Biomaterials for Bone Tissue Engineering Applications
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Module 6
Lecture No 31

In this module we will discuss this, uh, Additive Manufacturing of Porous Scaffolds for Bone Tissue Engineering Applications.

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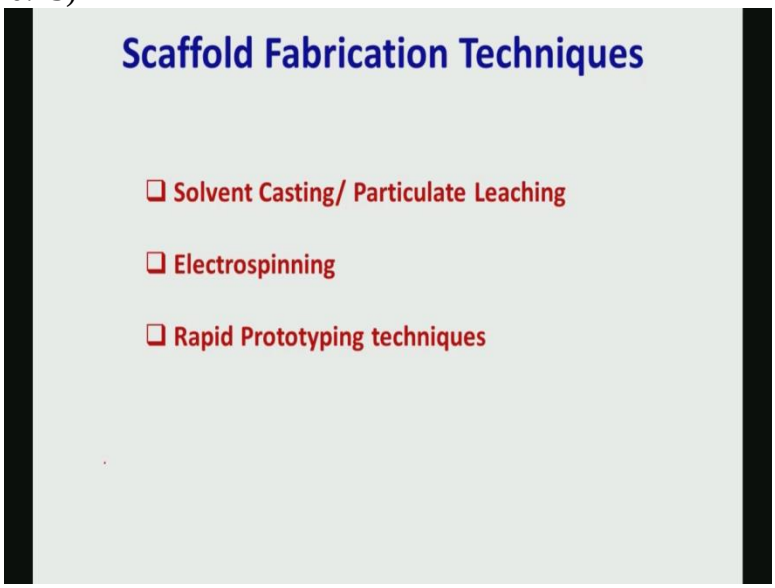

So what, what I will give some brief idea about the what is the different Scaffold Fabrication Techniques that is Solvent Casting and Particulate Leaching, second one is Electrospinning and third one is a Rapid Prototyping Techniques.

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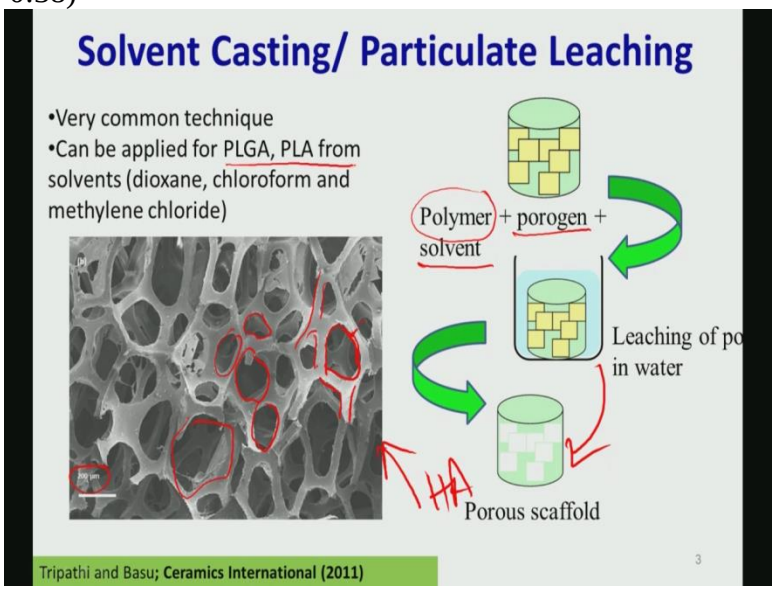

So, the solvent Casting or Particulate Leaching It is a very common technique. What you do here? You start with a particular polymer which is the base polymer. You use some porogen, this porogen can be some sacrificial agent, like, you know, Sugar, or kind of salt crystals, and all NaCl crystals and all. And then after you add this, then you put it in the water and then you see that how this Leaching takes place. And this Leaching is done, then it gives you a Porous Scaffold.

These are typical example of a Porous Scaffolds which is made of the Hydroxyapatite. This is from our own research as you can see these pores that is forming here these pores are of different size. And if you look at cancellous bone structure, which is highly porous, there the also pore morphology is not uniformly identical for all the pores, but there is a distribution of the pore size and pore shape into the Scaffold.

And that is what is very typical of any biological origin materials like cancellous bone, for example. The other things you do we do notice here that is called Pore Wall Width or Strat Width. Then again that very nice Strat Width also you can see. That is also That is also different if this is the 200 micron, so this is like, you know, 50 micron or so. Now, this Strat Width is important.

Why Strat Width is important? Because that Strat Width will determine that what is the mechanical strength properties of these materials. How fast this Porous Scaffold will collapse under load? If the Strat Width is very small, then one this Scaffold cannot experience or cannot sustain higher mechanical load. Now, this Solvent Casting and this kind of Scaffold Processing Technique can be applied to different materials like Polylactic Calculic Acid, Polylactic Acid and so on and so forth.

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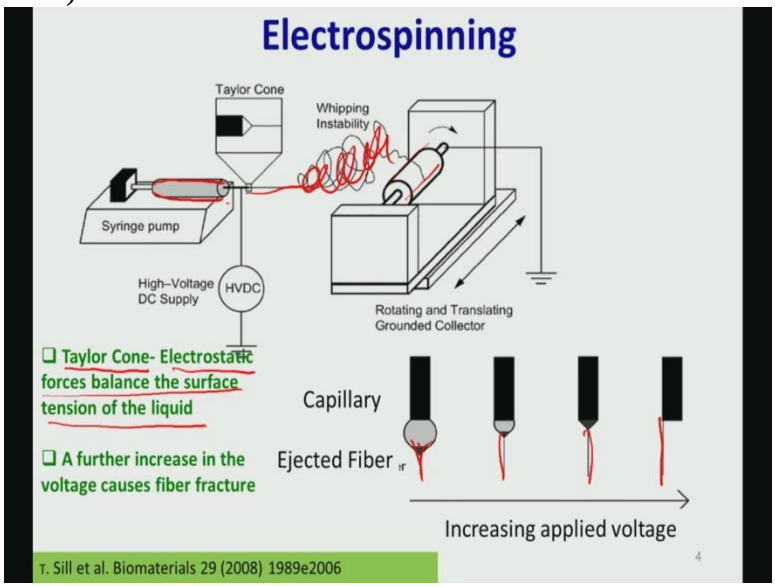

The second one is the Electrospinning Technique. Electrospinning is what? That you take the Polymeric Precursors Solution in a Syringe Pump here. And in a syringe pump, then you make a Taylor Cone, and this Taylor Cone, then you make it this kind of uh then it form that instable uh Tailor Cone, and then that will be uh deposited either on this kind of rotating and translating collector or it can be deposited simply on a on a plate, Receiver Plate.

Now, this is essentially shows that how these capillary forces are important and it forms a cone and this is also with increasing applied voltage like the voltage you essentially you essentially apply between the uh Syringe Pump and the Collector Plate. Now, Tailor Cone formation is a fundamental importance in the process of Electrospinning, and it essentially uh originated from the Electrostatic Force Balance and then Surface Tension of the liquid.

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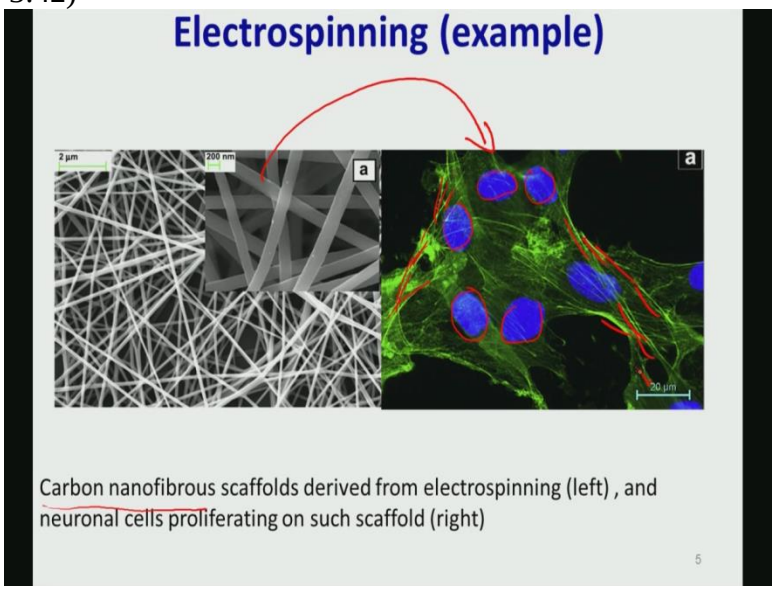

This is some of the examples of the Electro-spun Scaffolds. This is the again from our own research result. These are Carbon Nano fibrous Scaffolds and what you see in the right hand side is that some of the neural cells which have proliferated on this Scaffold. The blue stained region is the darkest stained nucleus of the live cells and the green stained region is that actin and do you see these actins?

They are kind of oriented very nicely on this kind of fibrous scaffolds. And this essentially the fibres will provide certain kind of physical anchorage or fibres will provide some kind of physical structure on which the actins hyto skeleton can reorient and re-organize and can expand on the surface.

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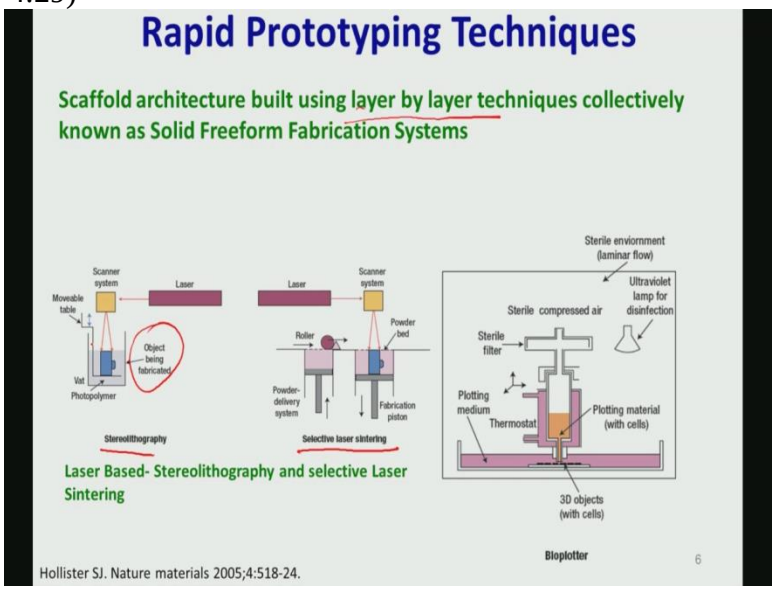

Now, coming back to the Rapid Prototyping Technique, the fundamental definition of Rapid Prototyping must include that layer by layer deposition technique. That is the generic description in whatever Rapid Prototyping Technique that you are using, whether it is for Scaffold fabrication for paramedical application or whether it is for structural applications like a dense component, non-porous component, everywhere these things is used.

Two techniques has been mentioned here. One is a Stereolithography and one is a Selective Laser Sintering. What you see here in the Stereolithography, a laser is focused on scanner system. Then when that object being fabricated here, that you can put it here. And there is a fat and photopolymeric solution is there. And there, your structure would be fabricated in a layer by layer technique.

Second one is a laser Uh Selective Laser Sintering. You start with a powder bed, you focus your laser system here and on the structure. Then powder bed will be moving one layer by one layer and then it will be heated up, then it will be sintered and solidified in this selective Laser-based Sintering Technique.

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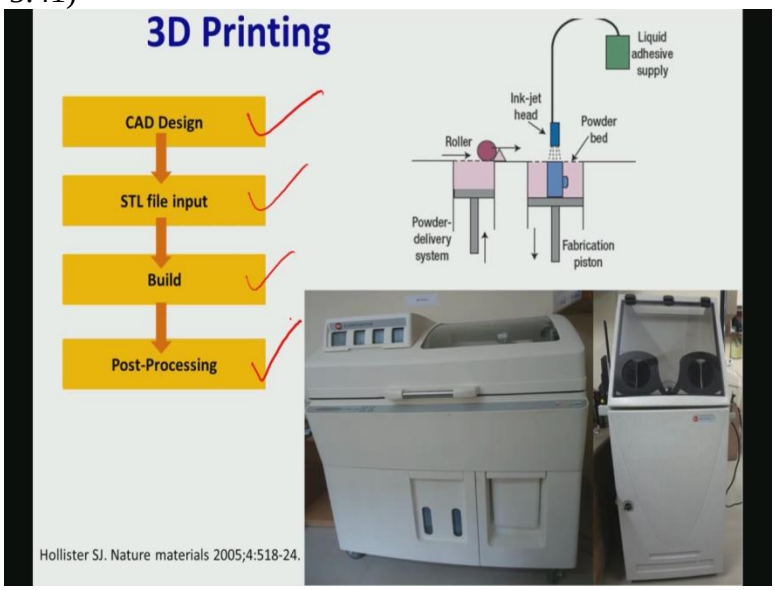

Now, in the 3D Printing Technique, again you start with a CAD Design, so that is computer aided design. You make the Stereolithography file as an input file. Then you have a build Piston and you have a Post-processing, okay? So in the Build Piston and Post-Processing, what you see here that how this structure is being built and then further how it will take a particular concept.

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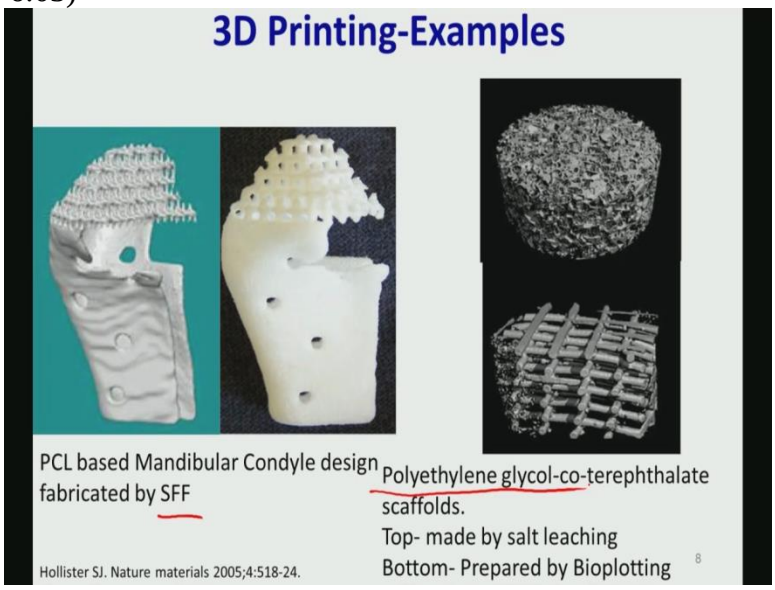

So, this is some of the examples of this 3D printer structure like Polycaprolactone-based Mandibular Condyle Design fabricated by Solid Freeform Fabrication. SFF stands for isolid Freeform Fabrication'. And also PEG Polyethylene Glycol based Scaffolds like Salt leaching and prepared by Bioplotting.

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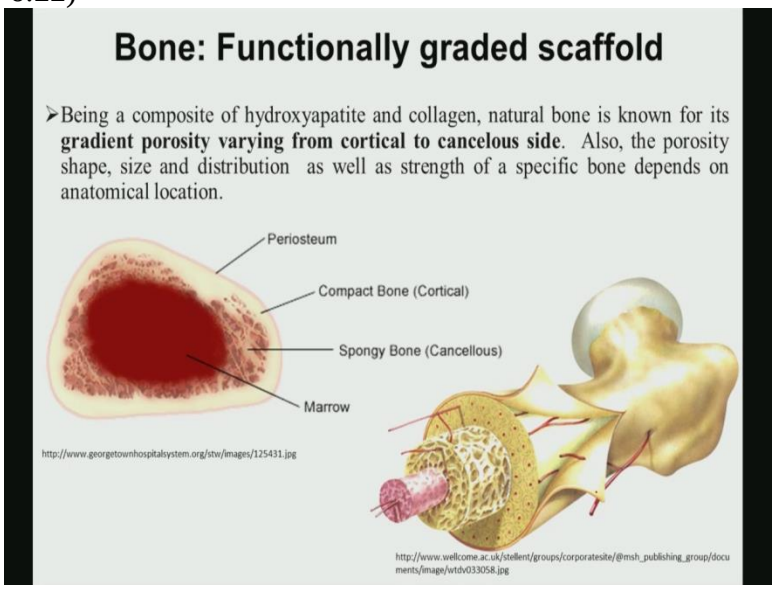

Now, in the context of biomedical applications. Um this additive manufacturing or 3D printing based techniques have received wider attention or have received uh have attracted lot of attention simply because that bone is a natural bio composite which is made of Hydroxyapatite and Collagen, and bone has different varying porosity in the structure. And this gradient porosity varies from cortical to cancellous side.

And this cortical to cancellous side that pore shape, pore size and their distribution as well as strength also depends on what is the anatomical location of the structure. It is very difficult to mimic this kind of gradient porous structure in any artificial, synthetic materials. And this gradient, porous structure is what drives this 3D printing to adopt in case of the tissue engineering application.

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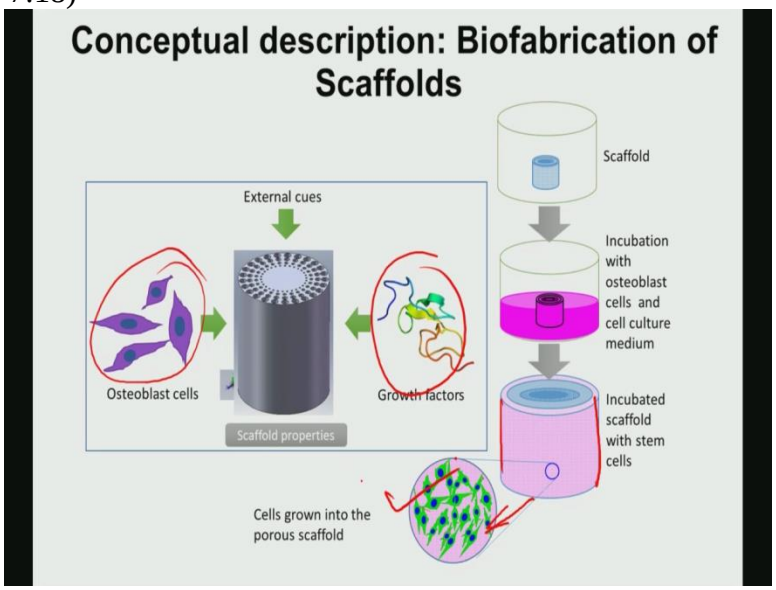

Now in more is the Tissue engineering applications like this is called Biofabrication. Why is the 'Bio'? 'Bio' word is used simply because you are using certain growth factors and then cells, together uh with that material itself from which you are making the Scaffold during the fabrication itself in situ. That means that with the help of growth factors and cells, Osteoblast cells like bone forming cells for bone tissue engineering, now these cells can be any type.

And you give some external cues like you can give some electric field stimulation or some mechanical stresses or you can give some fluid sheer for the cartilage applications or you can give some mechanical stress applications for the bone applications during the uh biofabrication of the scaffolds. So in this unique approach, you can integrate both the cells growth factor so that growth factors can stimulate the cells towards that more functionalized form.

And that is what has been shown here. Once you make these scaffolds, these scaffolds if you can see in two dimension, their cells are also interconnected with each other. So cells are kind of very much integrated into the scaffold structure and that is what is more important.

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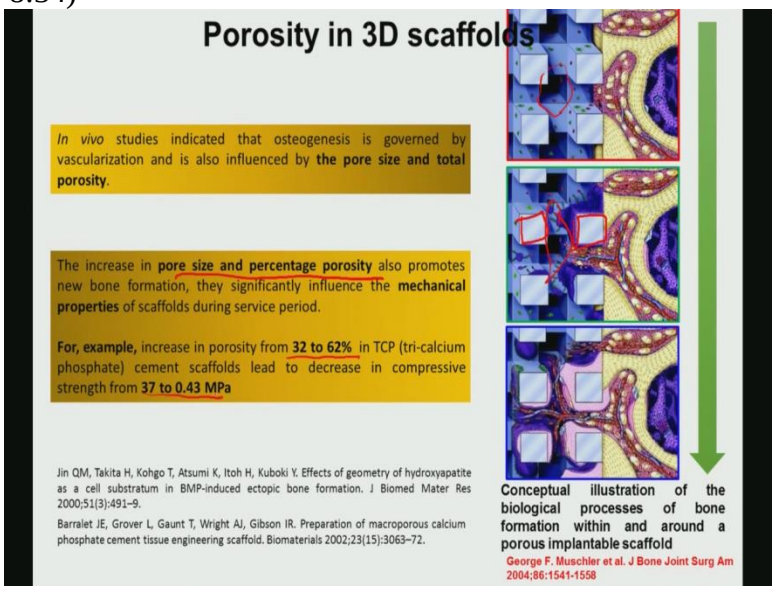

Why porosity is important in the 3D scaffold? If you see that the rectangular strat here, and this rectangular strat, once you gave this scaffolds then you have there is lot of pore spaces in between, right? In this pore spaces what you see is sequentially that as the arrow is going down, that means you are you are essentially culturing them in a 3D bio-reactor. With some angiogenic factor, of certain scaffolds, with the help of angiogenic factor blood vessels they form then get penetrated, they are penetrating into the well into the porous architecture here.

So essentially you are facilitating vascularization process and angiogenesis process together in this 3D scaffolds. But one cannot one cannot arbitrarily change the porosity fraction. For example, the increase in pore size and pores uh percentage porosity, promotes newer formation but at the same time significantly reduce the mechanical properties.

So just to give an example, if the porosity increases from 32 to 62 percent, in one of the implantable bio-serums, that is, tri-calcium phosphate, then your strength goes down order of magnitude from 37 to 0.43 Mega Pascal. So it comes down from 37 Mega Pascal to less than 1 Mega Pascal the porosity increases just double, 32 to 62 percent.

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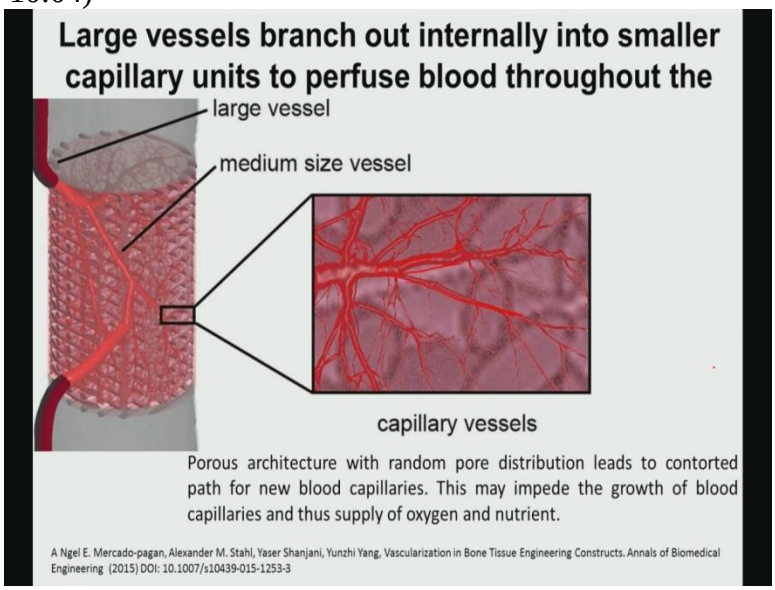

Now this vascularization and angiogenesis process has been more described here, as you can see that vessels that forms, these vessels are also of different size. These vessels are smaller size but some of the vessels are larger size. So you have some primary vessels and you have some secondary vessels which are of much thinner size. So the idea is that by providing different pore size and shape, you are essentially allowing these random distribution of different sized capillary vessels. Because capillary vessels are also will not have simply same size and therefore they need to be accommodated in this vessel structure.

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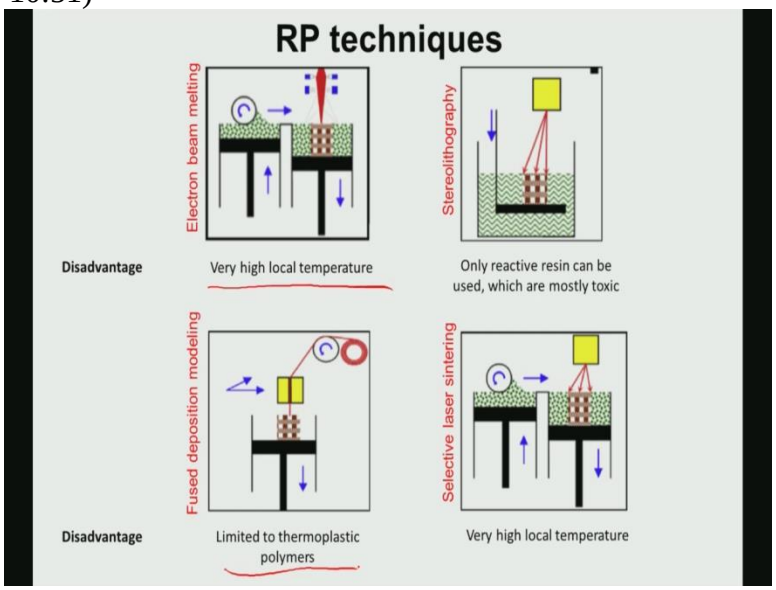

So, before we discuss little bit more specifically on the 3D printing or some of the additive manufacturing technique, let me just show you some of the other competitive techniques, or other competing techniques like Electron Beam Melting, Stereolithography, Fuse Deposition Modelling that is FDM and Selective Laser Sintering, SLS. So, in all these techniques as you can see, that there are certain disadvantages what we had been mentioned for example very high local temperature, or Fuse Deposition Modelling is only limited to thermoplastic polymers and so on.

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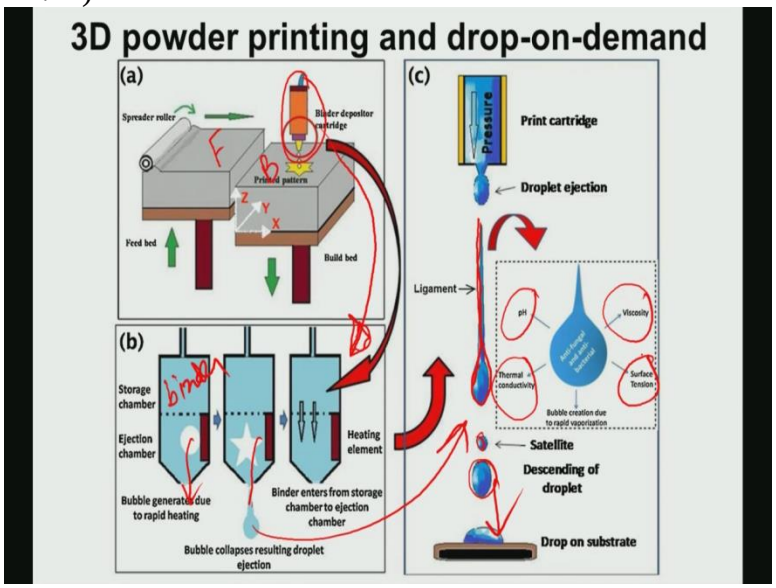

So these kind of techniques and that gives some idea that gives some rationale for the use of 3D printing which essentially uses uh two beds. One is the Feed bed that is the powder bed, so this is called F. And this is called Build bed that is that where the scaffold is fabricated in a layer by layer process. And you see this kind of cartridge and this cartridge if you zoom it up, see that what is happening, here you put your binder, in the cartridge, okay? So this binder it is now forced to get into the orifice.

Now you can see that more expanded part. Once this binder gets out of this orifice, it forms a large tail. And towards the end of the tail uh towards the end of the ligament you you see that it can disintegrate very easily once it is elongated to a larger extent simply because there is two forces that is acting here. One is viscous forces and one is a Surface forces.

Now, therefore, certain Fluid Mechanics terms will also come into picture because of your 3D powder printing. And there it forms a Satellite small drops and these drops will descend and then it will interact. Now what are the factors that which will which will which will actually are important for determining the binder properties? One is the PH, second one is the thermal conductivity, third one is the viscosity and fourth one is the Surface Tension. So these binders, once this droplet is ejected, then it will be integrated into the powder.

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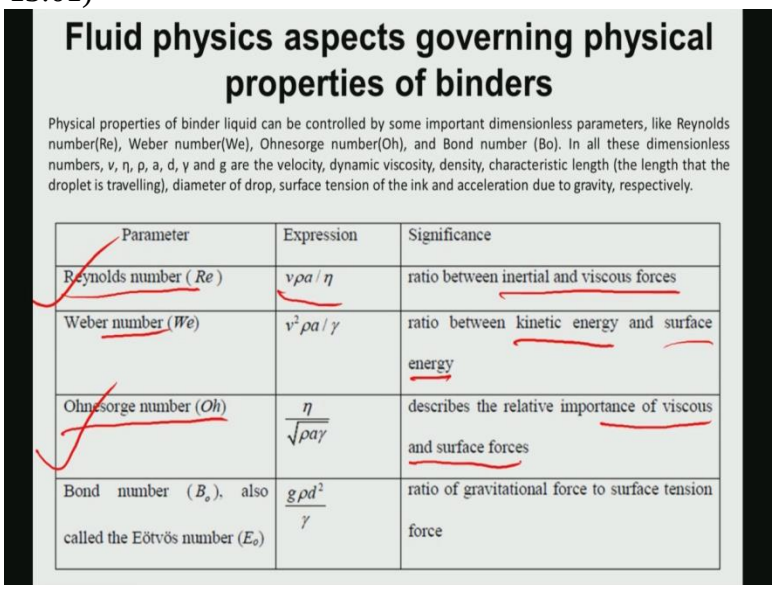

So, from the Fluid Dynamics part, there are certain dimensionless number which most of the mechanical engineers are aware of, that is one is the Reynolds number. And Reynolds number essentially the ratio between inertial and viscous forces. Then there is called Weber number that is essentially ratio between kinetic energy and surface energy. And third one is that Ohne Ohnesorge number. This Ohnesorge number is kind of more important and Reynolds number is equally important as I will show you in the next slide.

So, Ohnesorge number is essentially describe the relative importance of the viscous and surface forces, so Ohnesorge number. And fourth one is the Bond number that is gravitational force to the Surface tension force.

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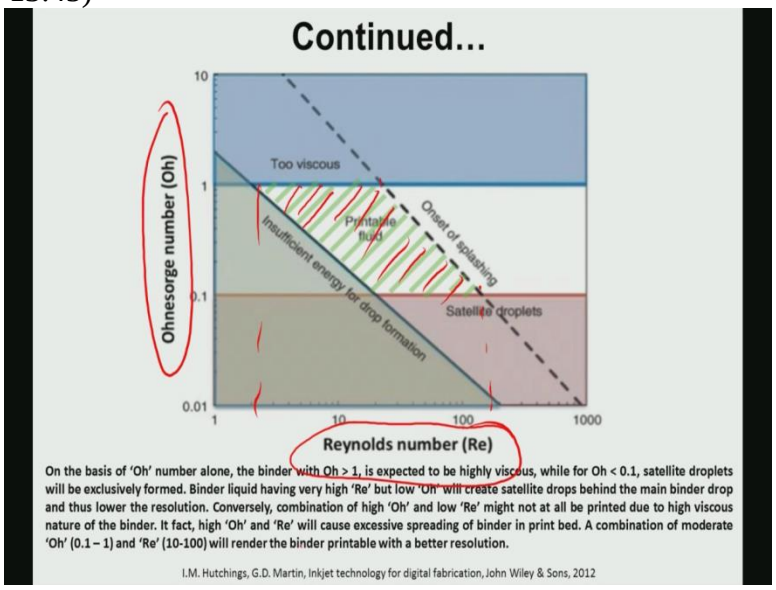

So in this in this particular case, Ohnesorge number once it is plotted against Reynolds number, it will give you certain uh printed printable fluid characteristics. So what I am trying to say that binder properties is very important. A binder which will have an optimum combination of these two dimensionless numbers, one is called Ohnesorge number and one is called Reynolds number those binders can be printed. So that your chemistry or your binder formulation, need to be tailored to that extent so that it will have a particular combination.

For example, Ohnesorge number should vary between 1 to 0.1, whereas uh whereas Reynolds number can vary between a large area between somewhere between 5 to 200 or 300. So, within

that range and this particular area that is that is what is called ‘Printable Fluid’. You cannot arbitrarily select any binder and you do not expect that binder will be optimum for printing that particular material.

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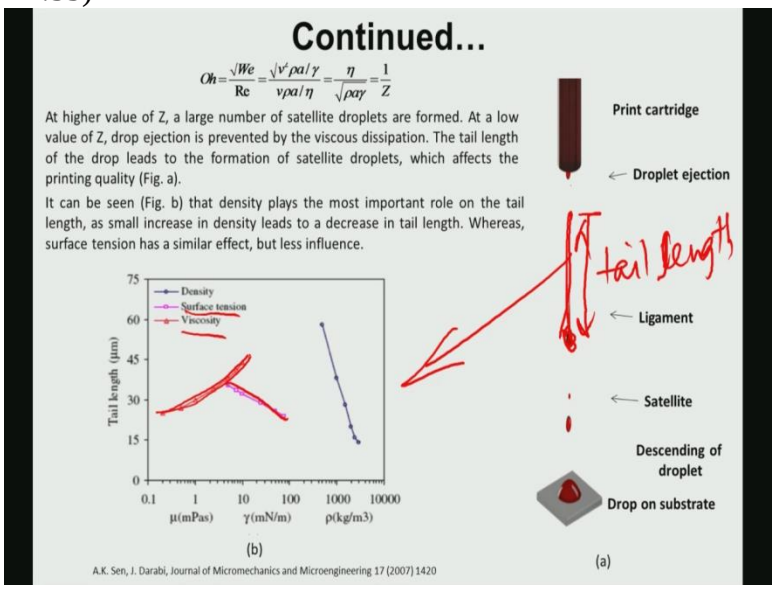

Here again, as I said that this ligament that is formed and then ultimately disintegrated to the drop, this ligament characteristics, what we call ‘Tail length’. So this is your Tail length here. This tail length how it will be dependent on the surface tension forces as as well as that viscosity of the material. And you see that surface tension and viscosity, they have a different, opposing trend. So if you increase the surface tension, then your tail length decreases. If you increase the viscosity, your tail length increases.

So what it means? You need to have a balance between these two competing terms like surface tension and viscosity and that leads to a very printable uh binder formulation.

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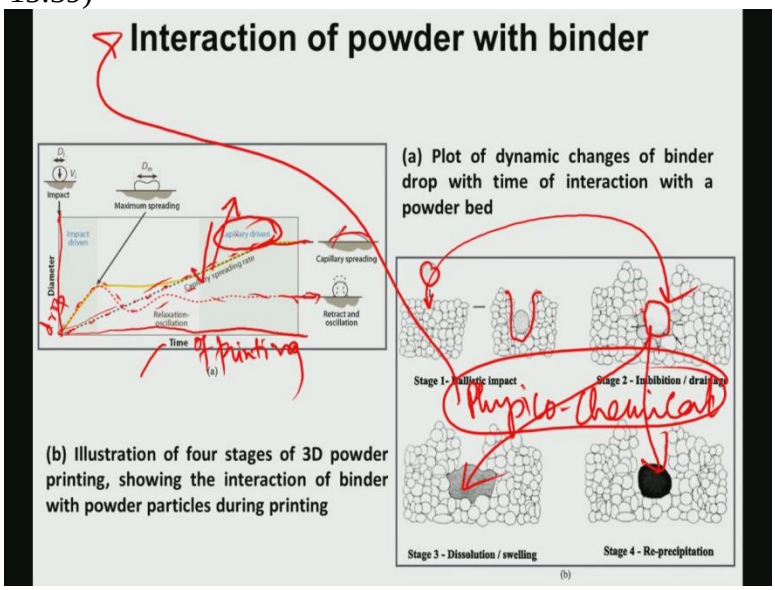

Some physicochemical interaction, physicochemical interaction essentially means that once your drop comes here, and then this is your powder base, the drops first to be wetted. So there should be wettability of the powder with that binder. Once it is wettable, then drops can be integrated into the powder bed and then this will dissolve. So it has to be dissolved like some chemical interaction. So this we call ‘Physico-chemical interaction’, ok?

So this physico-chemical interaction is very important that should go into the slide title. So physico-chemical interaction will lead to the more effective interaction of the powder with the binder.

Now, little bit qualitative description of that. Now, if you have that binder droplet here, if your ‘Droplet diameter’ and if you just this is your ‘Time of Printing’, okay? Along the X-axis. Along Y-axis, this is a drop diameter, binder drop diameter and if you plot it there then what you see here? You see that this kind of different variation and also there is a Capillary Spreading Rate.

So in one case your Capillary Spreading Rate you can see here, and in another case when it is follow this red one, then simply it will retract and oscillate. So it will not give rise to effective interaction. So what it will be interesting for us, whether this is what I have put it ‘right’ mark, so that droplet will change, increase in diameter and as you can see here this droplet will increase in diameter, droplet will increase in diameter.

Once drop will increase in diameter, that will give rise to more capillary-driven process. And this capillary driven process will be more useful for effective interaction of the binder with that of the powder bed.

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This entire thing has been summarised in a recent uh review paper which is published somewhere this year in Materials Science and Engineering R.

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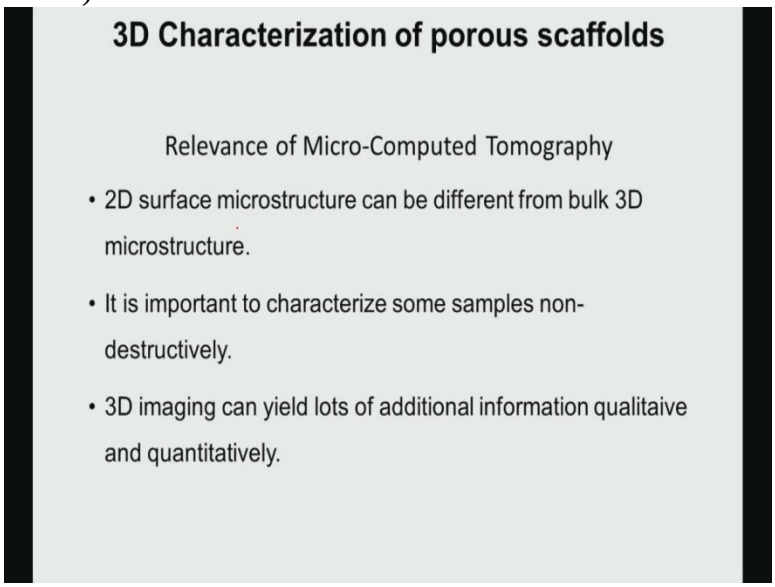

Now this once you make this 3D Scaffold, the next level of interaction come, next level of challenge comes that how you characterise this 3D fold scaffold? One of the one of the uh characterization tool is called Micro-computed Tomography and this Micro-Computer Tomography, you we have this very large facility in Indian Institute of Science, uh which is from Versa XRM-400.

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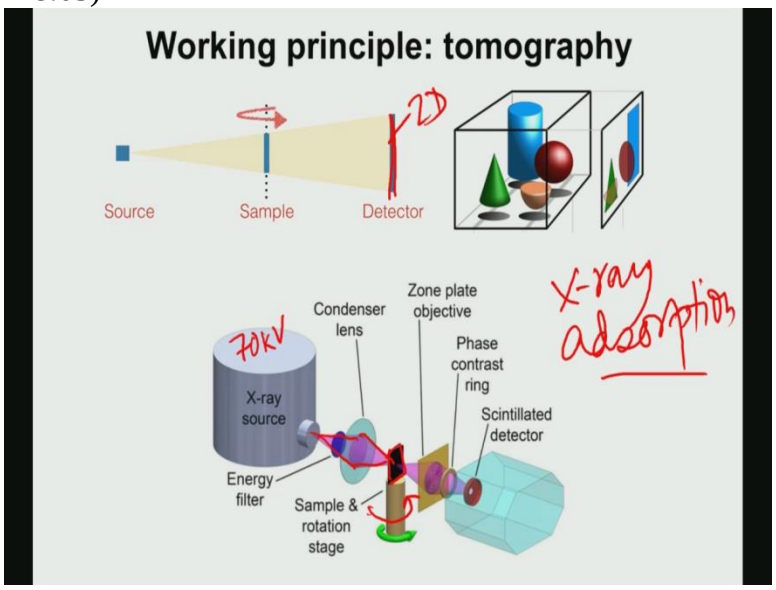

So essentially this Micro-Computer Tomography involves very high energy X-ray beam. Like X-ray beam here the energy is around 70 KV, just to give you some numbers. And typically if you

do that X-ray Diffraction Technique, in the X-ray Diffraction Technique it is essentially the accelerating voltage of the x-ray beam is around 20 Kilo Volt. So here you are using very high energy X-ray beam and then this particular technique is essentially based on X-ray adsorption based technique.

So this is that X-ray adsorption based technique, this and then you have a 3D Scaffold here. And then it comes this sample and this is rotated in 360 degree, this scaffold will be rotated in 360 degree. So each time that X-ray beam will come, it will make at the detector some 2D slice.

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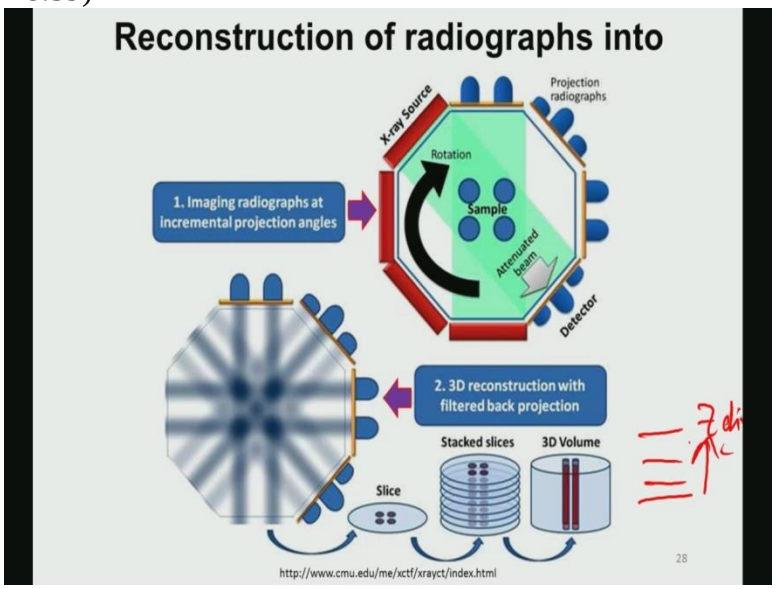

Now these 2D slices, as I am showing in the next slide, these 2D slices, it can be stacked. So several 2D slices can be stacked can be stacked in the Z direction. And once you get into the Z direction, then it will make this total scaffold in these materials.

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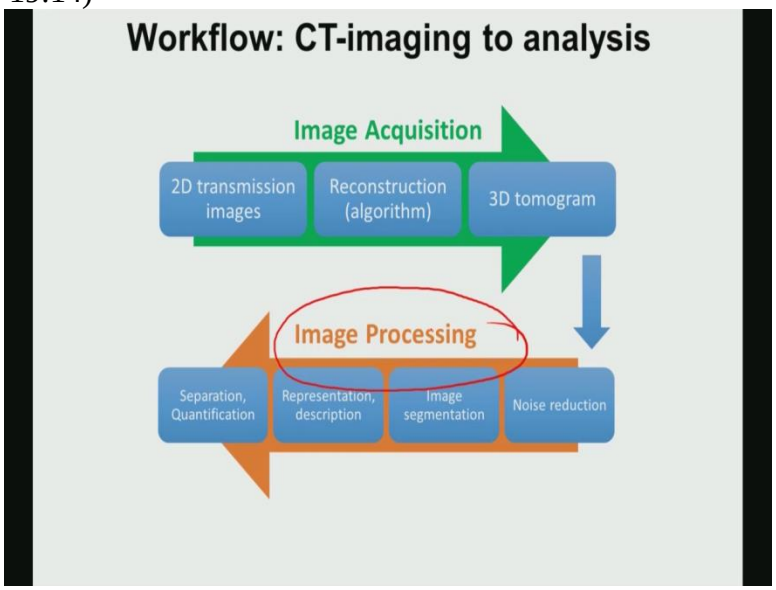

So one of the important things that I must highlight here in the micro CT that image acquisition is not that difficult uh only thing that you have to play around with that is what is that typical accelerating voltage, what is the resolution that you want and so on and so forth. What is much more difficult and I have put it in different colour-- that is 'Image Processing'. Image Processing means how you can get meaningful data out of that Micro CT uh Micro CT Micro CT results and that requires extensive expertise in the Image Processing software and so on.

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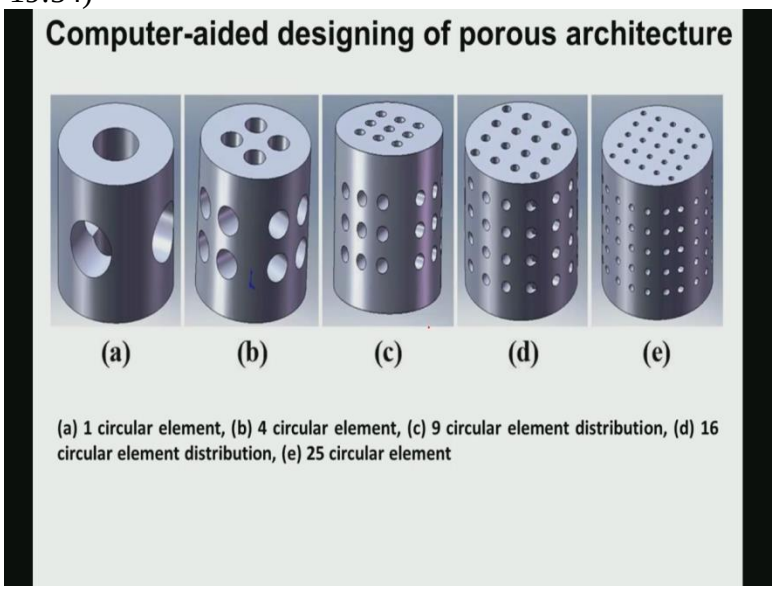

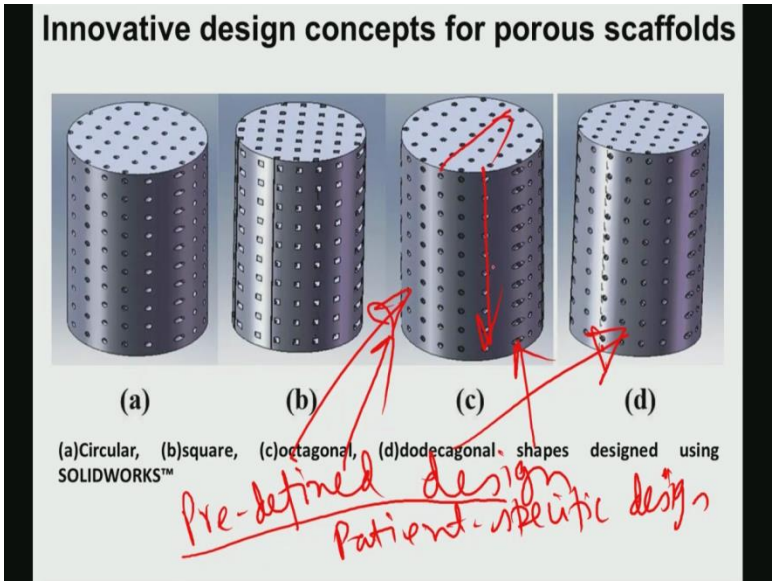

So I will show you some of that uh work from our own group so that you know that how to make these scaffolds in real life. So first thing is that how to how to conceptualize the design design aspect at the beginning. So this design can be whatever pre-defined design or it can be patient-specific CT data. So let me repeat it. So pre-defined design means like it can you can just get it or it can be patient-specific design.

So, patient-specific design means from any part of the diseased part where you want to may where you want to put your implant, so you can take that CT data from that implantation site and

then you can give that STL file to the 3D printer. Alternatively, if you are developing new material and trying to see that whether this new material can be printed using 3D printing, you can use some of the simplistic design files like these ones.

And this is what we call pre-defined design file like you are defining that your design file at the beginning with certain pore shape and certain pore size distribution. So, for example, your dodecagonal shapes is like this, your octagonal pore shape is like this and then you can see that it can be uniform distribution like along this length and along this length, pore size all are same, both along the radial as well as that along the height direction.

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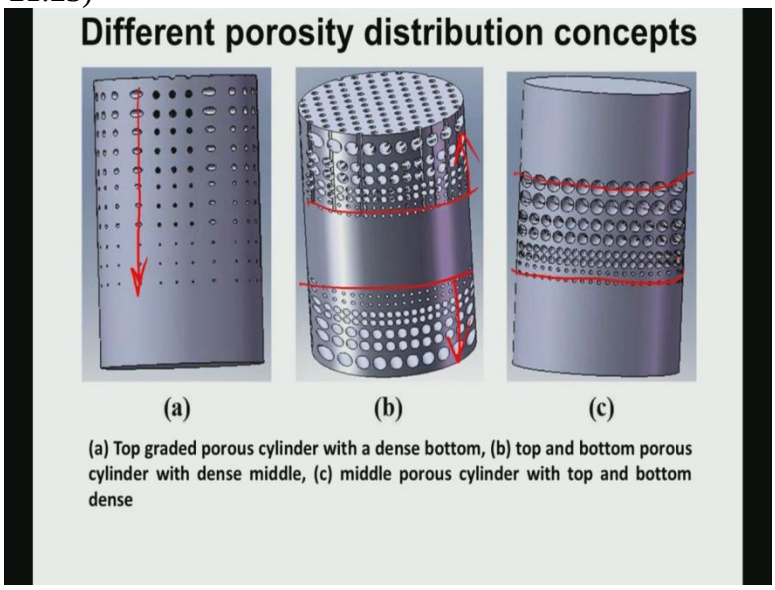

The other thing can be you can also gradient the pore size like along this direction as you see that the pore size decreases as you go along this direction. Or you can have one of the solid structure and one is the porous structure in both the ends. Or one can have a central region is the porous structure. So this is how you can define at the beginning of your 3D printing experiment that what kind of porous structure or porous scaffold that you want in that in this particular applications.

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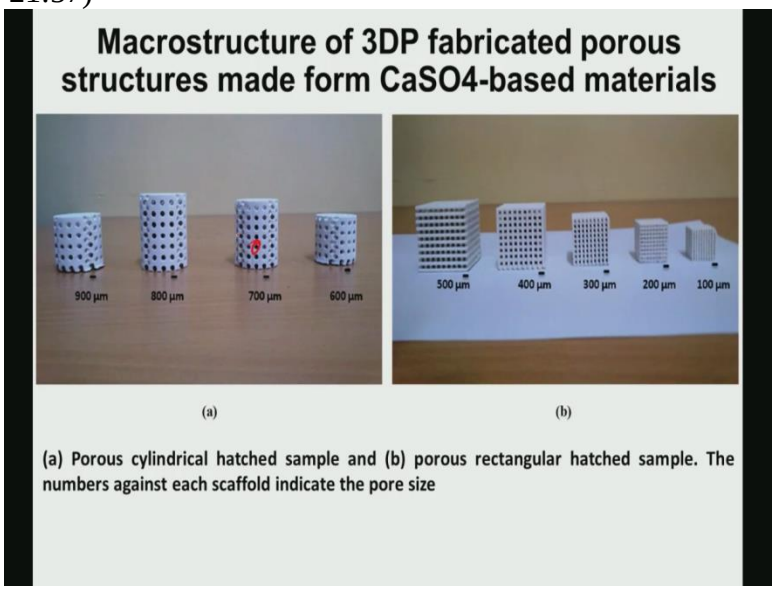

And then you can do some one to one correlation. So one to one correlation means, Suppose this is your input file, what is the output scaffolds that you are getting? So input file your pore size may be 100 micron, your output file your individual pore size can be 90 micron. Or your output file your pore size could be 120 micron. So you are not getting essentially what is the 100 micron pore that you are giving it as an input file, the pore.

Or you can give certain pore to pore distance, this kind of pore to pore distance, you define let us say 50 micron. That is the input file. But in output file, the pore to pore distance might be 40 micron. Because your pore sizes has already increased, so those kind of design to product, that kind of one to one ratio one has to see that how you can translate that.

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Conceptual design of three-dimensional scaffolds of powder-based materials for bone tissue engineering applications

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Abstract

Purpose – The purpose of this paper is to investigate the possibility to construct tissue-engineered bone repair scaffolds with pore size distributions using rapid prototyping techniques.

Design/methodology/approach – The fabrication of porous scaffolds with complex porous architectures represents a major challenge in tissue engineering and the design aspects to mimic complex pore shape as well as spatial distribution of pore sizes of natural hard tissue remain unexplored. In this context, this work aims to evaluate the three-dimensional printing process to study its potential for scaffold fabrication as well as some innovative design of homogeneously porous or gradient porous scaffolds is described and such design has wider implication in the field of bone tissue engineering.

Findings – The present work discusses biomedically relevant various design strategies with spatial/radial gradient in pore sizes as well as with different pore sizes and with different pore geometries.

Originality/value – One of the important implications of the proposed novel design scheme would be the development of porous bioactive biodegradable composites with gradient pore size, porosity, composition and with spatially distributed biochemical stimuli so that stem cells loaded into scaffolds would develop into complex tissues such as those at the bone–cartilage interface.

Keywords Design, Bones, Computer-aided design, 3D, Porous materials, Prototyping

Paper type Research paper

1. Introduction

Tissue engineering covers a broad field that applies the principles of engineering and life sciences to the development of biological scaffolds with the aim of improving tissue or organ functions (Byrne et al., 2008). One of the major challenges in the field of tissue engineering is to design and fabricate complex porous scaffolds to mimic structure and properties of natural bone. Such scaffolds are expected to provide appropriate microenvironments to cells and tissues, both in vitro and in vivo. Numerical simulations of bone growth into scaffolds based on mechanobiological models are relatively new. For example, Sanz-Herrera et al. used a two-dimensional finite element model to analyse bone growth within a scaffold implanted in the femoral condyle of a rabbit. Similarly, another approach proposed by Adachi et al. attempted to model bone regeneration in a unit cell of a three-dimensional (3D) scaffold microstructure. At present, the computer simulations to predict tissue formation use mechano-regulation algorithms that predict how mechanical forces modulate tissue differentiation and bone remodeling (Byrne et al., 2007; Sanz-Herrera et al., 2009; Boccaccio et al., 2011). The fabrication of such designed porous scaffolds in line with the mechanobiological property requirements demands the use of advanced fabrication technique, which can translate design to product.

Rapid prototyping (RP) refers to the layer-by-layer fabrication of 3D physical models or objects directly from computer-aided design (CAD). This additive manufacturing process facilitates the designers to print their ideas in three dimensions, and RP-based techniques can be used for direct production of machine parts or consumer products (Yan et al., 1996). As shown in Figure 1, RP involves several process steps from CAD design to post-processing. RP includes a number of manufacturing techniques and a multitude of experimental technologies in developing 3D architectures. Each technique has specified applications and has its own limitations. Shortly after its development, RP techniques have been used for manufacturing of biomaterials and tissue engineering scaffolds (Liu et al., 2006). Table I summarizes the established RP techniques, commonly used for fabricating 3D scaffolds for medical applications (Rengier et al., 2010).

In the early days, RP was primarily used to produce models and prototype parts and was in the domain of mechanical

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Authors acknowledge the financial support provided by the Department of Biotechnology and Department of Science and Technology, Government of India to carry out the research. The authors gratefully appreciate the critical and thought-provoking comments of the anonymous reviewers. Ramakrishna Vasireddi also acknowledges the help of Dr Alok Kumar in cell culture experiments.

Received 28 April 2013
Revised 2 December 2013
10 March 2014
10 June 2014
Accepted 11 June 2014

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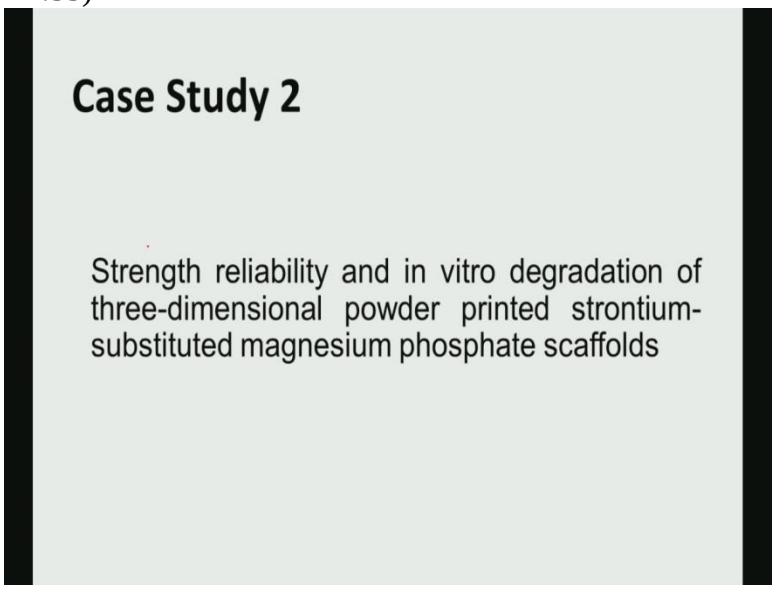

Now before I finish that other things that I want to mention is that another case study says istrontium-substituted Magnesium Phosphate scaffolds'. Now, why Strontium? Strontium is one of the strontium-based drugs like Strontium Ranelate, it is a very well-known drug for treating osteoporosis. So why Magnesium? Magnesium is bio-degradable. So we want to develop some Magnesium Phosphate scaffold, which is bio degradable nature and which also can be used for the osteoporosis treatment.

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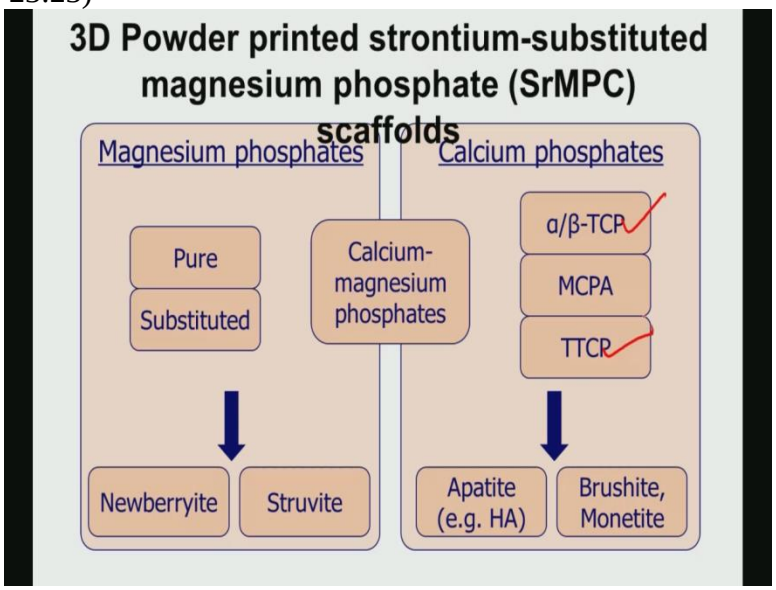

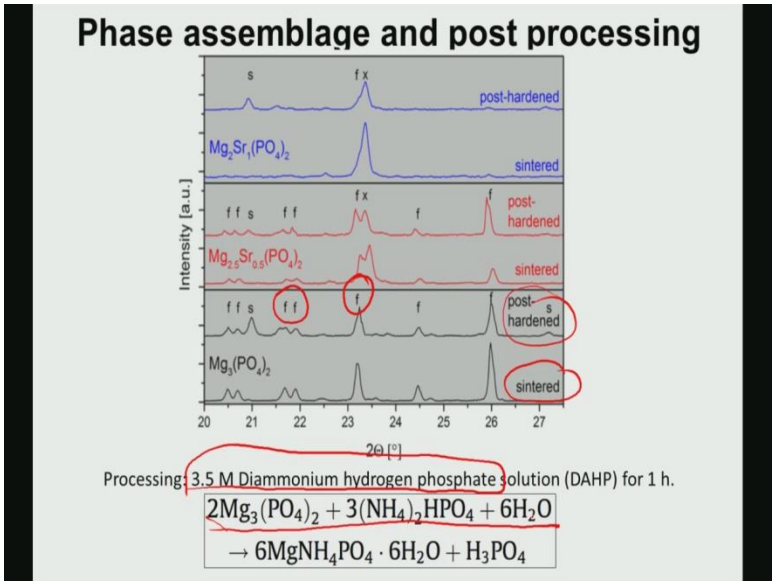

And then we started with the Calcium-Magnesium phosphates, that kind of scaffold and this Calcium-Magnesium Phosphate you have that tetra calcium phosphate, you have Tetra-tri calcium phosphate, you have alpha-beta tri calcium phosphate. Then you give this kind of phosphates, then you do the 3D printing and some post processing technique. And then post processing can be either by done sintering or can be post-hardened.

Now in sintering or post-hardened, depending on what the post hardening treatment essentially means, like you use these kind of 3.5 molar Di-ammonium Hydrogen Phosphate solution and

you immerse this scaffold for one hour. And once you immerse the scaffold for one hour, then after that you do this X-ray diffraction technique, and you use whatever is the phases that is forming that is either two (())(24:11) phase.

And then what is the reaction that is working that is working that is taking place during the um chemical conversion, your Magnesium Phosphate will react to sodium Di-ammonium bi-phosphate and then it gives MGNH4PO4 H2O, that is a product that is a reaction product.

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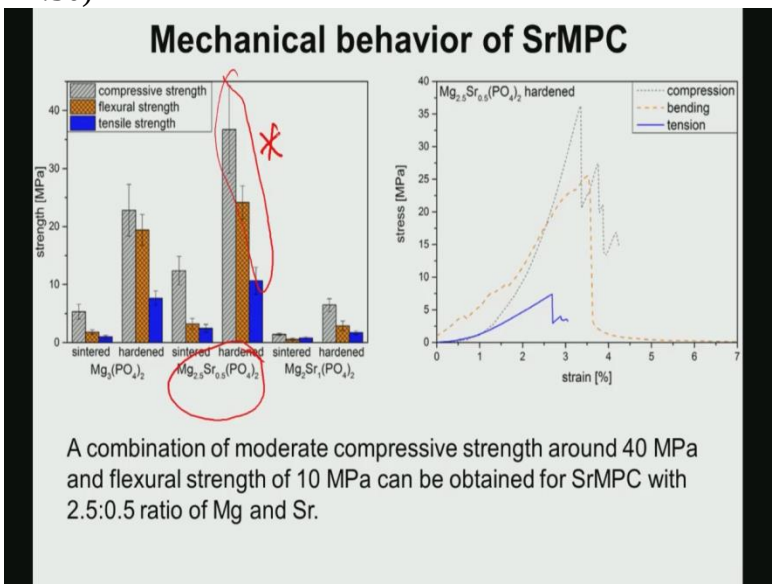

Now depending on whether you are doing sintering or whether you are doing Post Hardening, what you see here you are getting a different strength properties. Now, from our application point of view, this particular scaffold, MG2.5, Strontium 0.5, PO4 whole 2, is essentially Strontium is partially replacing Magnesium, and therefore, it is occupying the Magnesium site.

And then what you see here that the combination of compressing and flexible strength, the tensile strength is better than other ceramic materials here. And this is a typical tensile stress response here.

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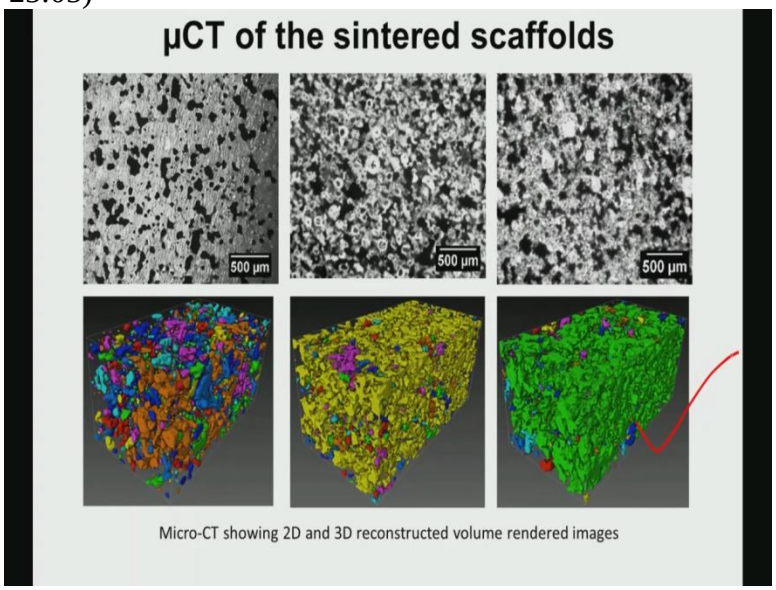

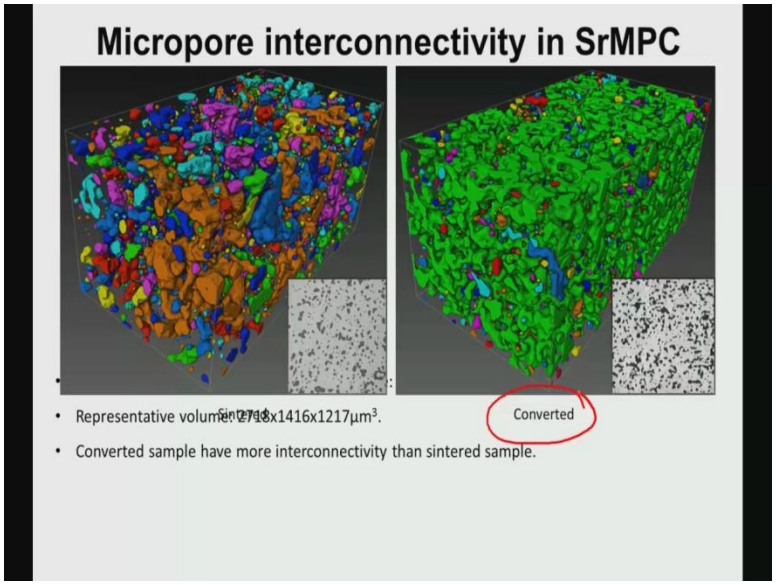

Now this is the micro-computer tomography data what you can see here. That this gives a very this last one gives a very nice, three dimensional porosity distribution in the 3D scaffolds. And this is more clear here, this is a chemically converted, as you can see very clearly that pore distribution is much more clear and why different colour contrast is coming? Different colour contrast is coming simply because these scaffolds have different types of phases, and each of the phase has a different X-ray absorption cross section.

And if your X-ray absorption cross section is different, it will give you different contrasts. And different colour contrasts is basically arises because of that.

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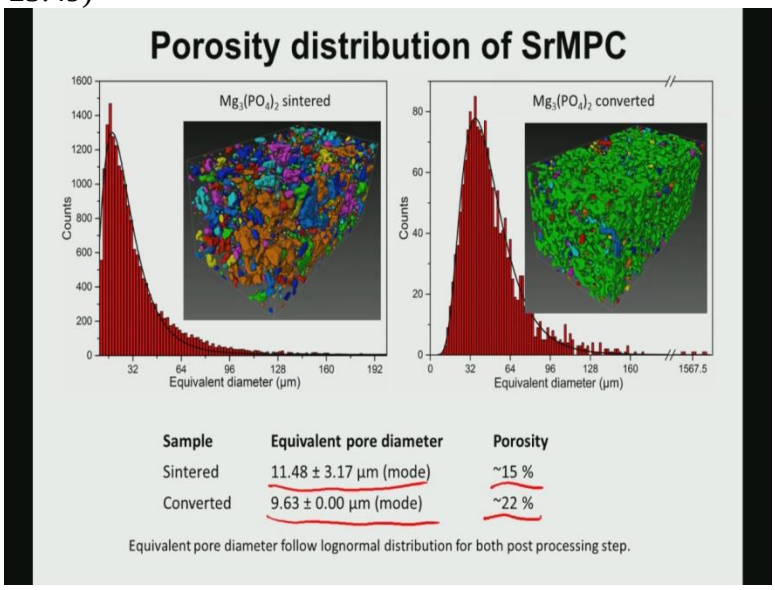

You can do lot of quantification also. Like pore size, pore diameter, like MG3PO42 sintered and converted. And this pore size it can give rise to up to 130 or 140 micron. And as you can see that is the pore size there is not much difference in terms of Sintered and Converted. Only the porosity fraction is different one is 15 percent and one is 22 percent. And in Sintered case it is 11.5 micron, Converted case it is 9.6 micron.

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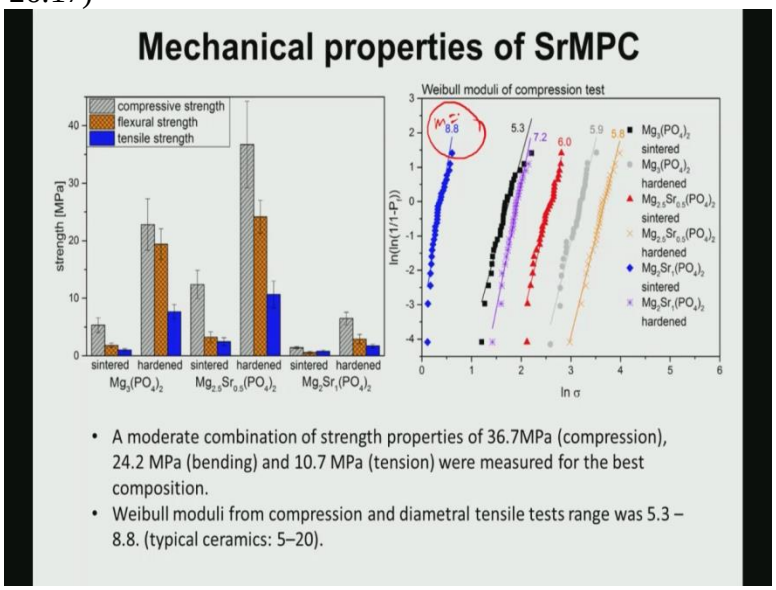

In the terms of Mechanical properties, you can do lot of other interesting things and that is called Weibull Modulus. Like Weibull Modulus means Strength reliability. And you can see the strength reliabilities values that is 'm' values. 'm' is Weibull Modulus, it is around 9 or 5. So these values are very important because additive manufacturing is one of the newer techniques. So here it is important to define what is the strength reliability of ceramics because which is the major concern for other conventionally sintered materials.

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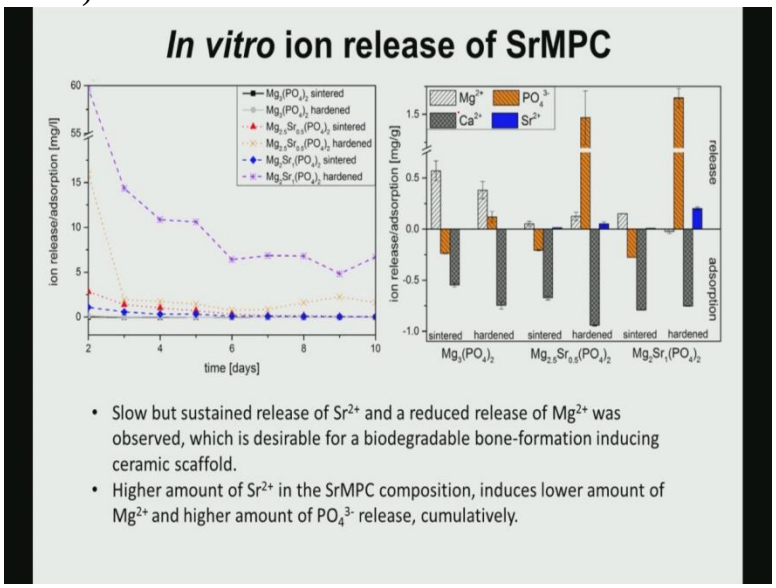

Now, in vitro ion release as I said in the beginning beginning of this particular case study, the strontium ion is expected to be released. And whether this strontium ions is released at all and this is released when this Magnesium 2 SR1PO4 whole 2 is put into the in vitro solution. Let us say it is a physiological medium and this release is important because this is not absorbed, this should be released. And once it is releases, it stimulates the balance between the osteoblast and osteoclast cells in the bone.

So if in kind of osteoporotic bone, your osteoclast cells are more in number than osteoblast cells. So strontium release actually will activate the biophysical processes to strike a balance between osteoblast cells and osteoclast cells.

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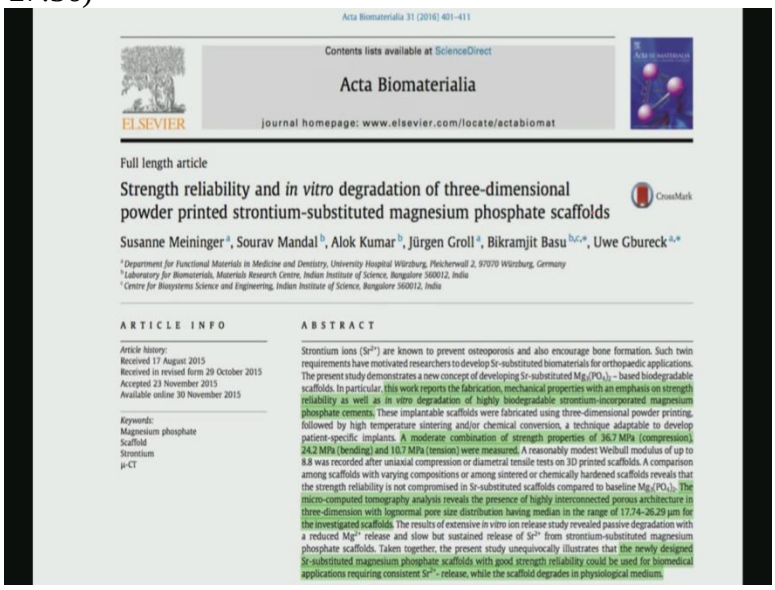

This work is now published in Acta Biomaterialia. And this one can give uh one can see more details and it is published in Acta Biomaterialia in 2016, and this is a collaboration of this work between IISc and a group in Germany. So that is all about this 3D printing of the scaffolds or additive manufacturing of the scaffolds. Thank You.