Biomaterials for Bone Tissue Engineering Applications
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Module 8
Lecture 38

Welcome to this module on this biocompatibility of 100 % strontium substituted silica alumina, (phos) P2O5, CO and CF2, glass ceramics. So what I am going to do in this particular module is to show you that, what how to conduct the pre-clinical study in the rabbit model using this femoral defect model and also, what is the, how the osseointegration of any material can be qualitatively and quantitatively established in, quantitatively established using an array of techniques which include, histology, micro computed tomography and so on and so forth.

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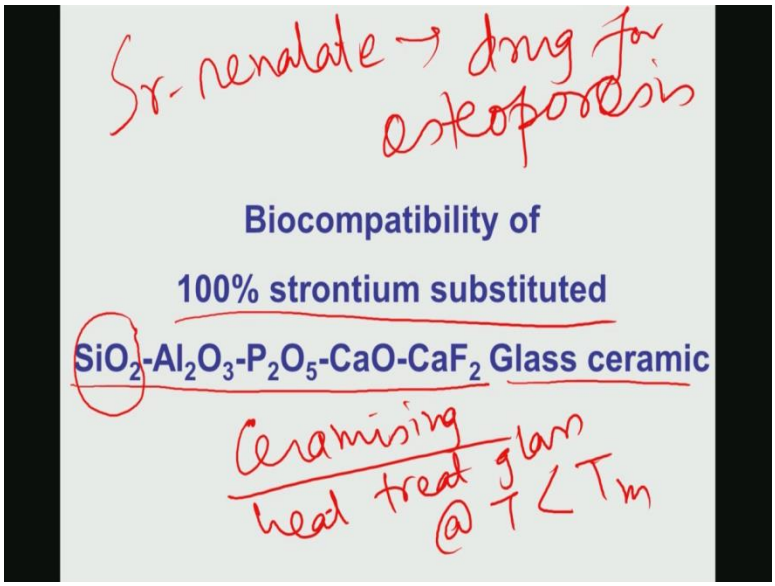

So first I will start with, briefly about this glass ceramic materials. In one of the earlier modules, I have mentioned, this glass ceramic materials let me iterate that. Glass ceramic materials is derived from a glass composition which contains certain characteristic shape of the crystals or which contains finite volume fraction of crystals with particular size and shape and this crystallisation of the glasses is possible because of certain heat treatment technique, which is known as ceramising treatment. So let me once again emphasize this technique called ceramising treatment.

Ceramising treatment essentially means you heat, you heat treat the glass materials, heat the glass at T less than much lower than TM, TM is the melting temperature of the glass and this process will involve the ceramising process will involve the nucleation and growth of the crystalline phases within the glass matric itself. So any specific composition may not be a glass forming composition. You need to have some composition which is called, which should have glass forming ability. Like silica for example, SIO2 has a glass forming ability. So, and this particular material also contain silica, now the question is that, when Y2 use strontium and Y 100% strontium substituted glass ceramics. Now strontium is one of the materials, I think in one of the earlier modules I had mentioned that strontium renalate is one of the commonly used drug, so it is a most commonly used drug for osteoporosis treatment.

Now to briefly mention what is osteoporosis. Osteoporosis is essentially bone loss and this bone loss essentially triggered because of the larger population of the osteoclast cells and it is more than the osteoblast that is the bone forming cell. So that osteoporosis is essentially triggered by the osteoclast cells. And as a result that that older patients actually they have much weaker bone and so on. So many orthopaedic surgeons they normally prescribe this commonly used drug that is strontium renalate and in spite by the use of strontium renalate there is some limited research activities in my group as well as elsewhere in the world where people have tried to dope certain glass ceramic materials with strontium.

The idea is that rationale for developing this kind of material is that, that when the glass ceramic materials when it will undergo in vitro dissolution process, then slowly they will release strontium also. Now the strontium can strike a healthy balance of the osteoblast cells in one hand, between osteoblast and osteoclast cells. So therefore the use of strontium can be rationalised to treat osteoporosis treatment.

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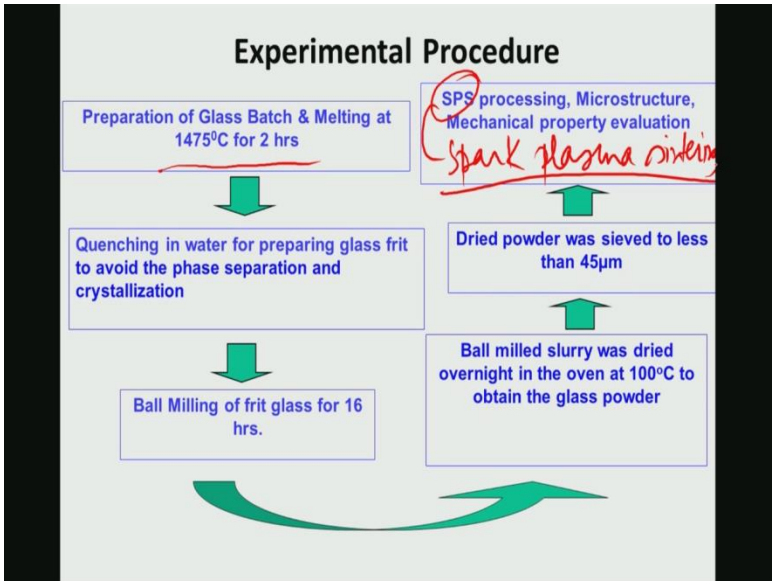

So having said that this particular glass ceramic materials were sintered by the falling root. So essentially glass melting takes place at 1475 degree for 2 hours and as I said, in the last, in one of the earlier modules that typically in a multi component glass composition one needs to use sequential melting or repeated melting technique to homogenise the glass composition. So this frequent melting (oppe) frequent melting is used to homogenise the glass composition and once this glass material is done, glass melt is prepared in a conventional furnace then you can simply quench the glass composition to prepare a glass frit to avoid phase separation and crystallisation.

And once you get the glass frit you can just ball mill it like in a conventional (())(5.39) process for 16 to 24 hours. Then after that you ball mill it and this powder, ball milled powder you can dry it and the dried powder is sieved and then you can do spark plasma sintering, SPS stands for Spark plasma sintering or you can do conventional sintering just to get the glass ceramic materials.

Now once you get the conventional sintering, Spark plasma sintering the next step is to do ceramising. So once you make this glass materials ceramising treatment will help to develop the crystalline phases in the glass matrix.

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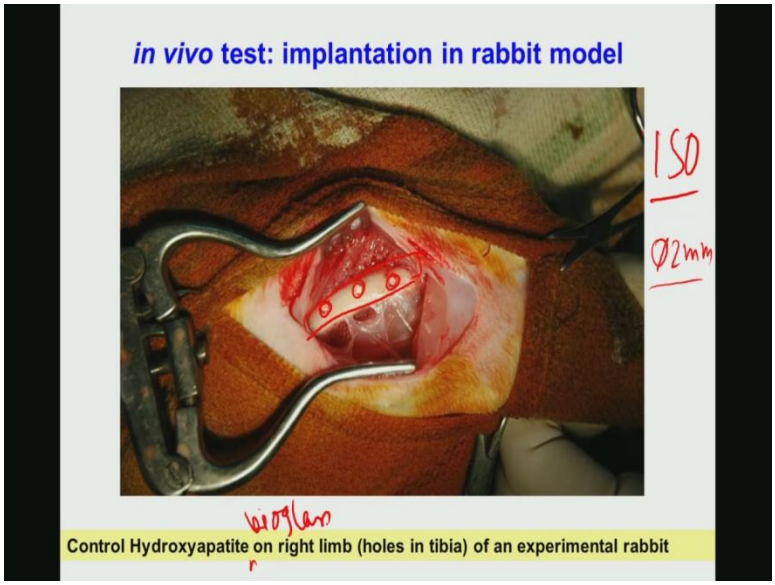

Now without spending much time, on this physical properties and other properties of the glass materials, I will be restrict, I will restrict, this module on the in vivo biocompatibility of this materials. So what you see, this is that femur of a rabbit and in this femur what you see is that, there are three holes, it is like evenly spaced holes not like very to (ofe) too close. So evenly spaced holes, spaced holes were drilled, using the precision ultra-microtome and this within microtome you can make that (())(06.50) 2 mm, that is as per the, you know ISO Standard. So as per the ISO Standard 2 mm holes is drilled and the length of the rod or the cylindrical rod that goes inside which is made of glass ceramic materials, it is typically 5 to 6 mm in length.

So this kind of dimension is to be closely fitted into this femoral defect and this, in this femoral defect, that, after that, after you put the space, the next stage is just to stitch the wound. The, once the wound is closed, you have to take care of the animal sufficiently and appropriately so that you can observe their natural behaviour of the animal. You can monitor the weight change of the animal after this implantation is done, and you can also see any abnormal activity and inflammation which you can see, very easily from outside, just for the macroscopic view. All those things you do, and then, after that desired time period, that, you can sacrifice the animal as approved by the Institutional Animal Ethical Committee, animal committee. So this is shown that, you know that, one can do this hole in the hydroxyapetite bio glass.

So this is that, and in every animal (expe) every animal testing or pre-clinical experiments I have explained to you before with sufficient details, that you have to use some control implants. The use of the control implant is to make sure that you do this, you implant the control, implant in the same animal itself so that you can compare the osseointegration behaviour of a material which is already proven to be biocompatible with that of the test implant. In the present case test implant is your 100% strontium substituted silica alumina phosphate with glass ceramic and your control implant is the hydroxyapetite bio glass materials.

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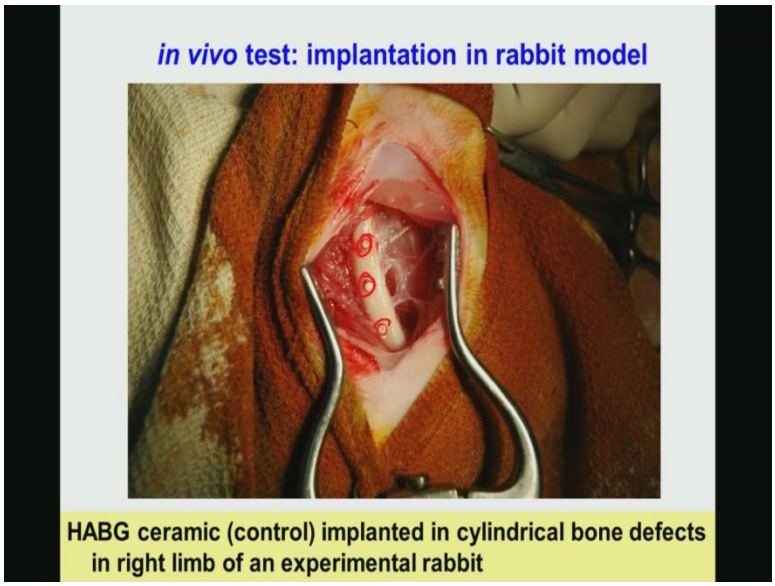

So this is what you can see, another view of the bioglass material which is used in the control implant that is closely fitted into the femoral defect cavity and in this femoral defect.

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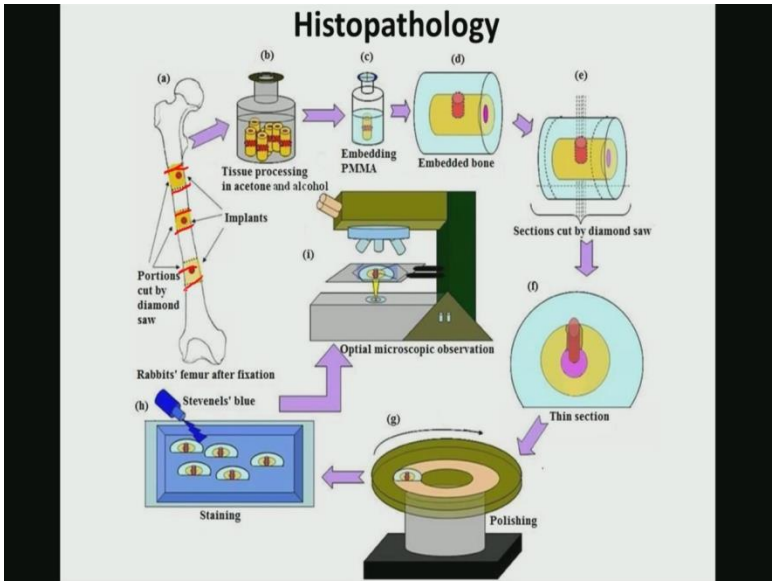

After this, all this test, implantation is done, animal is scarified then you take the explant and you take a small tissue sample, in this and then you can do further processing like tissue processing like, you do embedding, embedded bone, then sections cut by the diamond saw, then you make thin sections, you can polish it slightly then you can stain it in a (())(9.37) blue staining region and then you can do fluorescence micro optical microscope observation.

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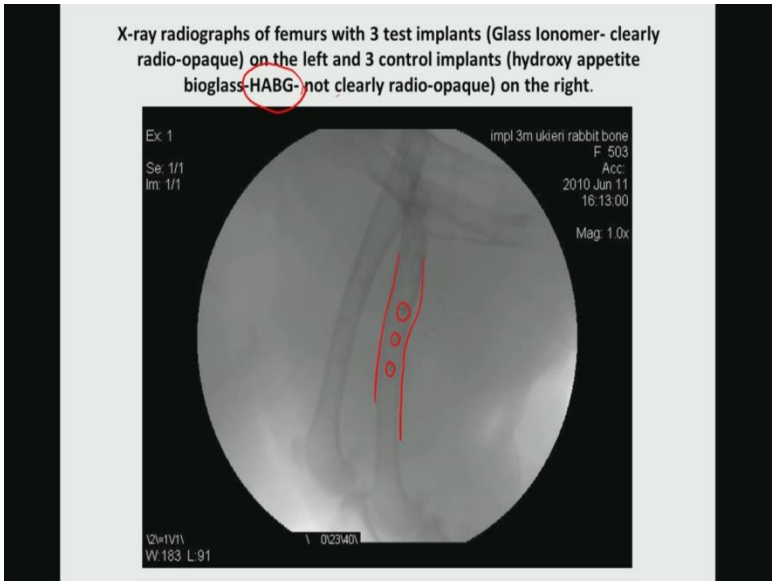

Now X-ray radiographs of the femur, this is shown, you can see then how this femurs are being placed, this is one implant, this is second implant this is third implant and from these difference in the density of this X-ray this one that you can see that how this material can, you can identify. So in this particular case, hydroxyapetite bioglass. That is one of the material with known, one of the material with a proven biocompatibility property that is not radio opaque. Whereas glass Ionomer or this particular, this particular silica based glass, these are actually clearly radio opaque.

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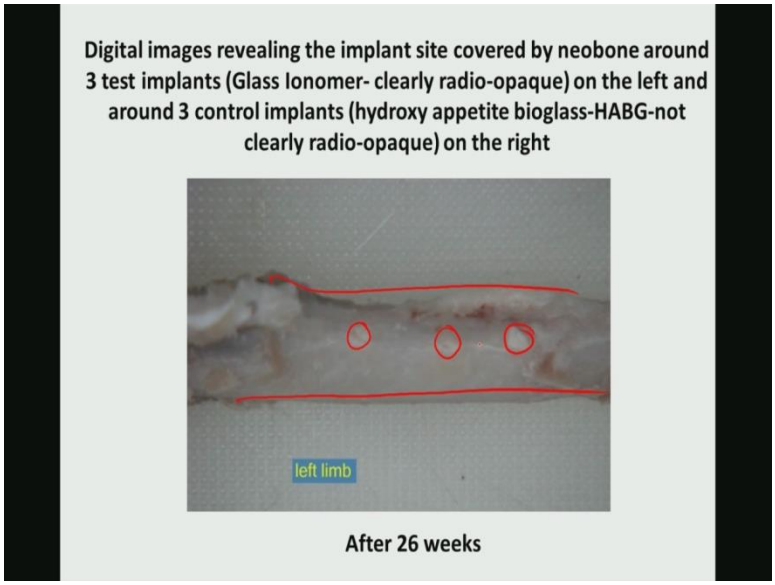

This is the macroscopic image of this explant and then you can see the three implants you can, not very clear but you can somehow able to identify the three implants are there and around that the bone has been regenerated so that it kind of, it shows that, integration without histology section it shows first indication or first (())(10.50) of the, the osseointegration of this materials.

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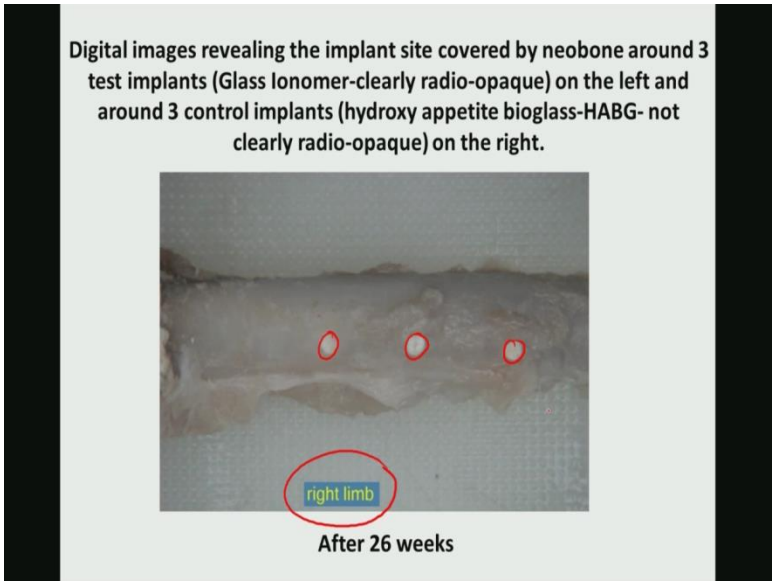

This is more clearly seen because one is radio opaque and one is not radio opaque, you can see this three implants, very clearly, seen along with this femur, and you can see that there is no obvious gap or there is no obvious discontinuity around the implant, this is the right limp. The other things I must emphasis here is that, as far as possible all the control implants and all the test implants are to be implanted in the same animal, in the same strain itself.

So like you cannot do like three implants, in one animal although the specious is same and you cannot do in the next animal the three other implants, simply because the moment you change the animal, their health status, although they belong to the same specious, same strain the health status may be different their age may be different, their sex maybe different. So if all these things change, it is not fair on somebody's part to compare the biocompatibility property of the test implant or the control implant.

Now, to avoid any extraneous influence which is not related to material itself, it is important for somebody to put the test implant and this control implant in the same animas.

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This is the histology images, now you can see, this is how it is, how it shows that this is your thin slice and this is your implant here. And around the implants you can see that, all the cellular activity here and you can also see that this implant is not very dense, you can see that there is also porosity in the implant.

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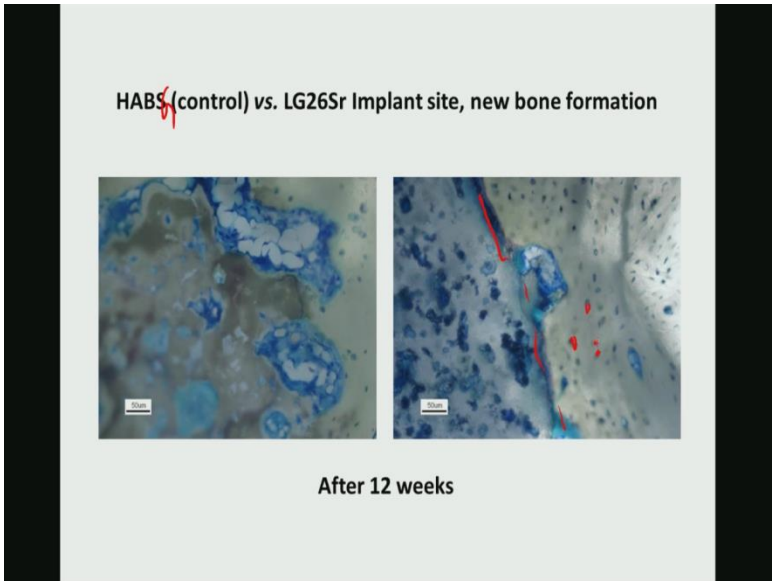

The same is seen can be seen in the test implant side, new bone. This is like, more histology images, and this histology images essentially show you that this histology images essentially

show you that there is some place here, and you can see this is some kind of indication of osteoblast cells around the implantation around the bone implant interface.

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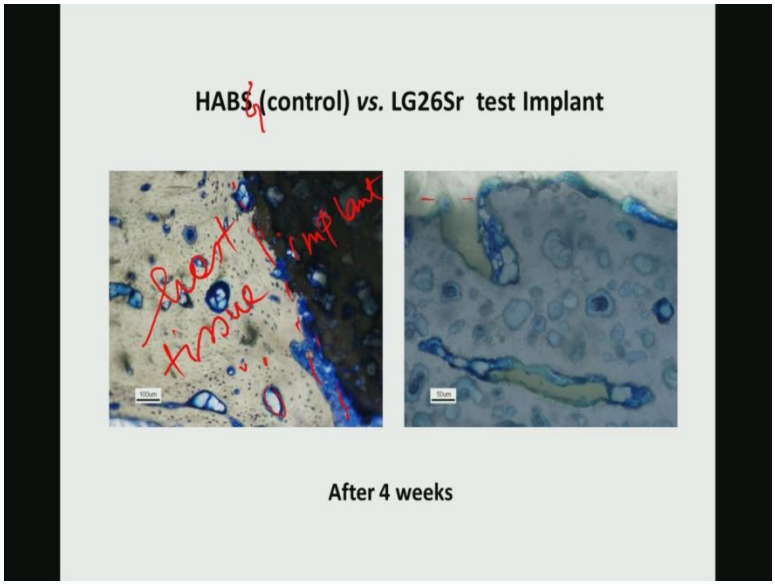

So this hydroxyapetite bioglass composition again you can see that how this cells and then host tissue, and then this is your implant part and this is your host tissue part, you are just seeing the, you are just seeing the cross section image here in the optical microscope, so here you can see, that there is a very continuous, implant, continuous interface and also see certain cellular features in that host tissue around the implant.

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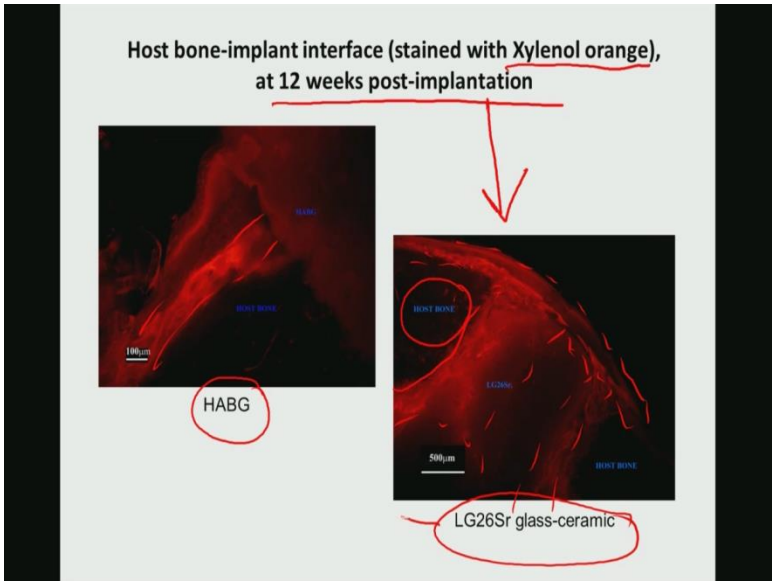

Now, one of the one of the additional apart from histology, one of the themes one can also do is that to see the progression of the bone formation in vivo. So for that what people do is that, the veterinary surgeons can inject, certain stains like Xylenol orange during, in the post implantation period not at the time of sacrifice but before the point of sacrifice, before the time point of sacrifice. The idea is that those stains will go diffused to the implant and tissue implant interface and then they will deposit on the implant tissue inte rface and then when you will do some fluorescence microscopy you can see it will give you certain contrast which is characteristic of that particular staining agent.

In this particular case when you see at 12 weeks post implantation what you see here, this is your host bone structure, this is your implant, and it is certainly show this Xylenol red stained image and this Xylenol red stained image essentially show when there is bone formation has been initiated. So the similar kind of features of the bone formation also has been shown in the hydroxide bioglass but qualitatively from these two image what you can see here, that qualitatively the bone formation perhaps is little bit more here in this case of that test glass material.

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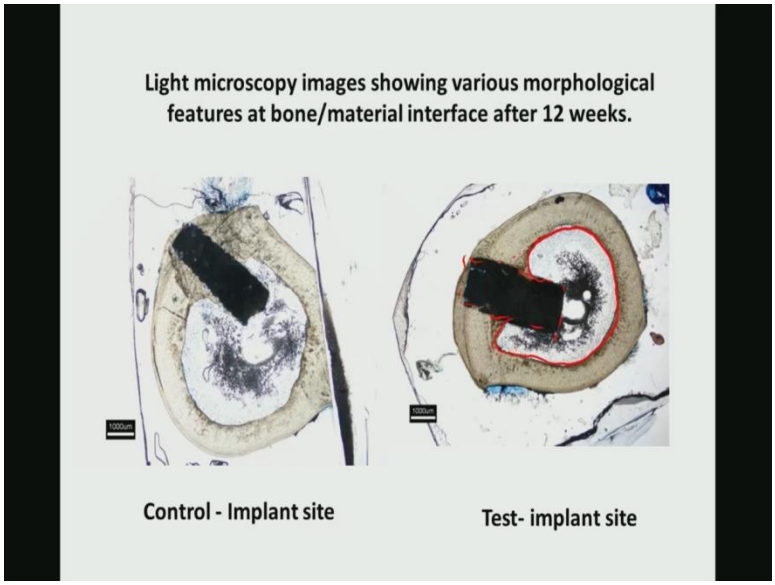

This is just a comparison of the control implant and the test implant and what I would like to bring to your attention is that, that this is your implant or test implant and around that (())(15.23) very characteristic region the way I am sketching. So these characteristic region and this particularly where I am making dotted region, this dotted region, so these dotted region is the area where new bone has formed. So this dotted region is characteristic or it indicates this bone formation. This is very uniform you don't see any discontinuity in that (bo) implant and bone interface.

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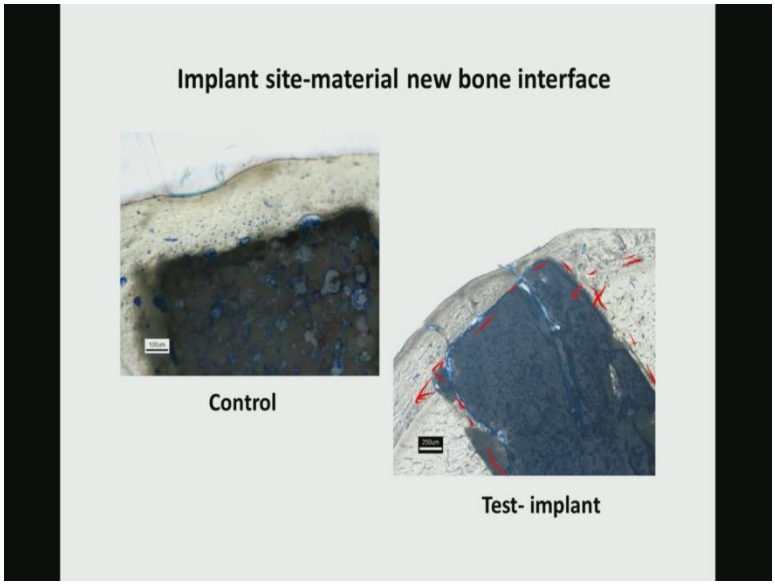

The second thing that you observe here that again in this separate set of histology images this is your implant here and this is the region, in the different region of the bone formation you just see at a different magnification how this they appear.

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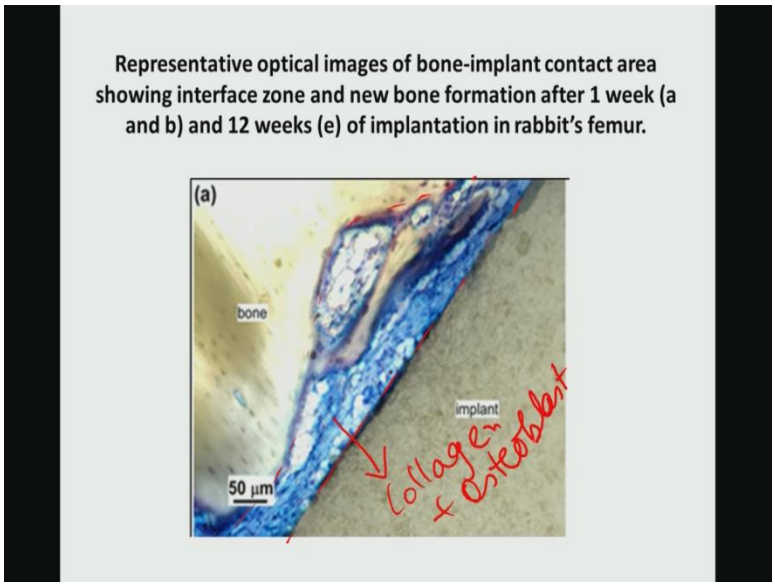

Ok, so this is, the other thing that you see, this is your test implant here and this is after 12 weeks and this is the region that histology stained image. Now this blue region there you can see the collagen as well as some osteoblast cells like forming cells, their activity at the bone implant interface.

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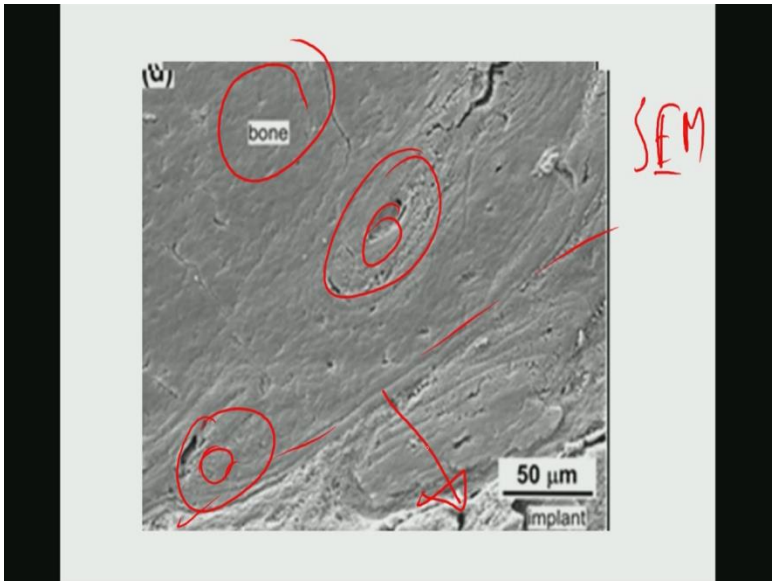

This is a scanning electron microscope image, just to show, that in that osteons and so on in the, in the host bone structure, and this is the host bone structure and your material is somewhere here down this side.

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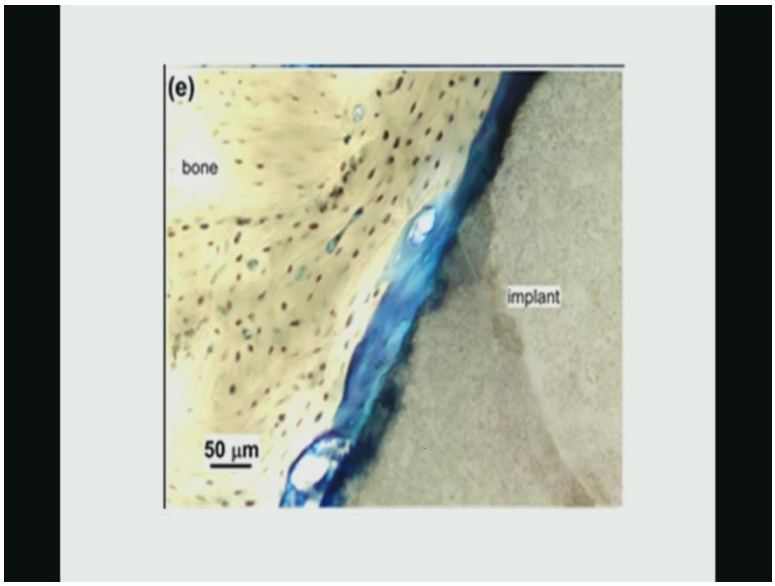

This is some more histology images, just to show characteristics cellular morphology in that host bone around the implant.

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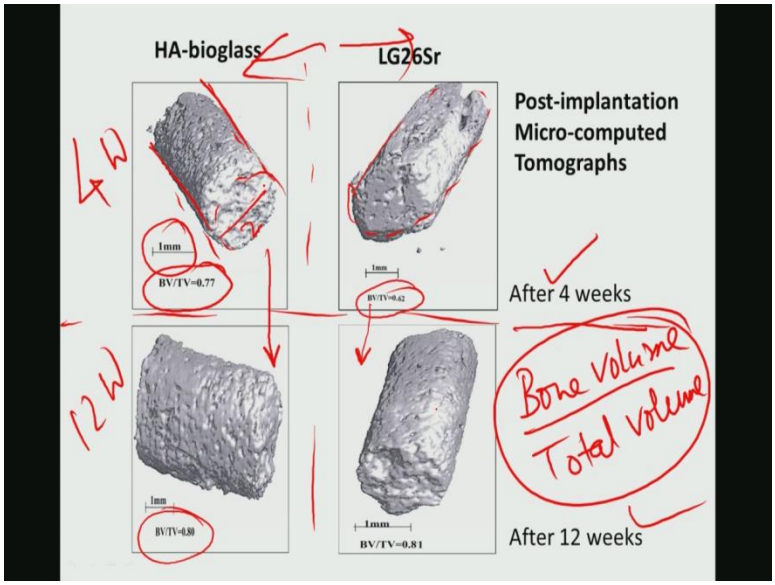

One of the things that I have mentioned while discussing the, the pre-clinical study that micro-computed tomography is been now extensively used to quantify the bone regeneration process also to develop better qualitative understanding of the bone formation in 3 dimension, and this is just to show you that this micro-computed tomography you can use directly on the explants. So after 4 weeks you take the explant and you scan it in the microcity this is what you get in the 3D image.

So what you get in the 3D image you remember that you started with a cylindrical cross section sample or a cylindrical sample like, I told you the 2 mm diameter and this is the 1 mm micron bar here or length scale. So this is your 2 mm diameter and this is your around 4 to 5 mm in length. So whether the implant A, that whether implant can retain the shape at different time point in the post implantation. B, if the shape is not retained whether there is any shape distortion or whether there is any sign of the degradation of the implant in vivo. So if the implant changes its size and shape that will essentially indicate that degradation of the material in vivo, ok and also one can do certain analysis to obtain bone volume that is BV divided by total volume of this (mat) of this implant.

Ok, so bone volume by total volume that ratio one can measure or determine from this microcity values. Now higher the bone volume to total volume or if the bone volume, total volume is more than .95 certainly that implant is very effective to regenerate the bone. If the bone volume to total

volume is less then what will happen, that implant is not that effective at that time point for bone regeneration purpose. Now, let us look at this data little bit carefully, so this is your HA bioglass this side and this side is your strontium substituted glass ceramic materials. Now what you see that, after 4 weeks and after 12 weeks.

So this total, these top panel is 4 weeks, bottom panel is 12 weeks microcity results. Now what you see here is that if you compare this to this only with the control (mat) control implant. So after 4 weeks this is 0.77 bone volume to total volume, after 12 weeks it is 0.80, compared to that in the strontium substituted materials at that initial period of 4 weeks it is 0.62 and bone volume to total volume it is 0.81. So although after 4 weeks bone regeneration is not that high like a control implant but certainly it is comparable when you compare the 12 weeks result, ok.

So what are the implications of these results, the implications of this results is that quantitatively that 100% strontium substituted glass ceramics may not be as effective to induce early osseointegration however at the long time osseointegration is concerned it is as effective as the control implants.I am mentioning these so that you understand how to interpret and how to signify this microcity result from quantitative perspective.

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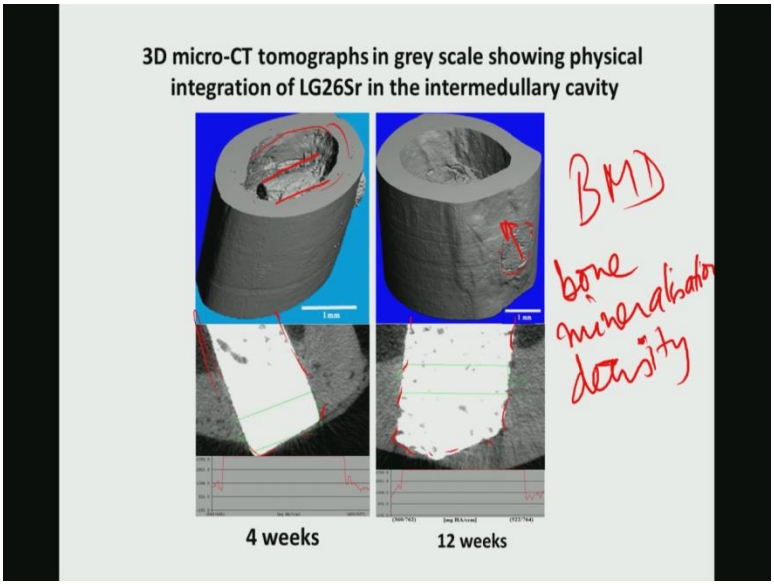

This is this, more, more view of this 3D microcity images of this glass ceramic material what you see here. This is a grey scale image so you see that this is your intermedullary cavity here, this is the outer shell so you just cut a section and then you see it, this is your implant. This

implant is kind of put it inside and then you can see here is the femoral bone defect from this angle ok. So this is the implant which is inserted and this implant goes in, ok and after you put in (inter) intermedullary cavity, after you see how is the shape of the (im) implant in the 3 dimension, ok and this is the 2D slices of this material and from these you can see the 2D slices, there is certain bone mineralisation density what you call BMD.

So what is bone mineralisation density? Bone mineralisation density quantitatively, quantitatively will tell you that what is the mineral content in that host bone around this material or what is the mineral content in the new bone.

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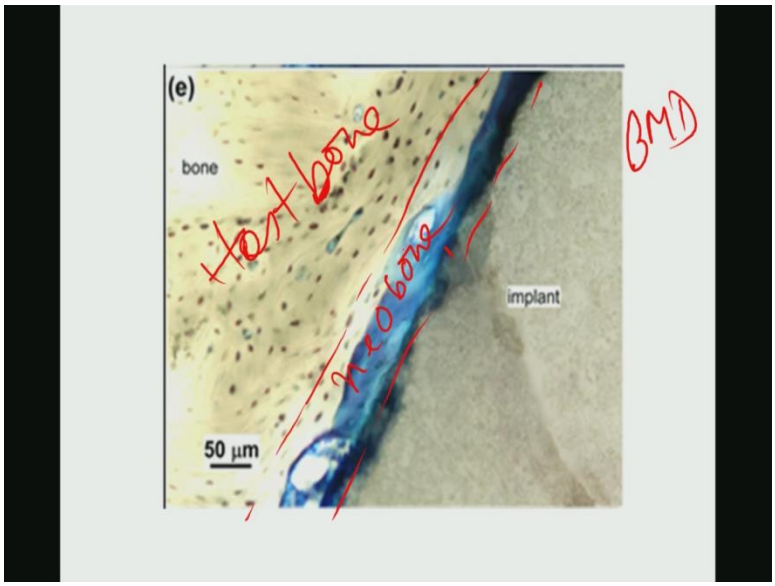

Now, why bone mineralisation density is important because when, let me go back to some histology image just to show you. Now you have this implant ok, and this is your host bone structure. Host bone means which was kind of part of the osseous structure of the experimental animal that is your rabbit.

Now this region you can see this dotted region. This region is the region where there is a new bone formation, ok. Now in this new bone you see that osteoblast cells and so on and so forth. Now what do I mean by BMD, bone mineralisation density is that once this bone starts forming around the implant or in the small cavity of the implant this bone may not be matured enough at different time point of the implantation. That matured bone means which will have composition

wise or property wise to some extend similar to that of the host bone will have bone mineralisation density equallent to that of host bone.

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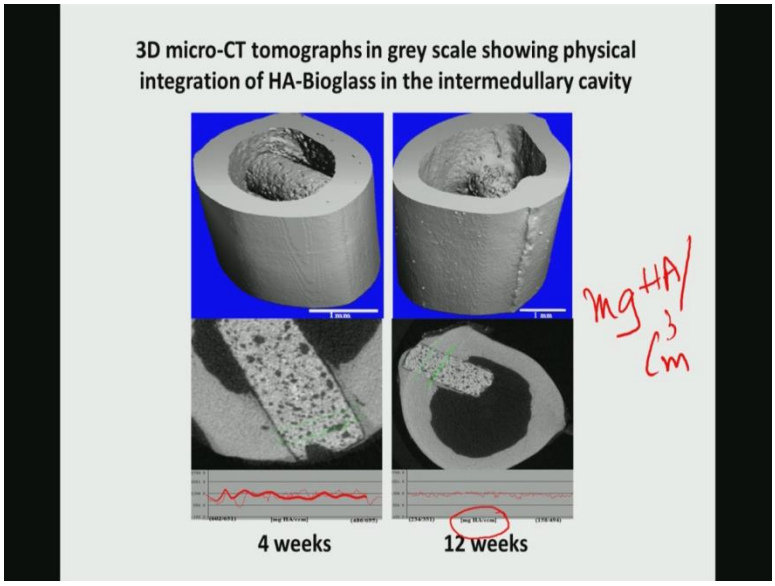

But during the process of bone maturation this bone mineralisation density increases and that is what exactly happens in this particular case that your BMD values what you are getting here. You can see the BMD values is really fluctuating and at different times and then the BMD values the unit of BMD values is that milligram of hydroxyapetite bar centimetre cube. So it is like what is the equallent to, value of the milligram of hydroxyapetite that is present in the natural bone and what is the milligram HA that is present in this new bone structure that is forming.

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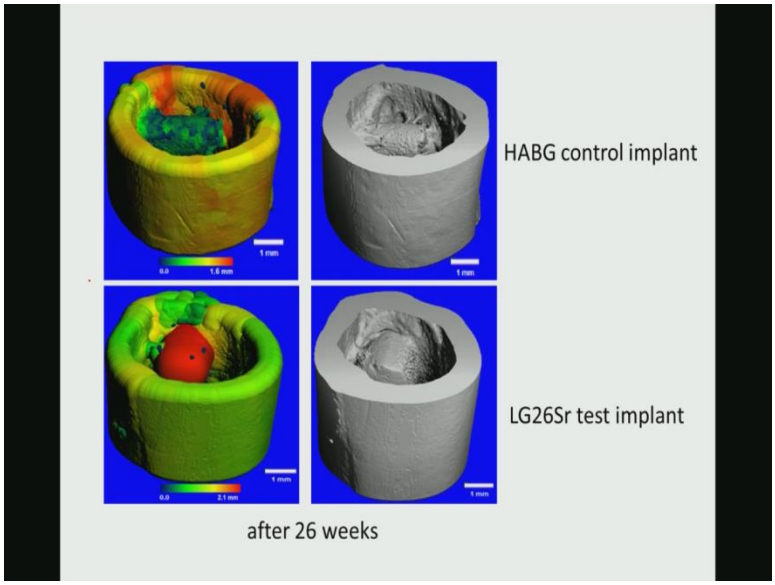

This is certain interesting result in the micro computed tomography what you see it gives a colour contrast at this left hand panel. Now this colour contrast also you can see that this different colour contrast tells you that what is the thickness of the tissue. Ok the tissue thickness s showing different colours. For example 2.1 mm, this 2.1 mm is the more red, so around 2 mm tissue thickness is there. Now if you look at this particular case, if you look at this particular case that almost all the, almost all the implant that is the cylindrical shape, that is covered with this thick tissues around it and this thick tissue typically have a thickness somewhere around 2 mm.

The same is however not true for the control implant and this is after 26 weeks and you see that all kinds of different kind of mostly green green colour contrast you can see around the implant essentially meaning that tissue thickness or bone tissue thickness on these control implant is not certainly towards the region but it is less than that. So from the tissue thickness also quantitatively you can understand that what is the quantitative tissue or bone regeneration around the implant.