Biomaterials for Bone Tissue Engineering Applications
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Module 1
Lecture 3

So welcome to the third module of bio compatibility, so I will carry forward the discussion that I had the in the last module on the bio material definition and so on. So let's refresh our mind that what is a bio material. This is an important definition and these definitions are mostly like textbook type definitions. A student or a researcher in the field can't afford to change certain key terminology or phrases used in this definitions, and that will be valid for both bio material, bio compatibility, post response, tissue engineering and so on and so forth.

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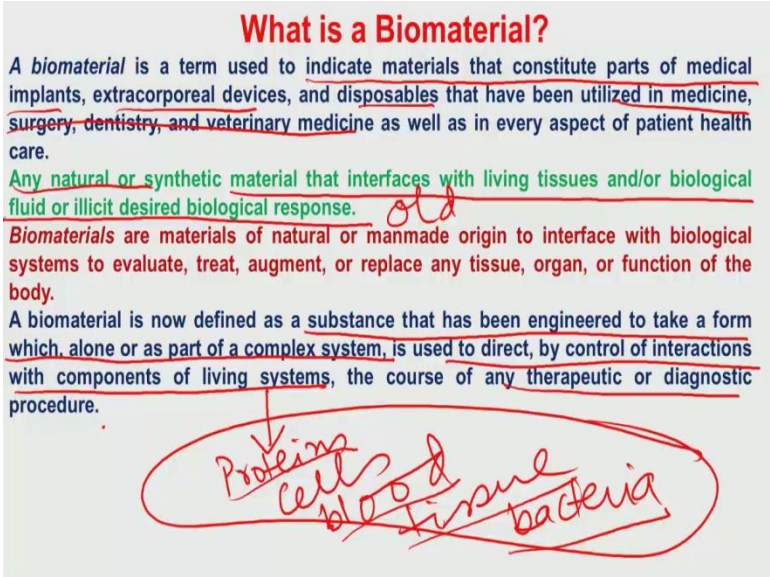

So a bio material is defined as the substance that has been engineered to take a form which alone or as part of a complex system. Let me substantiate this part of the definition. Typical drug which people use when they are not well like penicillin is a drug, or a simple saridon is a drug or some of the anti inflammatory drug, we cannot clarify the drug as a bio material, however if the dug is incorporated in a polymary bio material scaffold, with an intended purpose to be delivered at a targeted site in an animal or a human patient then we can call this as a complex system , this as a bio materials containing drug.

Similarly a bio material should have a specific shape, I've already explained to you before that natural bone has a very complex shape and this shape can't be described by regular geometric features or regular geometric shape. So therefore bio material necessarily needs to have a

specific shape and this bio material, the shaping of bio material requires one to adopt specific manufacturing protocol or manufacturing techniques.

For e.g. Aditi manufacturing is one of the techniques, or 3d printing is one of the technique which I'll also discuss to some extent in the later modules. Now one this bio material is fabricated or processed it's expected to treat certain therapeutic or diagnostic procedures or to facilitate certain therapeutic or diagnostic procedures by control of interaction with components of living systems.

I have explained to you in the last module that living system essentially contain proteins, cells, blood, tissue, bacteria and this interactions like interactions with the living system essentially means that depending on what is the targeted application, like if the targeted application is orthopedic or cardio vascular then accordingly one needs to see that how a given material will interact with the tissue specific cells, for e.g. if the targeted application is orthopedic then one has to use bone forming cells like osteoblasts or osteoblast osteoclast interaction. Osteoclast means bone reduction cells, so whether a bio material can facilitate the growth of the osteoblast cells, that should be the primary question that a researcher should address.

Similarly if a bio material is to be used for cardio vascular application so there cardiac specific cells for e.g. cardiomyocites, one has to assess that whether that particular scaffold or bio material can facilitate or can support the growth of the cardiomyocites cells. So with these two examples I think it should be clear to you that this entire this bio material definition, the inherent concept is more like application specific. Now with the targeted application in mind one has to find out that how a material can establish a beneficial interaction with the components of living tissues, living systems.

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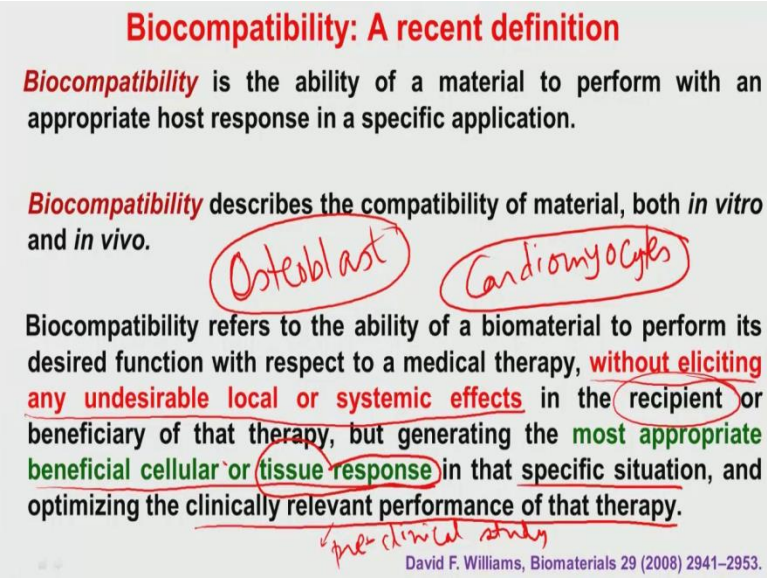

Now having defines bio material let me define now bio compatibility. As I said before what bio compatibility is the core concept which drives the development of a bio material and bio compatibility should be of prime concern or should be critically assessed before selecting any material for biological applications. So first two definitions are kind of very old definitions. First one is the, it's defined as the ability of a material to perform with an appropriate host response in a specific application. So this statements or this definition has some correlation with the, my last discussion on the, while I was defining the bio material so essentially the bio compatibility needs to be defined in a application specific manner.

The second definition essentially tells that it describes the compatibility of a material both in vitro and in vivo. I mentioned in the last module in vitro and let me remind it again so that you can, so that you're feeling more and more comfortable with this biological term. In vitro means testing conducted with petri dish or glass, glass wares or test tube. That mean that in most of the, all the in vitro testing, all the in vitro assessment is to be done in physiologically simulated environment, in a standard laboratory culture conditions.

In contrast in vivo essentially means the experiments conducted in an animal model. So this in vivo testing is also known as pre clinical study, pre clinical means, prior to the clinical trail in human patients, one has to see the compatibility of a material, of a synthetic material with the osseous structure in an animal model. Now these two definitions do not reflect on certain specific aspects on bio compatibility, therefore David Williams who happens to be the editor in Chief of the most prestigious journal in the field that is bio material. He was the edition chief for more than 5-8 years and he just stepped down recently.

He defined that bio, he defined the bio compatibility in a much more matured manner and the definition is it refers to the ability of a material, bio material to perform it's desired function with respect to a medical therapy, with respect to a medical therapy without eliciting any undesirable local or systemic effects, in the recipient or the beneficiary of the therapy. Recipient means this is the human patients. But generating the most appropriate beneficiary of tissue response. Let me underline this particular term here. What I want to mean here, beneficial cellular of tissue response.

Now coming back to my two examples that I've given back. Now if a material is to be used for nine replacement application, if a material is to be used for bone regeneration applications, then the cells that you've choose is the osteoblast cells like the bone forming cells. Osteo means bone, osteoblast means bone forming cells. So therefore a material which is intended to be used for bone regeneration applications must support the growth of this specific cell line.

But if a material is intended for cardio vascular applications then the cells that one has to select is cardio myocytes. And then one is to do in vitro asses to see that whether a given material can support the growth of the cardio myocytes. And let me also tell you that cardio myocytes is a

typical example of one of those cell lines which is very difficult to culture, which do not grow very easily in a standard culture conditions.

So let me come back to the bio compatibility definitions, so essentially a bio material is expected to elicit the most desired or beneficial cellular or tissue response in a specific situation and optimizing the clinically relevant performance of that therapy. So, now here the clinically relevant performance of the therapy means that it should have, it should have, it should demonstrate appropriate bio compatibility response in pre clinical study as well.

So therefore bio compatibility doesn't mean only in vitro studies, bio compatibility means in vitro and in vivo together. So in vitro only can indicate that whether a material can have good cellular response. But in vivo essentially would also tell you that whether a material will also have similarly good tissue response. So cellular response on one hand is important, tissue response in another hand is also equally important.

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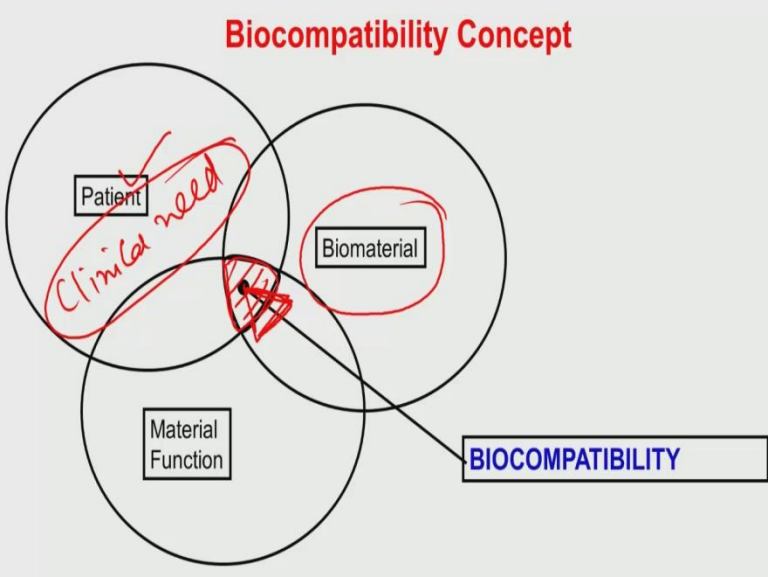

So now, since bio compatibility is very important in bio materials development, let me speak more time in defining this bio compatibility or explaining you little bit more details. So this is a three interesting circles as you can see here and three circles that common area is this area which I'm shading it here. So one circle is essentially saying that what is the patient specific need or what is the clinical need. And typically this clinical need is defined by clinicians or medical practitioner.

Now on other side you have a bio material, is in bio materials it's not that all the properties of that bio material would have direct relevance to clinical need. But may have some peripheral relevance. Certain chemical elements which is essentially defined as a bio compatibility property

So this bio compatibility would be much more relevant to the clinical need, so therefore bio compatibility can be better described as a common area of intersection among three circles, with one circle being patient, another one is bio materials any third is bio material function.

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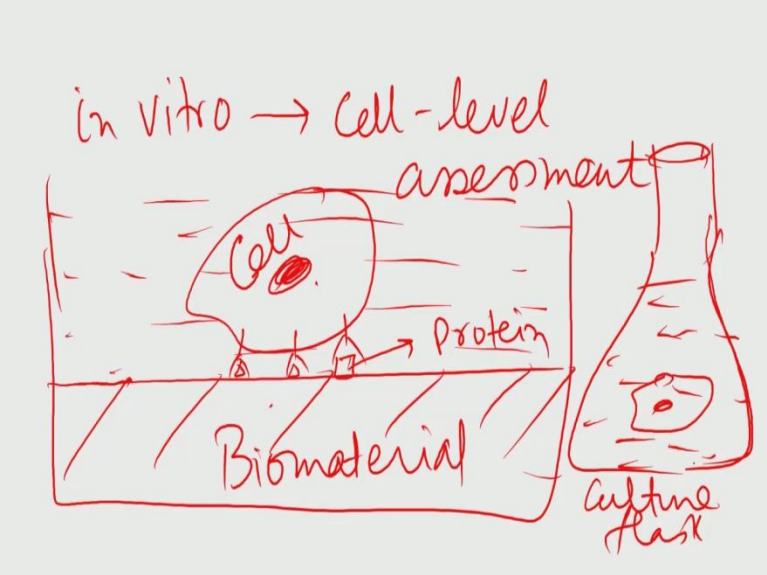

So bio compatibility in, so let me explain it, little bit more details. So bio compatibility needs to be first assessed at the in vitro level that means the cellular level or cell level study or cell level assessment. So one would like to know, that when you want to grow a cell, let's say cell as a connecting cell, cell as a nucleus and this is your bio material substrate, this is your bio material substrate. In another case you've a culture flask, and in culture flask you have a certain culture media, and in culture media similar cells you're trying to grow.

I'm just, for simplicity, I'm just showing you, this is a culture flask. For the simplicity I'm showing you one single cell but in practice you'll have thousands of such cell which are growing in a culture flask or a bio material substance. Now as I'll discussed with you later that, cell typical, cell surface has certain cell surface receptors and in a bio material substrate when you're essentially putting in in culture medium. So this bio material substrate will also have protein absorbed. So these are the protein which are absorbed on a bio material substrate.

Now the difference between growing cells on a bio material substrate and growing the cells in the culture flask is that essentially here cells is growing in material substrate which has certain elastic property, elastic stiffness property, which also has a surface roughness and also has a surface vertibility property like hydrophilic property or hydrophobic property, so essentially the cell material interaction or the way cellute function on a bio materials substrate would be quite different from the way cellute essentially grows in atypical culture flask. So having said this that typical growth pattern or functionality of a bio material substrate.

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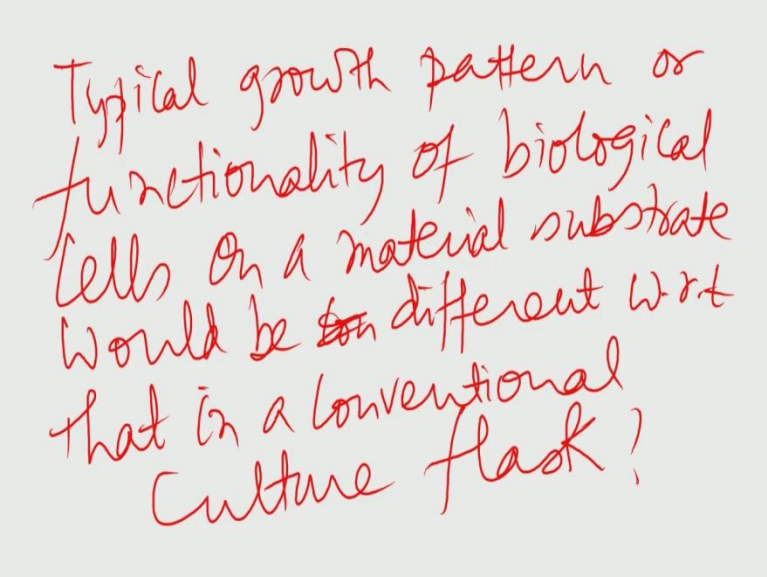

Let me write done typical growth pattern or functionality of biological cells on a material substrate would be different with respect to that in a conventional culture flask.

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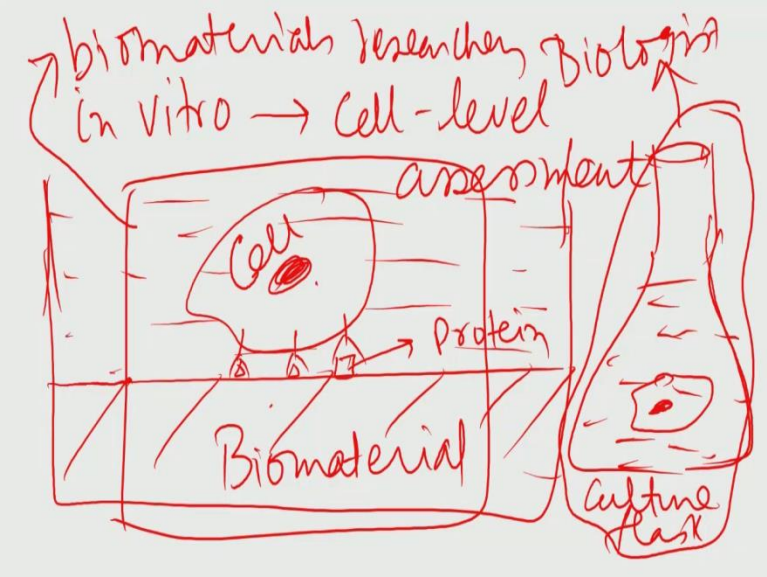

So this growing cells on a culture flask is in the domain of a conventional biologist. This particular case that growing cells on a bio material substrate is essentially, these things I'm emphasizing it, so this is that more in the domain of a bio materials researchers. So we'd like to understand that how a biological cell would adhere and function on a bio materials substrate and certainly this functionality would be quite different and we'd get a better understanding as we go through the course, thought subsequent model as part of this course.

Now this is the cell level compatibility one has to study at in vitro level. Now once a material is, a material exhibits certain level of functionality and which is appropriate or which is essential reflects in the good in vitro compatibility, in vitro cell compatibility then the material can be chosen for further pre clinical study.

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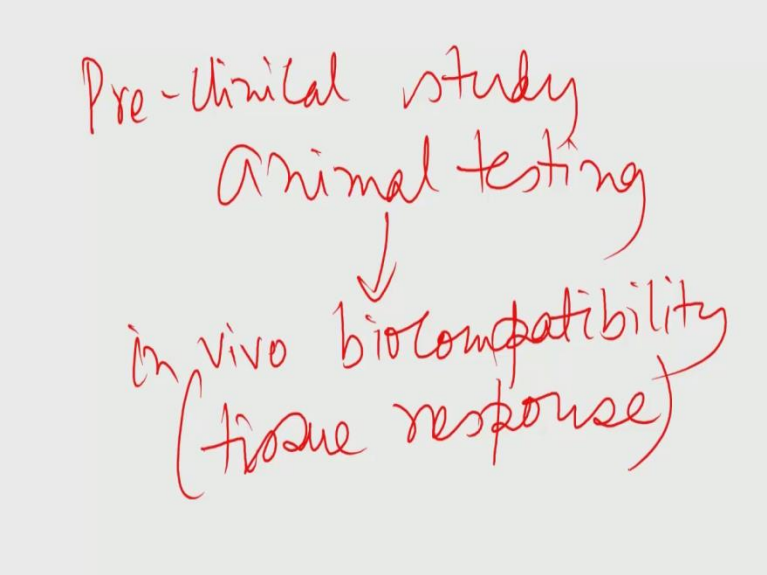

And this pre clinical study essentially means that is the animal testing. And this animal testing is to be done to confirm its in vivo bio compatibility, or tissue response. So you remember in the formal definition of a bio compatibility I've mentioned that a bio material is expected to elicit beneficial cellular and tissue response, so cellular response I've explained to you in the last two slides. And here I'm mentioning that pre clinical study and animal testing is essential to assess the in vivo bio compatibility or in other words the tissue response.

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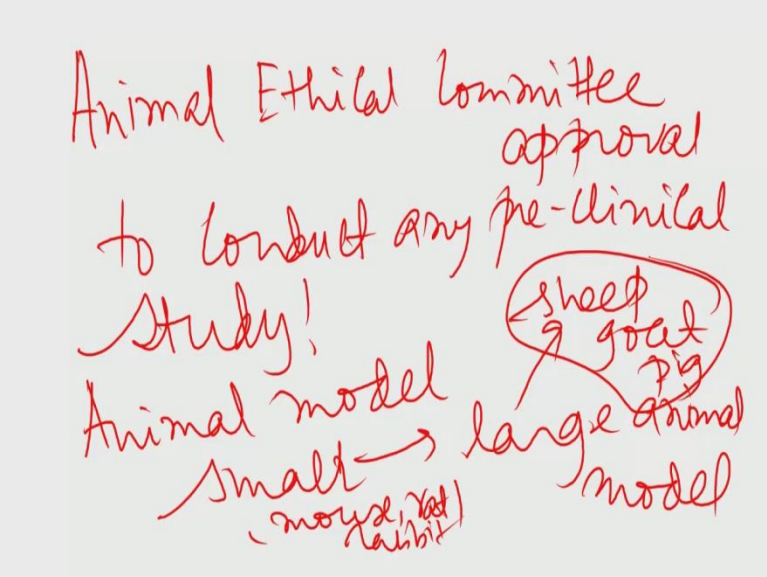

Now prior to the pre clinical study on what's to have some understanding that Animal Ethical Committee approval is required to conduct any pre clinical study. So here on what's to write the study proposal that how to conduct this pre clinical study and what is the type of animal model like whether it's a small animal model or large animal model, so to start with, one has to do only the small animal and then one has to go slowly to the large animal model. Now small animal means it's mostly mouse and rat and large animal means it is mouse, rat and rabbit, large animal means it's essentially sheep, goat and pig model. So these are like different animal models which are largely used.

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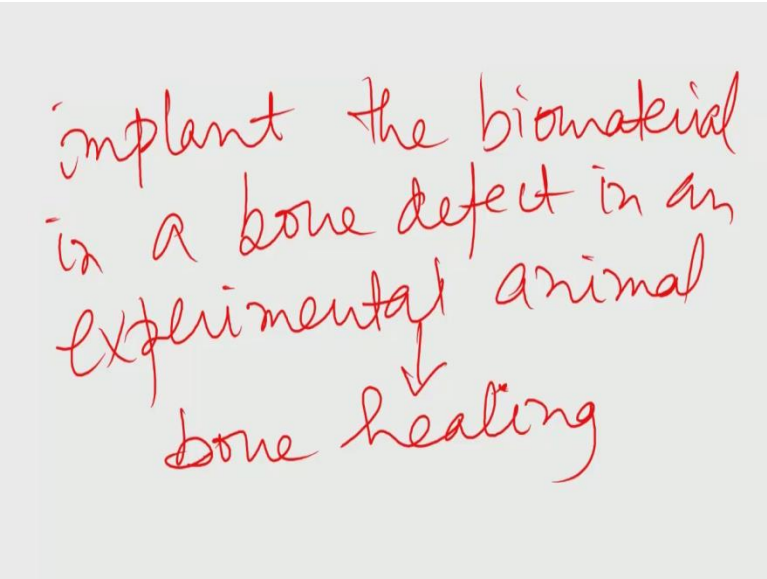

So what you do, one has to implant then bio material in a bone defect, in a experimental animal and then see that how this material and the one has to see that how this material essentially facilitates the bone healing. We first you've fabricate the material, do all the in vitro testings, hydro compatibility and so on and then you've to see that how this material when implanted into animal model can lead to bone healing.

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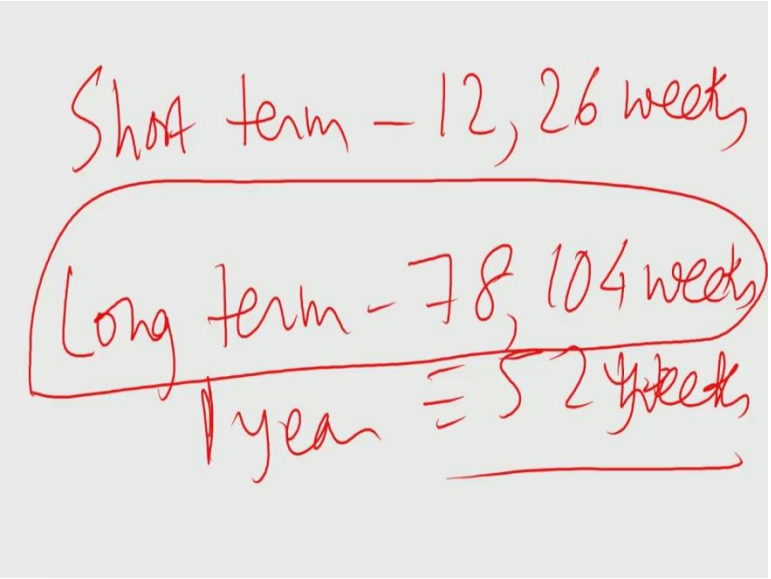

So as you can see that all these biological processes are extremely slow in nature, kinetically slow so therefore this animal experiments needs to be done over different time periods, one is called short term animal experiment implantation and one is called long term implantation, so short term implantation is typically extended up to 12-26 weeks and long term implantation is typically extended up to 78-104 weeks.

So typically in a year you have 52 weeks so essentially long term experiments can be extended up to two years maximum. And long term experiments can be only conducted with large animal model, not with small animal model, simply because the life span of small animal model like mouse, rat and all, they don't survive such a long time period therefore one can't do these short term experiments, one can't long term experiments with small animal model.

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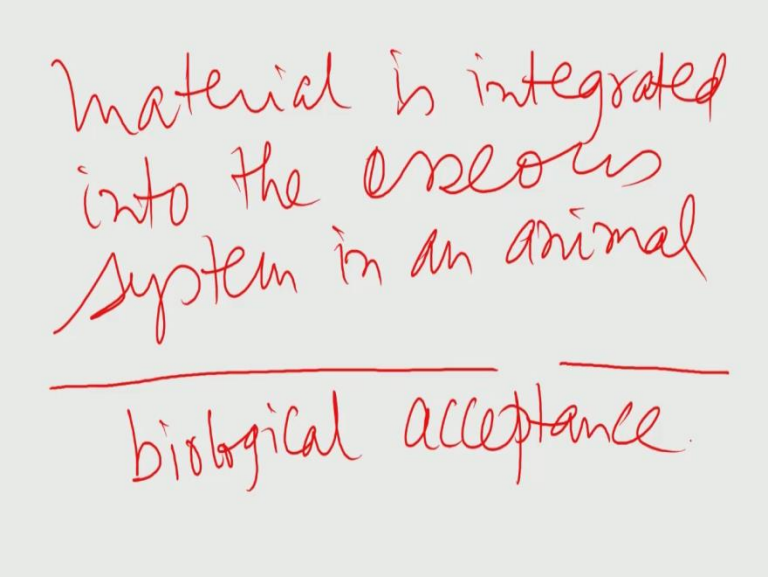

Now one you doing this animal model testing, first thing you've to see that how this material is integrated into the osseous system in an animal, so this I'll formally define more in terms of osteo integration and osseous integration later, but at this point of time it's important for you to realize that if you put this material in an animal model then how this animal osseous system is taking or accepting the material. So it's kind of, one can also explain this in terms of biological acceptance of the materials in an animal system. So this acceptance is important.

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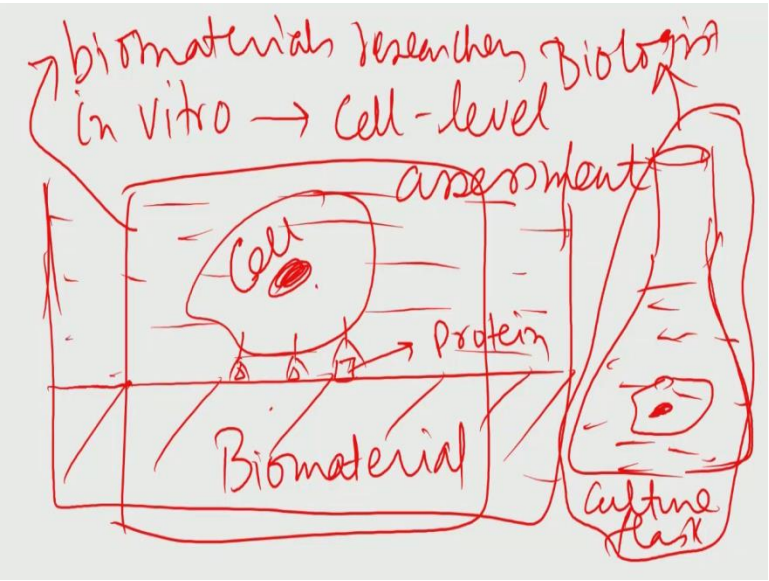

So essentially to go back to all our discussion is that, at a more cell biology level or more fundamental level, one has to first see that whether a material can elicit good cell level response

and in other words whether the application target specific cell lines when they will be seeded on a bio materials substrate, they not only survive but they also function in an appropriate manner while being attached to a bio material substrate. So if that question is being addressed through in vitro, then one can say that this material is in vitro cyto compatible.

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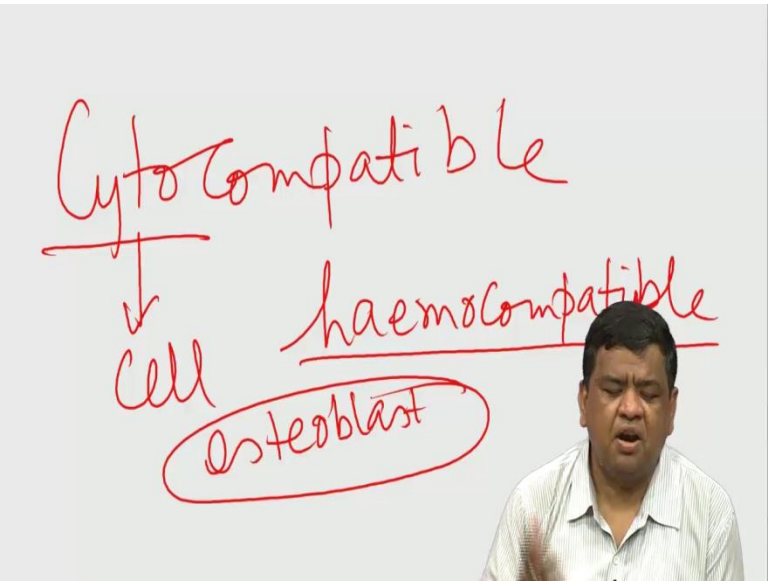

So there is two things is that if material is assessed at the cell level only then you can only call that materials is a cyto compatible material. Cyto means cell and compatible means cell level compatibility is established. If a material is tested in a flowing blood, or of a material is tested with blood and see whether this material doesn't cause any thrombogenic effect and so on then you can call that this material is hemo compatible or it has certain blood compatibility.

In other thing is very important, that one has to realize that in biology one should not make any over stretching statements in a sense like if you use only specific cell lines and you use certain functionality assessment on a bio materials substrate then you can safely state that material x is cyto compatible with respect to this particular cell line or this particular primary cells.

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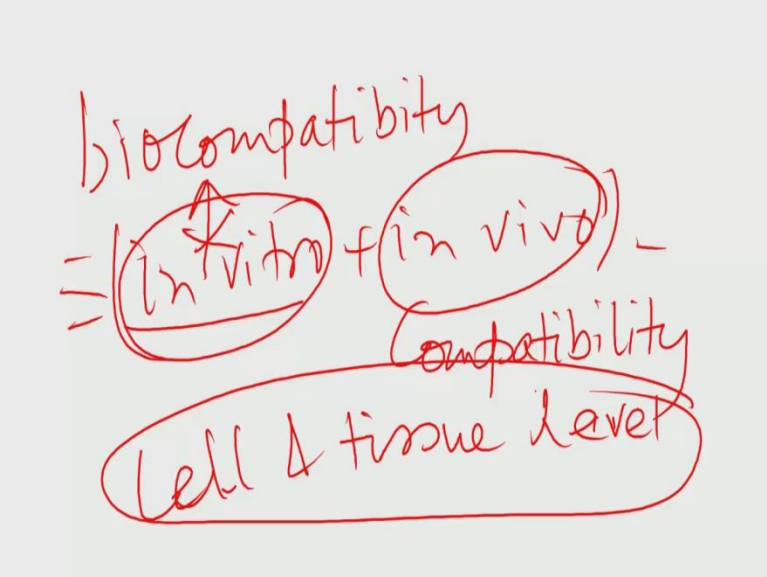

Now if a material is cyto compatible with, let's say osteoblast, one cannot claim this material is globally compatible with all the cell types. Because all the cell types means their functionality would be different unless you test their different cell types in culture medium, unless you their functionality is being changed or not, one should not comment or one should not make any over stretching statements.

Now bio compatibility essentially now can be defined that when in vitro and in vivo level compatibility. So only by checking that in vitri cell functionality one cannot claim that it's a bio compatible material, no. Once these both in vitro and in vivo testing is over and it's shows appreciable compatibility at both cell and tissue level both, cell and tissue level both, then only one can say that this material is indeed bio compatible.

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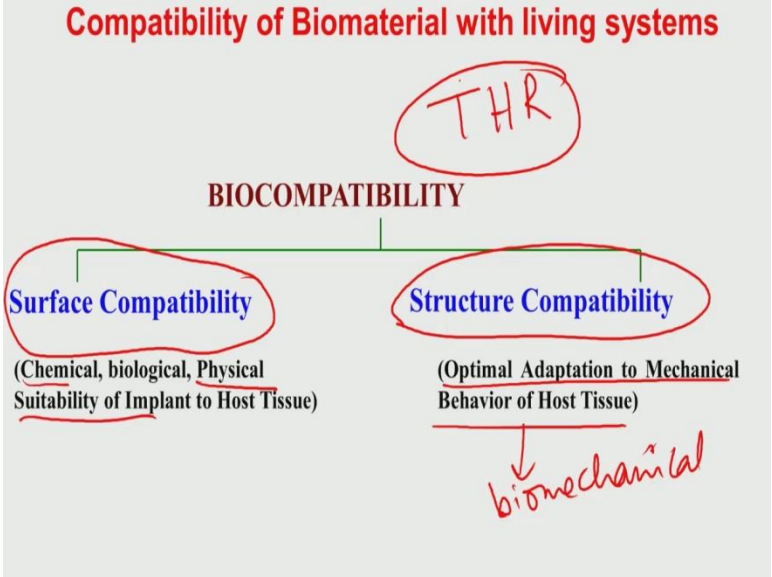

So from the proceeding discussion I think that I can now move on to the next definition. From the proceeding discussion one can also say that bio compatibility essentially means with the …. With the living systems in terms of both surface compatibility, surface means you've a certain chemical composition, you've a physical suitability to implant host tissue and another one is the structural compatibility that is optimal adaptation of the mechanical behavior of the host tissue. And there bio mechanical property is for relevance.

Now optimal adaptability means like for example as I've shown you in the last to last model. If you remember the typical example of total hiptual replacement. When a femural ball head is being attached to the assatebural socket and this whole assembly has to go and fit into the hop region of the human patient so it should have optimal adaptability to the mechanical, bio mechanical properties of the host tissue around the total hip joint replacement. This is what I meant, I referred to as a bio mechanical compatibility.

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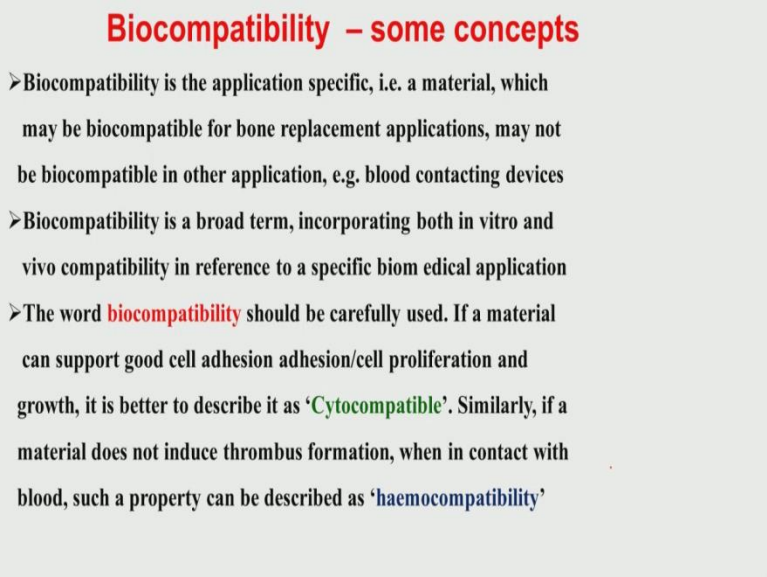

So all these discussions, I think can be summarized in this light and I've made some 2-3 points here, so bio compatibility is a application specific, that is a material which maybe bio compatible for bone replacement applications may not be bio compatible in other applications like blood conducting devices. Second one bio compatibility is a broad term incorporating both in vitro and in vivo compatibility in reference to a specific bio medical application.

And third term is that what bio compatibility should be carefully used, if a material can support good cell addition or proliferation or growth, it's better to describe this as cyto compatibility property. Similarly if a material doesn't induce thrombus formation when in contact with blood, such a property can be described as a hemo compatibility property.

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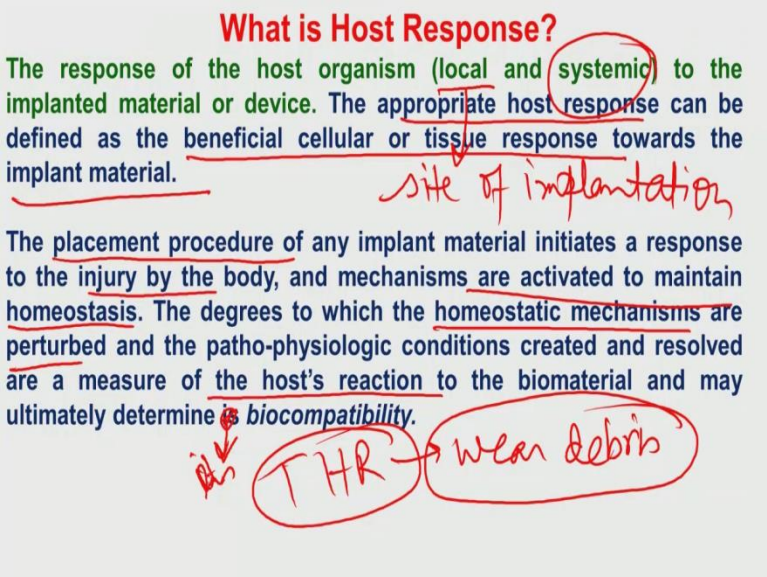

So last definition before the break is what is host response. So the response of the host organism which can be local or systemic. Local means like at the point of the implantation or at the site of implantation. And systemic means for example a total hip joint replacement during walking and running, there's a friction and where fumeral ball and assatebural socket interface. Now once it releases the wear debris it may not have any localized toxicity to the wear debris, but once it's transported to the other parts of the human system then it can course toxicity to the vital organs like spleen, heart and other places.

So one has to carefully asses that what is the level of toxicity of this final debris particles which are essentially generated from the physiological action of this total hip replacement system. To the implanted ma chello device and the appropriate host response can be defined as the beneficial cellular or tissue response towards the implant material. So one side you have the bio compatibility another side you have host response. So these both the concept together will define that what is the level of acceptance or biological performance of a synthetic materials which is not synthesized within the body but which is synthesized outside the humanbody and how it can potentially be accepted on a human system.

Just one more point that placement procedure, the way that you implant the material and that also initiates a response of the injury to the body and mechanism are activated to maintain the homeostatic. For e.g. whenever any material is implanted in animal system, it must initiate certain inflammatory response. This inflammatory response can't be avoided particularly when any artificial or non living material is implanted into a living system. Now the degree to which hemostatic mechanism are part up that means this homeostatic mechanism are part up and pathophysiology condition created in result are a measure of host's reaction. Host means that is the animals model to a bio materials and it may ultimately determine its bio compatibility.

So this is the last slide on the bio compatibility concept. I hope with this detailed discussion on this bus to compatibility now you have much more broad, specific and clear idea about the bio compatibility as an important concept. Thank you.