Introduction to Biomaterials

Prof. Bikramjit Basu

Prof. Kantesh Balani

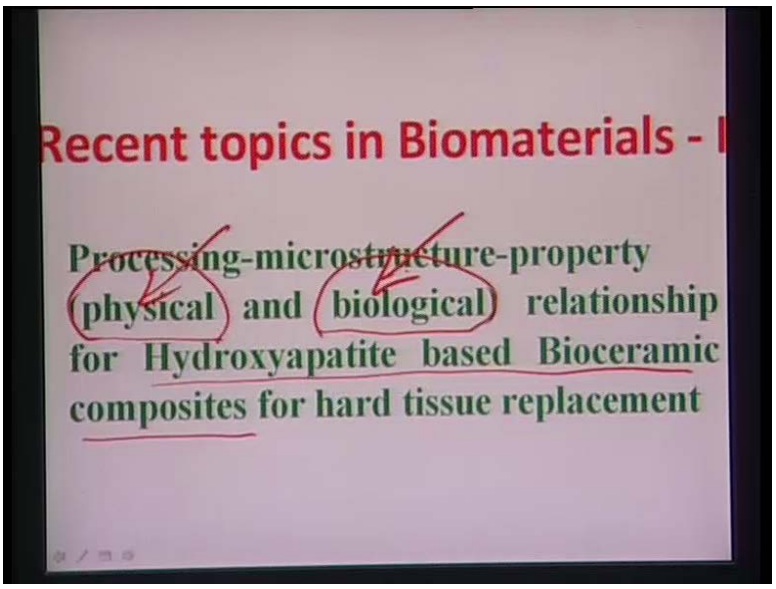
Department of Materials and Metallurgical Engineering

Indian Institute of Technology, Kanpur

Lecture No. # 23

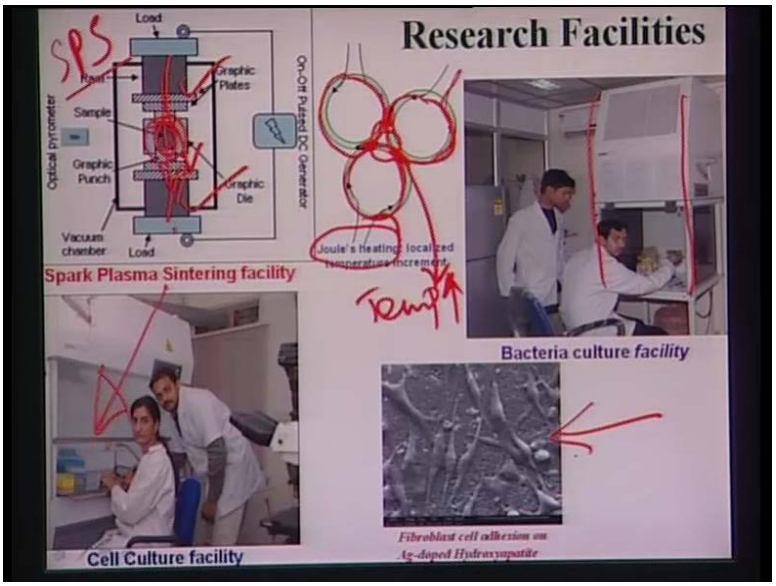
Development of hydroxyapatite based bioceramic composites for hard tissue replacement

(Refer Slide Time: 00:22)



In today's lecture and couple of next few lectures, I will just cover the some of the recent research results that are published in the area biomaterial, so that you can have a feel as how the material development in the area of biomaterials are driven by certain issues, which are related to both physical properties as well as biological properties. So, this is the physical properties as well as the biological properties. So, certain properties as far as the physical properties like strength, toughness is concerned or biological properties like in vitro cytotoxicity or cell growth, cell polyphase and sulphate processes; as well as the in vivo biocompatibility property, those we will try the development of hydroxyapatite based bioceramic composites. Now, the fundamental understanding or the basics of the physical properties or biological properties of tissues as well as synthetic material that were already discussed in the last several lectures.

(Refer Slide Time: 01:27)



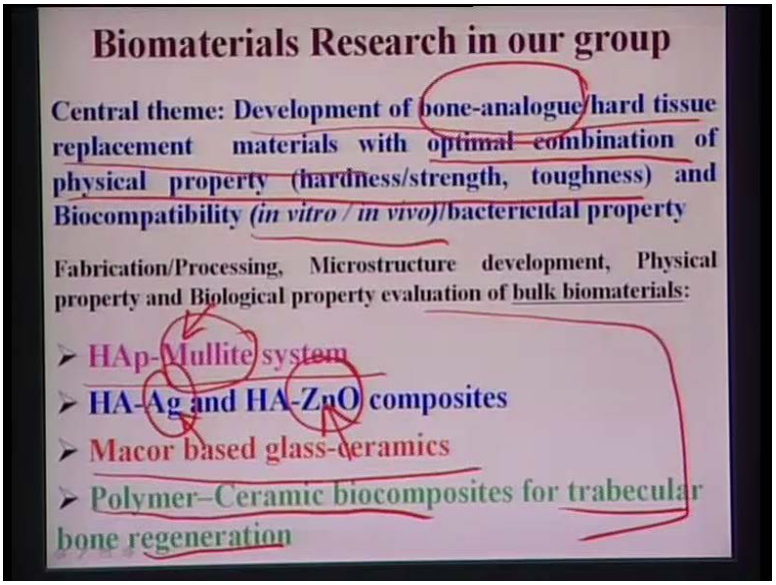
So, these are the kind of equipments like which are these are the facilities, which are used in this biomaterials research, so this is called the SPS that is called Spark Plasma Sintering machine; essentially, this Spark Plasma Sintering machine involves putting a powder compact here, in this graphite dye punch assembly, and then you are flowing a high current both through this graphite dye wall as well as through this powder compact. Now, this is the anode, this is the cathode; and then high current, when you till pass through this powder compact, that will help in the densification of consolidation of the material.

It shows that you know three particles, which are in contact with the each other, and then how this current will pass through the particle powder surface, and this is the spots, where this heating will take place, because that is the spots contacts spots, that will occur in resistance to the current flow; and as a result joule's heating will take place, and that will lead to the large temperature increase. So, here the temperature will increase to a high value, and that will help in the enhanced mass transfer.

Now, this is the fracture culture facility, so this is this particle laminar flow, where you can do culture of the different ductile strengths like E coli bacteria or (( )) species and so on. So, this is the cell culture facility, which were also used; and this cell culture facility has several equipments, which includes the microscopes like Ph 1 plus microscopes or fluorescent microscope; as well as this laminar flow would where people do this people

use it as a weight bench, we have this CO 2 laminar, CO 2 incubator for incubating the cells. This is the SEM image, typically showing that how the cells proliferate and silver doped hydroxyapatite.

(Refer Slide Time: 03:21)

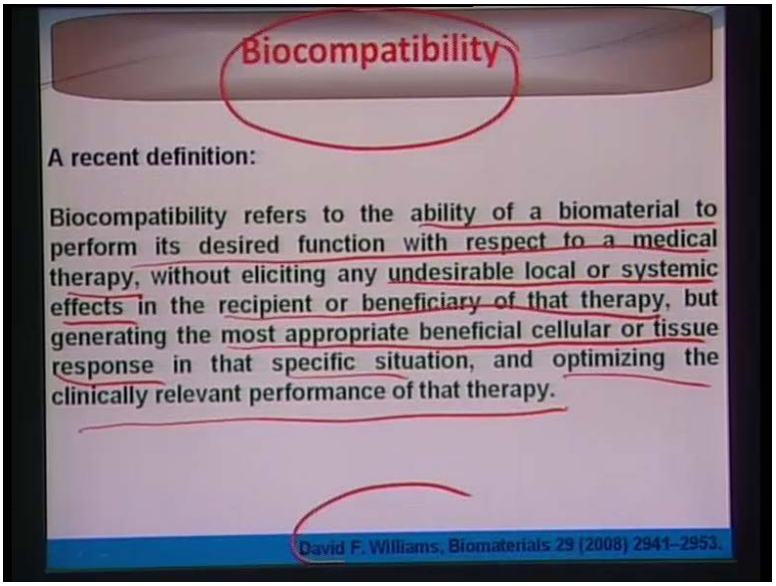


Now, what was the central theme of this biomaterials research? Central theme is to develop the bone-analogue or hard tissue replacement material; bone-analogue means it is like a, the materials which will have set of properties, which will be just sufficient to mimic a bone properties; or hard tissue replacement, hard tissue in other words the bone, so that hard tissue replacement is that like for any orthopedic applications, if you want to replace the hard tissue, then the materials, which it can be used, those materials are need to have optimal combination or physical property that is hardness strength and toughness and biocompatibility.

Now, there are certain terminology aspects; in the material science, the hardness, strength or toughness is called the mechanical properties. But in biological literature, many times in the many standard text books, these material properties like hardness, strength and toughness, they are mentioned as physical property. So, physical property in material science, they are mostly mean the density, thermal conductivity, resistivity, all those are physical properties. So, this is just that the nomenclature or designation, the way people tend to describe certain properties in the two different community, one is the material science community, one is the biological community.

Now, through that you know, next 50 or 60 lectures slides, I will just grow through some of the results, which are obtained in the hydroxyapatite based systems, which are in reinforce with mullite or silver or zinc oxide. So, this addition of this different second phase or either ceramic phase or metallic phase, they serve some purposes. Then third one is the Macor based dental glass-ceramics; this glass ceramics are used for this dental (( )) applications; then I will show you that how this different properties that you can obtained in this glass ceramic materials. The fourth one is that polymer-ceramics bio-composites that is for trabecular bone generation, and also we have developed another set of polymer ceramics composites for hard tissue replacement applications.

(Refer Slide Time: 05:33)



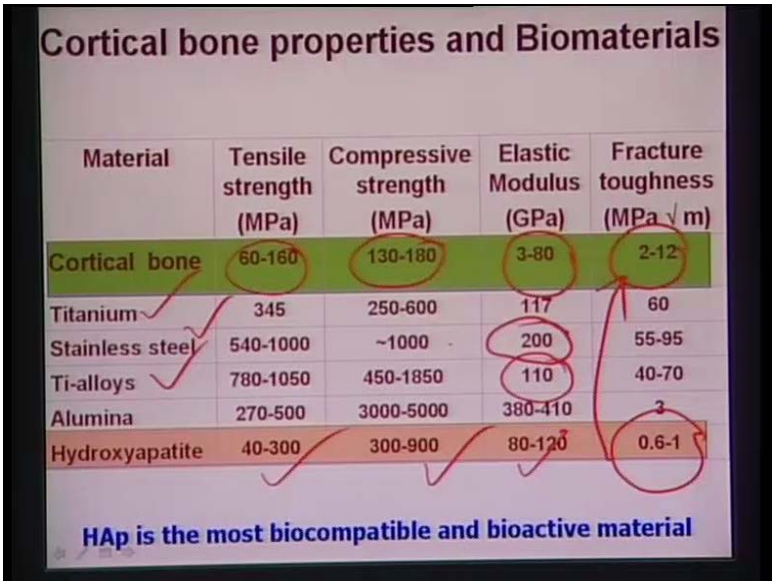
Now, the biocompatibility it has been dealt earlier also, but at every lecture, certain important concept or certain important definition, I feel like reminding you, so that you are absorbed with this important concept or important ideas. So to back to the very fundamental definition of biocompatibility, which is proposed by David Williams, who is the editor in chief of biomaterials channel; biocompatibility refers to the ability of biomaterial to perform its desired function with respect to a medical therapy. I again repeat biocompatibility such an important concept, it really depends on the application point in mind.

So application means, if it is a bone replacement orthopedic applications, then you require a set of biocompatibility property; if it is for blood contacting devices like heart

valves, you require a set of biocompatibility properties, which is different from heart to knee for the bone replacement applications. Now, without eliciting any undesirable local or systemic effects, if you remember what I meant by systemic effect; now systemic effects can be acute, can be sub acute, can be chronic or can be sub chronic. Depending on how this host response that is observed or realize within certain time frame right.

Now, those details I have already you know, discussed in details, in the recipient or beneficiary of that therapy, who is the recipient or beneficiary that is the human being, but generating the most appropriate beneficial cellular of tissue response; most appropriate beneficial cellular tissue response means like you know that how these cells will be held, when they are coming in contact with the biomaterials, that is what is meant by most appropriate beneficial cellular or tissue response; whether that (( )) capsule will fall in vivo, when this material is implanted in specific size; in that specific situation and optimizing the clinically relevant performance of that therapy. Now whatever was the application point of view in mind, your biomaterial should be able to perform in a clinically relevant manner, so it is like a more basic definition of the concept biocompatibility.

(Refer Slide Time: 07:51)



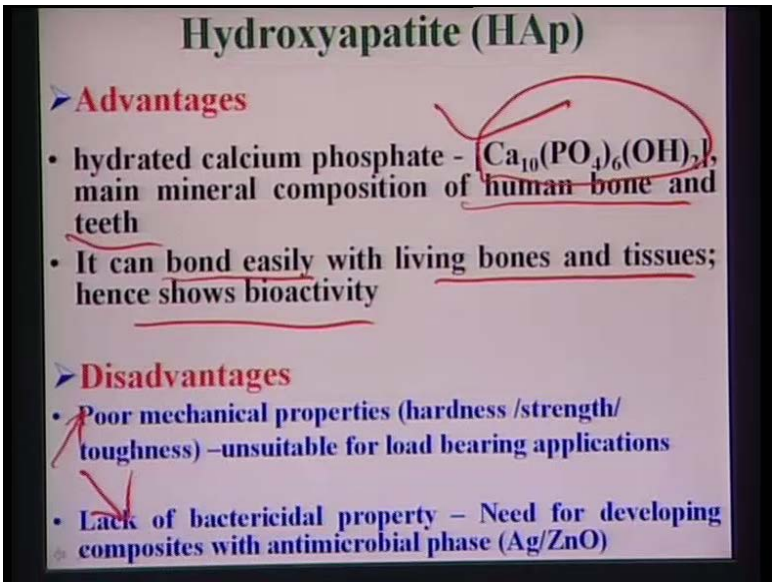
Now, as I said that you know that development of the new generation biomaterial, it is driven by certain factors. Now what are those factors? First one is that if you look at this cortical bone, the green row. What you notice that depending on anatomical location,

you have the range of properties, which includes tensile strength 60 to 160 megapascal, compressive strength 130 to 180 megapascal, fracture toughness 2 to 12 MPa square root meter, and elastic modulus 3 to 80 gigapascal.

Now, if you compare the cortical bone property with that of the hydroxyapatite, what you notice here; then in case of hydroxyapatite, you have this tensile strength, compressive strength as well as elastic modulus, which are just matching with them or which are even higher than that of the cortical bone properties. However, if you look at this cortical bone, fracture toughness and the fracture toughness of hydroxyapatite, then fracture toughness of the hydroxyapatite, even does not touch the lower bound toughness of the cortical bone. So that was the point we noted here and why I am putting so much emphasize on the hydroxyapatite, because hydroxyapatite is the inorganic major inorganic component of the natural bone. So, therefore, if the hydroxyapatite has good biological properties, but you have to also know that hydroxyapatite lack certain physical properties, and that is the reason why hydroxyapatite cannot be used as a bulk implant materials for several bone replacement applications.

Now, if you go to the other metallic materials for example, titanium or stainless steel, titanium alloys; again if you see that elastic modulus is higher than around 110 or 120 gigapascal, which is higher than the cortical bone. So, if the difference is too high, as I have mentioned in earlier lectures, then it can cause the aseptic loosening, because this materials itself will bear most of the load. If you look at the stainless steel, now stainless steel has a elastic modulus of 200 gigapascal, that shows that stainless steel elastic modulus is more than double that of the cortical bone even the highest range, highest bone modulus. So, that is the reason that you know stainless steel in many case, except the stem of the total hip replacement cannot be used as a bone replacement materials.

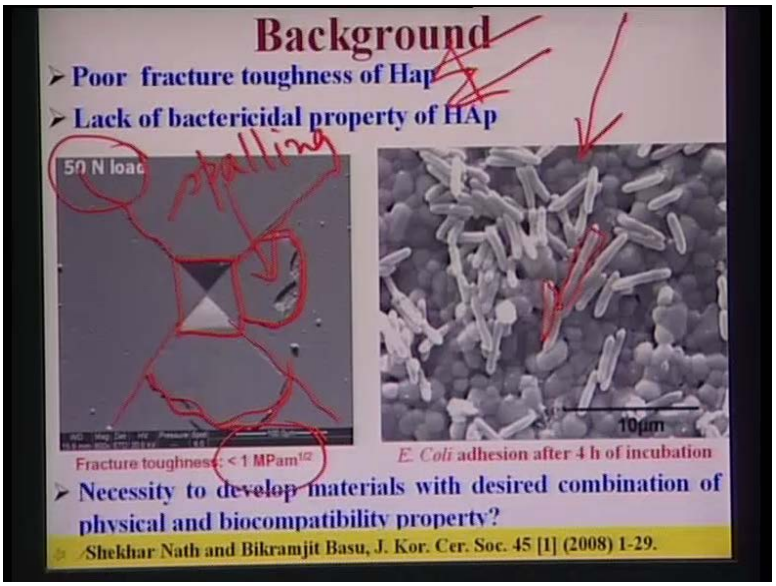
(Refer Slide Time: 10:21)



Now therefore, if I summarize that what are the advantage or disadvantage of hydroxyapatite; in hydroxyapatite is a typically, it has a chemical formula of Ca 10 (PO 4) whole 6 (OH) whole 2 and that is the main mineral composition of human bone and teeth. So, if you have this teeth, teeth also has a large amount of the hydroxyapatite, and because it is the major mineral composition of the human bone and teeth, that is the reason it can easily born biologically with living bones and tissues, and hence hydroxyapatite shows bioactivity.

Now, what are the disadvantages of hydroxyapatite? The first one is that it has a poor mechanical properties, that is the hardness, strength and toughness variation, and definitely it is unsuitable for load bearing applications. The second one, it is more critical, because when you put the material as an implant inside the in vivo condition, other factor that needs to be considered as the prosthetic infection; prosthetic infection means like if you put this implant at the specific sites, lot of bacteria they will be attracted, and if they are attracted and if they stay happily on the implant surface and that cause prosthetic infection. Now, in order to avoid the infection, you have to develop some material, which has good antimicrobial property; unfortunately hydroxyapatite does not have antimicrobial property. Now, that has been shown in the next slide.

(Refer Slide Time: 11:51)



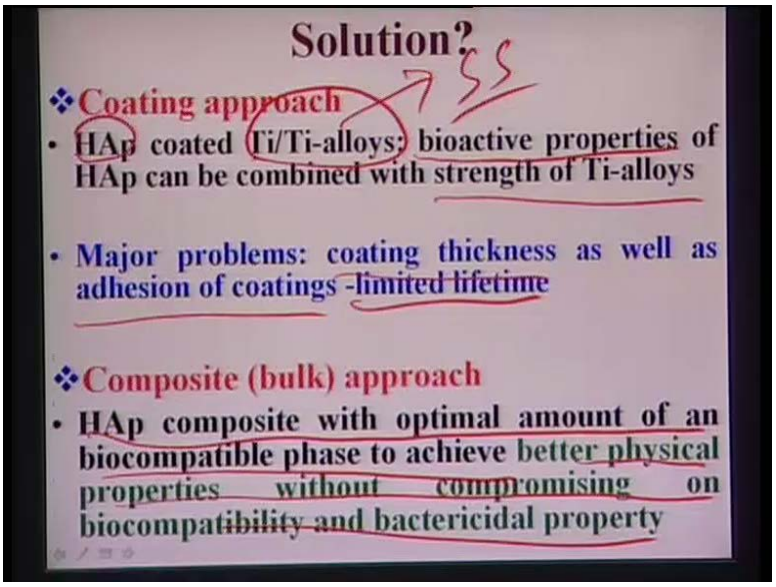
Now if you see this particular slide or this SEM image is actually taken after the 4 hours of incubation or E coli bacteria on the hydroxyapatite surface. Now, this E coli bacteria is the gram negative bacteria, which has a typical aspect ratio of 5 to 10 that is aspect ratio means length to diameter ratio; and this is a typical rod like bacteria, and they are actually happily surviving on this material surface. So, that shows that hydroxyapatite does not have a very good antimicrobial property.

Other things that important things the hydroxyapatite that it has very highly, it is highly brittle. Now, this is your vickers intent; now if you look at this intent, and if you look at the region surrounding this intent, then what you notice here that region just adjacent to the vicker's intent, this is the region where spalling take place; spalling or chipping up materials that take place, and because this cracks are extensive that this extensive crack formation leads to spalling.

However this crack length, which is called radial crack length, which actually from all the corners of the vicker's intent, those cracks are also relatively larger in length at the 50 Newton load, which is indicate that there fracture toughness is essentially very low, and if you determine this fracture toughness, this fracture toughness is less than 1 MPa square root meter. So, the two aspects which are important, which drive the development of hydroxyapatite based composites; the first one is the poor fracture toughness

hydroxyapatite, the second one is that lack of bactericidal property, bactericidal means there is a lack of ability to kill the bacteria in contact with that particular material.

(Refer Slide Time: 13:40)

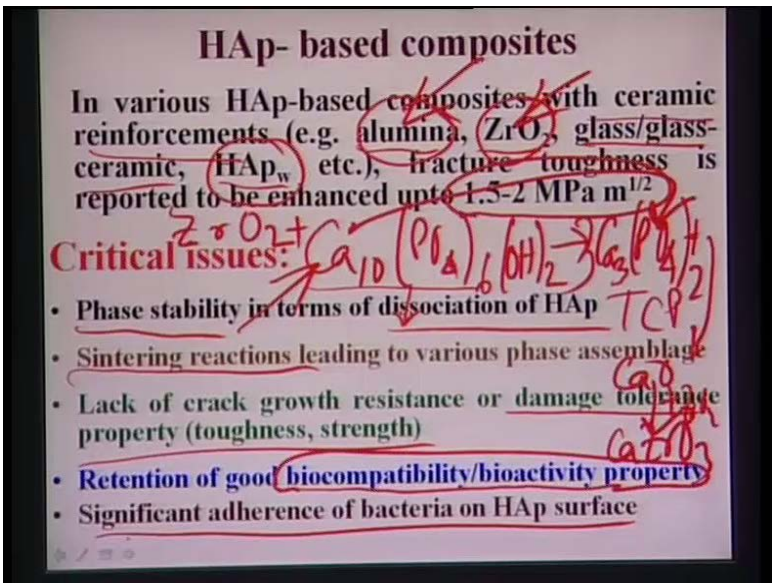


Now, the question is that what are the solutions; there are two solutions; one is the coating approach; coating is like you know, you can coat this hydroxyapatite on the titanium and titanium alloys or stainless steel substrate, whichever it is true. So, you can coat this stainless steel substrate or titanium or tritanium alloys. So, thereby you are trying to combine the good bioactive property of the hydroxyapatite with good strength property of the titanium alloy. So, you are essentially, combined good mechanical or physical property of the metallic alloys with a good bioactive properties of the ceramic coatings that is hydroxyapatite.

But there are major concerns, major problems is that coating thickness as well as the adhesion of the coating. Now, coating thickness typically means, if the coating is not very thick, then the coating does not have a longer life time; and the if the coating is very thick, then the coating can be ruptured or the coating can be delaminated from the substrate. So, both the aspects actually create limited lifetime to this hydroxyapatite coatings. Now, the composite approach; composite approach is that hydroxyapatite composite with optimal amount of an biocompatible phase can be developed to achieve better physical properties without compromising on the biocompatibility on the bactericidal property.

Now, what this statement means, this statement means that you can add the second phase x or y in a particular amount provided that x and y are non-toxic or non-biocompatible in nature and with the ultimate aim to if better physical properties, better physical means better toughness properties or strength properties. However that increase in the physical properties should not take place at the expense of biocompatibility or the back residual property. So, this is the major thing that is important for this composite or bulk approach.

(Refer Slide Time: 15:47)



Now, some more information on this hydroxyapatite based composites; now in various hydroxyapatite composites particularly with ceramic reinforcements; now, if you see the ceramic reinforce like alumina, zirconia, glass or glass ceramic or hydroxyapatite twisters, fracture toughness is reported to enhanced up to 1.5 to 2 MPa square root meter, because as you have noticed that in the last two last slide, I have mentioned that hydroxyapatite fracture toughness is around less than 1 MPa square meter. So, what this statement means, this statement means if you add alumina or zirconia or hydroxyapatite twisters, the fracture toughness can be modestly improved, and this modest improvement is shown in the final toughness values of up to 2 MPa square root meter.

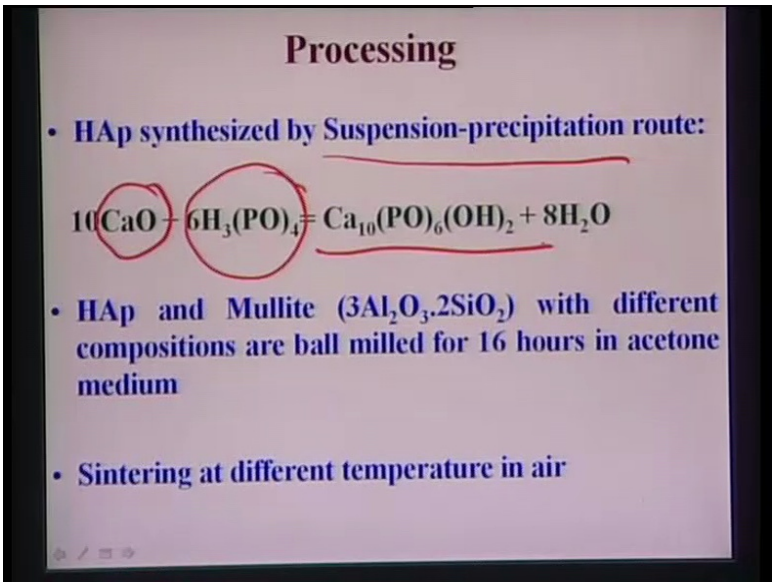
Now, what are the critical issues that should be considered in the development of hydroxyapatite? The first one is the phase stability, in terms of the dissociation of hydroxyapatite; now hydroxyapatite as you know that it has a formula that Ca 10 (PO 4) whole 6 and (OH) whole 2; now this can be transformed to Ca 3 (PO 4) whole 2; now Ca

3 (PO 4) whole 2 is the called tricalcium phosphate. Now this tricalcium phosphate is popularly known as TCP; now, this has two polymers; one is the alpha TCP and one is the beta TCP. Now these transformation of hydroxyapatite two alpha TCP or beta TCP that take place more easily or more favorably in some of the composites, where you add this alumina or zirconia has a reinforcement. Other problem is that with this hydroxyapatite, suppose you add zirconia to hydroxyapatite, during sintering if you do not control the sintering conditions value well, then what happens - this zirconia can react with now.

First of all, this hydroxyapatite can release this TCP or can be dissociated with TCP, and if you balance these reactions, then you will end up having 1 CaO, 1 mole of CaO from 1 mole of hydroxyapatite; and this CaO can further react with zirconium oxide, and this can form calcium zirconium. So, what I am saying here that these sintering reactions also can take place depending on what kind of reinforcement you add to this hydroxyapatite. And if your sintering reactions take place, then you cannot take full advantage of 100 percent advantage out of the fact that you have the hydroxyapatite in the material.

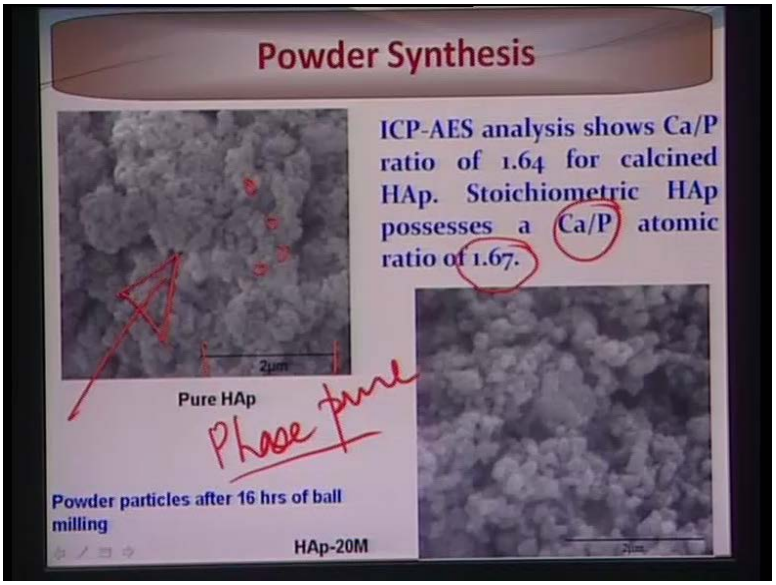
Third one is that it has a lack of crack growth resistance or damage tolerance property, and this damage tolerance property is mostly the toughness or strength property. The fourth one is the retention of good biocompatibility or bioactivity property; now this good biocompatibility or bioactivity property is lost, when your hydroxyapatite is transform to tricalcium phosphate or it undergoes sintering reactions. Fifth point is that significant adherence of bacteria on hydroxyapatite surface, and that essentially leads to prosthetic infection or infection at the implant cells.

(Refer Slide Time: 19:10)



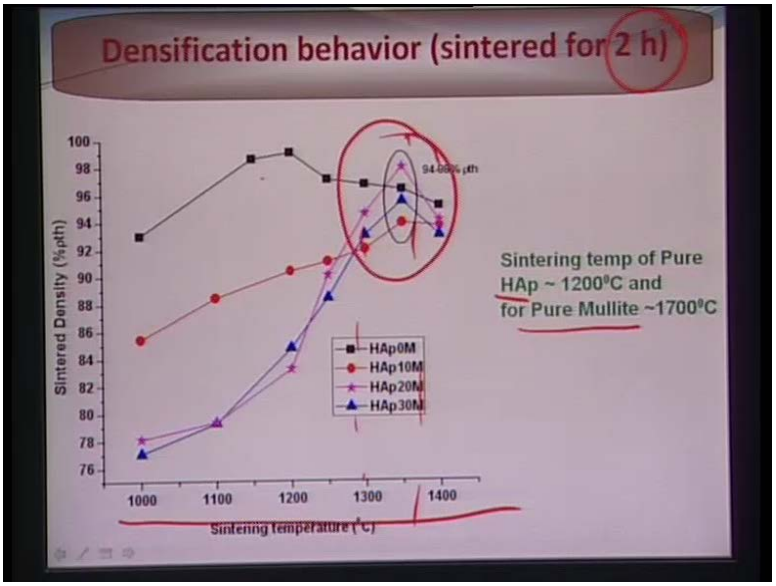
Now, typically in the laboratory, scale synthesis route, hydroxyapatite can be synthesized be what is known as suspension-precipitation route. Now, what is suspension-precipitation route? You can start with the calcium oxide as a precursor; another precursor can be orthophosphodic acid; now this calcium oxide an orthophosphoric acid you can allow them to react, and that reaction produces hydroxyapatite. Now, some of the experiments that was carried out to improve the hydroxyapatite toughness that was to add mullite to hydroxyapatite surface.

(Refer Slide Time: 19:36)



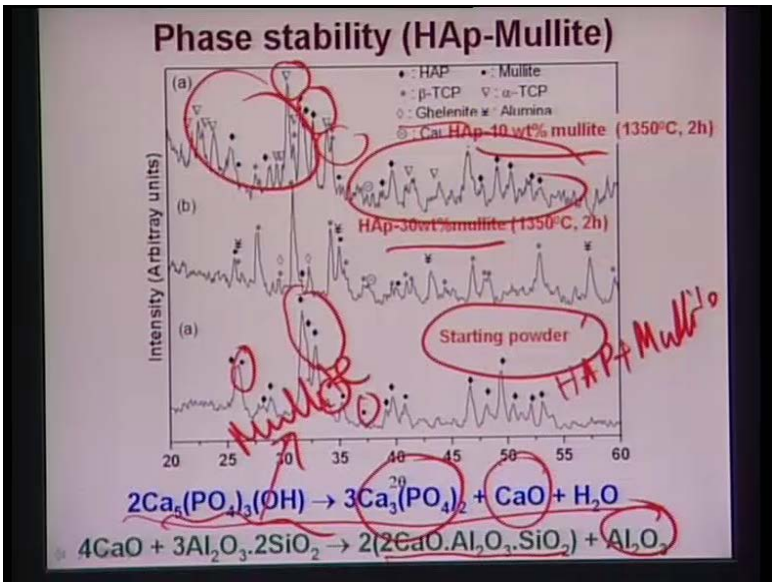
Now, this is the as synthesis hydroxyapatite surface; now if you look at this bar, this is 2 micron here. So, therefore, individual particles there, which is largely spherical in nature; this individual particles is certainly in the range of nano size of 100 nanometer or little bit higher or lower than 100 nanometer. The other important thing is that whether you have the phase pure hydroxyapatite or single phase hydroxyapatite that can only be confirmed by the Ca by P ratio; and this Ca by P ratio, you can you can measure by ICP that is inductively coupled plasma, atomic emission spectroscopy, and from that you can find out the Ca by P ratio, if it is 1.67, then in then only you can say that this hydroxyapatite is the phase pure hydroxyapatite.

(Refer Slide Time: 20:37)



Now, if you do this normal sintering, pressure less sintering of hydroxyapatite with the mullite for example, just to improve the fracture toughness, I will show you why mullite increases the fracture toughness; how mullite content increase the fracture toughness. Now, the these sintering experiments were carried out for 2 hour in simple air, and different temperature right from 1000 to 1400 degree celsius. It was not known that at what temperature, you can obtain sufficiently high density in the hydroxyapatite mullite, and that was the reason that is why sintering temperature were needed to be optimized. Now, if you look at this around 1300 to 1350 degree celsius, you get close to 90 to 95 percent theoretical density.

(Refer Slide Time: 21:22)



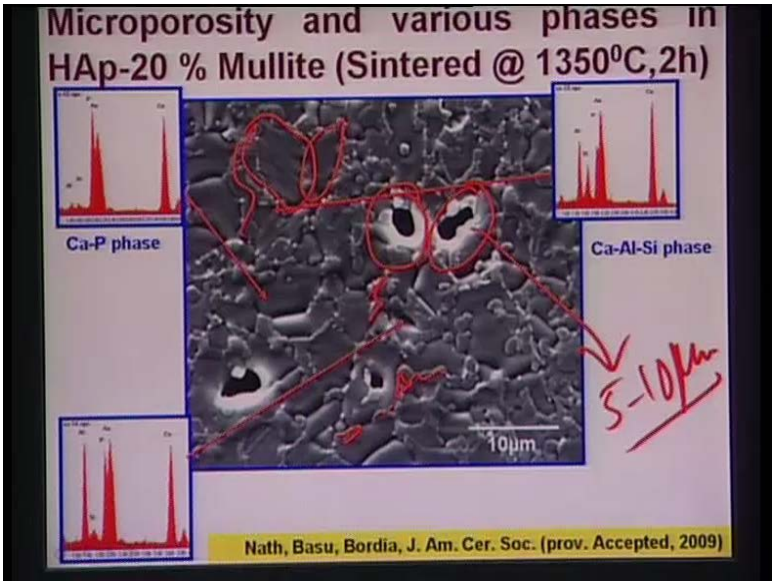
Now, this is exadispectra; and this exadispectra essentially tells you that when you have a starting powder, you have this characteristic hydroxyapatite peak, and you have also the mullite peak. Now, when you add this 30 weight percent mullite or 10 weight percent mullite to this material; and sinter then at 1350 for 2 hours, what you see here - multiple peaks like you have this TCP that is the alpha TCP peak, you have the hydroxyapatite peak, you have this star one that is a beta TCP peak, you have the ghelenite peak etcetera, etcetera.

Now, what is this Ghelenite? Ghelinite is C 2 As; C 2 As means 2 calcium oxide alumina and silica that is the ternary compound. Now you are starting with your only hydroxyapatite and mullite; now, when you are seeing that all this multiple phases are formed, what it means? It means hydroxyapatite must have dissociated to form this alpha and beta TCP that is number one. And subsequently there are some other sintering reactions must have taken place to explain the formation of this ghelinite and other phase.

Now, what we are proposed is that if you start with this Ca 10 (PO 4) whole 6 (OH) whole 2 that is the hydroxyapatite, it forms this tricalcium phosphate - TCP it releases CaO. Now this CaO can react with this 3 alumina 2 silica, which is nothing but mullite. So, mullite is the solid solution of alumina to silica and that is 3 is to 2 ratio. Now if you look at these reactions, then CaO reacts with this mullite, and then it will form calcium

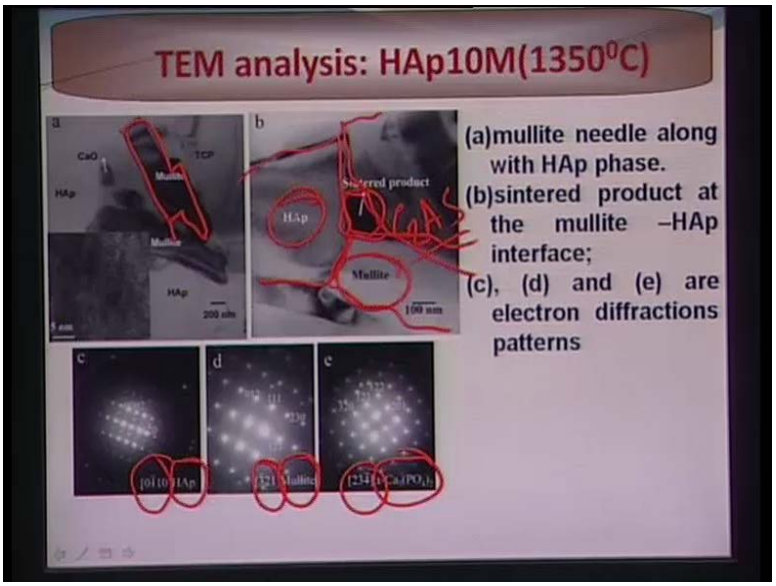
alumino silicate and this calcium alumino silicate, the formation is also associated with one mole of alumina. So, all these sintering reactions essentially explain that why this multiple stages are formed during this sintering.

(Refer Slide Time: 23:19)



Now, typical SEM image essentially tells to that you have a micro porosity in the structure; and this porosity is roughly 5 to 10 micron in size. And this micro porosity is also associated with certain crane boundary phase, and most likely it is calcium aluminosilicate phase, which is formed along this grain. So, this is your tricalcium phosphate or hydroxyapatite Ca P grains.

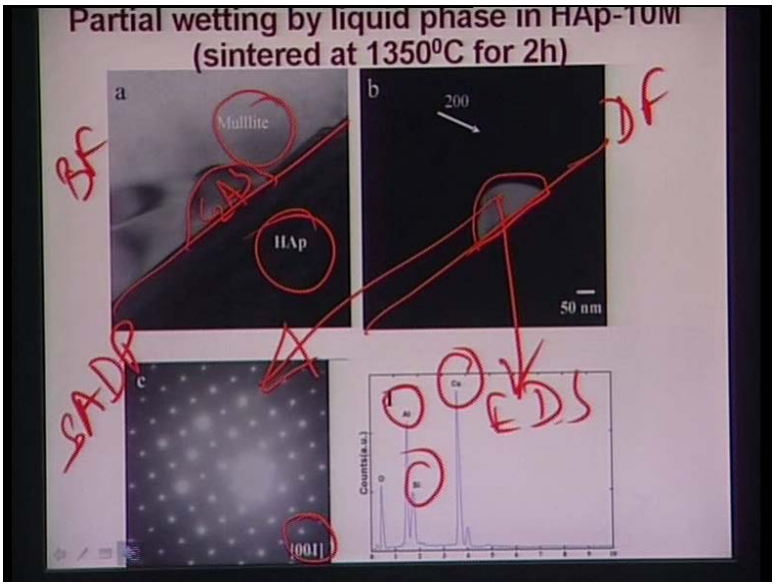
(Refer Slide Time: 23:48)



Now, more analysis was carried out using the transmission electron microscopy; now what you see here that mullite is the needle like structure; and these mullite particles are dispersed in the micro structure. And there is sintered reaction product, and this sintered reaction product is nothing but C 2 AS that is the 2 calcium oxide alumina and silica that is calcium aluminosilicate. And what you see here further, this this C 2 AS is formed at the interface of hydroxyapatite, mullite, and you have the another tricalcium phosphate.

Now if you know the microscopy well, then if you take the selected either diffraction pattern from individual hydroxyapatite or mullite or the sintered reaction products, then you can confirm the presence of all these phases. You can index it, just like you can index the extra diphases, similarly you can index from the selected you know, diffraction pattern, and that is what has been done here; you can see this the zone axis, and this is the hydroxyapatite; from mullite, this is the zone axis; and from alpha tricalcium phosphate, this is the zone axis. Now, this spots essentially tells you this kind of spotty pattern essentially tells you all these phases are crystallized in nature. If there is a ring pattern in the transmission of microscopy, then that will essentially indicate that this amorphous in nature, lack of crystalline in nature.

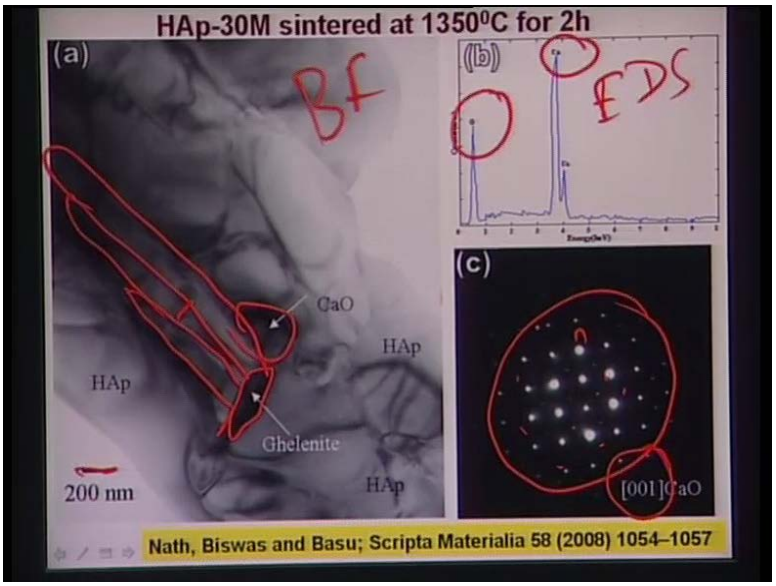
(Refer Slide Time: 25:11)



Now this state of this is the dark filled image, and this is the bright filled image. So, these set of bright filled and dark filled image essentially tells you that this there is a partial weighting of this C 2 AS phase, this is the C 2 AS phase that is formed due to the reaction, this is your mullite particle, this is your HAp particle. Now, essentially what you what it shows you that this is the part partial weighting of this green boundary by the C 2 AS phase; and this C 2 AS phase if you take a selected a diffraction pattern, then this is 001 zone axis, and along that you can find that there are spot patterns, and then this is the spot patterns, then again this C 2 AS phase is crystalline in nature, not an amorphous phase.

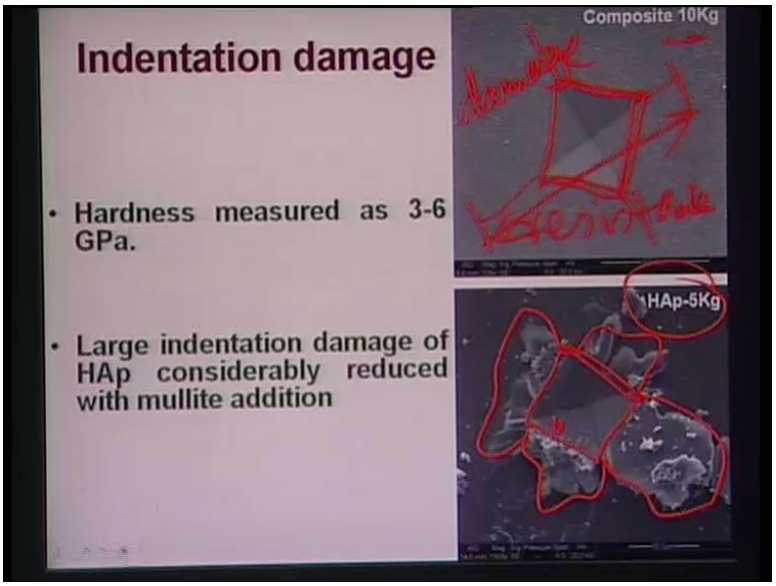
Other thing that you can notice this is a EDS spectra that you can get in the PM, from this C 2 AS phase, if you take it show the calcium peak - strong peak, because it is 2 CaO; so that means, the calcium peak should be strong. You have reasonably aluminum peak, because it is A l 2 O 3 is also there; and also you have SiO 2, so that silicon peak also comes from that. So, essentially the combination of SADP pattern that is Selected Area Diffraction Pattern coupled with the EDS analysis confirms the presence of C 2 AS phase as the grain boundary region in this microstructure.

(Refer Slide Time: 26:38)



Now, this is more bright filled image, essentially tells you that there is a gelignite phase here, which is a C 2 AS phase; you have these needles, these needles are essentially mullite needles; and these needles are mullite needles, which has a very high large aspect ratio of 8 to 10; and if you look at this, this is 200 nanometer, so essentially this width of the mullite levis is 200 nanometer. And also you have this CaO phase, and how to confirm the CaO phase? If you look at this EDS pattern it only shows you calcium oxygen, so that means, there is no doubt undoubtedly, this is CaO phase. Other things it is also confirm that this, it takes that SADP that Selected Area Diffraction Pattern, and that 001 zone axis, you can see again this pores reflection planes, it essentially tells you that this is actually the coming from CaO.

(Refer Slide Time: 27:36)

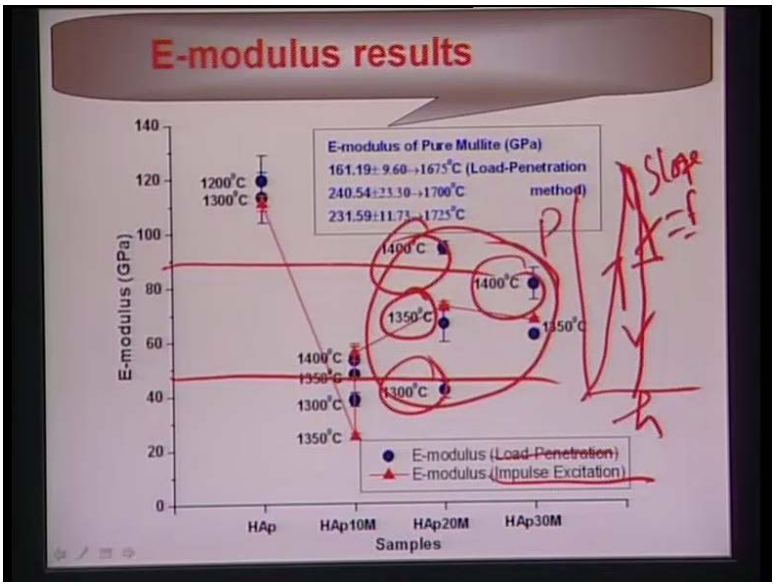


Now I have shown you one of microscopy, now let us discuss that mechanical properties or physical properties of these materials. Now, as I shown you at the beginning of this lecture, that hydroxyapatite is essentially brittle phase, this has been proven again, this is been proved again. Now if you take the 5 Kg load vicker's indent, then you can see there is a lot of this falling or indent induce damaging this material.

But when you make these composites even at the 10 Kg load, you can see that this is sharp vicker's indent that means, it is essentially damage resistant; if you look at that, so what I am trying to say here, if you take the 10 Kg load in the hydroxyapatite; it will be even much more severe damage of the indent induce damage, but even at the 10 Kg level in the composite, you can see the perfectly sharp, and very well distinct vicker's image; essentially, tells you that this material must have greater damage resistance property. Clear?

The second thing that you notice that there is also some cracks, which are forming, which is not that clear, but I can see on the image very closely that you know, there are certain cracks, which are propagating from this vicker's indent corner. And based on this crack length essentially you can measure the fracture toughness, I will show you later.

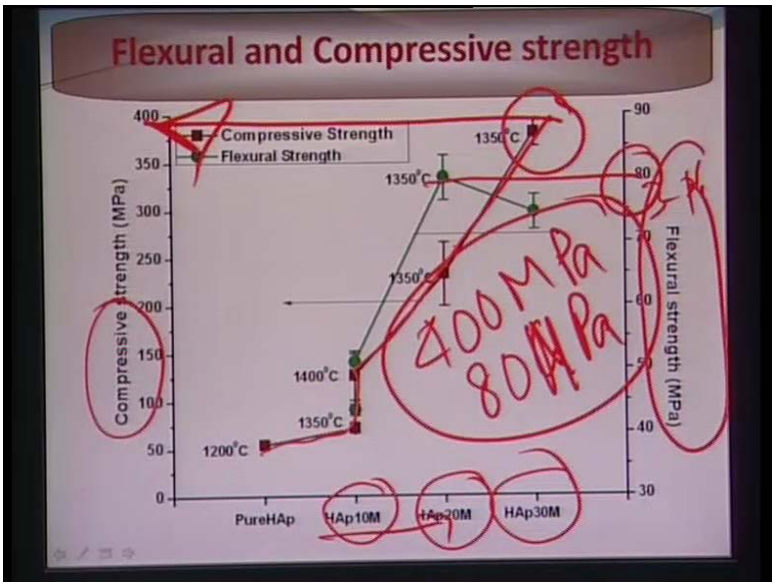
(Refer Slide Time: 28:59)



Now, elastic modulus - elastic modulus can be measured from two ways; one is the load penetration and one is the impulse excitation. Load penetration means like you take a micro indent, this is the loading curve, and your unloading curve will be like this. Now from the slope of this initial phase of this unloading curve, from this slope, it will give you the elastic modulus. Now this is the load, and this is your penetration depth that is h. So, from that you can find out that what is the elastic modulus of this materials; another way you can measure this elastic modulus is the impulse excitation, like you take a bar samples, you can impulse it with some hitting with the some steel balls or something (( )) by balls; and then you can measure the resonance frequency, you apply certain general formula, you can get the elastic modulus of these materials.

Now, independent of what measurement technique is used, the message from this slide is that that you can get this range of this elastic modulus, which is roughly around 45 to 85; and this 45 to 85 gigapascal elastic modulus, which will closely correlate with that elastic modulus values of the particle bone that is the natural bone. So therefore, this hydroxyapatite mullite materials that depending on the sintering temperature that you can find out that you know, what is the elastic modulus values, which you require for real applications.

(Refer Slide Time: 30:38)

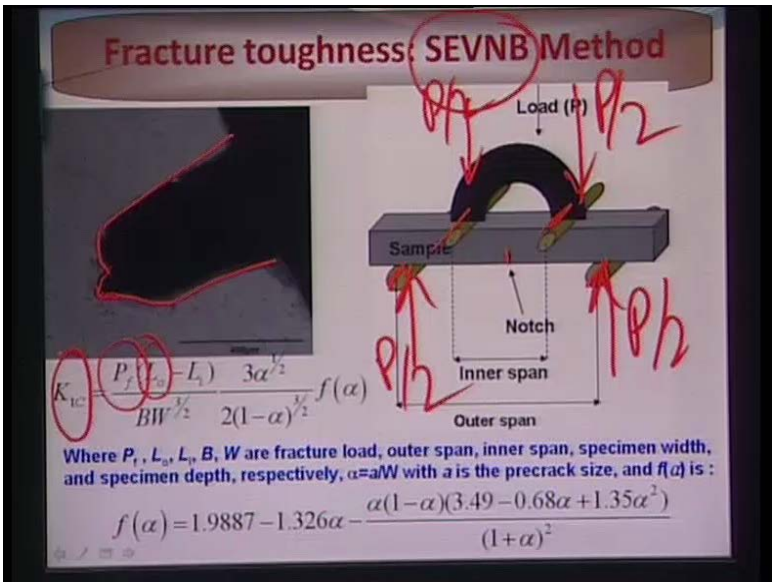


Let us look at the strength properties; now this strength properties for ceramic materials, you can measure only the compressive strength; compressive means you can take a sample, you can put a pressure from both the ends, then you can measure the what is the load at which sample is broken, from that you can calculate what is the compressive strength? And we have also measure the 3-point flexural strength; 3-point flexural strength means, you can take a bar separate sample, you can put it, you can you can place this bar separate sample on two support rolls; from the top roll which is placed at the half of the span length here, you press there as it, you break the bar, you measure that what is the load at which the bar is broken; and based on this geometry of the sample and the load at which the load at the fracture point, then you can calculate that what is the flexural strength of these material.

Now, what you can notice here that with increase in mullite content like HAp 10 m, HAp 20 m and HAp 30 m; that means, essentially means 10 at percent mullite, 20 percent mullite, 30 at percent mullite; your compressive strength is subsequently increases, and this increased value of compressive strength can go as flows as 400 megapascal; now 400 megapascal compressive strength in ceramics is quite a respectable number. Now, if you look at the flexural strength, because hydroxyapatite itself is very poor and flexural strength, you can get a maximum of 80 megapascal. So, you are getting close to 400 megapascal compressive strength, and you get close to 80 megapascal flexural strength

in this material; and this combination of strength properties are quite good as far as the application of these materials is concerned.

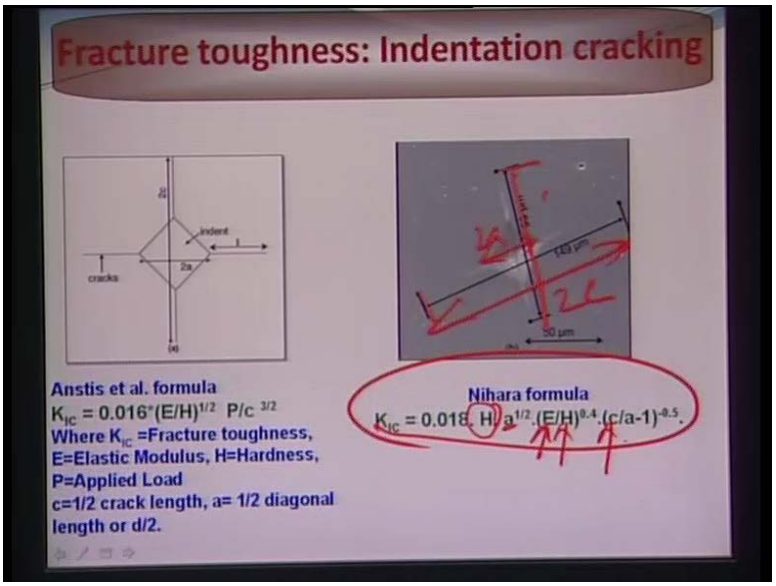
(Refer Slide Time: 32:14)



Now, fracture toughness fracture toughness essentially means, what is the resistance to the crack growth in these materials. Though the properties, which describe the resistance to crack growth, that is what fracture toughness means. Now, we have measure this Single Edge V-Notched Beam technique; now single edge v-notched beam means, you make this v-notch here at this root of this one, and then you can put this v-notched beam samples in the tensile side; and then you put it under this two support role here, and from this two top role, you can apply this P by 2 P by 2 load. Then you can find out that at what load from the equilibrium of this point, these two support rolls also must support the P by 2 load at each point; now this is called 4 point flexural configuration.

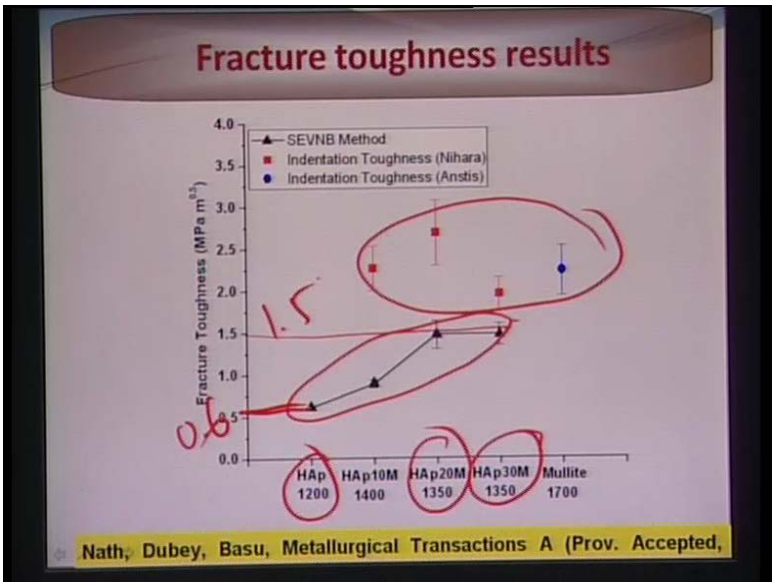
Now, based on this different crack length as well as the fracture load and so on, you can actually calculate that what is the mode one fracture toughness; K 1c stands for critical trace intensity factor under mode one loading; K 1c, often students pronounce it K Ic, which is wrong; K 1 c stands for critical stress intensity factor under mode one loading.

(Refer Slide Time: 33:36)



Now, the other way you can measure the fracture toughness for brittle materials like ceramics is that by crack length measurement; and this crack length measurement it has been shown for example, if this is a 2C that is the total crack length; in both longitudinal transverse direction, you can measure this 2C crack length; and then based on this diagonal 2a, then you can adopt different formula like formula proposed by (( )) Niihara tells you that K 1c is nothing but 0.018 multiples by 8, 8 is the hardness, a is the half diagonal, vicker's diagonal, E is the elastic modulus, H is the hardness, C is the half of the crack length. Now, if you apply this formula, you can get a value of the fracture toughness of these materials.

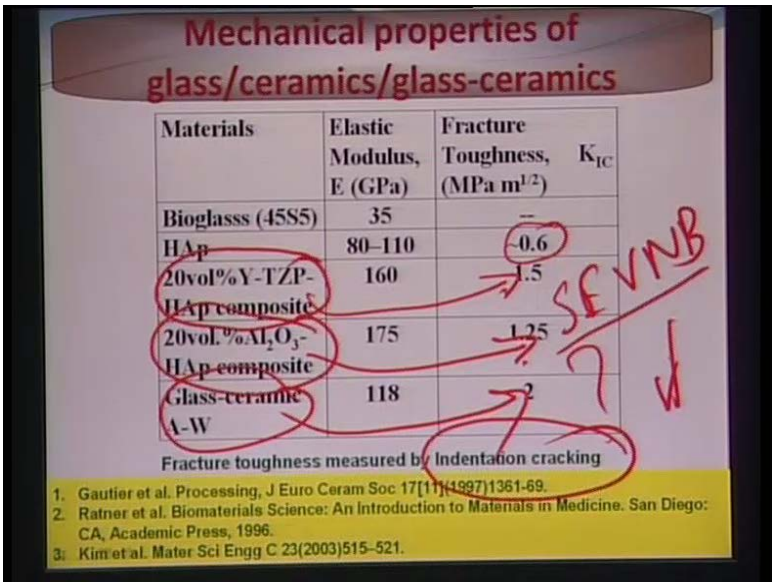
(Refer Slide Time: 34:22)



Now, we have measure this two properties fracture toughness using both the value like you know, both the indentation toughness as well as the SEVNB value; what you get here in the SEVNB value, the fracture toughness value is does not over estimate, but in the indentation toughness, fracture toughness values over estimate. And what you get your fracture toughness is 1.5 MP a square root meter, that is the SEVNB value Single Edge V-Notched Beam value, and that is the value you can get it both 20 percent mullite and 30 percent mullite; in pure HAp, your fracture toughness value close to 0.6 MP a square root meter, so this is 0.6 and this is 1.5.

Now, if you look at this value, if you compare the what is the toughness of the base material that is hydroxyapatite, you can immediately realize that there has been modest improvement in the fracture toughness values. The other thing that I must mention here that if you go to the SEVNB value, this value is most reliable. And many times in the community - ceramics community, people have questioned the measurement of this fracture toughness using the indentation technique, so this is a question mark. So, therefore, whatever value you are getting from indentation that is also question mark; however, this value that what you get in the Single Edge V-Notched Beam technique that is the most reliable, because it does not over estimate the toughness properties of these materials.

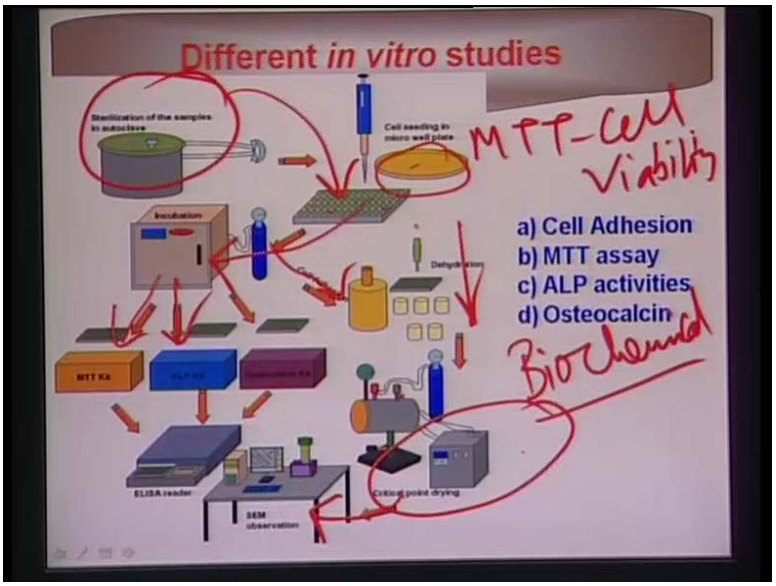
(Refer Slide Time: 35:53)



Let us see some table, which essentially tells you that what is the typical toughness values of the different materials, which are developed, why they are different research group. Now, if you take the hydroxyapatite based materials, it is a 0.6; in the 20 volume percent Y-TZP HAp composite it is 1.5; 20 volume percent alumina HAp composite it is 1.25; in the glass-ceramic, it is 2 MPa square root meter. Now unfortunately, all these materials, all these toughness values are obtained by the indentation toughness only or indentation cracking on.

So, essentially again I can put a question mark here, because if you measure the SEVNB toughness value of these materials, I can very real tell you that these values will further decrease. So, whatever you are getting 1.5, it will not be 1.5, but it may be below 1 here, MPa square root meter. So, what I am trying to say essentially that the toughness values that which have been measured with the hydroxyapatite mullite values, certainly tells you that you can get a good SEVNB toughness properties from this materials, which are not being even achieved with any other materials, which are reported till there.

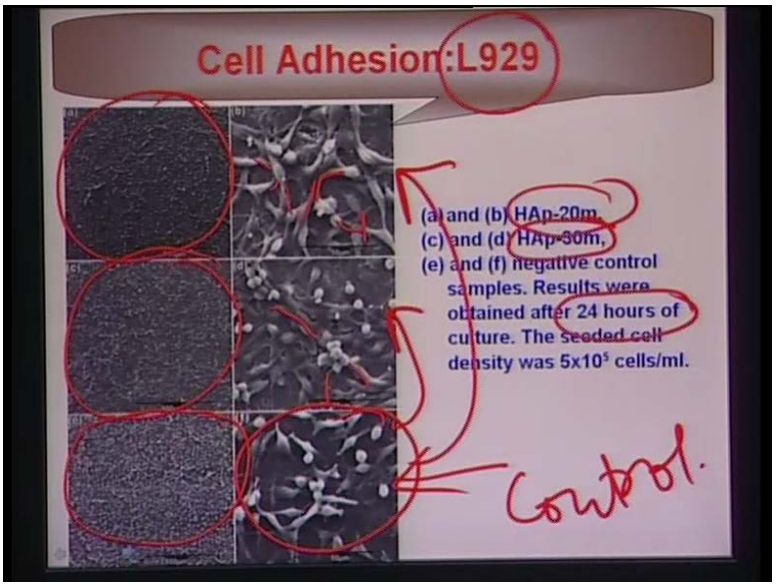
(Refer Slide Time: 37:07)



Now in vitro properties; this is a kind of standard protocol for these in vitro properties; first you have to prove the steam autoclave, and either you can do autoclave by steam autoclave, you can do autoclaving by gamma radiation. Now the ceramics like hydroxyapatite based so on that steam autoclave is okay. Then you have to seat the cells on these materials, you can take the 96 well plates or you can take that 6 well plate and so on. So, this is the micro well plate, and then you can put these materials inside the CO 2 incubator, where there is atmosphere is maintained at 5 percent CO 2 95 percent air, and then also you have the good amount of humidity that is maintained inside the CO 2 incubator.

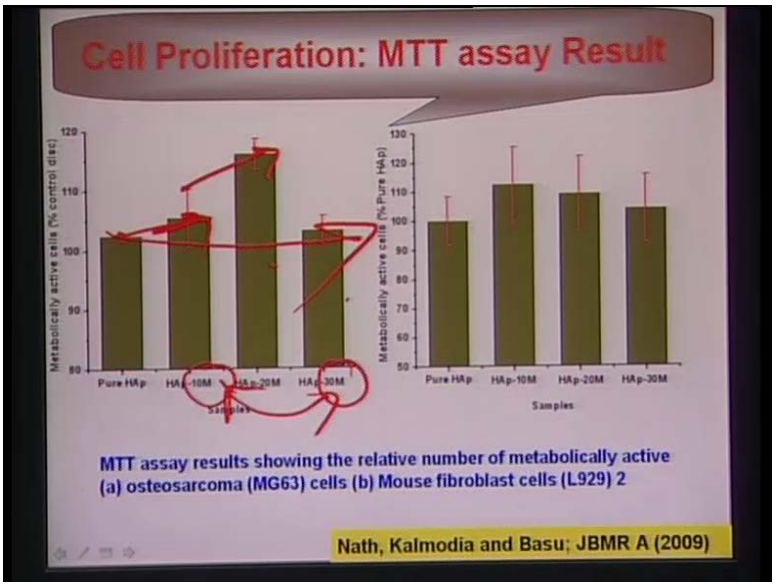
Now, you can do the cell adhesion test, then you can do the dehydration or the serial dilution; then you can further use the critical point dryer, and you can proceed towards the SEM of the flourasence microscopy to see the morphology of the cell. Now, alternatively you can use some biochemical assays, so these biochemical assays essentially will quantify certain cellular fate processes; what are the cellular fate processes? MTT that will give the cellular viability, then ALP that alkaline phosphate that will tells you the osteoblast differentiation capability, and osteocalcin tells you the bone mineralization capability.

(Refer Slide Time: 38:39)



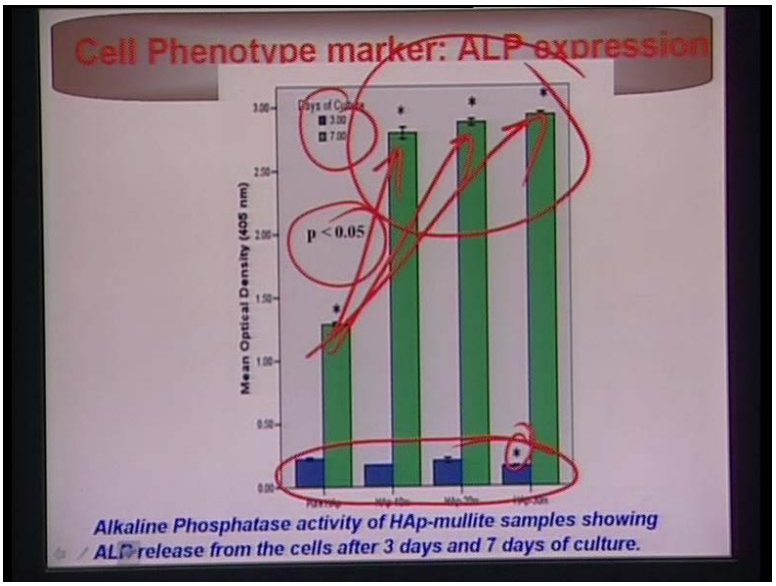
Now, L929 is the mouse fibroblast cells, which is the connective tissue cells, so cells of the connective tissue of the mouse. So, these are cultured for 24 hours on different hydroxyapatite 20 percent mullite, 30 percent mullite, and this is your control sample. Now if you compare the cell adhesion as well as cell proliferation with this one as well as with this one, you do not see much difference in terms of the cell adhesion and cell proliferation. Interesting pictures that you can clearly see here that is cell to cell cellular network; and this cellular network you can see very clearly here, and this is summary, it is called cellular bridges, cell bridges; and overall all the hydroxyapatite mullite surfaces are completely covered by this L929 cells, and this shows good biocompatibility in vitro cytocompatibility property of this materials.

(Refer Slide Time: 39:32)



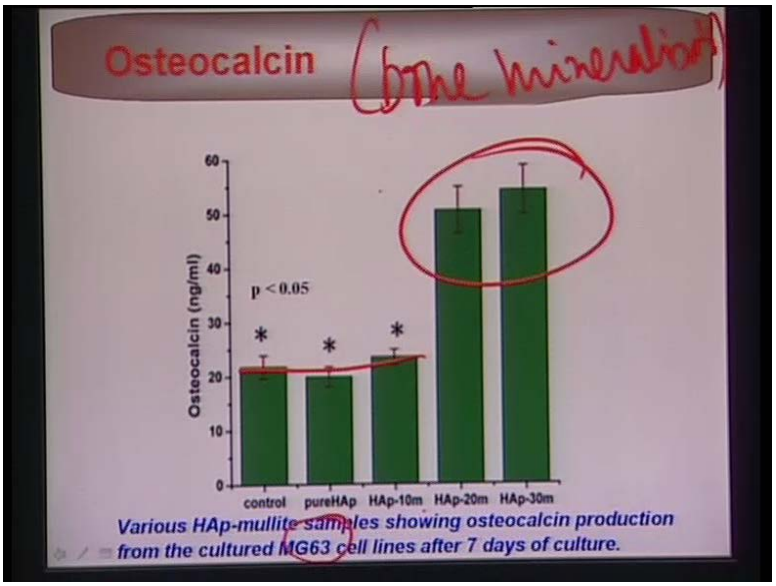
Now MTT assay was perform prime to quantify that cell viability of these materials, and as far as the cell viability is concerned, you can see that this is for the pure hydroxyapatite, this is for the hydroxyapatite 20 mullite, this is for the 30 mullite. Now you see here this error bars overlap; and from this to this again it is roughly the error bars overlap, and this and this error bars overlap. So, what I am trying to say here that for this as far as the MTT assay concerned this hydroxyapatite mullite, they have similar MTT values like the pure hydroxyapatite; and what it means? That means, when you add 10 to 30 percent of the mullite to hydroxyapatite, you do not compromise anything as far as the cell viability is concerned, that means you are not reducing the cellular viability by adding this mullite to hydroxyapatite samples.

(Refer Slide Time: 40:26)



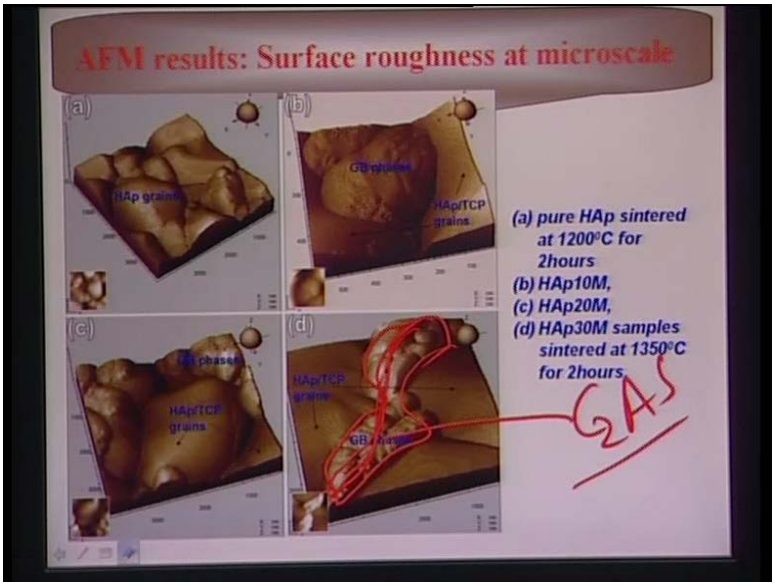
Now as far as the ALP expression is concerned that alkaline phosphatase activity, and these tests were done for 3 days as well as 7 days; and this is the statistical analysis of perform for P less than 0.05. And what you see here that for the 3 days, this pure hydroxyapatite and 10, 20, 30 mullite does not show any statistical of this significant difference except these one; but here in the 7 days, the difference is quite significant; if you see, compare to this hydroxyapatite to all these materials in 10, 20, 30 that significant difference is obtained; that means, that these materials they show better osteogenic differentiation property in this when L929 cells were cultured on this material. So, these are like MG63 that is osteoblast like cells were cultured on this material.

(Refer Slide Time: 41:13)



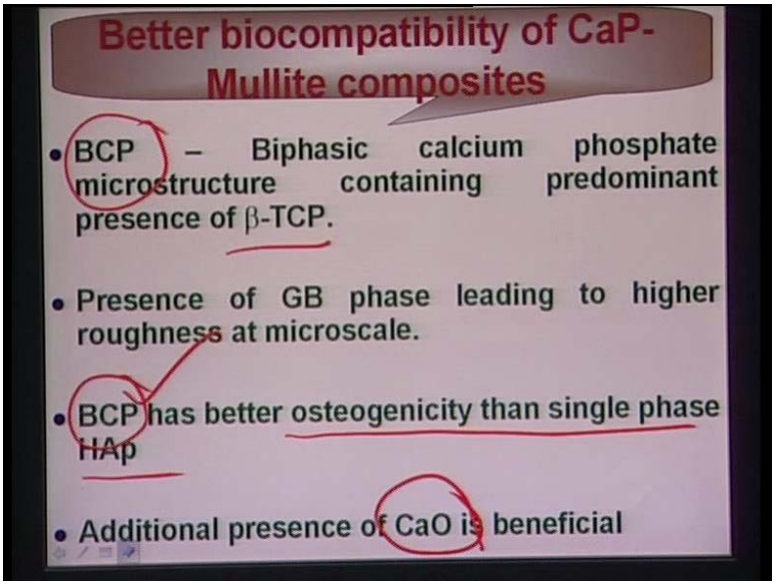
Now, osteocalcin, which is the bone mineralization, so essentially this bone mineralization… So, bone mineralization property were evaluated, then again for the this pure hydroxyapatite and hydrogen 10 percent mullite, this 20 and 30 they really stand out; that means, this 20 and 30 percent mullite material, they have better ability for the bone mineralization; and therefore, they have better osteocalcin expression, when MG63 that is osteoblast like cells were cultured on this material surfaces.

(Refer Slide Time: 41:52)



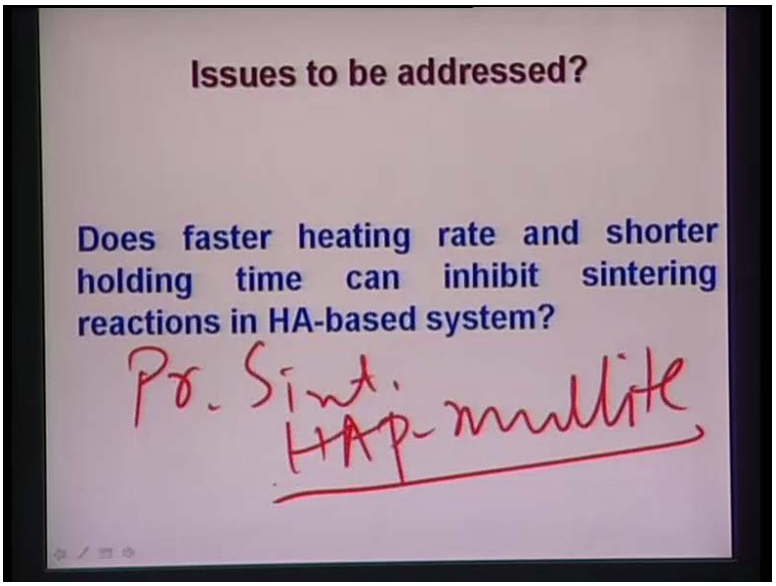
Now, what was the reason for this difference in these in vitro cytocompatibility or in vitro I mean, in vitro that biomineralization property etcetera. Now, first of all you have to look at these AFM images of these materials. Now atomic force microscopic images tells you that in the grain boundary region, you have a micro scale roughness that means, this grain boundary phases, which are formed that is C 2 AS phase, that is 2 2 calcium oxide 1 alumina, 1 silica; the C 2 AS phase essentially formed, and that increases the micro scale roughness of this material.

(Refer Slide Time: 42:29)



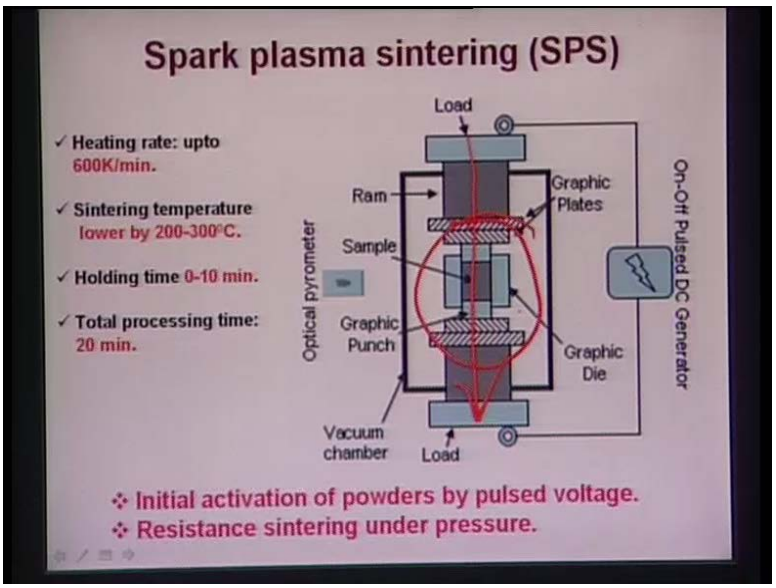
The second thing is that these materials actually they are like BCP phase; BCP means that is like Biphasic Calcium Phosphate Microstructure; and they contain beta TCP as well as the hydroxyapatite phase. And then other thing that BCP that is Biphasic Calcium Phosphate phase has better osteogenicity than single phase hydroxyapatite. So, that is also important, because if the BCP phase is preferred than hydroxyapatite, then as per the biological compatibility property is concerned, people always deserve this BCP phase in the microstructure. Third one is this, as you remember the TM image, the Transmission under Microscopic image that these materials, they have a calcium oxide and the additional presence of calcium oxide also influences the biocompatibility property.

(Refer Slide Time: 43:19)



Now (( )) sintering of this hydroxyapatite they always leads to multiple phases. And these phases actually also influence the mechanical properties to some extent. Now, next set of experiments that are show the results of this experiments, that are show that to demonstrate that how this sintering reactions can be avoided in this hydroxyapatite based system.

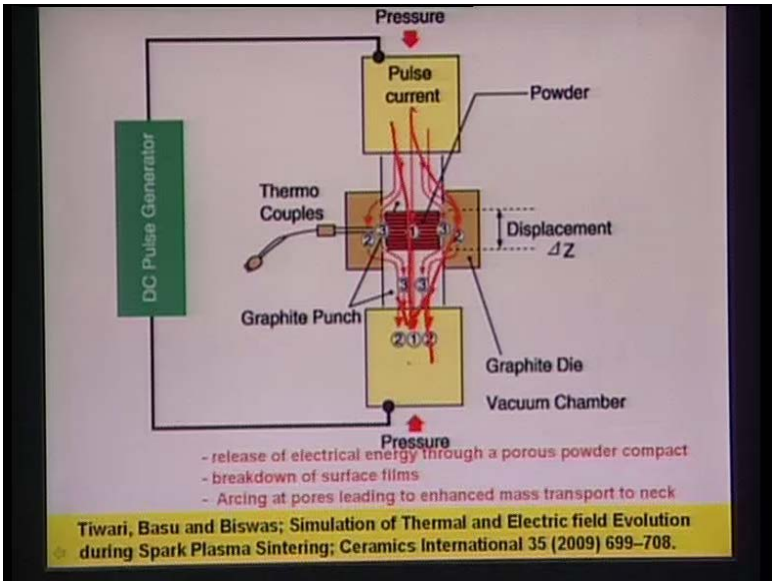
(Refer Slide Time: 43:46)



Now, for that we have carried out Spark plasma sintering experiments; and this Spark plasma sintering as I explained to earlier, so it is like a hot phrase by the fundamental

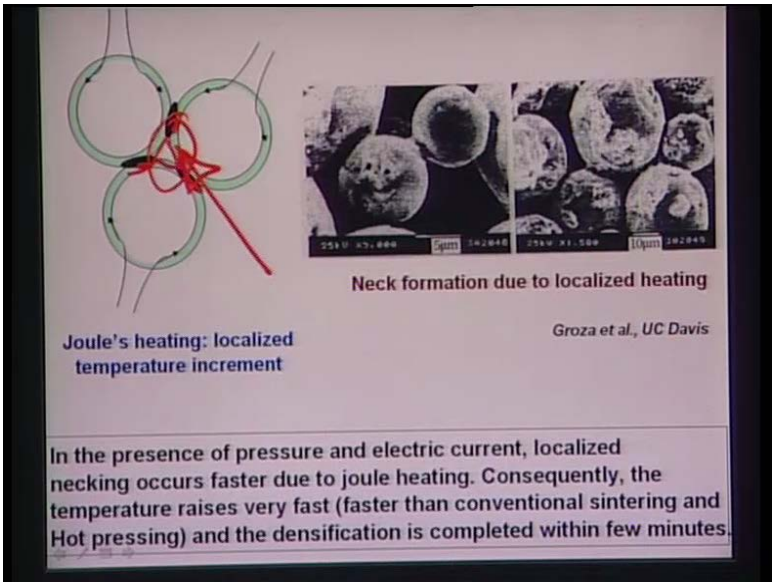
difference is that large amount of current is flow, you make to flow from the anode to cathode, during this spark plasma sintering; and this current is around 1 to 1.5 kilo ampere, which is larger than that of the welding current that typically used in industry.

(Refer Slide Time: 44:10)



Now, this current will flow either to the graphite dye wall or to the powder compact, depending on the conductivity of the powder compact - porous powder compact.

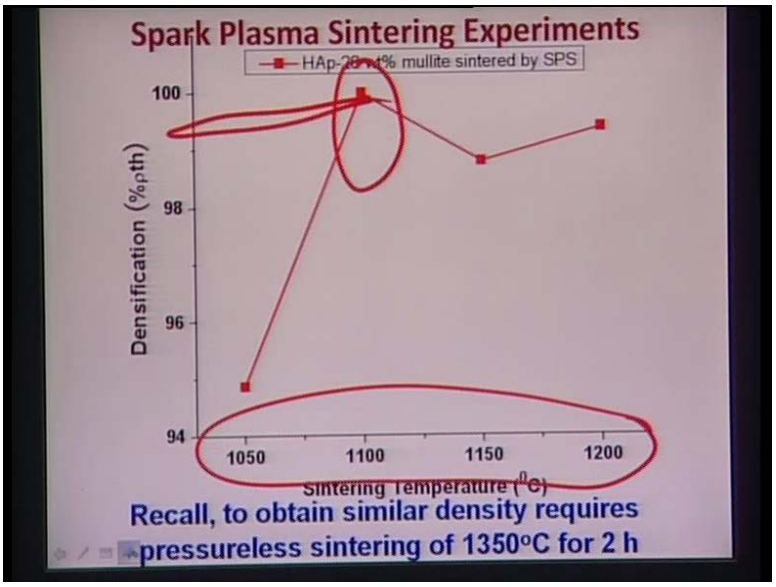
(Refer Slide Time: 44:20)



And this current will influence this high temperature generates around this neck region or the triple pocket region. And also because this contact-contact resistance of the particle-

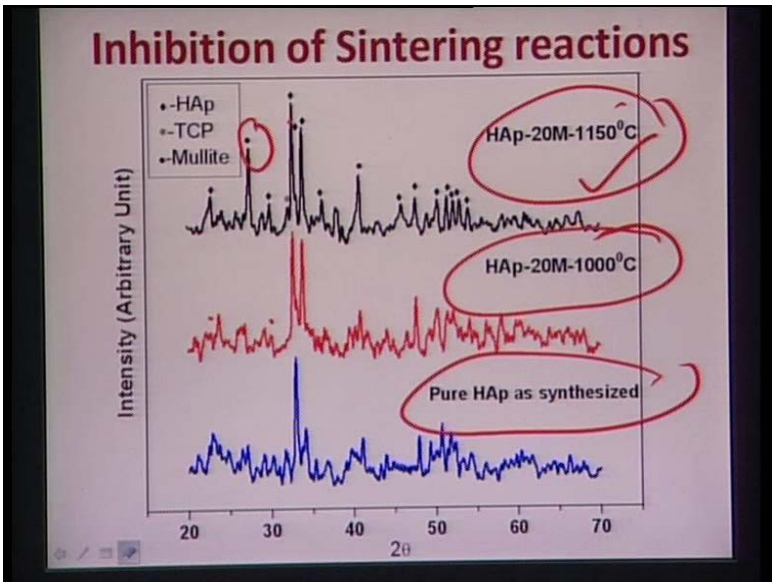
particle contact, they offer some kind of resistance to the current flow that also increases the total heat generation at this neck region; and higher the temperature, more is the diffusion coefficient; and therefore, more will be the diffusional mass transport process, which is required for this sintering to take place.

(Refer Slide Time: 44:50)



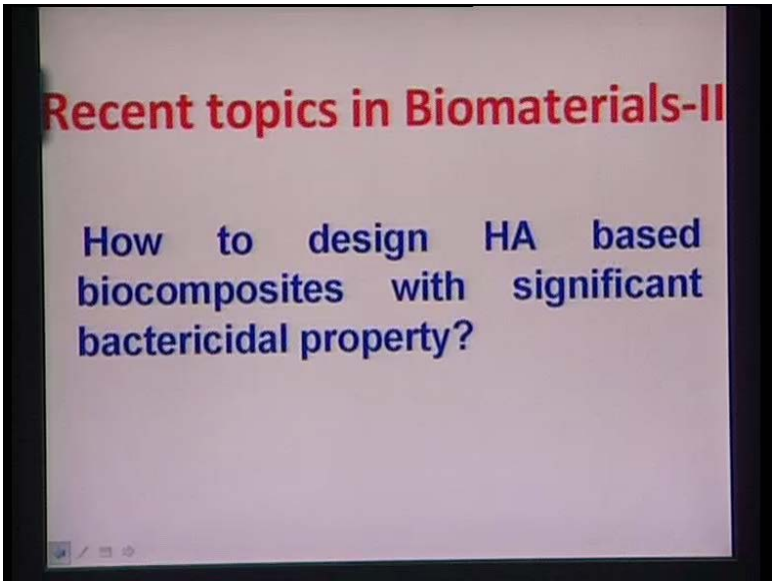
Now, we have done experiment still from 1000 to 1050 to 1200 degree celsius; and what you see at around 1100 degree celsius, these materials we can get roughly around 100 percent theoretical density.

(Refer Slide Time: 45:04)



Now, this is that hydroxyapatite as synthesized, and then when you sintered this HAp, this at 1000 or 1150 degree celsius, what you see? We do not see any presence of the hydroxyapatite tricalcium phosphate except that 1150 degree celsius samples; and there is no other reaction product like calcium aluminosilicate or alumina or silica, those kind of reaction product is not there. So, it is very clear that if you use a Spark plasma sintering; you can avoid those sintering reactions, and you can retain both hydroxyapatite and mullite phase in the microstructure.

(Refer Slide Time: 45:45)



So I think, I will stop here; and then in the next lecture, I will start with this hydroxyapatite based bicomposites, how to design this hydroxyapatite biocomposite with significant bactericidal property.