Biomaterials for Bone Tissue Engineering Applications
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Module 7
Lecture No 32

So in last few modules I have covered the processing aspects of different materials particularly metals, ceramics and polymers. And also I have discussed one of the advanced manufacturing technique particularly rapid prototyping. And there I have discussed salient features of 3d printing, what is the physics of the drop and demand based 3d printing technique and some examples of the 3d prints materials.

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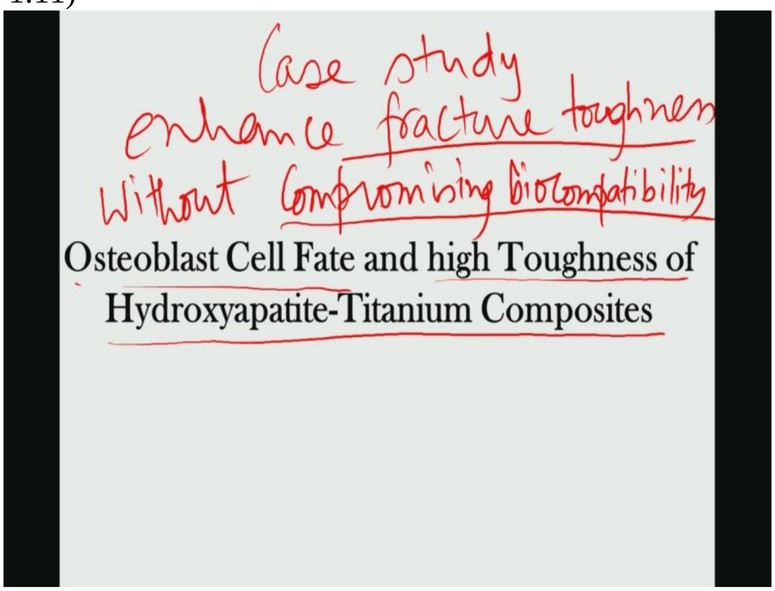

So in this module and then subsequent modules I will present some case studies, essentially highlighting the different aspects of bio ceramic development to address how one can enhance fracture toughness of this material. Now this is that perennial problem of ceramic based materials that they are extremely brittle, essentially they have very poor fracture toughness. And while one has to enhance the fracture toughness that one of the things that one has to take care that one should not compromise bio compatibility property.

So this is kind of a very key concept in that bio ceramic development that you can enhance the physical properties without compromising the bio compatible properties. So every time some new composition or some new materials are developed, while one has to carefully measure the different physical properties but at the same time ensure that how this new composite would also retain the uncompromised bio compatibility property with respect to some of the conventional or clinically used material like hydroxyapatite.

So therefore what I will do actually, I will show you that one of the material science based approach as how to develop that hydroxyapatite titanium composite with better toughness properties without compromising that osteoblast cell functionality in this kind of materials.

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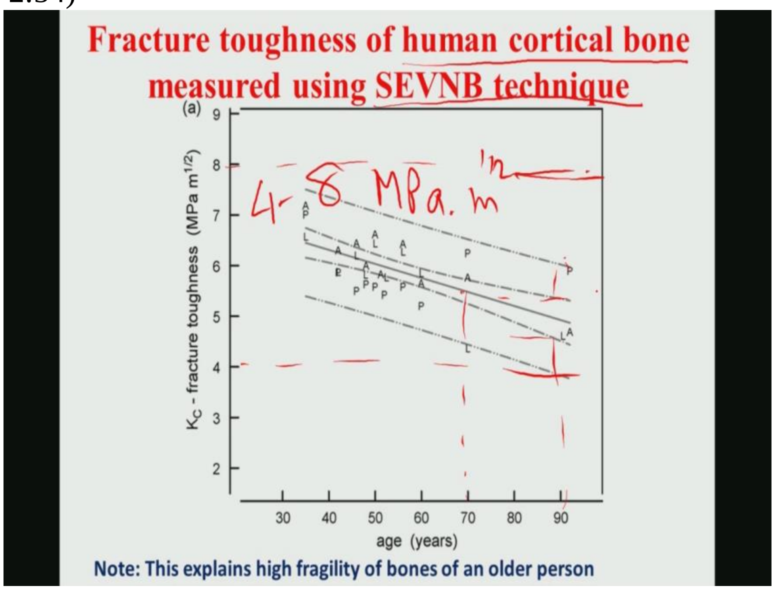

Just to give you some background as well as to refresh your mind that what is the typical fracture toughness values of natural bones. So this is that human cortical bone. People have reported ,or people have used single edge v notch beam technique to measure the fracture toughness of the human cortical bone. Now and they have taken the cadaver bone from different patient of different age ranging from somewhere is between 35 to 90 and what you see here that this fracture toughness of the human cortical bone varies in the window of somewhere between 4-8.

So essentially this window is 4-8 mps square root meter. That is the typical value of the SEVNB long crack fracture toughness of that cortical bone. So therefore any material which is to be developed for the intended bone replacement applications they should have fracture toughness somewhere close to 10 mps square root meter. Because you know that higher the fracture toughness better is it is reliability in certain applications in their applications like clinical application.

So this slide also tells you that in the older patients like patients of the age 70-90 if you see that their fracture toughness is much lower compared to the adult, healthier patients of lower age group like below 50 and so on. So therefore that older patients are more prone to bone fracture during accident or if they slip or sleep on the floor and so on so forth because their fracture toughness is very low compared to the adult healthy person.

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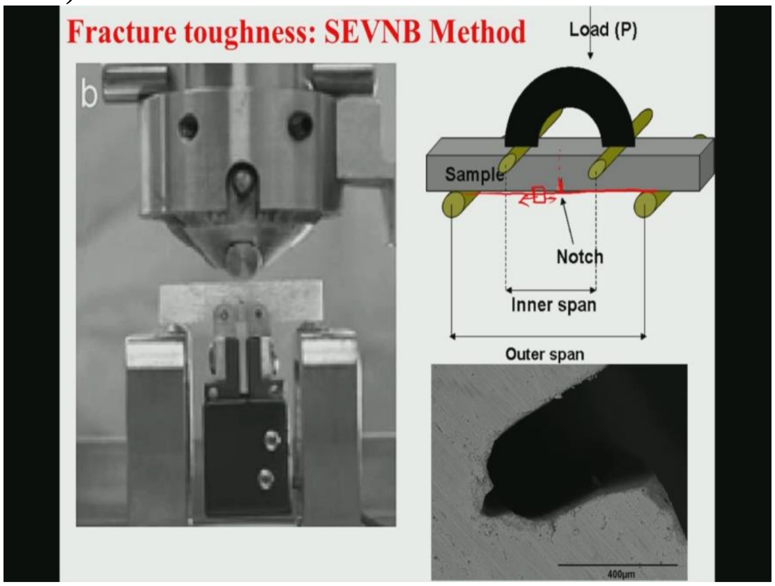

And this is how this single edge v notch beam technique is used to measure the fracture toughness. So this is the 4 point flexile configuration you have introduced a v notch here and this notch is introduced in the tensile surface of the or the tensile face of v notch samples. So that the cracks, once this notch is introduced this cracks will essentially go further leading to two pieces of the samples.

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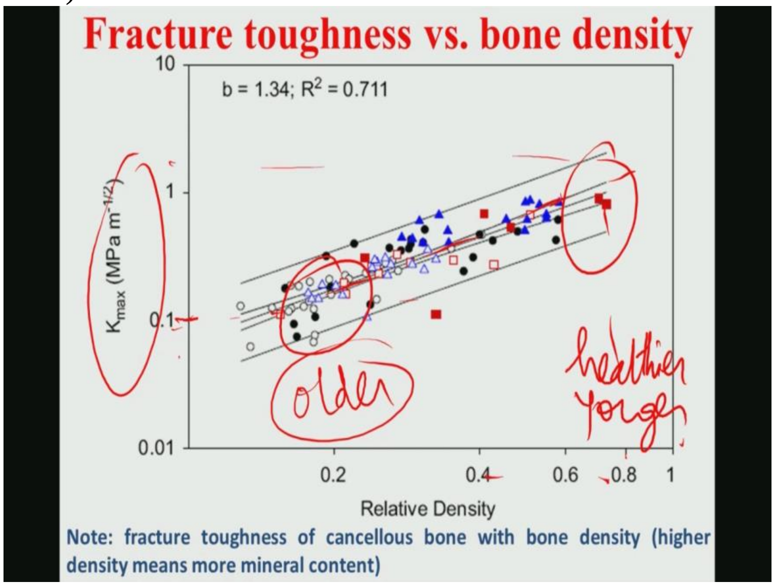

So bone density is also important in case of a natural bone and this essentially shows that this fracture toughness scales with the it goes bone density, so this is for the cancellous bone, it goes from .1 to somewhere above 1 mps square root meter. Now bone density if your remember, bone density essentiallydepends on the hydroxyapatite content or calcium apatite content in the in the natural bone. So higher the relative density of the bone means higher is the mineral content.

If lower is the mineral or calcium apatite content lower is the elastic modulus. So if you now place this plot with respect to the age of the patients so this region would be the older patients and this here will be that healthier and younger patients ok? Younger patients because younger patients the bone density is relative density is higher so that fracture toughness value is higher, older patients bone density is lower so that calcium apatite content is lower so density is lower and then fracture toughness is also low.

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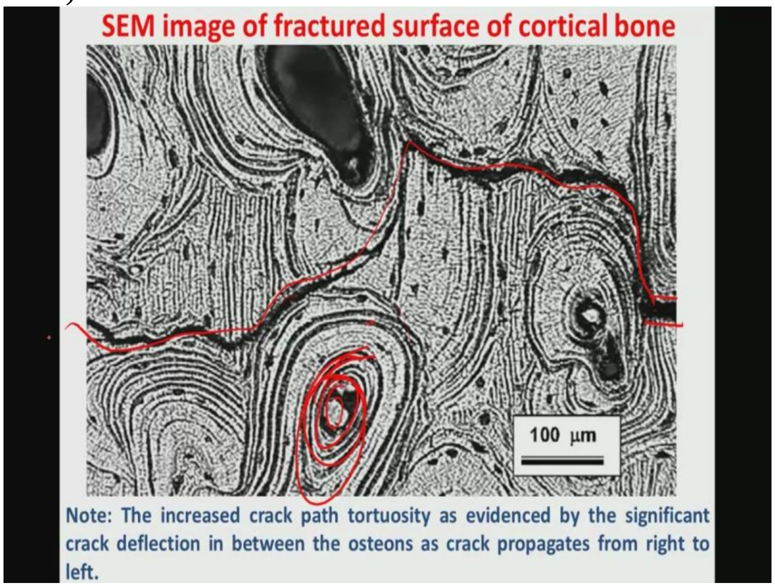

nd this is how the fracture takes place in the cortical bone. As you can see that we have introduced some v notch, then the cracks will go like this. In then this is your osteons. Different concentric circles and then cracks will essentially go through at the periphery of the osteon surface and go toward the other end of the bone structure.

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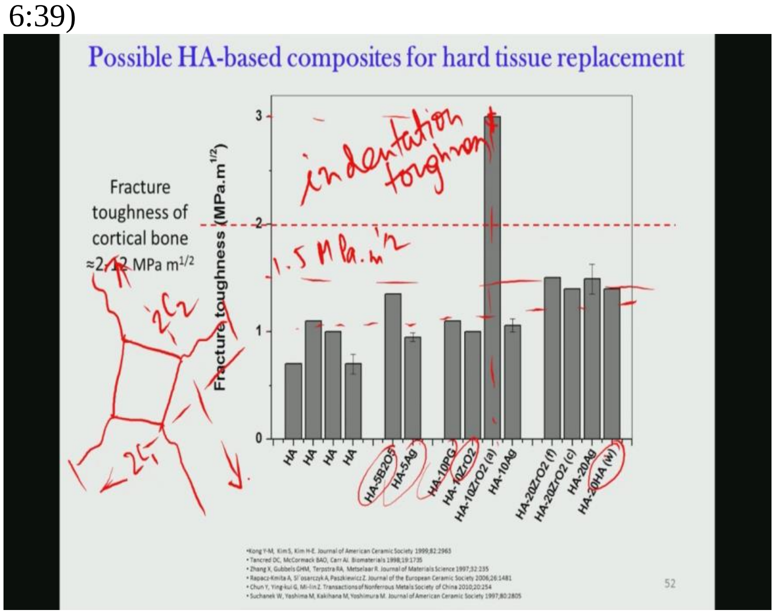

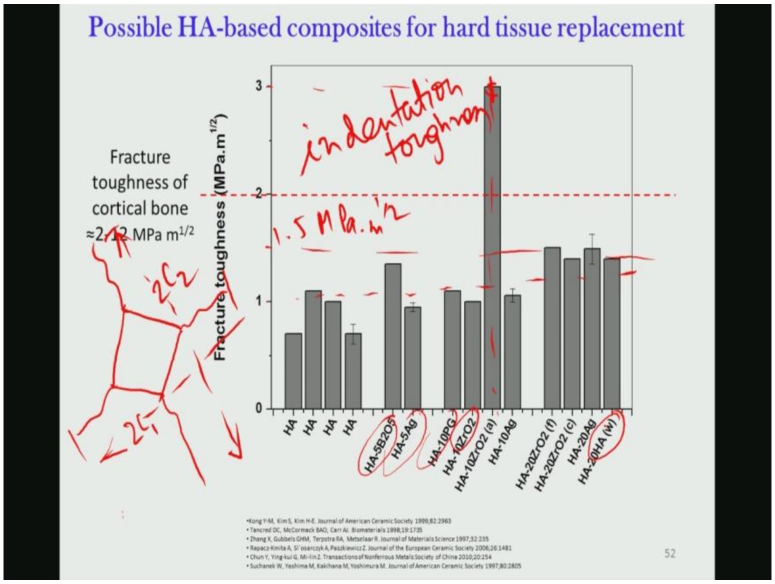

k. Now what is the driving force for development of the hydroxyapatite based composites in the bio ceramic applications? Essentially as I said that most of the hydroxyapatite based materials, if you plot their fracture toughness properties, what you see here, we have tried to summarize here. So this is B2o5 has been used as a reinforcement. Silver has been used as a reinforcement. Phosphate glass, zirconia and various other materials, hydroxide whiskers has been used as a re enforcement.

But whatever there is material what you see here except that one single report of hydroxide zirconia that all of them are below 1.5 mps square root meter, so 1.5 is somewhere between that more higher hydroxyapatite based composites, and then second thing that I must mention in this plot, this toughness is measured using indentation toughness. Now indentation toughness is doesn't give a very reliable measure of toughness.

Indentation toughness means you take the weakest indent, in brittle material this weaker indent produce some cracks in the, from the 4 edge of the corner. Then after that at any given load you can measure that what is the crack length, 2c1 and this is 2c2, what is the crack length, so you take average crack length then based on that you can measure the indentation toughness. So (())(8:13) very briefly, higher is the crack length lower is the fracture toughness or in reversely or conversely lower is the crack length higher is the fracture toughness.

So physically if I want to explain, this lower crack length essentially means the material is tough enough no to allow crack propagation in that micro structure. If the fracture toughness is high or if the fracture toughness is low that measure that material doesn't have enough resistance against fracture toughness. Because remember fracture toughness means resistance against crack propagation. So if a material has high fracture toughness that means has higher resistance to crack propagation.

And therefore higher fracture toughness materials you won't be able to see much cracks whereas lower fracture toughness materials you will be clearly seeing this crack generation even at the lower indent load. This drives us to see how to make hydroxyapatite materials with high fracture toughness property, so this is what has been mentioned here.

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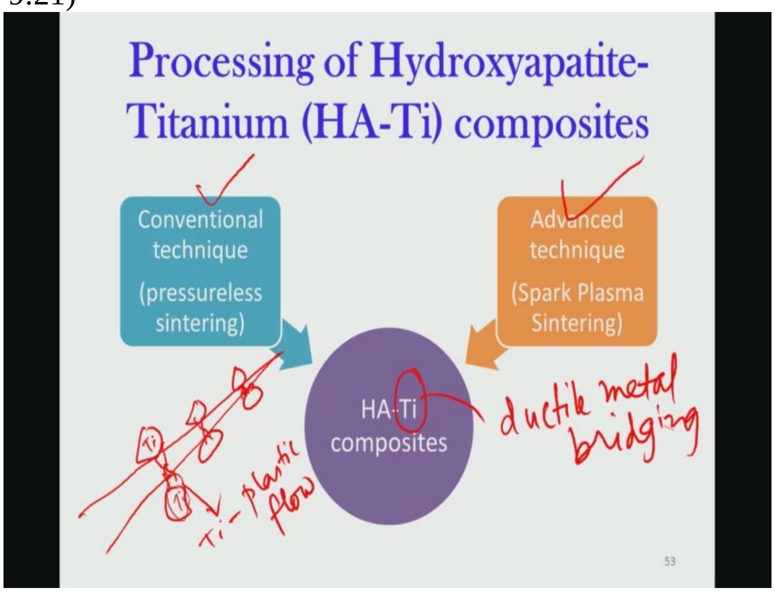

So what we use is the titanium as a metallic re enforcement. Now why titanium, because titanium being a metal, it can enhance the fracture toughness through the mechanism is ductile metal breezing. So ductile metal breezing essentially is a concept is that, suppose you have a titanium particle somewhere here and the crack is going to pass through this kind of micro structure.

Now in if this is the titanium particles this is a titanium particle, so what will happen? This titanium will undergo plastic flow, or plastic deformation at the crack tip. Because crack tiphas certain stress distribution here. So this is your titanium, this is your titanium, and once it will ductile flow then it will flow like this. And if this will flow from both the sides, then what will happen?

There is this cracks will, this crack tip will be closed because of the flow of the titanium particles in the crack tip. So what we do, we have us both the techniques, one is conventional sintering like pressurized sintering and one is spark plasma sintering. What is conventional sintering? You mix the hydroxyapatite with different proportions of titanium like 5 percent, 10 percent, 20 percent, and then you make the palette and you throw it inside the furnace like a conventional furnace like you know vacuum furnace or argon furnace, furnace which is heated in the argon atmosphere.

And then at higher temperature you see that whether densification takes place and then you make the composite. In adverse sintering like spark plasma sintering, which I have mentioned before, like it is more like flowing very large amount of current lie 1-1.5 kilo Ampere through this furnace and then you essentially heat the powder compact by Joule is heating and therefore you can make this composites.

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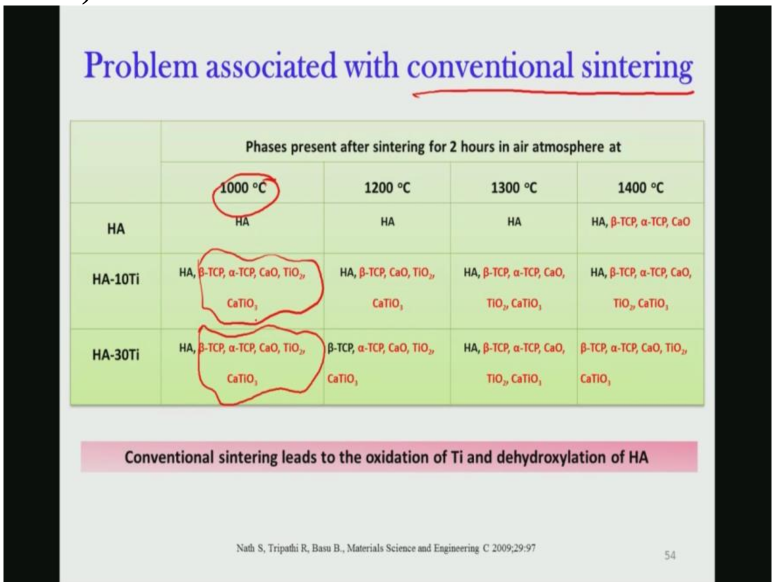

Now there is certain problems associated with conventional sintering when you particularly sintering here. What you see that is red phases, these are like different phases which is forming, when it is sintered at 1000 degree Celsius. Like what you see calcium titanate, essentially reacts titanium with calcium oxide and so on tio2. Also (())(11:43) 1300 or 1400 degree Celsius you see several other phases are also forming. So what it shows essentially thermo, what it shows is that chemical reaction or sintering reaction taking place hydroxide and titanium both are reactive.

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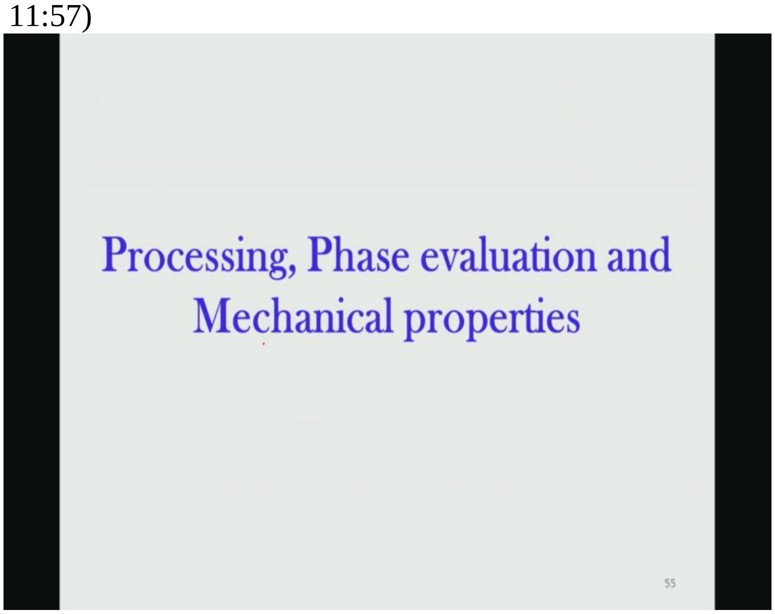

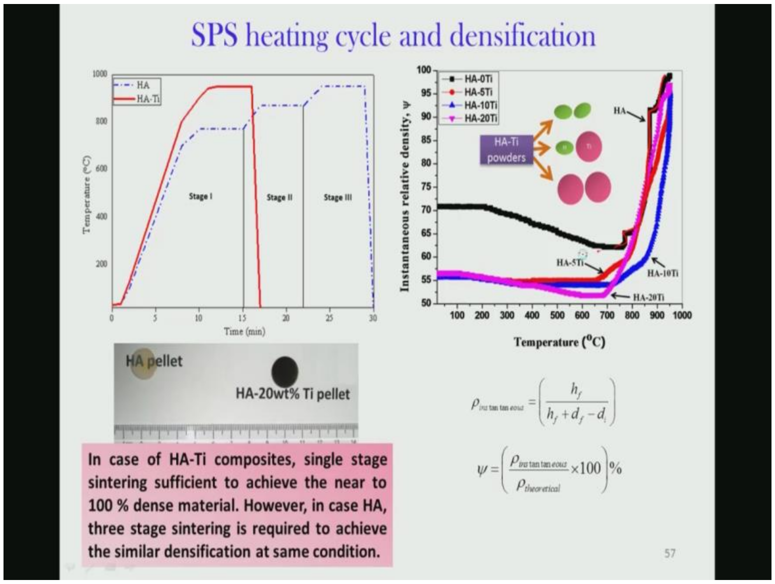

So what we do, we try to sinter them in spark plasma sintering in argon atmosphere at a temperature of 950 degree Celsius and so on. And then idea is that if you heat them very fast and if you hold them for smaller time period then you are not allowing the hydroxide titanium to react stop so all the conventional related sintering reactions can be avoided and you can achieve higher densification.

The same thing is true here, as you can see that hydroxyapatite is going, this is going towards 100 percent densification then when you give titanium and so on, this densification is actually is taking place at a much lower temperature and as a result and as a result that titanium actually aids in the shrinkage or densification process.

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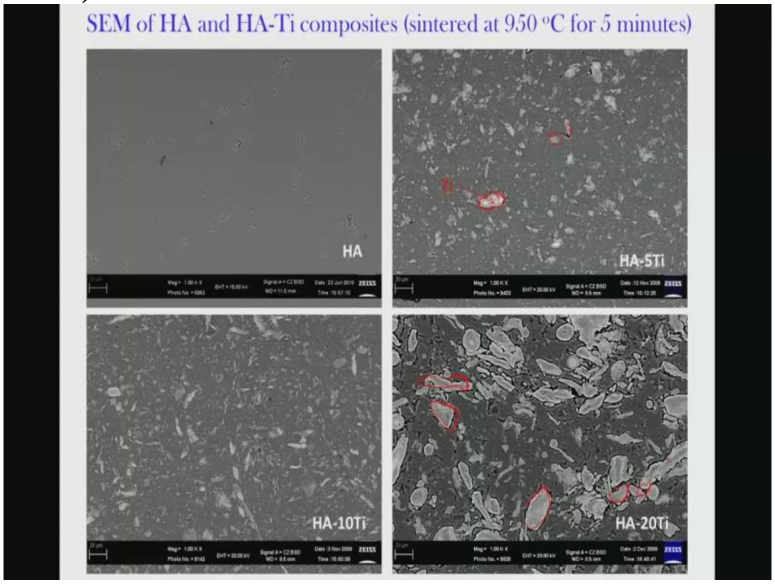

Now as far as the acm images of this titanium distribution is concerned, what you see that titanium particles are distributed quiet uniformly but at higher titanium quantity like 20 percent titanium and so on, you can see that there is a plaster of titanium particles which is distributed in the hydroxyapatite matrix.

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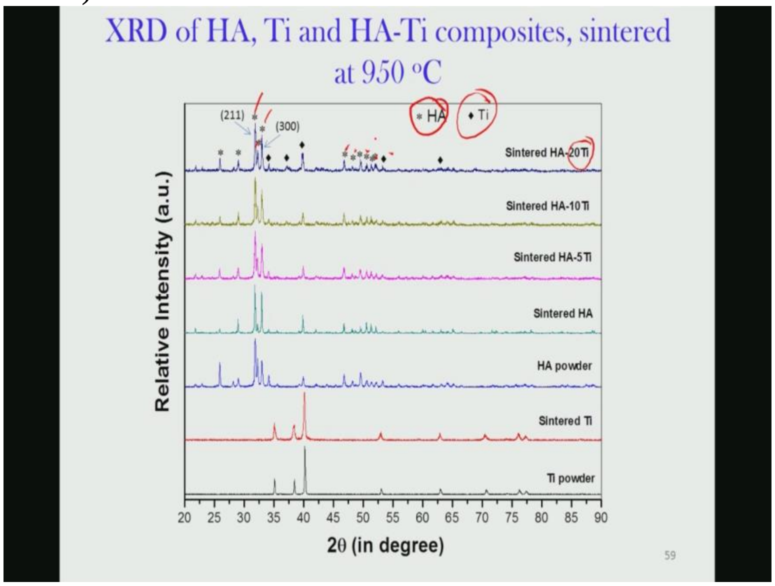

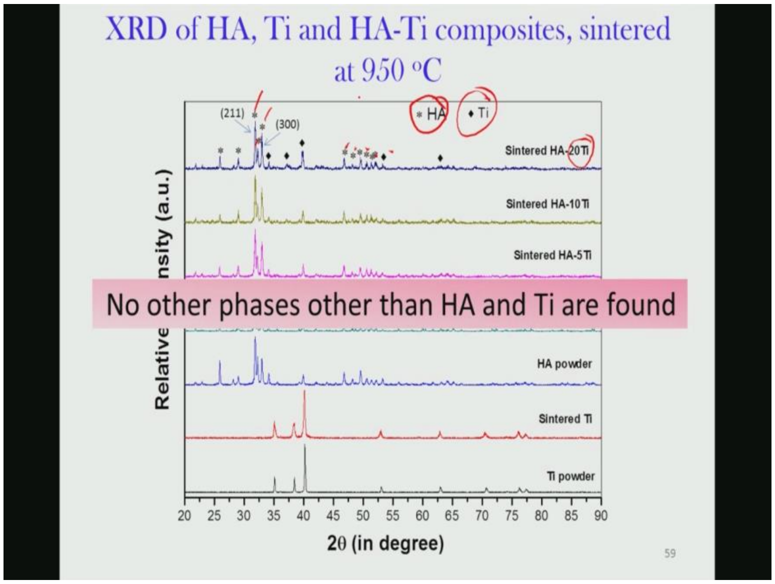

Now this hydroxyapatite and titanium it is both retained very predominantly in 20 percent titanium and 30 percent titanium. I think when we published this paper in (())(13:30) layer that was the first proof that both hydroxyapatite and titanium can be indeed retained in the micro structure which is otherwise very difficult if one uses the conventional sintering technique and as you can see all the characteristic peaks of the hydroxyapatite, all these are lined up at a particular 2 theta angle which is essentially follows very closely with the speediest file (())(13:51) file of this particular phase.

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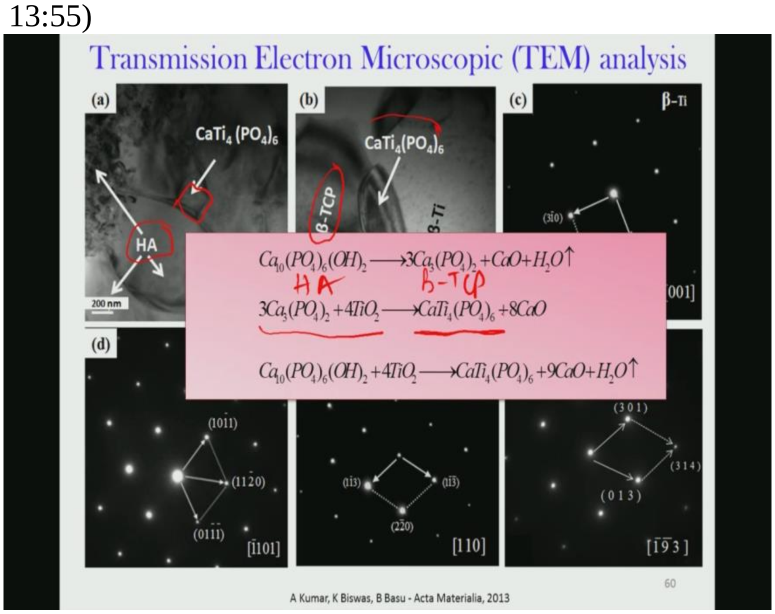

Now although this any any indication of the second phase formation could not be detected within the detection limit of the extra diffraction, but we did observe some second phase particularly in the grain boundary region or grain boundary triple pocket here. As you can see the grain boundary triple pocket here. Now this phase when we did that selected area diffraction pattern what you see here it is the cati4po4 whole 6. That is very unusual phase which doesn’t from during that conventional sintering experiments but it forms during that spark plasma sintering experiments. And except that we see that very clear evidence of hydroxyapatite and beta titanium and small amount of beta tricus and phosphate.

All these phases are uniquely identifies or analyzed using the selected area diffraction pattern from the individual phases. Now what is the specific reactions that perhaps is taking place in this one? The specific reaction are ca10po4 whole 6 that is the stoic metric ha and this ha essentially going to beta tricus and phosphate what you see ca3po4 whole 2 leaving conh2o and this ca3po4 whole 2 can react with titanium dioxide to from this sintering reaction product that is cati4po4 whole 6.

Why tio2 comes into picture? All these metals titanium and so on, they are always covered with a very thin layer of oxides like titanium dioxide and so when calcium phosphate reacts with titanium dioxide then it there is a possibility of cati4po6. It is very difficult to assess the thermo dynamic feasibility of this reaction simply because they do not have all this Delta g values that thermo dynamics gives to energy. Change of formation of these compounds are not available in literature.

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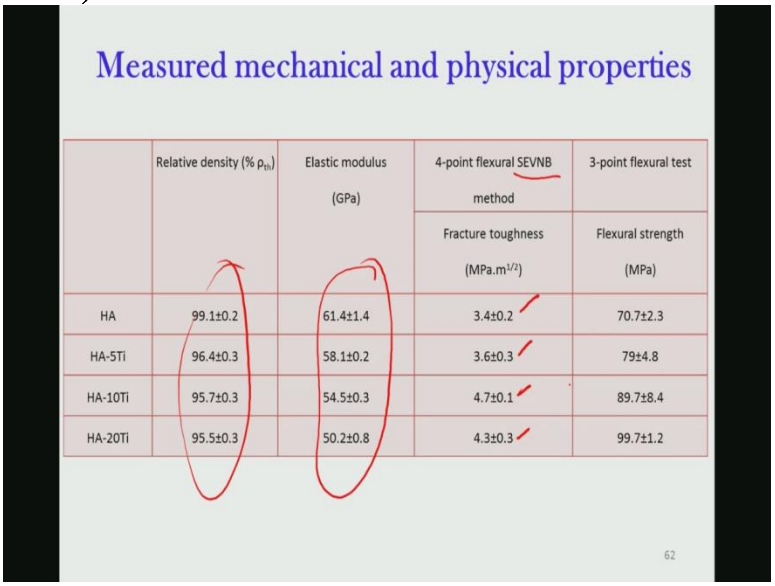

	Relative density (% ρ_{th})	Elastic modulus (GPa)	4-point flexural SEVNB method Fracture toughness (MPa.m$^{1/2}$)	3-point flexural test Flexural strength (MPa)
HA	99.1±0.2	61.4±1.4	3.4±0.2	70.7±2.3
HA-5Ti	96.4±0.3	58.1±0.2	3.6±0.3	79±4.8
HA-10Ti	95.7±0.3	54.5±0.3	4.7±0.1	89.7±8.4
HA-20Ti	95.5±0.3	50.2±0.8	4.3±0.3	99.7±1.2

Now coming to the mechanical properties of these material, first of all these material are sintered more than 95 percent theoretical density. Elastic modulus also is fairly low. It is I mean although a little this is 50 to 60 giga Pascal. SEVNB, single edge v notch beam technique when you measure, this fracture toughness values are relatively high 4.3 MP square root meter.

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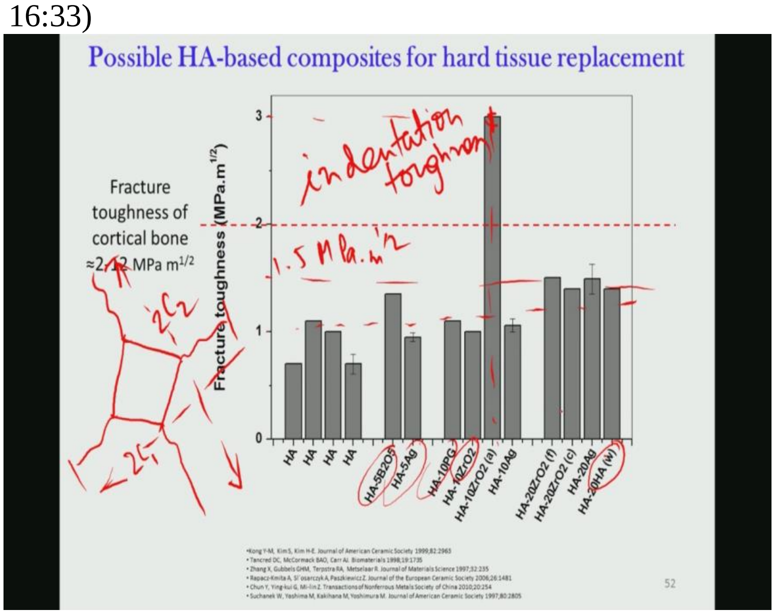

Remember that earlier, remember that in one of the earlier slides I have mentioned that which drives the development of hydroxide based material, that most of this earlier investigated, earlier developed hydroxide based composites, they have a fracture toughness when measured using the indentation technique is somewhere around 1.5 mps square root meter or below with the exception of one literature case where they have reported close to 3 but that again was measured using the indentation toughness.

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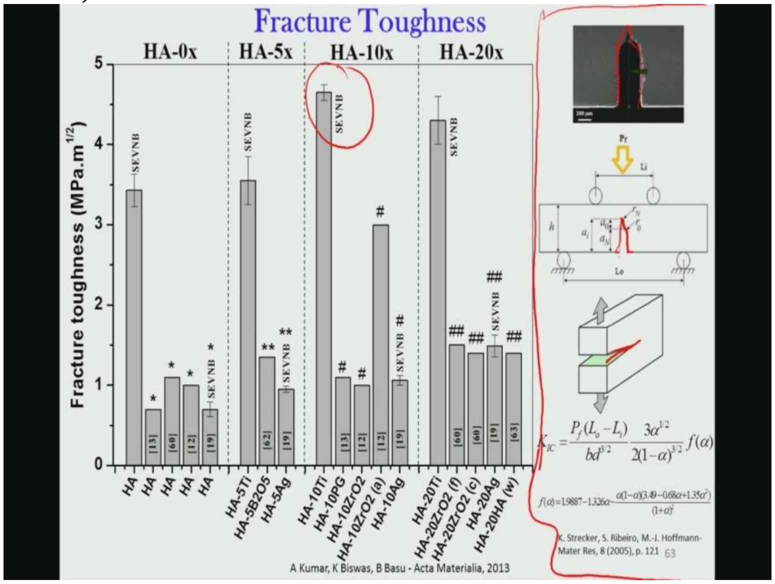

Ok. Now this is little bit more clear picture about this how this material stand with respect to earlier investigated material. On the right hand panel I have explained that SEVNB technique. So we have used that single edge v notch beam technique, we introduce this notch here. This notched surface is placed in the tensile side of the SEVNB. In this we have used that 4 point bending configuration, the flexural configuration. And this is the you know v notch here.

And then v notch and then you measure that k1c values that is that critical crack tip and stress intensity factor under mode 1 loading and this is that particular equation one can use and then when you do this SEVNB measurements here, even the hydroxyapatite itself has very fairly high fracture toughness and then in the 10 percent titanium one can go very close to 5 mps square root meter. Remember the cortical bone fracture varies somewhere between 3 to 8 mps square root meter. So this 5 mps square root meter certainly we are almost half way in the range of that natural cortical bone toughness.

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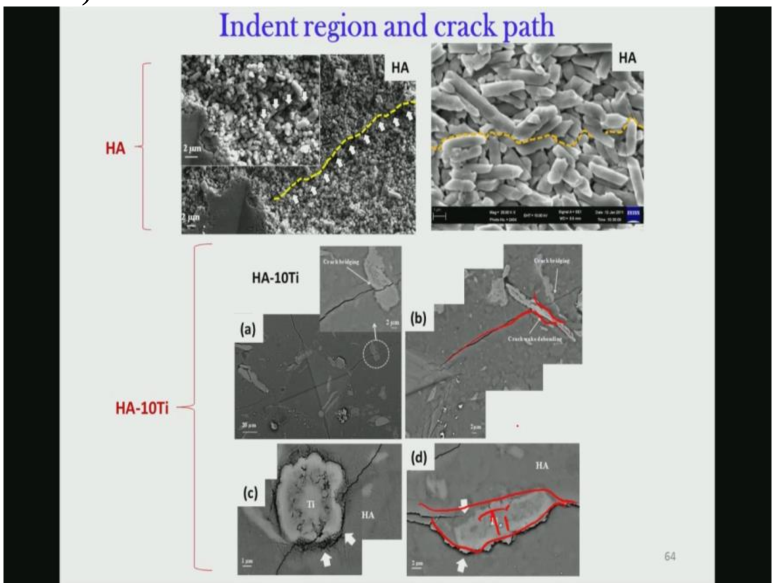

Now as far as the toughness is concerned the what we have observed that when you have titanium on the way this crack is going and then crack there is a evidence of the crack going debonding and crack breaching. And this is kind of very clearly, here you can see titanium here and around the titanium there is some evidence of the crack breaching as a additional toughing agents.

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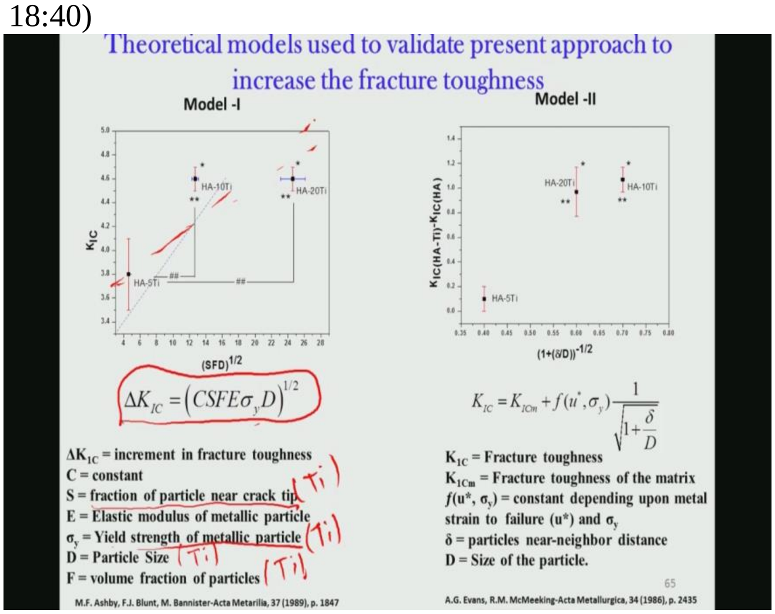

So we have used therefore some of the well known (())(18:45) model or the Evan is model just to show that how fracture toughness can be correlated with some of the parameters which can contribute to the fracture toughness in these materials. So these parameters, one of them is s that is fraction of particle near the crack tip, that is titanium here were talking about. And second one the yield strength of the metallic particle again titanium because titanium is retained predominantly, d is the particle size, again titanium and volume fractions of the particles. So what you clearly see that all these things are linearly correlated linearly correlated with delta kc, because Delta k1 is the toughness enhancement because of the presence of the titanium.

And they are indeed proportional to square root of each of these term in a linear manner. So higher these values if these parameters, higher will be the fracture toughness enhancement. And that is indeed shown here in our case, only things within the limited data points what we see, it is a kind of clearly linear trend. Although they do notfollow exactly linear but they show some increase fracture toughness enhancement of these things.

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On the toughness enhancement in hydroxyapatite-based composites

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Abstract

Among various biologically compatible materials, hydroxyapatite (HA) has excellent bioactivity/osteointegration properties and therefore has been extensively investigated for biomedical applications. However, its inferior fracture toughness limits the wider applications of monolithic HA as a load-bearing implant. To this end, HA-based biocomposites have been developed to improve their mechanical properties (toughness and strength) without compromising biocompatibility. Despite significant efforts over last few decades, the toughness of HA-based composites could not be enhanced beyond 1.5–2 MPa $m^{1/2}$, even when measured using indentation techniques. In this perspective, the present work demonstrates how spark plasma sintering can be effectively utilized to develop hydroxyapatite–titanium (HA–Ti) composites with varying amounts of Ti (5, 10 and 20 wt.%) with extremely high single edge V-notch beam fracture toughness (4–5 MPa $m^{1/2}$) along with a good combination of elastic modulus and flexural strength. Despite predominant retention of HA and Ti, the combination of critical analysis of X-ray diffraction and transmission electron microscopy investigation confirmed the formation of the $CaTi_4(PO_4)_6$ phase with nanoscale morphology at the HA/Ti interface and the formation of such a phase has been discussed in reference to possible sintering reactions. The variations in the measured fracture toughness and work of fracture with Ti addition to the HA matrix were further rationalized using the analytical models of crack bridging as well as on the basis of the additional contribution from crack deflection. The present work opens up the opportunity to further enhance the toughness beyond 5 MPa $m^{1/2}$ by microstructural designing with the desired combination of toughening phases.

Keywords: Composite; HA/Ti; SPS; SEVNB; Fracture toughness

Now this work was published in (())(20:01) in 2013 value so if you are interested you can look at this paper for more details.

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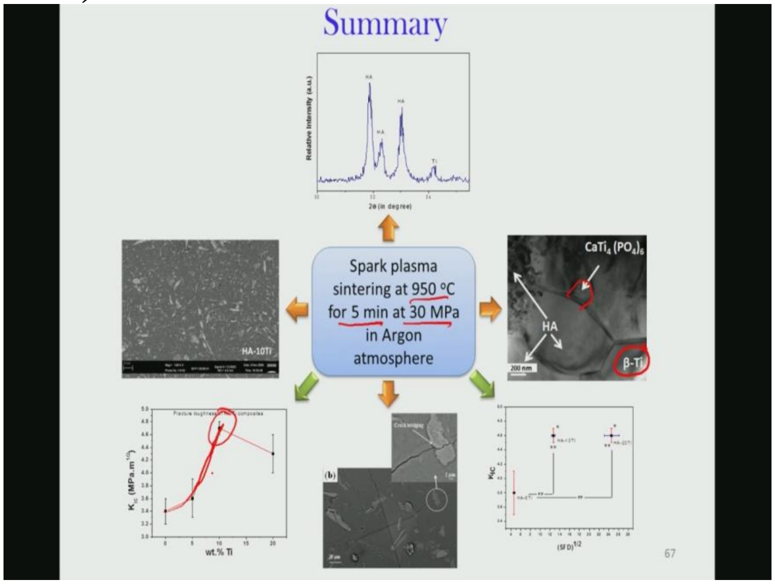

So this is kind of a summary of this spark plasma sintered materials at 950 degree Celsius in 30 mega Pascal and for 5 minutes, it is with indeed very short sintering and then you do see that evidence of this second phase formation of the grain boundary triple pockets, we have explained that some reactions that how it is occurring and apart from that you have a beta titanium. And k1c is the function of the percentage titanium, you see that it goes through a very high, relatively higher values like 4.8, close to 5 mps square root meter.

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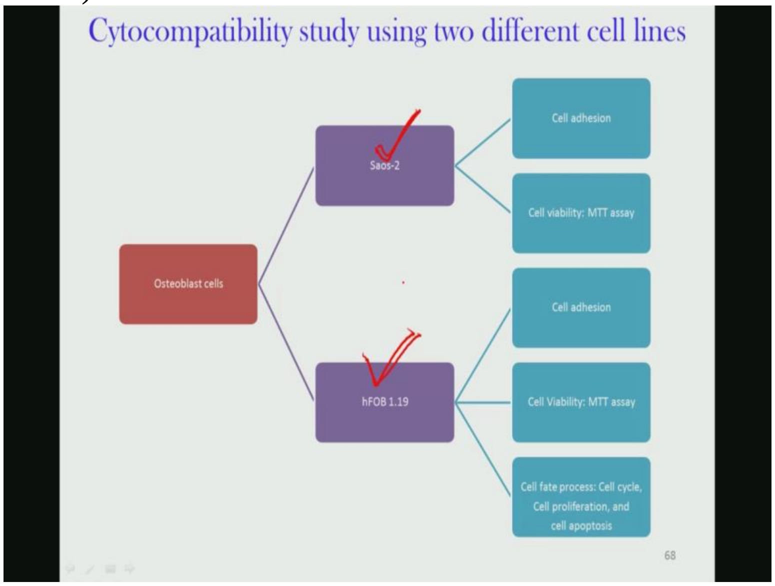

Now cyto compatibility or cell level compatibility of this materials was established using two cell lines one is human fetal osteoblast and it is is sos2 means sircom osteosarcoma. And this is hfob means human fetal osteoblast. And different assays are done addition, viability and so on.

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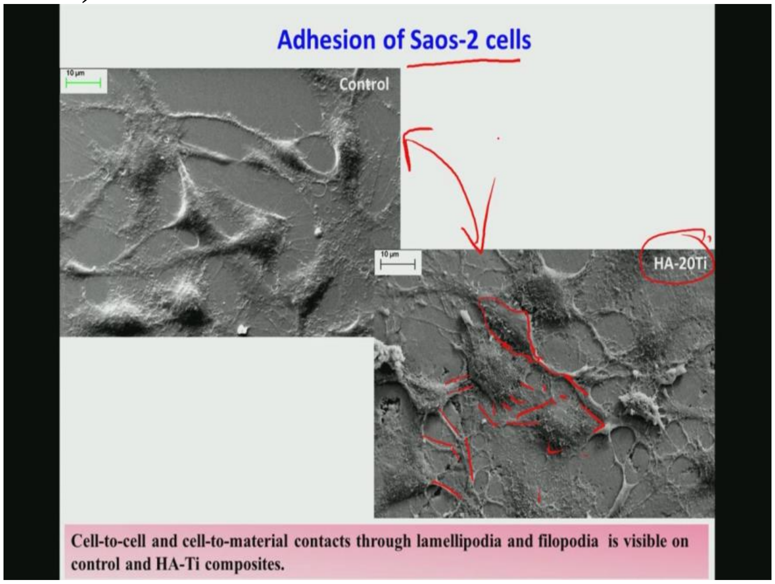

So first one is that addition of sos2 cells, so this is ha 20 percent titanium surface on the cells, now certain features you noticed that first of all this osteoblast cells, they show more like a flatten morphology which they should show while adhering on a surface. Second thing we do observe cellular breach formation like cell to cell contacts and this is a very characteristics of osteoblast and there are some evidence of extra cellular matrix formation on this material and there is very clear interaction of the filopodial extension and filopodial and lamella podia.

And then there is not much significant difference between the controlled sample and the hydroxyapatite 20 percent titanium in terms of that cell morphological changes. And then extensive extensive extension of filo podia, lamella podia also kind of provides some signatures of the or some signature towards the evidence of the cell migration.

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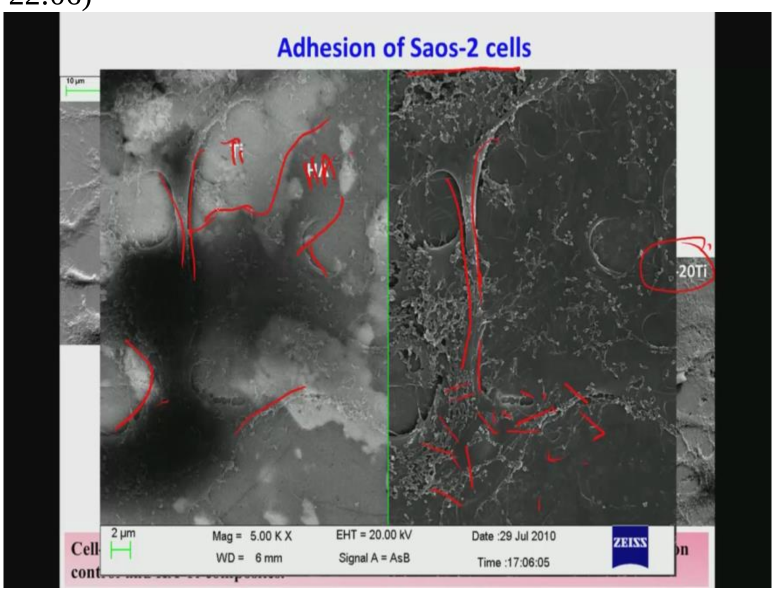

This is more evidence of this how this cell morphological changes had taken place on this particularly sos2, and then we do see certain evidences of titanium and hydroxyapatite as expected cell are adhering more on the hydroxyapatite breach region because hydroxyapatite as you know that is that kind of a it it is inorganic composition of the natural bone.

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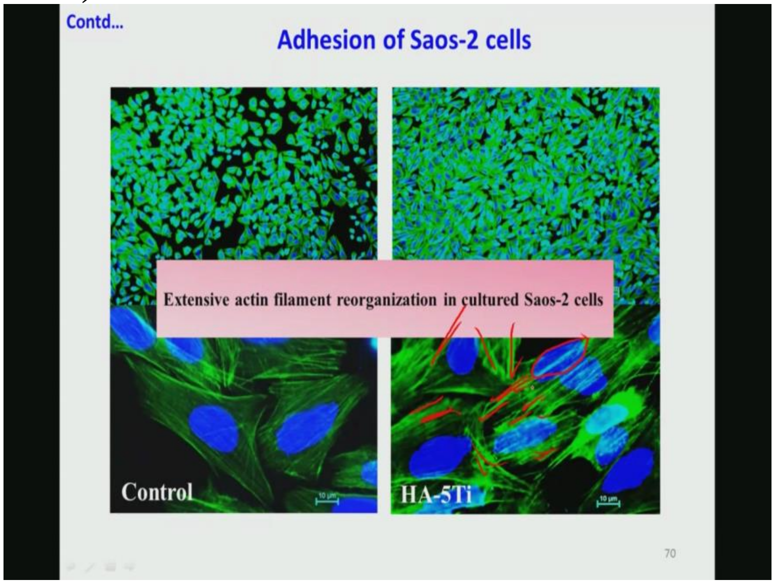

Now this is the fluorescence fluorescence micrograph representing fluorescence micrograph of the sos2 cells and that hydroxide 5 percent went titanium surfaces and what you see this is that actin which is stained in the green, and the nucleus which is stained in the dapi, remember the dapi intercollects with that DNA of the nucleus and therefore it also gives a blue blue fluorescence and then other things that you can see that there is the clear evidences of the cyto skeletal reorganization and cyto skeletal stretching, acting filament stretching on this materials on the cells which have which have adhered on this material substrate.

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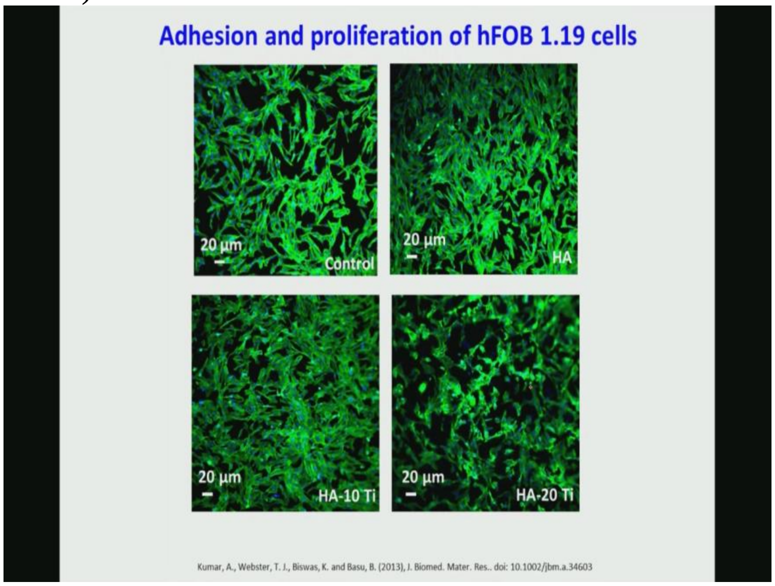

Now human fetal osteoblast cells when they are grown on this materials for three days in culture they do exhibit similar kind of proliferation behavior and forming a very dense cell colony on this materials. And this also activating filament reorganization in the culture.

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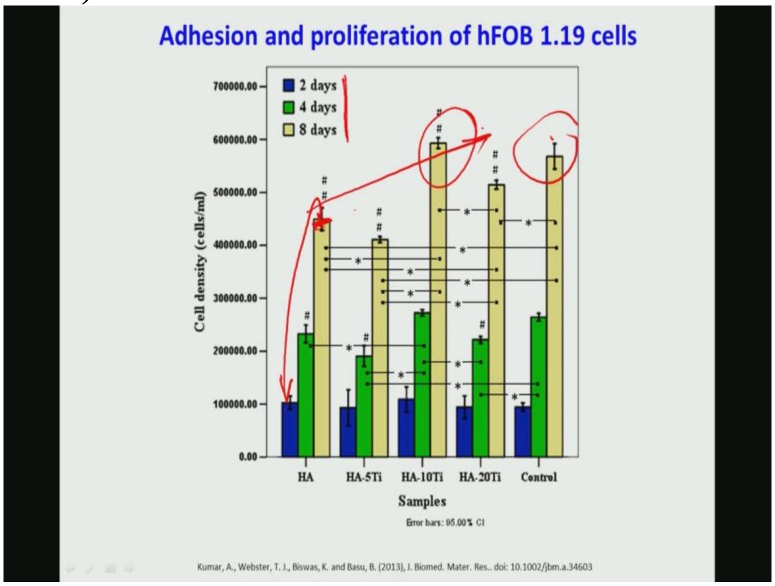

Now in terms of the numbers we have done this experiments for the two days, four days, and eight days in culture because this human fetal osteoblast cells are relatively slow growing cells. In all these cases you see that compared to control certainly there is a significant, if you consider this hydroxyapatite this one of the control, compared to control that ha 10 percent and ha 20 percent titanium, these are significantly higher values even after 8 days of their culture of these cells in this substrate.

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Society For Biomaterials

In vitro bioactivity and cytocompatibility properties of spark plasma sintered HA-Ti composites

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Abstract: The present study reports the results of the detailed *in vitro* bioactivity and cytocompatibility properties of the hydroxyapatite (HA) and the HA-titanium (HA-Ti) composite with varying amount of Ti (5, 10, and 20 wt %), densified using spark plasma sintering process (SPS). Using this technique and tailoring suitable processing parameters, it has been possible to retain both HA and Ti in the sintered ceramics. Importantly, the uniquely designed SPS processing with suitably chosen parameters enables in achieving better mechanical properties, such as higher indentation fracture toughness (~1.5 MPa $m^{1/2}$) in HA-Ti composites compared with HA. X-ray diffraction and scanning electron microscopic (SEM) observations reveal good bioactivity of the HA-Ti composites with the formation of thick, flaky, and porous apatite layer when immersed in simulated body fluid at 37°C and pH of 7.4. Atomic absorption spectroscopic analysis of the simulated body fluid solution reveals dynamic changes in Ca^{+2} ion concentration with more dissolution of Ca^{+2} ion from the HA-20Ti composite. However, the measurements with inductively coupled plasma spectrometer do not record dissolution of Ti^{+4} ions. Transmission electron microscopic analysis indicates weak crystalline nature of the apatite and confirms the formation of fine-scale apatite crystals. MTT assay, fluorescence, and SEM study demonstrate good cell viability and cell adhesion/proliferation of the Saos -2 cells, cultured on the developed composites under standard culture condition, and the difference in cell viability has been discussed in reference to substrate composition and roughness. Overall, HA-Ti composites exhibit comparable and even better *in vitro* bioactivity and cytocompatibility properties than HA. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 101B: 223-236, 2013.

Key Words: HA, Ti, SPS, bioactivity, cytocompatibility

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Now this work is again published in jbmr B journal bio medical research part B.

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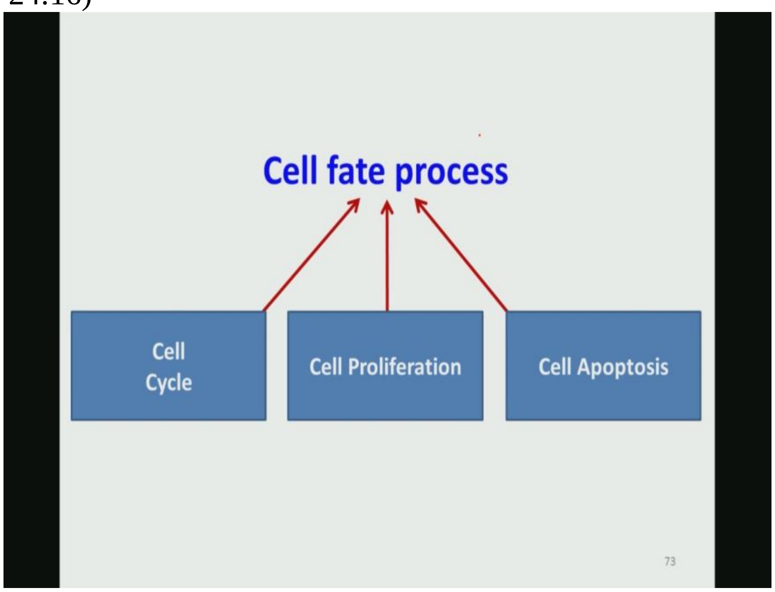

Now third aspect is the cell fate processes. Now this thing I have mentioned earlier that is the cell cycle, cell proliferation, cell apoptosis, all this cell fate processes can be quantified using fluorescent activated cell sorter, which has been discussed before.

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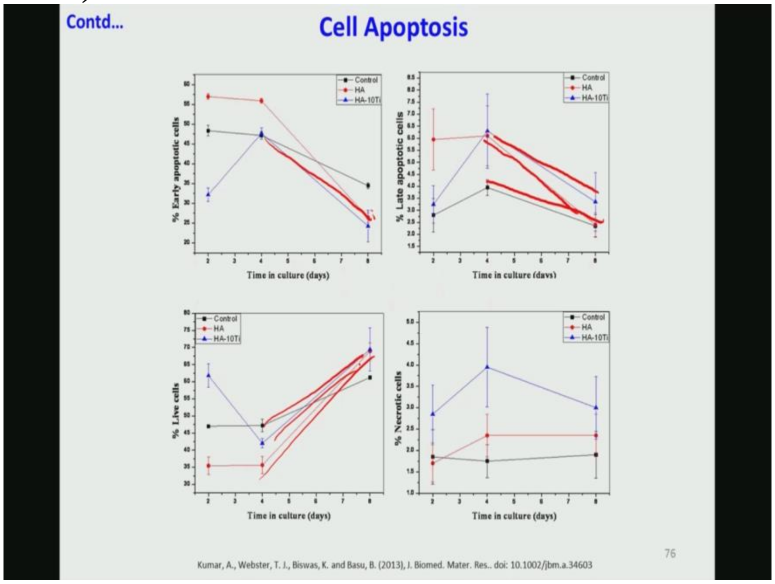

And in case of this hydroxyapatite 10 percent titanium, when we have compared with that controlled what you see here that earlier apoptotic cells that kind of reduces with days in number, with days in culture. Similar thing late apoptotic cells also shows certain decreasing trend in terms of time in culture. Percentage necrotic cells and percentage live cells, live cells also that increases with more time in culture.

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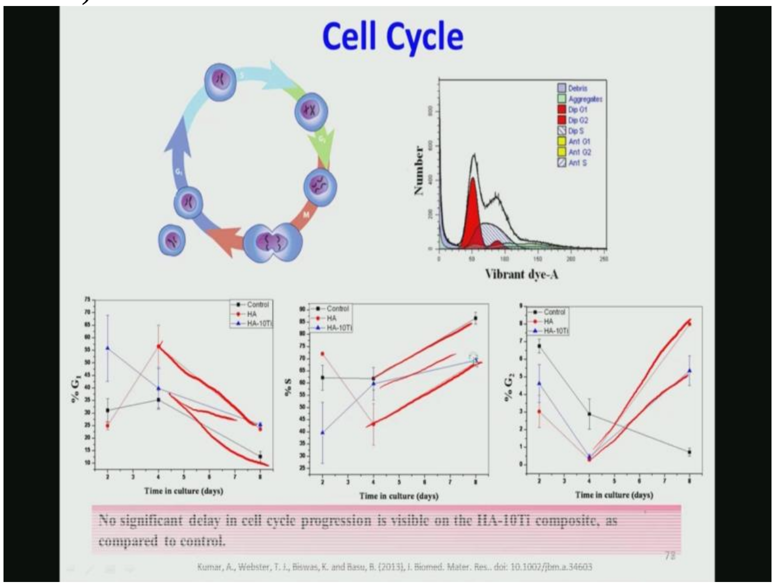

Now in terms of cell cycle we have done some analysis in terms of what is the percentage of cell in g1, s and g2. And what we have observed that g1, percentage g1 it is is reducing with more days in culture that means more cells are going into the next phase that is the g2, and certainly except with with the exception of the control both hydroxyapatite and hydrogen 10 percent titanium, cells are increasing, that number of cells in g2, phase increases and same is true for the s phase that number of cell increases. So what it shows that cells are not stuck while growing them on this substrate and certainly cells are able to cross the check points while adhering or while growing on this particular substrates.

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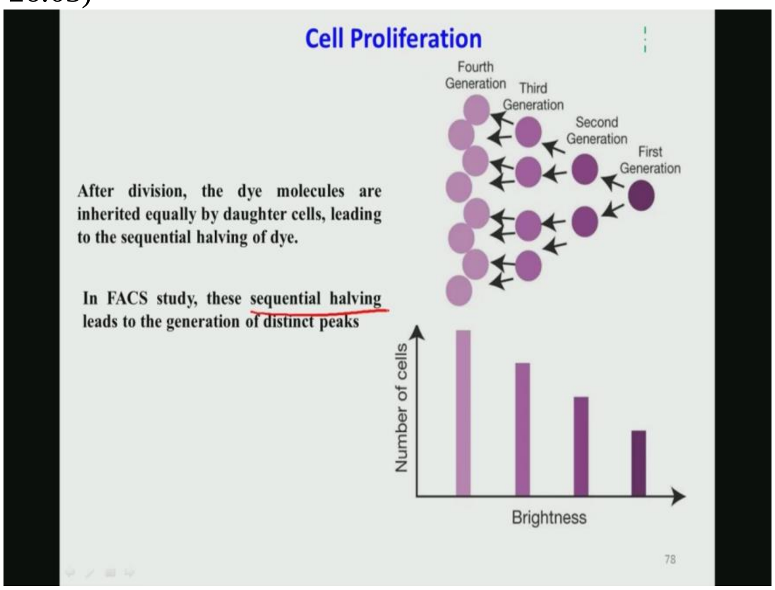

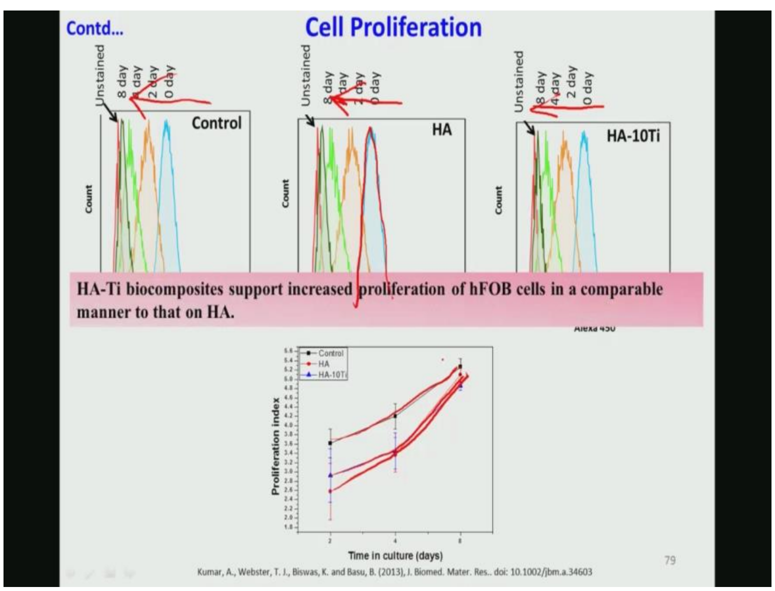

Now in terms of proliferation when cells go from first generation, second generation, third generation and there is a concept called sequential hoving . So therefore it leads to the generation of distinct peaks and that is what has been shown here that it goes from 0 day and every time it, more the time in culture it goes to more on the left hand side. So this is that 0-8 days. And based on that one can calculate that what is the proliferation index.

And interestingly all this number shows that proliferation index systematically increases. That means these substrates while enhance in the fracture toughness they indeed actually can be used as a cell growth substrate also. So this is flow cytometry based analysis that has been again published in journal bio medical research part B.

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Society For Biomaterials

Review Article

Hydroxyapatite-titanium bulk composites for bone tissue engineering applications

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Abstract: The research work on bulk hydroxyapatite (HA)-based composites are driven by the need to develop biomaterials with better mechanical properties without compromising its bioactivity and biocompatibility properties. Despite several years of research, the mechanical properties of the HA-based composites still need to be enhanced to match the properties of natural cortical bone. In this regard, the scope of this review on the HA-based bulk biomaterials is limited to the processing and the mechanical as well as biocompatibility properties for bone tissue engineering applications of a model system that is hydroxyapatite-titanium (HA-Ti) bulk composites. It will be discussed in this review how HA-Ti based bulk composites can be processed to have better fracture toughness and strength without compromising biocompatibility. The advantages of the functionally gradient materials to integrate the mechanical and biocompatibility properties is a promising approach in hard tissue engineering and has been emphasized here in reference to the limited literature reports. On the biomaterials fabrication aspect, the recent results are discussed to demonstrate that advanced manufacturing techniques, like spark plasma sintering can be adopted as a processing route to restrict the sintering reactions, while enhancing the mechanical properties. Various toughening mechanisms related to careful tailoring of microstructure are discussed. The *in vitro* cytocompatibilty, cell fate processes as well as *in vivo* biocompatibility results are also reviewed and the use of flow cytometry to quantify *in vitro* cell fate processes is being emphasized. © 2014 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 103A: 791–806, 2015.

Key Words: hydroxyapatite, titanium, composite, fracture toughness, biocompatibility

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I think with this we have also written a review paper on this hydroxide titanium based composite on bone tissue engineering application those who want to read more about it, they can follow this review paper.

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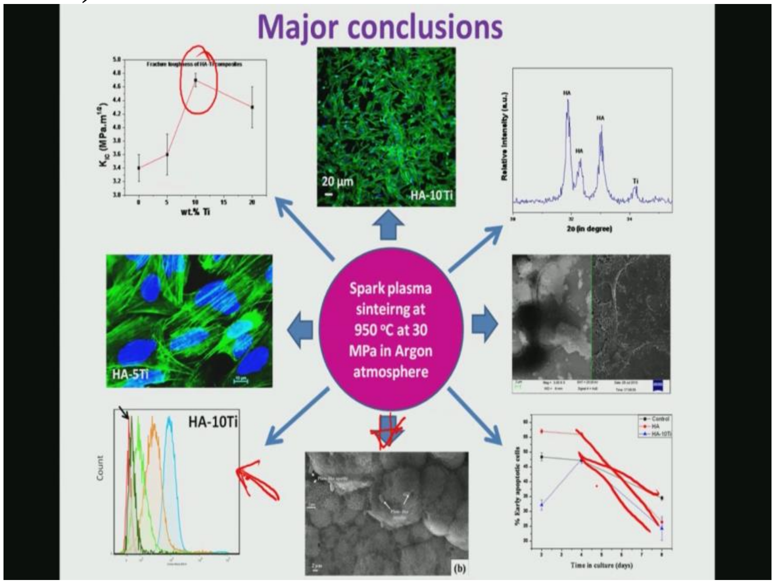

And this is this entire summary of this one. That we have sintered this material in 950 degree Celsius. What I have not shown that we have also done some bio mineralization experiments just to show this apatite crystal formation which is not influenced because of the presence of the titanium. We have shown that how cell fate processes is not compromised, fracture toughness values importantly increased to quite some extent and also early apoptotic cells also decreases because and that shows that this substrate do not induce much of this necrotisor apoptosis.