Introduction to Biomaterials
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Module No. # 01

Lecture No. # 14

Structure and Properties of bone as well as in vivo testing and his to compatibility assessment

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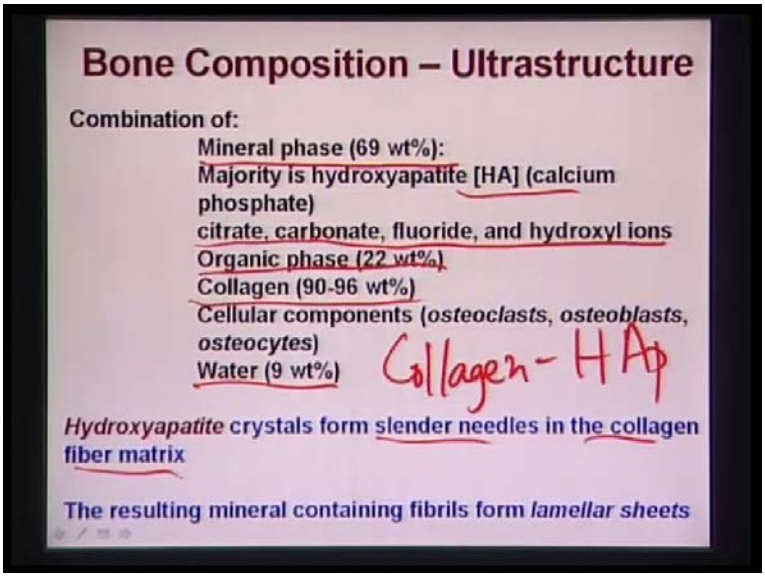

Earlier, I have already discussed that, what is the structure, morphology, or composition of biological cell. I also discussed that, what is the properties of the cells, like cellular adaptation, like atrophy, hypertrophy, metaplasia, hyperplasia. I also discussed that, you know that, how protein, what is the, you know, structure of the protein molecules, like amino acids, and how these protein molecules are formed, and how protein molecules, they interact with each other, and as well as the collagen; what is the composition of the e c m extra cellular matrix and so on; and, at the same time, I have focused on the part, that tissue, that bone is an example of the hard tissue. What is the composition of the bone? This is a combination of the mineral phase that is 69 percent. So, 69 percent means that, your natural hydroxyapatite, or calcium phosphate particles

which are in nano size in the bone, which is contain 69 weight percent; majority is hydroxyapatite, as I said; then, you have citrate, carbonate, fluoride and hydroxyl ions.

Then, you have organic phase and organic phase is 22 weight percent and water is your 9 weight percent. Now, among these organic phase, your collagen is 90 to 96 weight percent; that means, majority of the organic phase, or most of the organic phase is your collagen. So, that is the reason bone is a composite and this composite is known as collagen-hydroxyapatite composite. So, collagen is your organic phase. So, that is like a polymer; hydroxyapatite is ceramic phase. In other words, you can describe bone as an unique example of the polymer ceramic composite. Now, hydroxyapatite crystals, that form a slender needles in the collagen fiber matrix and the resulting mineral containing fibrils form lamellar sheets.

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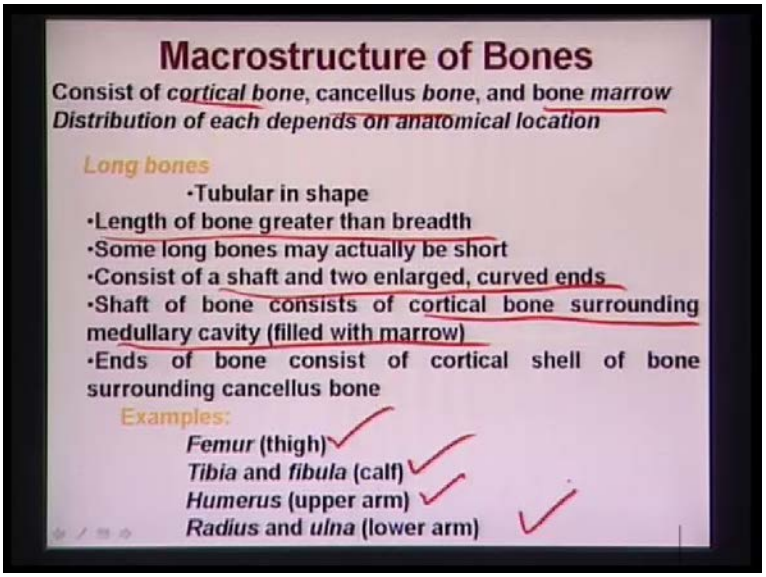

Now, let us see that, you know what are the different examples of the bones, based on the, their shape or size. Now, this typical bones, they consist of cortical bone, cancellus bone and bone marrow. So, I think, in one of the earlier lectures, I have mentioned that, cortical bone is the outer surface of the bone, which is very hard and strong; cancellus bone is the porous structure are called trabecular bone. So, porous structure means, whenever bone has a porosity, that means, the properties are inferior compared to the cortical bone. So, property-wise, cortical bone is stronger, compared to the cancellus bone. Then, you have bone marrow. Bone marrow is that

region, or matrix region, inside the bone. Now, many times, if you say that, a patient is having a cancer, and people say bone marrow cancer; bone marrow cancer means, there are some cells inside the bone marrow region, that is proliferating at an abnormal phase.

So, cancer is nothing but, cell growth, or abnormal growth of the, a group of cells and which cannot be controlled, or which is not natural. Long bones; long bones is typically tubular in shape, and this length of the bone is greater than the breadth. So, if you consider the aspect ratio, aspect ratio of the long bone is much greater than 1; that is the length to the breadth ratio. Some long bones can be, actually be short, and this consists of a shaft and two enlarged curved ends and shaft of the bone consists of cortical bone surrounding medullary cavity. I think, I will show you some slide, what is meant by medullary cavity. Now, examples of the long bones are the thigh bone, that is called femur, tibia and fibula, that is the calf bone, humerus that is the upper arm, and radius and ulna, lower arm. So, you have the femur, tibia and fibula, and you have the humerus, and you have the radius and ulna.

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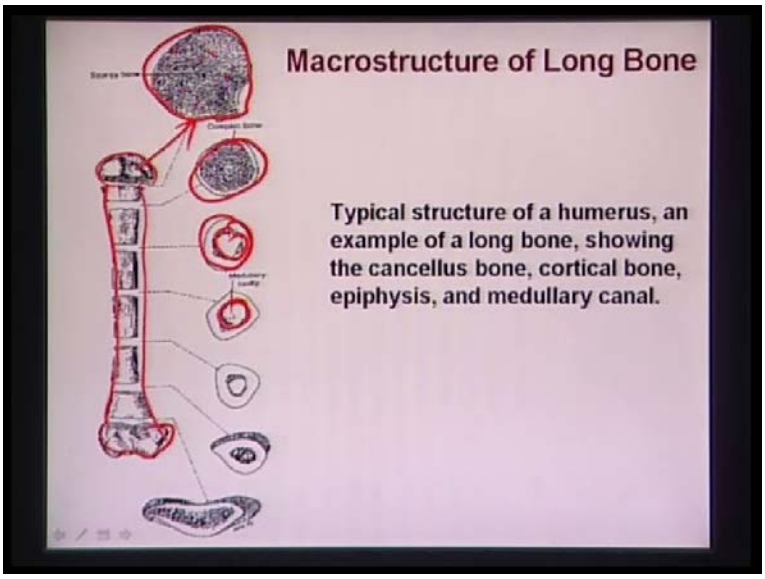

Now, let us see that, how this macro structure of the long bone, they look like. Now, this is a typical structure of a humerus and if you see that, humerus' entire structure, you have a kind of a cap here and you have that long end also; so, you have a cap that is the, that you know, long compact bone and compact bone is the outer surface of the compact bone; and inner surface, you

have a spongy bone; spongy bone means, cancellus bone. So, cancellus bone has a different name; one is the spongy bone, because it has a porous structure; other name of the cancellus bone is called trabecular bone. So, trabecular bone, spongy bone, cancellus bone, they are synonymous; synonymous means, they carry same meaning. Now, cortical bone, that is the harder part of the bone. Now, if you take a cross-section, what you can see in the cross section? This is called your actual medullary cavity. Now, through this medullary cavity, you have a blood, proteins, everything, they are circulated throughout, because blood is the tissue which carry all this oxygen to different parts. So, if the blood flow is not there at any part, that means, oxygen is not transported; if oxygen is not transported, cells will die; cells will undergo apoptosis; and therefore, tissue will not perform; means, automatic function tissue also will die.

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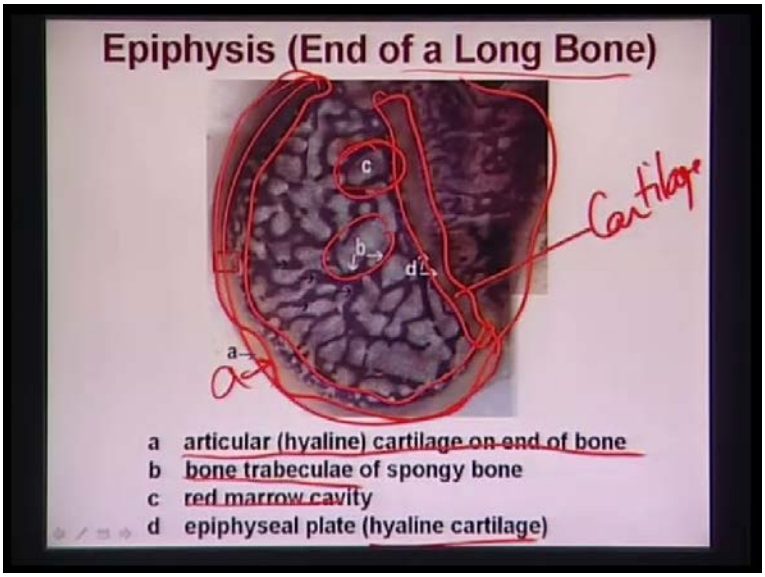

Now, epiphysis. Epiphysis is the end of a long bone and this is the, you can see, that is, that how this long bone end, it looks like. So, this is the region, I am now…So, this is the region, we are now focusing here and these are different parts; that a, is the articular cartilage at the end of the bone. So, this region is that articular cartilage. Now, b is that bone trabeculae; bone trabeculae or the spongy bone. Now, c is the red marrow cavity; that is the region, that is red marrow cavity and d is the hyaline cartilage. Now, d is the, again, this part. So, cartilage is another tissue. So, what it say, what it shows here, both the outer region and the inner region, you have the cartilage tissue and inner part, you have that cartilage cancellus bone or the trabecular bone.

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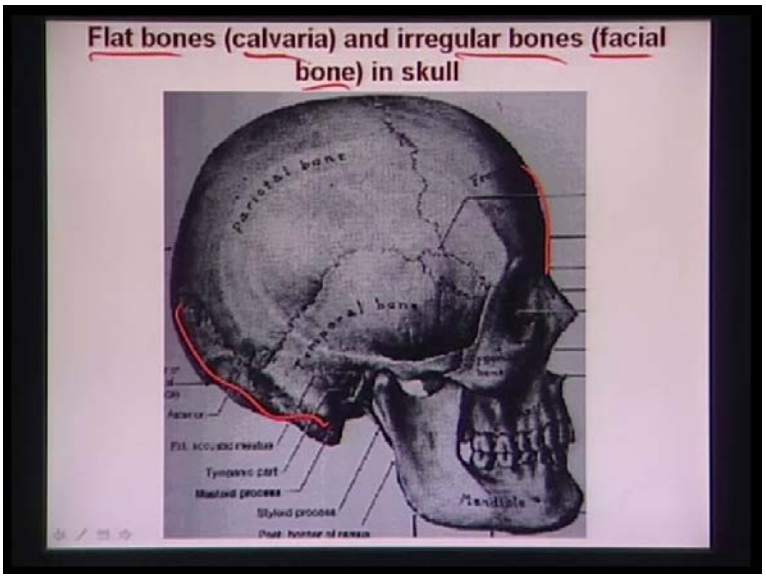

Then, what is known as the flat bone? Flat bones is that calvaria and irregular bones are the facial bones. So, facial bones, you can see, that is in the skull, that is called the facial bones, or irregular bones. These are the examples of the irregular bones and flat bones have name is that called…

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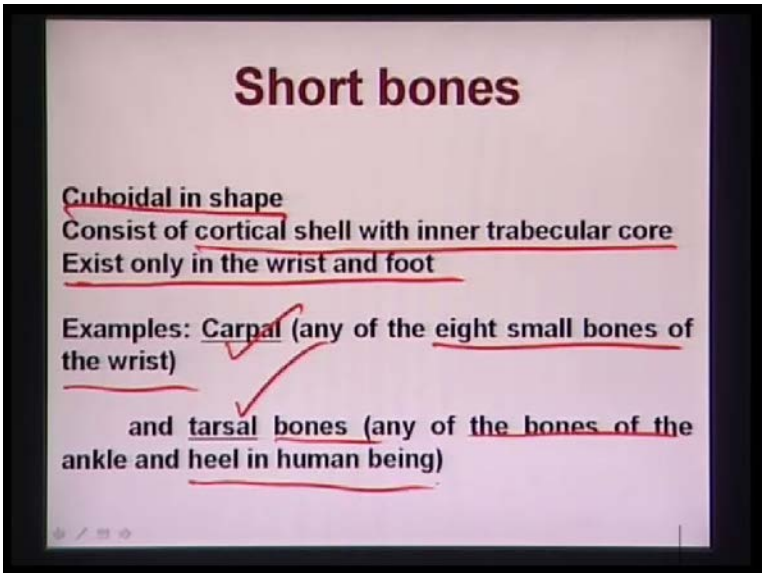

Now, short bones. Short bones are typically cuboidal in shape. If you remember that, how these bones are classified into different types, long bones, if I can summarize, long bones are actually, length is much greater than the width; that means, aspect ratio is much greater than 1; and you have seen that, long bones typically consist of cortical bone, trabecular bone or spongy bone; third one is the bone marrow region or medullary region. Then, you have called flat bones and you have called irregular bones. Then, you have called short bones. Typically, it is cuboidal in shape and it consists of the cortical shell with inner trabecular core. So, typically, what you see, generically, bone always contains cortical shell; that means, that is the hard surface and the hard surface actually protects the internal part of the bone.

So, that is the reason, you know, that this concept is very natural; that is the reason you people always put coatings on the surface; coatings means, the coatings are typically harder than the substrate. So, you, whenever you put coatings, like titanium nitrate coatings on titanium, or some other examples, then, titanium nitrate has much higher hardness, much better wear resistance, than the titanium. So, that is the reason that, why coatings are placed on a soft oxalate. Similarly, all the bones, they have a cortical shell like outer surface, and inner trabecular core and exists only in wrist and foot. So, these are like short bones; wrist as well as foot, they are called short bones and examples are the carpal and tarsal bones. So, carpal is the, any of the eight small bones of the wrist and tarsal bones are the, any of the bones of the ankle and the heel in the human being.

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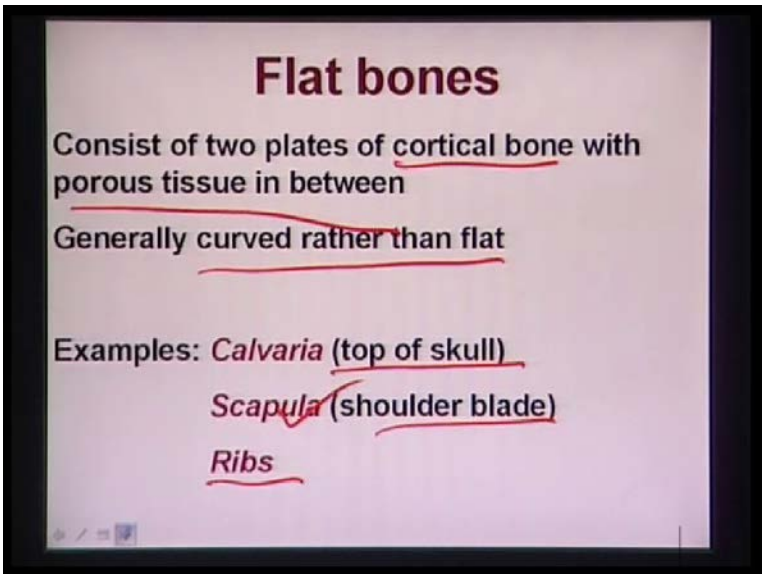

Then, you have called flat bones. They are essentially consists of two plates of cortical bone with porous tissue in between. Again, if you come back to the, just the description that I have mentioned to you, that is the generic description, that you have always the cortical bone which is at the outer surface, and inner material is always the cancellus bone. So, that means, the hard surface which is protecting the spongy material, or spongy surface, and this is generally curved, rather than the flat and calvaria is the top of the skull, scapula is the shoulder blade. So, that is also the examples of the flat bones and the ribs are also another examples of the flat bones.

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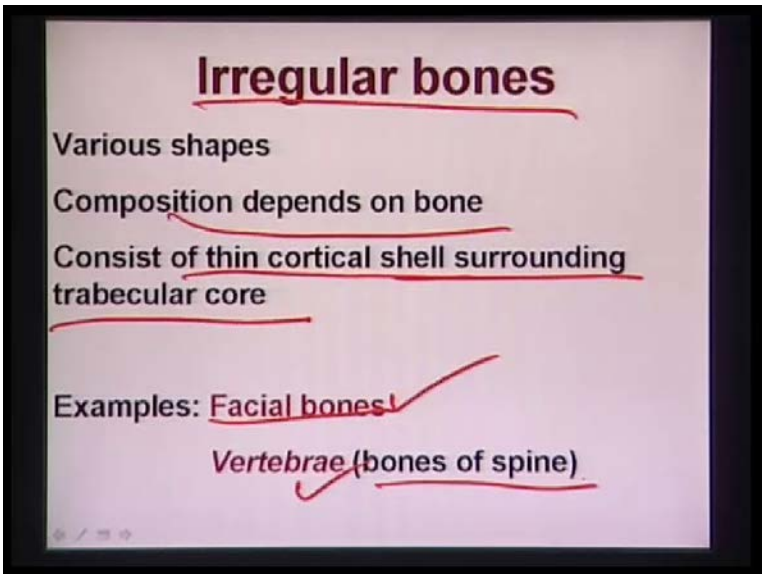

Irregular bone. As the name suggests, irregular bones means, it has a various shapes and it also, the composition also depends on bone; but, it consists of a thin cortical shell, surrounding trabecular core. Again, the same principle; some hard surface, protecting the weaker region, that is the trabecular core. And, the examples are the facial bones and vertebrae, that is the bones of the spine; that means, that is at your spinal cord, or spine. These are the examples of the irregular bones. Irregular means, that does not have any specific shape, like long bone, or flat bone; they do not have that kind of specific shape; like cuboidal shape, that is short bone. So, irregular bones, like, it is always, it does not have any specific geometrical shape, let us put it that way, so that, you understand that, why it is meant by irregular shape.

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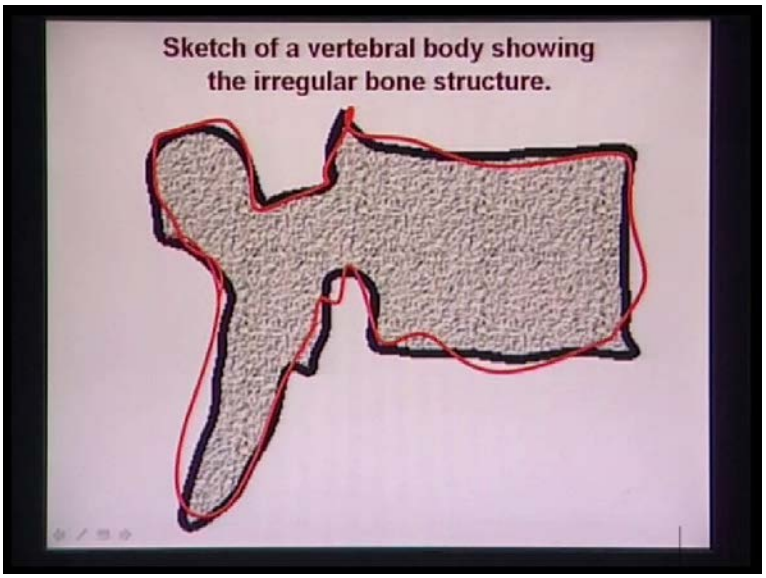

Now, this is a sketch of the irregular bone structure; as you can see, that it is really of different, very irregular shape and this shaped materials is called irregular shape.

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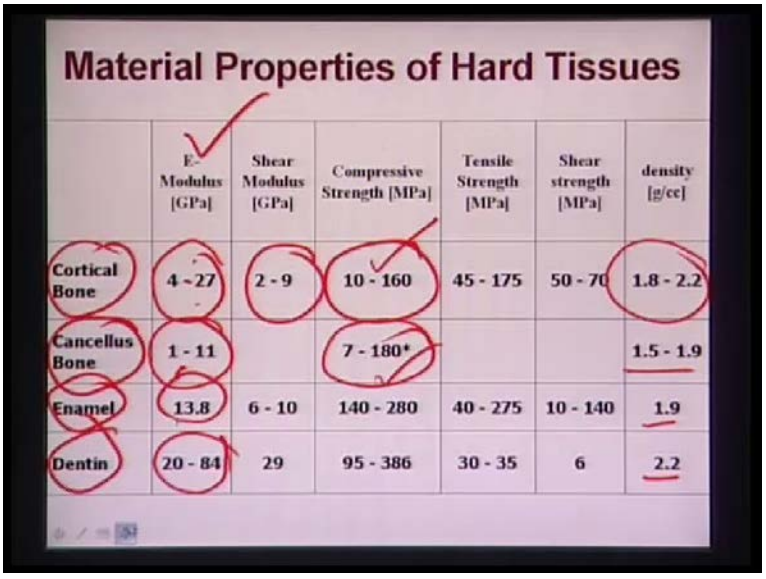

Now, this slide actually gives you a table, which gives you the range of properties that is typically measured, with different parts of the bone, like cortical bone, cancellus bone, enamel and dentine. Enamel and dentine are the hard tissues of the teeth, right; enamel is the outer part;

dentine is the inner part. Similarly, cortical bone and cancellus bone, again is, the cortical bone is the surface; cancellus bone is the inner core. Now, first thing I must mention here that, you know, you, you see all the ranges of values; now, that means, all this bone properties, they cannot be characterized by unique value, unique property value; you cannot say, elastic modulus of the cortical bone is 10 gigapascal; because, depending on the anatomical location, like, whether it, you are taking from the wrist, whether you are taking from thigh, whether you are taking from leg, your bone property is also different; because, the composition of the bone is also different, depending on the anatomical location; and, if the composition is different, composition means, that is the proportion of the collagen and the synthetic and the collagen and the natural hydroxyapatite.

If this compositions varies, you know, the composite properties depending, depends on, typically depends on the ratio of the two phase, like alpha beta; if you change the alpha beta ratio, then, the composite made of alpha and beta must change. Similar rule is, can also be applied to the bone, because, bone is a polymer ceramic composite. So, if the ratio of the collagen to hydroxyapatite changes, that will also change the bone properties; and, that is what, what I am trying to hint here that, this bone has a range of the properties, be it cortical bone, be it natural bone, be it enamel or dentine and the range of properties is already is shown here. Now, density of cortical bone is somewhere around 1.8 to 1.2; cancellus bone, because it is porous in nature, is less and density of enamel and dentine is 1.9 or 2.2. Now, when you talk about the elastic modulus, cortical bone has a 4 to 27 gigapascal; cancellus bone is much lower, because cancellus bone has a porosity. So, that porosity reduces the elastic modulus. You have a compressive strength 10 to 160; cancellus bone can have a lower compressive strength; then, you have a, enamel has a 13.8 and dentine has the 20 to 84 gigapascal, which is close to 100 gigapascal, ok.

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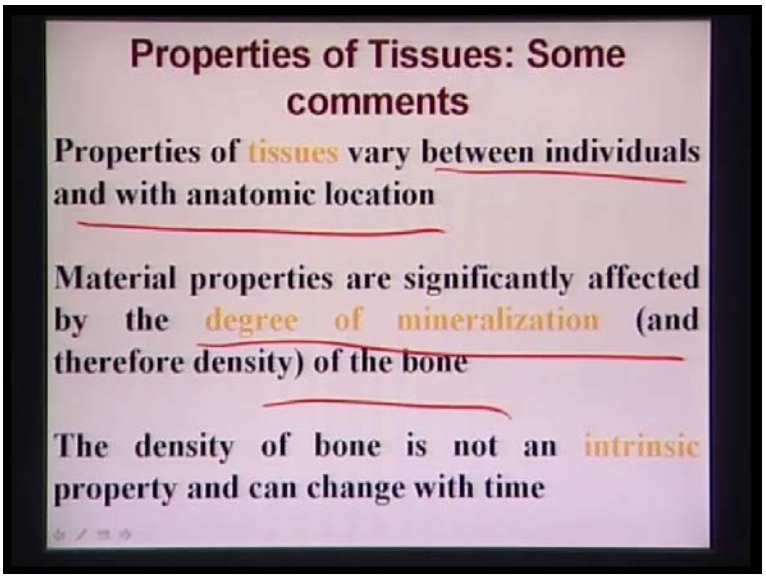

Now, as I was describing just now, the properties of tissues, some comments here, that has been mentioned; that properties of tissues, vary between individuals and with anatomic locations; that is what I just mentioned; and between individuals means, like, you know, two persons of the same age can have different bone properties; that is what I am trying to say; material properties are significantly affected by the degree of mineralization of the bone. Degree of mineralization means, what is the mineral content, means, what is the content of the hydroxyapatite in the collagen matrix.

So, that is what is meant by degree of mineralization. The density of bone is not an intrinsic property and can change with time. For an old person, many times, if you, the doctors say, the bone density is decreasing. Bone density is decreasing means, number of bones at the unique length, or along a particular length, that number of bones is reduced. Why, because, bones will slowly dissolve in the human body, so that, total volume occupied by the bone is reduced and that is cause the bone loss; and that is why, you can see that, a aged person, or a person of 70 years age, he cannot walk like a 10 years old, or 20 years old person, right; because, that walking capability, or running capability is reduced; because, your tissues and bones cannot perform the same function, at the same speed, like what a young person can do, because, their, their tissue, or the bone is losing it is capability, because of the bone loss, or the bone density.

Now, some more comments. Mineral stores within a bone can change dependent on, on the physiological demand. Mineral stores means, again, it is a hydroxyapatite content. So, it is essentially, Hap. Now, pathological processes can affect that degree of mineralization. In normal and healthy bone, the range of bone and bone mineralization is small. What it means, in a normal and healthy person, the bone mineralization, that means, synthetic, or the amount of the hydroxyapatite content varies only within a small window; let us say, total hydroxyapatite content is 69 weight percent; so, it can vary over 68 to 70 weight percent; not like 60 to 70 weight percent; that is a large window. Now, what is pathology? Pathos means, like, it derives from Greek words, like, most of the things are derived from Greek words; pathos means disease and logos means what reasons. So, it is the study of the links between the disease and the basic sciences. And, what is the disease? A disease is a physical or functional disorder of a normal body system, that places an individual at an increased risk of adverse consequences.

So, like, when you have fever, you cannot do your, or you cannot perform your normal functions, at the same speed, like, when you are in healthy conditions, because your tissues will not perform its normal function. So, because, that there must be some functional for physical disorder of the normal body system and disease are typically diagnosed by physicians, or other health care provider, through a combination of tools. Now, whatever you have disease, that can, you can only get to know, only when you do the blood test, or urine test, or other pathological test, ok. Now, let us discuss something on the mechanical response of the human cortical bone. Now, what you do, what, what has been plotted here, stress versus strain. Now, this is that very standard way of plotting the mechanical response of any material, let it be metal, ceramic, polymer, etcetera. So, that mechanical response is always expressed in the plot of stress versus strain, and that is what has been mentioned here.

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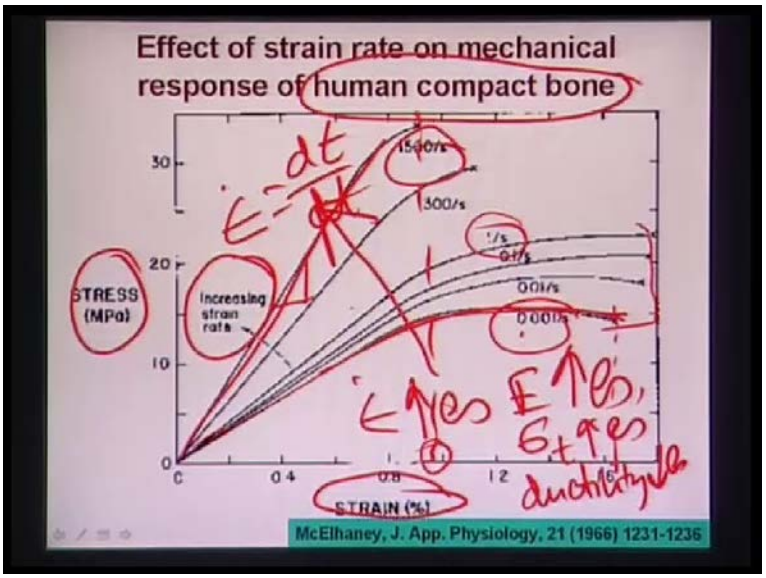

Now, this is a human compact bone means, compact means, it is like a cortical bone; mostly, it is a cortical bone. Now, what you can see from this graph? This graph, you can see, if you increase the strain rate, strain rate means, if you increase the epsilon dot; epsilon dot means, this is d epsilon by d t, and that, you can increase by varying the (( )) speed. Suppose, you have a tensile specimen; you are pulling it with two (( )) speed and that would, (( )) speed if you increase or decrease, or if you vary, then, your strain rate also will vary; and, if you increase this strain rate from 0.001 per second, here, it is the flat one, to 1500 per second, what you see, you see that, curves become much more steeper. Now, how this elastic modulus is calculated? Elastic modulus is calculated from the slope of the linear path, right; and from here, what you can see, when the strain rate is increased, so, if your epsilon increases, then, your elastic modulus increases, right; when epsilon increases, your strain also, that is, sigma t, or whatever, that is also increases; but, what decreases, that is your ductility, that is, decreases; and how this ductility is measured here? Ductility is measured by the total strain at failure. Now, total strain at failure is roughly 1 percent and when you are doing the test at 0.001, then, your total strain is 1.6 percent.

So, as you can see that, it has a linear region, followed by a flat region, at the low strain rate. Similar thing is observed upto the strain rate of 1 per second, but, when you go to very high rate, strain rate experiments, then, it becomes flat; so then, it becomes very steep and the non-linear part, or the flat part is also reduced; and accordingly, your elastic modulus increases; your

strength increases, at the expense of decrease in the ductility; that means, your strain to failure is significantly reduced, compared to the low strain rate phase. So, in other words, major message from these plot is that, that with increase in strain rate, your ductility decreases; and then, mechanical properties of the, mechanical response of the human compact bone is highly sensitive to strain rate. Again, mechanical properties of the human compact bone cannot be described by a unique value, because, that depends on the strain rate, as you can see from this particular slide. Now, the question is that, why it depends on the strain rate? The answer is, bone has a complex composition; like, it has some water molecule, about 9 weight percent; you have the majority is the hydroxyapatite, that is 69 weight percent; then, you have the collagen content, around 20 to 8 percent or so on.

Now, it is a polymer-ceramic composite. Now, polymers, the mechanical response of polymers are time-dependent and that leads to a time-dependent response of the bone, because bone essentially contains the polymeric molecules also; because, for ceramics, the mechanical response is not time-dependent; you do that test at 0.001 per second, or you do the test at 1500 per second, I do not think, the elastic modulus will vary much; but, for polymers, it will vary; because, polymer essentially, the chain micro molecular kind of structure; the faster you do that, your, you are not giving enough time, that chain de-orientation to take place during the polymeric mechanical behavior; that explains why, bone has a time-dependent mechanical response, or strain rate-dependent mechanical response.

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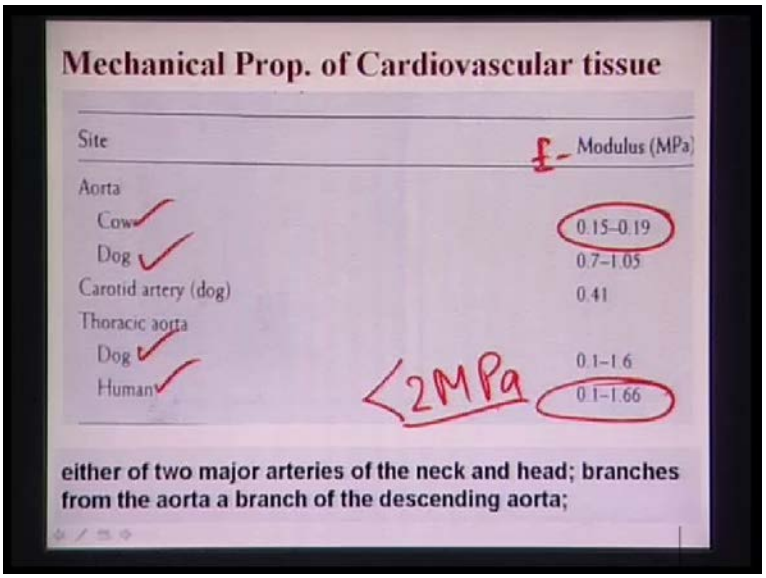

So, this list tells you actually, some mechanical properties of the cardiovascular tissue. Now, what you can see here, this is the elastic modulus. Now, elastic modulus, you have seen from metals and ceramic, that elastic modulus is the level of gigapascal, 10 to the power 9 pascal, or 10 to the power 9 Newton per meter square. Now, what you see here, aorta for the cow or dog or thoracic aorta for dog or human, it is always around less than 2 megapascal; so, that means, it is extremely soft tissue; soft tissue means, the properties are very much less. So, megapascal versus gigapascal means, it is three orders of magnitude lower elastic modulus, when you are considering the soft tissue.

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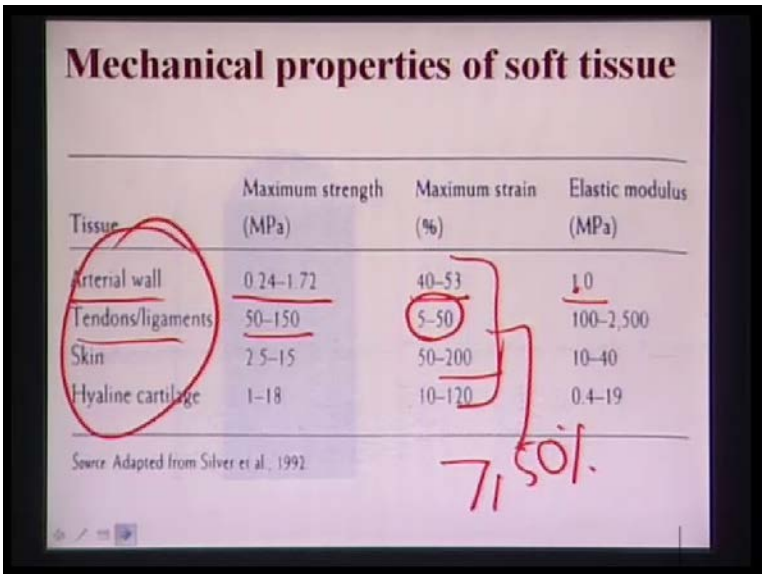

Now, this is the kind of, some other, you know, complete range of the soft tissues that are mentioned here; like, arterial wall, it has a maximum strength of around less than 2 megapascal; maximum strain is 40 to 50 percent, elastic modulus is 1 megapascal; it is so low. Tendons or ligaments, it has a maximum strength of around 200 megapascal and this maximum strain is 5 to 50. Then, what you can notice here that, in both, all this cases, at least, these three cases, your maximum strain is less, greater or equal to 50 percent. So, it is a large strain, fracture, or strain to fracture that is possible; you cannot get this kind of strain to failure in most of the metals, or ceramic material; only in the polymers, you can get this type of large strain to fracture. What it means? That means, this most of the soft tissues are (( )) the polymer base; that is why, you give such a large value of the strain to failure.

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I will be describing the typical, a protocol that is followed in in-vivo implantation as well as histopathological evaluation of biomaterials.

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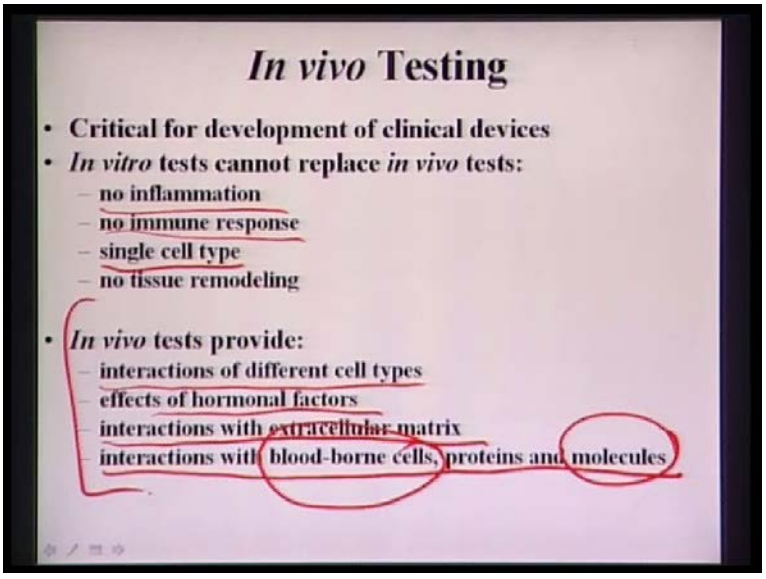

So, before I do that, let me remind you that, why in-vivo testing is required, because in-vivo testing is critically important, or it is very critical, for development of clinical devices. For last two lectures, I have described that in-vitro test, like, what is the in-vitro biocompatibility

assessment, or how different in-vitro test, like hydrotoxicity, genotoxicity, haemocompatibility, they can be performed in laboratory scale environments; and laboratory scale means, that is the in-vitro test. However, as I said before, in-vitro test cannot replace in-vivo test; because, in the in-vitro test, you do not see any inflammation and then, second thing is that, there is no inflammation and there is no immune response. So, because this test, in-vitro test are not carried out inside the animal model, so, they cannot show, they cannot tell you, in-vitro test cannot tell you, whether a given biomaterial can cause inflammation, when implanted inside the human body, or any animal; or whether there will be any immune response to this biomaterial.

Third important point is that, these kind of in-vitro cytotoxicity test, they are always carried out with single cell type; single cell type means, like, it may be either fibroblast cells, that is, connectivity cell lines, or it can be osteoblast cells, that is, the bone forming cells; they do not involve multiple cell types. But, when you put this material inside the in-vivo environment, then, multiple cell types will come in contact with the biomaterials; or, in other words, the biomaterials will come in contact with certain tissue, then, the, those tissues can contain multiple cell types. Then, fourth point is that, there is no tissue remodeling that is possible, or that cannot, or that can be assessed in the in-vitro testing.

Tissue remodeling means, that extra cellular matrix composition, during in-vitro tests are largely, largely do not change with time; but, inside the in-vivo environment, extra cellular matrix composition, E C M composition continuously changes; because, in-vivo environment, there is more dynamic changes in pH; there is more dynamic changes in composition of the E C M, as well as cell proteins, etcetera. Now, those kind of things, you cannot really simulate in the in-vitro test. Therefore, in-vivo tests are necessary and they provide information on the following aspects; like, first one, interactions of the different cell types. As I mentioned that, you know, during in-vivo implantation, that you know, a material will come in contact with different cell types. Effects of hormonal factors; those factors cannot be assessed in the in-vitro conditions. Interactions with extracellular matrix, that is also important things. Interactions with blood-borne cells, proteins and molecules. Now, this, although you use some serum protein, or other kind of proteins in the in-vitro test, but, you know, number of proteins that are experienced, or those are exposed to biomaterial in in-vitro are limited; and different biological molecules also, biomaterial will come in contact. Biomaterial also will come in contact with a leukocytes or

erythrocytes, because there will be always blood through, at that any given locations. So, all those things are not possible in the in-vitro conditions, and therefore, in-vivo tests are necessary.

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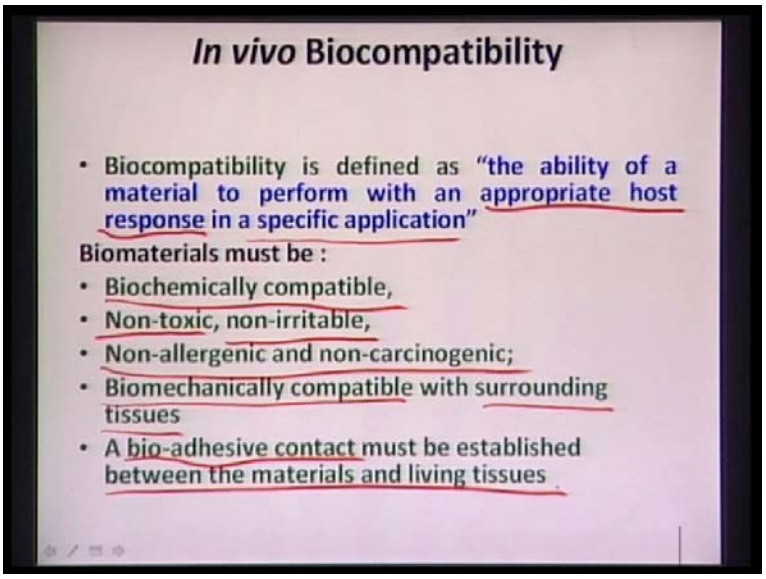

So also, let me remind you that, what is defined as the biocompatibility? That is the ability of a material to perform with an appropriate host response to a specific applications. Appropriate host response means, like, host here, it is that human or animal. And therefore, appropriate host response means, that in the implanted area, where the material is being implanted, the host area should not show any sort of inflammation, or any toxicity, to, just because, that material, synthetic material is implanted in that position. And, in a specific application means, as I said, this biocompatibility is a broad term and that has a different meaning for different applications, right. For blood contacting devices, it is the haemocompatabilty; for bone forming, or bone replacement, it is the cytotoxicity, tissue formation ability, all those things are important.

Now, therefore, biomaterials must be biochemically compatible; that is the first important point, that is biochemically compatible. It should be non-toxic and non-irritable. Non-toxic means, like, you know that, cell level or gene level, there should not be any toxicity. Non-irritable means, it should not cause any irritation to any part of the body. Third one is that, it should be non-allergenic and non-carcinogenic. Certainly it should not cause any cancerous effect; like, you know, it should not activate the cancerous cells. And once, and if the cancerous cells are

activated, that means, uncontrollable multiplication of cells will take place, and which will cause the cancer to spread. Also, biomechanically compatible with the surrounding tissues.

Now, what it means? It means that, every tissue, like, whether it is a hard tissue and soft tissue, you have seen in earlier lectures, they have a range of properties, in terms of elastic modulus, in terms of mechanical properties. Now, whenever you are putting the biomaterial for a given applications, the tissues that, whether it is bone, which is surround that material, there should not be large difference between that material and the bone, which is in the neighborhood of that material. And, if there is a large difference in mechanical properties, particularly, in terms of elastic modulus, then, what will happen? There is a biological process called aseptic loosening; that is, that means, this biomaterials can be detached from the neighboring hard tissue, or bone; because, if the biomaterials has a larger elastic modulus, then, biomaterials will carry most of the load. If the bone has a larger elastic modulus, then, bone will carry most of the load; but, there should not be large difference in terms of the elastic modulus. Then, last point I have mentioned here, the bio-adhesive contact must be established between the materials and living tissues. Bio-adhesive contact means, there should be some kind of biological contact; that means, when a material is implanted, then, if it, if a capsule tissue is formed around the material implant, then, that is what is desirable for good in-vivo biocompatibility.

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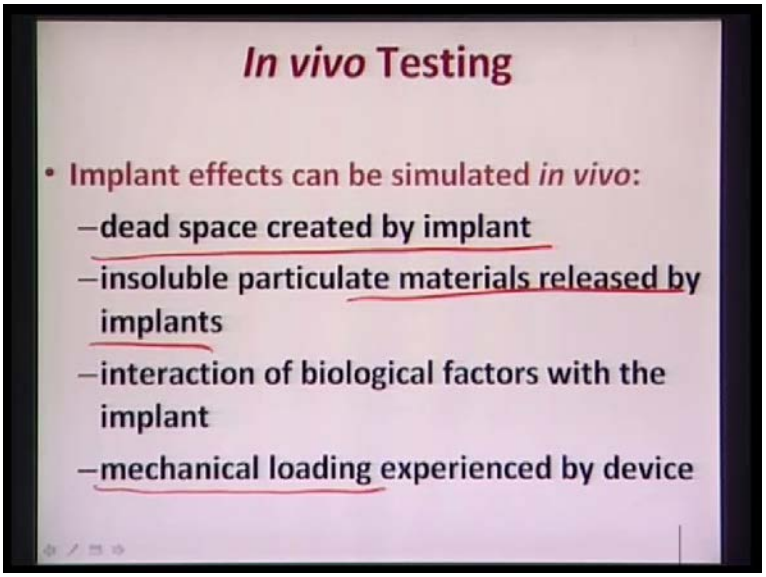

Now, this implant effects can be simulated in-vivo; that is dead space can be created by the implant and insoluble particulate materials released by implants. Now, these are the factors which you can easily assess during the in-vivo testing. The first one is the, dead space created by implant means, like, suppose you have a very long bone structure and a long bone, you cut some pieces of the bone, then, you put your implant. Now, dead space means, if the interface is not uniform, interface means, that is the space between the implant as well as the natural bone; if the interface is not, not continuous, then what will happen, there will be some dead space, which will be available, where there is neither material, nor any natural tissue, ok. So, that is what is not desirable. Then, in those cases, there will be problem with a mechanical behavior of the bone.

Second thing is that, insoluble particulate materials released by implants. Now, insoluble particulate materials means, certain particulate materials, when it is finite debris particles and so on, then they can be easily dissolved in the body fluid or body plasma. But, however, if there is a insoluble particulate which is released during the in-vivo degradation, in-vivo degradation means, like, inside the implant materials, the material also will undergo some kind of degradation; and under those conditions, they will release some material; they will be leaching some materials, and if those materials can cause some undesirable toxic effect around that tissues. Then, third factor is the interaction of biological factors with implants, and fourth one is the mechanical loading experienced by the device. Now, mechanical loading is, it can be either compression, or flexural. Now, compression means, when a body will be, at certain part, which will be loaded from two opposite directions; or flexural means, it is like a bending type of situations. Now, these kind of, two type of mechanical loading that are generally experienced by the device.

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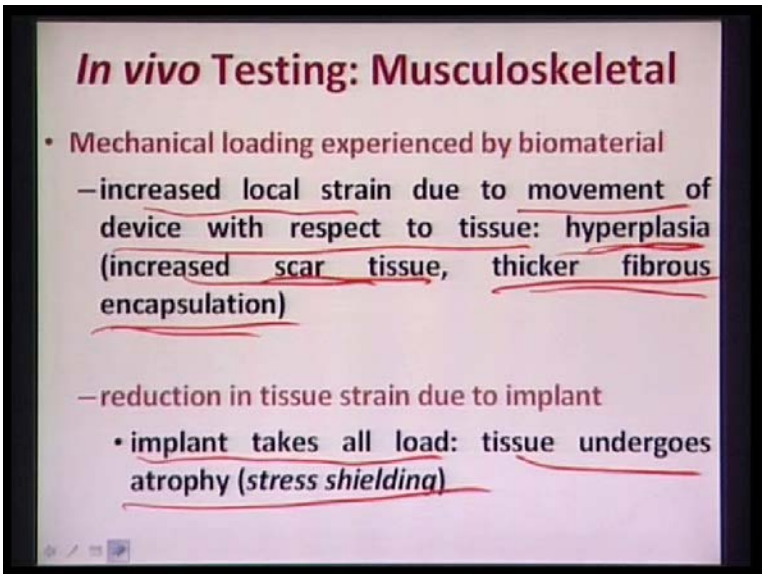

Now, it is more kind of explained here that, increased local strain due to movement of device with respect to tissue and hyperplasia means, increased scar tissue, thicker fibrous encapsulation. You remember, that is the cellular adaptation process; cellular adaptation process means, there are four types of cellular adaptation processes. One is the atrophy, that is, the decrease in cell size; one is hypertrophy, that is, increase in cell size; one is hyperplasia, that is, increase in cell number; one is metaplasia, that is, the change in cell type; like, osteoblast to osteoclast transformation, that is the called metaplasia.

Now, what is mentioned here that, you know, whenever a biomaterial is implanted inside the body, then, the cells which in the neighborhood, in the tissues, or the bones, those cells also can, can see that, some foreign material is implanted at that place. As a result, there will be, cells also will experience some kind of different surrounding environment, which is not natural; you try to understand what I am saying. So, the cells in the tissues surrounding the biomaterial, will see kind of environment, where a foreign material is implanted inside the body, and which is quite different in terms of composition and some terms of shape, compared to the natural tissue, or natural bone, if it would have been there.

So, therefore, there will be cellular adaptation process. Like, those cells which are in the neighborhood, those cells will undergo some cellular adaptation process, and the one cellular

adaptation process that I have mentioned is the hyperplasia; that is, that will increase in the cell number and if it is in large number of cells will form, then, what will happen, they will form kind of scar tissue; like, you might have heard this word scar. So, many times, some scar will appear at the patient, at the implanted area, or thicker fibrous encapsulation; thicker fibrous encapsulation means, if this is a implant, so, around that implant, there will be, fibrous tissue will be forming, and this fibrous tissue will encapsulate the implant from the surrounding natural environment, or surrounding bone environment.

Now, second point that has been mentioned here that, reduction in tissue strain due to implant. Like, if implant takes all load, then, tissue undergoes atrophy, like stress shielding. What it means, as I said that, if there is a large mismatch in elastic modulus between the tissue and the implant, and if the implant has much higher modulus than the tissue, then, what will happen, implant will carry most of the load; not the tissue. And, as a result, that, the tissue will undergo atrophy. Tissue means, the tissues which is surrounding the implant, will undergo atrophy. Atrophy means, decrease in the cell size. And, if this decrease in cell size continuously takes place, then, there will be a effect called stress shielding will take place.

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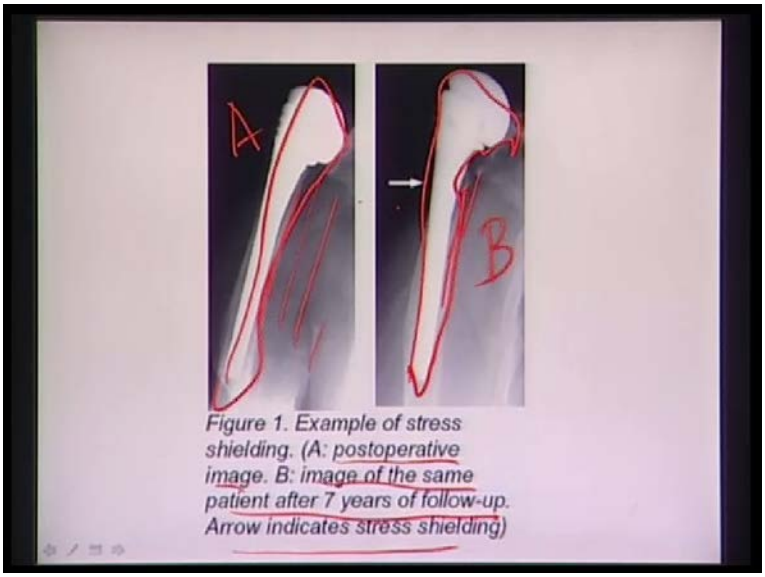

Now, these are the examples of the stress shielding. Now, A is that post operative image, that is the called A and that is the B is the image of the same patient, after 7 years follow-up. Now, this

is B. Now, what you see that, this is the implant material, right; and, this implant material remain the same, but, what is the, notice, you can see that, it is now firmly adhered through this neighboring tissue and neighboring bone; but here, it is somewhat, you know, get detached, or loosened because of this aseptic loosening factors that I just mentioned.

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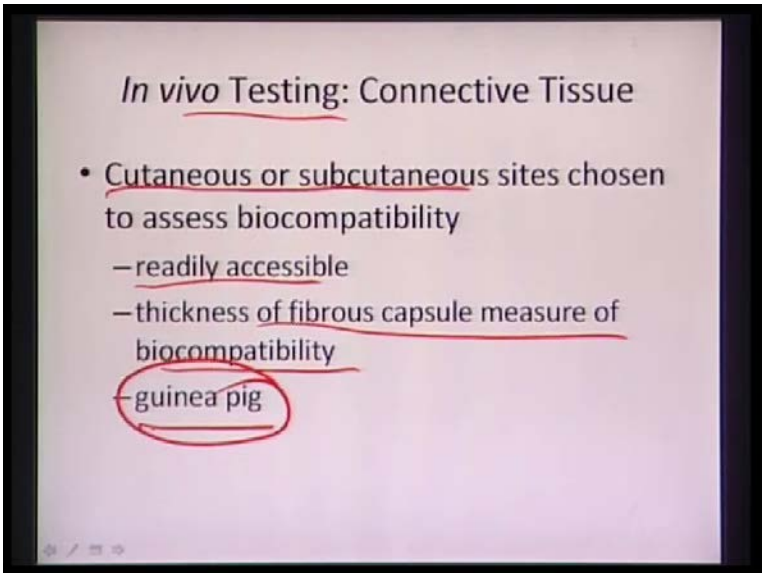

Now, connectivity tissue. Connective tissue is one of the examples of the fibrous tissue and then, that is the cutaneous or subcutaneous sites. Cutaneous or subcutaneous means, that is, you have the skin; subcutaneous means, it is just under the skin; that is called subcutaneous region. These sites are chosen to assess the biocompatibility and this type of testing is readily accessible and thickness of fibrous capsule is a measure of biocompatibility, what this mean and typically, this tests are carried out in guinea pig. What I am trying to say here that, if you want to do some implant test, or in-vivo testing in guinea pig, the best possible way, is to just put the implant just under the skin; then, you leave the animal for, let us say, 4 weeks, or 6 weeks, or 12 weeks. Then, at regular interval, you take some sample out; then, you try to find out that, around the implant, what is the thickness of the fibrous tissue that has formed.

Now, if the fibrous tissue has formed progressively in larger amount, then, the mid material is in-vivo biocompatible; but, if there is no fibrous tissue formed, even after prolonged time of implantation, that this material is not in-vivo biocompatible. But, that remember, same material

can be in-vitro biocompatible; as I said in the earlier lectures that, this is the fundamental thing, that you must remember, that if a material is biocompatible in-vitro, there is no guarantee, that the same material will be, will be found to be biocompatible in-vivo, ok.

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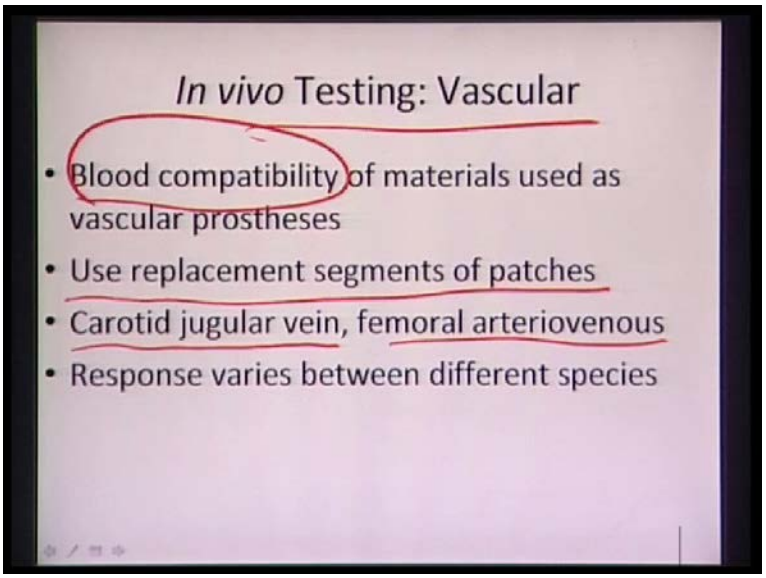

Now, in case of the vascular implants, like blood compatibility of materials used as a vascular prostheses. Now, what happens that, you know that, if you put this implant, you can put this implant as carotid jugular vein, or in some other replacement segments of the patches and for this kind of materials, it is not that, what is the thickness of the fibrous tissue that is formed, that is the indication of the in-vivo biocompatibility; instead, if the material has good bio-blood compatibility in-vivo; blood compatibility in-vivo means… In the last lecture, I have categorically mentioned that, there should not be thrombus formation, when the material will be coming in contact with the blood, and if any, the thrombus is form, then, this thrombus should be carried away from the material by some continuous blood flow. So, in those two possible scenarios, you can tell, the material is blood compatible.

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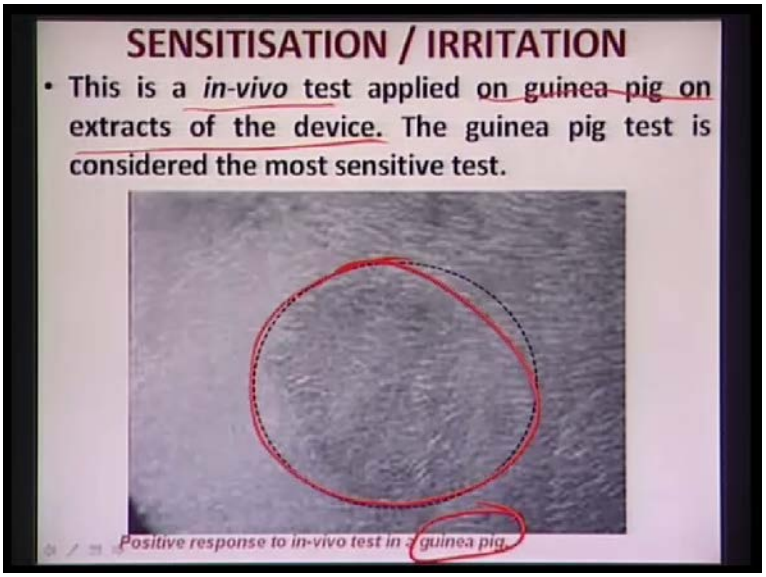

Now, this is that guinea pig experiments, just, I just mentioned. Now, what you see that, this is the typical in-vivo test applied on guinea pig, on extracts of the device and guinea pig test is considered the most sensitive test. Now, what is called extracts? Extracts means, you have a biomaterial which you can ground into very finer particles, and those finer particles, you can inject them, just as a drug delivery thing that is injected in the subcutaneous region. Now, if there is a inflammation, like the way it has been mentioned here, that this is the area, if there is a inflammation on the skin, that you can clearly take an image and see, then, this material, you cannot say that, it is a in-vivo biocompatible.

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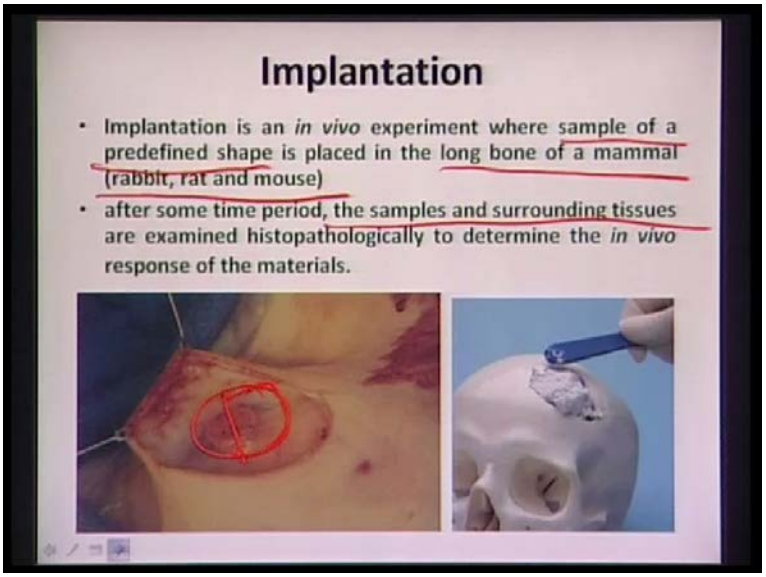

Now, implantation. So, implantation means, that is a sample of a predefined shape. Now, predefined shape means, it can be of cylindrical cross-section; it can be square cross-section, it can be rectangular cross-section; all this typical geometrical shape can be placed in the long bone of a mammal. So, typically, all these materials were implanted into a mammal, like rabbit, rat, mouse. Now, as I said that, this is the philosophy behind choosing the particular animal model in the in-vivo experiment. If a material that you develop for bone replacement, or the load bearing bone replacement materials, implants, then, the first thing, you should do the implantation test in rabbit, not in the small animal; because, small animal means, rat or mouse, their bones, that strength of the bones is not as strong as the way you get the intermediate animal model, like in rabbit.

So, this load bearing capability, or the effectiveness of this material as a load bearing implant, that you cannot assess scientifically, if you use the rat or mouse model. So, selection of the particular animal model is important in the in-vivo test. Other thing that I have mentioned, I think, couple of lectures back that, larger animal, like rat is the small animal; mouse is similarly, small animal; rabbit is little large animal; then, goes to dog, sheep and then, goes to human. Now, as the size of the animal progressively increases, the complexity of the in-vivo environment also continuously increases. Like, complexity means, the changes in pH, changes in

temperature, changes in the number of proteins available, changes in different cell types, etcetera, etcetera. In terms of biological complexity also, subsequently increases, if you go from small animal, to intermediate animal, to large animal, ok.

So, if you choose the intermediate animal model, that what we call the rabbit model, or rabbit experiments, there, it is, you can choose, you can say that, this material is the in-vivo biocompatible in the rabbit model. Again, if a material is successfully found as a in-vivo biocompatible in rabbit model, there is again no guarantee, that the same material will be in-vivo biocompatible in the human being. Unless and until, you do the test in the human, you cannot safely state that, this material can be used in the human body. You understand, this is the kind of philosophy of the in-vivo test. Now, after some period, the samples and surrounding tissues are examined histopathologically to determine the in-vivo response of the materials. That, what it means that, suppose, you implant the materials at a particular area. So, you take the cross section of the material along with the tissues. Then, you do that standard histopathological analysis, just to see that, whether in the surrounding the material, whether there are cells, or tissues, that has formed very successfully, after a certain period of time. Now, how this is done, it will be more clear in the next couple of slides.

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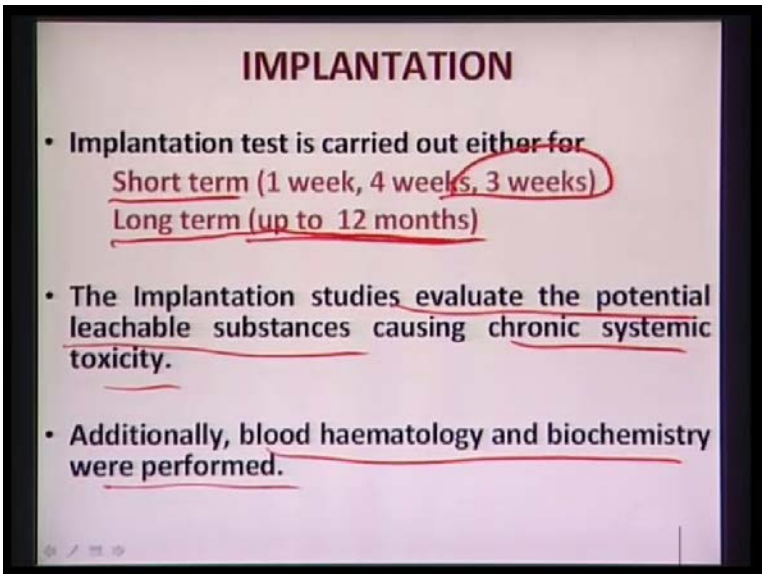

Now, first thing is that, you have to know that, according to the ISO standards, or FDA protocols, ISO means, International Standards Organization; FDA means, Federation of Drug Approval. Now, this implantation test has the two types of implants; that is that, one is the short term, and one is the long term. And, in short term, test can be done to, upto 3 weeks to 12 weeks, and then, whereas long term implantation test can, needs to be done for up to 12 months. Now, why this two tests are necessary, this two types of tests? Now, if you want to screen a certain biomaterial, whether it is in-vivo biocompatible, you can do for short term period and long term period. What happens, if some materials, some leaching or biodegradation takes place over the long term period, then, that cannot be assessed, if you do short term period like upto 3 weeks, or 4 weeks, or upto 12 weeks. Therefore, if you do the long term test, then, you can be more sure that, this material is in-vivo biocompatible for the long term basis. So, implantation studies also evaluate the potential leachable substances, using chronic systemic toxicity and then, blood haematology and biochemistry are also performed in many cases.

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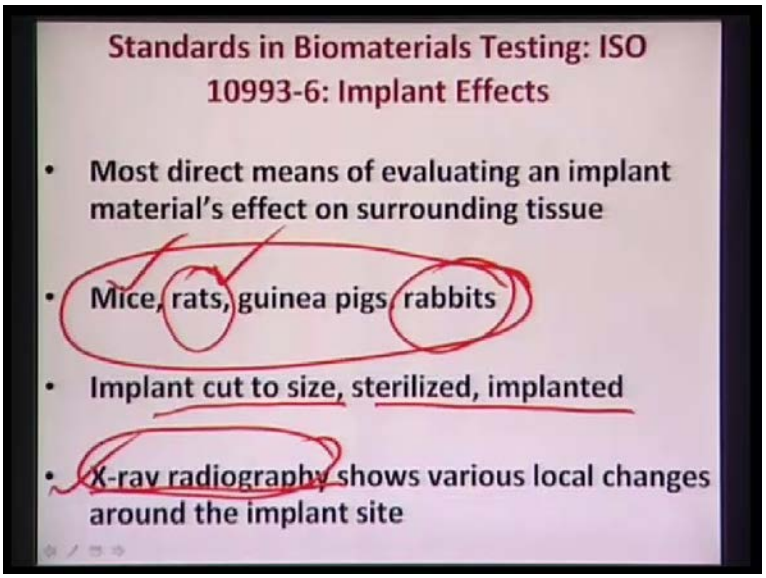

So, as I said that, different animal model which can be used, or which are used, like mice, rats, guinea pig, rabbits. As I said, the rabbit model typically used for the kind of materials, like ceramic based materials, or biopolymer based ceramic composites. Those kind of materials, rabbit model is used; however, if the material is entirely polymer based, where the mechanical

properties is much inferior compared to ceramic base materials, in that case, smaller animal models like rats, or mice also cannot, can be used. Now, implants, this is first cut into size, sterilized and implanted, and then, x-ray radiography needs to be carried out. And, this x-ray radiography means, like, you have to take that x-ray image; just like, you know, you take x-ray image for that, when your leg is fractured, or your hand gets fractured; similar x-ray image you have to take at the implant site. You have to store it. Then, you have to see after 1 week, this is the x-ray image; after 3 weeks, this is the x-ray image; like that, you can compare this x-ray image.

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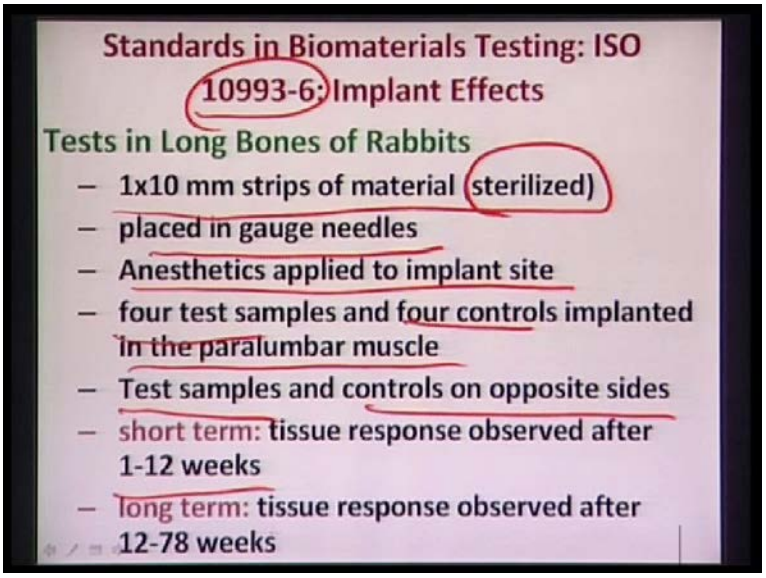

This is like, you know, standard protocols; what is defined as that ISO 10993-6; that is, the implant effect and test in the long bone of the rabbits. Now, this is like, 1 into 10 millimeter strips of the materials that you can, that can be cut and sterilized; this is placed in gauge needles. Anesthetic is applied to the implant site. So, it is like, you know, every test is done in a OT, like Operation Theater. So, you have to give anesthesia to the, local anesthesia to the animal at that site and then, four test sample and four controls of the each composition needs to be implanted in the paralumbar muscle. So, what it means, like, you have to use the test samples like, your materials which are testing; at the same time, you have to put the control samples also.

Now, why control samples is necessary? Control samples means, the samples where you already know, that is the in-vivo biocompatible; you understand what I am saying? So, that, with respect to the control sample, you can now assess, whether a given material, or the material that you have developed in your laboratory, is in-vivo biocompatible or not. So, that is why, the control samples is necessary. And, you already, you have seen that all, in the in-vitro experiments also, that is the always positive control, or negative control sample, is always used. Now, test samples and controls on the opposite sides; that means that, if you take one leg of the rabbit, you put your test samples; if you take another leg of the rabbit, then, you put your control samples. So, these are like, on the opposite legs. Then, short terms, as I said that, there are 1 to 12 weeks; long term, you have to do this tissue response upto 78 weeks.

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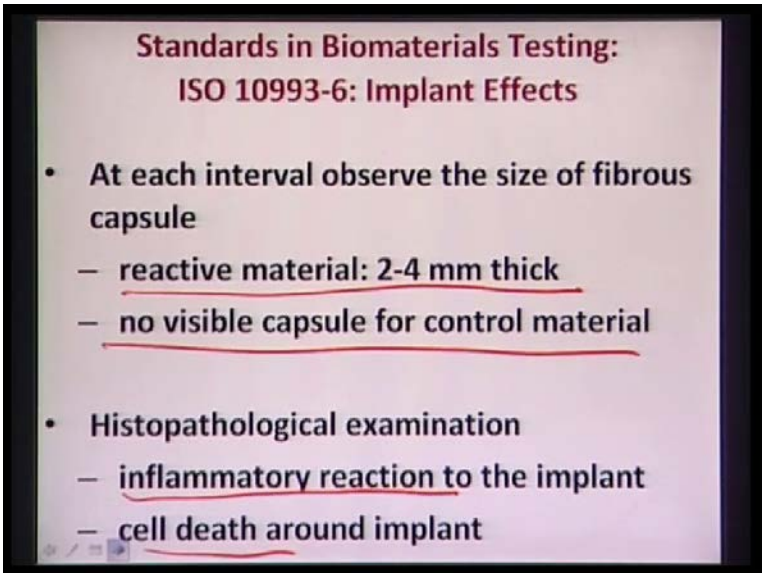

Now, at each interval, now, what would be the interval that you will be observing? That depends on, whether it is a 1 week, 4 weeks, 8 weeks, 12 weeks, or it will be 1 week, 4 week, 12 weeks. Now, this reactive material is typically 2 to 4 millimeter thick. What is reactive material here? That is the, just the material and the tissue surrounding, just adjacent to the material, that is called the reactive material. And, no visible capsule on the control material. And, histopathological examination will actually tell you that, what is the inflammatory reaction to the

implant, and whether it is a cell death around the implant. Now, if that is a cell death around the implant, then what will happen, that, the material cannot be used in the real life applications.

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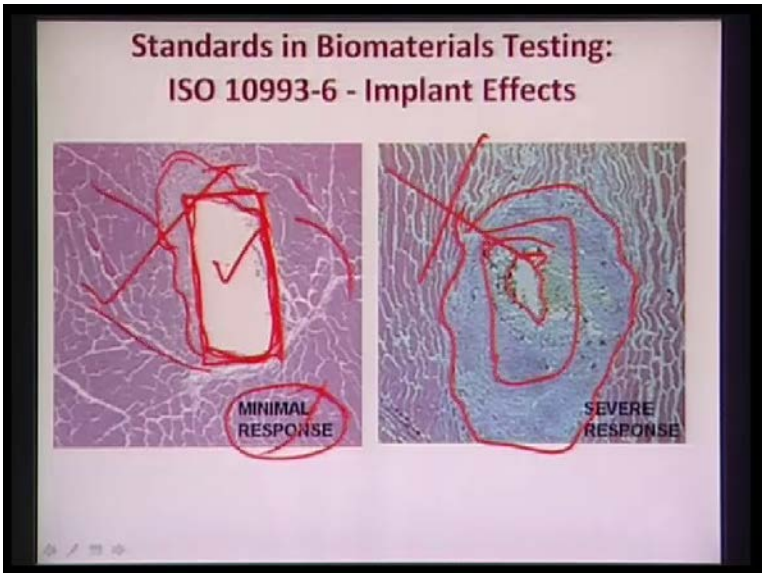

Now, this actually tells you that, how you can qualitatively assess that, whether a material, or whether two different materials, how good, or how bad they are, as far as the in-vivo biocompatibility is concerned. Now, you see, this is your implant materials. This, quite contrast materials, these are, it is the different shape. What I presume that, possibly this material, when it was implanted, this was like a perfect geometric shape, of a rectangular shape; but, these edges actually indicate that, irregular edges actually indicate, that some part of the material has been leached away during the in-vivo implantation. This is the surrounding tissues around the implants and if you see that, this is the region, where this fibrous capsule has formed around the implants. Now, it is called minimal response. So, response is not extensive.

But, a composition, similar shaped material of different composition were also placed here, and what you can see, entire material has been dissolved in-vivo; you are left with, only this small parts of the material, and there is an extensive inflammation reactions around the implanted zone. So, that means, this material, you cannot use; this material, you can accept as the (( )) in-vivo biocompatible. Now, you understand that, how, qualitatively you can assess, whether two different materials, which one you will choose for the in-vivo biocompatibility.

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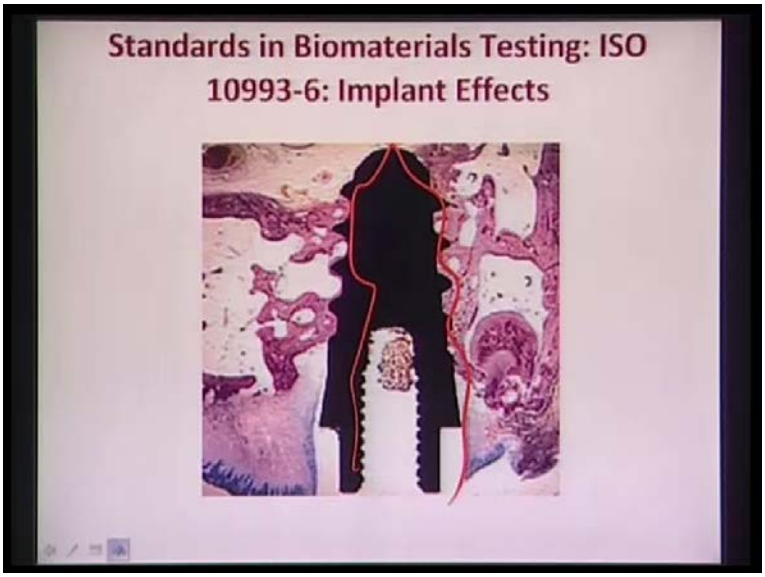

This is like, you know, dental screws, which are actually put it inside the, you know, as dental restorative applications.

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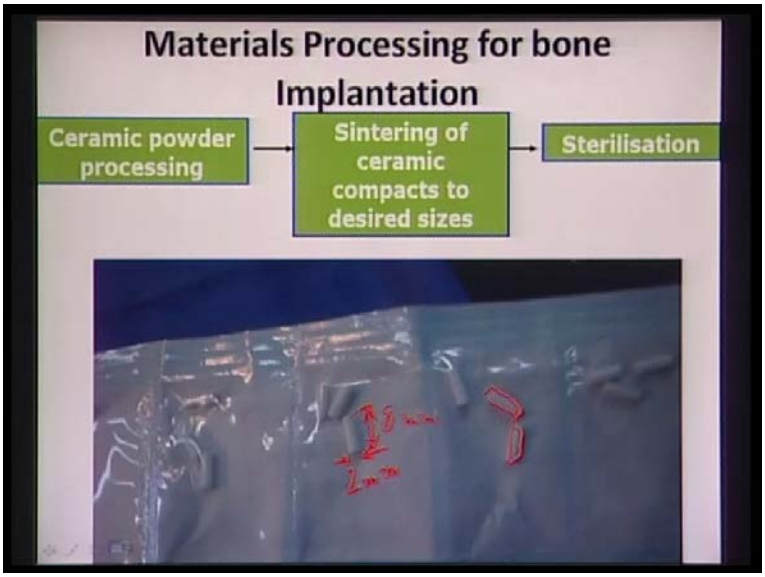

Now, I will show you, some of the experiments that we recently carried out. Now, these are the material shape, typically we used in our experiments. As you can see, these are like, cylindrical shaped specimens, and I know, their diameter is around 2 mm diameter. So, it is extremely small

size diameter, a small size and this is, aspect ratio is around 3 to 4 aspect ratio. So, 2 millimeter diameter and 8 millimeter length, that is, in cylindrical cross-section, those samples were used in this in-vivo experiments.

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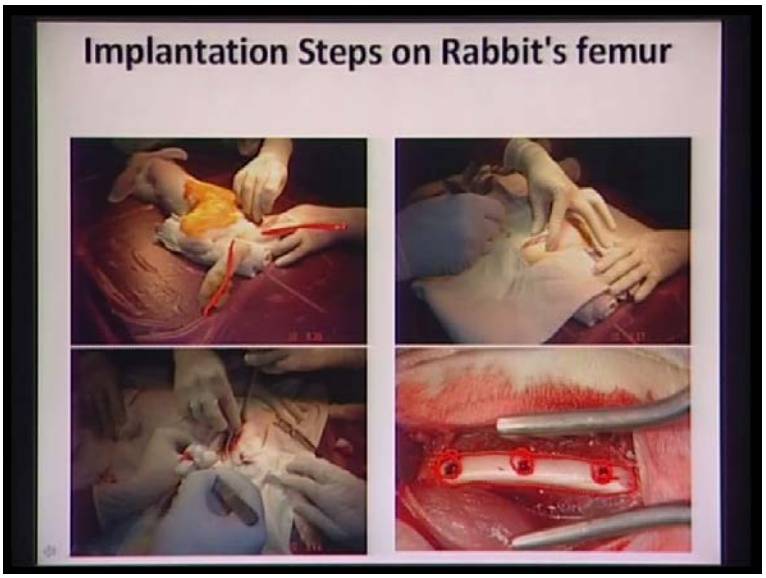

Now, these are the experiments which are going on in rabbit. Now, this is the long bone of the rabbit. As I said that, this long bone, you have to put some holes here. These are like, three different holes; and these holes, the moment you drill the holes, there will be also blood coming out, right, through this holes; and you have to put the material here, at three holes in one leg; this is one leg; that is the another leg, which you cannot see very clearly. So, one leg, you put the rabbit; another leg also, you put the control specimen.

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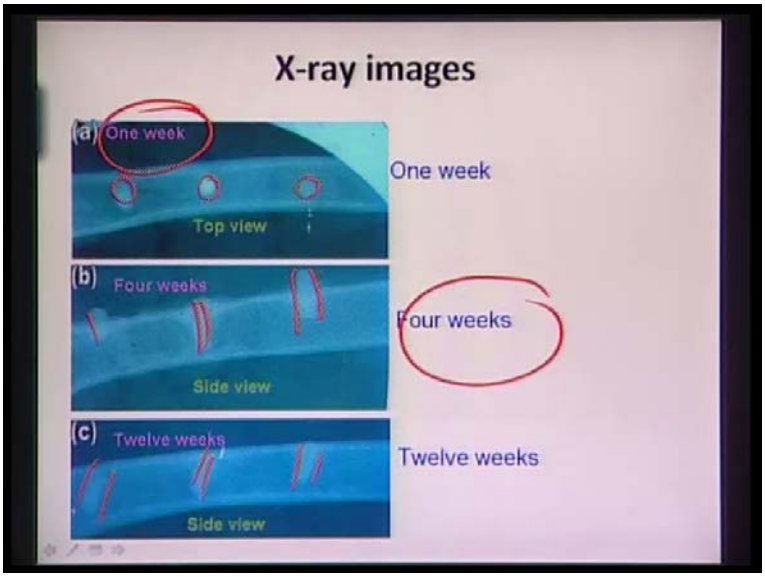

Now, what you do here that, after 1 week, this is the x-ray images. Now, what you can see, this x-ray images, this is your sample; you can clearly see your samples, right; that is your hydroxyapatite, mullite, or whatever samples it is; after 4 weeks, you can clearly see the samples here; and after 12 weeks, you can see the sample is almost of the same size. What it means that, there is no visible in-vivo degradation from the samples. If it would have been degraded in the in-vivo environment, you cannot see the same 2 mm diameters' cylindrical specimen after 12 weeks; because then, samples would have (( )) to half the diameter, or half the length, whatever it is, ok.

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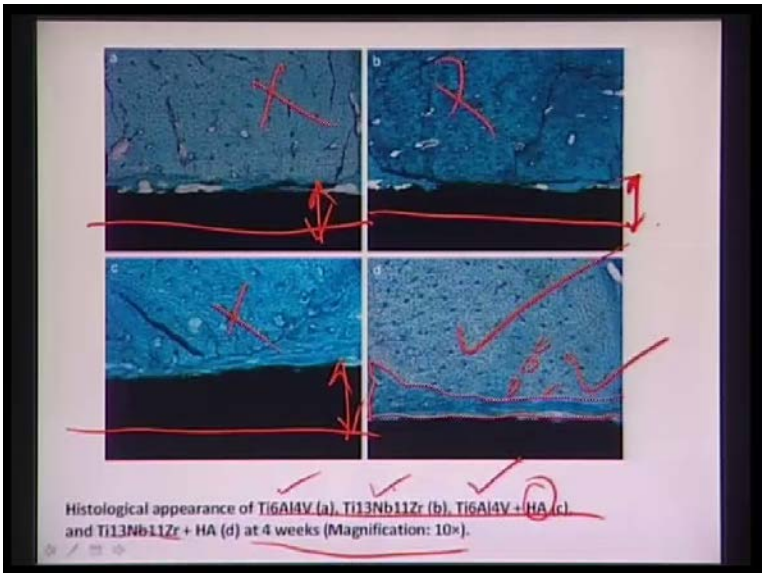

Now, this is one of the way to find out that in-vivo degradation. I will also show you, before going into more details about the recent results, I will also go through the, some of the literature results, where people have done several experiments, on all different type of materials. Now, titanium based alloys, as I mentioned, titanium and titanium based alloys, they are highly desirable for this stem applications of this Total hip Joint Replacement. Stem means, that is the long rod. And, why titanium is preferred? I said that, from the point of view of the corrosion and wear, titanium is more corrosion resistant, compared to stainless steel stem, for example.

Now, if you see here that, after 4 weeks, if this materials are implanted in an animal model and these are like four different composition; one is, Ti 6 Al 4 V, that is titanium 6 percent aluminum 4 percent vanadium; Ti 13 Nb 11 zirconium, that is titanium 13 percent Niabium 11 percent zirconium; Ti 6 Al 4 V, that is titanium 6 percent aluminum 4 percent vanadium plus hydroxyapatite means, hydroxyapatite means, this is, is in the form of hydroxyapatite coating. Then, you have this Ti 13 Nb 1 zirconium plus hydroxyapatite, like, you have this same alloy, but with hydroxyapatite coating, after 4 weeks. And, what you see in this, Ti 13 Niabium 11 zirconium plus hydroxyapatite, here, it does not show much toxicity response, and you have this very fibrous tissue forms and you can see, there is different cells; these are like osteocytes, and osteoblast cells. But, in most of the other cases, the same size implant, because the implant size

were the same in all the cases, ok. However, this is the region, this is the region that, the material has been dissolved in the in-vivo environment; that means, all three materials you cannot use that well; these materials, yes, you can be consider, you can consider it as a in-vivo biocompatible.

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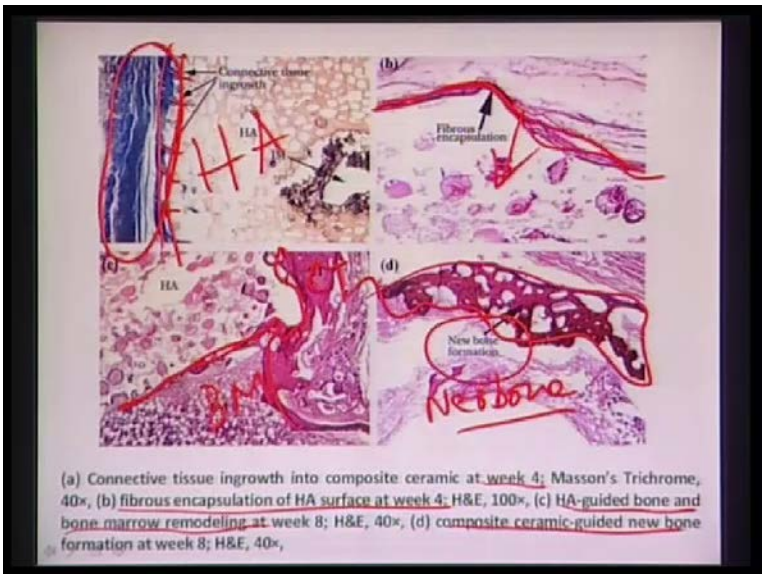

This shows that connective tissue ingrowth into the composite ceramic at week 4 and this is the fibrous encapsulation of hydroxyapatite week 4, and hydroxyapatite guided bone and bone marrow remodeling at week 8, and composite ceramic new bone formation at week 8. Let us go through one by one. Now, this one, actually connective tissue. Now, this connective tissue, you can see that, after straining, this connective tissue actually is growing inside the hydroxyapatite; this entire material is your hydroxyapatite; this entire region is your natural tissue. Now, fibrous encapsulation means, as I said that, if a material is in-vivo biocompatible, around the material, there will be a fibrous tissue and this fibrous tissue will be formed and they will encapsulate the material from the surrounding natural tissue or bone. The third one is that hydroxyapatite bone growth. Now, this is the bone marrow and this is the cortical bone c t and you can see that, there is a nice bone formation even after 8 weeks and this is shown as the newer bone formation, newer bone or new bone formation at the implanted region. So, hydroxyapatite is well known as the in-vivo biocompatible material and this is the proof that, yes, under the in-vivo conditions, even after 4 weeks, or 8 weeks, you can clearly see that new bone formation.

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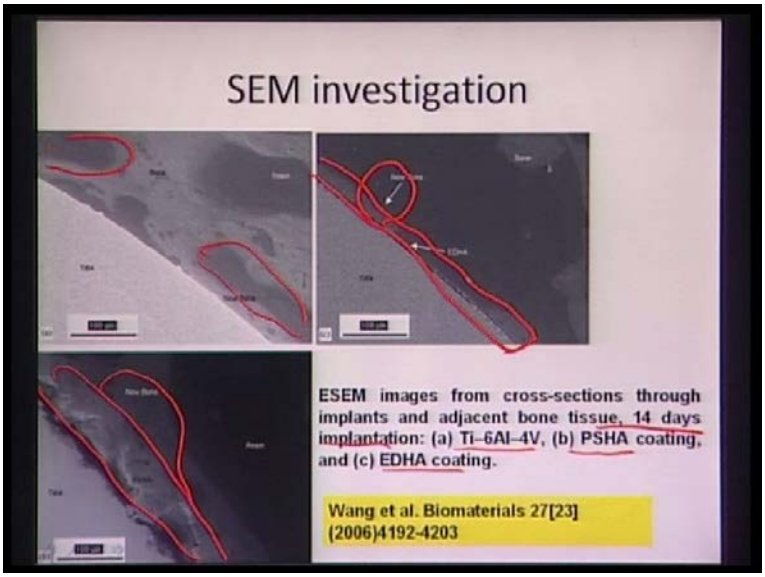

So, this is after 14 days, or 2 weeks of the implantation, on different materials like T i 6 Al 4 V, PSHA coatings and EDHA coatings. Now, in all this cases, you can see, that is a newer bone formation and this is also the region bone formation. Now, Ti 6 Al 4 V, this is your implant; this is the region, that is the, where newer bone has been formed; this is also that, another Ti 6 Al 4 V and on that, there is some coatings, hydroxyapatite coatings is there and this region, newer bone formation. So, what has been shown here that, hydroxyapatite, putting the hydroxyapatite coating on this titanium 6 Al 4 V also enhances this bone growth, or bone formation in-vivo.

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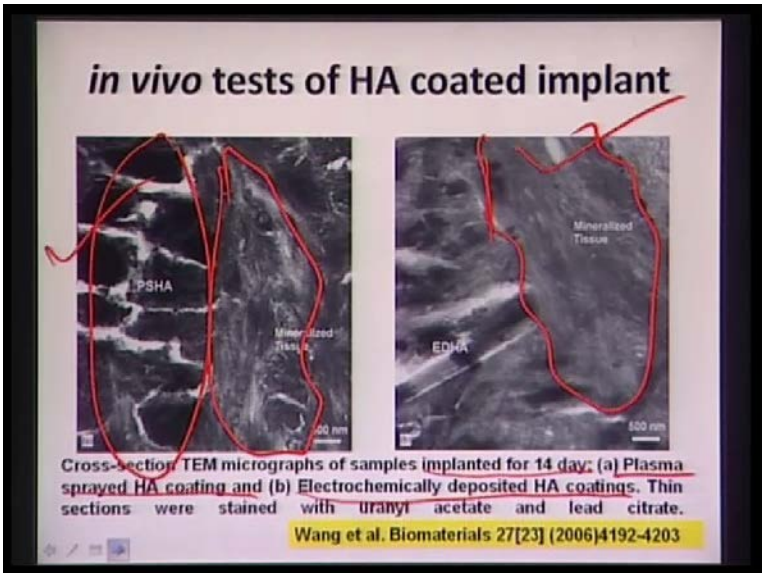

This is the Transmission Electron micrographs and this Transmission Electron micrograph essentially tells you that, what happens after implantation for 14 days. So, here, the two materials were investigated. One is the plasma sprayed hydroxyapatite coating, and another one is electrochemically deposited hydroxyapatite coating. So, plasma sprayed hydroxyapatite coating, this one; electrochemically deposited is this one. Now, this is your coating structure and this is your mineralized tissue. Mineralize tissues means, like, this tissues has lot of hydroxyapatite content. Mineral means, that is again the inorganic component of the bone, that is the hydroxyapatite; and, this is the mineralized tissues which are formed here and that, you can clearly see after the staining.

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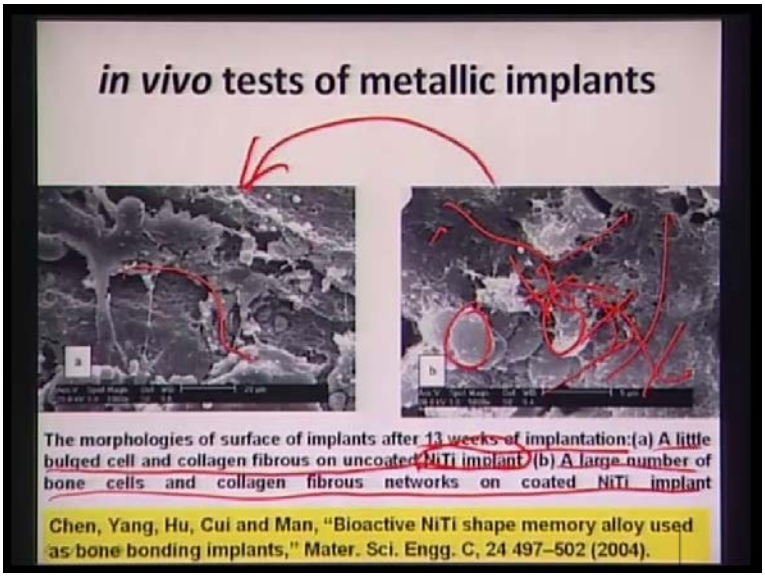

Now, there are several other metallic implants, that which are used in the in-vivo condition; that is, Nickel titanium. This is the shape memory alloy, Nickel titanium. And, this Nickel titanium implants, when it is put it for 13 weeks of implantation in a particular animal model, then, what you see here that, large number of bone cells and collagen fibrous networks that you can see and the little bulged cell and collagen fibrous on uncoated nickel titanium implant. Now, here, you can see large number of osteoblast cells and this fibrous networks you can see; this fibrous networks are essentially collagen fibers. So, both the collagen fibers, osteoblast cells, if they are present in the neighborhood, that will clearly indicate that, you are essentially enhancing the extracellular matrix formation, as well as the bone forming cells are activated, so that, you can have newer bone formation. However, if you compare this, with this, you can clearly see that, when you are, when you do not put in hydroxyapatite coating, then, you do not see that well, the bone cells, different bone cells, as well as this collagen network is also not that much prominent, or more visible, or more extensive, like that in the presence of the hydroxyapatite coating.

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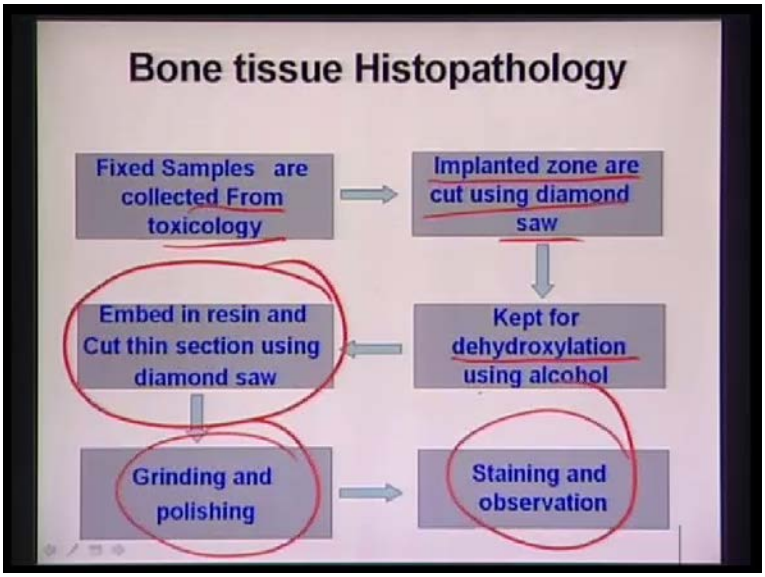

Now, how this bone tissue histopathology, that is carried out in real life? Now, you have to first get the samples in the toxicological lab. Then, implanted zone are cut using diamond saw. So, using the diamond saw, you can cut the implanted zone. Then, you have to do it for the dehydroxylation using alcohol. So, that means that, serial dilution using alcohol you do the, you get rid of that, all the water molecules which are adsorbed. Then, you embed in resin. So, you put it in resin and then, you cut the cross-section-wise, using the diamond saw. Then, you can grind and polish these cross-section samples and subsequently, you can stain and you can observe them under the microscope. Stain means, it is just like etching of the metallic materials. Stain means, if you do etching in the metallic materials, ceramic materials, you can see grain boundaries. Similarly, if you stain, that means, you can use some particular chemical reagent and those chemical reagent actually will stain certain cells, or the extracellular matrix, or the actin filaments, or the nucleus, or the mitochondria. Depending on what kind of stain you use, what concentration of stain you use, you can clearly see those cells and their particular features.

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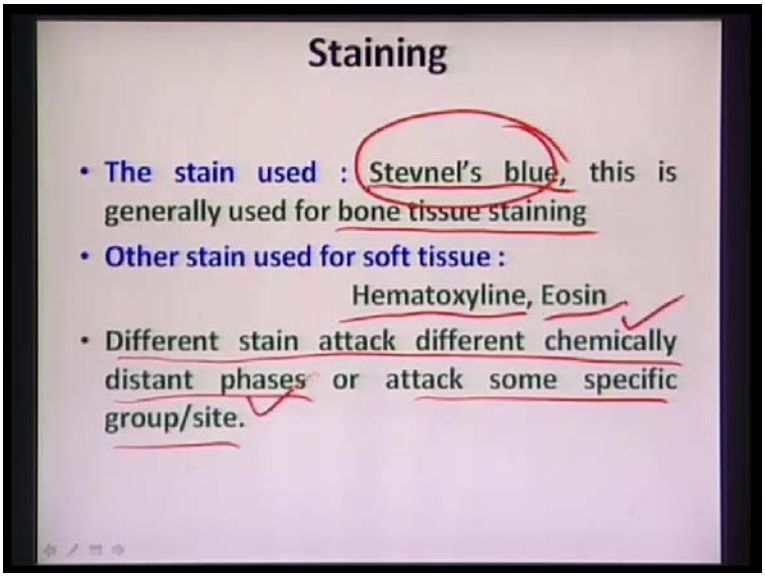

So, typically, the stains those are used in this histopathological investigation, this tells the stains that are widely used as Stevnel's blue. This Stevnel's blue, it is generally used mostly, for this bone tissue staining. And then, other stain used for soft tissue is that, eosin and hematoxyline. The soft tissue of, this soft tissues, these are like, different tissues like, these are not like hard tissues, or bone cells; for the bone tissue engineering, this is always Stevnel's blue, which is widely used. Now, as I said just few minutes ago that, you know that, staining agents is just like etching or etchants in case of the metals. Now, different stain, they attack differently, chemically distant phases, or attack some specific group, or site. What it means? It means that, like an etchant, in case of metals and ceramics, they also chemically attack the different grains and then, they will reveal the grain boundaries. Similarly, here, this stains attack different chemically distant phases; like, mitochondria has different chemical environment, compared to the nucleus per per se.

So, nucleus will stain differently, compared to mitochondria, or nucleus will stain differently, compared to the extracellular matrix, or the actin filaments of the cytoskeleton; because, all this phases, although they are bound in the same cell, but, they have different chemical composition and accordingly, they will be attacked in a different manner by the stains.

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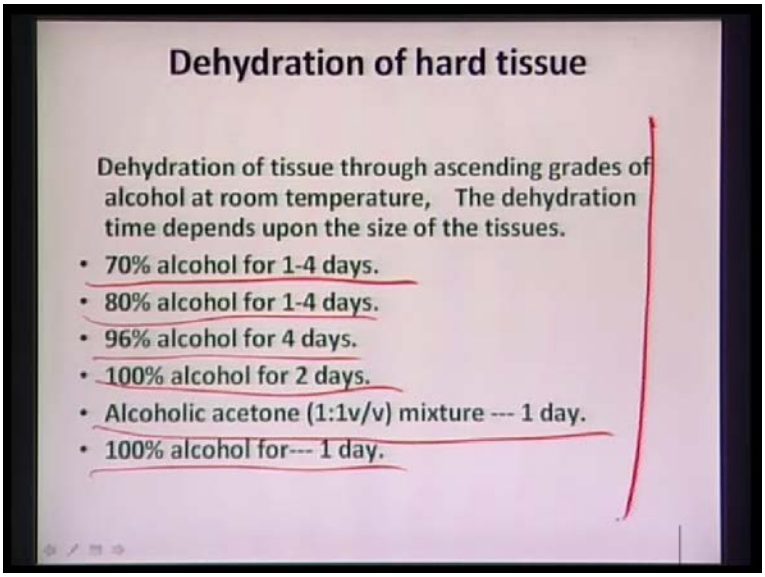

Now, what is the dehydration? How this dehydration takes place? This dehydration, first you have to, you know, use the 70 percent alcohol for 1 to 4 days; 80 percent, 96 percent, and 100 percent alcohol for 2 days and then, alcoholic acetone mixture, that is 1 to, 1 is to 1, that is 1 day, and finally, 100 percent alcohol for 1 day. So, that is the way, you can dehydrate, or dehydroxylate this entire tissue material interface sample.

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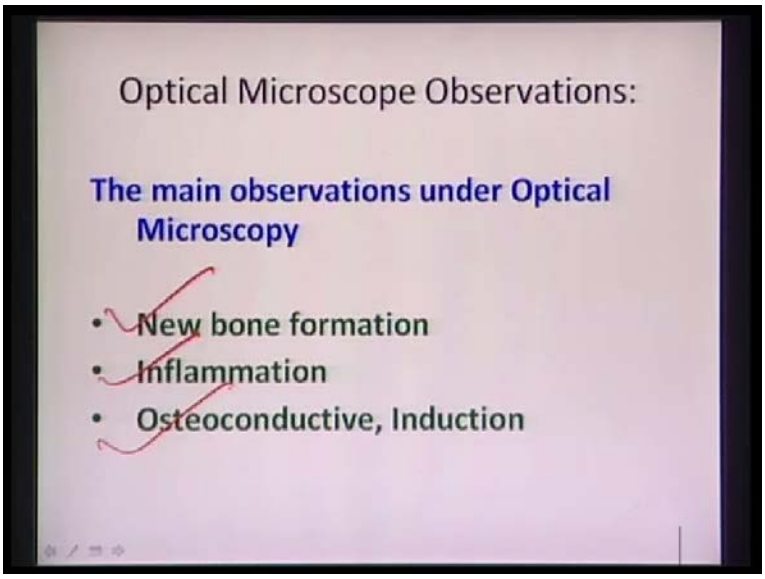

Now, what are the things normally you should look for, when you look this bone tissue samples under the microscope for in this optical microscope, or fluorescent microscope, or what kind of microscopy, or SEM. Then, you want to see first, this new bone formation, whether that take place around the implants, that is the number one; number two, whether there is an inflammation around the implant, and number three, whether there is a bone is the osteoconductive and it has also induction property.

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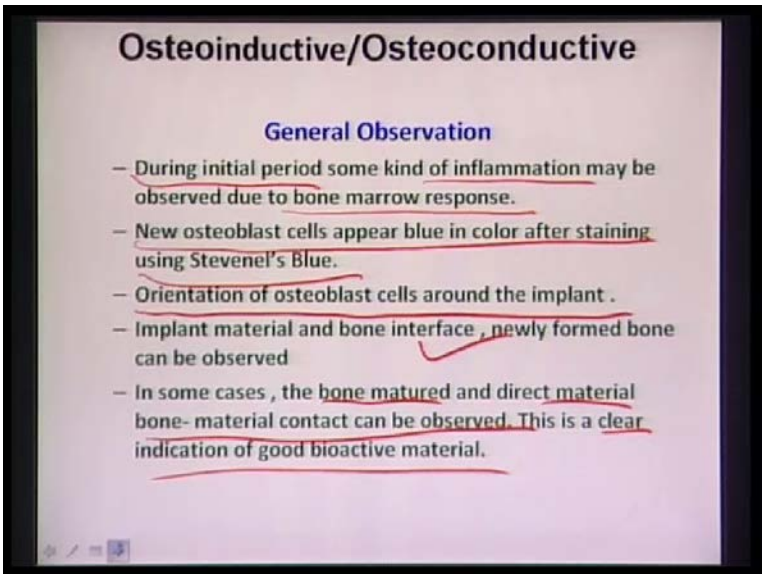

Now, some general observation is that, during initial period, let us say, some, many times, what people have reported in the literature, like, it is a first, after the initial first 1 week, some kind of inflammation may be observed and that is due to the bone marrow response. But this inflammation should not sustain for 12 weeks, or 78 days. This will go away as you give more and more time for the animal to heal from that wound. Essentially, whenever you cut an long bone, essentially you are wounding, or you are giving some wounds, or you are imparting some wounds to the animal. And then, every wound healing process biologically will takes time. Suppose, you get some, you know, your, you are hurt in this, in your legs. So, there will be blood clotting and everything.

So, this blood clotting will not go just 1 hour; it will take may be 1 day. So, similarly, in the animals also, this wound healing process takes place, possibly a week or so. And, new osteoblast

cells, they appear blue in color after staining using Stevnel's blue and you have to also see that, what is the orientation of the osteoblast cells around the implant. Osteoblast cells are not like, you know, epidermidis cell lines, like, they are just lined one after another. So, they are like, connective tissue cells. So, they are dispersed in the extracellular matrix, but, they are this, they are actually, there are a lot of distances between this 2 or 3 osteoblast cells. Now, also, you have to see that, implant material and the bone interface, that is the new bone formed, can be observed; and, in some cases, some matured bone, or direct material, bone material contact also, can be observed. This is a clear indication of a good bioactive material. Bioactive material means, bioactive in-vivo, then, this kind of observation you can make.

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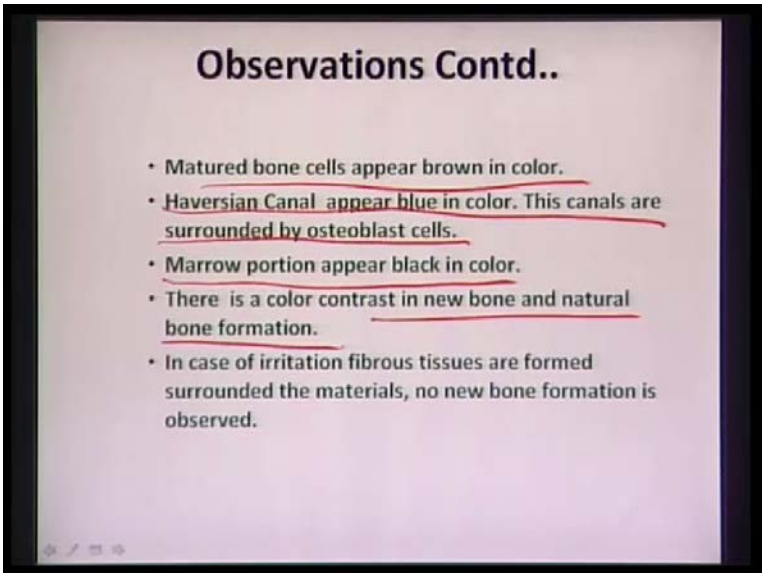

Then, matured bone cells appear brown in color. Then, if you see that, matured osteoblast cells, then, you see the different colors and Haversian canal appear blue in color; this canals are surrounded by osteoblast cells. Now, what is the Haversian canal? Haversian canal is like a, more or less like a cylindrical channel like (( )), through which nutrients and blood and foods are supplied to the cell. Marrow protein appear black in color. Now, all this color sequence, you have to know that, how they will appear in the fluorescent microscope. Then only, you will be able to identify the different images. And, there is a color contrast in the new bone and natural bone formation also. That is important, because, how you can see that, whether it is new bone or

natural bone. Natural bone means, what it has existed before implantation and new bone means, what bone formation has taken place, after you put the implant, or the particular animal cell.

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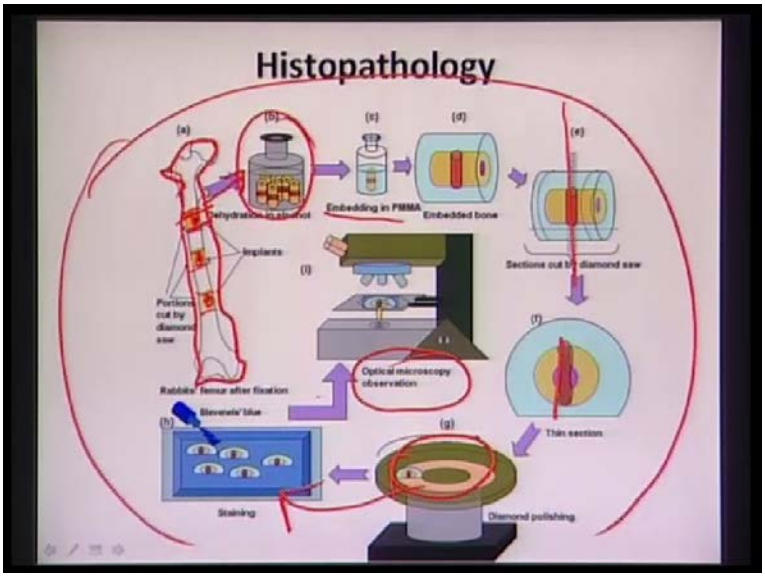

Now, this is the entire histopathalogical protocols, you know, you can, one has to follow, to get to study this kind of in-vivo biocompatibility aspect of biomaterial. Now, what it shows here, this is a long bone. As I have described in last lecture, that is, a long bone typically has a large aspect ratio, like, it has a large length to diameter ratio. Now, in the long bone, you are cutting at different region and then, you put your implants. This is the red circle, that is the implants you have put in the long bone. And then, after that, you take this long bone, that you can cut it by the diamond cut, diamond cutting machine. Then, you put it in the dehydration, that is in alcohol. So, that is like serial dilution, that you, that you give the treatment to this bone tissue sample, serial dilution. Then, you embed it in some kind of PMMA, that is, poly methyl methoxilate, that is a polymer.

Then, after you embed, then, you cut the cross-sections by that diamond saw, and if you cut this cross-section in the diamond saw, then, essentially, you are seeing that, both the implant and the implant bone interface. Then, you take a thin section. Then, you polish it in that standard polishing well and then, you do the staining. This staining is done by Stevnel’s blue, because of the bone tissue engineering. And, if it is for soft tissue engineering, other stains like eocene can

be used. Then, you can use the fluorescent microscope to observe this, what are the different features of the neighboring bone and then, neighborhood of the implant. So, that is the entire protocol for this bone tissue engineering, or histopathological response.

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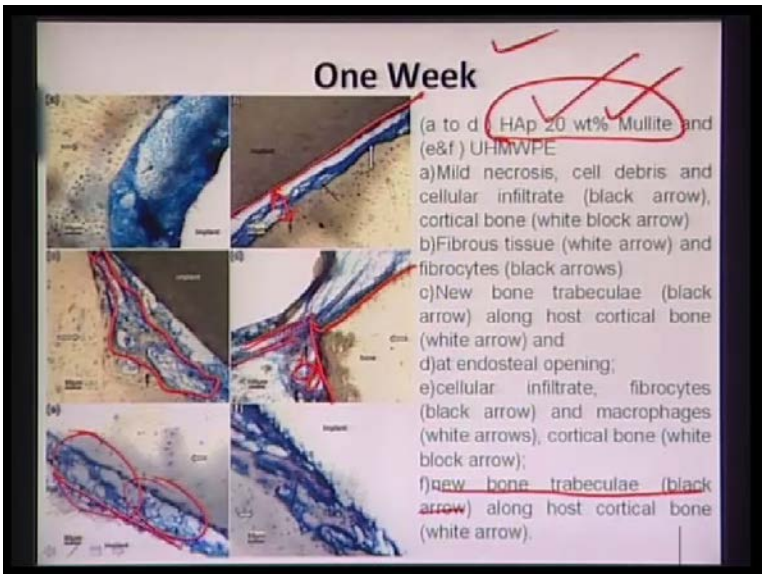

Now, I will show you some of the results that were obtained for this hydroxyapatite-mullite composites. Now, these tests were carried out (( )) medical science and technology, that is in Trivandrum down south, and that test was done for short term implantation, like upto 12 weeks. Now, after 1 week, this the hydroxyapatite 20 percent mullite; because, why we have chosen this of particular composition because, this particular composition given a stated better combination of mechanical properties and better combination of the in-vitro cytotoxicity, bone mineralization, etcetera.

So, therefore, we thought that, we will do the test only on this hydroxyapatite 20 percent mullite, because, when you are doing this in-vivo experiments, then, you have to also be careful; because, there is a animal ethical committee, or animal welfare committee. You cannot use capital n number of samples and you cannot kill that capital n number of animals in, during that, any in-vivo study. You have to minimize the number of animals that you want to sacrifice, because of your scientific study. So, therefore, the selection of a particular material for in-vivo tests, also

needs to be made very carefully, with some scientific rationale, or with some logic. So, therefore, we have only chosen this particular composite and what you see here, this is your implant.

Now, around 50 micron, that is some tissues are forming and this is also at different, you know, different, after doing this Stevnel's blue, you can see the connective tissues, as well as fibroblast cells around the implant materials, which is formed. And, these are the examples or newer bone; this is the new bone trabeculae, that is the black arrow; and then, new bone trabeculae means, that is, the trabeculae means, spongy bone, and that spongy bone structure you can see here; that is a like a mould like a (( )) structure. So, as you know, the trabecular bone, spongy bone and cancellous bone, they are synonymous words, right; they carry the same meaning.

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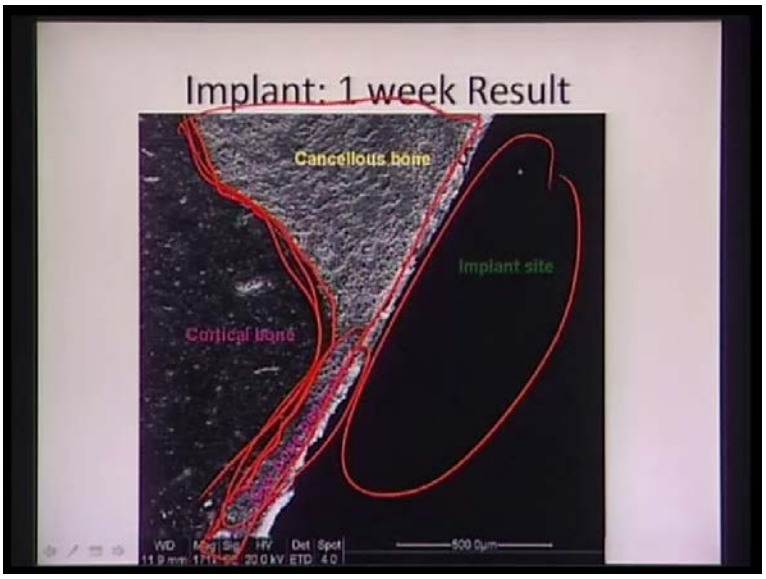

Now, here, you can see, this is the like, mould like cancellous bone that forms. So, and this is the newer bone that is formed in that, and this is the cortical bone and this is the implant site.

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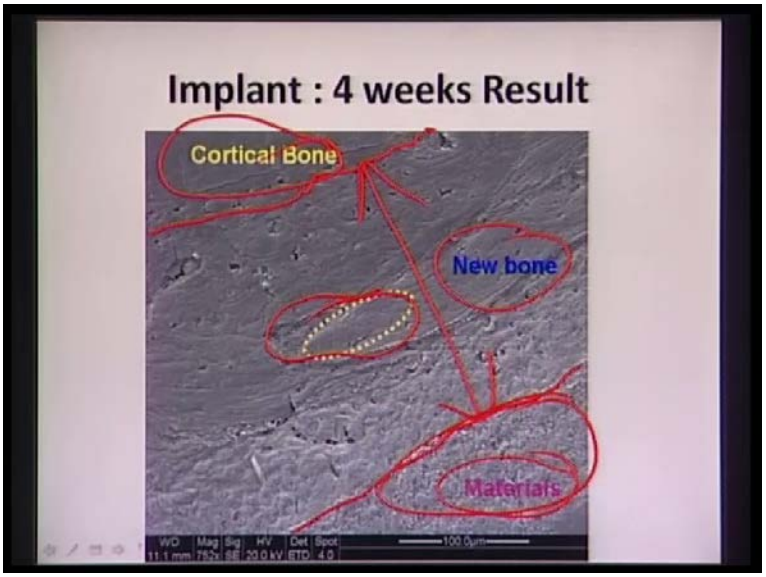

This is after 4 weeks; what you can see here, this is your material; this is your new bone. There are some regions, where the Haversian canals are there and you have this cortical bone, that is your natural bone. So, you have this natural bone; this is the entire interface, that is the bone material interface, and this is your material. So, that means, this material shows good in-vivo bio compatibility.

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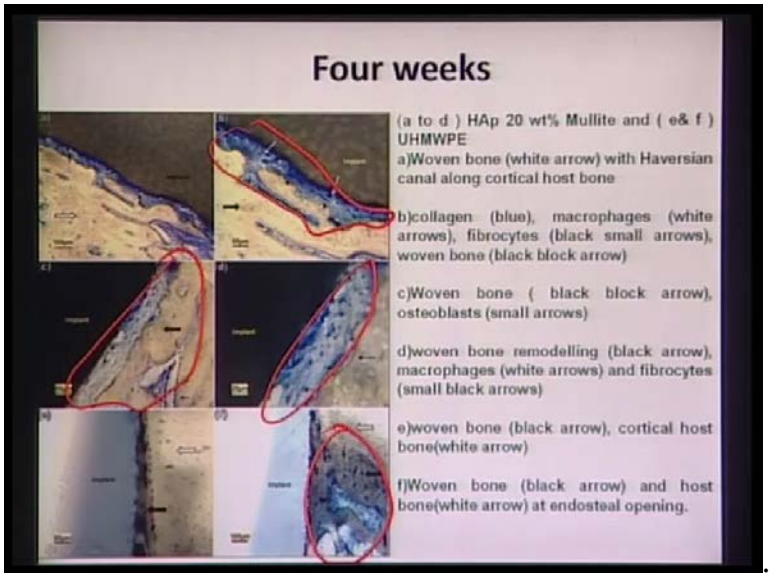

And, you can see here that, around this implant shown here and this part, you can see that clearly, that tissue formation and there are also multiple difference cell types, which stain differently in the, when you use this Stevnel's blue; you will see some blue color cells; also, you can see some differently colored cells, here.

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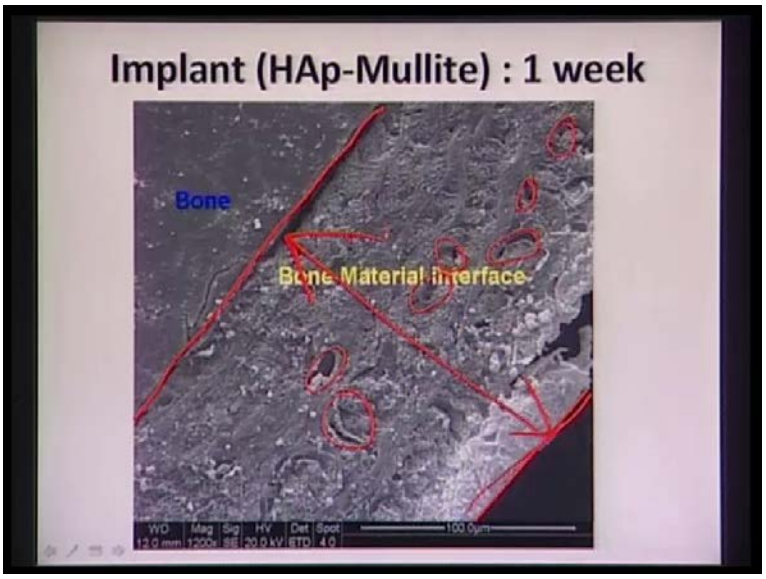

This shows that, you know, how this bone material interface, they look like in the Standing Electronic Micrograph. If you closely observe, then, you can see, there are some canals here, right, and these canals are essentially, what I called as that Haversian canals. And also, this interface is continuous; you do not see any gap in the interface, or there is any loose part in the interfacial region.

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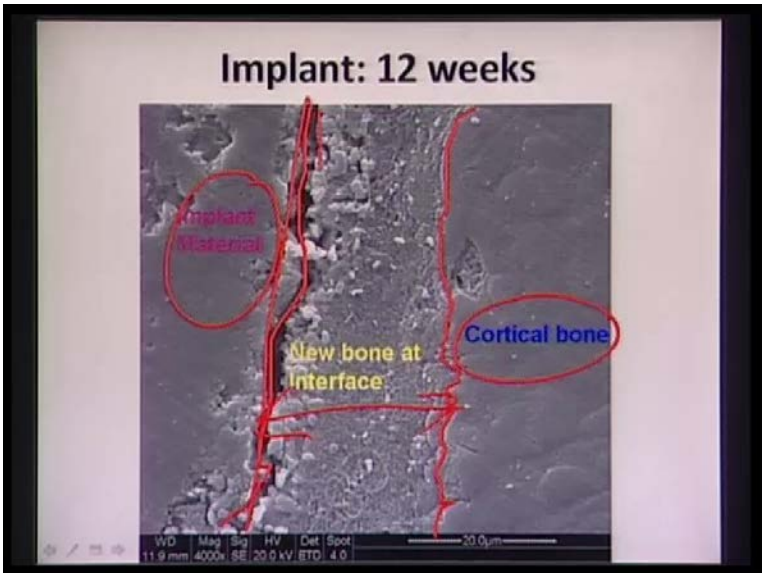

This is again, some new bone formation and this cortical bone is formed, and this is your implant material. Often, if your sample preparation is not good, then, this is a brittle material, you are handling. You can cause some crack at the interface. These cracks are not necessarily formed, because of the any in-vivo reactions. So, you have to also analyze it carefully; because, you are handling the ceramic based material, which is brittle. You are handling some bone tissue at the interface, that is also brittle. So, if your sample preparation is not done with extreme care, often, you do see some kind of cracks at the interface.

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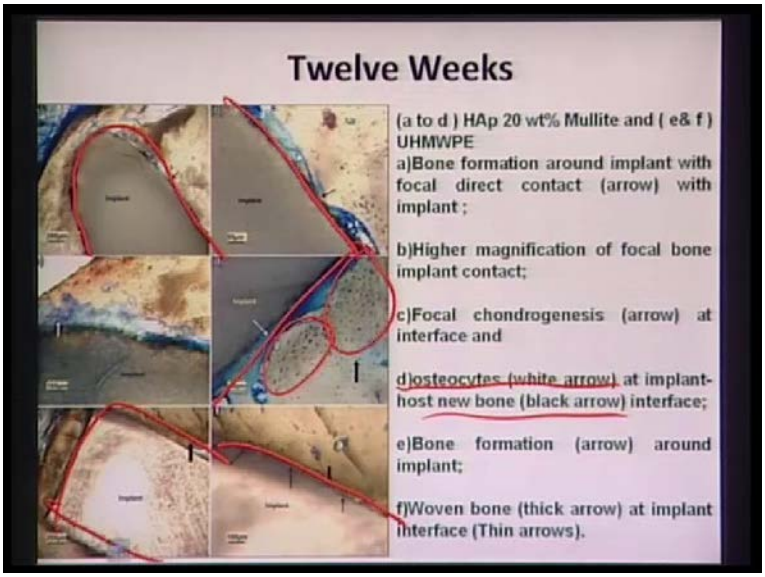

This is after 12 weeks, and 12 weeks also, you can see, this is, this is the shape of the implant, and then, from this shape of the implant, you can notice that, this material does not undergo any in-vivo biodegradation process. And, these are like different cells, like, these are like osteocytes, osteoblast cells. Now, osteocytes is the white arrow and new bone is the black arrow, in different images, that is fluorescent images, that you see here.

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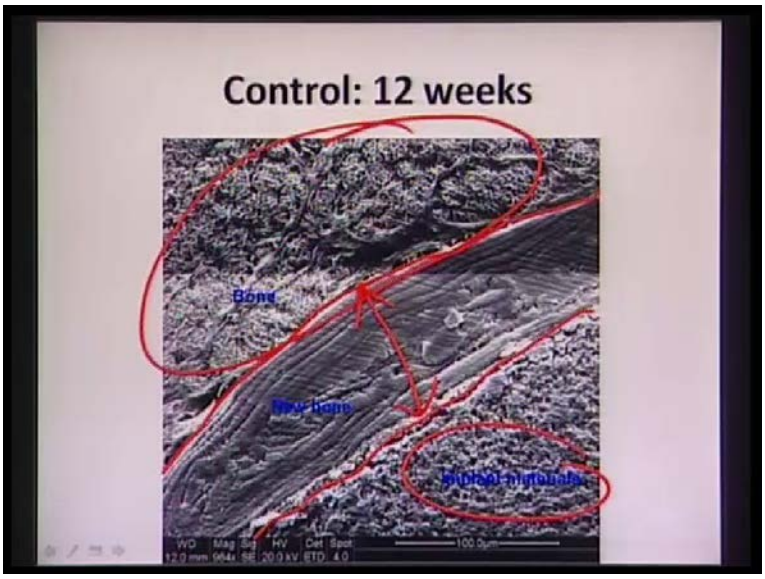

This is 12 weeks. This is very good picture. What you can see, this is a material; this is your new bone and that appears in different contrast and this is your, actually, natural bone. Natural bone, again, natural bone contains, both trabecular bone, as well as the cortical bone. Typically, the surface, outer surface is cortical bone; inner surface, inner area is the trabecular bone, or the spongy bone structure.

So, I think, I have finished here, that is, the discussion on the in-vivo implantation. And then, in the next lecture, I will start with some of the results that you have, for experimental results, that we have got on this hydroxyapatite based different biocomposite and some of the polymer ceramic composites.