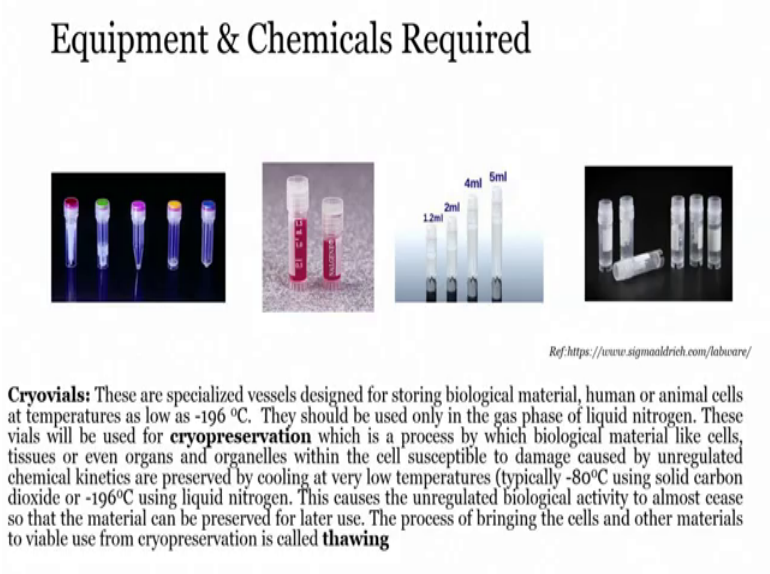

So, let us go to the next slide. And next slide is Cryovials, what are cryovials? Cryovials, these are specialized vessel designed for storing biological material human or animal

cells as temperature as low as minus 190 degrees centigrade. They should be used only in gas phase of liquid nitrogen; you see they should be used only in gas phase of liquid nitrogen. These vials will be used for cryopreservation to preserve the cells for a longer time which is a process by which biological material like cells tissue or even organs and organelles within the cell susceptible to damage caused by unregulated chemical kinetics are preserved by cooling at very low temperature. This causes the unregulated biological activity to almost cease, right.

So, if the cells are going to get affected they are susceptible to damage caused by unregulated chemical kinetics. Then all biological activity then what we can do? We can preserve it at a very low temperatures such as minus 196 degree centigrade. Yeah, typically minus 80 degree centigrade can be used for solid carbon or minus 196 for liquid nitrogen. Now, using this is what happens unregulated biological activity to almost ceases and so, that our material can be preserved for later use.

The process of bringing cells under materials to viable use from cryopreservation is called thawing, thawing, thawing is nothing, but heating, thawing. So, these are (Refer Time: 38:18) walls the in which you can load the cells and you can preserve it for longer time. This images are taken from Sigma Aldrich so, Sigma Aldrich is a company which makes the there are lot of companies, but these are the some of the images and these are the cryovials as you can see in the figure of different volume, you can say 1.2 ml, 2 ml, 4 ml, 5 ml, right. So, these are the, another set of tools or equipment that you can know or a or a preservation vessel that is there in the biology environment.

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Now, the most important thing is to give the nutrition to the cell. So, the cell can stay longer time without any significant affect. So, cells would not die, right. So, to preserve the cell we use something called media hm. In our body blood provides nutrition to the or to the all the cells, right. So, necessary nutrition and other things are provided by the blood. However, cells have complex nutritional requirement and that must be bit in the, in the body it is met, but in the in vitro platform this is met with the help of media.

Now, earlier people use chick embryo extracts plasma, sera, etcetera as a growth media, but they varied in their growth promoting characteristics. And hence, they affected the reproducibility of the experiments, alright. So, using these earlier techniques like sera or embryo growth factors right extracts plasma they can all, these all things were tried earlier. However, what is the problem the consistency and the reproducibility, in the growth promoting factors these are the problems.

So, what happened? Now, you can see here today number of chemical defined formulations are available that suppose the consistent and reproducible growth of several primary and secondary cell lines and some of these chemicals are Eagle Basal Media, Eagles Minimum Essential Media EMEM. Then, there is another one which is Dulbeccos Modified Essential Media; this is DMEM which is extremely widely used generally in the laboratory. You will find DMEM Iscoves Modified Dulbeccos Medium, IMDM. Finally, there is HAM's F 12 media. There are several other medias, but, but these are the

basic, most frequently used medias, in this also in these also the, the number 3 which is DMEM is widely used.

So, what are the various nutrition. So, when I say there as the nutrition is provided by the blood, what are the nutrition? Nutritions are glucose, fat, fats, fatty acid lipids, phospholipids, right sulpho phospholipids as well as ATP and amino acids., it can be vitamins minerals and also sometimes antibiotics to prevent the growth of unwanted micro, microorganisms. So, these are all the, the nutrition's when we talk about nutrition, there are several nutritions like we just discussed.

So, this is the combination of all the nutritions are there in the media and that is why the cell can sustain for a longer period of time. So, let us come back to the slide. Another major constitution of media is serum. So, what is the role of serum? It provides growth factors hormones cell adhesion factors because the cell has to adhere to the base of the T 75 plus or a tissue culture flask. And other essentials required by the mammalian cells for the long term growth and metabolism. Some common serums are FBS, FCS, CS, HS and HOS.

Now, another point is the PH, the PH should be between 7.2 to 7.4 for the mammalian cells to survive, right. So, phenol red is used as an internal indicator as the cell consumes the nutrition. So, if this nutrition if you, if you load the cell, right, load the cell and load the nutrition on the cell which is your DMEM, the phenol red is added as an internal indicator. What does that mean, that as the cell consumes the nutrition this red color changes to, changes to yellowish or light brown in color, you got it.

So, initially it is red. Air is the cell growths and consumes the nutrition from the media, the color of this media changes from red to yellow or golden, golden color or light brown color. This changes the color of solution and gives an indication to change when to media. So, when you see this, you have the indication that the, the nutrition from the media has been consumed by the cell and the time to change the media, right.

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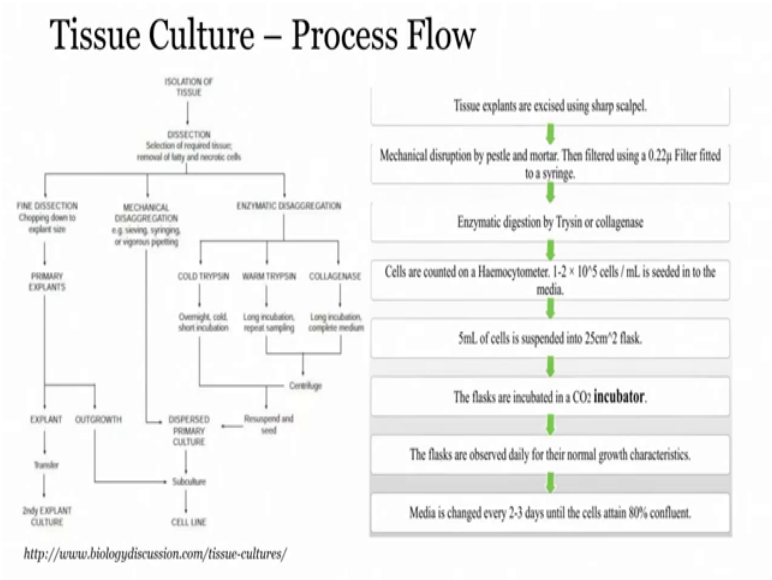

So, this is how the tissue culture process flow is that if you, if you go here, let us see here right side, tissue explains are excised using a sharp scalpel and then mechanical disruption by pestle or mortar, right. This we have also seen in the drug screening device, right then the filter using 0.22 micro filter using a syringe then enzymatic digestion by trysin is can be trypsin or collagenase cells are these are counted on hemocytometer, right. And then 5 ml of cell is suspended in 25 centimeter square flask, the flasks are incubated in CO 2 incubator flasks are observed daily for the normal growth media is change every two to three days still the cell attains 80 percent confluency, right, this is the process flow of a tissue culture.

Now, if you go further in detail, then this is how it occurs which is in your left side that you have the isolation of tissue; then there is a dissection selection of required tissue, removal of fatty and necrotic cells. And then, it goes for fine dissection, you can go for mechanical aggregation or you can go for enzymatic disaggregation, right. If you talk about mechanical fine dissection is chopping down to explain size, primary explained, explained and a transfer to a secondary explained culture, if it is outgrowth it can go to subculture and to the cell line. When mechanical way of this aggregation that likes syringing, vigorous, pipetting, sieving then after that is dispersed primary culture subculture to the cell line.

While enzymatic way of this aggregation is cold trypsin, warm trypsin, collagenase. So, our cold trypsin, we had to put it overnight in cold short incubation, this warm trypsin is long incubation and (Refer Time: 45:37) to repeat sampling while the collagenous is long incubation complete medium. And then it had to be centrifuge this has to be directly go to resuspend and seed and then goes to the dispersed primary culture followed by subculture and cell line, right. So, this is how the tissue culture process flow is that.

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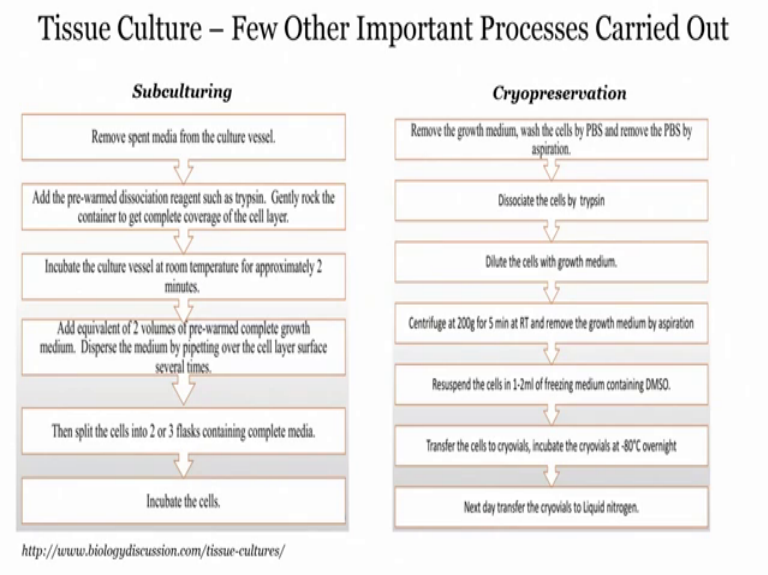

Now, other important processes are carried out such as subculturing and cryopreservation. So, if it is a subculturing, how it is done, ok. So, remove spent media from the culture well, our vessel, then add pre warmed this dissociation reagent such as trypsin, gently rock the container to completely cover the entire layer, right which is cell layer, then you have to incubate the vessel at room temperature for approximately 2 minute followed by adding equivalent to volumes of pre warm complete growth media. Disperse the media by pipetting the over the cell layer then split the cell into two to three flask containing complete media and then you have to incubate a cell.

So, this is more of a subculturing process. You can see this in a in a YouTube video, that is right sub culturing and you will understand, how it is done? Same way goes for cryopreservation, once there the growth medium is removed we have to watch the cell by PBS and then remove PBS by expiration followed by dissociation of the cells by trypsin, this is called trypsinization. Finally, after that it is a diluting the cells with growth media

then centrifuges at 200 for 5 minutes at room temperature and remove the growth media by aspiration. Final next step would be resuspending the cells in 1 to 2 ml of freezing medium containing DMSO and then transferring the cell to cryovials incubate the cryovials at minus 80 degree overnight followed by transferring the cryovials to liquid nitrogen, right.

So, this is how the cryopreservation works, right. So, if you want to do sub culturing, you have to follow a particular process if you want to do cryopreservation you do perform another process, right. So, what are the application of tissue culture, right. Let us see what are the application of tissue culture.

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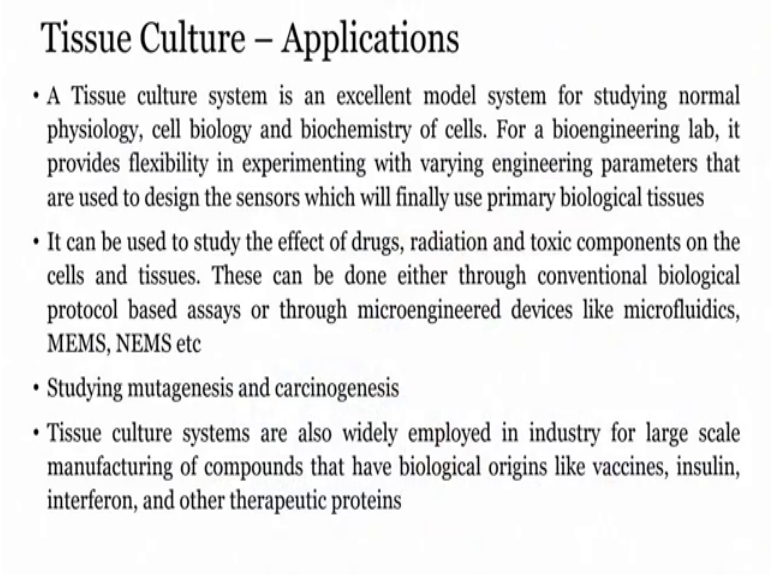

A tissue culture system is an excellent model, excellent model for studying normal physiology cell biology and biochemistry of cells, ok. So, a tissue culture can be used for studying normal physiology cell biology and biochemistry. Now, what we were looking at is the physiology of the tissue, we are also looking at the biology of the tissue. All cells, where if you, if we recall immunotherapy, we were understanding, how CD 4, CD 8 concentration changes if you recall electro thermo mechanical properties, we were understanding the physiology of the tissue.

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, correct. We have used it, it can use to study the effect of drugs, we

have seen right drug screening, radiation toxic components on the cells and tissue. This can be done either through conventional biological protocol based assay or through microengineered devices like micro fluidics MEMS, microelectromechanical systems, nano electromechanical systems, etcetera, hm.

It can also be used for studying mutagenesis and carcinogenesis whether it is carcinogenic or not or. And then tissue culture systems are also widely employed industry for large scale manufacturing components like biological origins like vaccines, insulin, interferon and other therapeutic proteins. So, the, the application of tissue culture is really, really wide. Not only for understanding the properties, but also for, for creating new vaccines and therapeutic and the in therapeutics, right. So, that is a important of tissue culture.

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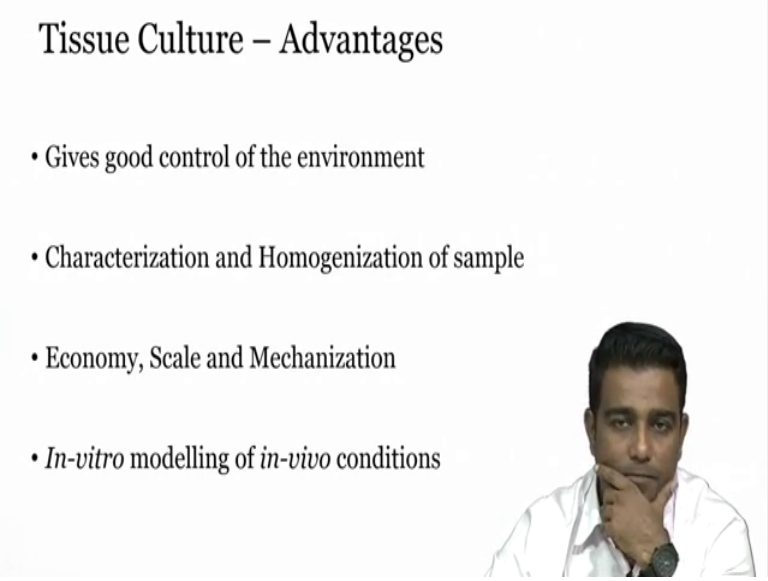

Let us see advantages, quickly, it gives good control of the environment, the advantages of tissue culture, ok. Characterization and homogenization of sample is possible, it is economical a scale and mechanization is also possible in vitro modeling and in vivo conditions we can study by taking the cells from the body and placing it in the, in the device that we have created in the laboratory and studying it further.

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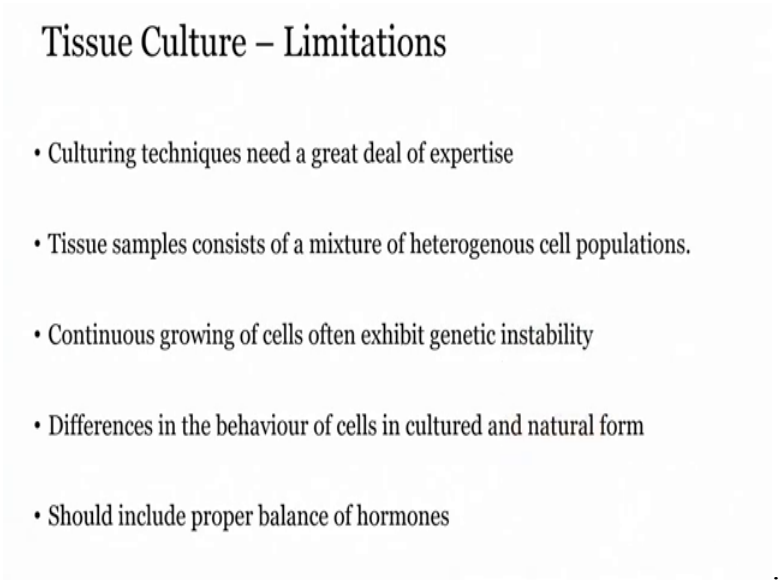

Then there are limitations, what are the limitations of the tissue culture? The limitation of the tissue culture are culturing techniques need a great deal of expertise. Tissue sample consists of mixture of heterogeneous cell populations, right.

See, culturing techniques it requires (Refer Time: 50:47) expertise, but once you know, its becomes easier. Continuous growing of cells often exhibit genetic instability, right, so, you cannot use many time the cells. You cannot just keep on regrowing it, difference in the behavior of cells and culture and natural form that is another point. Finally, it should be include proper balance of hormones. So, these are few limitations of the tissue culture technique, right.

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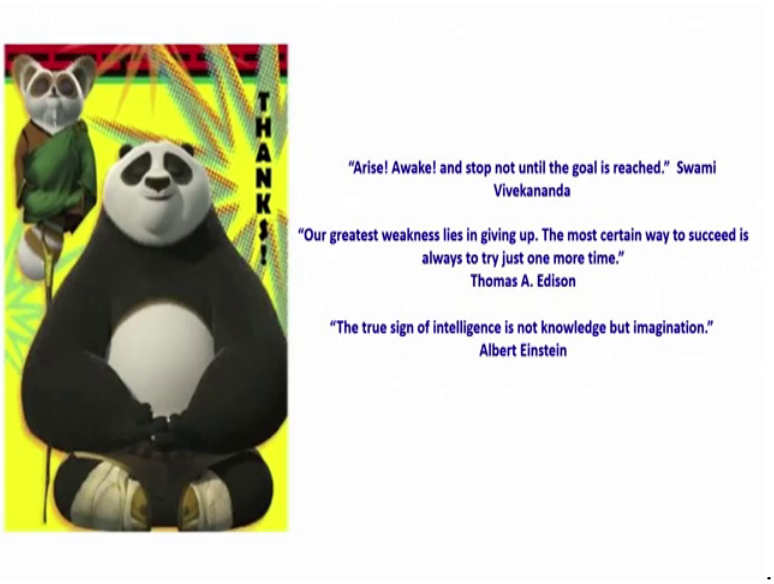

So, this is the end of this particular module and end of this particular class and just go through this technique, I do not want to go in depth about tissue culture and cell culture, but this was the kind of information of how the cells are preserved and grown in the lab environment. There can be another thing where the spheroid is a grown from the cells. The organides are taken out from the tissues; there are several techniques, right. Then you can see that how you can, make mesenchymal cells and how you can have epithelial cells.

So, if you go into cell culture and tissue culture in depth there are a lot of things to understand. What we are interested is let us get the glimpse of how the biology lab looks like and what parameters we should learn so, that all we should understand, so, that we can create a device, we can manufacture a device that can be used to solve a problem existing in clinics. There is a clinical problem, for example, we talked about immunotherapy, we talked about drug screening, right.

Now, in the next class, we will be talking about extremely important problem and that is called nt or it is called bacterial resistance. Let us, let us put it in this way bacterial resistance ok. So, bacteria is resistant to what? Bacteria is resistant to antibiotics. How we would know or how you can devise technology that can be used to understand whether a particular bacteria would be resistant to antibiotic or not; that means, antibiotic

can be effective to kill the bacteria or not, if not then that antibiotic should not be given to the patient.

So, are not there any techniques available, right now? There are, but those techniques takes about 24 to 48 hours. So, we will see how an engineering approach to solve this particular issue of time, time taken to capture and to grow and to understand the resistances of the bacteria can be reduced from this 24 to 48 hours down to 2 to 3 hours and I will tell you in that lecture why it is so, important, right. Till then just look at this module, I will see you in the next lecture, bye.