Introduction to Biomaterials

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Module No. # 01
Lecture No. # 30
Glass-ceramics for dental restoration applications

In today's lecture, I will be dealing with mostly that glass ceramics material for biomedical applications.

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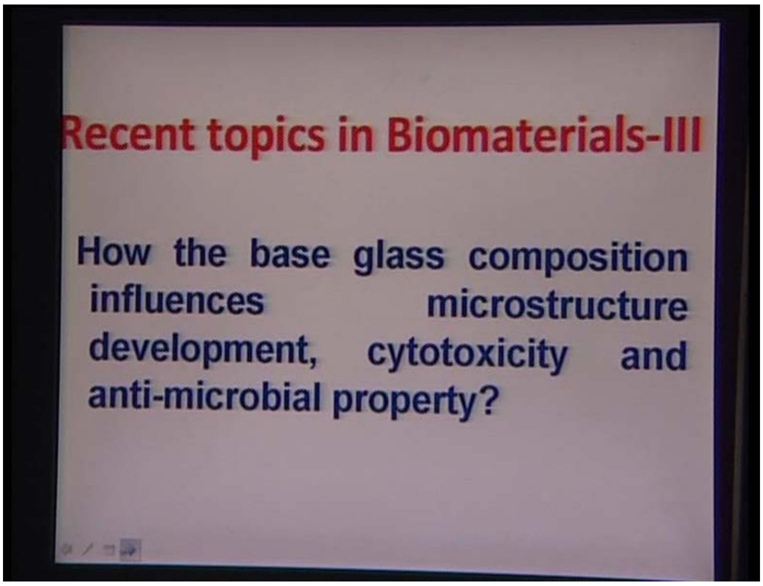

Particularly, I will address the issue that you know if one changes a base glass composition, how it will influence microstructure, cytotoxicity and anti-microbial property.

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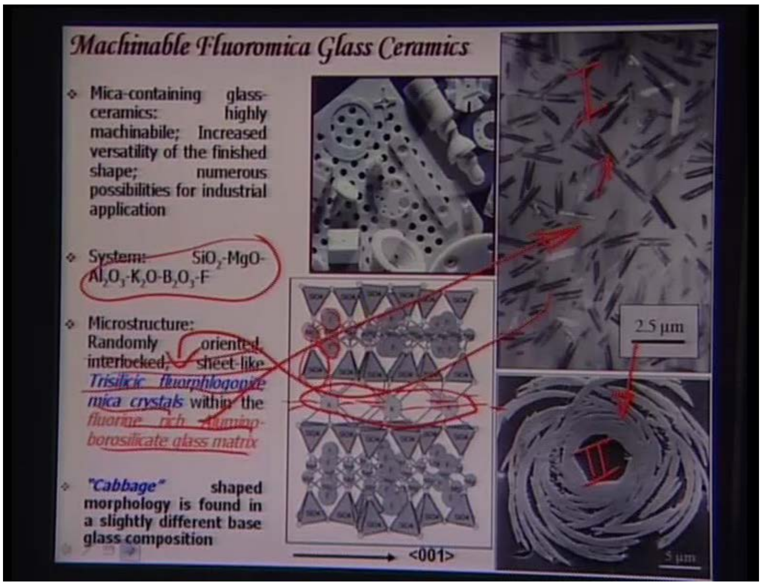

Now, there are in number of glass-ceramics which are commercially available. But my lecture will mostly focus on machinable fluoromica based glass ceramics. Now, this glass ceramics have particular relevance for dental restorative applications, because these materials are largely machinable, whereas most of the ceramics they are not machinable, because they are extremely brittle in nature. Secondly, that these dental materials now tooth's are of differential. So, if the materials can be easily machined then these materials can be given different complex shapes as desired for different types of tooth. And that is the reason that, why this materials has received much attention in the biomaterials community. That is the other glass-ceramic that I must mention that is 45 S 5 glass which was originally discovered by Professor Larry Hench, and these materials have 45 percent silica and then, you have that different other oxides. And these materials Hench and coworkers, they have actually done the testing up to the clinical stage.

Now, this particular machinable fluoromica glass ceramics, they have a particular crystal structure which make them machinable. Now, if you look at this crystal structure here, what you find that you have the potassium atoms which appear at the central region and these forms a central row of atoms. Then, on the top of it potassium atoms, you have that silica tetrahedron that SiO 4 tetrahedron, they are arranged in a particular manner, and then, in between the two silica tetrahedron which are there in the top and bottom, you have the magnesium atoms and you have that fluorine ions also. So, this magnesium and fluorine ions, they are also these ions are also arranged in a symmetric manner, and if you see the top part and bottom part is ideally symmetric to each other.

So, because of this kind of particular characteristic crystal structure, what happens? During machining operation, these potassium planes they act as a weak cleavage plane and the materials can be broken along the potassium plane here, and that leads to important characteristics that is known as the high machinability. And the system that it have this particular fluoromica glass-ceramics are observed in a particular system and this is multi oxide system, and if you see that multi oxide system like SiO 2, MgO, Al 2 O 3, K 2 O, boron oxide and fluorine. Now, boron oxide you one has to add some precursor and this precursor is H3BO3 that is boric acid, and fluorine you cannot add directly, but again fluorine has to be added to this material via precursor, and this precursor is ammonium fluoride c NH 4 F or CaF 2 calcium fluoride.

When you synthesize these materials first you one has to obtain the first and primary stage for a glass-ceramic production, one has to get the base glass composition. Now, how this base glass is obtained? So, like conventional, applying the conventional ceramic process includes one has to makes this different precursors powders in a desired ratio, then it has to be molten. And during the melting process this all this oxides they are mixed with each other, and this mixed oxide melt when it is cool to room temperature, and when it is cast and typically the casting is done in a platinum crucible. Why platinum crucible, because platinum is extremely inert, it does not participating any kind of chemical reaction with the other oxides. So, because of the noble nature and because of the extremely inert nature of the platinum (( )) crucibles are used and where this molten glass is cast in a particular shape.

And then after that once this base glass is obtained then one has to crystallize or one has to do the crystallization heat treatment or this is in scientifically note as ceramasizing. Now, ceramizing means that you are actually base glass is essentially amorphous in nature, and then after the crystallization you expect different volume fraction and different shaped crystals to be formed in the base glass matrix. And then the material which is characterized by base glass matrix where the crystalline ceramic phase are distributed in different volume fraction or different shape. That material is known as the glass-ceramic material. People have observed and people have reported in number of multiple systems that typically glass-ceramics have better mechanical properties compared to the base glass. And this better mechanical properties has been possible, because of the presence of the ceramic phase in the glass matrix.

Now, I have been said this at this particular glass-ceramic that SiO 2, Mg O, Al 2 O 3, K 2 O, B 2 O 3, F adopting different heat treatment procedures. People have been able to develop different kind of microstructures, and these microstructures if you look at at the right hand side there are this microstructure has either it can be randomly oriented interlock sheet like fluorophlogopite mica crystals within the fluorine reach aluminoborosilicate glass matrix. So, this glass matrix is essentially aluminoborosilicate glass matrix and these randomly oriented crystals are what is known as the trisilisicate fluoromica crystals.

Now, this is that the microstructure of type one let us say. That the another type of microstructure which resembles more like a cabbage type of microstructure, this actually that cabbage shaped morphology is found in with a slightly different base glass composition. Now, this cabbage actually it exactly resembles more like a cabbage vegetables. So, this crystalline phase which forms it has like you know cabbage flower or cabbage leaves and those kind of morphology it has. So, what I am trying to say here that within the same or almost similar base glass composition, if you add up different type of heat treatment; in one case you find random crystalline features, in another case you find more characteristic cabbage shaped morphology. So, the message is that one can adopt different heat treatments schedule and thereby one can obtain different shape and different volume fraction of the crystals in the order wise amorphous glass matrix.

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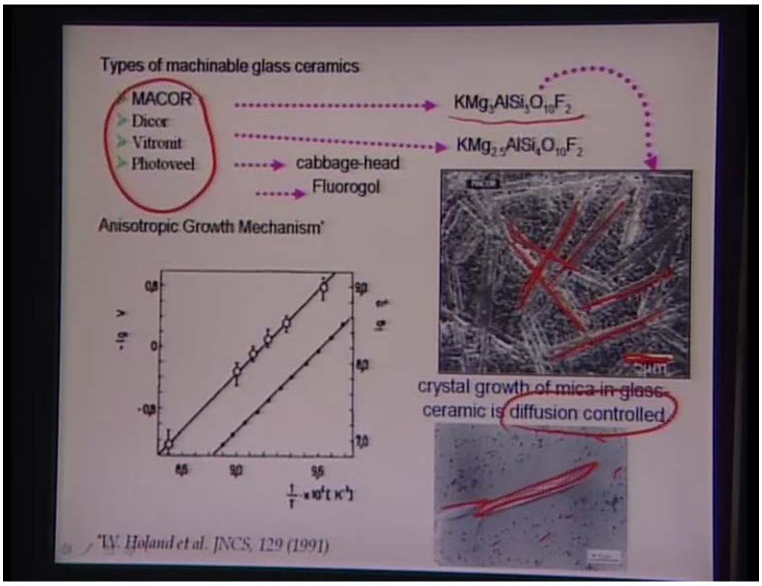

Now, these are the different types of commercially available glass ceramics of similar base glass composition. Now, commercial trade name is MACOR and where MACOR the glass ceramic the crystal composition is K Mg 3 Al Si 3 O 10 F 2. Now, if you can see that this particular crystals, they have a very complicated chemical formula. Then these type of crystals essentially reflect that they are essentially generated from the base glass matrix, and this base glass matrix has a multiple oxides. And because of the phases of multiple oxides, this crystalline phase also has a very complex phenomenon. And you can see here, this is like interlock type of mica crystals which are present in an amorphous glass matrix.

If you look at this micron bud here, this micron bud is 5 micron. So, typically length of this crystalline phase is roughly around 50 micron or so, whereas width of the crystalline phase is around somewhere 2 to 3 micron. So, roughly the aspect ratio of this kind of crystals may be somewhere around 15 to 20 of aspect ratio. The other things you can see here that this crystal growth of mica in glass ceramic is essentially diffusion control. And this you can see that this is that amorphous phase and where the crystalline phases they are formed, and this is like very high resolution transmission electron micrograph.

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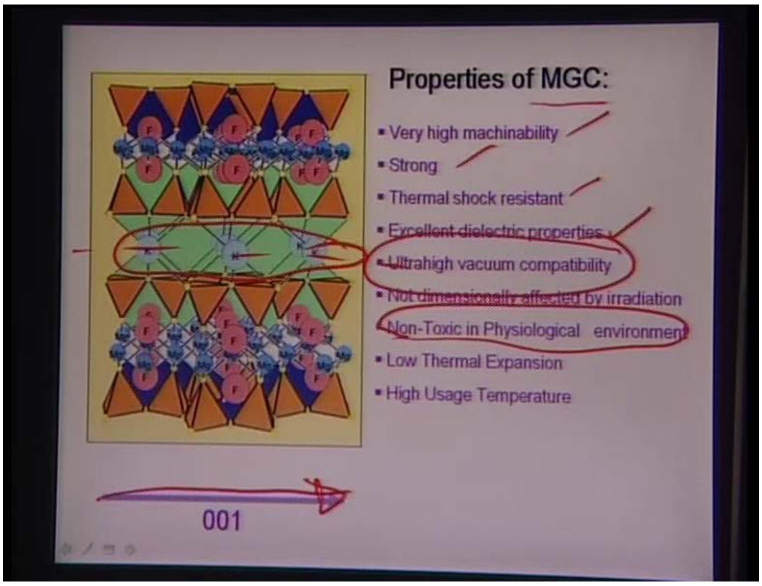

Now, this to summarize the different properties of the MGC, MGC stands for machinable glass ceramic, they have very high machinability, they are relatively strong, they have god thermal shock resistance and excellent dielectric properties, and they are non-toxic in physiological environment. These properties actually of relevance to biomedical applications and they have ultrahigh vacuum compatibility. Now, here you can see this is the 1 0 0 plane that is that atomic arrangement, and you can see that as I said earlier this potassium plane across which the top and bottom this different silicate arrangement, and fluorine and magnesium ions or atoms they are they are located, so this potassium plane acts as a mode like a weak plane and that access a mode cleavage plane here.

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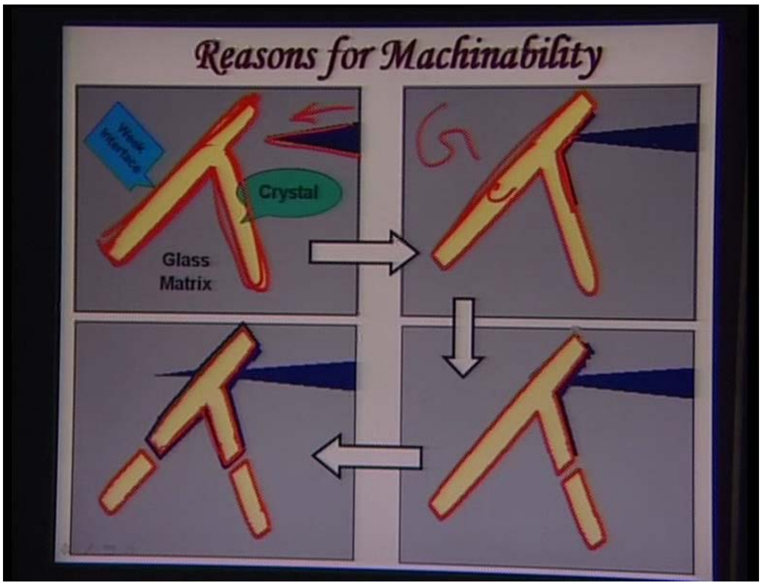

Now, the reasons for machinability, this is the schematic. Now, suppose you have a crack here, this is like half crack that has been shown which is propagating from right to left for example. And you have this interlocked type of mica crystal. This interlocking mica crystals, now when this cracks will come and they will interact with the interlocking mica crystals, then what will happen? This is like a weak interface. Why it is a weak interface, because this is the crystal and this is your glass and this is your crystal. Now, glass is a amorphous matrix and crystals is your crystalline morphology. So, this interface typically acts as a mode like a weak interface.

Now, cracks will initially trying to be getting deflected around this glass matrix interface, but subsequently it should be it would be able to break the crystals and then it will propagate. Now, each time the crack will come and interact with the crystalline phase, some energy will be lost in breaking the crystals and further propagation. Now, if the crack tip energy is lost in this process then what will happen, the driving force for the crack propagation also will be reduced. And if the driving force is reduced then brittleness or the more machinability property can be imparted to this kind of glass ceramics.

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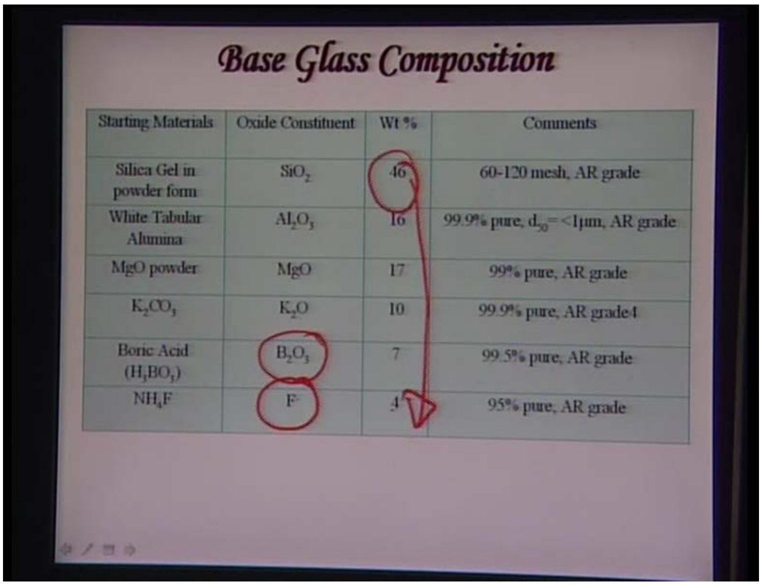
Base Glass Composition

Starting Materials	Oxide Constituent	Wt %	Comments
Silica Gel in powder form	SiO_2	46	60-120 mesh, AR grade
White Tabular Alumina	Al_2O_3	16	99.9% pure, d_{50}=<1μm, AR grade
MgO powder	MgO	17	99% pure, AR grade
K_2CO_3	K_2O	10	99.9% pure, AR grade
Boric Acid (H_3BO_3)	B_2O_3	7	99.5% pure, AR grade
NH_4F	F	4	95% pure, AR grade

The other things that is the typical base glass composition. Now, if you see that different type of precursor powders as I said the boric acid is used for boron oxide and ammonium fluoride is used for fluorine. Now, these are different weight percent of the different crystalline different powders they are mixed.

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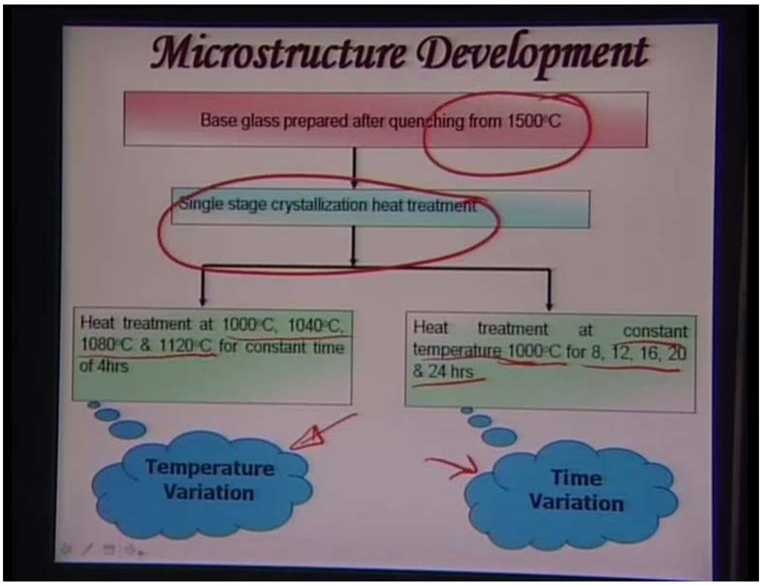

Now, after they are mixed this base glass typically melted at 1500 degree celsius. From this 1500 degree celsius, you can quench it or you can cast it in platinum crucible. After that you can do single stress crystallization heat treatment and this heat treatment either can be temperature variation or can be time variation. In the temperature variation case, you we have chosen that four different temperature 1000 degree celsius to 1120 degree celsius for constant time of 4 hours. And another one is that heat treatment at the time at a constant temperature of 1000 degree celsius for different time frame from 8 hours to 24 hours. So, in one case, you are actually varying the temperature, another case you are varying the time at a constant temperature, and that is why you had saying in one case time variation sample, in one case we are showing temperature variation samples.

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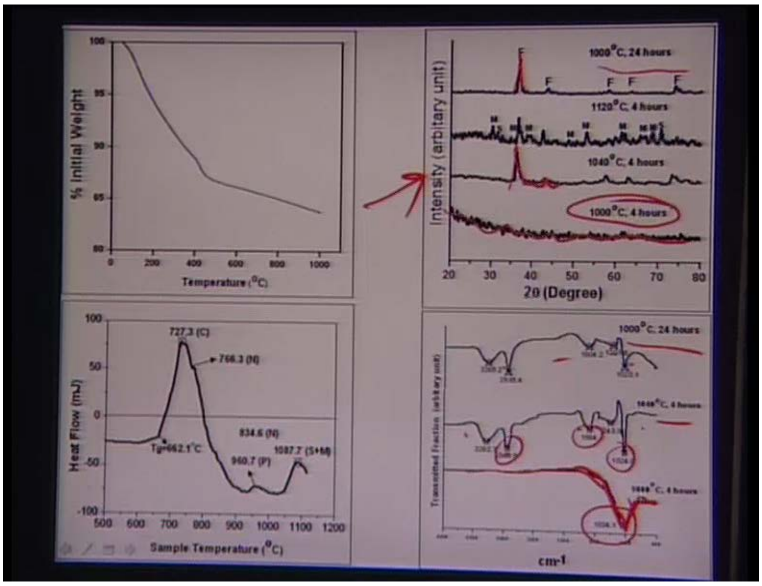

Now, this is the x (( )) plot which essentially says that at 1000 degree celsius 4 hour samples you have that amorphous matrix. Now, when you crystallize it 1040, 1120 and 1000 degrees of 44 hours, what you see, it has a various characteristic fluorophlogopite peak, so that is a crystalline peak. So, this crystalline peak comes from the crystals that are formed during the heat treatment conditions. And from the FT-IR are also one can find out that they are a different crystals bands, because this is 1000 degree celsius 4 hours, you can see that FT-IR has only these particular FT-IR band. Now, because of the presence of the crystals, these different FT-IR bands are formed at different numbers here at different heat treatment conditions.

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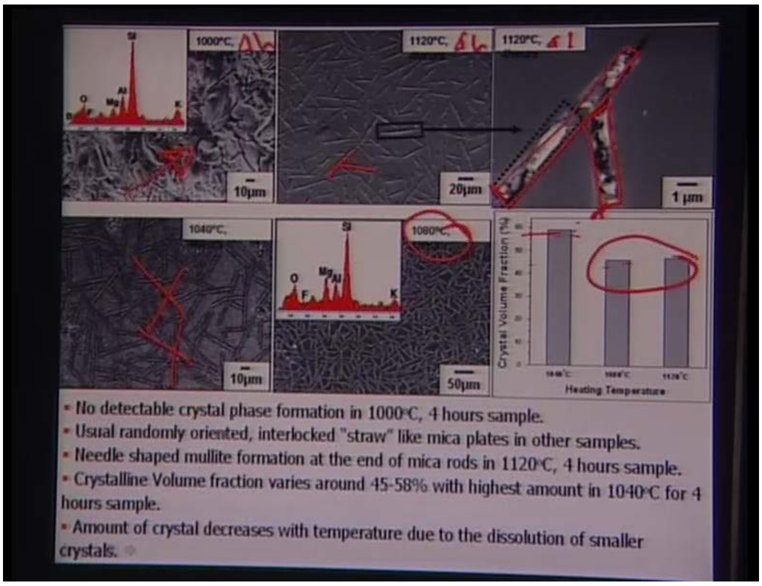

Now, this is the microstructure that was generated after heat treatment at different temperature for with for constant time frame of 4 hours. Now, these now, initially 1000 degree celsius 4 hours, this is an amorphous matrix. Now, at 1120, 4 hours what you see here, this is the randomly oriented crystals or interlock type of crystals; this crystal is going like this, another crystal is going like that. And that you know one of the crystals if you want to see, you will be able to see this is one mica crystals, this is another mica crystals and they (( )). At 1040 degree celsius also you will see that different mica crystals, they are growing at different directions, similar observation has been made at 1080 degree for 4 hours.

So, this is the crystal volume fraction based on this SEM images, one can measure the crystal volume fraction, and what you find here at 1080 and 1120 there is no change, so the crystal volume fraction is roughly around 45 percent, whereas at 1040 degree celsius crystal volume fraction is roughly around 58 percent or so.

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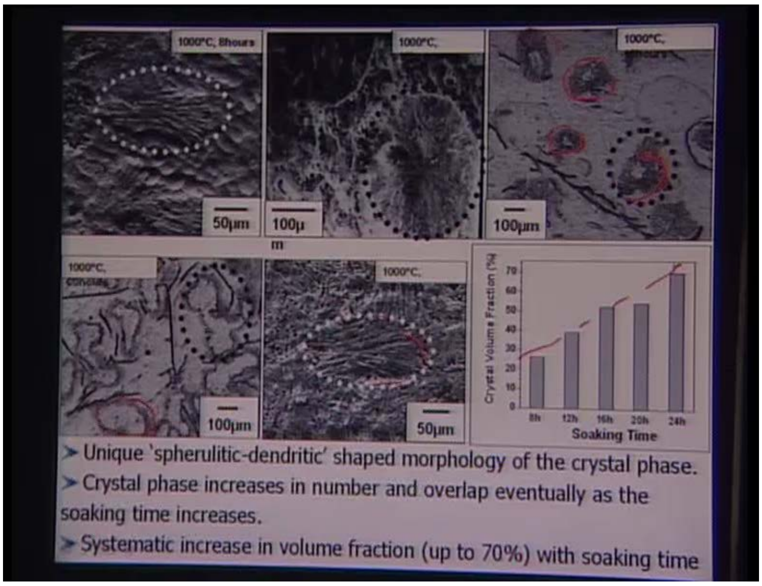

Now, when are do the heat treatment at different time scale for same temperature, let say 1000 degree celsius, what you see here, you will see essentially the butterfly shaped type of crystals and this butterfly shaped crystals are what we called spherulitic-dendritic type of crystals. They characteristically formed at 1000 degree celsius. And if you increase the soaking time, you can essentially see that somewhere around 25 volume percent to 70 volume percent after soaking for 24 hours or 1000 degree celsius and this is a like a systematic increase in volume fraction with time.

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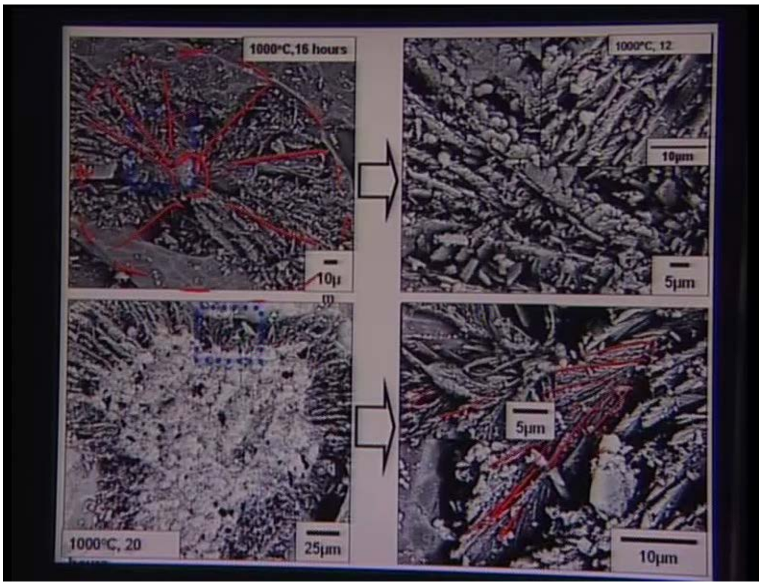

Now, this microstructure you can see it more clearly here. Now, this is the crystal domain let say, and in this crystal domain if you focus that this is a kind of nucleus, and from this nucleus this butterfly crystals they are growing in different directions. And you can see that it vary characteristic crystal morphology here, it is more like a far tree or it is more like a tree leaves kind of pattern. Now, you can consider this as male leaf and these are like different branches which are formed during this crystallization process.

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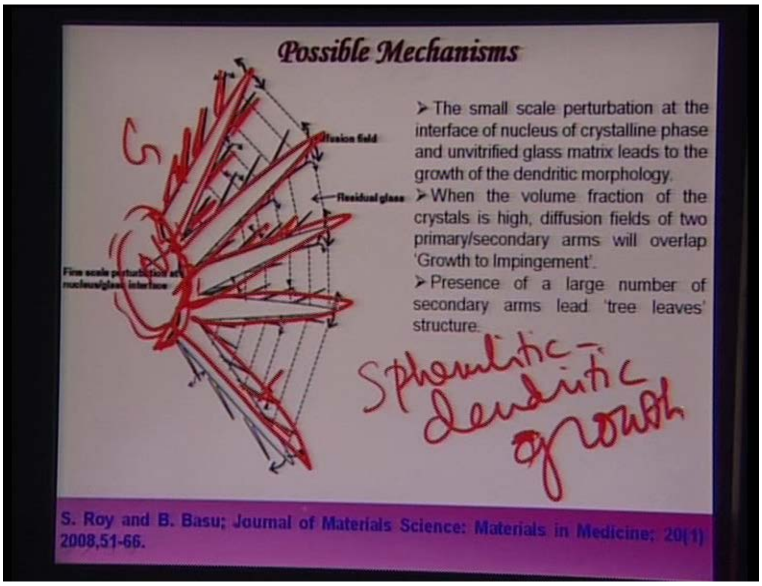

Now, what we believe that during this crystallization process in the base glass you have this nucleus initially forms, and this when this heat treatment takes place from this nucleus some solid that, this is the nucleus and this is the glass matrix, nucleus glass interface some kind of perturbation actually grows. And this perturbation leads to the crystals in different directions and these crystals are formed, and this main branch of the crystal is what we called primary arm of a dendrite. Now, from this primary arm, the secondary arms they are generated and this secondary arms they are formed, and if there is sufficient concentration gradient or diffusion field they are, from the secondary arm that tertiary arms also can grow. Now this type of characteristic morphology what we called as a spherulitic-dendritic type of growth. Now, spherulitic and spherulitic type of growth is quite common in many of the glasses, but spherulitic and dendritic growth combination is kind of unique in this particular glass ceramics case.

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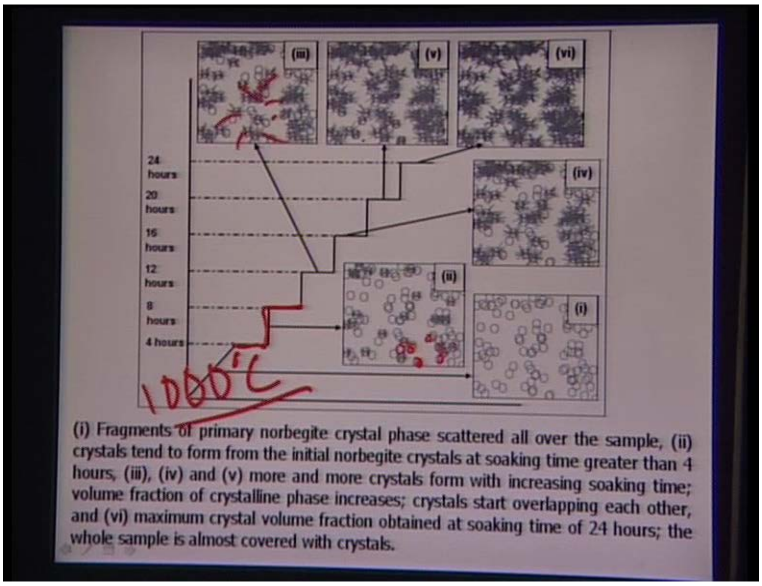

Now, this is what is schematically has been shown here like you know when you start this heat treatment at 1000 degree celsius for different time scale, then what you have at initially during this 4 hours and after that when you go to 8 hours sequence, then all this droplets and this nucleus they form. And from this nucleus you can see that it is growing in different directions or multi-directional growth of the crystals that takes place from this spherical shaped nucleus. And subsequently you till form a typical what we called as characteristic spherulitic-dendritic type of morphology.

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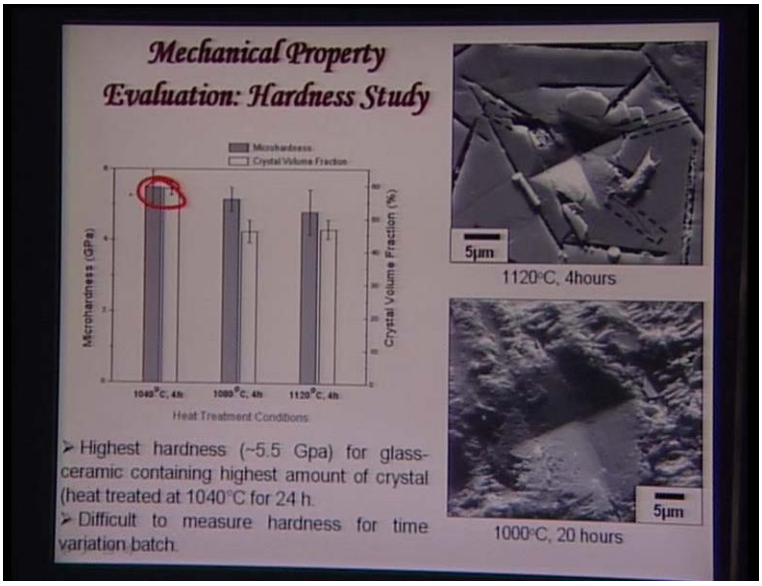

Now, these materials when we have done this mechanical property characterization, what we have observed that depending on that heat treatment conditions, one can get the hardness something around 5.5 gigapascal that is the highest hardness, and this 5.5 gigapascal hardness is obtained when at the maximum crystal volume fraction of close to around 60 volume percent. So, higher the volume fraction of the crystals higher is the hardness data, and roughly the error box overlapped with each other. So, there is no statistically significant difference between these three different values of the hardness.

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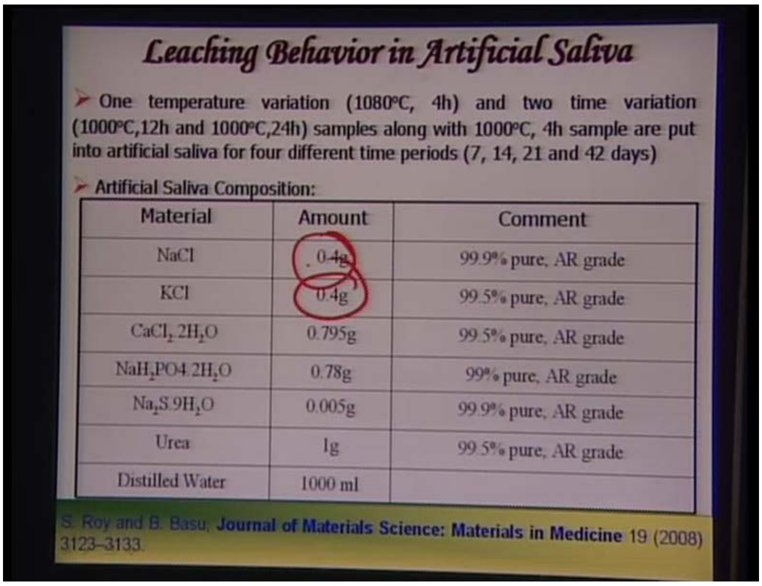

Now, we have done the in vitro biomineralization test for these materials, and what we have done? We have prepare the artificial saliva, because these materials are to be used for dental (( )) application; so, we do not use the simulated body fluid, but we use the artificial saliva which is present in the oral environment. So, be so that you can simulate the composition which is existing at the oral environment. And this artificial saliva it has in part 1000 milliliter or 1 liter of the artificial saliva, NaCl is 0.4 gram and KCl is 0.4 gram. As you can see that it is much sodium chloride, potassium chloride, calcium chloride and there are different sodium containing salts are also present as well as the urea is 1 gram.

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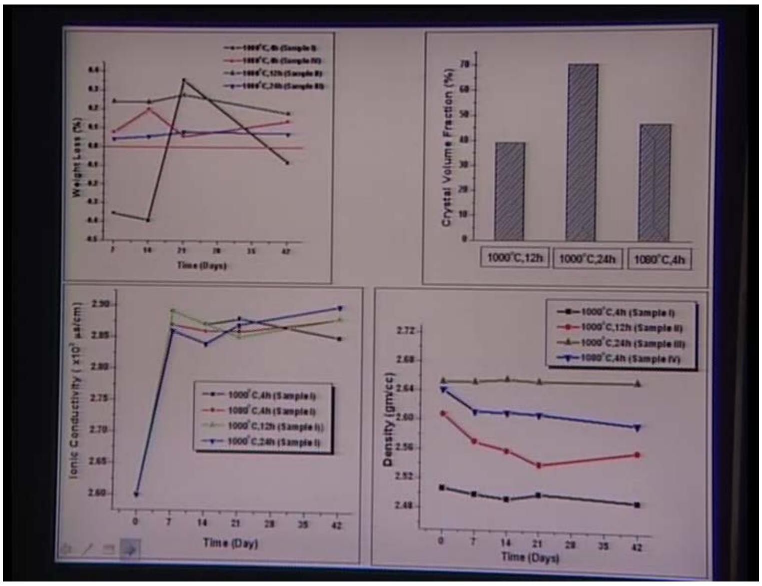

Now, when you use these artificial saliva, now what we have found.

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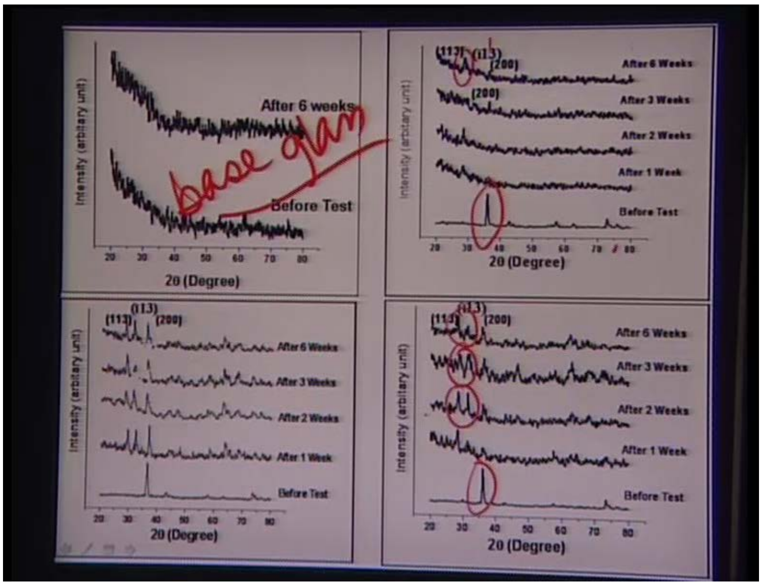

After testing for a different 6 weeks for base class, this is the base class, before test and after test the glass remains amorphous. But when you do this test for the different other samples before test and after test, you see that there is some texturing effect, because this main crystalline phase somehow vanishes when you do this test for different time scale and new peaks are actually appearing.

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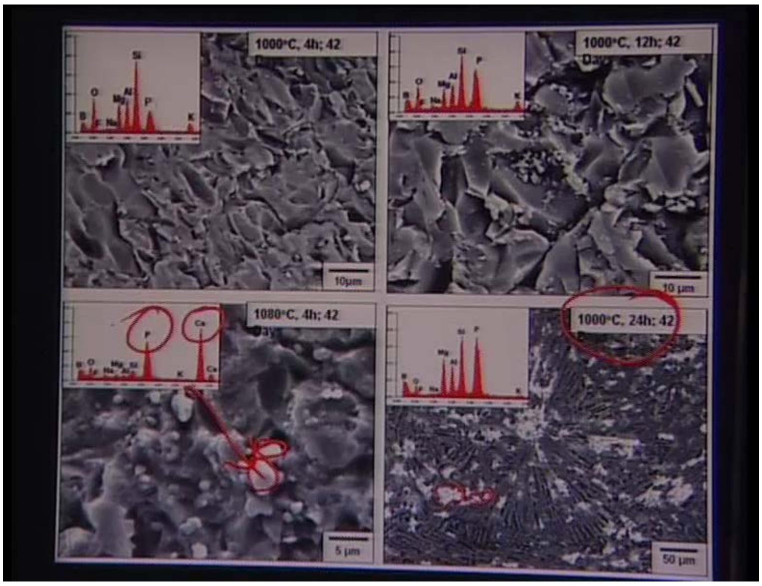

Now, new peaks are appearing from different crystal phases here. Now, this is what you can see at 1000 degree celsius 24 hour sample, after 42 days you can see this bright contrastive phases as well as this kind of spherical shaped deposits. And when you take these edax spectra from these spherical shaped crystals, what you see that is the presence of calcium and phosphorous significant presence. So, essentially what it tells you that these products are essentially calcium phosphate rich bio-mineralize products, and this calcium phosphate rich bio-mineralized products are formed after 6 weeks of SBA femurs.

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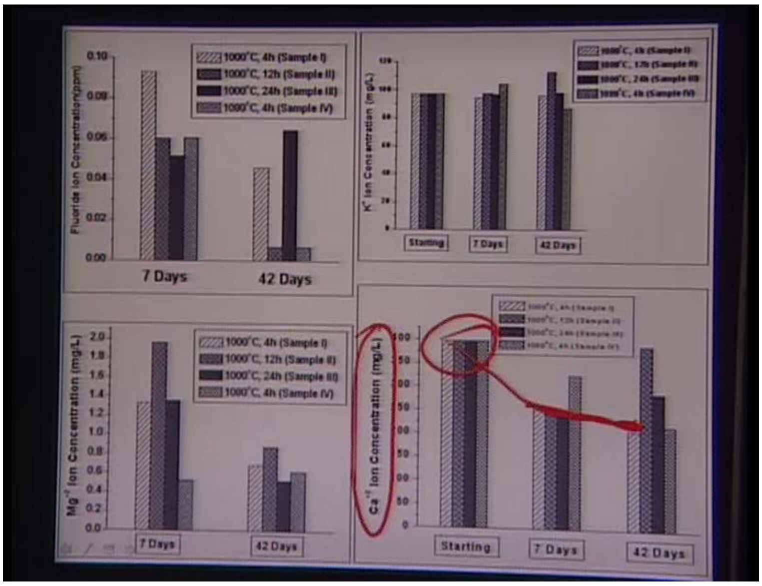

Now, this is what you can see here the calcium ion concentration which is most important. At the starting you have this calcium ion concentration. After 7 days and 42 days depending on what kind of material you are choosing. There is a decrease in the calcium ion concentration in the solution.

Now, if there is a decrease in calcium ion concentration in the solution that should be reflected in the increase in the calcium ion deposition on the material surface. Because total volume total volume percentage of the ion should be (( )) before test and after test. And that is that commensurate well with your calcium phosphate rich product formation on the material surface.

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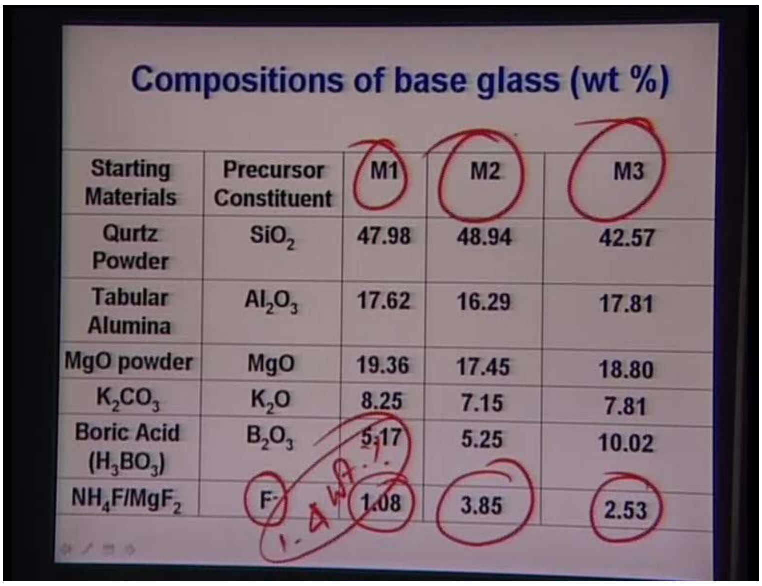

Compositions of base glass (wt %)

Starting Materials	Precursor Constituent	M1	M2	M3
Qurtz Powder	SiO_2	47.98	48.94	42.57
Tabular Alumina	Al_2O_3	17.62	16.29	17.81
MgO powder	MgO	19.36	17.45	18.80
K_2CO_3	K_2O	8.25	7.15	7.81
Boric Acid (H_3BO_3)	B_2O_3	5.17	5.25	10.02
NH_4F/MgF_2	F^-	1.08	3.85	2.53

Now, in the second set of experiments what we have done, we have changed little bit of the fluorine content in the starting powder right from 1.1 to 2.5 and 3.85, so roughly around 4. So, essentially what we are doing, we are changing this fluorine content is 1 to 4 weight percent. And accordingly we call it as M 1, M 2 and M 3 glass ceramic composition.

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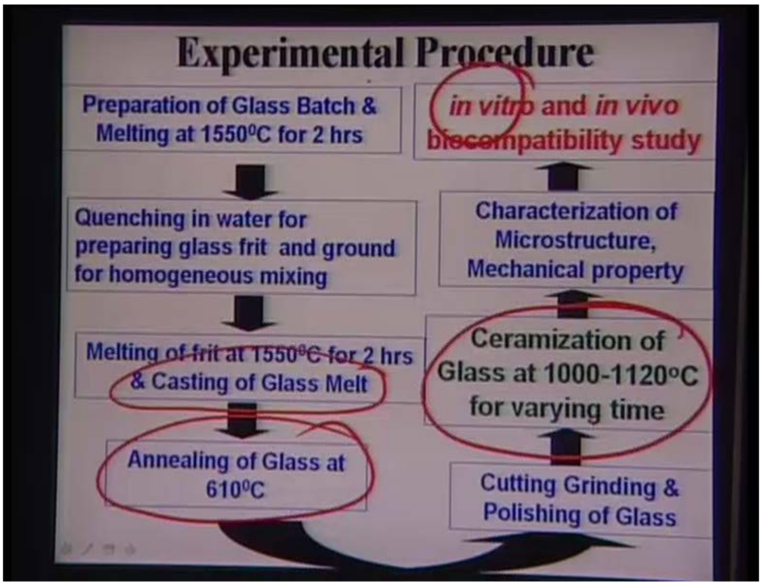

And this glass ceramics when they are melted at 1550 degrees 2 hours, then you can quench it in water and cast it in platinum crucible. After that you again re-melt this glass ceramic; why this re-melting is done? That is to homogenize the composition, because it is a multi-component multi-oxide system.

If you do not re-melt this glass ceramic, all this different oxide they will not be mixed properly. Now, to ensure that they are mixed properly, you re-melt this entire glass ceramic composition, and then you can cause this glass ceramic melt in the platinum. After that you can anneal this glasses at 610 degree celsius, you got polish and ceramize the glass ceramics at different temperature right from 1000 degrees to 1120 degree celsius. You can characterize the microstructure mechanical property and then in vitro property were evaluated. I will go through all this properties one by one.

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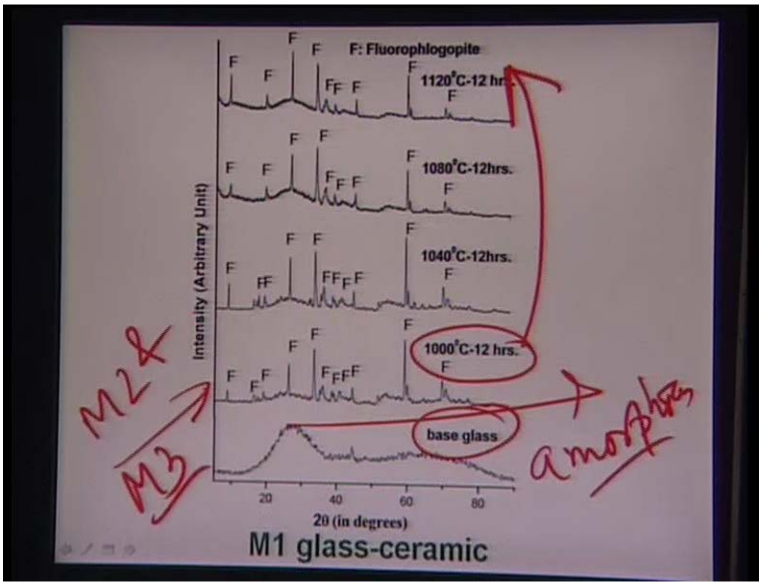

Now, this is the base glass M 1 composition that is the base glass ceramic. Now, if you see the base glass, here this is this shows essentially amorphous peak; very broad peak, no crystalline is observed. But the same base glass when you heat treat at 1000 degree celsius for 12 hours then what you see, very characteristic sharp crystalline peak. And this F stands for fluorphlogopite; fluorphlogopite is the crystal phase that formed when you do the heat treatment in this base glass.

And when you do this heat treatment from 1000 degree celsius, 1120 degree celsius for 12 hours that the constant heat treatment conditions. This fluorphlogopite peaks are also much more intensity they increase and essentially it says that fluorphlogopite volume fraction also should increase, and this kind of results is also observed for M 2 and M 3. So, this is just a representative phrasal that I am showing for M 1 glass ceramic.

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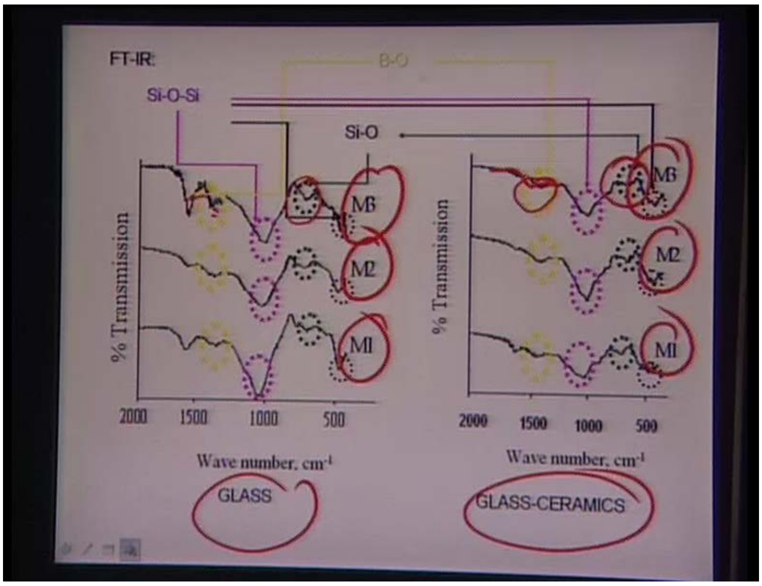

Now, what happens in this FT-IR spectrum when we do this FT-IR analysis? This is the best glass and this is the glass ceramic; base glass M1, M2, M3 composition and this is the glass ceramic M1, M2, M3 composition.

Now, you have this Si-O-Si band, you have this Si-O band and if you have this B-O band. Now, B-O bands is much more sharper here in this as well as the Si-O-Si band also; B-O band is much more sharper in this base glass wherever it is much more diffuse and make broaden here in this glass ceramic that is one observation you can make. Other observation is that you have this Si-O band is also present and then there is a combination of the third peak that appears here which was relatively weak in the case of the base glass.

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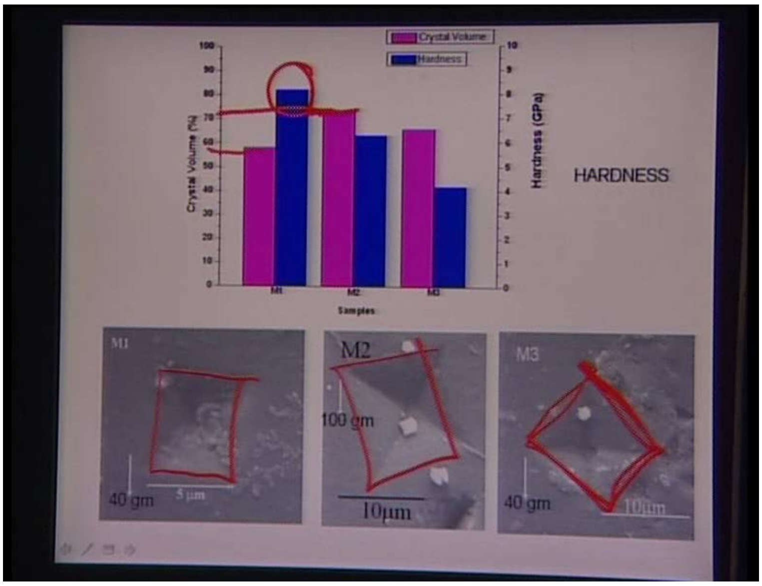

Now, interesting thing that has to observed in this glass ceramics that one can obtain here much higher volume fraction of the crystals and this crystalline volume fraction can go up to 58 to 60 up to 70 volume fraction, so whereas, in the earlier case we can to obtain only up to 55 or 58 percent. And most remarkable result that we have got is that hardness of 8 gigapascal. Now, this hardness of 8 gigapascal is very high value as far as the glass ceramic is concerned. Just to give an example, the steel is mostly fully harden condition like when steel is fully martensitic condition, they are the hardness is around 7 gigapascal. So, these glass ceramics this has a hardness which is better than fully harden martensitic steel also. So, it is really hard and which is not commonly observed in many of the glass ceramic materials.

This is the SEM image of this different (( )) that was present on this glass-ceramic. And essentially what you see at the 40 gram or 100 gram indent load, you see that this material that indent has a good shape and then very stable indentation response.

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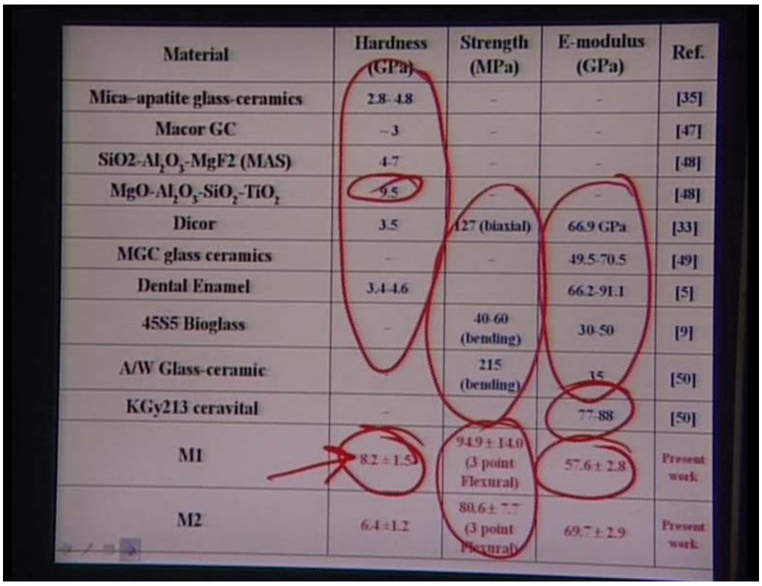

Material	Hardness (GPa)	Strength (MPa)	E-modulus (GPa)	Ref.
Mica–apatite glass-ceramics	2.8–4.8	-	-	[35]
Macor GC	~3	-	-	[47]
SiO2-Al_2O_3-MgF2 (MAS)	4-7	-	-	[48]
MgO-Al_2O_3-SiO_2-TiO_2	9.5	-	-	[48]
Dicor	3.5	127 (biaxial)	66.9 GPa	[33]
MGC glass ceramics	-	-	49.5-70.5	[49]
Dental Enamel	3.4-4.6		66.2-91.1	[5]
45S5 Bioglass	-	40-60 (bending)	30-50	[9]
A/W Glass-ceramic		215 (bending)	35	[50]
KGy213 ceravital	-		77-88	[50]
M1	8.2 ± 1.5	94.9 ± 14.0 (3 point Flexural)	57.6 ± 2.8	Present work
M2	6.4 ± 1.2	80.6 ± 7.7 (3 point Flexural)	69.7 ± 2.9	Present work

Now, this is the summary of the all the earlier we investigated glass ceramic materials. And what you see here that mostly that glass ceramic materials you with some exception of 9.5 gigapascal here. Mostly they have a hardness values roughly around 4 to 5 gigapascal or 4 to 6 gigapascal. In our case, we got in M1 composition 8.2 gigapascal which is quite good value. In most of the cases, people have not reported the combination of strength in the elastic modulus value, and some cases people have measure that bending strength or biaxial strength and which is roughly around 60 to 200 megapascal. In our case also we got close to 100 megapascal 3 point flexural.

In case of the elastic modulus we have got around 58 megapascal, in other cases we got in over 77 to 88 or around close to 90 gigapascal. So, these elastic modulus of 60 gigapascal is good for boundary placement application, because cortical bone also has a elastic modulus 3 to 80 gigapascal; 3 point flexural strength around 94 or 100 megapascal is also a good value. Hardness is certainly much better than many of the earlier investigator glass ceramic. So, this combination of closely matching elastic modulus as well as high hardness value that I we belief will make this material very viable for biomedical applications.

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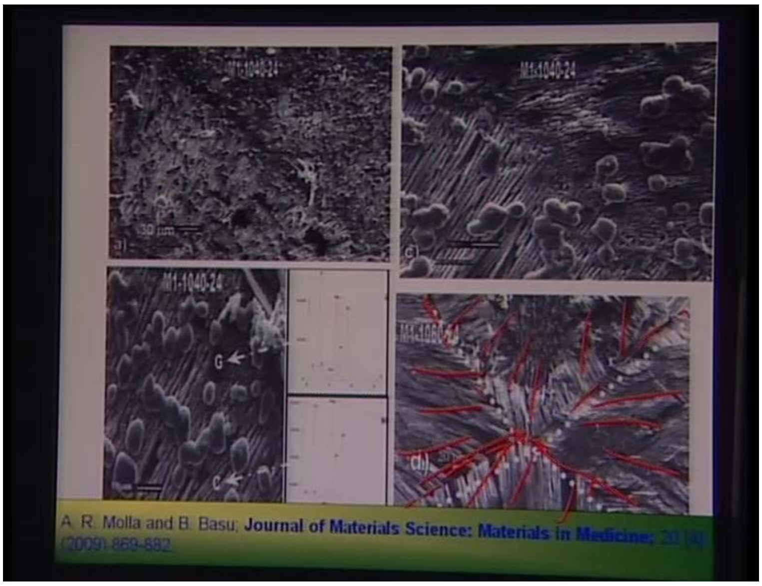

Now, let us look at the microstructure of this material and what is interesting as per the microstructure is concerned that they have what we call is a characteristic envelope type of crystals that are formed in this materials. And this envelope is exactly that letter envelope you can fold and you put it, and this each part of this envelope you can see the crystals are formed and they are growing in different directions. And that the boundary of this crystal domains these two crystals which are growing in two different directions they come and they meet and their growth is stopped.

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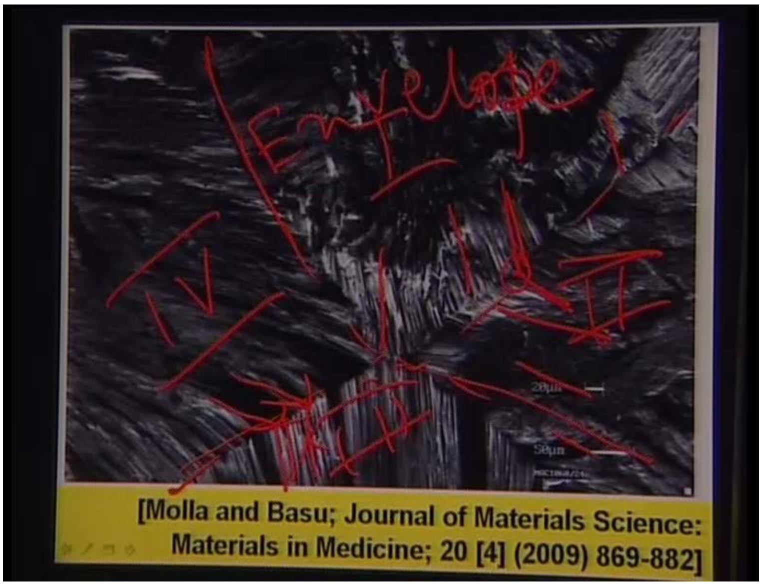

Now, this is a (( )) very characteristic microstructure of these materials. Here you can see that more clear few of this envelope type of crystals. So, this is domain one for example, this is domain two, this is domain three and this is domain four. There are four crystal domains and this is the four parts of the envelope crystals, and each domain the crystals are growing in different directions for example. Now, this domain this crystals growing this direction, this domain this crystals growing in this direction, was the growth front develop with each other, then the growth of this crystal as well as the growth of this crystal will be stopped. So, they will not grow further.

Similar things happen here; this is growing in these directions, this is also growing in these directions. The moment they interact then the growth of both the crystals they stop and this stoppage of this growth is taking place at the interface between the two domains. So, this should be very clear to you. So, this is a (( )) characteristic crystal morphology.

Now, if you go back to the introductory slide where I have told you that this glass ceramics they really provide good opportunity to develop different type of microstructure by changing either the base glass composition or the heat treatment condition. In this case you are getting the envelope type of crystals, in other case you have I have shown you that you can get that interlock mica type of crystals, in other case you you have seen that cabbage shaped crystals. So, all different kind of crystal morphology is possible in the similar glass ceramic composition just by adapting different type of heat treatment conditions. So, that is the major masses I thought that I want to share with you.

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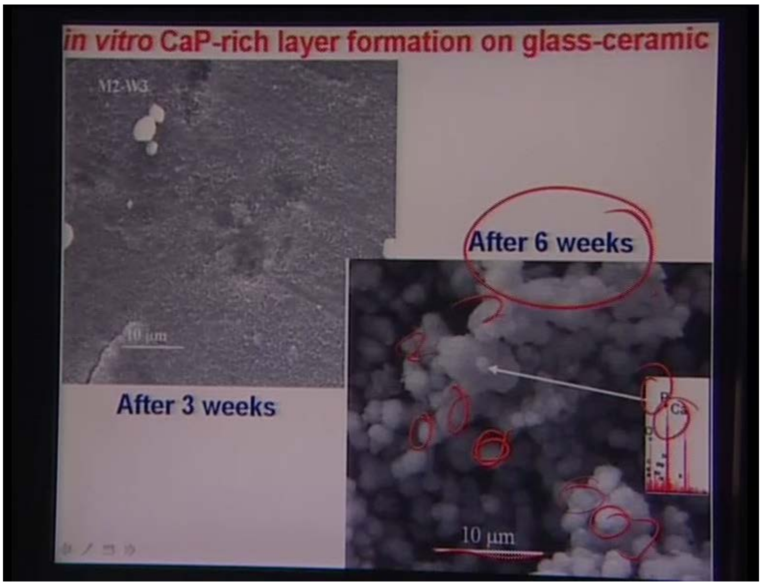

These materials we have done the bio-mineralization test and this bio-mineralization test after 6 weeks what you see here, this is like calcium phosphate rich layer formation, you can see that calcium and phosphorous peak very clearly. And this calcium phosphate rich dense layer that forms, and this is like spherical aggregates, and this spheres is of 2 micron in size or so. And then you can see they are like they are form a continuous layer here. And you can see after 3 weeks also this calcium phosphate rich layer formation starts.

Now, as I said earlier that this kind of calcium phosphate rich layer formation is essential for good bi-activity property of these materials. So, one this calcium phosphate rich layer forms, then this material when it is implanted in the body, then it will be quite good idea that you know this that a biological response of the human tissues to this kind of materials would be much more positive.

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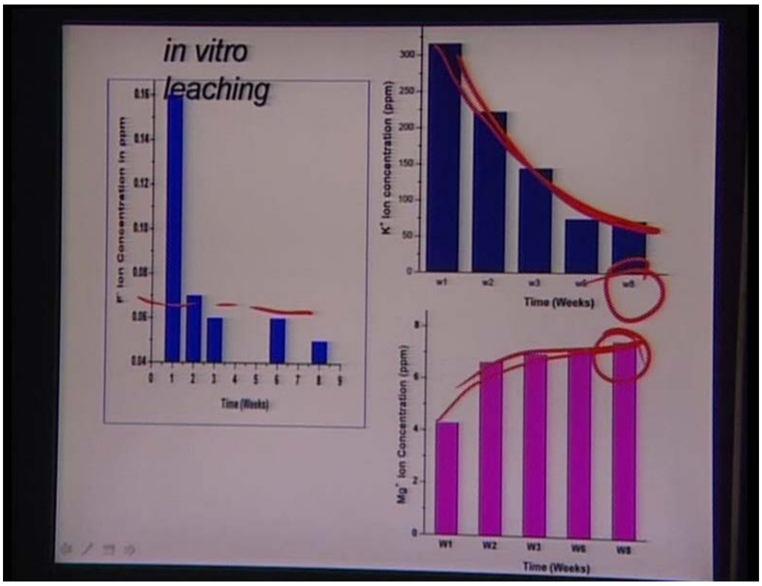

Now, fluorine ion concentration, this fluorine leaching we have measured at different time period like up to 6 weeks or 8 weeks, and what you see here the typical fluorine ion is leached less than 0.1 ppm. Now, typically according to the world health organization - WHO standard maximum fluorine level in the body that can be tolerate is 1.5 ppm. So that less than 0.1 ppm for the present set of materials is quite an acceptable value as well as the present materials is concerned.

Now, potassium concentration is also decreases with time like up to week 8 people have done we have done experiments. Magnesium ion concentration is increases then become stable up to the week 8. But all this is the amount of this leached ion is of the order of some PPM, so which is quite acceptable for this glass ceramics conditions.

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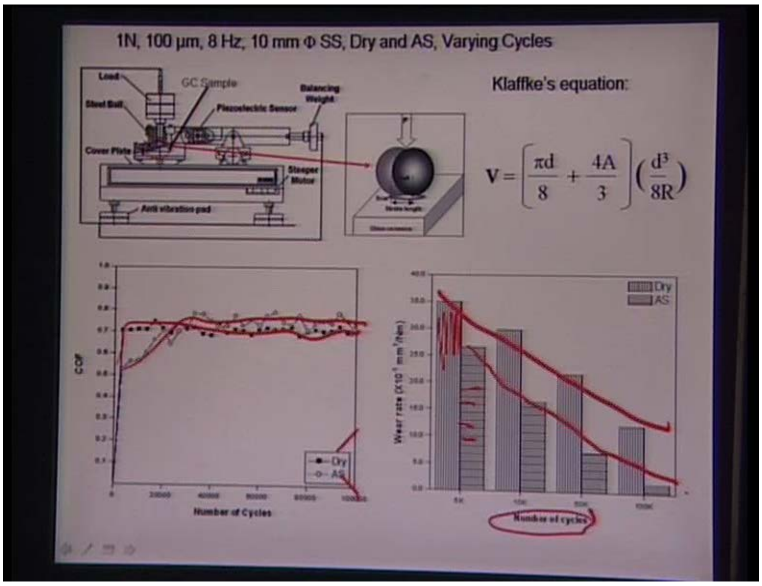

Well we have done some tri-biological testing on this M2 glass ceramics, and what we see here that after testing against the steel base materials that on the dry conditions and artificial saliva you have this COF response, and this COF we do not see any difference. So, in the both the dry as well as the SBF condition COF is roughly 0.7. But interesting thing has been observed when you measure the wear rate, and what you see in the dry condition that is the normal hatched that vertical hatch and that SBF condition that is the horizontal hatched column that - if you increase the number of cycles or number of test durations or the total test duration there is a progressive decrease in the wear volume. That means the ware volume decreases as you increase the total number of cycles that means wear resistance is improved we as you do more more number of cycles or as you increase the test duration. Similar thing has been observed also in the artificial saliva; here also you can see that this wear volume decreases with increase in number of test cycles.

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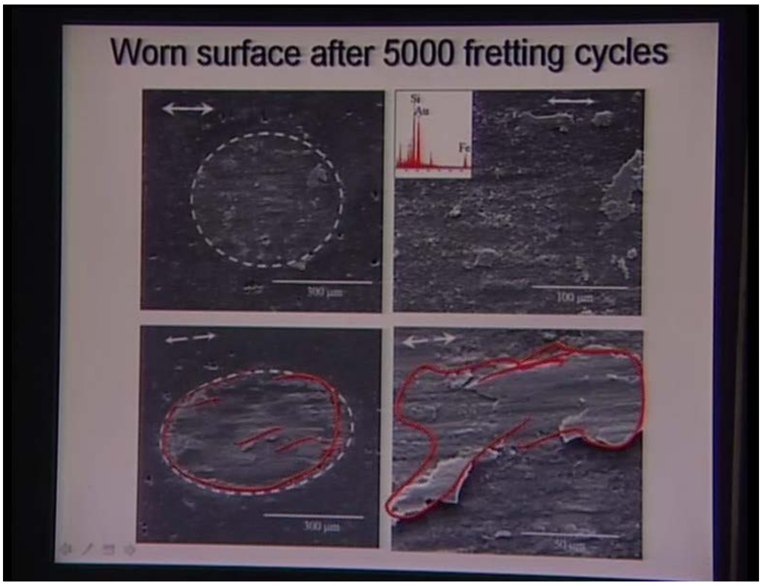

Now, what happens during that why wear volume decreases or why wear resistance increases, what we have observed with increase number of cycles that tribochemical layer forms in on the material surface, and this materials now they can be at this tribochemical layer is very much protective in nature and they can prevent the material from further wearing.

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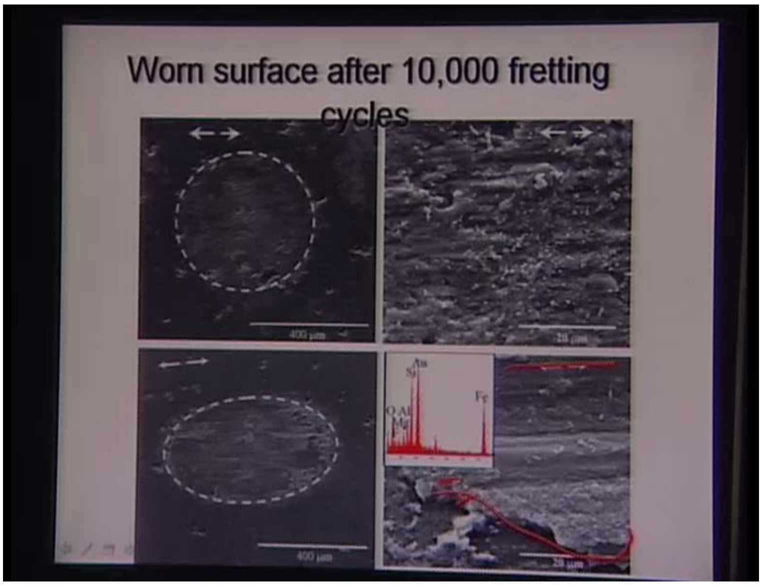

And that is what is happening here and you can see this thick tribochemical layer essentially protects the underlying material. And the thickness of this tribochemical layer and protectiveness of this tribochemical layer increases as you increase the total number of test duration or total test duration or total test cycles.

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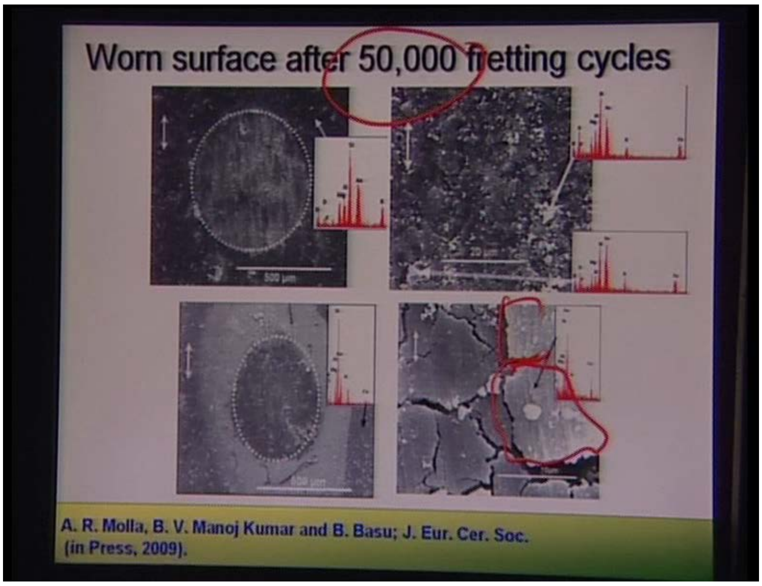

You can see after 50000 fretting cycles that this there is tribochemical layer, it is starts little bit breakage, but you can see that it is very thick as well as the tribochemical layer is concerned.

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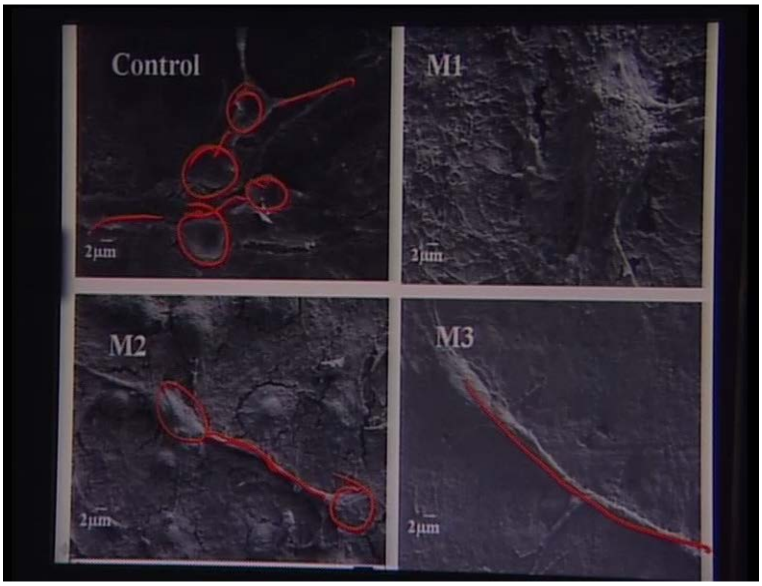

Now, we have done this in vitro cytocompatability testing using Saos-2 cell lines. Saos-2 is that osteosarcoma cell lines - human osteosarcoma cell lines, and this cells were culture for 24 hours on this glass ceramic materials. You can see that filopodia extension of the cells, this is cell number 1, this is cell number 2 and that leads to the cellular bridge formations. And also (( )) you can see that long filopodia extension. So that essentially indicates cells are in migrated in nature or migrated in conditions. And you can see that this difficult cell morphology here and then this cellular network formation also seen on the material surface.

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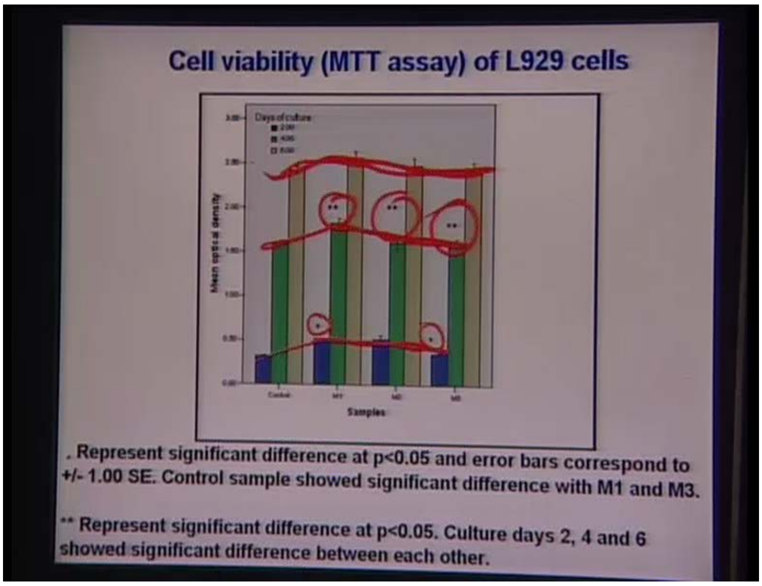

Quantification of the cell viability was carried out using entity as say and then statistical analysis was also carried out at using (( )) analysis at p less than 0.05 level.

Now, we have done this cell culture test for the 2 days, 4 days and 6 days - the different time scale. Now, these blue ones have for the 2 days culture and you see that there is not much difference, only thing this is statistically significant that is statistically significant for M1 and M3 samples. For 4 days the green bars actually you can see the green bars also they are double studies they are, and then 6 days that control samples and that M1, M2, M 3 there are almost like at the similar level. So, essentially all this samples they have similar cell viability as far as the control sample is concerned all the glass ceramics.

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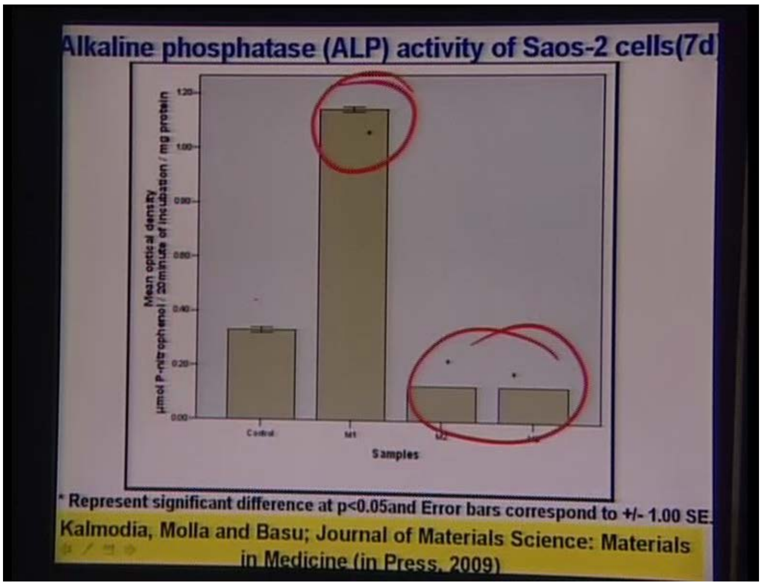

ALP activity that is osteoblast differentiation ability or this is the my phenotype marker of the osteoblast differentiation, and what you see here that again the (( )) analysis has been carried out and demon glass ceramic has the best ALP value, ALP production compare to the M2 and M3 as well as again the control sample.

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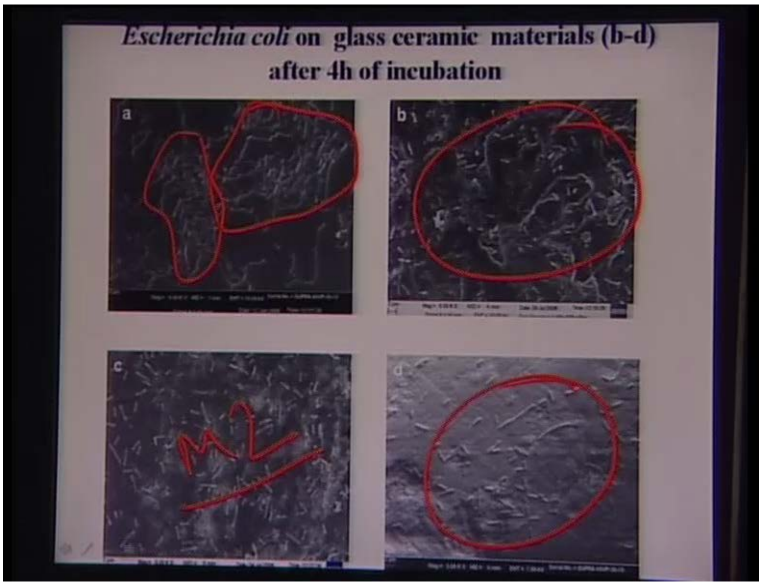

We have done this antimicrobial property evaluation for this glass-ceramics using staphylococcus epidermidis that is a gram positive bacteria. After 4 hours of incubation you can see on the control sample large colony formation. This colony formation is still observed in M1 samples, M2 sample which is lastly reduced and M3 we do not see much of this, see a few that colony forming unit on the material surface.

Similar observation has also been made for this gram negative bacteria that is the E-coli bacteria. This is little bit rod shaped bacteria and you can see that close colony very dense colony is been formed of the E-coli bacteria on the control sample. Now, for the M2 sample, there are this colony formation is much negligible and the M3 is much more reduced, whereas M1 also similar anti microbial properties observed.

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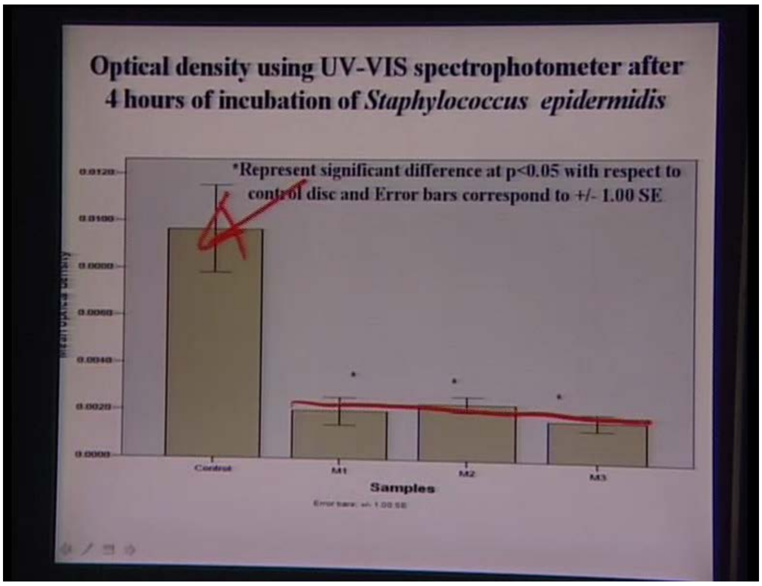

Quantification was carried out using for this staphylococcus epidermidis bacteria using UV-visible spectrophotometry. This is the mean OD value for this control sample, M1, M2, M3 value mean OD value is statistically significantly less compare to the control sample.

So, why these corroborates well with the our SEM observation. Essentially what it saying what it is what it tells you that both all the three glass-ceramic materials M1, M2, M3, they have acceptable level of anti microbial property compare to the control samples.