Biomaterials for Bone Tissue Engineering Applications
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Module 7
Lecture No 34

So in this module I will just continue my discussion on that hydroxyapatite based electro conductive composites. In the last module I think I have discussed on the hydroxide barium titanate.

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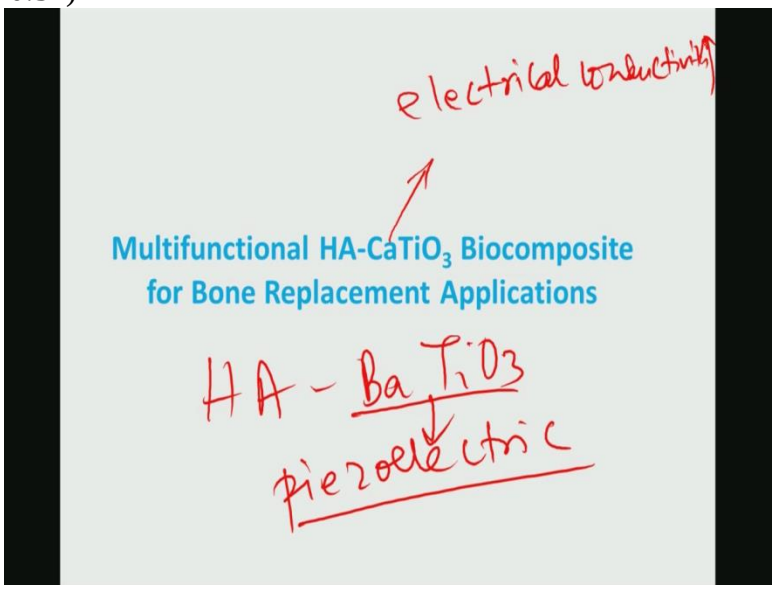

Now the use of the barium titanate was very useful in a sense that barium titanate can. So that earlier we have done hydroxide barium titanate. Now barium titanate particularly induces that piezoelectric properties in his hydroxyapatite composites. Now why piezoelectric properties is very important because bone has a piezoelectric because of the presence of collagen.

So in order to mimic the piezoelectric properties of the bone we have added this barium titanate to hydroxyapatite and we have shown in the last module that how the addition of barium titanate up to 30-40, percent can enable hydroxyapatite based composite to attain bone like conductivity and bone like piezoelectric properties.

Now extending the discussion today we will cover, or we will try to analyze what is the status of the development of hydroxyapatite calcium titanium based composites. Now why calcium

titanate? Again calcium titanate with the addition of calcium titanate it is expected electrical conductivity of the composites, of the materials will increase. And if the electrical conductivity increases then again it will have a bone like conductivity property. However calcium titanate doesn't have heretic properties like barium titanate.

So therefore only advantage of addition of calcium titanate is to enhance electrical properties. Towards the end of this module I will also show you that what is that bio compatibility property, the results of the bio compatibility properties in terms of establishing early stage Osseo integration of hydroxide calcium titanate in the rabbit animal model. And particularly to heal, the efficacy of this material to cause bone healing for that femoral defects in this kind of the femurs of the rabbit.

Other things I must mention that why calcium titanate has been added, calcium titanate is not only has good conductivity compared to hydroxyapatite but also one should remember that hydroxyapatite is kind of coated on the titanium 6 aluminum 4 vanadium substrates in their potential application as the stem of the, femoral stem in the total hip replacement. So when you coat the hydroxyapatite on titanium, during the coating deposition by established techniques or commercially used techniques like plasma vapor deposition and all this related thermal assisted deposition techniques, so there what will happen hydroxyapatite reacts to titanium and forming calcium titanate at the interface.

And this hydroxyapatite coated titanium materials has been clinically tested to be bio medically safe and they are in clinical product they are in commercial production and many of them have been tested in human patients. So having said this Calcium titanate is indeed anticipated to be bio compatible in nature and also additionally calcium titanate also has better electrical conductivity properties. So in view of these two this these properties advantageous properties we have assessed that whether calcium titanate can be added as a second phase additive to hydroxyapatite.

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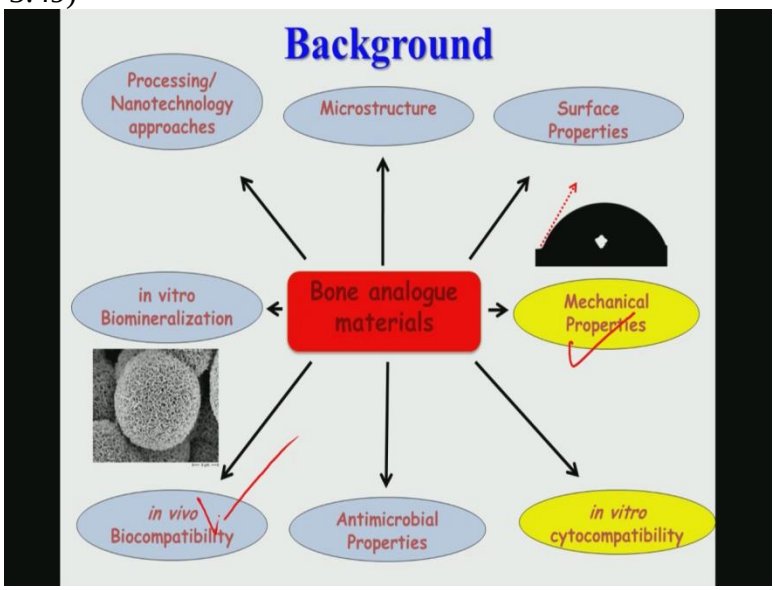

Now little bit of this background on the bone analogue materials. So some of the things I will be I will be dealing here one is that mechanical properties that I will show that how calcium titanate will enhance the mechanical properties and also the in vivo bio compatibility properties that that in terms of early stage Osseo integration.

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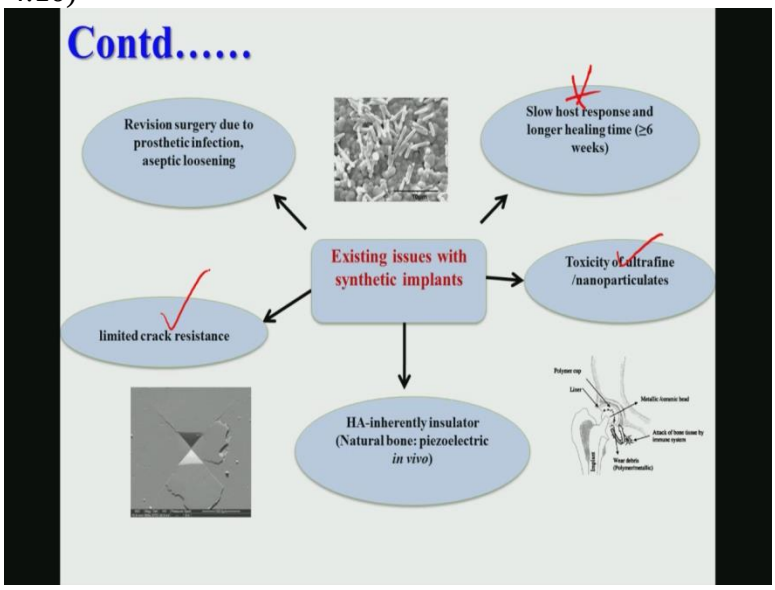

These are some of the summary slides of the issues that are related to mostly the synthetic implants for orthopedic applications which includes slow host response. So this is the things which is very important in the in the bone replacement application, bone analyze applications.

Also the toxicity of ultra fine nano particulates and limited crack growth resistance. Limited crack growth resistance means it has very poor fracture toughness.

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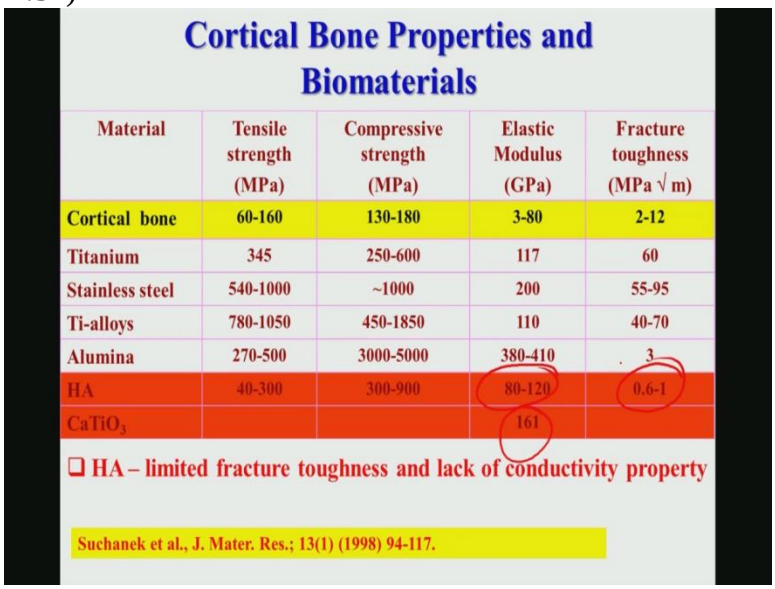

Now coming to that hydroxyapatite and calcium titanate if you see their properties, elastic modulus of hydroxyapatite is 80-120 whereas elastic modulus of the cortical bone is somewhere around 3-80 gega Pascal. Fracture toughness of hydroxyapatite is.621 mps square root meter.

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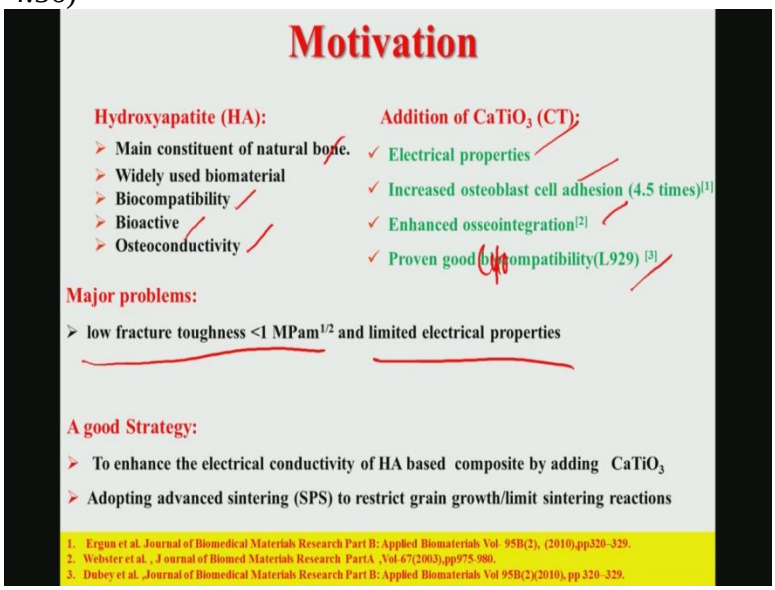

So these are the things that I have mentioned in the first slide while discussing the topic of today is module, so I have mentioned that calcium titanate has good electrical properties, it also is reported to enhance the osteoblast cell functionality at least 4.5 times than of the control. Enhanced Osseo integration. And proven good bio compatibility, cyto compatibility with respect to a 929 cells. Hydroxyapatite has good osteonconductivity, bio activity, bio compatibility and main constituent of natural bone. But the major problem with hydroxyapatite is low fracture toughness and limited electrical properties.

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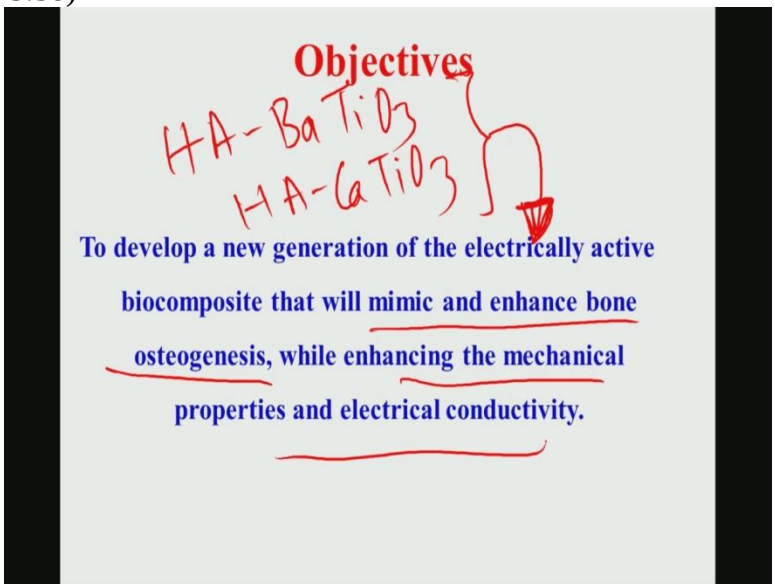

So therefore these two components if they are mixed together, if you make the composite then it is possible that you can enhance the mechanical properties as well as the, without compromising the bone, bio compatibility properties. So this is the statement that is very important. So that the overall be hydroxyapatite barium titanate or hydroxyapatite calcium titanate, this development of these two classes ofthis composites, of these two sets of composites essentially is driven to fulfill this objective, it is to develop a new generation of electrical active bio composites that will mimic and enhance bone ontogenesis while enhancing the mechanical properties and electrical conductivity properties.

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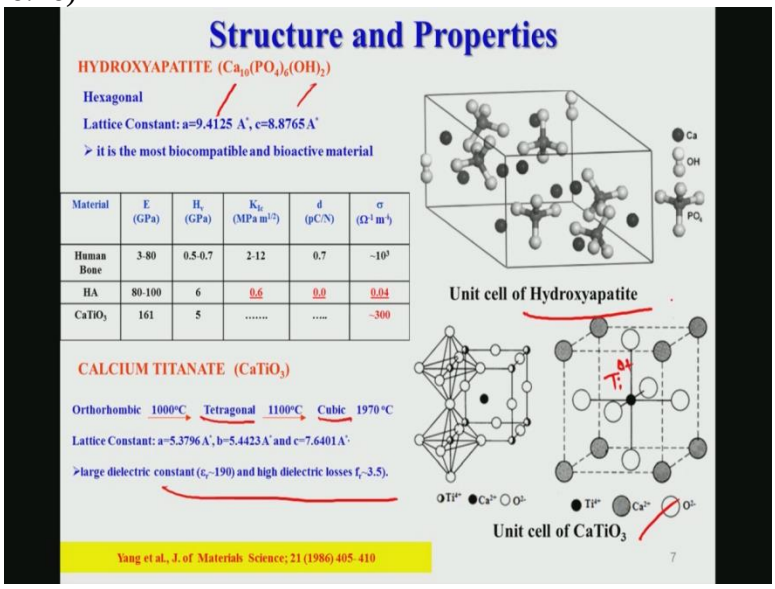

Now little bit background of hydroxyapatite crystal structure and calcium titanate crystal structure. Hydroxyapatite is more hexagonal crystal structure. Although hydroxyapatite can exist in the mono clinic form. And typical lattice constant is 94 Armstrong. A and c is 8., So ac is very short, c is shorter than a of this hydroxyapatite. Now calcium titanate it undergoes certain phase transitions.

At high temperature it is, so at room temperature it is orthorhombic or in orthorhombic symmetry then it goes to tetragonal at 11, 1000 degree Celsius, then it goes to 1100 degree Celsius, it is cubic. And lattice constant of the orthorhombic structure are also mentioned and it has a large dielectric constant like 19d and also high dielectric losses is 3.5. and this has been shown that calcium titanate hydroxide, calcium titanate crystal structure, what you see that titanium is

occupying that half, half, half, position, that is the body center position, oxygen is occupying that face centering position and then calcium goes to that cube corner of this structure.

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Now there are certain commercial available bone stimulators which are actually commercially produced by different companies in new jersey as well as in Texas. Texas companies orthofix, they call physio steam like and that is inductive coupling or pulsed electromagnetic fields which can be given to this particular sites where you need to stimulate that bone muscles and so on. There is also capacitive coupling based electrical devices and direct coupling based electrical devices that is OsteoGen and Orthoback. So these are some of the companies which gives that commercially available bone stimulators.

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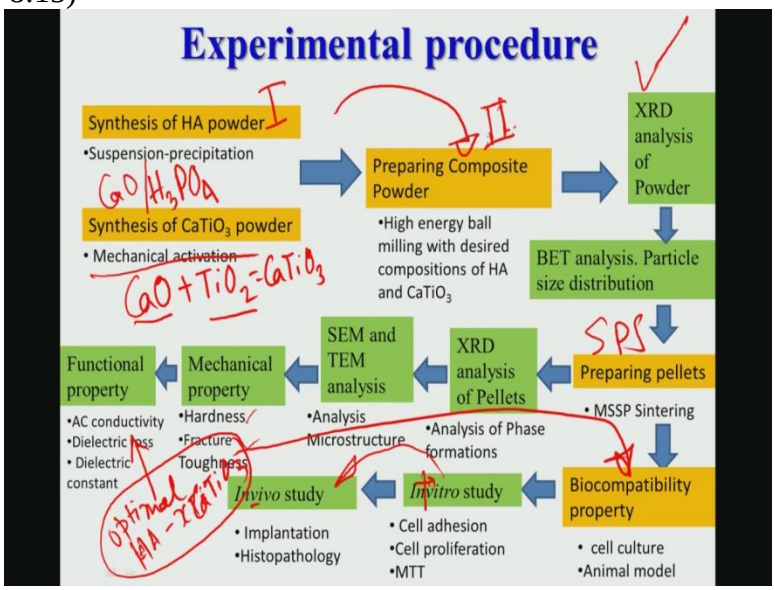

Now this is that some of the things that is very useful for the students just to show that how to make this composite. So first you have to start with the synthesis of that synthesis of the, this is the stage 1 of this total manufacturing process. First you have to start with the synthesis of the hydroxyapatite powder as well as the calcium titanate powder.

Now calcium titanate powder you can do it like this you can mix the calcium oxide and titanium dioxide, and then you do mechanical activation ball miring and then subsequently you sinter them at high temperature so that you facilitate that reaction synthesis or reaction sintering to take place to form this calcium titanate. So you can do 1:1 proportion of calcium oxide and titanium oxide to make this calcium titanate. It is called mechanical activation process.

Hydroxyapatite process synthesis that I have mentioned in one of the earlier modules. You start with the precursors of the calcium oxide and Ortho calcite. And then use the suspension precipitation route and this suspension precipitation route you can synthesize the hydroxyapatite powders. Ok. Now after that you can prepare the composite powder and once you, this is the stage two that is the ball milling, so you add certain percentage of calcium titanate to certain percentage of hydroxyapatite.

Let is say 20 percent, 40 percent, 60 percent, 80 percent like that then after he ball milling you just check that whether the powder are ok in terms of XRD. Then do this particle size

distribution. Then you do SPS technique that is the spark plasma sintering. What is spark plasma sintering I have discussed this while discussing that ceramic processing technique. Now after this so technique you do XRD to see that what are the different phases are there.

And then do SEM and TM analysis, subsequently mechanical property measurements like hardness and fracture toughness. Now functional properties ac conductivity, dielectric loss and dielectric constant. Now after you do all these things, then you optimize the sample, so from all these things you know that what is the optimal sample composition or optimal ha, x percent titanium composition and once you know the ha x percent calcium titanate, let is say that x value is 40 percent calcium titanate, then you do all this study in terms of that high bio compatibility study then in vitro study, so in vitro study there should be gap , and in vivo study there should be gap.

So in vitro study essentially at the cell level study and in vivo study using that animal model. Again after in vitro study whatever optimal composition you do then you do this in vivo study.

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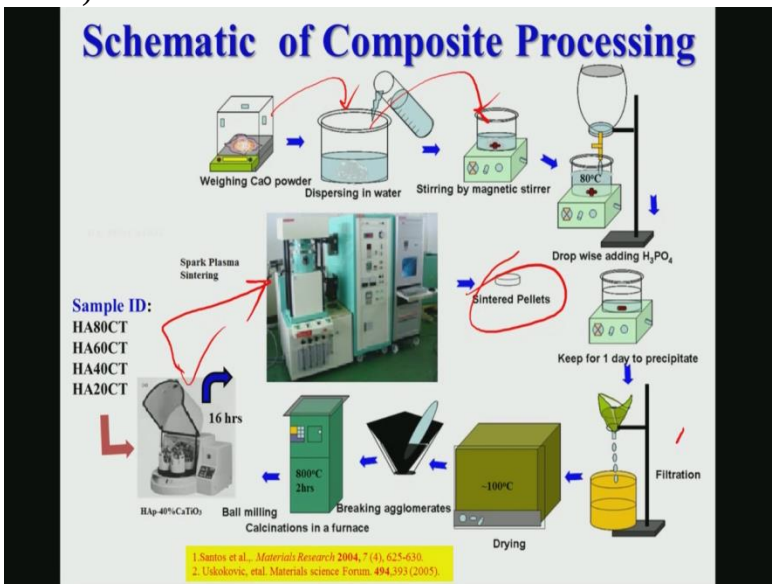

So this is little bit more pictorial presentation of this sample processing itself. So you start with that hydroxyapatite powder preparation. You do this calcium oxide powder, then you do this magnetic star in this calcium, in drop wise hydroxyapatite powder synthesis. Keep it for one day,

then you do filtration and then after that you do drying and then breaking the powders and ball milling and calcius in the furnace.

Then you do ball milling. Now after you ball mill then you do this spark plasma sintering and finally you end up having the sintered palettes. So essentially this is a complete scheme of whatever synthesis followed by the bulk sample preparation of this hydroxyapatite calcium titanate formulation that is useful.

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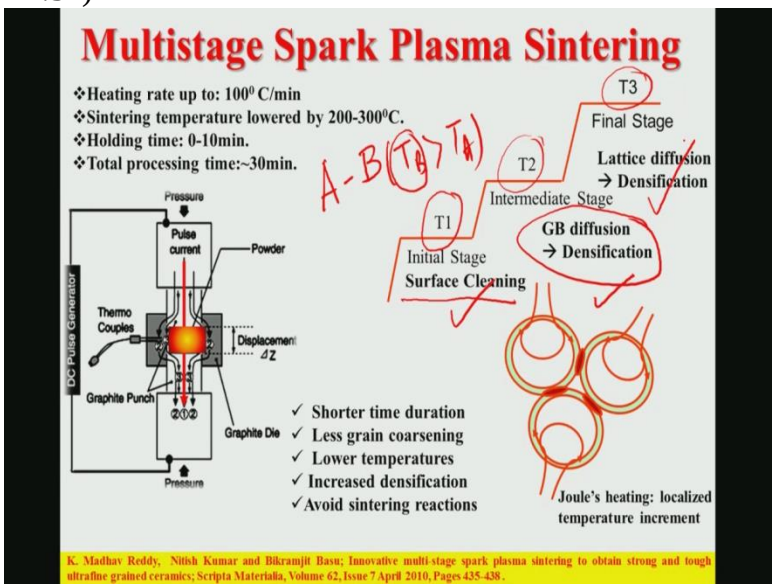

Now in terms of the multi stress spark plasma sintering that means that you are holding more than one different temperature before you do the final stage of sintering. So you start with the initial stage of sintering then comes the intermediate stage of sintering and then comes that final stage of sintering. Let me also tell you that t1, t2, t3, are essentially based on certain logic.

The logic is you you decide that which is the material which actually densifies at the high temperature, you take that melting point tm, then you consider.45 tm like you consider that you just do it at t1 so you set that t1 as that kind of less than.5 tm. T2 you can set at as little above that.5 tm, and t3 you set as.6 or something, of that posted 20 powder constituent which has high sintering temperature or which has higher melting point .

So that you allow that higher, more difficult to be sintered phases to be densified and consolidated during the spark plasma sintering. Let me reiterate this statement by giving some

examples. Let is say you are sintering a b two different powders. And a b two different powders, suppose b has higher melting point so tb is greater than ta.

So then your all this formulation of all this t1, t2, t3, should scale with tb not with ta. Because you know b element or b phase is more difficult to be densified. So your sintering temperature where you will hold this different temperature at t1, t2, t3 should be directly correlated or should scale with tb, not with ta. So therefore here in the calcium titanate, calcium titanate is more difficult to be sintered than hydroxyapatite.

Hydroxyapatite is typically sintered at 1000 degree Celsius or 1100 Celsius very easily. So therefore you just play around with this t1, t2, t3 by considering this one. And remember your sintering by definition is that consolidation of the powder compact to a sense solid by heating at a temperature of t greater than tm. And this sintering is defined essentially with respect to monolithic samples. Like you do not consider that any second phase addition to this monolithic sample.

So therefore and essentially by heating to more than t greater than.5 tm, essentially you are facilitating diffusional mass transport process during the sintering itself. So having said this , this t1, t2, t3 is not arbitrarily said but there is certain logic. Second logic is that as far as the mechanism is concerned initial phase will give you surface cleaning. Intermediate stage will give you that grain body diffusion and densification and then final stage will give you lattice diffusion and densification. So heating rate is typically 100 degree Celsius per minute. Sintering temperature lowered by 200-300 degree Celsius, holding time is 0-10 minutes.

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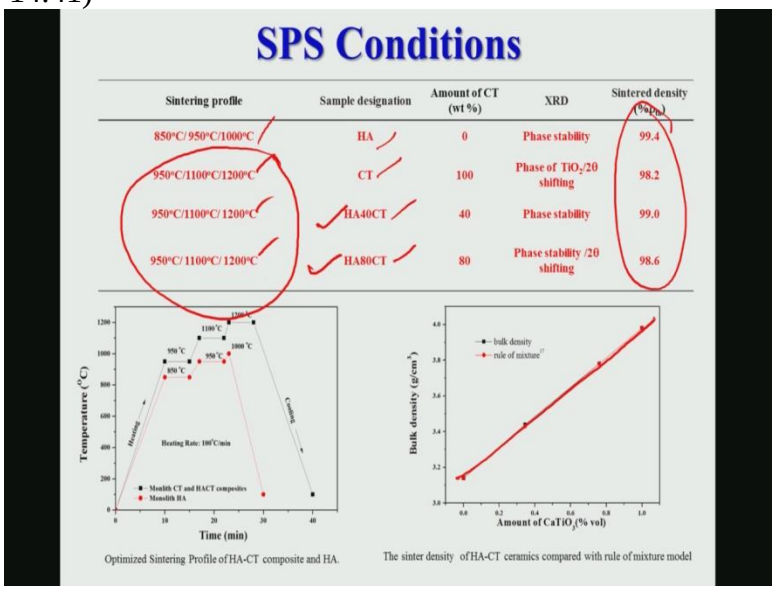

Ok. Now there is different sample designations given hydroxide 100 percent, calcium titanate 100 percent, ha 40 ct stands for hydroxide 40 percent calcium titanate, ha 80 ct hydroxide 80 percent calcium titanate and in all these cases you see that by optimizing the sintering conditions like 850, 950, or 950, 1000, 1100, 1200 as you see that calcium titanate, whatever we have set the sintering , that calcium titanate sintering schedule is also valid for 40 percent calcium titanate or 80 percent calcium titanate simply because calcium titanate is more difficult to be sintered.

So once it is scaled with the base line calcium titanate, the rest of the composition which contain calcium titanate will follow the similar schedule so that you achieve almost similar level of densification in the final sintered sample. So this is the scheme that has been shown here and bulk density to the rule of mixture density follows extremely in the co linear manner, essentially you say that it is theoretically densified as far as the composition is concerned.

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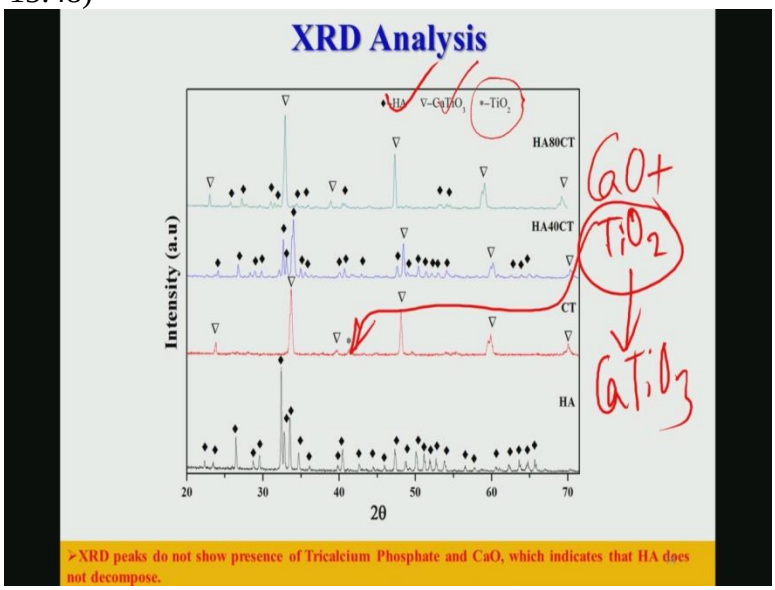

Now as far as the XRD analysis is concerned all these composites after sintering what you see that you you retain that hydroxide calcium titanate as a predominant phase only little bit of titanium oxide. Now origin of the presence of the titanium oxide can be attributed to the unreacted titanium oxide when you are actually facilitating this reaction calcium oxide plus t1o2 to form calcium titanate by mechanical activation process. So there some titanium oxide remain unreacted and this titanium oxide essentially shows off when you are doing this pure calcium titanate sintering.

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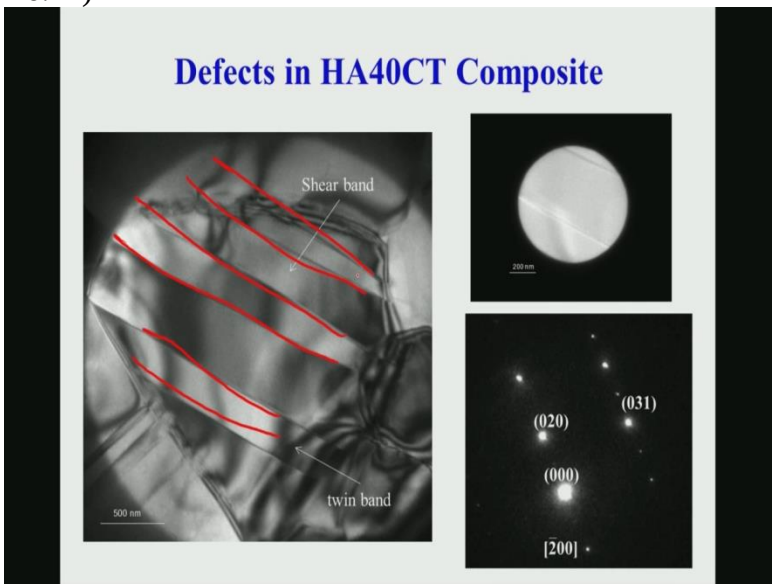
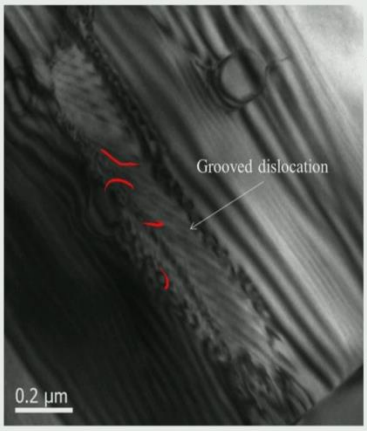
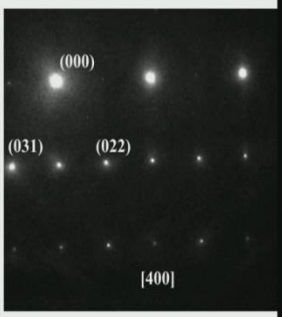
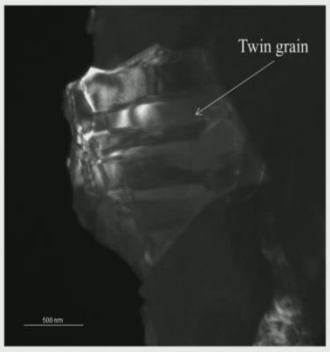


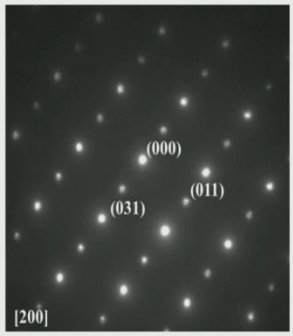
Contd..... Threaded dislocations





Twined $CaTiO_3$ grain





Now some of that transmission under microscopy shows that you know I will be showing very few calcium titanate transmission under microscopy it shows very characteristic twin morphology and this twins with characteristics interlamellarspacing is very typical of this orthorhombic calcium titanate phase and apart form twining you can also see this threaded dislocation so this is called threaded dislocation in this material and threaded dislocation essentially form the defect structure in this material.

And this dislocation also gave some characteristicsselected area diffraction pattern which has been thoroughly analyzed. Now this twin grains once again is more clear in this dark field transmission under microscopy images and you can see very clearly and this twin morphology needs to be analyzed in the more dark field so that this twin morphology becomes more prominent in the dark field transmission of microscopy images.

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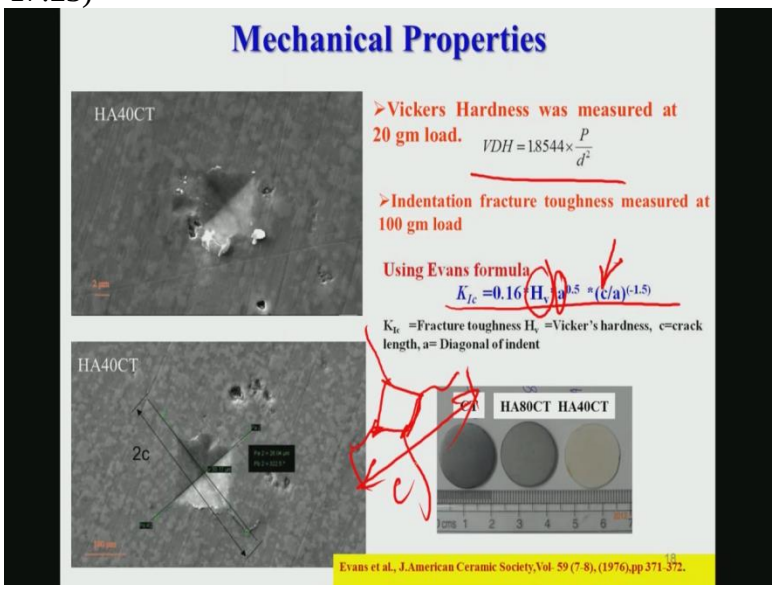

Now hardness and fracture toughness is measured using the conventional indentation technique. You do the Vickers hardness with Vickers hardness, then you measure the indentation length and from the indentation length you get this Vickers hardness. Then fracture toughness is essentially measures using Evan is formula which doesn't require essentially the elastic modulus and it is the formula.16,hv or hardness.

A is your indentation diagonal length, c is your crack length form indentation. So if you take the indent that in the brittle materials, the moment you take that indent, the indent will produce certain cracks. So you measure the cracks length. So total crack length is c, as I have explained in one of the earlier modules. And that c value you put it, then you get the indentation toughness.

Remember the indentation toughness has single edge v notch toughness is two different ball game. Because single edge v notch toughness gives you more reliable measure of the fracture toughness. Whereas indentation toughness gives you more qualitative estimate of the fracture toughness of this material.

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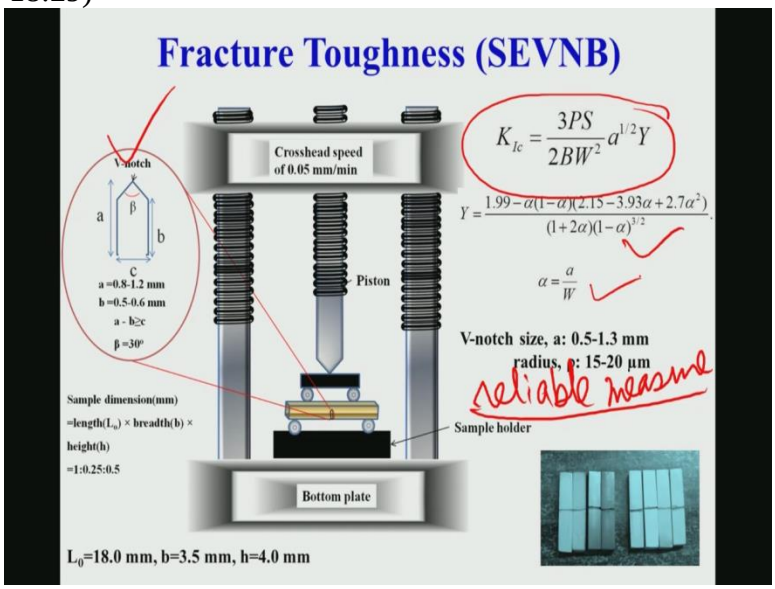

Now this is what has been shown in the single edge v notch beam what I said that it gives you more reliable measure of the fracture toughness. So that means clinically if you want to use it this material you must use that single edge v notch beam not the indentation technique. And single edge v notch beam technique as you might have seen in the earlier modules you put a v notch here and this v notch essentially is placed in the tensile surface.

Then you break the specimen in 4 point loading consideration and this notch geometry has been shown here. And this k1c you can measure it like 3ps by 2bw square and alpha to the per half and y and then y factor depends on what is the crack geometry and alpha is essentially a by w. a is your pre crack length and w is the width of the sample specimen.

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Fracture strength was measured again in the 3 point bending configuration and 3 point bending essentially shows you that this material is loaded in tension tension compression mode and this 3 point means concentrated loading condition then you measure that what is the load at which the sample fractures what is the spam length, what is the b, breadth, and then what is d is that the height of the sample.

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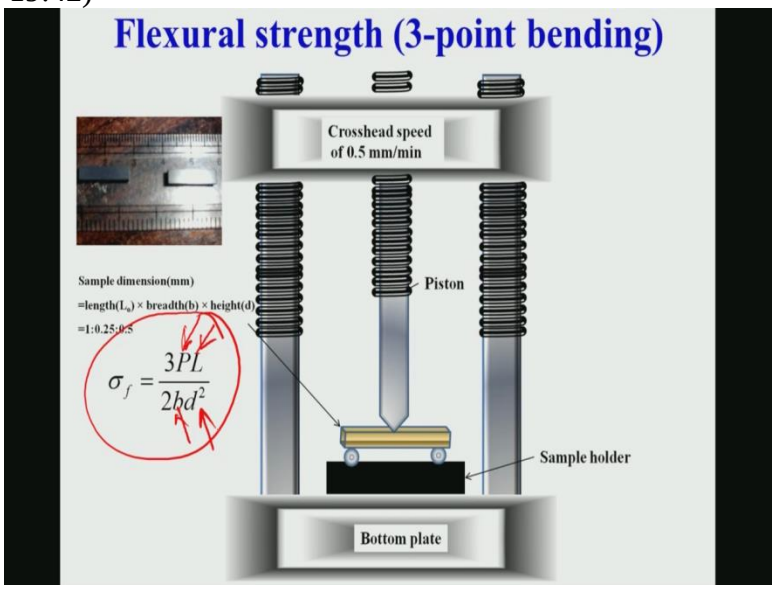

Diametral compression essentially gives you the tensile strength property of this material and diametral compression essentially means then you take that full disc of that material and then you break them in compression mode. Now if you do along the diameter essentially the sample will experience tensile stress.

If you do that little bit of that mechanic analysis although the name diametral compression suggest that as if the total material will be under compression load but you are not testing the material under compression load instead you are using instead the sample will experience the tensile stresses. And this diametral compression strain that is the tensile strain you can measure, It is simply by 2p by pie dt so d is the diameter, t is the thickness and p is the load at which the sample fractures.

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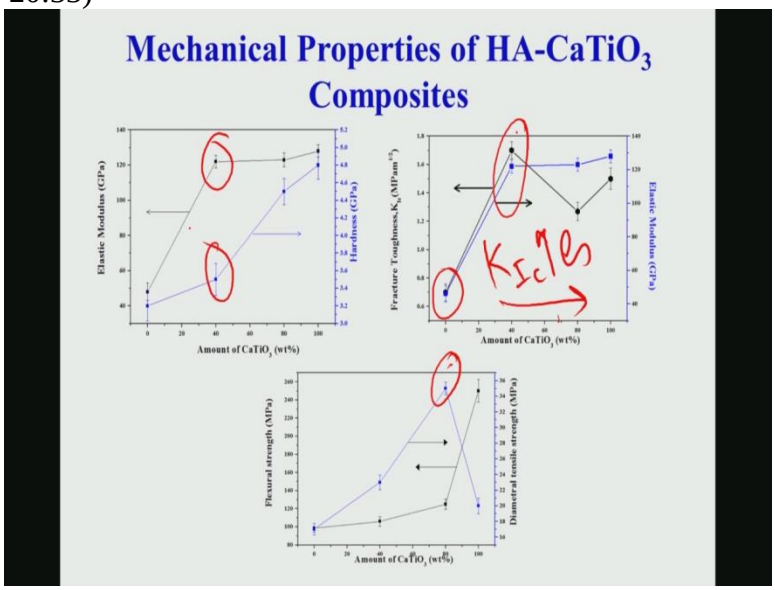

So this is the typical mechanical properties of this material. You vary this calcium titanium content, what you see, with the increase in calcium titanate you are getting little bit higher or at least two times higher than the fracture toughness of material so k1c increases quite a bit. And this toughness is essentially measured by single edge v notch beam technique. And in terms of the flexural strength also we see that the flexural strength there is a peak around 80 percent of the calcium titanate addition.

Elastic modulus and hardness, this also increases more or less systematically although not linearly and then it shows kind of increasing trend with addition of calcium titanate addition to hydroxyapatite.

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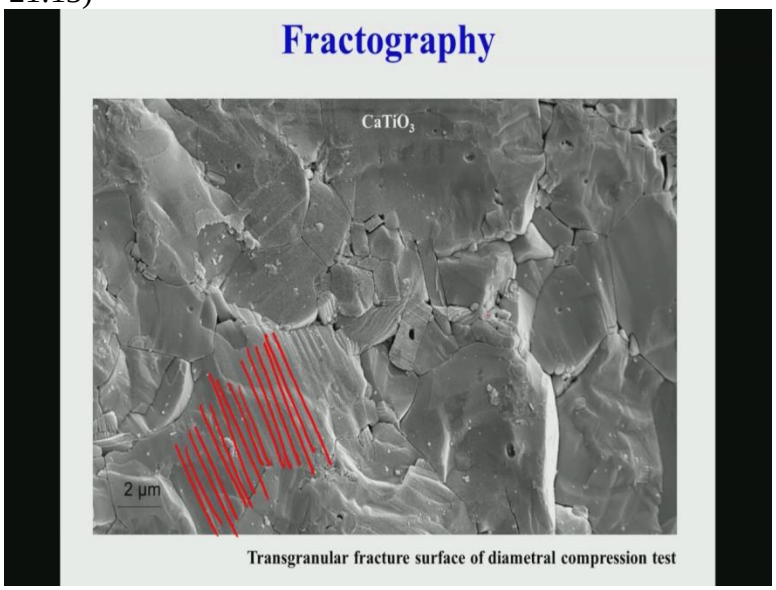

Once you break the samples you do see this kind of twinning morphology. And you can see this twins are actually appears as steps on the fracture surfaces and these are like very nicely oriented parallel twins in this material and then calcium titanate grain, and this calcium titanate grain it shows that characteristics twin morphology even on the fracture surfaces. So that means the twinning participates in this fracture process as well.

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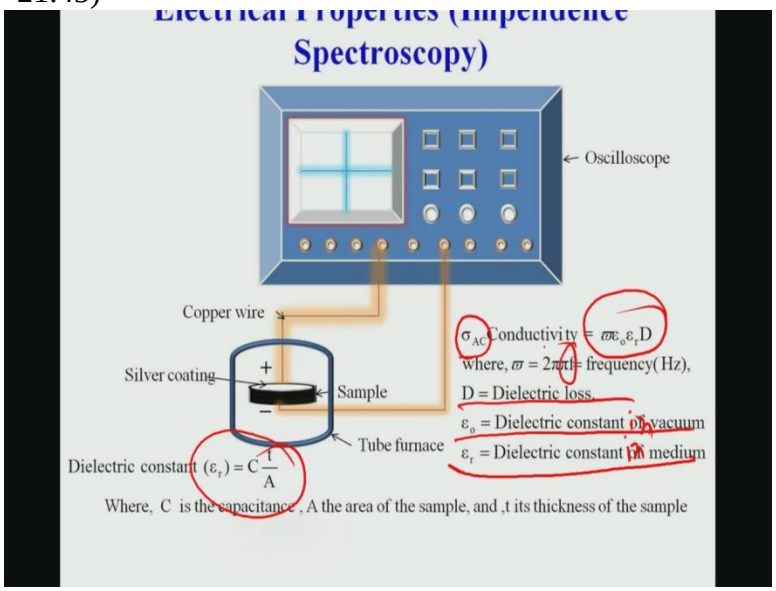

Now electrical properties particularly conductivity and dielectric constant, those are measured using the impedancespectroscopy. So impedance spectroscopy here you can measure the frequency dependent conductivity like in the ac mode and this is nothing but Omega Epsilon not Epsilon R multiplied by d so capital d is your dielectric loss, Epsilon not is your dielectric constant in vacuum and Epsilon r, dielectric constant in a particular solid medium.

So from that and dielectric constant how you measure, that is c into t by a, t is your thickness, a is the surface area of the sample and capital c is the capacitance of the sample. So from simple this reaction, relations you can use that frequency dependent and you can see that conductivity should scale with this frequency and it is Omega is equal to 2 pi f that is frequency.

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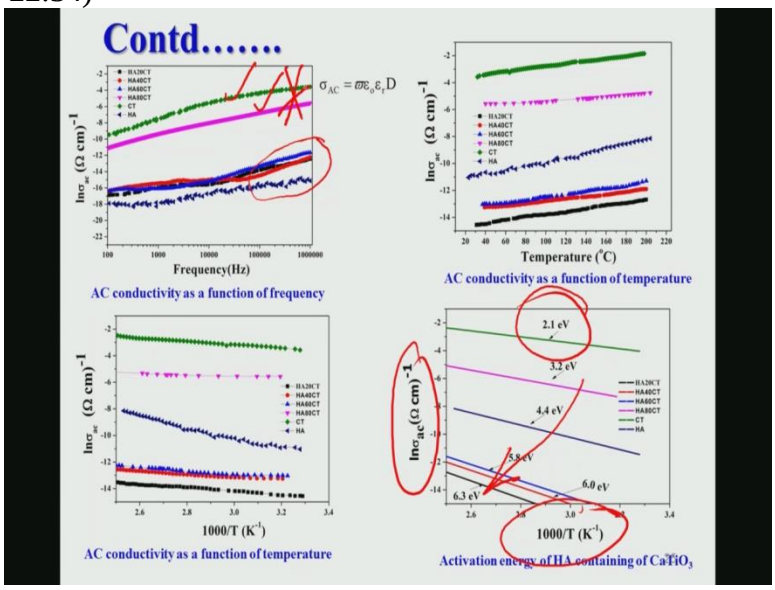

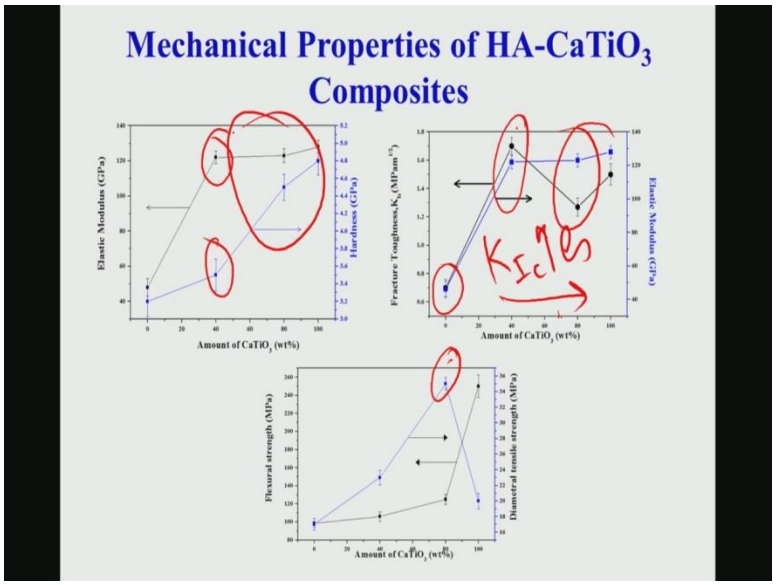

Now in terms of ac conductivity as a function of frequency when we plot we found that as a function of frequency that hydroxyapatite and hydroxyapatite 40 percent and hydroxyapatite 60 percent they are all lined up close to each other. but when you go to calcium titanate hydroxide 80 percent calcium titanate that is substantial increase has been noticed. So what it means that that when you add that 80 percent calcium titanate to hydroxyapatite, the conductivity is very close to that of the monolithic calcium titanate.

So that but when that hydroxyapatite when 40 percent , 60 percent or 20 percent calcium titanate is added then electrical conductivity doesn't extend to that significant extent or in other words matrix conductivity or matrix properties dominate. And matrix properties here is the hydroxyapatite, so you do not see very significant increase in terms of the conductivity when you add that 20, 40, 60 percent calcium titanate to hydroxyapatite .

However 80 percent calcium titanate indeed shows very high conductivity properties and close to this one. Then when we did some calculations in terms of the temperature dependent of the sigma and then you do this calculations with respect to 10 to the power 3 by t, temperature dependent conductivity properties, then you can find out that what is the activation energy of this conduction of conduction in this particular materials.

And it shows somewhere between 2.1 in case of pure calcium titanate fairly low and that increases as you go to the pure calcium titanate, it goes to almost 3 time like 6.3 electron volt. So

hydroxyapatite the activation energy for conductivity is 6.3 electron volt whereascalcium titanate it is fairly low that is 2.1 electron volt. That also shows that activation energy barrier in hydroxyapatite is much higher so that conductivity also is lower.

Now from these two slides one thing that you notice that ha 80 percent calcium titanate has good amount of conductivity and in another case you also see that mechanical property wise ha 80 percent calcium titanate has also reasonable mechanical properties in terms of hardness and fracture toughness.

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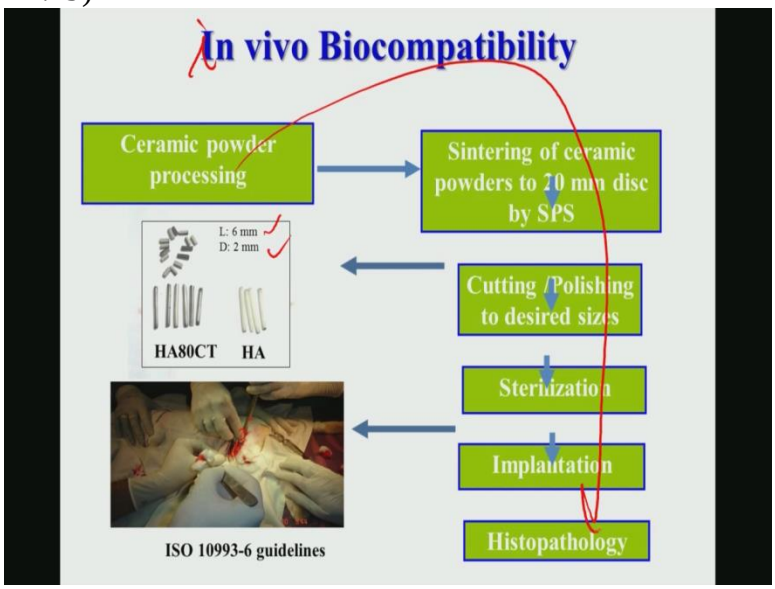

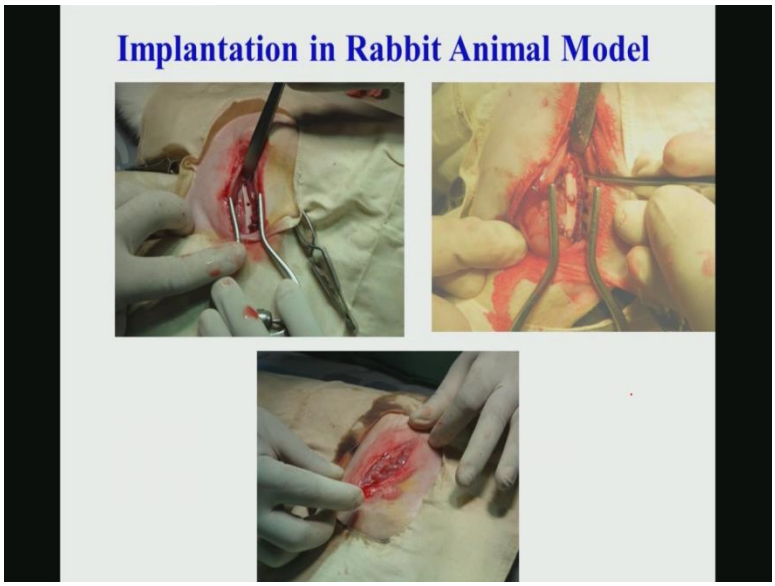

So therefore all this final in vivo compatibility experiments were conducted using rabbit animal model, so what we did, we made some femoral defects in that experimental rabbits, then we put this kind of rods like 6 millimeter length and 2 millimeter diameter of that hydroxyapatite and hydroxide 80 percent calcium titanate materials.

Then once we do this we do sterilization, implantation, histopathology, and this is that actually, when you do this femoral head femoral defects and in femoral defects you just put this materials and after that you just simply stich it. So after the surgery is over you stich it in the rabbit model, and then you close the wound, then you allow the animals to live or survive with sufficient feed and sufficient care, unless you do the sacrificing unless you sacrifice this animals .

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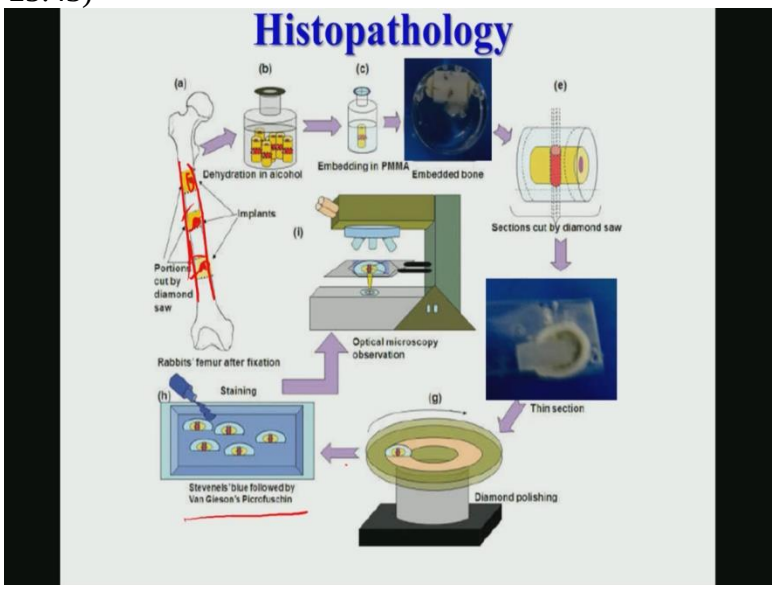

Now after the after the animal is sacrificed then you take out this femur of the, you take out the samples from the femoral defect along with that post born and then you do the standard histopathology sample preparation and which involves the section is cutting by diamond saw as well as thin section, then you do staining like either Mason is trichrome or that van gieson picro fuchsin or in that hematoxylin and eosin staining.

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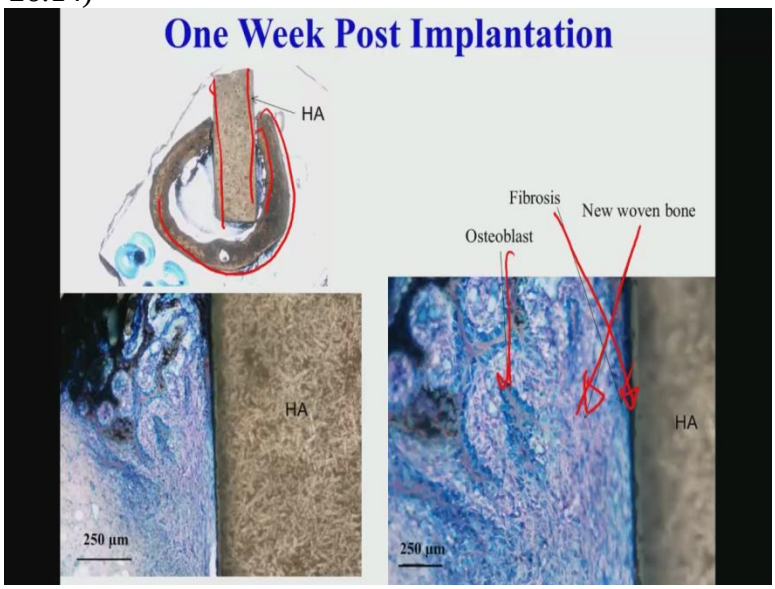

Then after one week post implantation you do see that there is osteoblast cell functionality here and then you see some evidence of the fibrosis and new ovum. And this is your implant and this is that thin sections which is stained in this during histopathology analysis.

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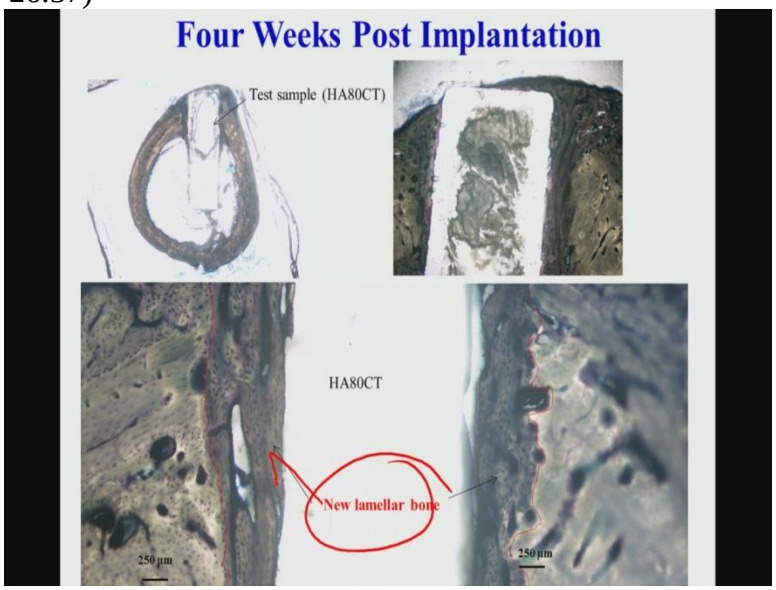

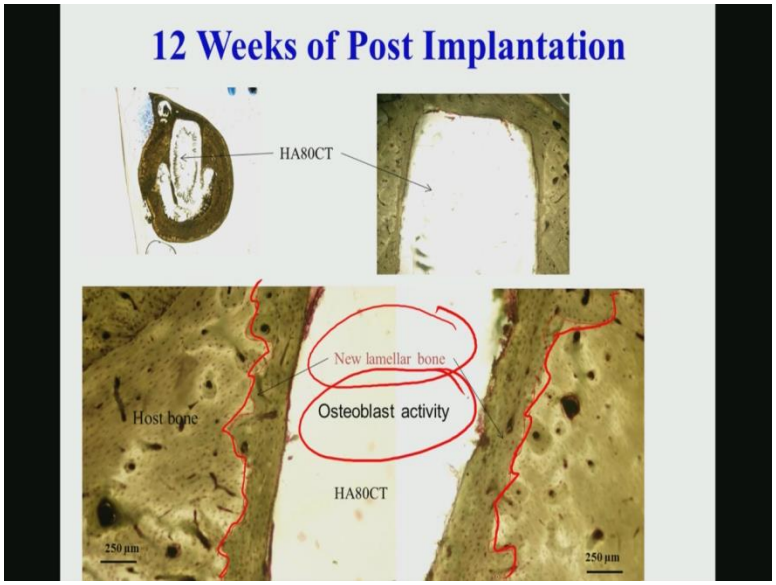

Now similar things also has been noticed for the test implant as well and test sample. So after four weeks post implantation, we also observed the new lamella bone which has been marked here, in both the cases of this implantation and then after 12 weeks of this implantation you do see that osteoblast activity and biggerlamellar bone has matured more to a larger extent and this is that sketched that is what I'm showing here. So this sketch is essentially, the way I'm sketching shows that this is the new lamellar bone which has grown post tissue or the implant post tissue interface and which you can essentially quantity in terms of the width of the new lamellar bone.

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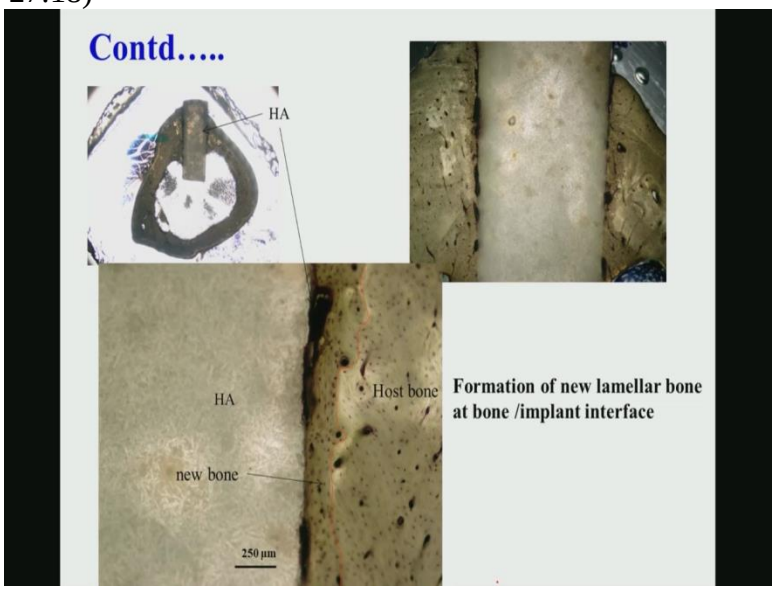

And here it shows the more clear picture of the new lamellar bone.

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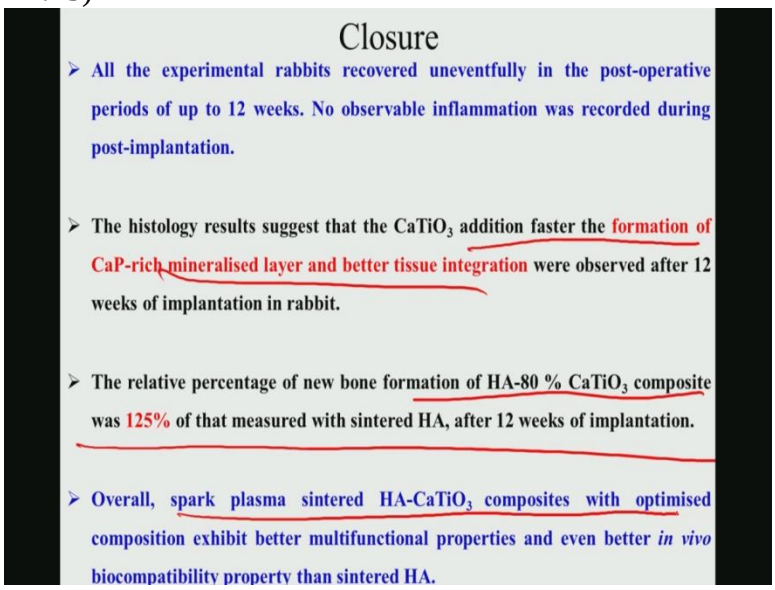

And this is the last slide so essentially all the experimental rabbits recover uneventfully in the post operative period of up to 12 weeks and then histology shows that calcium titanate adds more formation of better tissue formation and there is a new home formation of that ha 80 percent calcium titanate was 125 percent of that measured with sintered ha after 12 weeks of implantation and overall spark plasma sintered ha calcium titanate composite exhibit better multi

functional properties and even better in vivo bio compatibility property than sintered ha. Thank you.