Biomaterials for Bone Tissue Engineering Applications
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Week- 02
Lecture- 09

So, coming back to the properties of the bone. In the last module I have discussed that how to define the fracture related properties; particularly a fracture toughness. That is that critical stress intensity factor under mode one loading. So this module we will discuss some of the properties and we will we will try to understand that how the bone properties depend on the mineral content or bone properties can vary from patient to patient.

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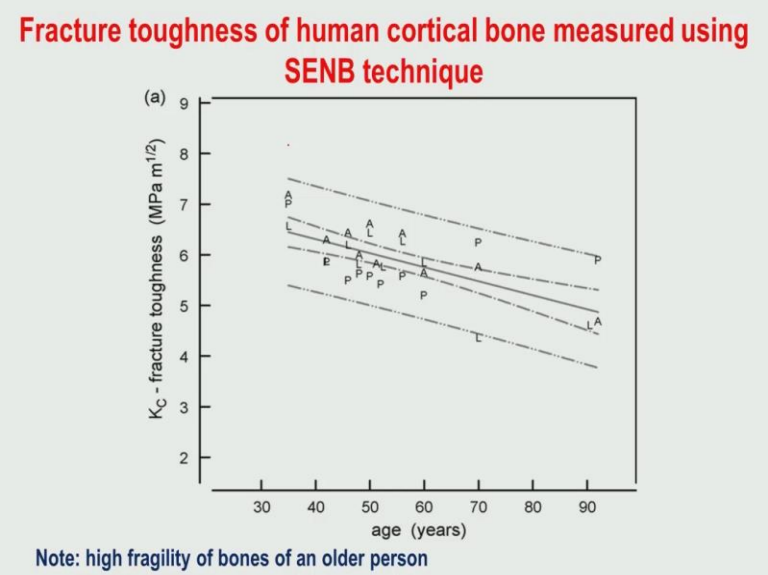

So, this particular slide essentially plots the fracture toughness values of the human cortical bone. Human cortical bone measured using, measured using Single Edge Notched Beam technique. And what you notice here is that this along x axis the age of different patients from which this cadaver bones were taken and then they were then they measured the fracture toughness or plotted.

So there is been a general trend that higher the age of the patients there is a general decrease so there is a decrease in the fracture toughness with increase in age. That means patient older patients the the bones in older patients will have more proneness towards the fracture or or the older patients will be more prone to bone related fracture than the younger patients.

The other thing that what you notice, that window over which this fracture toughness of that human cortical bone that they vary. And this window is somewhere 4 to 7 MPa square root meter. So this is the window across which this cortical bone fracture toughness is so if any material which you need to use that is the bone replacement materials.

If their fracture toughness somehow can be within this particular window 4 to 7 MPa square root meter although larger is the fracture toughness better it is. However 4 to 7 MPa square root meter that is the typical cortical bone fracture toughness and that is what one has to match with that kind of natural bone.

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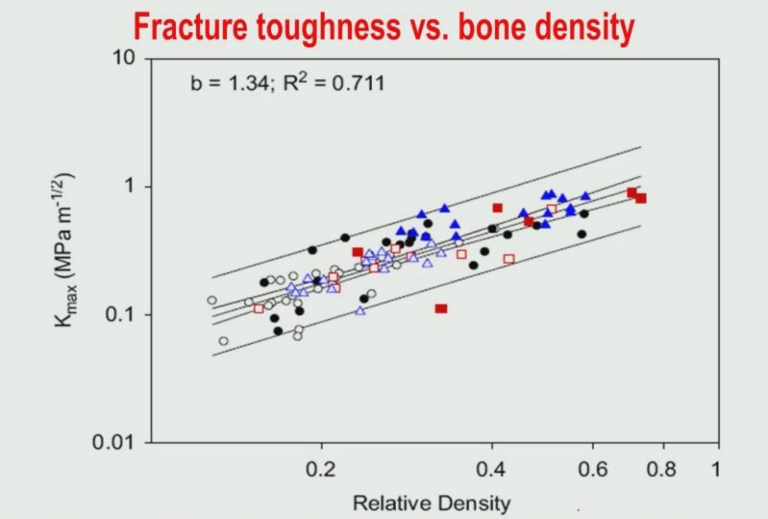

Note: fracture toughness of cancellous bone with bone density (higher density means more mineral content)

Now in terms of the bone density, as I mentioned earlier that calcium phosphate content in natural bone decides the bone density. So, larger the bone density means that this bone is much more healthy and adult bone. Lower the bone density means the bone has much lower calcium phosphate content or hydroxyapatite content and typically this lower bone density is observed with older patients.

Again there is a very linear trend with increase in the relative density of the bone. that maximum fracture toughness also increases. It is a lock scale point 01, point 1, 1, 10 so therefore it must be somewhere between 4 or 5 to more than. So, so this is the typically the cancellous bone structure. So, cancellous bone is much weaker. So cortical bone has a fracture toughness between 4 and 7 yet cancellous bone has a fracture toughness between point 1 to 1 MPa square root meter.

So essentially the cortical bone sorry cancellous bone has extremely weak in as far as the mechanical property is concerned and therefore it has extremely low fracture toughness. So it is extremely fragile extremely fragile as far as the mechanical loading is concerned. So this 2 bone; cortical bone and cancellous bone; they have a widely different fracture resistance properties in terms of the fracture toughness and also they so bone mineral density dependent fracture resistance properties and in both the cases you can see in the cortical bone also that Kc increases as the bone mineral density increases.

bone mineral density increases so because if you look at the age in the going to at the right hand side if you plot the bone mineral density that should go on the left-hand side. So larger is the bone mineral density, more is the fracture toughness. And here again you can see that more is the bone mineral density, more is the fracture toughness. This is also true for cancellous bones.

However the magnitude of increase or the absolute toughness values of cancellous bone is much less than that of the cortical bone and that is due to their inherent porosity that is contained within the cancellous bone structure.

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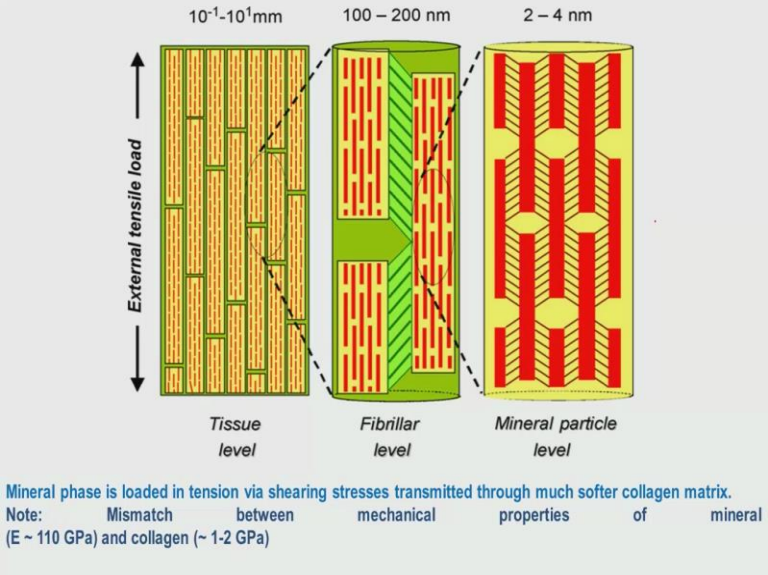

Okay now let us understand a little bit on the bone mechanical properties or what I have mentioned earlier that structure property co-relation. So therefore let me recall the structure of the bone at different length scale and if you look at this different length scale, typically the length scale increases along the arrow. So at the very (lower) lowest level of the structural description. Let us start from right hand most of the structural description.

So you have this collagen bundle, so this is the red block collagen bundle. So, and this collagen bundle are there are some sideways interaction is also shown here. So the sideways interaction essentially by weaker hydrogen bonding or van der Waals type of bonding. So therefore at any given stress conditions if necessary this collagen bundle can slide over 1 over each other. So this sliding of the collagen bundle it is possible and that will that leads to certain element of deformation in the bone structure.

So this is at the mineral particle level and this is that 2 to 4 nanometer this is the description but then individual this collagen bundles then in the fibrillar level these are like several; this numbers several of these collagen fibrils are present again between these fibrils again you have this kind of weaker bond structure and then if you go to the tissue level description then this this is that small regions of the tissue which are like magnified here and this is small level of fibrillar description that is being magnified here.

So now when you are applying that external stress sigma then what will happen; the shear stress that will be generated along the bundle circumference and that is what is being mentioned in the

next slides. So before that let me also underline some of the important concept. So you have a mineral phase that is the calcium phosphate or hydroxyapatite in this bone. So when you put them when this assembly of the collagen as well as hydroxyapatite nano platelets are place under tension.

Then what will happen. Calcium phosphate phase will be placed under tension by shearing stresses and that will be transmitted through softer elastically softer collagen matrix. And if you see that what is the typical difference in that elastic modulus values. Elastic modulus of collagen is 1 to 2 giga pascal which is like order of magnitude less than the elastic modulus of the hydroxyapatite as such.

So hydroxyapatite elastic modulus is 110 giga pascal. Collagen elastic modulus is 1 to 2 giga pascal. So because of this large mismatch of the elastic properties, it is quite mechanically it is quite feasible that hydroxyapatite will be loaded in tension and collagen will experience more like a shear stress under mechanical loading.

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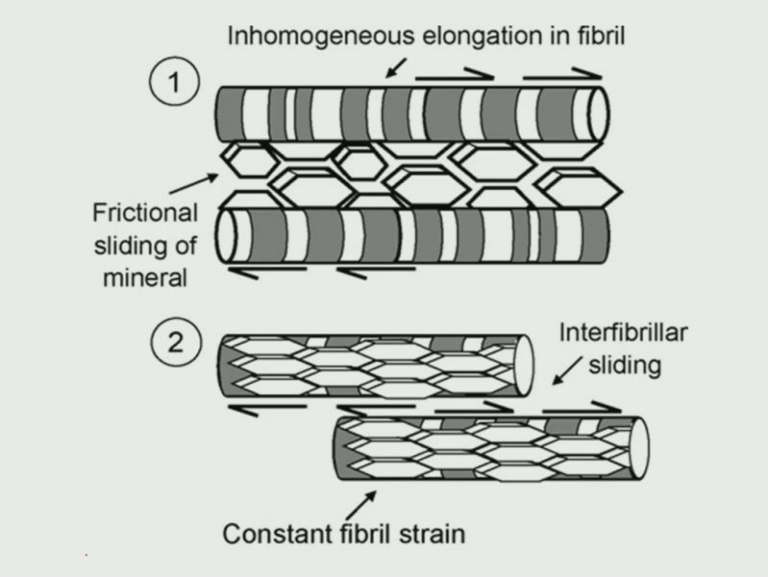

So there are 2 type of scenarios that are shown here. Scenario number one is that when hydroxyapatite or calcium phosphate phases their kind of present between the 2 collagen bundle here. So this is one collagen bundle and this is the second collagen bundle. So what is been shown here that this collagen bundle here, they will experience some kind of shear stress here and the shear stress through that that presence of the shear will make this collagen bundle to slide over each other.

slide over that hydroxyapatite platelets. These hydroxyl platelets although they will experience more like a tensile stress as I explained in the last slide. The another scenario is that you have the collagen bundle and this collagen bundle, within this collagen this collagen bundle you have the hydroxyapatite platelets here which are contained well within the collagen bundle and this collagen in the hydroxyapatite loaded collagen bundle, when they will be experiencing the global tensile stress so there will be automatically there will be sliding interfacial sliding which will allow this collagen bundle one to slide over the collagen bundle 2. And therefore this will have a very constant fibrillar strength which will be experienced by the hydroxyapatite which are contained within this collagen bundle.

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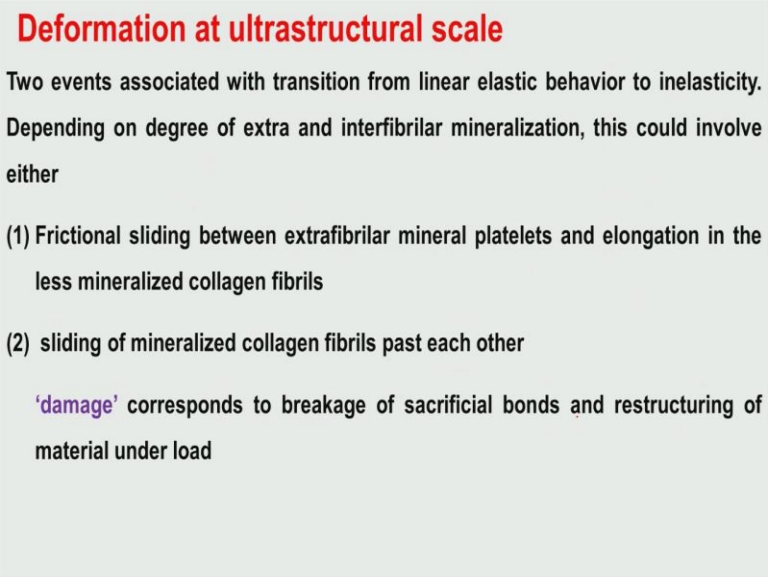

So therefore deformation at the ultrastructural level can be described by the 2 events as I explain to you in the last line that is one is the frictional sliding between extra fibrillar mineral platelets and elongation of the less mineralised collagen fibrils and second one is the mineralised collagen fibrils past each other.

So damage essentially of the bone structure corresponds to the breakage of the sacrificial bonds. So sacrificial bonds means these are the kind of sacrificial bonds I have mentioned here. So these are like sacrificial bonds. So this is the breakage of the sacrificial bonds and restructuring of the material under load.

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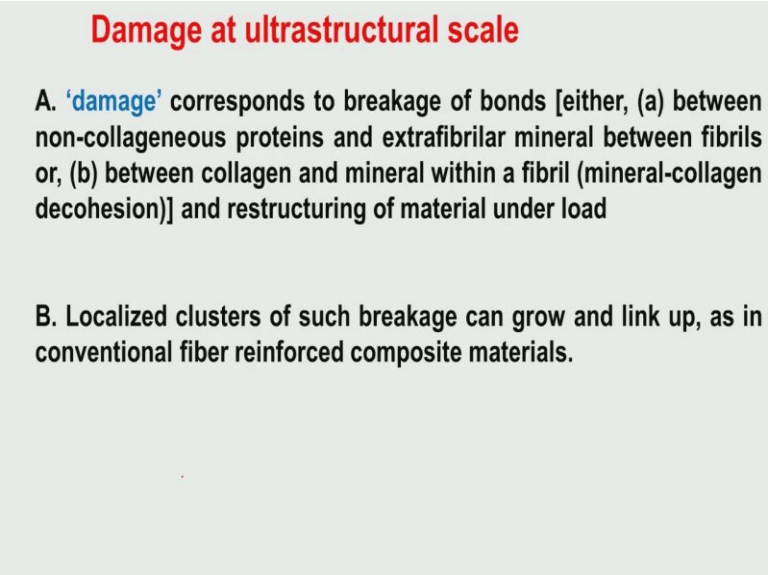

Okay, so damage again this this damage; so this breakage of the bonds has been explained here in more specific terms that is you have the non-collagenous proteins and extra fibrillar mineral between the fibrils and between collagen and mineral within a fibril and restructuring of the material under load. And also there is localised clusters of such breakage and that can lead to that can grow and link up as in conventional fibre matrix, fibre fibre reinforced composite material.

So in a way, from the basic description of the different scale of this bone structure it should be apparent to you that natural bone can behave more like a fibre reinforced composite which is the fibre component here. So this fibre is your collagen and what is the reinforcement here and reinforcement is essentially hydroxyapatite or the mineral phase.

So any fibre reinforced composites in that conventional material science you know that it shows much better mechanical properties compared to individual constituent. So if you extend that similar kind of idea here in describing the bone properties. It should be clear to you that because that collagen fibres dispersed in this bone structure and this hydroxyapatite platelets are equally present here. So hydroxyapatite platelets, presence of hydroxyapatite platelets is essentially reinforce the elastic properties of this bone structure. So I hope that I have covered both this bone structure and their properties and to some extent how this fracture properties can be measured experimentally.

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Now we will start with this some of the key concepts of the bio materials and essentially the definitions and certain discussion of some of the concept. So the aspects of the biomaterial science essentially deals or essentially integrates the concepts of the 2 widely or 2 remotely linked disciplines. One is that bio means biological sciences and materials being material sciences. So these 2 these 2 disciplines they are integrated and their ideas and their concepts are being drawn together to build up this discipline of this biomaterials.

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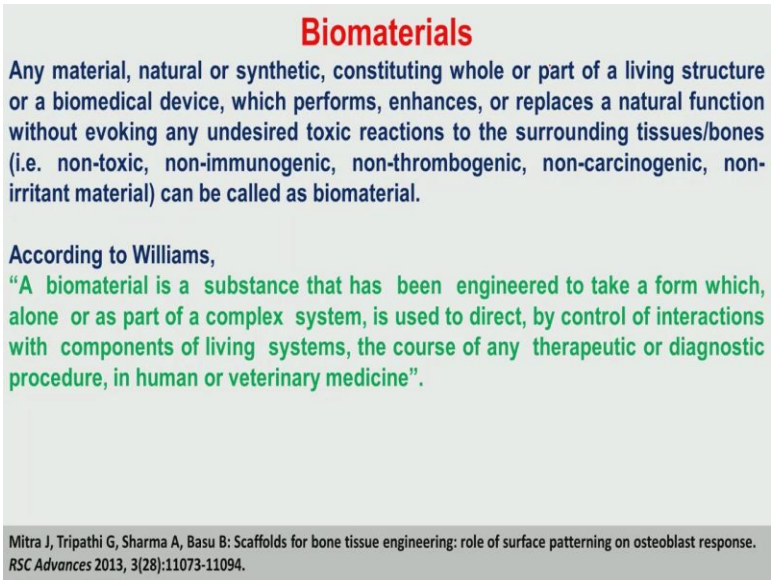

So this is like more formal definition of this biomaterials; so any material natural or synthetic constituting the whole or part of a living structure which performs, enhances or replaces the natural function of a natural function of a tissue without evoking any undesired toxic reactions can be called as biomaterial.

So, there are certain things that has been that needs to be underlined here. So one is the non toxic, second one is the non immuno non immunogenic, third one is the non thrombogenic, fourth one is the non carcinogenic and non irritant material. So therefore one has to assess each of this properties like how one can one can (qua) quantify or assess the non toxic properties or non (immune) immunogenic properties of an synthetically fabricated or synthetically produced material.

The second definition which is much more recent and this is given by David Williams. And which states that a material which is engineered to take up form which alone or a part of a complex system like a drug delivery system by is used to direct by control of interactions with components of living systems like living systems means cells, proteins, bacteria, blood, etc. the course of any therapeutic or diagnostic procedure in human or veterinary medicine.

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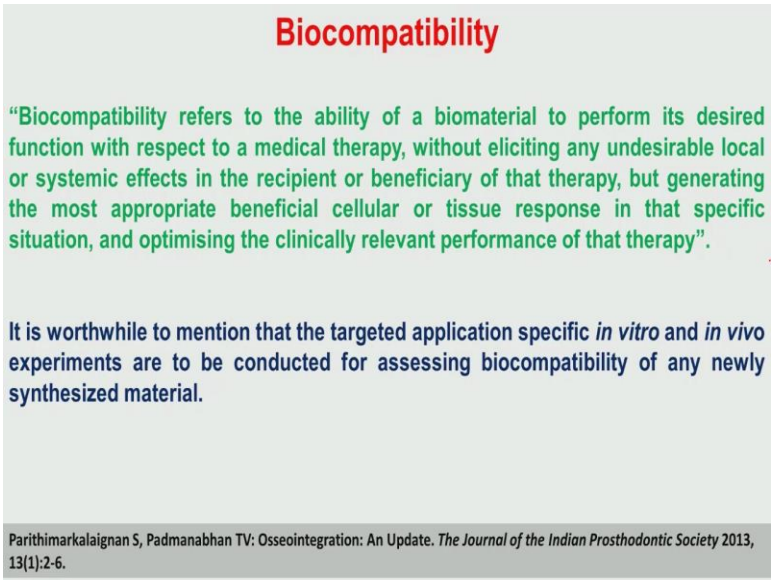

So one of the core property of (bio) biomaterials which has been briefly mentioned before also that is a biocompatibility and this is again a textbook type of definition. So, which essentially states that it is the ability of a material to perform its desired function with respect to a medical therapy without eliciting any systemic effects or local effects in the recipient or beneficiary of that therapy that is human patient, but generating the most appropriate beneficial cellular or tissue response.Most beneficial cellular or tissue response that means this is application specific biocompatibility property.

So in other words biocompatibility definition wise is more application specific. The endpoint objective is that, that material that biocompatibility property should enable clinically relevant performance of that targeted therapy. So this is a targeted application specific and it should involve both in vitro as well as in vivo experiments. In vitro means some, the experiments which are connected in the lab with glassware or petri dish; In vivo means experiments were conducted using animal models.

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So in vitro the Latin word means test tube, culture dish or, or glass and it is an artificial environment outside a living organism such as a test tube. Essentially you are simulating the living system in an test tube or in a glassware and these are the scientific experiments which are essentially performed with cells or biological molecules like proteins and so on in physiologically simulated environment but outside the normal biological context. So therefore since these are the experiments which are (contac) conducted outside the normal biological context, it cannot; although it wanted to simulate that exact environment which is present in a in any animal or human patient.

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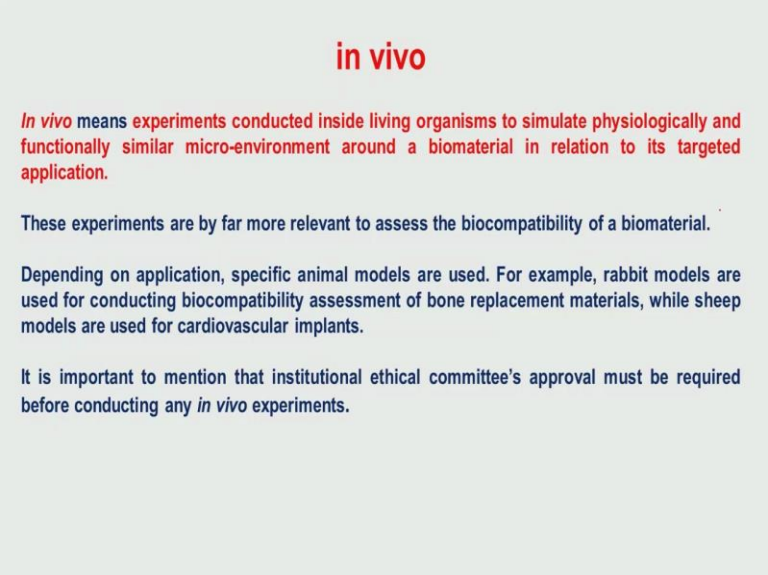

(())(18:04) is the first study, it is the first set of experiments which are to be conducted before one can go for the in vivo experiments which are again defined here as experiments conducted inside living organisms to simulate physiologically and functionally similar micro environment around a biomaterial, in relation to its targeted application. So therefore whenever you one has to do this in vivo experiments, there you have to have a different kind of defect model or different kind of animal model to simulate the in vivo environment to simulate the in vivo experiments.

Now specific animal model the rationale of choosing specific animal models is also disease specific for example rabbit models are used more for conducting bone replacement materials while sheep model are used more for cardiovascular implants. So therefore, for cardiovascular implants one is to use much larger sized animals like sheep or a pig model.

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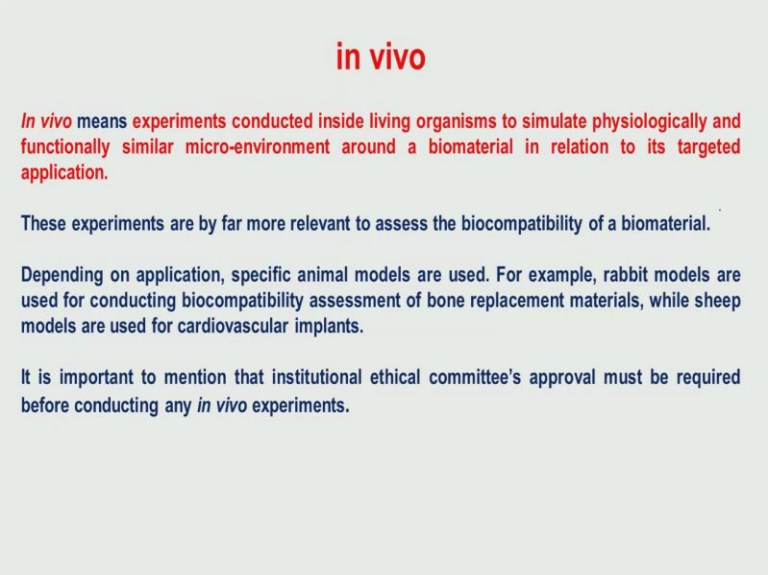

Okay. The, this third one is that host response or foreign body response so it is the reaction of a living system to the presence of a foreign material in vivo. So to start with, one has to remember that whenever you implant any biomaterial in a living system it always causes it always cause some kind of local or systemic inflammatory response. And this response in scientifically is known as the host response and it occurs irrespective of the way that you implant the material like either through surgery or through injection as all biomaterials are expected to cause a disruption in the local tissue environment.

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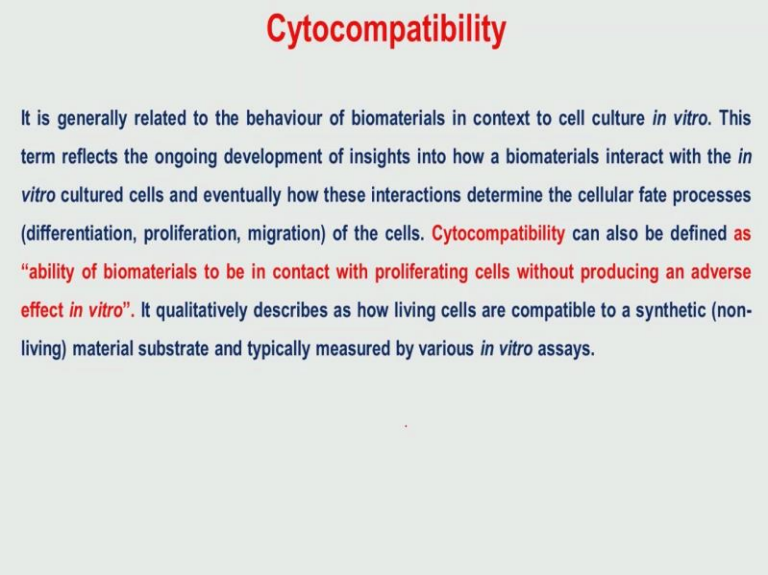

Cytocompatibility also has been mentioned before that is it is cell level compatibility so essentially ability of a biomaterials to be in contact with proliferating cells without producing an adverse toxic response.

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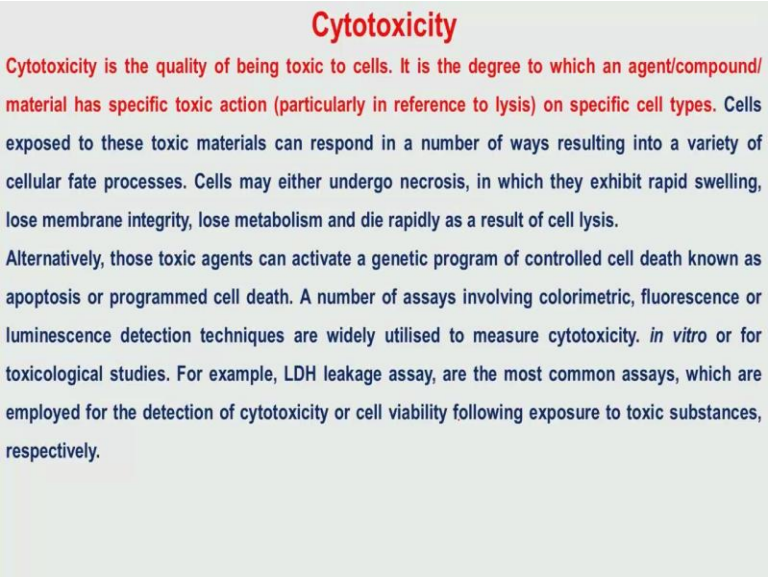

And this toxicity is quantified using a using different assays. Most popular assays that people use is MTT assay and a complementary assay also one can use is LDH assay. Now each assay one of the things that in cell biology one has to remember that each assay actually gives you certain specific information.

One cannot make any over stretching statement based on the results of one single assay; for example; MTT assay tells you the number of metabolically active cells. So if that x percentage cells are metabolically active in a given cell population, one cannot say that 100 minus 6 percent cells are dead simply because for, in order to quantify the dead cells one has to use that LDH assay. So this LDH assay when you do LDH assay then only you can say yes, y percentage of the cells in a given cell population is dead. It is not alive anymore. x plus y can be 100 or may not be 100 or less than 100. So in cell biology one cannot, kind of, make over stretching statement based on the information of 10 from a single assay.

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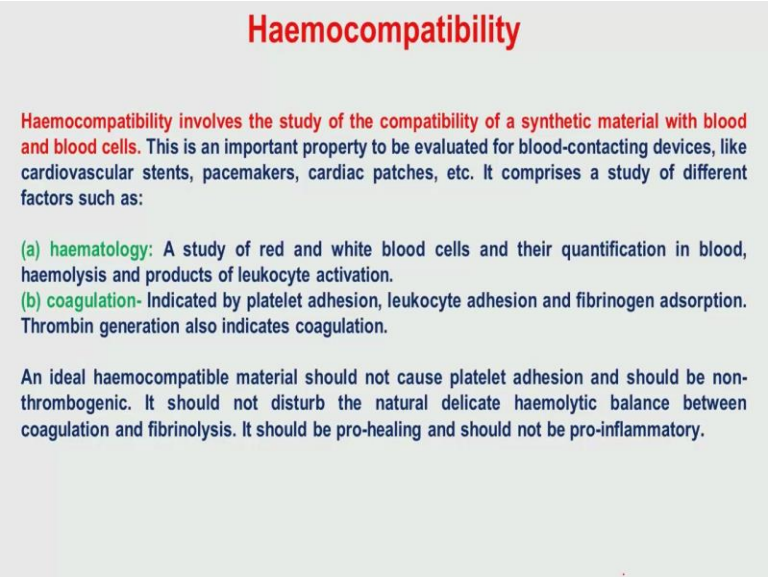

Haemocompatibility, haemo means (blood) haemocompatibility essentially means that blood level compatibility and it is a study of a compatibility of a synthetic material with blood and blood cells. Now this level compatibility is important for cardiovascular materials like cardiovascular stents, pacemakers, cardiac patches and so on. And it depends on the different factors like heamatology; a study of red and white blood cells and coagulation that is the platelet adhesion or leukocyte adhesion and fibrinogen adsorption; so which also takes place on artificial material.

Ideally haemocompatible, a perfect haemocompatible material should not cause any platelet adhesion and should be non thrombogenic in nature. In other words it does not disturb the delicate haemolytic balance between the coagulation and fibrinolysis.

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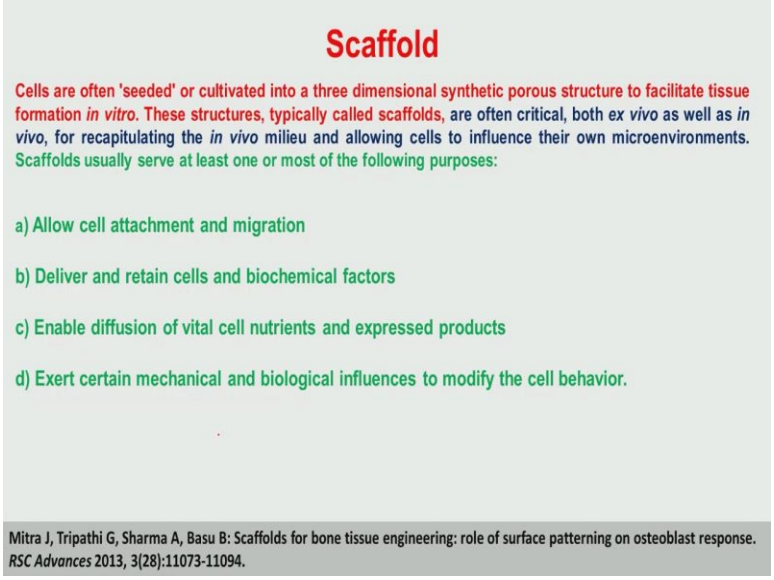

Okay. Now, having given this all this different definitions there are 2 things often people are confused in this biomedical applications or as far as the biomedical materials are concerned; 1 is called Scaffold and another is called (im) implant. Now these 2 things need to be defined with respect to their distinct with respect to their characteristics which distinguish themselves. Scaffold normally means, that is a porous material and this porosity is very helpful for the cell (colono) colonisation and cell growth and proliferation and also to many extent to some extents cell differentiation.

So this scaffold structures are essentially 3-dimensional synthetic porous structure as is been mentioned very clearly in the first line itself, to facilitate tissue formation in vitro. And this kind of interconnected porous structure which is present in many of the scaffolds which are experimentally studied in different research groups around the world; this interconnected porous structures are helpful for the osseointegration property like when you implant this scaffold structure in any animal model, that also helps in the helps in angiogenesis like blood vessel formation through this porous structure that also helps in the bone in growth into the porous structure.

Now those things we will (dif) we will describe it much more later but at this point you must understand that scaffolds usually sharp most of the ABCD properties or at least few of these properties. this allow cell attachment and migration. Now what is cell migration, I have explained to you before; that is essentially mortality on a material substrate deliver and retain

cells and biochemical factors enable diffusion of vital nutrients and expressed products and exert certain biological and mechanical influences to modify cell behaviour.

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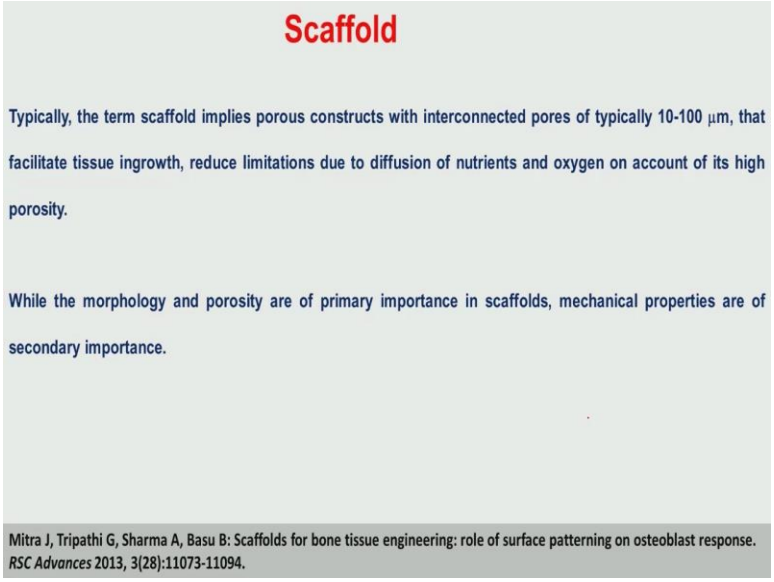

Now, typically the scaffold term implies porous constructs with interconnected pores more in like 10 to 100 micro meter which facilitates, which facilitates tissue ingrowth, reduce limitations due to diffusion of nutrients and so on. So the morphology and porosity are the primary importance in scaffold whereas mechanical properties are of secondary importance.

As I said since it is a synthetic porous structure 3-dimensional porous structure these cancellous bone you remember that cancellous bone also has a very large porous structure and cancellous bone are mechanically inferior than cortical bone. Similarly, since scaffolds are essentially 3-dimensional porous structure, scaffolds also have weaker mechanical properties and this scaffolds are important in tissue engineering before before defining other terms let me also explain that what is meant by implant.

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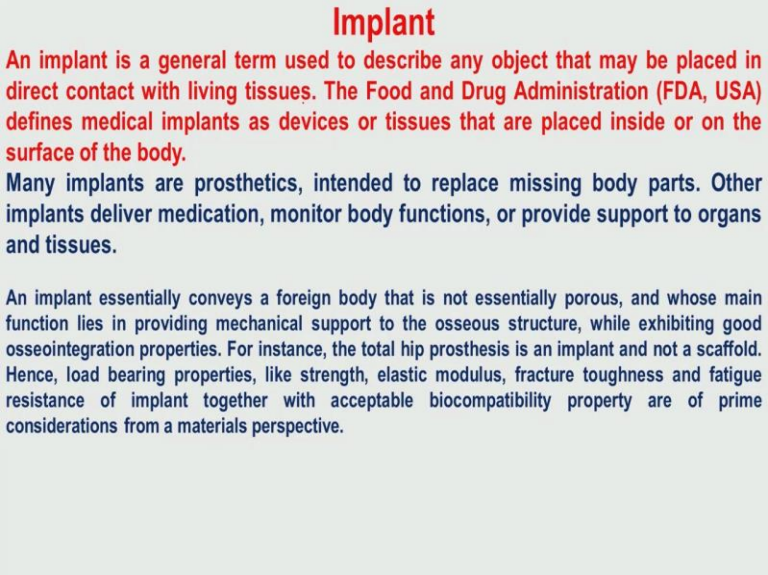

Implant is a general term used to describe any object that may be placed in direct contact with living tissues. That FDA that is US body that is Food and Drug Administration defines medical implants as devices or tissues that are placed inside or on the surface of the body. So it is used; many implants of prosthetics and this is intended to replace missing body parts. And other implants deliver medication or monitor body function. Those things we are not that does not come under the purview of this present course.

However this implants by definition as you see that these are like medical devices which are placed in direct contact with living tissues. Examples of the implants is THR, like total hip joint replacement; you have a stem that is made of either titanium or stainless steel; that is the stem. You have a femoral head that is either made up of steel or alumina and we have acetabular socket again that is made up of that polymeric materials like ultra-high molecular polyethylene.

Now all these 3 materials essentially at the bulk level, they are not 3-dimensional porous structures which is scaffold is but that is this THR which is a very well-known example of an implant, it is essentially a 3-dimensional solid structure, nonporous structure which is intended to carry lot of mechanical loads as well as it is intended to function under a large number of fatigue cycles during that mechanical loading conditions so therefore implants are by definition meant for more like mechanical load bearing applications.

Another examples of the implant is total knee replacement. So total knee replacement also has load bearing materials. So this load bearing materials are essentially nonporous and solid

materials like total hip replacement materials. So certainly the implants must have much better mechanical properties than scaffolds but with uncompromised biocompatibility properties.

Why implants are necessarily to have or its meant to they are meant to have better combination of mechanical properties with uncompromised biocompatibility properties because biocompatibility properties that is a bottom line for performance of any biomedical applications and therefore we need to understand that what is the distinction between the scaffold and implant and I hope that in last 2 slides I have made such distinctions little clear in your overall understanding.