**Introduction to Biomaterials**
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**Lecture No. # 12**
**Assessment of biocompatibility of biomaterials**

In the last lecture, I have discussed about the cell metal infraction, so I will continue with that and then I will discuss in this lecture on the in vitro testing.

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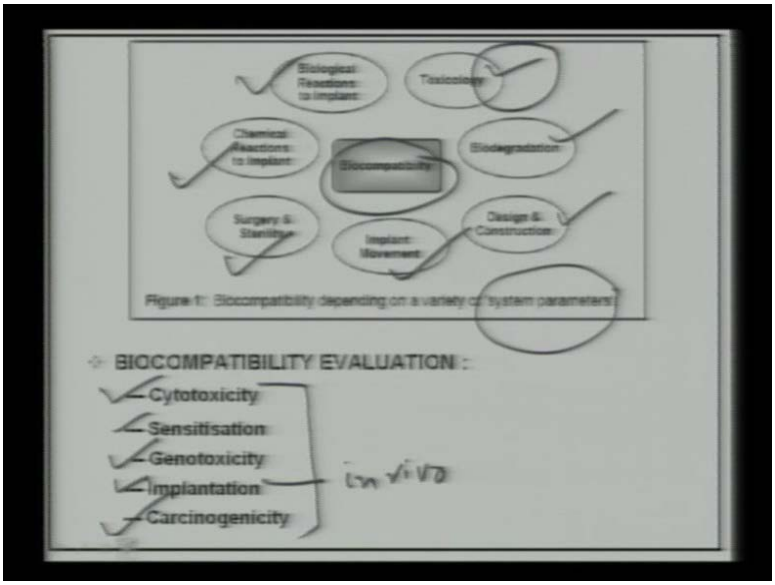


So, different in vitro testing that is required to assess the biocompatibility property of biomaterials. Now, as you know that biocompatibility is the co-requirement or principle requirement for assessing the whether a material can be used in the in vivo applications or whether a material can be used for a given biomedical applications.

So therefore, it is important to know more details that, how this biocompatibility property can be assessed. Now, first is that you need to know that biocompatibility is system property. System property means if your material is biocompatible for a particular applications, as I have mentioned couple of times during the last two lectures that if a material is biocompatible. For example, in the knee replacement that does not necessarily mean, that the same material is biocompatible for heart valve applications,

because your requirement is application specific; and therefore, your property requirement is also application specific.

That is the number one point. Number two point for assessing the biocompatibility property you need to do in vitro testing and in vivo testing. In vitro testing essentially means that when you are doing the tests in the laboratory stimulated environments. In vivo essentially means when you do the experiments in the animal body itself or inside the animal body.

Now, again if a material from the in vivo applications, if a material is found to be good biocompatible in the rat model or rabbit model that may not necessarily mean or they may, that may not necessarily tell you with guarantee that the same material will be successful in the human model experiments because, the in vivo conditions in the rat or rabbit is quite different from that inside the human being.

Because human actually is a large animal model and in the large animal model your fluctuation in PH, your fluctuation in temperature or in that different type of collagen or different tissues and their composition should be different compare to that the same thing in the rat or rabbit.

So therefore, in before one material can be applied in the human body, it is important that you do the test right from the small animal, intermediate animal right up to the human being before you can sell it into the market. So, this understanding is important and that is what I am saying here that biocompatibility depends on a variety of system parameters and it is essentially a system property. So if you change certain parameters in the system, then biocompatibility property also will be changing.

Now, some of the things that I have mentioned here one is the toxicology, now toxicology essentially means that whether a biomaterial is toxic to the gene or the DNA or is capable of damaging the DNA, inside the nucleus. Now, this test is part of the genotoxicity testing which I will be showing later. Now biodegradation biodegradation essentially mean whether a material can degrade itself in the biological environment that means so, if if a material before putting the in vivo conditions or before putting in the animal model has a certain set of properties like hardness properties, strength properties etcetera etcetera.

Now, you put the same material inside a animal and then after let say 2 months or 3 months you take out the material do the test, now if if you see that the material loses its strength or the strength is decreased, that means there is a fate of the biological environment on the degradation of the material properties.

So that is number 2. Number 3 design and construction now, it also depends on what is the shape of the biomaterial for example, if the shape is very much circular type of materials, then you do not have any stress concentration at any of the age of the biomaterial however, if you have a very sharp edge like a square of square type of implant that means, this corners are the potential sides where there will be stress rises or where the stress can be quiet high and accordingly the the implant when it is used for the load bearing applications those the presence of this square corners actually will effect on its biocompatibility.

Then implant movement, now implant movement is that for example, your knee implant is an example of that implant which will experience I mean more number of movements compare to for example, any other implants inside the body or knee will take more load or more load r they will take more load during its applications compare to any other material.

So, implant movement is also important and later all I will show you that how implant movement will cause the accepting loosening. Accepting loosening means whether how the bone or bone replacement materials or implants can be can can be detached from the natural bone or can lose may be can loosen out from the natural bone. Then surgery and sterility sterility is important and typically the sterilization is done either by gamma radiation for certain materials or by steam autoclave like you know steam treatment at typically at 121 degree Celsius in water vapor.

Chemical reactions to implant chemical reactions, to implant means, like whether a biomaterial leaches out some of the elements from its components and thereby those leached out elements can cause certain toxic reactions in in vivo conditions. The last one is the biological in reactions to implant. Now, biological reactions to implant means for examples when you out the any material in the in vitro conditions many times that in vitro environment or in vitro biological fluid can cause, the hydroxyapatite or calcium phosphate rich layer formation on the material and if it does that is very good because,

the presence of hydroxyapatite calcium phosphate rich layer formation can induced more biocompatibility in the materials.

So therefore, biocompatibility evaluation essentially will address or will focus some of the important properties that I have listed out here: cytotoxicity, cyto means cell and cytotoxicity means cell level toxicity, sensitization, genotoxicity I have already mentioned implantation means when you are putting the material as an implant in the animal body. So, that is the kind of in vivo experiments, Carcinogenicity whether the material can potentially cause the cancer inside the in vivo conditions.

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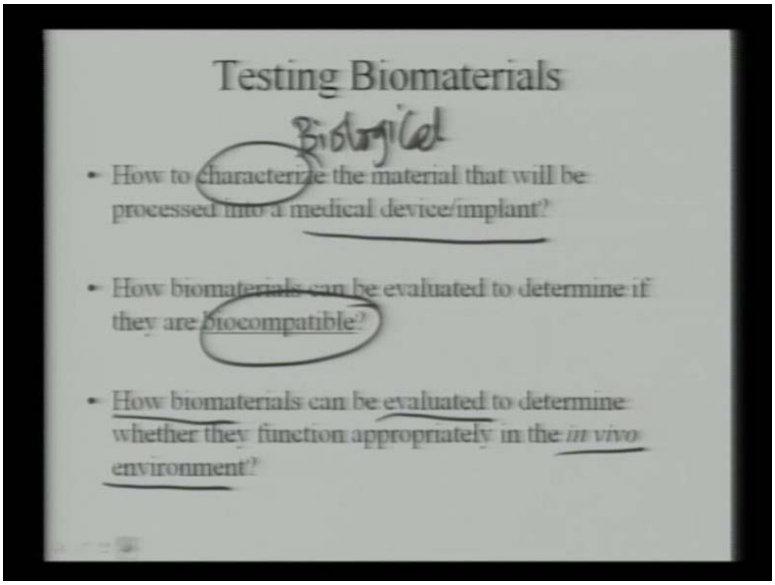


So therefore, testing of the biomaterials are this biocompatibility assessment will try to address the following questions that how to characterize the material that will be processed into a medical device or implant. So this characterization is more from biological characterization remember, second one how biomaterials can be evaluated to determine if they are biocompatible. Now if they are biocompatible as I said biocompatible is a broad parameter.

So, in the laboratory scale experiments people do only a set of particular experiments which are bad of the broad property which is known as the biocompatibility. So if you do the cell culture experiments, you cannot say on the basis of the cell culture experiments a given material is fully biocompatible, you can only say on the basis of the cell culture experiments whether a material is cytocompatible or cell compatible.

So, that is the cytocompatibility property is a much better description compared to biocompatibility if you use the word biocompatible right. Now, how biomaterials can be evaluated to determine whether they function appropriately in the in vivo environment. Now, again as I have mentioned that if a biomaterial passes through all the test of the in vitro conditions that even cannot guarantee that the same biomaterial can function very appropriately in the in vivo experiments unless, you carry out a particular set up in vivo experiments understand. So these things stand up here in mind all the time as long as you are dealing with the biomaterials.

So therefore, the question is that how biomaterials can be evaluated, to determine whether the function appropriately in the in vivo experiment. So that question is a kind of peak time question and then you need to address it very carefully.

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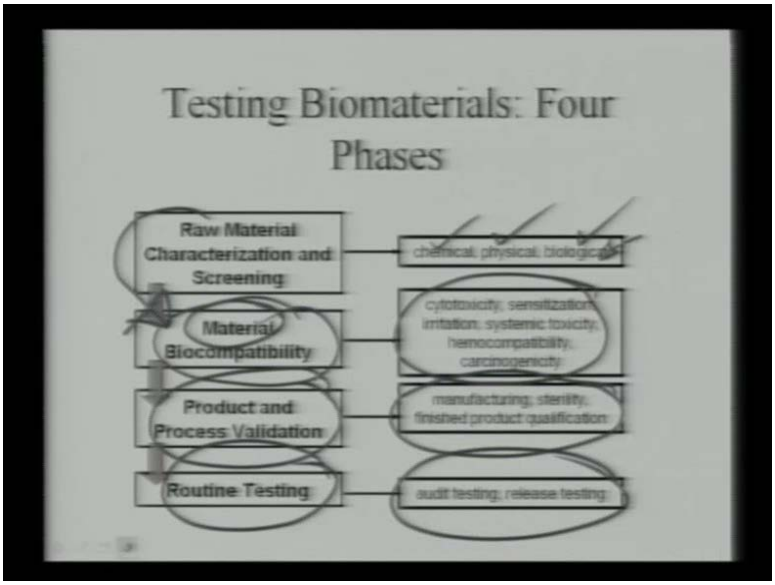


Now, testing a biomaterial four phases: first one is the raw material characterization in screening. So, raw material characterization means that what are the elements that you are using or what are the starting powders that you are using in the as a raw materials and they are chemical, physical and biological characteristics or biological properties. Chemical properties means, what are the elements that they contain like calcium, phosphorous, silicon etcetera. etcetera Physical properties means what is the size, what is the properties. Biological properties mean whether any of the starting material itself is biologically compatible or not.

Then comes, the material biocompatibility and here this material biocompatibility again I have mentioned that all the set of in vitro and in vivo properties that is cytotoxicity irritation, systemic toxicity, hemocompatibility: hem means blood, hemocompatibility means blood compatibility. Carcinogenicity: carcinogenicity means cancer level toxicity or whether it can potentially cause cancer.

Now, product and process validation: And product and process validation means like the way you prepare this biomaterial right from your raw material that is important and that depends on the manufacturing or sterility finished product etcetera and routine testing. Routine testing means all the ISO standard testing. ISO means, international standard organization so, tells you that certain set of test you need to do before you can release the product in the market. So, all those testing needs to be done before this product can be marketable.

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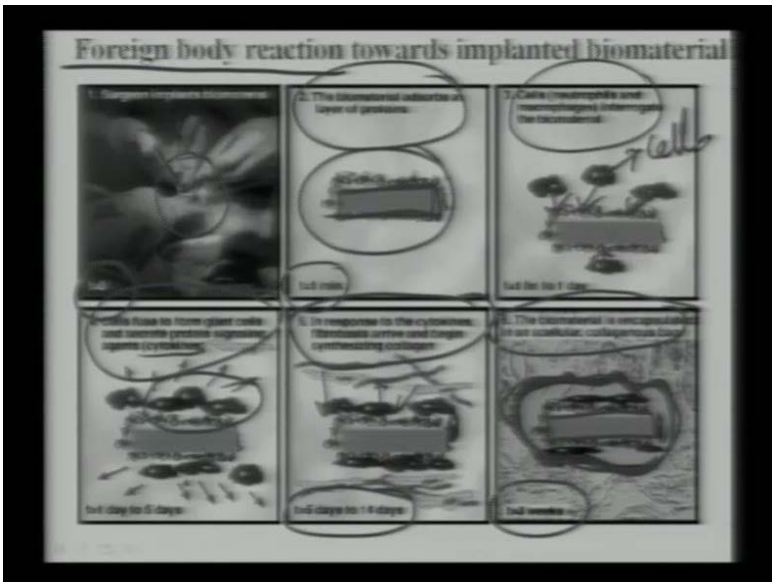


Now, there are two types of materials interaction. It is important that you know how this implant will look like or how the implant will react, when it is put in the host. Host means here the animal or any human being. So here the implant is shown as the gray rectangular slab. Now this is the doctors are performing this surgery doctors are performing the surgery here, that means your doctors are putting this gray rectangular slab at the specific sides inside the human body. Now, when you put this implant inside the human body or the any animal then within t is equal to 1 minute.

So, you look at the timescale also like what is the time frame along which these reactions formed, whether t is equal to 1 minute the biomaterial absorbs a layer of proteins as I said in the beginning due to the cell material interaction, that protein absorption is the first reaction or the first (( )) before any further cell adhesion takes place.

Now, within the first 1 minute or so that you biomaterial absorbs a layer of proteins then cells, cells means this is this is like fibroblast cells or macrophages. Now, these are your cells now these cells actually come and attracted get attracted towards the protein layer and you know from your basic in vitro study, that how this reaction takes place like cell surface protein they reacts with the absorb surface proteins on the biomaterial surface and therefore, these cells will get adhere to the protein layer there.

Then fourth one is that cells fuse to form joint cells and secrete protein signaling agents like cytokinesis. Now, cells these joint cells means when number of cells they aggregate together and this aggregation can be done in a self organized fashion. Self organized means cells will attach to themselves like in a in their own fashion in a self organized manner and they form a joint cells and what they do? They secrete a protein signaling now this signaling process I have mentioned briefly in the last lecture, that signaling is important because, through this signaling one cell will transmit the cell signal to the other cell.

So that cell movement, cell proliferation or cell differentiation or cell division can take place during the in vitro condition and this signaling can be transferred or this or or this signaling can go or this signals can go from one cell to another cell through E C M that is the Extra Cellular Matrix, now those things I have mentioned in the last lecture.

Now, in response to the cytokines fibroblast arrive and began synthesizing collagen. Now what will happen once these joint cells formed, then they sends the signals to this fibroblast and because, of this proteins signaling fibroblast cells. Fibroblast means that is the cells of the connective tissue and once this fibroblast cells they are getting attracted towards this implant material and that takes place during t is equal to 5 days to 14 days then what will happen that within that time period this fibroblast cells they are they will arrive at the cell surface near to the cell surface and then they will secrete the collagen material.

And collagen material is important because, collagen also will constitute or collagen is the major component of the Extra Cellular Matrix. Then after that the biomaterial is encapsulated in an acellular and collagenous bag, now it is kind of you know that entire material is now contained in a inside the bag. Bag means it is like you know it is a big bag which will contain the collagenous material as well as some cells and then protein molecules also.

So entire biomaterial is now is now contained in a biological bag kind of things. So, that is the way these two things that is the way this entire Foreign Body Reaction. Foreign Body Reaction means F B R that takes place towards the biomaterial. So, essentially its starts with t is equal to 0 and it it will go in the t is equal to 2 weeks so, entire process to take place it takes almost like a 2 weeks or 21 days and that is one of the reason that why many of the short coming plantation tends to see that whether a biomaterial is biocompatible in vivo, that people do up to the 12 weeks because, your initial thing takes place again 3 weeks then you see whether it causes inflammation to the neighboring tissues of the cells and that entire thing should be started up to a sufficient time period and that is the reason it takes 12 weeks time.

Now, if I can describe this then you will understand it much better, there are two types of materials interaction one is to create an inert surface and not allowing the adsorption of proteins and adhesion of cells and thus preventing activation of the immune system, blood coagulation, thrombosis, extracellular matrix deposition and other interactions of the materials and the surrounding environments.

So what I describe that to what happens from t is equal to 0 to t is equal to 3 weeks the entire process is not facilitated by certain material. So, what they call as bio-inert materials. Bio-inert means like it is biologically inert type of materials. Now, these kind of materials the examples are like for example, alumina or zirconia these kind of materials they cannot create and biologically active surface, they essentially creates an inert surface and they do not allow all these protein adsorption, cell adhesion and any activation of the immune system to take place.

Now, examples are the heads and cups of the joint prostheses, intraocular lenses and blood-contacting devices. Blood-contacting devices means like heart valves so, these are like blood-contacting devices. Heads and cups of joint prostheses means, you have the

stem you have the acetabular cup and you have the you have that acetabular cup and you have the femoral ball.

So femoral ball and acetabular cup they do not essentially require this kind of tissue formation because, there is essentially mechanical devices. So, those kinds of bones or tissues they essentially do not require any kind of the biologically active material so, within the bio active materials are safe to use that are perfectly.

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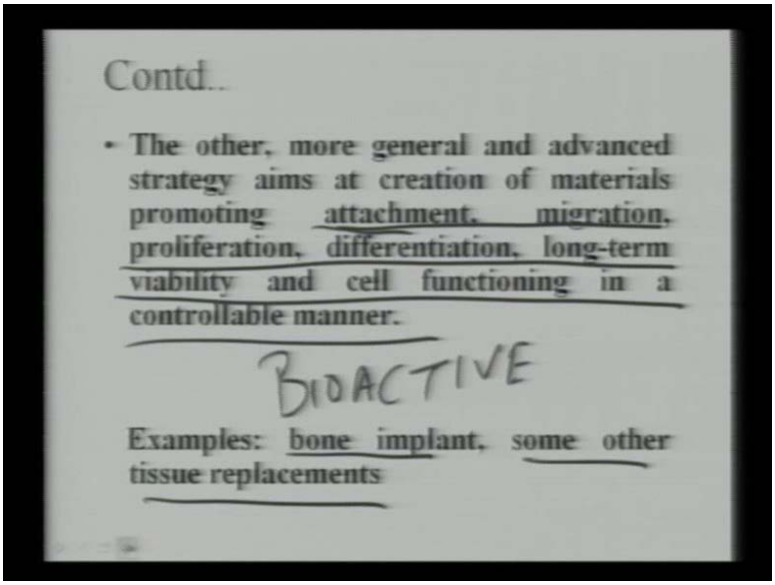


Now, this is the other that is more general and advanced strategy aims at creation of materials promoting attachment, migration, proliferation, differentiation, long-term viability and cell functioning in a controllable manner. So that is the other biomaterials that is called bioactive materials and these bioactive materials essentially are the materials which belongs to the family of hydroxyapatite or calcium phosphate type of material and their uses bone implant or some other tissue replacement application.

So, these materials in the in vivo environment they always create some surfaces which will promote the cell attachment or cell migration or cell proliferation. So, these are the examples of the cell-fate processes. Now these cell-fate processes are essentially promoted or essentially encouraged when this sort of materials like bioactive materials like hydroxyapatite, dicalcium phosphate they are implanted inside the animal body.

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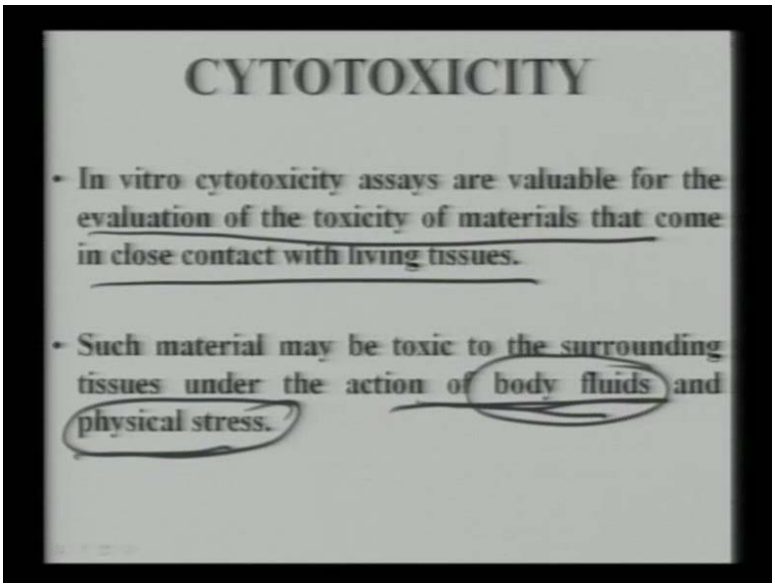


Now cytotoxicity, now, in vitro cytotoxicity as say there essentially important to evaluate the toxicity of materials that can cause in close contact to the living tissue. So tissues mean these are aggregates or the self-organized. Self-organized aggregates of the biological cells and therefore, if the biological cells himself experience any toxic effect because, of the material then entire tissue also will experience similar toxic effect.

Such materials may be toxic to the surrounding tissues under the action of body fluids or physical stresses. Now, one thing that I must mention here that typically cell culture experiments of cytotoxicity experiments, in the normal biology labs or molecular biology lab they do not involve any dynamic conditions.

Dynamic conditions mean they do not involve any flow of the fluid, these are called static test. Static test means you simply put the material inside the solution and you absorb the cell proliferation, cell attachment, but under the dynamic condition this cell attachment or cell proliferation behavior can be quite different. So, on the basis of your simple static cell culture experiments you can again cannot comment how the cell attachment or proliferation will take place under the dynamic condition.

So, these are the kind of things that you should remember so that you can understand that what happens in the entire process, the other thing is that physical stress. Physical stress means when you are putting that any bone implant materials so human body is not always in the rest condition or people do not sleep 24 hours right.

So, people sleep 8 hours and 16 hours in the working conditions, in 16 hours that entire things are moving right in the movement conditions ,again under the physical stress of the movement when the bone is on the moving movement conditions, then that will also affect the way cell proliferate or cell differentiation.

So, but however static test again cannot tell you how these two factors like action of the body fluids, like dynamic conditions or physical stress will affect the cell compatibility or the biocompatibility of the materials.

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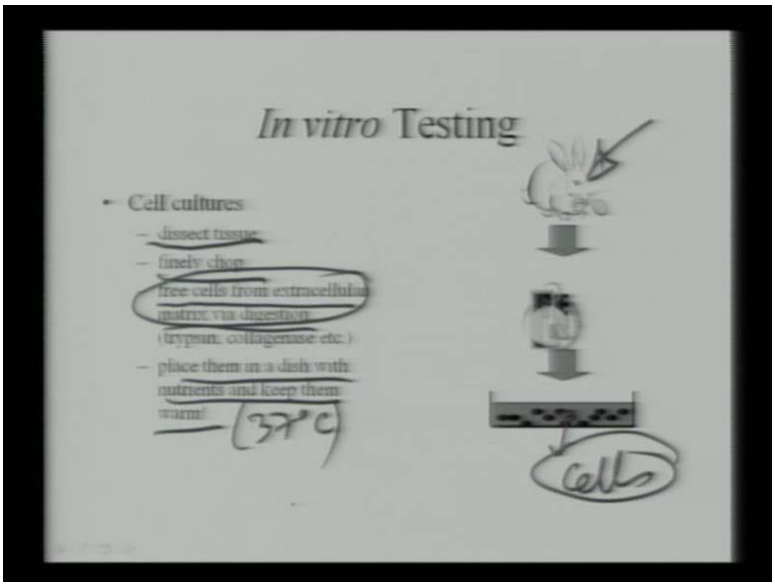


Now, in vitro testing now cell culture solution so these are like you know cells, so these are like cells with the disperse in the solution. So, let say you take any animal we said we dissect the tissue and you finally, chop. So, this dissection of the tissues and chopping of the tissues essentially require to extract the cells from that particular tissue. So if you are taking the connective tissue cells then you can chop it and you can extract biologically the fibroblast cells.

But this can be done in the laboratory, in the advance laboratory and normally all the researches they get the cells as if commercially in the growing conditions on the living stem, then the another things is to that you have to free cells in the extracellular matrix via digestion.

So, cells also will be essentially dispersed in a E C M or extracellular matrix, but you want only cells not the cells with the extracellular matrix so, essentially you have to extract the cells from the extracellular matrix also place them I a dish with nutrients and keep them warm, now warm is it is like 37 degree Celsius.

Now you have to always provide the cells with a environment which will be sufficient for it survival and sufficient for it survival means you have to give nutrients, like food like an human being cannot survive without foods similarly, cells also will not survive without nutrients and then second thing is that, you have to give them physiological environment which contains 37 degree Celsius, PH 7.4.

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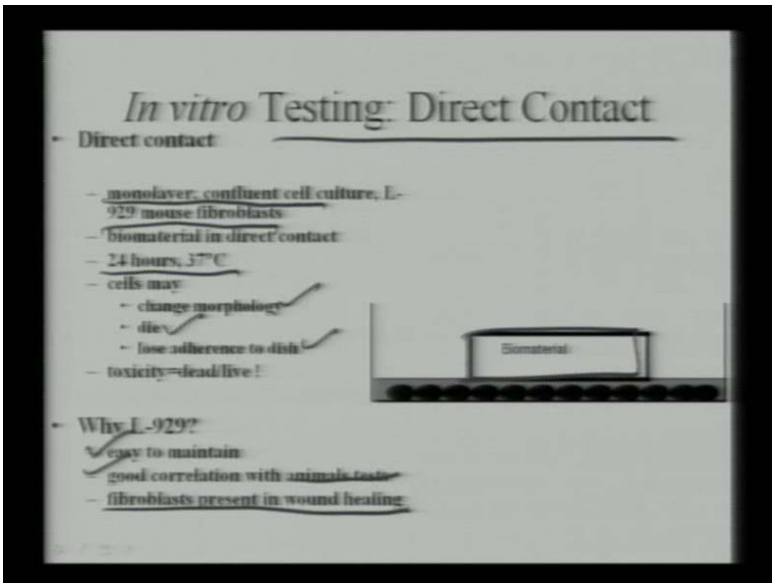


So, that environment must be given so that cells will survive. Now, this is the direct contact testing of the cytotoxicity and direct contact means this biomaterial you can directly mark and the surfaces that in direct contact with the cells and then cells are in the solution and this solution contacts D N A and phosphor buffer solution on all those things. Then you can have this monolayer and confluent so, monolayer means one layer the surface will contain one layer which is pool out in a particular cells and these cells can be L-929 fibroblast cells and again this culture conditions typically can be 24 hours depending on the cell lines and 37 degree Celsius.

Now, cells may change morphology that is important, cells may die or cells may lose adherence to the dish. So, what I am saying that there are three scenarios: one scenario is

that cells die like, cells as soon as in coming contact with the material, cells will undergo apoptosis that means cell death will occur and that will essentially tell you thus this biomaterial is toxic. So, that biomaterial is toxic.

Number 2 cells change their morphology, change their morphology means essentially cells which are in free condition in the solution they are like spherical shaped, now once it comes in contacts the biomaterials then adhered this spread and then cells change the morphology and that is very good as far as the cytocompatibility property is concerned.

Third one cells will lose adherence to that is so, sometime what happens you know that these type of that lower surface of the cell culture solution, cells will not cells will be freely suspending in the solution, but cells will not adhere to the material as the at the same time cells which are suspended in the solution they are not dead.

Do you understand what I am saying? What I am saying is that cells are surviving? Cells are cells will be dispersed in the solution, but cells will not adhere to the material itself. So that shows that the material is such that it the protein adhesion cannot take place or cell adhesion process cannot be trigger.

Again those materials, you cannot call them as a bioactive material. Now, in most of the cell culture experiments in the laboratory scale people use L-929 that is the mouse fibroblast cell lines. Now, this mouse fibroblast cell lines why they are used? The question the answer is, they are easy to maintain this cell and easy to maintain. Second one, people have found good correlation with animal test. Animal test means if you use the mouse fibroblast cell lines and you say that these material is in vitro cytocompatible, many times people have to observed or people have reported that the same material is also in vivo biocompatibility, that means it is mold like one to one correlation if it is having the good cytocompatibility to L-929, it has good biocompatibility in vivo.

The third point is that fibroblast present in the wound healing process. Wound healing means like essentially when you are talking some bones or joints in human body and put in the implants so, you are essentially you are essentially inducing some wound in the patient body and in the wound side the fibroblast cells are the first cells which will come and then they will form some tissue in contact with the implant. So, that is the reason that is why fibroblast cells are used primarily most of the cell culture experiments.

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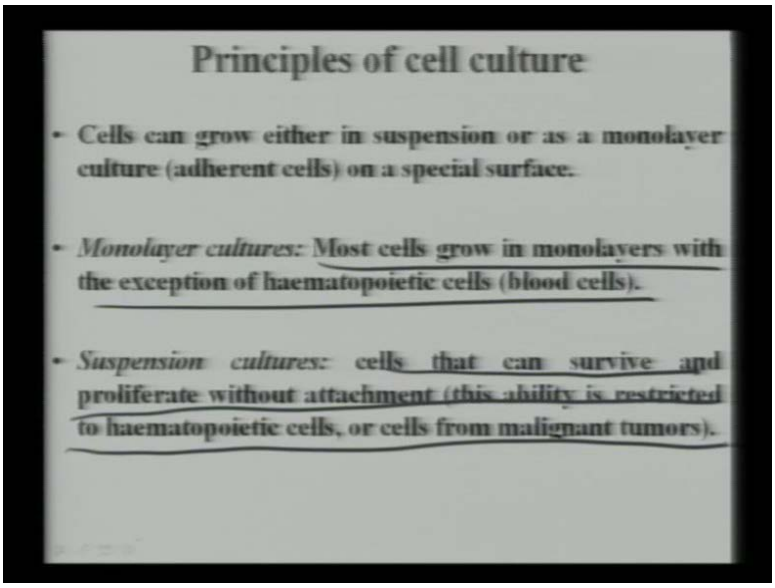


Now, principles of cell culture, cells can grow either in suspension or as a monolayer culture on a special surface. Now monolayer cultures means most cells grow in monolayer with the exception of the hematopoietic cells that is the blood cells and third one is the suspension culture, cells can cells that can survive and proliferate without attachment this ability is restricted to hematopoietic cells or cells from malignant tumor.

So, essentially what it means that is the other scenario that I have told the cells will not get themselves attached to the material, cells will still survive and they will suspend in the cell culture solution.

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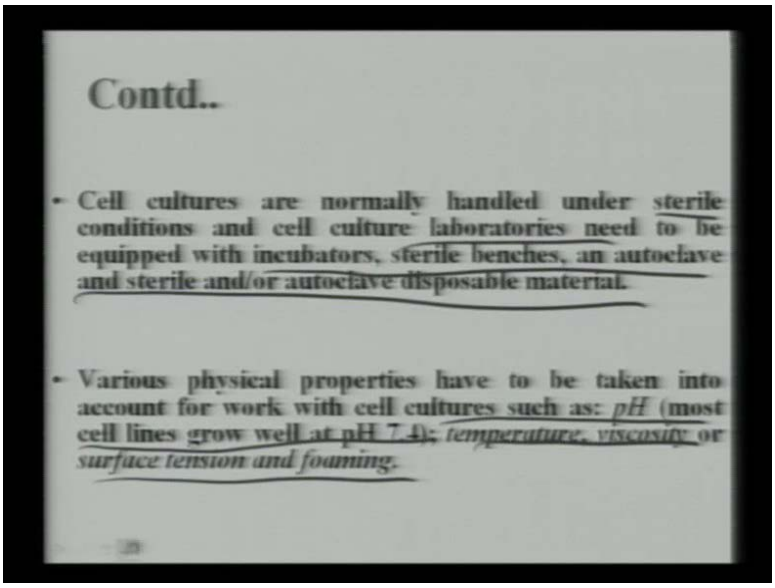


Now, cell cultures are normally handled under the sterile conditions and cell culture laboratories need to be equipped with incubators, sterile benches, autoclaves and all those things that you know, the other part is that various physical properties have to be taken into account to work with the cell culture, such as PH most cell lines grow well at the PH7.4 temperature, viscosity, surface tension and foaming.

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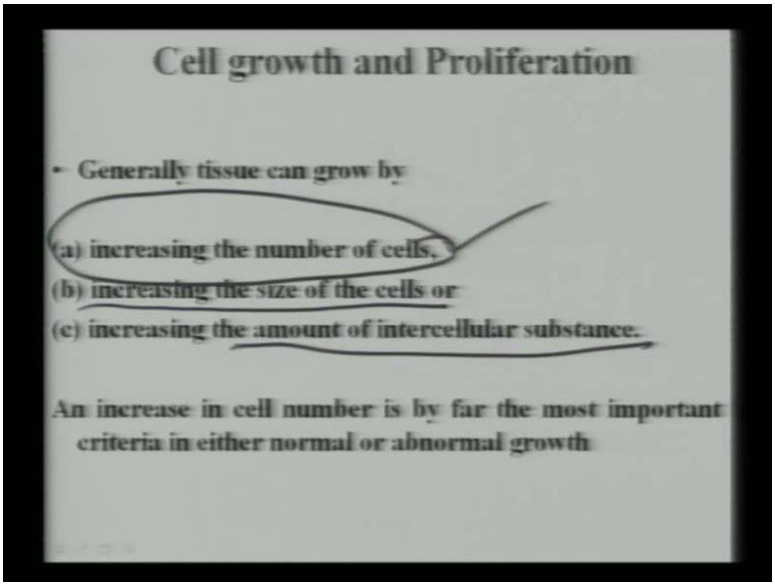


Now, how this cell growth and proliferation can take place so, tissue can grow by increasing the number of cells, now when you have to increase in the number of cells

then that process is known as the hyperplasia. Increasing the size of the cell that is the hypertrophy, not atrophy, increasing the amount of intercellular substances so when a cell will increase a cell size, will increase in size that essentially means that increase in cell size must be accompanied by the increase in the amount of the intercellular substance, if the intercellular substance is the not increased then cells will not increase, will not be able to increase in size.

And intercellular substance means like protein molecules if they are transported from outside the outside the cell body or outside the E C M or from other cells to the particular cells then cells will accumulate more and more number of protein molecules and then also it will increase in size. So, an increase in the cell number is by far the most important criteria in either normal or abnormal growth. So, what has been stated here that, when any process is accompanied by the increase in the number of cells and that increase in the number cells is important because, that will tell you the 12 cells that are in the growing stage or cells are proliferative pause.

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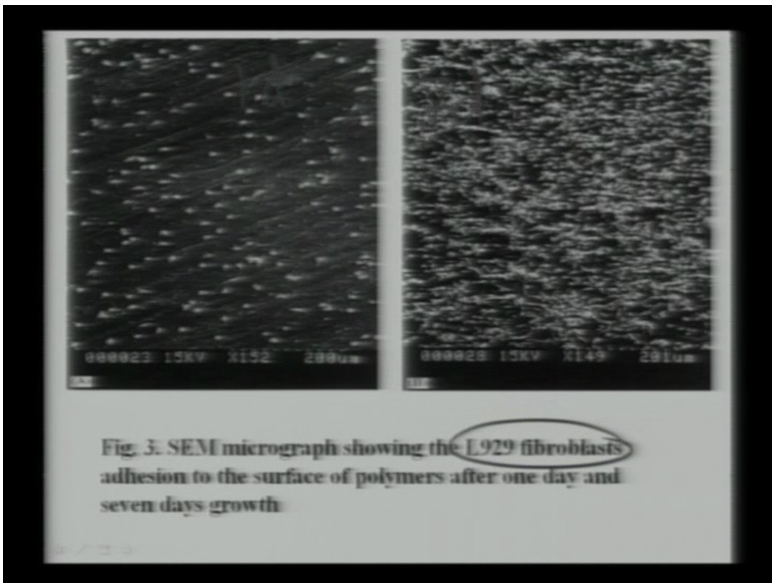


Now, cell proliferation also or cell growth also takes place over the certain time duration. Now, this particular S E M micrograph tells you that what happens after 1 day and then right hand side micrograph tells you, that what happens after 7 day. So at the end of the 1 day L-929 fibroblast cells they are adhering, but the number is much less, but at the 7

days, the cells are adhering at certain point cells are forming, some cellular network or cellular bricks.

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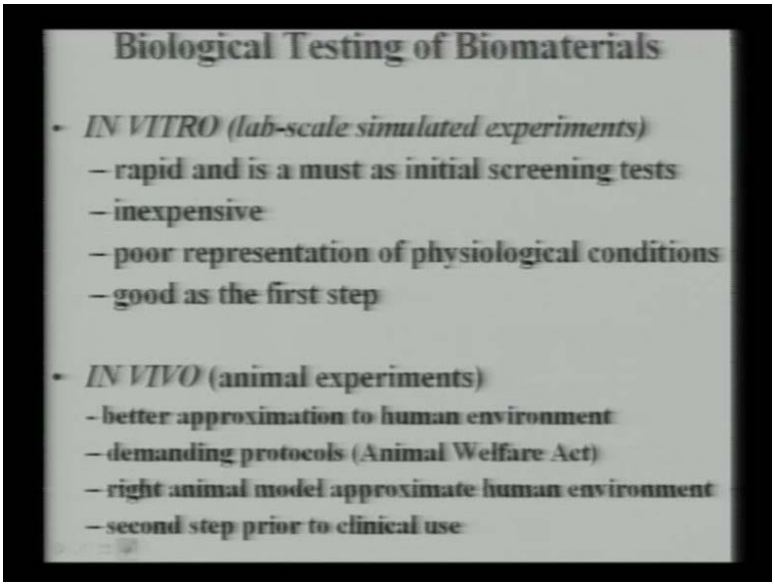


So, this you know that what is the meaning of the in vitro and what is the meaning of in vivo by now and why that both the of set of test are important ,

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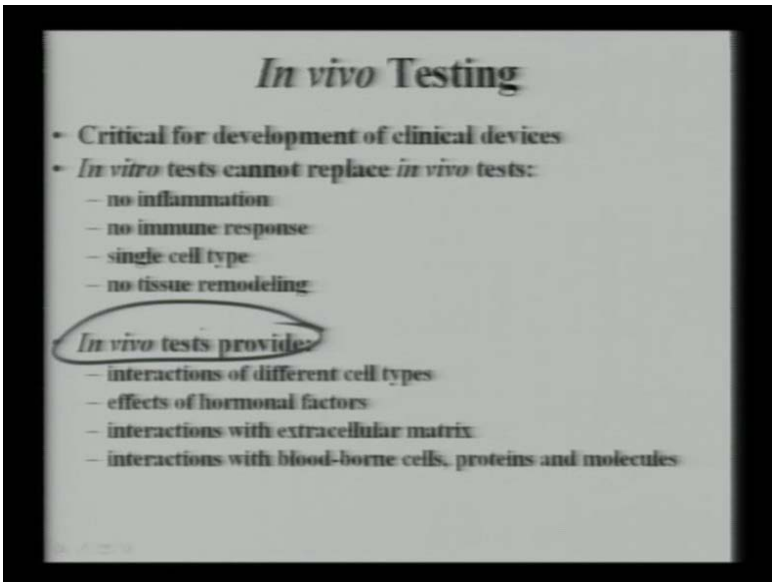


and what is the necessity of the in vivo test as far as I mean per say

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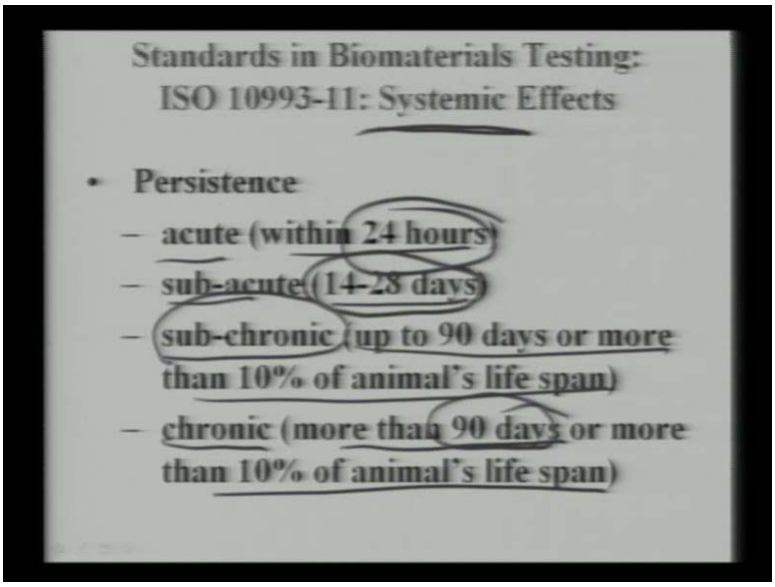


Now, there are certain types of terminology that you should know what is known as systemic effect. Systemic effect means whether a biomaterial when implanted inside the animal body and that biomaterial, causes some effect in a in the system which is surrounded which surrounds the biomaterial and it can be acute like within 24 hours, it can be sub-acute within 14 to 28 days and it can be sub-chronic like up to 90 days or more than 10 percent of animal's life span and it can be chronic that is more than 90 days and more than 10 percent of the animal's life span.

So, what is means by that suppose, it causes some toxic effect any biomaterial and this toxic effect is seen within the implantation of within the first 24 hours then we can call it as a very acute toxic effect. Now, if that toxic effect is observed in within 14 to 28 days then it is called sub-acute toxic effect. Now, it is called if it is a chronic, then it is typically it is observed after 90 days, months 3 months and within the 3 months if the animal or if the animal model is experienced that any toxic effect then is called sub-chronic type of effect.

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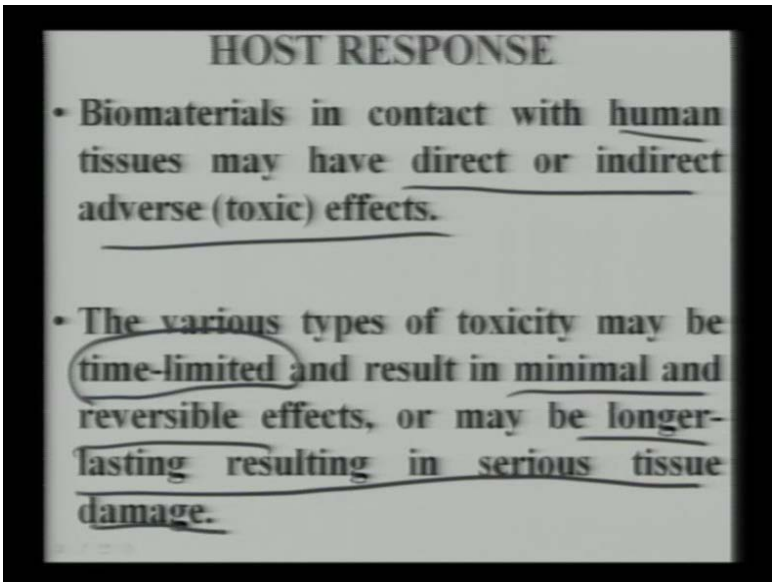


Now, host response as I mentioned earlier, host response means how the animal model itself will actually respond to the implant that you have put it inside that particular animal. So, biomaterials in contact with human tissues may have direct or indirect adverse toxic effect. Now, the various types of toxicity may be time limited and result so, this toxicity as you have seen in the earlier slide that it can be systemic means this toxicity effect may not be seen immediately after the implantation, like it cannot it it may not be very acute. Acute means what you see within 24 hours.

But it can be chronic also, chronic means after long time lets a 90 days this toxic effect is seen, then that types of toxic effect is known as the chronic toxic effect and this in case of the chronic disease or chronic toxic effect, that initially the implant may perform well, but you know after 3 months the implant may not perform biologically well in the environment. So that is why it is saying it is said that this host response can be toxicity may be time limited and result in minimal and reversible effect minimal and reversibleeffect and may be longer lasting resulting in serious tissue damage.

So, serious tissue damage means that within long term and were for a longer time interval and on your prolong basis, that material can cause some tissue damage. So this slide has been explained before,

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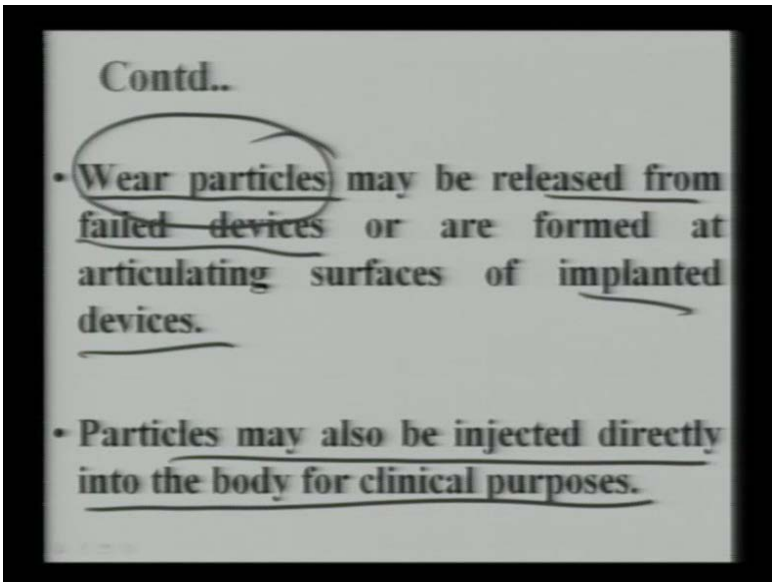


now the question is that, why a biomaterial or why certain types of biomaterial are shows or exhibit that kind of chronic disease or chronic toxicity after 3 months for example, now the answer is, is many times this biomaterials will experience some corrosion and wear in the in vivo environment and the wear takes place after long time and they generate some kind of particles, so that is what is mentioned here that wear particles may be released from the failed devices and are formed at the articulating surface of implanted devices.

And these wear particles they are typically micron size particles or can be nano sized particles, finer the particles more is the toxicity effect. So if the particles are nano sized then it will have more toxicity compare to particles which are in the micron size and the particles may also be injected directly into the body for clinical purposes.

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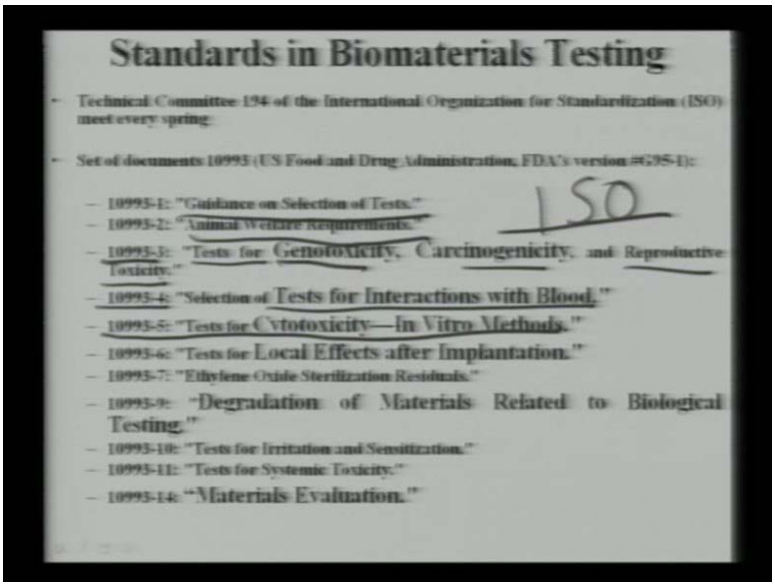


Now, these are the standards in the biomaterials testing this is like ISO testing, I S O and which is approved by FDA. F D A is the U S food and drug administration and ISO means international standard organization. Now they have this 1 0 9 9 3 set of documents and these 10 9 9 3 documents there is 1 2 3 like different subsets or set of documents like first one is the guidance on selection of tests, that tells you like suppose you are trying to develop a material x, for y application now for this material x, what are the kind of test a b c d that you need to perform for to satisfy or to say that this material x will be suitable or will be ideal for this biomaterial biomedical application y, I s 110993-1 will tell you that these you have to test from a to d or for example, a to g. Then second one is the animal welfare requirements like you know suppose if you put this material inside a particular animal or human being what is the animal welfare requirements that you must know those things will be mentioned in the 10993-2.

Now 10993-3 that is a genotoxicity, carcinogenicity and reproductive toxicity, that is important, I mean those days guidelines are mentioned 10993- 4 that is test for interaction with the blood that is the blood compatibility test, 10993-5 that is a test for cytotoxicity like in vitro method, so all these tests are essentially tells you that what are the guidelines that you need to follow when you want to do a particular set of tests.

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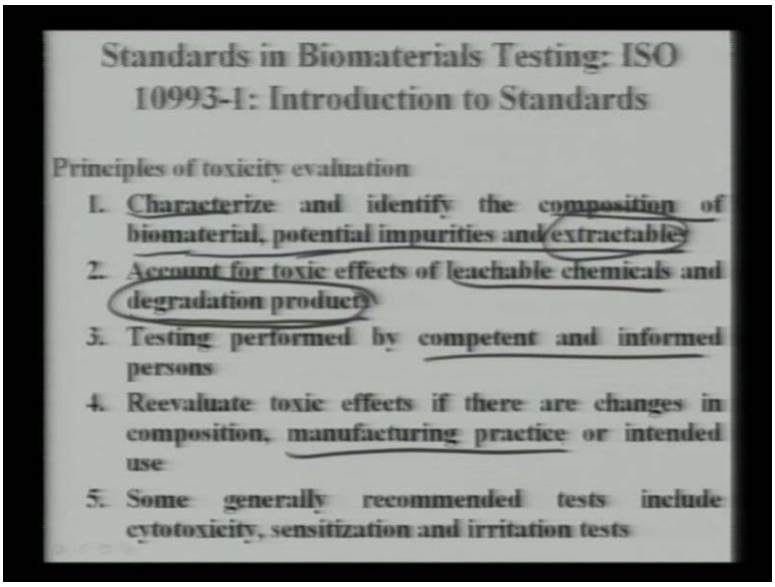


Ok We will start with the 10993-1 that is the set of guidelines which will tell you that what are tests that you need to carry out for a specific application. Now principles of the toxicity evaluation, that essentially characterized and identified the composition of the biomaterial and the potential impurities and extractables. Extractables means like when your material has a multiple composition x y z, now this you have to be judicious enough to be understand that whether x will be list out in the in vivo conditions or y will be list out in the in vivo conditions or the z will be list out in the in vivo conditions.

And what are the impurities those will have this kind of effect, this second one is that account for toxic effect of the leachable chemicals and the degradation product so, leachable chemicals and the degradation product so remember that these two things may not be the same because, your element can be list out from the material, but then it can react with the biological fluid then it can cause some kind of a product. So that degradation product may have a different composition from what the element that is list out from the material. Third one is that testing perform by competent that informed person so that is the medical professionals.

And fourth one is the reevaluate toxic effect if there is change in the composition manufacturing practice or intended use. Now, if you change your manufacturing process means the way you have the abrogated or you have sintered or you have compression

molded or you have injection molded particular material, if you change your process parameters ,it is advisable that you need to revisit your all these toxicities studies again.

Now, some generally recommended tests include cytotoxicity, sensitization or implantation test. Now, as I said that you know that in vitro cytotoxicity or that is the cell culture test that is under the guideline of 10993-5 and I will quickly go through that you know, what has been summarized to the last lecture?

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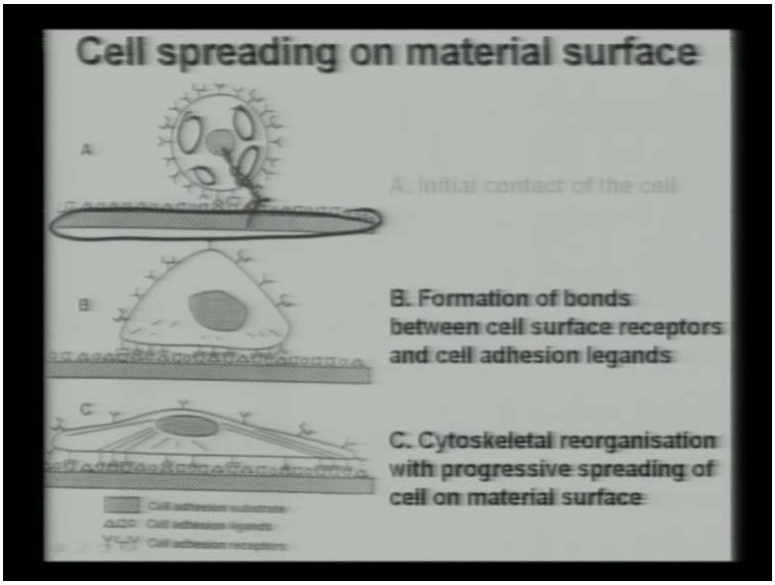


So, these toxicity actually effects like when this particular material, this is the hatch is the material, that is the biomaterial it will release some kind of chemicals and these chemicals can enter the material, inside the material they can either transported through the nucleus then cause the DNA damage or it can cause some death of this intracellular elements like mitochondria, golgi body, golgi appearance it can cause some damage.

So that, mitochondria cannot function like the way it performs in the normal healthy cells. Do you understand what I am saying? So your essentially culturing the normal healthy cells on the biomaterials. Now if the biomaterial leaches out some substances which can cause damage to the mitochondria which produces the energy in the inside the cell or which is the power house of the cell. Now, if the mitochondria cannot perform the way it should perform in the normal healthy cells and if it is damaged that means cells also will lose its normal functioning and essentially the cells will die over a time period.

So, that means that cells is causing a toxicity effect in vitro or if the material can be directly transported to the nucleus and then it has a toxicity effect, it can cause the genotoxicity or the DNA damage of the material.

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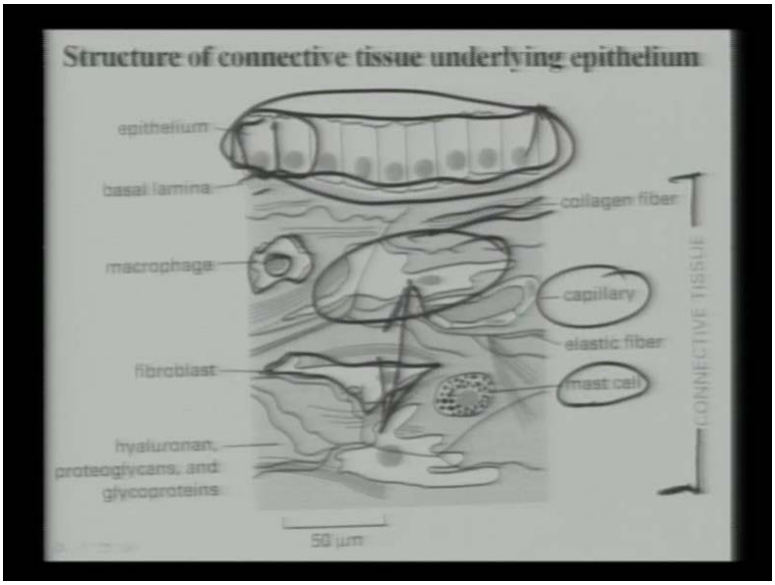


Now, this is the structure of the connective tissue and the tissue underlying epithelium, so, epithelium is the top surface of the top tissue and here you can see that all the cells, eukaryotic cells are connected to each other and you know very dense manner. So, that is what I have mentioned earlier that in a tissue cells organize themselves and then tissue can be discuss self–organized aggregates of the cells.

Now, here you have a basal lamina and here you have the macrophages. The macrophages another cells also type of eukaryotic cells and you have the fibroblast cells which has a different type of shape, which does not have the same shape as the cells of the epithelium and this entire green stuff as you can see that matrix, it contains the E C M that is the extracellular matrix and extracellular matrix it has collagen and it has the collagen as well as the hydroxyapatite particles and so on.

And you have that all other other type of cells like mast cells or capillary etcetera and this shows the collagen fiber, now this is called the connective tissue. So, the entire connective tissue contains a number of cells the other things that you should notice here that these cells they are kind of in direct contact with each other. However all the

connective tissues, these two connective tissues are not connected to each other, but they are separated by the extracellular matrix. So connected tissues therefore, have a different environment compare to the cells in the epithelium layer understood.

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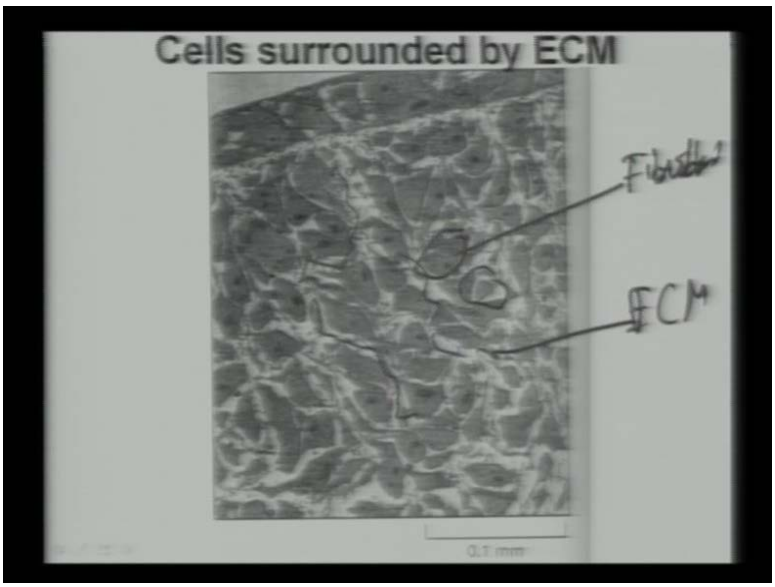


This is the cells how the cells which are surrounded by the E C M. So this is the part of your this white background or you know white matrix, this is the part of your E C M and you have the cell lines like you know connected tissues cells and so on. So this is the cell lines like fibroblast cells, so each all the fibroblast cells they are like separated from each other by the presence of the extracellular matrix. Now this shows how cell are surrounded by spaces which are filled by E C M.

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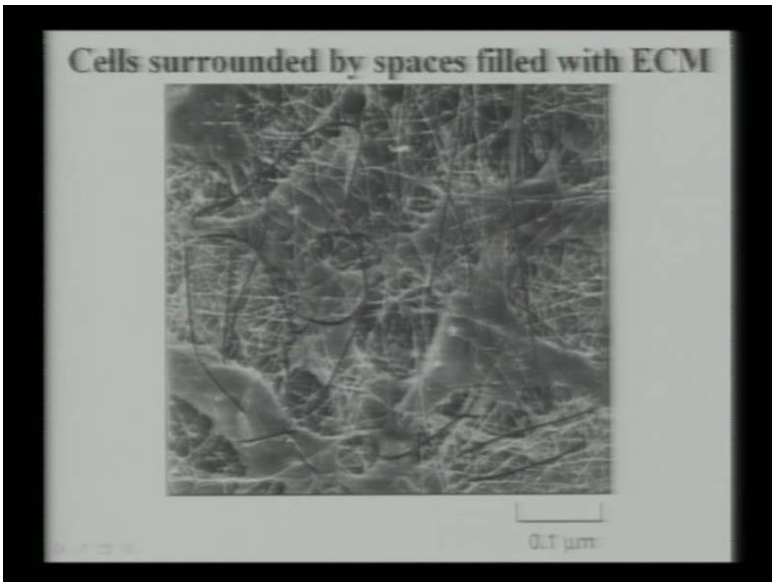


Now, this is the part of one cell and you have these fibers like structures you can see these are essentially like collagen fibers. Now this collagen fibers constitute your extracellular matrix and these fibers can be clearly seen and some fibers inside the cells do you notice these are the cytoskeleton that is the constitute of cytoskeleton.

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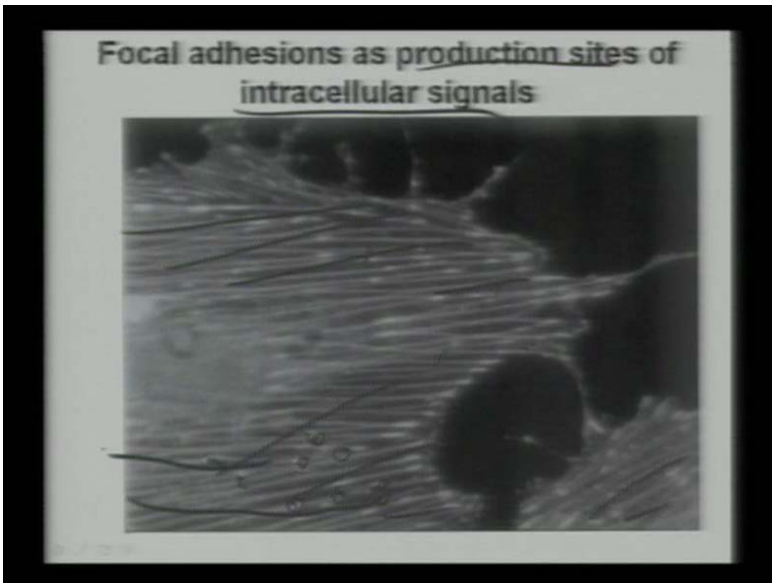


Now this recent image clearly shows you that, what are the focal adhesions as the production sites of the intracellular signals? Now these focal adhesions are present at different sites in the extracellular matrix and these are the collagen fibers which are

constituting the extracellular matrix. So these appears as the green and this focal adhesions means this is the points of the focal adhesions where the signals are generated and signals can be transferred to other target cells, through the extracellular matrix collagen.

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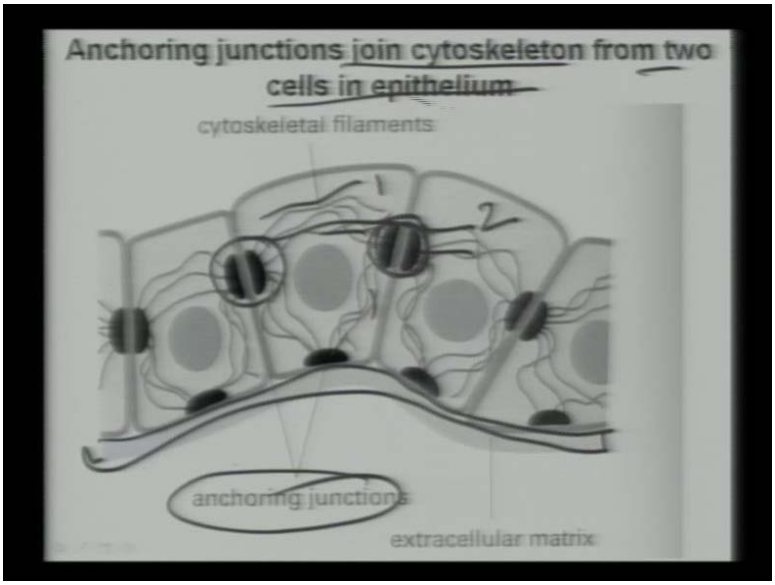


Now, this is the anchoring junctions and these anchoring junctions they join the cytoskeleton from two cells in the epithelium and these anchoring junctions are essentially these particular junctions are called anchoring junctions and these are the cytoskeleton filaments. Now what you see that cytoskeleton filaments from cell one, they are getting attached to the cell two and then they form this black level junctions and this black level junctions you can see here which are considering, which are known as the anchoring junctions. Now, the other thing is that you see that is that you have extracellular matrix E C M now, which is known as the labeled and this is the yellow junctions which is just outside the this epithelium cell lines.

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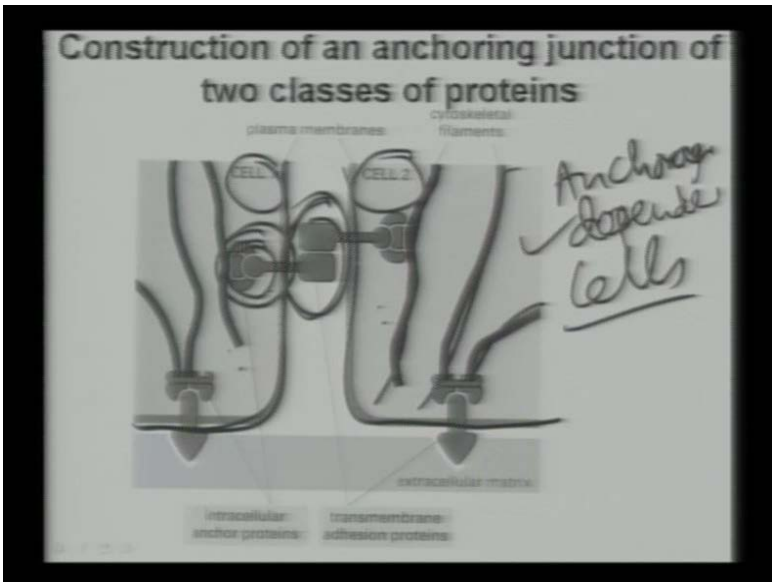


Now, this is the conditions that when the two cells are connected to each other as an epithelium, but when the two cells are separated from each other like the way it is separated here ,this is cell 1 and this is the cell 2 and this you have this plasma membrane which is the cell membrane.

And now you have the cytoskeleton filaments here this green one, red one and you have this intercellular actin pro, actin intercellular anchor proteins and you have also transmembrane protein. Now there are different type of proteins as you say as you noticed in the earlier lectures like you have the proteins the number of proteins can be as has 10 to the power 19, 10 to the 9 to 10 to the power 13 numbers. So this large number of proteins can be of different types, now different types is protein is called trans one is the transmembrane adhesion proteins. Transmembrane means they are essentially present between the two membranes and they are essentially participate in the adhesion process between the two cells.

Now this is mediated by this adhesion and this is your actually intracellular anchor proteins. intracellular Intracellular means from one cell to another cell it is helping in the anchor anchoring anchoring process. So these cells are kind of called anchorage dependent cells and the examples of the anchorage dependent cells are fibroblast cell. Anchorage dependent cells means like essentially two cells will be attached to each other by the intracellular anchoring proteins as well as the transmembrane proteins as you can

see that this is like a forming a bridge, between the two cells and therefore, the two cells will be attached to each other.

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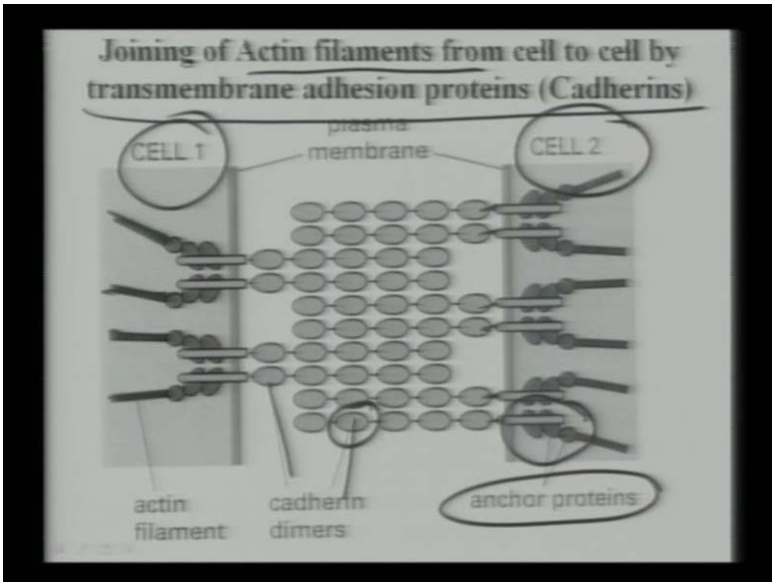


Now, other a way that two cells can be attached to each other is the joining of the actin filaments from cell to cell by transmembrane adhesion proteins that is called Cadherins. Now this is cell 1 again part of the cell 1 and this is part of the cell 2 and you have this actin filaments here. Now, this actin filament and you have these anchor proteins also, this anchor proteins is the blue one and you have this cadherin proteins that is the green one. So, what happens that cell to cell junction is formed here which is established by these anchor proteins which are getting connected to the cadherin dimers.

And you know that how protein complexes takes place, because protein has certain anchoring sites and through this sites the other proteins can be coming and can come together and two individual proteins can come together and form a protein complex. So similar protein to protein a junction is essentially develop between the cell 1 and cell 2.

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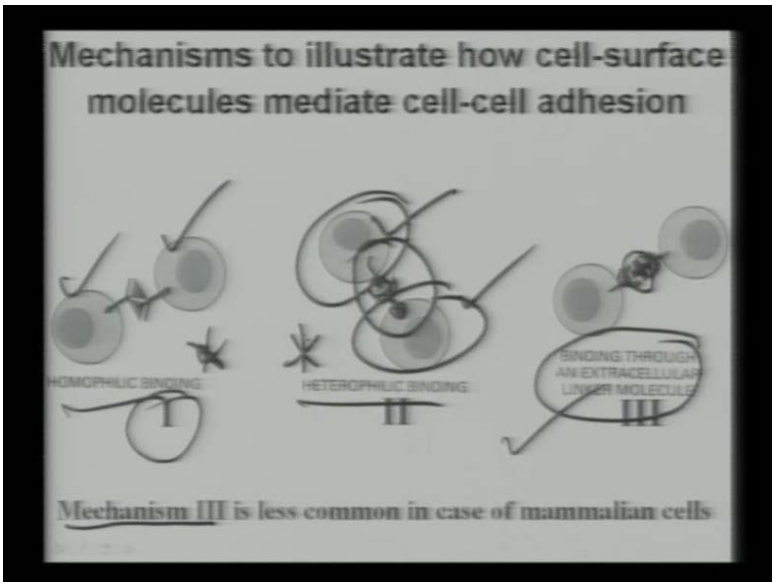


So, this is the mechanism to illustrate that how cell surface molecules they mediate, now this is there are three types of binding it has been shown that one is the homophilic binding. Homophilic means this is the two same type of cells and then from this one cell to other cell this similar type of protein molecules or cell adhesion molecules they come out and they make a bonding together the other is called heterophilic binding. Heterophilic means like this is again two different cell lines or it can be same cell lines and then this is the one type of protein molecules coming so if it is the protein molecule type x, from other protein molecule type y, they form and they form a bond here.

The third one is the binding through an extracellular linker molecule like two surface proteins which are coming together, but they are not capable of binding to each other at site, this binding is essentially mediated by this positive this plus molecule and this plus essentially molecule from the extracellular matrix. So this binding here is mediated by the extracellular matrix molecule. So these are like homophilic binding, heterophilic binding and then whether this binding is actual when its extracellular linker molecule.

The statement, that has been written here that mechanism 3 is less common in case of mammalian cells like whatever biological cells or mammalian cells that you are using in the laboratory or it is of relevance to the various biomedical applications, it is mostly the mechanism 1 or mechanism 2 which are mostly common or which are kind of hominine.

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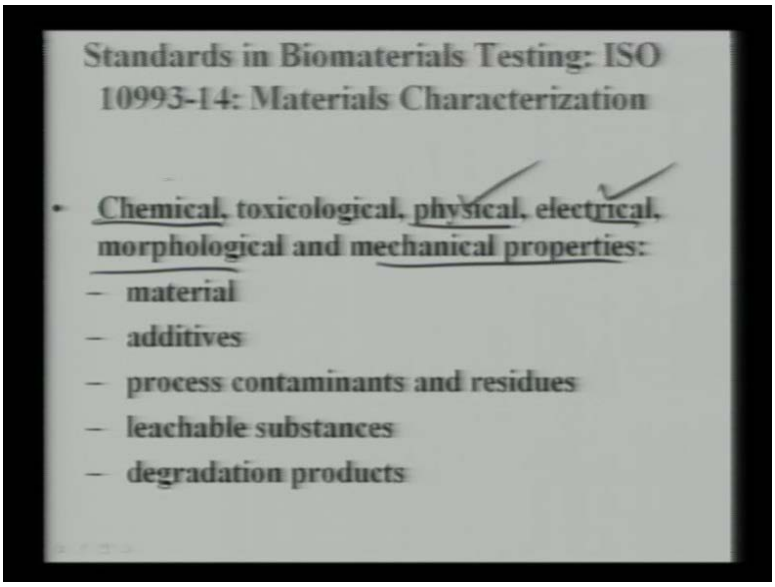


Standards in biomaterials testing that ISO 10993-14 that is the materials characterization, now here you have to find out that what is the chemical, physical, electrical, morphological and mechanical properties of the materials that you are using. So, most of these things some of this things I have already mentioned that what is really mean,

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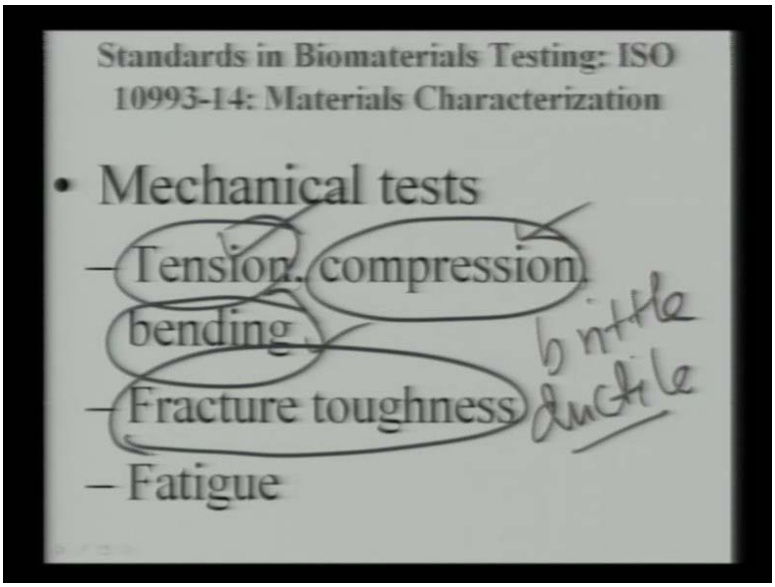


now mechanical tests essentially means you can do the test as a tension, compression, bending depending on whether you have a brittle material like ceramic or whether you have a ductile material like metal. If it is a ductile material you can do that in tension

test, if it is a brittle material you can you have to do compression tests or bending tests. And accordingly you calculate what is elastic modulus? What is the string? What is ultimate denses? Etcetera. You can measure the fracture toughness, now this toughness can be measured from the simple tensile test from the area under the curve or this fracture toughness can be measured for the ceramic by single (( )) or put in the indentation measuring the crack length and so on and other things are equal to.

Now, fatigue test is also important, but I thought metallic materials like you know you have the (( )) curve. so fatigue testing means when a material is under repeated stress cycle what is the maximum stress level, that the material can which stand before fracture so when you do simple tension tests, simple tension test means you have a material you are pulling in tension from two ends.

Now, you are you are giving the uniform stress throughout the destiny, but fatigue testing means you are not giving uniform stress but, your stress cycles are going like sinusoidal or cosine or whatever typical repeated stress cycle or reverse stress cycle etcetera and then you were trying to assess that what is the property is of this material, under that repeated stress cycle and typically if your material is under uniform stress then the material will have higher load bearing capability compare to the material which is under the repeated stress cycle or reverse stress cycle.

So, fatigue strength is much lower compare to your typical tensile strength of material. So that is the message that you should remember.

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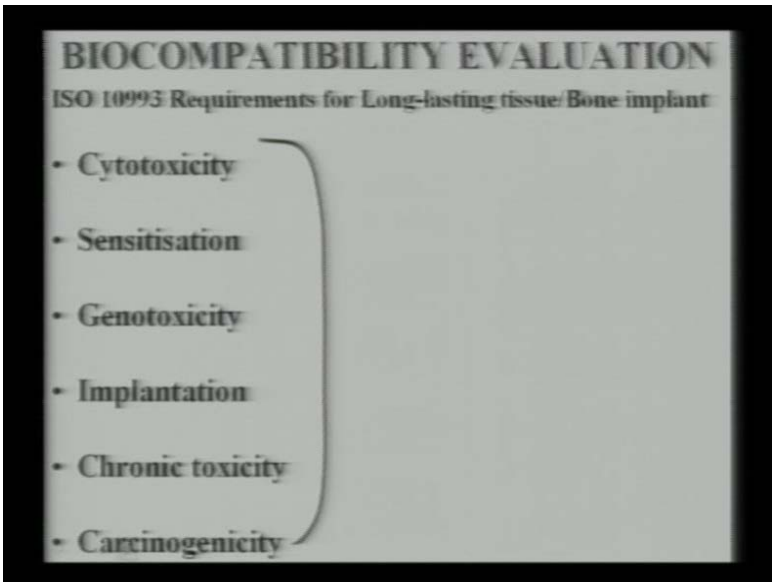


Now, as I this has been mentioned early that these are the set of biocompatibility testing that one is to evaluate here.

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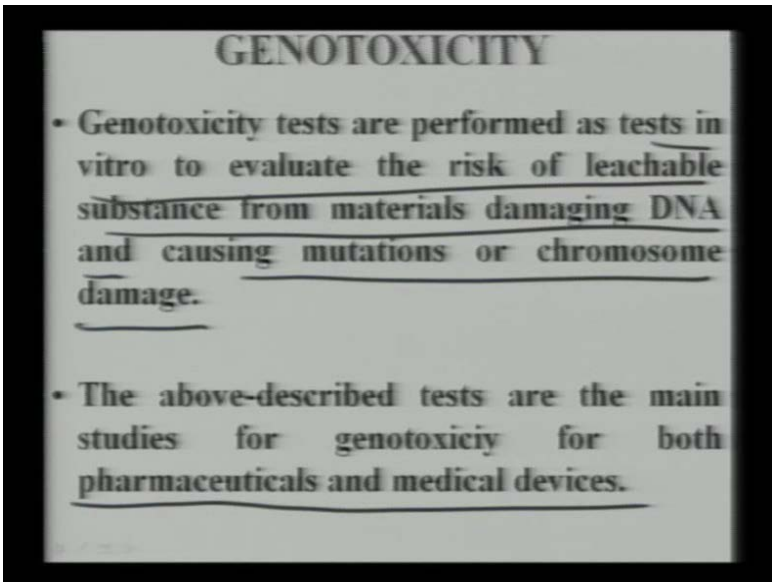


Now, genotoxicity that is the next five slides I will teach and before I finish today, now genotoxicity tests are performed as tests in vitro to evaluate the risk of leachable substances form materials damaging D N A or causing mutations or chromosome damage. So, that is the core idea of the genotoxicity test and these are very important for pharmaceuticals as well as medical devices.

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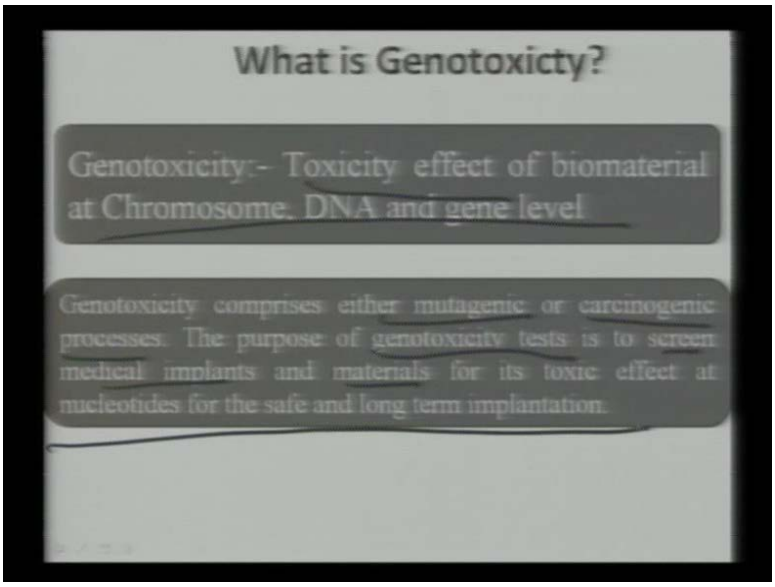


Now, what is genotoxicity this is repeated here again that is the toxicity effect in the chromosome gene D N A and gene level and genotoxicity compares as either mutagenic or toxinogenic processes and up these tests essentially screen medical implants and materials for its toxic effects at nucleotides for the safe and long term implantation.

So, what it means that is the long term level this material should not wear out and produce some wear particles which will cause directed DNA damage. So, that is what is essentially investigated here.

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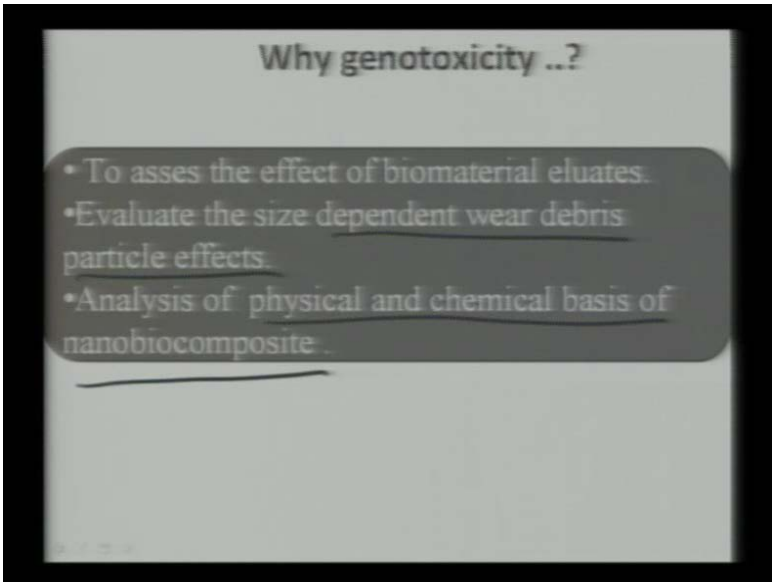

Now, why genotoxicity to assess the effect of biomaterial eluates? To evaluate the size, dependent wear debris particles and third one analysis of the physical and chemical basis of nanobiocomposite. So size dependent wear debris particle effects as I mentioned to you earlier that finer the particles, more is the potential for the particles to cause genotoxic effect. So, if you are comparing the effect of 10 micron persists 10 nanometer. 10 nanometer particles definitely will have more genotoxic effect, compared to 10 micro meter particles.

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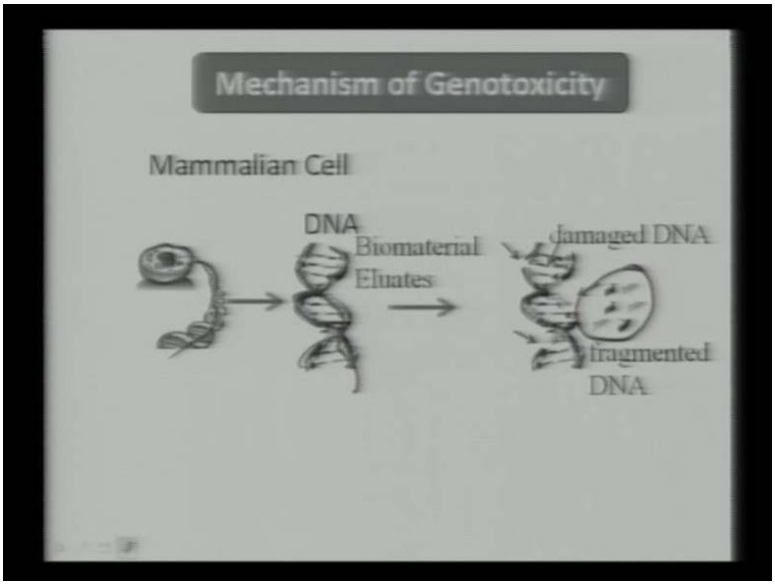


Now this slide essentially tells you that you know the basically the concept of this genotoxicity effect, now you have this half of the cells here and in the half of the cells, this is the nucleus and nucleus contains this DNA. Now, DNA has a typical double helix type of patterns and this double helix patterns when it is exposed to biomaterial eluate. Eluate means you have this wear debris particles which is in solution like d a e m a and phosphate buffer solution and so on. So this entire solution is called eluates.

Now, when this D NA is directly exposed to biomaterial eluates what will happen some particles may damage this hydrogen bonds which are essentially linking between two helix and if the hydrogen bond is broken that means you are essentially causing some damage sides on the D N A and what it will have what this will lead to this fragmented DNA, that means part of the DNA that will be immediately disperse in neighborhood of

the D N A. So, this is essentially cause by the debris particles and as I repeat finite the debris particles more is the chances of dies genotoxicity effect

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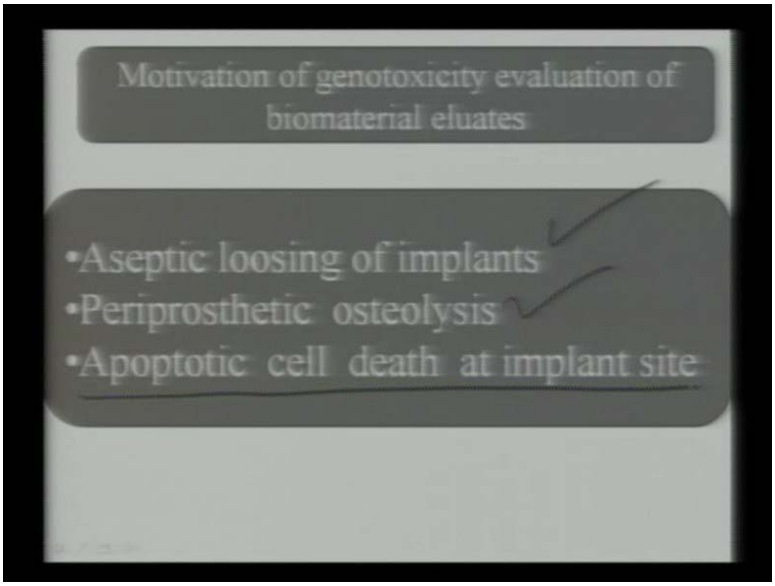


Now, what is the motivation for genotoxicity evaluation number 1 is the aseptic loosening of implants and periprosthetic osteolysis. Aseptic loosening means when implants are put it inside the animal of and then after long time then implants can be loose by can be detached from the neighboring tissues or the bones.

Periprosthetic osteolysis also is caused by the aseptic loosening third one is a apoptotic cell death at the implant site. So, that is also another thing which will happen

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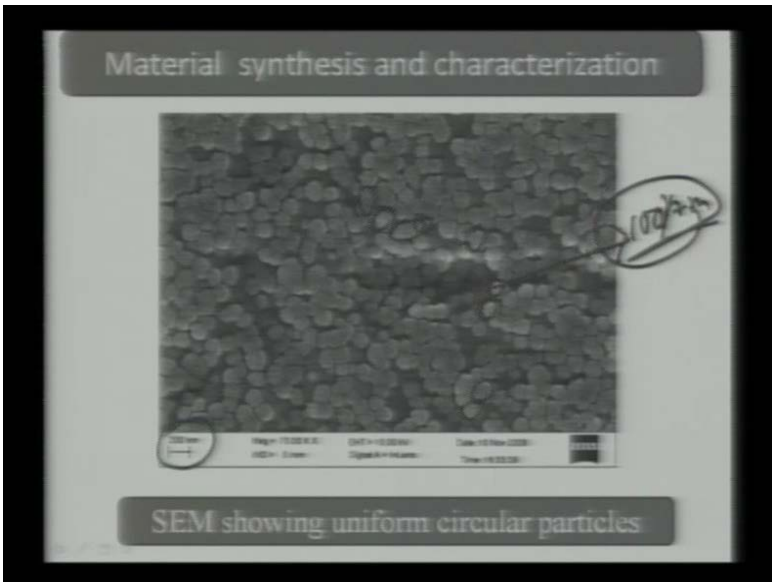


now this individual particles you can see these particles are coated by DMM that is the dimeth (( )) medium (( )) medium and these DMM solution is statistically codes preferentially all the particles and if you see this micron bar here these particles is roughly around 100 to little bit higher than the 100 nanometer. So, essentially you can call it as ultra fine size particles are all most like nano sized particles.

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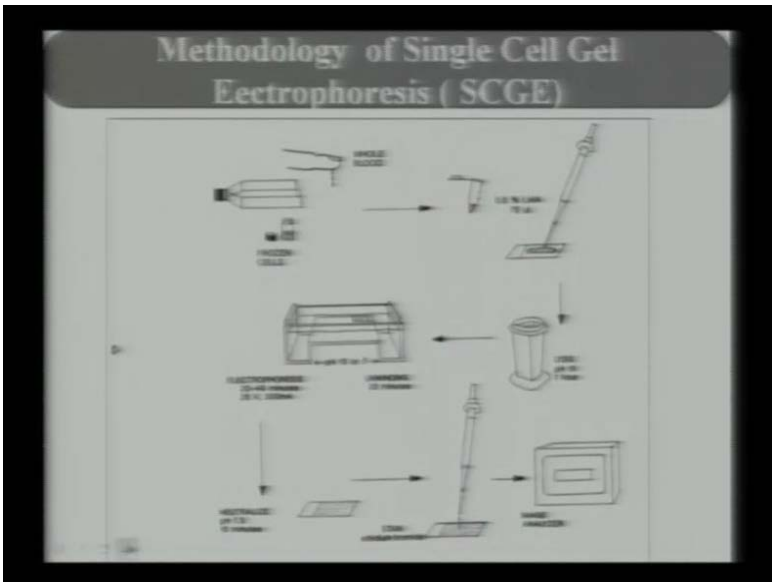


Now when you put these materials

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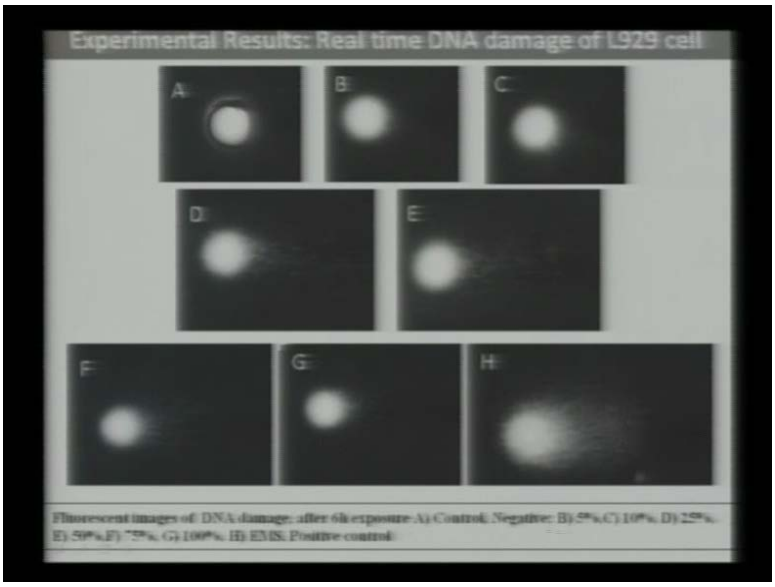


in the solution and then there is the amount of these eluates and then see that what is the D N A damage of the L-929 cells now this is the case for the control material and this is the condition of L-929 cells Now you do not see any damage here, but if you increase this concentration of these eluates here now if you go to A and E for example, in both the cases what you notice here that this is the fragmented DNA region which shows these concentrations have the genotoxic effect and is the positive control, positive control means it is a reference sample which will definitely have the genotoxic effect and that is what has been illustrated here that it has a long tail.

Now, if you take multiple images by the fluorescent microscopy and then what you can do you can measure the length of this tail and this tail length is first proposed by scientist called Olive and accordingly this is known as Olive Tail Movement, this is O T M.

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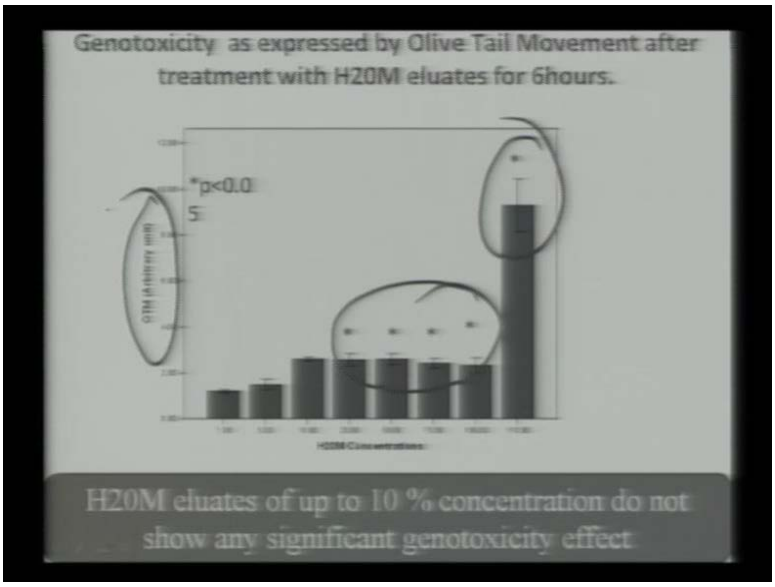


Now, if you measure this OTM for different conditions and put it in a graph, what you can see here, that if this particular concentration more than 25 percent, they have a statistically significant genotoxic effect. And the positive control has a much more genotoxic effect. So, this is the O T M that Olive Tail Movement for this particular biomaterial eluates. So, I think I will stop here, and in the next class I will start with the blood compatibility.