

Advances in Additive Manufacturing of Materials: Current status and emerging opportunities

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Lecture 59

Welcome to this last lecture of this NPTEL course where I am going to briefly mention the key definitions, key concepts and also major information that I have shared over last several lectures as part of this NPTEL lecture. therefore, the title of this lecture is Summary of Key Concepts in AM. Topics covered include basic definition, overview of AM processes, cellular and lattice structures, process science of major AM processes, some of the illustrative case study and bio(material) ink formulation strategy. one of the fundamental things that we have learnt in the initial one or two lectures is the definition of additive manufacturing. I would like to revisit this definition so that all the students who have registered for this course, they essentially remember this by heart. it is a computer controlled manufacturing process involving layer by layer building of materials that is very important this particular phrase in a predefined manner that means you have already defined the way different layer by layer will be built up as part of the construction of the design solids as per the design CAD model or digital model.

to be constructed leading to creating three-dimensional objects often with complex geometry and topology. I would say mostly with complex geometry and topology. this is the definition that we have learnt in the beginning of this lecture. in the beginning of this NPTEL course.

And most of the key phrases that I have highlighted you can never change this kind of key phrases whenever you are asked to define what is additive manufacturing. Complex geometry and topology that is very important. And you have also learnt as part of the one of the lectures as part of this NPTEL course what is the meaning of topology optimization and how different lattice structures, cellular structures can be constructed, what are the fundamental basis of this lattice structure, cellular structure on which this kind of structures are being built, those things also you have learnt which I am going to also cover that very basic concepts as part of this lecture too. evolution of additive manufacturing if you start from 1950 more than 74 years ago that initially immediately after 1950 continuous inkjet printing was invented. Then between 1960 and 1970 or after 1965 first cell sorter was essentially developed.

Then comes laser printing close to 1970, inkjet printing was one of the first one to be invented close to 1980 by MIT professors. Then after the 1980s, chondrocytes re-differentiate into 3 dimensional environment, chondrocytes is the cartilage cells. That were shown to re-differentiate in a 3 dimensional environment and that raised lot of promise for how 3D microenvironment can influence the cellular functionality. Then comes SLA, stereolithography, which has been quite widely used. Tissue engineering was coined close to 1980 by Bob Langer at MIT.

And this tissue engineering whole concept actually has revolutionized the field of healthcare and this tissue engineering based on the tissue engineering concept, we have also seen that how 3D bioprinting and 4D bioprinting can perhaps revolutionize the field of healthcare as well. Then comes FDM, Fused Deposition

Modeling, then micro contact printing and then protein printing, hydrogels with RGD sequence, this hydrogel is essentially Gelma, gelatin. So, this Gelma we have essentially learnt a lot about Gelma as an hydrogel and then what is the definition of hydrogel I am going to come to that. after few slides in this part of this summary lecture. And then one of the reason that why gelma was quite widely investigated because it has an RGD sequence because that RGD sequence enhances the cell attachment as well. Then between 2000 and 2010 if you see this is the very busy period and why it is very busy period as far as additive manufacturing field is concerned.

Because that is the period you know organ printing was coined or organ printing as one most impactful aspect of the 3D bioprinting that was essentially envisaged. Then comes robotic dispensing of cell laden hydrogels like you know how robotically their dispensation process can be manipulated. Then comes bio laser printing of cells first in vivo use of AM, additive manufacturing cell containing gels by SLA. And then inkjet printing of cells, laser direct writing of cells, tissue engineering scaffold by FDM. these decades of this additive manufacturing has also generated significant interest in the scientific community and that has led to the establishment of International Society for Biomanufacturing. They have their regular scientific conferences, also strong and bioactive journals.

There is a specialized journal nowadays available in bioprinting, biofabrication and so on. And as I mentioned as part of this NPTEL course, every month hundreds of papers are being published across the scientific disciplines in different journals. Whether it is related to the biomaterials, whether it is related to tissue engineering, every journal if you open you will find quite significant number of papers are being published in this area. all these things essentially reflect tremendous enthusiasm in the scientific community on the advances and progresses being made in the field of additive manufacturing. Now, why additive manufacturing is so important and this has been and what is the rationale for additive manufacturing that I have discussed at length with respect to the conventional manufacturing.

Conventional manufacturing like metal casting for example and this particular slide essentially shows the consideration of cost versus complexity and what you see here. this essentially straight line it is for the metal printing. metal printing you know the cost per part almost remains the same. in terms of the more and more complexity that your structure has. it is independent of the complexity.

Now if you follow this dotted lines that essentially traces the traditional manufacturing. in case of traditional manufacturing which is either machining based or drilling, turning, so it is essentially subtractive manufacturing. You remove the material while shaping the material to your desired design and shape. essentially this kind of complex parts when you try to manufacture using subtractive manufacturing processes, what you see that there is a progressive increase in the cost of the materials, cost of the part. therefore the key message is that when it is a low volume complex part manufacturing you see that this particular simple parts are cost effective to traditionally low volume manufacturing.

simple parts are very cost effective to fabricate traditionally, whereas complex parts are more cost effective to do 3D printing. And typically, when in the industry also people are trying to manufacture very complex parts, there 3D printing is much more cost effective. Now, right hand particular plot what you see estimated cost of the unit part and then here you see that what is the material, what is the design, 3D printing setup and printing and what is the post processing, how the cost is increased. post processing this is referred to particularly for the metal printing, when this is the finishing cost or post processing cost is perhaps the highest. in case of the metal printing and it is almost like 5 to 6 times higher than for example design cost or material cost.

material cost, design cost, setup cost, printing cost if you add them together, post printing, post processing cost even still is higher than that. You are talking about the unit part manufacturing the major message is that 3DP can manufacture implants with complex geometries and now we will see that what kind of complex geometries we are talking about, in order to substantiate this statement what I have been showing that here this is the FDA approved designs. for some of the metallic implants. these are like FD approved design. it is not that every additive manufactured parts with the complex to complex geometries can be used as implants for human healthcare.

there are certain FDA approved designs for example, this is called corpectomy cage, this is more for the neural implants, there is ALIF, the cervical spine truss, this is the osteotomy utility, this is the posterior spine truss, so these are like some of the complex geometry that FD is approved and if it is manufactured in a GMP compliant additive manufacturing machine then this can go through FD approval process provided the part quality is not compromised. And some of the things you can clearly see for example this particular geometry It is very difficult to conventionally manufacture this kind of strut geometry because you know that you can see that this is made up of the different struts as well as the nodes. You learn later as part of the lattice structures or cellular structures how this different combination of struts and nodes can make the structure even complex complex design is one thing. manufacturability is another aspect. it is not necessarily that all complex designs can be manufactured very easily, it also depends on what kind of machine you are using and that is one of the reason that why 3D printing or layer by layer manufacturing process can be pursued in a different type of machines, with different type of precursors or with different type of starting materials.

For example, as you have learnt in this course, you have the metal printing itself, there are different type of printing options, You can either use selective laser sintering melting. You can use directed energy deposition. You can use electron beam melting. If one machine fits all the options then there is not much necessity to invent the other machines. the fact that we are constantly improving on our machine aspect, the fact that we are developing newer ways to manufacture this kind of 3D printed parts that essentially indicates that these particular machines should in principle allow the construction and manufacturing of more and more complex components.

that implant design with intricate geometry is possible only using AM techniques. One of the things early in the course that we have also learned that in the context of the 3D printing of soft materials like gels or hydrogels that there is two terms that one is to distinguish one is called bio-inks and one is called bio-material inks. Biomaterial inks are essentially the inks which does not contain cells. essentially additive manufacturing of biomaterials as inks and on that you can grow the cells like these are cells. Bio-inks are essentially the soft hydrogels or soft materials which necessarily contain biological cells or biological component.

It can be either proteins, growth factors, cells and so on. cells as a mandatory component. bio-inks essentially means it must contain cells. And optional is that combined with materials and then processing with a biofabrication technique. Again, the word biofabrication essentially means layer by layer manufacturing of materials with biological cells, in the context of 3D printing of biometric scaffolds without biological macromolecules or cells are to be called as biometric inks, Now steps involved in this 3D printing, first is the virtual platform.

This is the designing of 3D CAD file, conversion into STL, slicing into 2D planes, this STL file in many of the 3D printing machines like, you know, extrusion 3D printing and all, they can read only on the STL file. And designing the CAD file you know there are several options are available. I have also mentioned throughout this

at some specific time point of this course that what are the different computational platforms one can use to make this CAD file. Then comes 3D printing, layer by layer materials deposition, then comes post processing like removal of support materials and also machining, curing, infiltration and heat treatment. And some points I have shown that how infiltration is done for the ceramic 3D printing like zirconia, how the heat treatment can be pursued for metallic 3D printed structures.

depending on what kind of materials you have also learnt that materials engineering materials can be broadly classified into metal, ceramics, polymers and composites. depending on what kind of materials you are printing this post processing options also can be different. Largely for metals it is heat treatment. For ceramics, it can be infiltration, it can be also sintering and in some cases for the metal 3D printed structures in order to get the exact desired surface roughness or dimensional tolerance, one has to do very limited amount of machining of the 3D printed parts. one of the concepts that I have also mentioned very early on in this NPTEL course is the patient specific 3D bioprinting.

what you see? You get that for example, if you are a very ambitious scientist like you want to do a 3D bioprinting of this functional heart, the way I am mentioning it is not that easy. what you need to do for the patients to which you have to implant the heart or patient who undergoes the heart transplant. you have to get the MRI scan or CT scan, then you can see that what is this CAD model of that patient specific heart. Then you have to choose what is the soft gel materials or hydrogel materials that you want to use along with the cardiomyocyte cells. For example, this is one of the cardiac tissue specific cells and growth factors.

Then you construct the scaffold. with cells using the 3D bioprinting technique either laser based extrusion process, stereolithography or inkjet. You can see that human patient specific heart is being constructed or being printed. And then when the cells are being encapsulated in this 3D extrusion printed gels, then you have to put this in the bioreactor for maturation purpose. And this bioreactor maturation purpose, you can see that what is the final printed tissue. We expect that after this bioreactor maturation that the cells which are being encapsulated in this hydrogels they will have that for example, beating performance like you know if this will beat exactly at the 72 beats per minute that how that human heart functions.

human heart specific functionality needs to be obtained in this final 3D printed tissue. A number of challenges are involved during each step of fabrication to develop patients with fully functional artificial tissues or organs with clinically acceptable results. And this is rationale for the fact that 3D bioprinting is still not used for patient care in clinics in many advanced nations. Biofabrication, what is the definition that we learnt? It is a computer controlled layer by layer manufacturing of complex living and non-living biological products using live cells, biomacromolecules, extracellular matrices and biomaterials as per the design. Now, here comes the key terminology what we learnt in some of the early lectures of this NPTEL course is the computer-aided design.

This is the application of specific software platforms and these software platforms can be very different on computers or workstations for the creation, modification, analysis and optimization of the design of a 3D structure. CA Doutput is often in the form of electronic files for print, machining and other operations. For machining normally we give the G-code, computer controlled programming language which is meant controlled automated machine tools. standard triangle language STL that is essentially describe a raw unstructured triangulated surface. it will essentially define that what is the x, y, z coordinates of any triangle and this triangle you can essentially put it all together then you form a particular structure of material.

of the unit normal and vertices is ordered by the right hand rule using the triangles using 3D Cartesian coordinates like x, y, z coordinates. And as I said before G-code is the code that any CNC machine knows how to read, how to interpret and how to essentially implement in machining of any components. what is the thumb rules that we learnt? One is the vertex rule. Vertex rule is that so this is 1, 2, 3, 4 triangles 4 triangles essentially will meet at a point. Each triangle must share 2 vertices with its neighbouring triangles.

for example this is not a correct configuration. because what you see that must share 2 vertices with its neighboring triangle. if you see between this triangle, top triangle, this triangle the way I am tracing and bottom one of the triangle these particular vertices only one vertices are shared . only two vertices are shared. here again these two vertices are being shared but between these two triangle this triangle and this triangle.

2 vertices are not shared. whereas in this particular case, central vertices are shared by all 4 triangles. Orientation rule, the direction of the normal should point outwards. This is the correct orientation. All positive octant rule, this is also one of the rule that is clearly followed and that must be followed in constructing the STL file. The coordinates of the triangle vertices must all be positive.

What are the computational platforms? This has been mentioned in little bit more details like SOLIDWORKS. This is a software that is used, AutoCAD, Autodesk Fusion, Rhinoceros 3D, nTOP. These are the computational platforms which are used for engineering structures. And in case of biomedical implant designing, mostly in many hospitals or some of the bioengineers, they use Mimics and 3-matics. Out of that I must mention 3-matics is less explored compared to mimics and this is most widely explored, SOLIDWORKS.

cellular lattice structures, this is some of the most creative design philosophies that were used to make this kind of a cellular and lattice structures. How the cellular structures are defined? It is a solid materials containing high volume fraction of pores. this high volume fraction of pores if you see that key requirement is a combination of material and space like this is your material strut and these are like pores. you can see this is the unit cell strut and node And repetition, unit cell is repeated in 3 dimensional space and here I have also taught you some of the additional concepts which are used in material science in constructing the crystal structure for example unit cell.

for example lattice and motif. this very definition of lattice and motif and unit cell were taught to you so that you could understand this concept of cellular structure and lattice structures much better and particularly this kind of additional concepts were taught whether it is in the context of how the crystalline structure is made in materials or whether it is a material characterization, how you can characterize the materials or how to measure the properties of these materials or how to visualize the three-dimensional structure of the materials, those are additional things were taught in this particular NPTEL course which make this NPTEL course very sustainable and also and to be understood by students from a broader engineering and scientific community. Because I believe that this course is taken by students from mechanical engineering, from material science, from biotechnology, from biomedical engineering, from bioengineering and maybe perhaps from chemical engineering. this kind of broad disciplines, they may not necessarily come with the concepts, with the knowledge of the concepts from deep into the material science, deep into the engineering design. this course will ideally suit for all of them. Then I have also mentioned about the different examples of the lattice structure like Schwarz-Diamond structures, Schoen-Gyroid structure and Neovius structure.

Here one of the main concepts that was taught to you is the TPMS, Triply Periodic Minimal Surface. That means surface is minimal and it is a triply periodic and these are characterized by having the smallest possible surface

area while filling a given volume in 3 dimensions without any self-intersections. one of the concept that has been kind of quite extensively discussed is the Voronoi diagram in the way when I was teaching, when I covered the lattice structure and cellular structure. what is the Voronoi diagram? If we recall, so this is a random distribution of the seeds within a design domain like, so this is your design domain.

or people say ROI, region of interest and so on. you consider the random distribution. Then you start connecting these points to form a triangle. this is another triangle.

This is another triangle. you can make this triangle. Then there is something called Delauney triangulation, then comes identification of the bisector lines. you take each of the sides of a triangle, there is a bisector lines And these bisector lines must meet at a point. Then you consider that point and these particular points then you start connecting? what are the bisector points? And then you can get this kind of a structure. and this is called Voronoi tessellation. it is a mathematical logic with the key features of the nearest adjacency and skillfully reflect many expression features.

It is expressed in both 2-dimensional and 3-dimensional space. Topology optimization is being taught as a mathematical method that specially optimizes the distribution of material within a defined domain like size, shape and weight by fulfilling given constraints previously established and minimizing a predefined cost function. you have a CAD model, you have a topology optimization for additive manufacturing. If you have FEM design verification, then you do additive manufacturing and do mechanical and material verification. In the overview of the different additive manufacturing processes, some of the processes were highlighted like powder-based fusion process, selected laser melting, directed energy deposition, inkjet printing and also binder jet printing so on, right.

selective laser melting and direct energy deposition were actually given more importance and emphasized primarily to give the more scientific case study which are based on the experimental study carried out at my group. Then comes 3D binder jet printing of Ti6Al4V and here what I have mentioned is that it is a polymerisable ink and this polymerisable ink must satisfy certain physical parameters like viscosity, surface tension and more importantly some of the dimensionless number like Reynolds number, Ohnesorge number and what we learnt That is a combination of this Ohnesorge number if it satisfies that particular ink can be used to 3D binder jet printing. And some of the printing parameters were printing speed, resolution, layer thickness, powder deposition modes. Then I have shown you using two different kind of binders. One is the starch paste binder and one is called in-situ polymerizable binder and they And simple geometrical calculations we can see how the binder material interactions take place and particularly in-situ polymerizable binder how this binder essentially will undergo in-situ polymerization that will trap the powder particles in an effective manner.

We have given two case studies on the Ti6Al-4V and I have shown that one can get strength reliability which is quite good at around 8.1 considering the fact that these binder jet 3D printed materials have 28% porosity. and 98.4% interconnectivity of the pores. although it is a metallic materials that makes the materials more prone to failure and therefore weakest link fracture statistics were adopted and then I have shown you that how experimentally we have done this analysis and Therefore, these materials have Weibull modulus which is close to that of the engineering ceramics because most of the engineering ceramics like whether you consider alumina, zirconia, silicon carbide and all they are Weibull modulus or strength reliability or M value is typically 3 to 10.

Now laser based additive manufacturing process, one of the things that we learnt is the powder characteristics, whether it is a spherical shape or whether it is irregular shape that is important. Then comes laser characteristics.

Laser characteristics means how this power distribution whether P_d is equal to small s capital P , f is the fraction of the laser power that is being absorbed and that brings us to the next parameter called laser powder interactions. And when you consider the laser powder interactions, I have shown how this mass balance, energy balance and momentum balance, all these 3 kind of parameters were essentially considered to get into a very, very complex mathematical formulation or mathematical framework to describe laser based additive manufacturing process. What you have learnt this laser beam is being essentially concentrated on a powder bed and the molten pool there is a Marangoni convection, heat conduction and heat radiation all these things that take place and this is a complex combination of all these 3 phases of all these 3 contributions that were captured in their highlighted mathematical framework.

Now metal 3D printed parts actually from the structural point of view in many cases they are not the ideal parts to be used in many engineering applications unless they are inspected, unless the proper quality is maintained because these parts often have different kind of a different kind of defects, for example, lack of fusion or balling. This is like two majorly occurring defects and also there is a failure due to the residual stresses because it is a very high intensity laser beam which is being concentrated. Keyhole means when the laser beam penetrates very asymmetrically deep into the surface and in this particular pool if there is a material which is vaporized then it is keyhole with porosity that will be one of the defects that will be generated. Now coming to the rheological aspects it is the modulus like you know that we have learnt this rheometer. parallel rheometer and in this parallel rheometer one can measure G prime.

this is the G star that is the modulus, the overall resistance to the deformation of material. One is called G prime, one is called G double prime. G prime is the storage modulus, G double prime is a loss modulus or viscous modulus. The $\tan \delta$ is the loss factor that is nothing but the ratio of G double prime to G prime. Why it is important because when you moved from metal printing to the soft hydrogel printing then what we have learnt in case of the soft hydrogel we have to tune the G prime, G double prime, viscoelastic modulus, storage modulus, viscosity and then shear thinning properties and so on.

those properties are very important in the soft hydrogel. Now shear thinning and thixotropic properties, these are also equally important and what we learnt is that η is equal to $k \dot{\gamma}^{n-1}$ and here you can see η is the viscosity of the fluid, k is the flow consistency index and $\dot{\gamma}$ is the shear rate, it should be $\dot{\gamma}$. Now, when $n < 1$, then it is a pseudo plastic flow. When n is equal to 1, it is a Newtonian flow. If n is equal to 1, that means η is equal to k , viscosity is a constant.

It is a Newtonian flow. And when n is greater than 1, then it is a shear thickening behaviour. what you see here it is a shear thinning behaviour. And what we have emphasised that it is the shear thinning behaviour it is very important. And all the hydrogels, if they are to be 3D printable and if they are to be 3D printed, shear thinning properties, they must possess the shear thinning properties. That has been emphasized time and again while I have covered various case studies in this particular course. Then comes thixotropic behavior and typically thixotropic behavior is another prerequisite for hydrogel to be 3D printed and what you see here viscosity consistently decreases with time and that is called thixotropic behavior.

opposite to thixotropic it is the rheopectic behavior, the rheopectic material becomes more viscous with increasing time of the applied force. hydrogels are defined as the viscoelastic gel composed of 3D network of one or more hydrophilic polymeric components with ability for significant water retention. And what is the hydrogel, so it is a dry polymeric network, so if you put lot of water molecules, they can absorb, they can swell. The swelling

is one of the major important things in the hydrogel and what you can do in this hydrogel structure, you can cross-link. You can do photo cross-linking, you can do chemical cross-linking like if you immerse the hydrogel structures in calcium chloride, calcium ions essentially can cross-link the hydrogel networks.

next comes is the 3D extrusion printing. extrusion printing the concepts that we have learnt is that essentially a hydrogel or soft gel will undergo or will be forced to be extruded through this particular nozzle under pressure. this is the pressure that is applied. This is a hydrogel based bio ink. I mentioned bio within bracket ink. it can be either biomaterial ink like without cells or it can be with cells that is a bio ink.

some of the properties which are important and that is why I have taken one or two lectures on the rheology itself to define what is viscosity, what is gel strength, what is yield properties, what is shear thinning and post-printing recovery properties. then what is also we have done is that not only printability but also buildability. These are the two things that I have essentially emphasized in several case studies in this particular NPTEL course. In case of bioprinting that has been emphasized that what is the cell fate like whether cells are viable, to what extent cell death takes place and to what extent cell viability is compromised after the 3D bioprinting that was one of the major concern and that has been addressed while discussing several case studies. 4D bioprinting , after 3D printing in the cell encapsulated scaffolds if they will experience some physical stimuli or they are exposed to physical stimuli with this temperature, magnetic field, electric field or light or chemical stimuli for example pH or magnetic stimuli for example enzymes then if there is a change in the shape or functional transformation If the both the shape changes and functional changes is taken place then only you can call this a 4D printing.

essentially you are adding an extra dimension to the 3D printing by exposing the 3D printed or 3D bio printed structures to either physical stimuli or chemical stimuli or to biological stimuli there are 3 different ways that you can do this stimuli. And as a result of that external stimulation, if the shape or functionality is transformed, then you can call it as a 4D bioprinting. And one of the major things in the 4D bioprinting or 3D bioprinting that we have learned, how to cross-link the hydrogels. Whether it can be ionically crosslinked, then it is a calcium chloride solution. It can be UV crosslinked photo hydrogel, then you can use the LAP or you can use that Igacure that is the photo crosslinker and typically EGA cure is used more extensively than LAP, perhaps cost is one of the concern, LAP is much more costly.

LCST or UCST hydrogel. dual cross-linked hydrogels, you can subject the 3D printed structures hydrogels to either to chemical cross-linking first followed by physical cross-linking first followed by chemical cross-linking and then I have shown in one of the case study like where I have shown GGMA that is one of the hydrogels that has been developed as an alternate to Gelma. how these hydrogels when they will undergo dual crosslinking, how their properties are different compared to if they undergo only physical crosslinking or chemical crosslinking. The next crosslinking approach that has been discussed is enzymatic catalyzed crosslinks and crystallization crosslinked hydrogels that is the crystallization region. And mechanism of dual crosslinking as I mentioned that you know that if you take this GGMA, the gelatin glycidyl methacrylate, then you can use igacure, you can use the UV at 365 nanometer, you can get the crosslink structure, in the context of GGMA, you can essentially, this is the chem draw design structures, you can use essentially the dual cross-linking. Now, if you do that alginate, alginate essentially helps in that gelatin glycidal methacrylate alginate GGMA structures.

If you add this alginate then you can use the calcium chloride as a cross-linker and this is so called egg box kind of model that you can essentially generate by using the dual cross-linking methods. And you can see that from 7.5% GGMA to going to 7.5% GGMA, 2% alginate, 0.5% oxymethyl cellulose and if you do the dual cross

leaking the gel stability is much better.

Now collagen versus gelatin and this what you learn gelatin is a denatured form of collagen. Hydrogel definition I have already mentioned and multiple times in the NPTEL lecture I have emphasized that hydrogel definition. Gelatin is one of the widely used materials in hydrogels for tissue engineering biofabrication. animal tissue you first get whether it is a porcine source, whether it is a cow, pig or fish, any of the three animals for the particular fish scale, when you get first the collagen, then you denature, you form that gelatin. And then from gelatin, you can essentially further do heat treatment or enzyme treatment, you can get collagen peptide.

one of the things that you learn that how to modify the hydrogel structures by adding some secondary cross linker, adding viscosity modifier. And in this context you have learned that if you use carboxymethyl cellulose CMC which is this kind of a structure and if you do and if you add them to the gelatine methacrylate you can see that this is the gel stability is not good. If you do this 1% modified carboxymethyl cellulose, gel stability is better. But if you do that 5% Gelma, that is a very low concentration gelatin methacrylate, you add this 2% modified carboxymethyl cellulose, then you can get very good gel strength. And this the modification the signatures of this modification one can use using nuclear magnetic resonance spectroscopy NMR and as you can see this is the area where it can get the signatures of this modifications of this gel ma.

And this is that complete chemical formulation aspect that what we discussed when you do UV crosslinking, how that UV crosslinking from Gelma to Gelma mCMC essentially make the crosslink structure more stable, much stronger compared to uncrosslinked structures. other things that we have learnt is that how this scaffold printability and buildability, they are essentially modified by changing this 5% Gelma, this low concentration of Gelma. And low concentration of Gelma is much less investigated compared to larger concentration of Gelma. And you can see that 5% Gelma, it is not good at all. But while if you do this larger concentration 5% Gelma, 1% modified methyl acrylate CMC and hydroxyapatite, the scaffold buildability is much better.

And this is what you have learnt that this is a Gelma, it is not good. To some extent it is okay but I will put a double tick just to show that how buildability is improved and what is the number of layers is 45 layers. Printing speed of 10 millimeter per second and layer height is 0.3 millimeter. What we learnt more fundamental point is that in case of 3D bioprinting, it is important for you to recognize that whether we can retain and whether we can use the similar 3D extrusion printing parameters. Encapsulate and use it to print cell encapsulated hydrogels and still maintain good cell viability, still maintain good printed resolution, still maintain good buildability of the 3D printed structures.

That is the major challenge And also major challenge is to retain the cell viability to the maximum level. to maintain the cell functionality to the highest level in the 3D bioprinted structures. Thank you.