Biomaterials for Bone Tissue Engineering Applications
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Module 1
Lecture 4

Ok. Coming back to that discussion on the bio compatibility and the host response.

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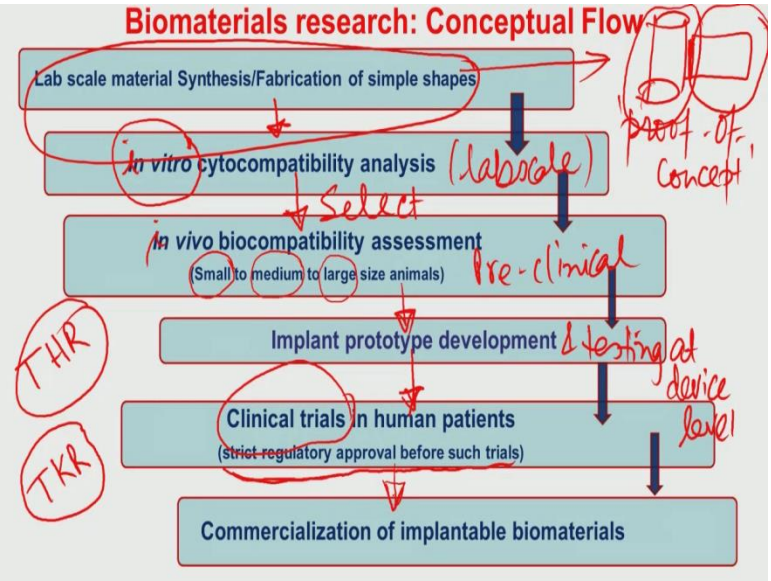

The previous discussion has been summarized in this slight to some extent so; one has to start with this lab scale material synthesis of fabrication of the simple shaped examples. Now simple shaped examples can be either cylindrical shaped or can be rectangular shaped and so on, which may not be the ultimate shape of an implant which will be put it inside human patient.

So, let me recorrect the statement, what I am saying is that in the laboratory whatever biomaterial or scaffold be synthesized normally for the In-vitro testing purpose or for physical property measurement purpose, these samples are of simple geometric shape which in no way can be (impl) can have any similarity in terms of the size or shape of the implant that are to be placed in human patients.

So, however that file we start with the simple shaped material because simple shaped material is required for standard material testing like whatever string property were testing or hard disc property or (())(01:47) or even microstructural phase simulate study you need very simple shaped material and so that you can establish what I call as a proof of concept.

Proof of concept essentially means like first of all you one has to know for that the material is have been appropriate compatibility and sale and tissue level, so now to prove that point you can do this testing with a simple shaped material. After that you need to do is that in vitro compatibility test, Hydro compatibility analysis we we using various biological assess, now this biological assess are actually a kind of experiments where one can use the elements of biological system, be it proteins, cells, blood sample or bacteria with an end point objective to determine its functionality change or its survivability in contact with the bio material or artificial material of scaffold.

This is to be done with the glass wares or test tubes or petri dishes as I told you before. And this is done in a typical lab scale experiments ok? Next level once you select some of the material so, you select a material from a group of material which will have the best combination of both physical property and In-vitro cytocompatibility property. You select that material and go to the next stage of research and that is called In-vitro compatibility assessment and this is also known as as I said before a preclinical study.

Now the rational of doing this preclinical study I have mentioned you before one has to start with the small animal model then go to medium and large animal models to validate the bio compatibility this is the feature obtained with the small animal model. So, here that animal ethical committee approval is a must. And each institutions, each institution where ever wherever in this world once to carry out this animal testing must have the institutional animal ethical committee which has members from different disciplines as well as socially concess a person as well as person who is remotely linked to this study.

So, that particular committee is given the approval to any animal study and that approve with that approval one can contact this ani preclinical study in the animal model. And this approval becomes much more complicated or even much more strict if one goes from small to medium to large animal model. For example, if one has to carry out the experiments in a monkey model the approval is most difficult. But if one has to carry out the experiment in a rat or mouse model the approval can be little easier provided the study design is appropriately framed or study design is appropriately rationalized in the within the frame work of the guidelines of the standard bodies like ISO guide lines British standards and so on or if the protocol one has to use.

So, once the preclinical studies done, animal survive bone healing has been established or there is no undesired tissue response. Because of the implantation of a synthetic material, that you are investigating. The next level is that one can do this proto type development of the biomedical devices and this proto type development has to be and after this proto type development you wanted to for the testing and what I called this testing at device level. Now just to give you two examples in the context of orthopedic or bone

tissue regeneration application or bone tissue regeneration applications one is THR and another one is TKR.

THR stands for Total Hip joint Replacement and TKR stands for Total Knee Replacement device. Now in both the cases THR and TKR the final device has a much more complex and complicated shape and size than one you can start with a simple shaped test sample at the laboratory scale. That is the step number one in your inter biomaterial research. So, once this femoral ball head or acetabular socked assembly fabricated they are being assembled and so on one has to carry out device level testing using Joint Simulators.

And there one can simulate that various parts and works what is the stress level and what is the stress cycle than that and that a device is expected to experience. To that stress cycle one has to simulate within the Joint Simulator and there are two Joint Simulators which are relevant. One is that Hip Simulator that is relevant for total hip replacement and then another one is knee simulator that is relevant for total knee replacement devices.

So, once the large number of fatty cycles are contacted using the seep simulator and the materials survived, materials do not fracture they do not wear to a significant extent and their wear debris particles are taken and their toxicity has they are they are non non-toxicity or cell or tissue level established then only all these preclinical test stages is further is way of the is for that transferred to the next level of study that is called clinical trials.

As I said clinical trials essentially is extremely difficult because of the requirement of the strict regulatory approval. Now, clinical trials again one has to write a proposal and that proposal has to be approved not only by the institutional ethical committee but also national level ethical committee in India we have the DCGI that is the Drug Controller General of India. So, DCGI kind of approves all the clinical trials that are carried out on Indian human patients. And similarly, every country own national level approval committee for clinical trials.

Because clinical trial often come to the newspaper because if the clinical trials are contacted in not an appropriate manner or not a clinically acceptable manner it may cause kind of very undesired results and it can lead to the death of the uh uh human volunteers which is not at all desired. Often it comes to the newspaper this uh that wrong clinical trials been contacted in different parts of the world. Now once the clinical trials are done, then the devices read with the technology can be transferred to the industry and therefore that commercialization is possible.

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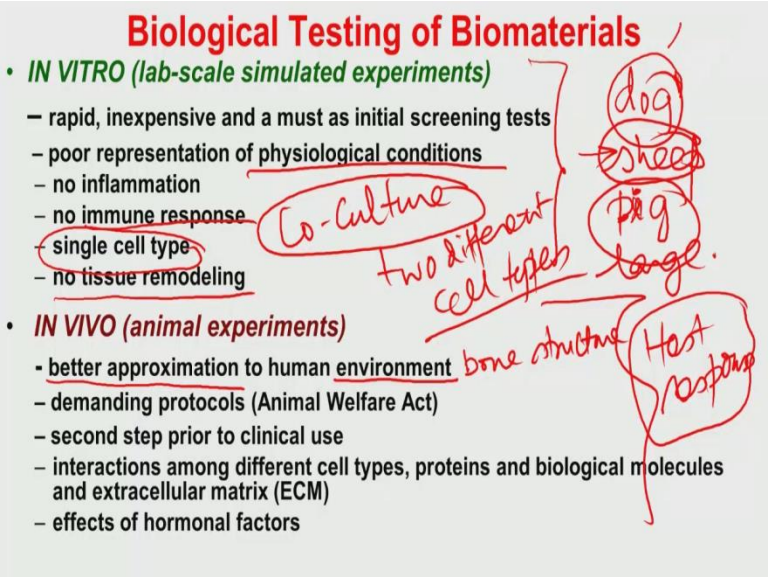

Ok. So, before the host responds before one can assess the host response one has to do in vitro testing and in vivo experiments one can assess the host response right? So, this like is kind of important in a sense that the slide essentially tells you that what is the difference between in vitro and in vivo experiments. To repeat in vitro means lab scale simulated experiments. Uh however it is a poor representation of a physiological condition that is no inflammation that you can start to hear there is no human response largely in vitro experiments have conducted with single cell type.

Either you culture as tubular cells or you culture fibroblast cells. But there is a new concept or recent trend people tyy to use there is called co-culture. Co-culture means two different cell types are cultured together ok? Uh so, this is in fact this is still not being regularly used in different biomaterials laboratory. The other things that you cannot do or you cannot assess in the in vitro experiment is tissue remodeling simply because you are handling with the biological cells uh only. In contrast, in vivo experiments that is the animal experiments a better approximation to human environment.

Let me spend some time uh on this particular first point that to what is better approximation of human environment. Essentially, human physiological environment or human bone structure or bone composition or bone characteristics, now the way that human assess structure can be defined depending on the anatomical location. Natural bone has different has slightly different variation in terms of the position as well as the properties and certain characteristics. Now the several animal models are used but out of that three animal models I must mention which closely mimic that human bone structure.

One is called dog, second one is called sheep and third one is called pig. Now dog, sheep model and pig model these are all as you see these are all large animal models. Now dog model has a bone composition and bone structure its and and animal weight and all that is kind of coming close to that of that the human uh human model or it can be used as a representative human model. Here again bone micro structure and bone remodeling of the dog structure is quite different from that of the human system.

Pig again is a good model because pig uh that pig as a structure, pig bone structure and remodeling is quite good, but pigs the problem is their bone size or that femoral size or TBL size is rather small. So, therefore experimentation or accessibility to that bone structure while conducting pig model in an operation theatre is quite difficult. Also difficult is how you can uh manage the pig models during experiments. So, these are the real bottle necks in using pig model.

The third one is a sheep model. Sheep model is very good because sheep model has a bone structure is and the micro structure as well as bone remodeling is very close to that of that human uh human system and essentially, many people they use that sheep model.

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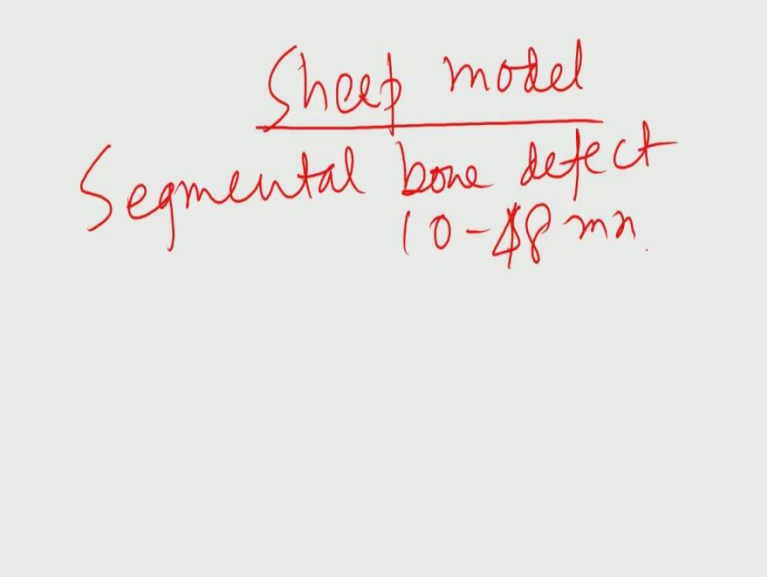

And sheep model one can use very large segmental defects and if one goes to literature then you can find that typically in the sheep model people have used, people have used large segmental bone defect. And these defects can have size somewhere between 10 to 48 milli meter.

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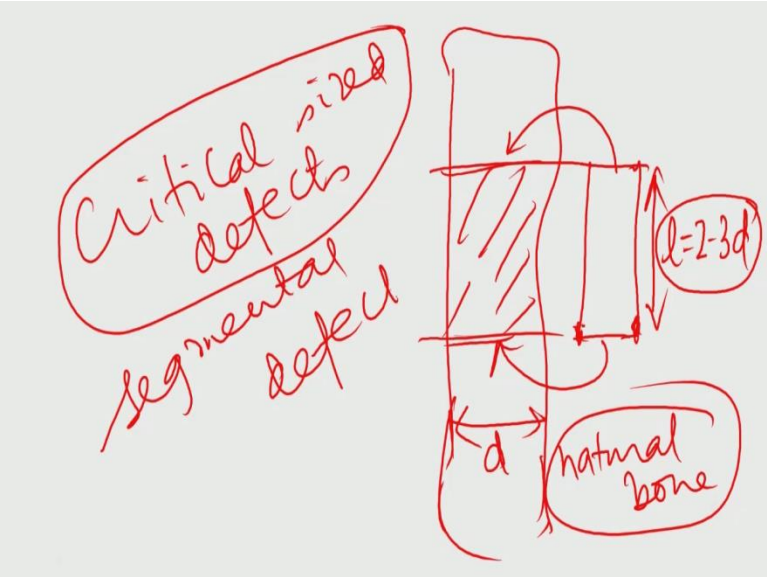

So, this is the large size. So, suppose you have, so we have a large irregular bone structure you have and a large bone structure suppose you cut this of the bone then you have a cylindrical implant, this implant you can insert it at this bone defect. And this there is something called critical size defect so, that the definition of the critical size defect is that if you cut a large piece of the bone structure and try to replace between the implant size.

The size of this implants, the length of the implant the `L' should be somewhere between 2-3 times the diameter `D' of the host bone of the diaphysis of the host bone. If you maintain this kind of dimension then it is called critical size defect. Now, one of the challenges in the orthopedic research is to is to establish that further this critical size defects can be healed using synthetic biomaterials or using artificial biomaterial. So, I repeat so, this is your natural bone structure so, if it is if it is a large long bone structure like femur it will be femur structure now you are parting this small segment of the material, small segment of the natural bone and in this gap you are putting your implant material which I am now shading.

Now this implant material, the length of this implant material can be 2-3 times that the diameter of the host bone structure where it is been cut, then you can call it as a Critical Size Defect or these kind of defects are also known as Segmental Defect.

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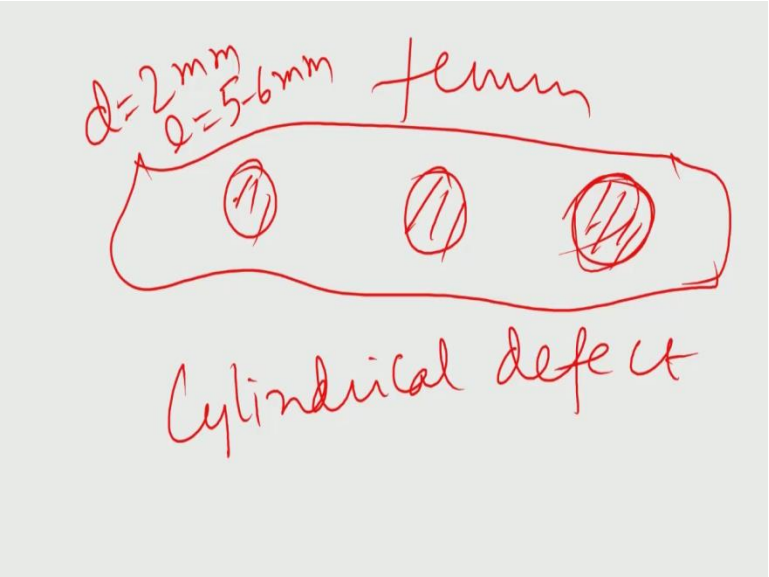

And the segmental defect model in general is kind of a little different from the Cylindrical Defect model. Now, Cylindrical Defect model what you have, you have a femur of let us say rabbit. In this femur you are drilling some holes and this typically the diameter of this hole is 2 mili meter, the length of this implant which you can put it inside this put it inside here it can be somewhere 5 -6 mili meter.

So that aspect to show is around 3 length of the implant which can go and get inserted into this defects Cylindrical Defects is around 5-6 mili meter with their diameter of the implant is 2 mili meter and then you see that around this Cylindrical Defect model how this bone regeneration takes place and how and how long does it take to heal this kind of bone defects. So, that is the whole (())(17:37) of this host response in this particular defect model.

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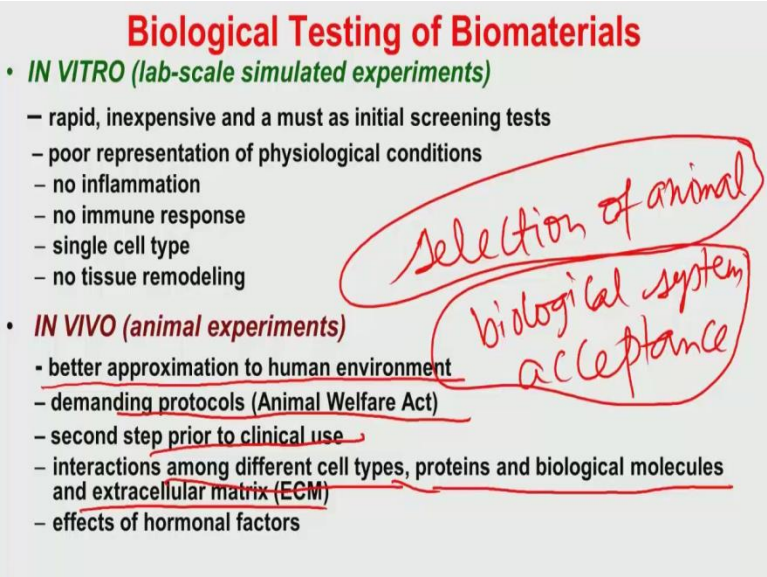

Ok. So, I hope I have kind of explained to you is little bit more details on how you can choose animal model. So, selection of the animal model is very important. And here one has to find out that what is the typical bone structure of that material of that animal and how this bone structure composition of the microstructure is close to that that of that human natural bone. Second one is the demanding protocol so, whatever protocol that you have to use that should be acceptable with the society also. Uh this is .second step prior to the clinical use very important and the interactions among different cell types, proteins and biological molecules and extra cellular matrix. These can be investigated together in any animal experiment.

So, this is by far I would say largest and scientific advantage in terms of assessing the what I mentioned earlier that biological acceptance or biological system acceptance to a synthetic material. So, it is sufficiently being assessed through in vivo experiments one can finally do the medium or larger size animal experiment to validate what results you have got at the small animal model.

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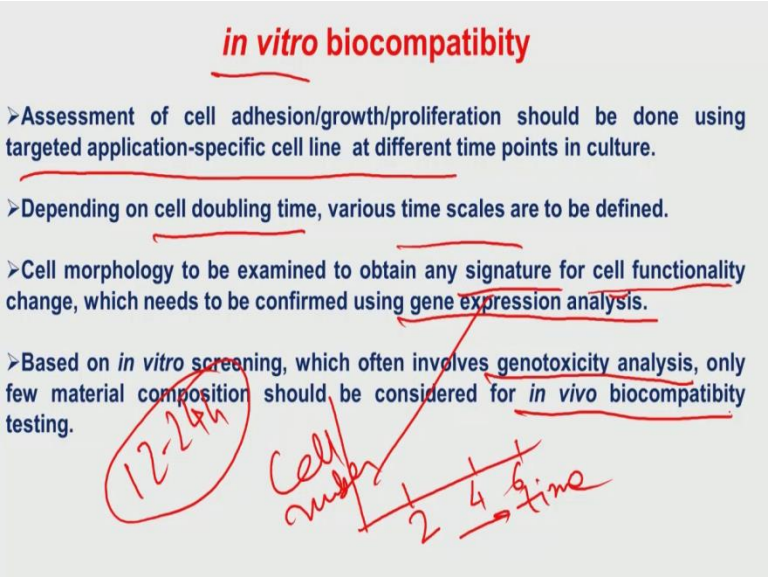

Finally the hormonal effects also the harmonal factors also can be assessed in this in vivo experiments. Let know also summarize some of the earlier discussion in this slide particularly in the context of the In-vitro cytocompatibility. So, this assessment of the cell addition growth proliferation should be done using targeted applications specific cell line at different points in time points in culture.

For example, you want to do the timing culture lesser 2 weeks or 4 weeks, 6 weeks that is the typical timing culture and you can see that how the cell numbers are increasing. So, if it is in seasonal linear manner then that mean cells are able to grow, multiply or divide while being attached to the biomaterial substance. And how to find select that what is the different time lines that you are need to be using that depends on the cell doubling time. So, typical human cell lines has a doubling time of 20-24 hours when it longer then depending upon the cell doubling time the different time points and culture needs to be defined.

Cell morphology to be examined using different microscopic techniques like fibrosis microscopy, confocal microscopy. This microscope let me tell you at this point are different than widely used than the microscope which we use in the material science discipline. Because in material science discipline, we use mostly scanning electron microscope or transmission electro-microscope. But for the cell biology related one has to use the process microscope or confocal microscope and also one has to do gene expression analysis.

Based on the In-vitro screening which often involves genotoxicity analysis. Genotoxicity means that is DNA level toxicity only few material compositions would be considered for the in vivo bio compatibility.

See, all these in vitro, in vivo things are inter linked so, we have to understand these things in a much more greater details that's why I keep coming back to some of the discussion points again and again through this module discussion.

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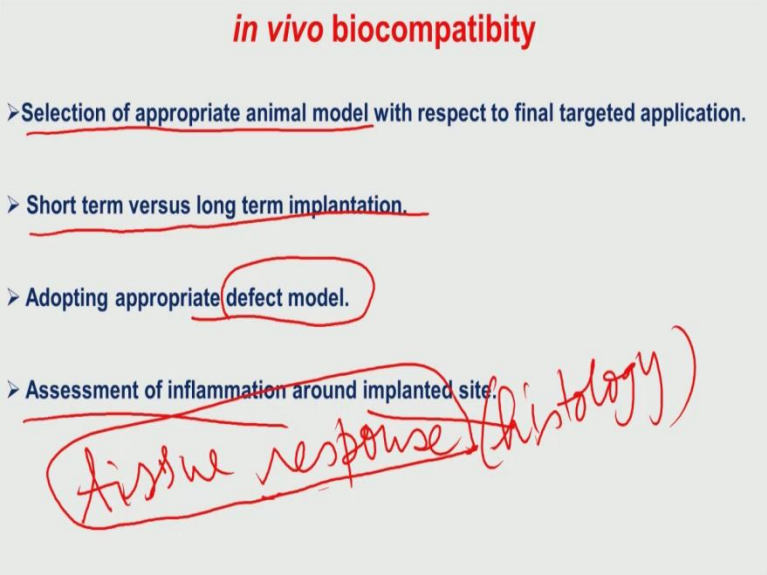

Now coming to the In-vivo bio compatibility as I said the selection of an acrobat animal model with respect to the final targeted application is the first key point and one has to address. Second point, one has to outer one what is the sort of long term implantation and whether what kind of defect model you are using whether it is a Critical Size Defect , whether it is a segmental defect, whether it is a femoral defect all those things. And assessment on inflammation around implanted time and also the tissue response.

Now, the tissue response here, one has to do some different analysis what is called Histology. Histology means that is the scientific analysis of the structure and morphology of the different tissues in a in a thin Histology slide. And these tissue responses is important because there you can see that got an implanted material whether it can cause toxicity to the cells in a in the neighbouring tissue and so on.

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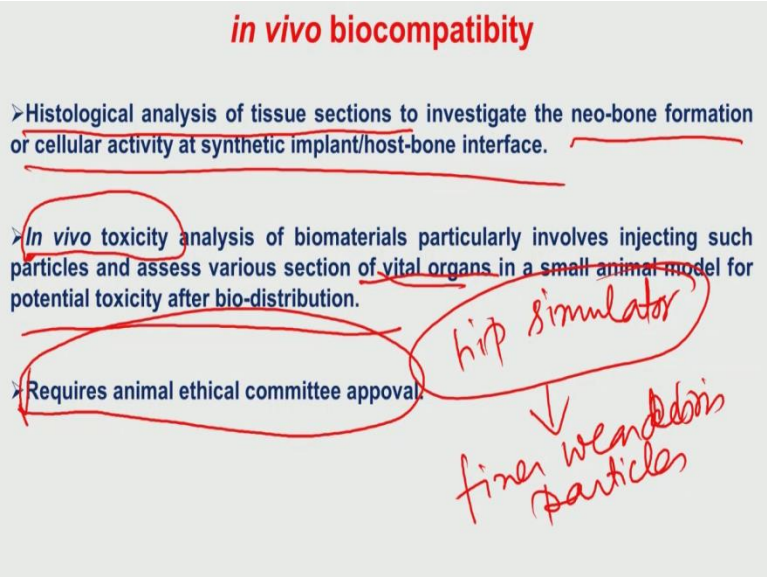

So these points have been mentioned here that is Histological analysis of the tissue sections to investigate the neo bond formation or cellular activity at any synthetic implanted bone host bone interface needs to be conducted. Now, these points I have mentioned very briefly in an earlier slide that is in vivo toxicity analysis. In vivo toxicity analysis means that Hip stimulated testing I have mentioned just few minutes ago. I will discuss it little more details later. Now, Hip simulated testing always generates some finer wide debris particles. Finer wide debris particles essentially generated because of the continuous friction and wear at the interface.

Now, these wide debris particles when they are transported to different tissue structure at different organs. They can cause toxicity of those vital organs and this potential toxicity after by distribution needs to be evaluated. And then one of the key things in the preclinical study is that it requires animal ethical committee approval. Without approval one should not conduct this kind of animal testing.

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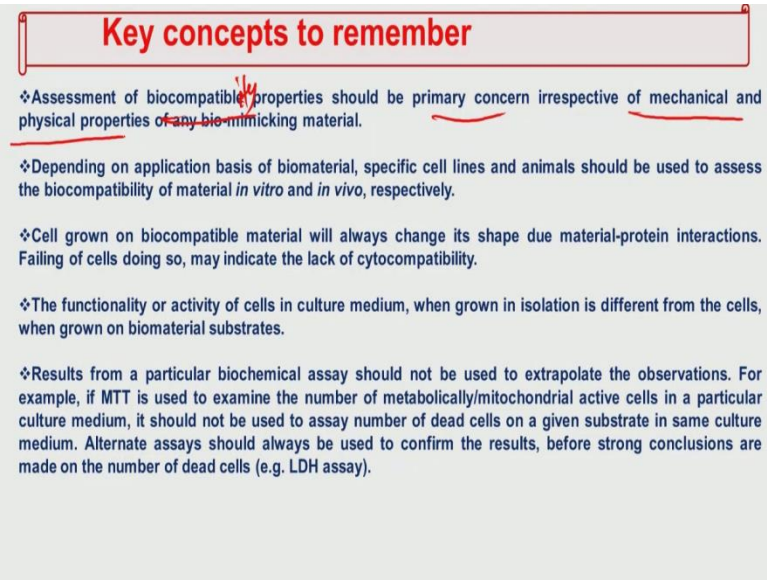

Ok. Now, two some of the other key points which has been discussed in this module and the previous module are as follows. The assessment of the bio compatibility properties should be a primary concern irrespective of the mechanical and physical properties of the material.

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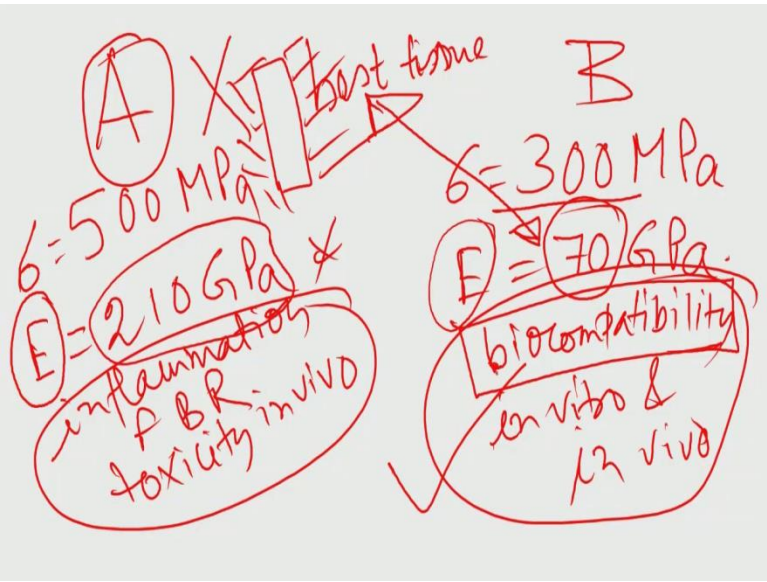

This point has been mentioned before also so, what I have mentioned suppose you have two different types of materials A and B. Suppose A has very good let us say strain properties of 500 mega pascal, B has a relatively less strain properties like 300 mega pascal. This is your strength typically we define in material sciences sigma.

Then there is the elastic stiffness we typically define in the material science E. And E the elastic modulus of let us say A has an elastic modulus of around 210 mega pascal. And here E has an elastic modulus of 70-80 giga pascal, let us say 70 giga pascal. This is sigma is a 300 mega pascal. Now A material cause some inflammation major inflammation or foreign body response and toxicity in vivo. I put a star, however B material cause acceptable bio compatibility property both in vitro and in vivo. If you are purely materiologist and material scientist then definitely you will choose the material A not B.

But that option is wrong as far as the bio compatibility or bio material is concerned. It does not matter whether the strain property little bit inferior or elastic modulus what is more important is that whether this material has a good compatibility both at the cell level and tissue level. With this very simple example and very hypothetical example I hope I have been able to convince you that how this bio compatibility property should be given more priority.

Now, one more point that needs little bit more discussion is that on the elastic modulus. Now, elastic stiffness essentially means that the ratio of the stress to strain. Now, if the elastic modulus is why it is more important your material always surrounded by host tissues or host bone structure, right? So, you have your implant material and surrounding this is your shaded region is a host bone structure. Now, if there is a large difference between the host tissue and in your synthetic material then what would happen?

If the implant material has a large elastic modulus then implant will carry most of the weight or most of the load, where as the host tissue will carry much less load under given by mechanical stress situation and that is not desirable because that will lead to the implant loosening or aseptic loosening. So, there should not be large difference in terms of the host bone elastic modulus and implant elastic modulus. That is very key. So, 210 giga pascal is certainly the bone stiffness is much less than this 210 giga pascals. So, this is the typical stiffness of steel based materials like stainless steel or any steel for that matter has an elastic modulus 210 giga pascal.

So, two things I have mentioned here, one is that you have to give more importance to the concept of bio compatibility that is point No.1 or Project no.1. Once the material is bio compatible, and if you have to select from a group of bio compatible materials like PQRS.

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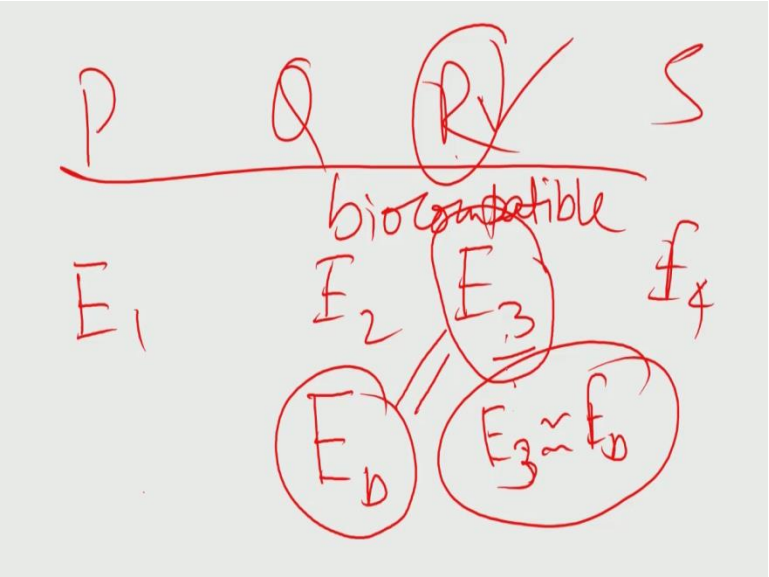

If among the PQRS if so, now if you have this four material PQRS and this all are bio compatible material, then but certainly this PQRS depending on the different material compositions. They have a different elastic stiffness value or elastic modulus value E1,E2,E3,E4. And your bone elastic modulus let us say EB. Now, you have to see that which one is very close to EB. And if for this hypothetical example if the material R elastic modulus E3 and E3 is very close to that of the EB that is a bone elastic modulus, then you have to choose the material R and the material R I should take.

Because it is expected for this kind of orthopedic application or bone regeneration application which is essentially done for restoration and repair of any bone defects or any Osteochondral Defects then you have to see that whether this material has elastic modulus or elastic stiffness which is extremely close to that of the human natural bone.

So, I hope I have put some emphasize on the on what mechanical property one should look at and how biocompatibility property should be given its due and central importance in selecting a bio material for bio medical applications. So, with this we will come back to next module for more discussion or continuing discussion on tissue engineering and scaffold and so and so.

Thank you.