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

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Welcome back to this last series of lectures on challenges and opportunities in additive manufacturing. We have discussed quite significantly the 3D and 4D bioprinting and not only the concepts but also several scientific case studies to demonstrate the fact that how to design the bio ink and then how to assess the printability and buildability relevant hydrogel properties. And also I have shown our experimental results to illustrate that you know how the cell viability can be compromised to a modest extent in the 3D bioprinted scaffolds. I will refresh your mind with some of those basic concepts first. This will be followed by the scientific insight as how the cell viability is compromised in the 3D or 4D bioprinted constructs. And then I will give one such example as the stresses that are generated or stresses that are experienced by cells.

when being encapsulated in the hydrogel and also undergoes extrusion printing for example. Then what are the clinical translation challenges? And then fourth one, I will be showing you the results of the biocompatibility study of the 3D printed scaffolds. Essentially to show these printed scaffolds when they are implanted into the animal model what are the responses of the tissues to the 3D printed scaffolds. if you recall this particular slide which has been shown to you before that tissue engineering conventionally is defined as a interdisciplinary discipline which integrates the concepts and ideas from biomaterial science and also biological sciences and medicine.

For example, this is a tissue engineered scaffolds which is being prepared using the biomaterials and or hydrogels or the macromolecular network structure or kind of a 3D printed structures. Now, these scaffolds when you will incorporate the cells and growth factors and bioactive molecules, you essentially are giving the cells the appropriate microenvironment not only for the sustenance for their viability but also for their functionality modulation. And in many of the examples I have shown also in this part of this study, part of this course that you can also incorporate functional nanoparticles. idea is there that you know these particular nanoparticles when they are incorporated into these tissue engineer scaffolds and then they essentially they are being incubated in a bioreactor system, 3D bioreactor system. this is your 3D bioreactor system.

then you will essentially allow the tissue maturation to take place. And this tissue maturation, this bioreactor systems is essentially for tissue maturation. And then once this tissue maturation is taking place, then you can put these scaffolds in the bone cavity. For example, this is like a segmental bone defect. Now you can also give kind of external stimulation like either mechanical stimulation or electrical stimulation or acoustic stimulation.

Now all these biophysical stimulation has a critical role to play in terms of triggering the tissue maturation process. Now in the context of that what I have just shown you that was the very fundamental premise of the tissue engineering as it has grown from the early work by Bob Langer at MIT and subsequently over by many researchers from Langer's group as well as elsewhere in the world. Now, that what in the context of this particular NPTEL course, what I have also taught you that 3D bioprinting. 3D bioprinting essentially one of the main thing is this bio-ink formulation or biomaterial ink formulation. biomaterial ink and bio ink you remember that fundamental difference is that in case of bio ink you have not only the viscoelastic hydrogel but also it must contains the biological cells or biological macromolecules.

This has been mentioned to all of you multiple times through at different time steps at the different time frame of this course. biomaterial inks you have uncross-linked polymer. and in bio ink it has a cell as a mandatory component. Then it goes to 3D bioprinting and then you can use many of the commercial printer and then this particular 3D bioprinter when you have this hydrogel formulation chemical and physical cross-linking can be done and then after that chemical and physical cross-linking you can grow cells on this particular 3D printed scaffolds. during the in vitro cell culture, essentially cells are grown on a 3D printed scaffolds.

This is like different struts, you can see different struts and this is like cells which are being grown on these scaffolds. Now, when you do bioprinting, so you can see this particular hydrogel, you can see here, this particular hydrogel now contains cells. cell is a mandatory component and then cells embedded and this hydrogen formation and chemical and physical cross-linking same thing is done and you remember this is the chemical cross-linking is typically done by calcium chloride, calcium plus 2 ions that helps in chemical cross-linking or in case of physical cross-linking you use the Igacure as a photo cross-linkable compound, igacure and then you can use that UV, if you expose UV then it will essentially allow the physical cross-linking to take place. Now, when you do again in vitro cell culture here in the 3D bioprinted scaffolds, you essentially expect the cells which are well impregnated or well encapsulated in the scaffolds They will have appropriate cellular microenvironment for cells to essentially grow and also modulate their functionality. you can see that this again these are like hydrogel scaffolds and within hydrogel scaffolds these

cells have impregnated.

Now what is the fundamental difference between 3D bioprinting and 4D bioprinting? Fundamental difference is that 4D bioprinting in the 3D bioprinted scaffolds, cell encapsulated scaffolds, you add additional dimension by giving them the external stimulation like either temperature or magnetic field or electric field or light. what are the main things that we have mentioned in the 4D bioprinting? If you want a process to be termed as a 4D bioprinting, then this kind of stimulation or chemical stimuli like pH changes for example, this kind of stimuli whether it is a chemical, electrical, electric field, magnetic field or thermal or photo stimulation like you know light or biological stimulation like you add some enzymes. essentially you want to see that there is a shape and functional modifications. the shape you know you can clearly see that it has been there is a permanent deformation to the structure of this scaffolds and also there is a shape related transformation and also there is a functional transformation and these particular things it is known as a 4D bioprinting. these are the two things but now I will show you some examples which we have seen before and then I have not explained at that point of time because I thought I am going to cover it towards the end of this course and I am going to show you examples.

For example, that in this 3D bioprinting and 4D bioprinting, when the hydrogel which contains cells or without cells, see one case you call it is a biomaterial ink, another case you can call it is bio ink. this hydrogels this has a viscoelastic property, G prime, G double prime, tan delta. Tan delta is a loss modulus, so G prime is your shear modulus, G double prime is your loss modulus and then you have a viscosity is eta. shear thinning behavior essentially means that when eta, viscosity eta as a function of shear rate gamma dot, it shows a constant decrease and this can be mathematically explained by eta is equal to k gamma dot to the power n-1 and n is the shear thinning coefficient. this is an important property or attributes that all bio 3D or 4D bioprintable hydrogels must have.

Second one is the viscoelastic recovery or thixotropic behavior. Here G prime and G double prime as a function of time should behave essentially going down, going up. in a particular it showed that you know periodic up and down and then G double prime essentially goes in a reverse manner. when G prime essentially goes down G double prime essentially goes up. just a reverse manner.

this is a thixotropic behavior. both these things I will put a star that a biomaterial ink and bio ink must have the attributes, these attributes like thixotropic behavior and shear thinning behavior so that they can be 3D bioprintable. The other thing that I have also mentioned that when I have defined hydrogels, so hydrogels are essentially I reiterate here, hydrogels are essentially viscoelastic gel which is made up of hydrophilic macromolecular polymeric chain. with the ability to retain large amount significantly large amount of water

molecules. that is kind of a fundamental definition of hydrogel.

And viscoelasticity in this particular case you know if you plot the shear stress versus shear rate and if you see this a Newtonian fluid which is a very straight line then you have a dilatant fluid then you have a pseudo plastic fluid and then you have a Bingham plastic. pseudoplastic fluid and dilatant fluid you can see that this kind of shear stress versus shear rate behavior it shows a non-linear behavior whereas Newtonian fluid it shows a very clear linear kind of dependence. The other things that I have also covered when I was covering the fundamental concept of process science of the 3D and 4D bioprinting is how the G prime and G double prime is kind of vary with that of the shear stress. Now if you look at that G prime that you know that it goes down and then there is a flow point. In the yield point is essentially defined when it starts going down or up to some shear stress it is constant.

When it starts going down it is a flow, it is a yield point. essentially the viscoelastic gel yields. they cannot sustain that constant shear modulus such which is real-time component as constant and then it goes and flow point essentially when G double prime starts kind of you know decreasing with increasing shear rate. that is your flow point. this flow point and yield point is very important because when you want to computationally model that hydrogel behavior under stress in the 3D extrusion printing setup, then you can essentially see that all these parameters need to be essentially considered.

while understanding that what is going on to the hydrogel under pressure You know that this particular place you have to place P, P is the pressure. this is the extrusion pressure and under the extrusion pressure how all the physical properties of the hydrogel they vary under pressure and then that means whether shear stress or viscoelastic modulus and with time. time is important because you know you have to store this bioprinted scaffolds over time and then if they will have viscoelastic recovery in terms of thixotropic properties and if the hydrogel does not have the characteristic shear thinning property it cannot be 3D extrusion printed. Now these are the things that if you recall that I have also explained to you in much more details and since in this last series of this concluding lecture it is pertinent that I must revisit some of the fundamentals as a kind of a point of summary. that you recall that what is most important for you to for you to consider as the fundamental take home message at the end of this course and that was the reason although these slides are repeated but believe me these are very very important scientific concepts that you cannot afford to forget at the end of this course.

These critical parameters which influence printability, it is like you know what is the extrusion pressure and then how extrusion pressure typically is a kilo Pascal, it varies up to 40 kilo Pascal and the distance from the separating location in the nozzle and then if you

see that they actually show a linear behavior and then show you know increasing behavior at some point from 20 kilo Pascal onwards. Now printing speed if you look at the line width in the printed structure line width as a function of nozzle feed rate and if you see that line width actually it shows a inverse relationship. So higher the nozzle feed rate the lesser is the line width. Then comes the line width versus printing distance then it shows a constant increase with the line width with the printing distance. Printing angle, now if you change the printing angle to acute angle towards more obtuse angle that is also going to influence the printability.

And you can see that how the gel, hydrogel actually flows when the angle also changed quite a lot. Infill patterns like people they have investigated some researchers have investigated by changing the different infill patterns from 1 mm to up to 4 mm and then that actually shows in the case of 4 mm for example you can very clearly see well structured grids which are formed at the end of the 3D printing which is not very clear when the the infill pattern has a dimension of 1 millimeter. Now what are the different ways that we have learnt to evaluate printability like when a hydrogel ink formulation that whether it is printable or not. in case of the printability one must know that filament must be extruded like this is a very very consistent pattern and you can see this is a consistent and stretched pattern. And then low filament quality means this it becomes like a more and more thick and then if this is a like a extruded as a kind of a drop like pattern that is certainly not good.

Now how to do that? One is that filament diffusion, so in the filament diffusion test you know this is your line width, this is your kind of individual strand, this is the strand with W and this is your ART that is the area within particular 3D printed strands and in between the two strands this is a DL. here you can see clearly in the 3D printed scaffolds this is the W that is the width and this W essentially find out that you know we can find out that what is the filament diffusion here. Now filament collapse test, filament collapse test essentially you have a multiple pillars which are separated by characteristic distance. And if this distance actually increases in proportion like 1, if this 1 is at some unit like it can be 10, the next would be 2 times. So, 1, 2, 3 like you know 1, 2, 3, 4, 5, 10, 20, 30, 40, 50.

and 60 millimeter for example. Now in the perfect case that you know if your filaments which you will be depositing on this particular setup, customized setup as part of the filament collapse test, there should not be any sagging. Filament should be extremely stable. That is the primary thing. And then if there is a sagging area for example if you see this is sagging area this is Atc and what is Aac, Aac is essentially this particular area. that in the filament collapse test this particular constant term CF is calculated by the ratio multiplied by 100.

Third one is the printability factor. Now, if it is a proper gelation you should be able to get

this kind of perfect strut structure. If it is under gelation, you can see there is a shrinking. If it is over gelation, you can see there is a bead formation. in all these cases, you can see that what is the interconnected channels.

If there is interconnected channel, PR should be less than 1. And what is Pr? Pr is nothing but pi by 4 into 1 by C that you have learnt before. Pr is equal to 1, then it is a perfect proper gelation of this particular hydrogel. And if it is over gelation, you can see that is also not nice in terms of this particular, this is also not acceptable form for this particular hydrogel composition. Now, I will give you two such examples.

And one such examples I will give you that I have already told you before is that if you take this Gelma. gelatin methacrylate and then I have mentioned that why Gelma why not gelatin because Gelma has a RGD sequence. Again this scientific case study was introduced to you before and the I have discussed the scientific case study at much deeper extent as part of the one of the earlier lectures. here the purpose is to bring this only very selected 2 or 3 slides to essentially show you one particular results that is the cell viability results and that will make you think that why this kind of a 3D printed or 3D bio printed scaffolds the cell viability is compromised and what could be the specific reason that I am going to explain and then we need to develop along this line how our future scientific understanding to get more insights into the fact that you know that how the cell viability is compromised. gelatin methacrylate gelma we have what we have learnt as part of the earlier scientific case study there is something called carboxymethyl cellulose.

This carboxymethyl cellulose you know you can add directly to gelma but what we have learned that if one can chemically modify the carboxymethyl cellulose by adding the methacrylate anhydride in deionized water while controlling the pH 8 to 9 in the ice water conditions like ice bath in 0 degree Celsius for 2 days then essentially you can introduce this particular functional group and this we call is a methacrylated carboxymethyl cellulose. Now on the right hand side this is that FTIR spectra, you can see this red one is your carboxymethyl cellulose. it has different fingerprints or IR bands. Then you have the blue one is this middle one that is uncross-linked carboxymethyl cellulose. And then you have the top one that is the cross-linked carboxymethyl cellulose.

You do see there is a specific kind of a bands either they evolve or they disappear or their intensity does change due to this particular methacrylate modification. If you look at this particular NMR, nuclear magnetron resonance spectroscopy, in the NMR you can see again this methacrylation, that particular signatures HA, HB and HC that you can see very clearly in this particular shaded region. these are like signatures of methacrylic modifications and that was very useful for us to confirm that indeed methacrylic modifications has taken place. Now, this is 5% gelatin methacrylate with 2% carboxymethyl cellulose. You

certainly can say that this gel is not stable and it certainly is not acceptable quality.

Now, when you simply do 1% modified carboxymethyl cellulose, then if you see this particular video, then you would be able to recognize that this particular gel is much better compared to the 5G2C. Now, when you look at further particularly 5% gelatin methacrylate with 5% gelma with 2% modified methacrylated carboxymethyl cellulose, then you will see, then you will be essentially be convinced that this is indeed the best hydrogel that one can think of in this particular system in terms of the gel strength and gel stability. this is the point I am trying to drive you back home, now these methacrylate modifications, it also when you do this UV cross-linking, organic radical which was introduced in methacrylation that actually helps in the physical cross-linking when you use that Igacure and then UV cross-link. there may be other photo cross-linker, it may be Igacure, it may be LAP or other cross-linker which I have also mentioned to you before. And when you add this methacrylate, this is the mCMC-mCMC cross-linking.

There is also GELMA-mCMC cross-linking. When you add this mCMC cross-linking and you do UV cross-linking, then essentially you can see this particular organic radicals are introduced in this particular case. Now the point that I was trying to emphasize earlier is that when you encapsulate the cells in this particular 3D bioprinted during the 3D bioprinting of the scaffolds. And what you have, what I have shown this is simply live cells. this is the live cells in green is a 5% gelatin methacrylate. This 5G1MC is 1% modified methacrylate carboxyl methyl cellulose.

1H means 1% hydroxyapatite containing hydrogel. This is 5G5P means 5% Gelma, 5% Pegda, 1% modified carboxymethyl cellulose. Then 5G5P1MC1H that is the hydroxyapatite. Now when all these cell encapsulated hydrogels were 3D extrusion printed. under the identical conditions under which those corresponding biomaterial inks also were printed, we recorded there is a decrease in the cell viability.

let me explain to you in little bit more details. for the moment you just forget about this cell encapsulated hydrogels. What I am saying this 5G, 5G1mC, 5G1mC1H, 5G5P1mC, 5G5P1mC1H, these are 5 scaffolds or hydrogel composition or hydrogel biomaterial in compositions were selected based on their combination of strength and ductility or UTS and strain at failure. And those were discussed when I have essentially discussed at length it is part of the scientific case study. Now, I have also shown that time but perhaps I did not get into very significant details.

I have just shown the results. Now, I thought it is most relevant if I can bring back these particular results and explain to you much more details. Now, what I am saying is that those biomaterial inks when they were 3D extrusion printed and if you grow. the human

mesenchymal stem cells for example HMSC stands for human mesenchymal stem cells then we can certainly record most of the cells are viable. Now when you do when we 3D print when you encapsulate these cells. and then you start 3D bioprinting and then grow these cells let us say for few days and then start doing this live staining and then you can see the cell viability varies between 85 to 92%.

what it means? It means that there is a moderate decrease in the cell viability when cells are encapsulated within the hydrogen. This is the point I was trying to make to you. let us accept this experimental results is the right indication that indeed cells experience specific cellular microenvironment where their viability is compromised to moderate extent I am not saying to a drastically it is reduced but certainly it is 85% to 92%. in the biomaterials science community typically we consider anything above 80% is kind of acceptable value as far as the sale viability is concerned. The more the better it is but above anything above 80% is certainly a good number.

Now, I will give you some other, I will get back to you to explain that why the sale viability is compromised, that just wait for couple of minutes, I will show you some more examples. Now, another things that I have also mentioned is that if you do that Gelma and Pegda and the Biomaterial Ink and Bio Ink. this is like a more chemical crosslinking and UV crosslinking basically base things. essentially this is the crosslinked structure. This by doing this crosslinking you can do the crosslinking in situ during the printing itself.

Or you can do cross-linking ex situ after the printing is over. if you do in situ then essentially you allow the hydrogel to get stiffened right due to the cross-linking. And if you do ex situ that means the printed structure will be stiffened after the hydrogel is exposed to UV. this is another thing, this is a Neuro 2a cells when it is being grown or encapsulated in the 7.

5% Gelma. as you know that we have discussed mostly that low Gelma concentration, 7.

5% Gelma, 7.5% Gelma 0.1% carbon nanofiber or 7.5% Gelma, 7.

5% Pegda or 7.5% Gelma, 7.5% Pegda 0.1% carbon nanofiber. or 7.5% Gelma, 7.5% Pegda, 0.5% gillen gum and 0.

1% carbon nanofiber. Now, similar experiments were conducted. Now, first we have optimized the hydrogel biomaterial ink composition based on their basic mechanical properties and also electrical conductivity properties because basic purpose was here to add the carbon nanofiber to enhance the electrical conductivity of the biomaterial ink so that the bio ink becomes or biomaterial ink becomes electroconductive bio ink. Now, when you

grow this Neuro 2a cells into this capsule Neuro 2a cells into this biomaterial ink and then you do 3D extrusion printing, after that you allow the cells to remain viable and then we have seen the viability is kind of varying in the window of 80 to 96 percent. certainly in some of the scaffolds the cell viability is compromised and drops to 80 to 82%. Now question is that why cell viability is compromised? What are the underlying reasons for that? That I am going to explain to you now. When we do this 3D extrusion printing, now what you notice here that this is the schematic illustration that you are applying the pneumatic pressure.

This is the hydrogel so that the red chains are polymeric hydrophilic polymeric component of some composition black one is can be polymeric component another composition yellow one can be another composition . then you can see the cells here this eukaryotic cells truly nucleated cells. when the cell containing the hydrogels when it will experience the pressure now what will happen? way top above this nozzle at a distance much further from the nozzle tip, these cells will experience shear flow dominated behavior. And shear essentially means that is the equal forces in two opposite directions, this will essentially change the cell morphology and cells will experience shear flow, shear stress. towards this nozzle wall at this part again the slipping of the chains will take place of the hydrogels because they are very soft viscoelastic hydrogen.

Now when you go towards the nozzle end what you notice that there is in this particular region there is a more extensional flow. Extensional flow essentially means that these particular hydrogel compositions and these particular cells will essentially being stretched right. at some locations at regions away from the nozzle tip, the cells will experience compressive and shear type of forces and the region close to the nozzle tip the cells will experience extensional force that is under tension. it is very clear that depending on the location, depending on the spatial location of the cells within the hydrogel, the cells can experience either compression and shear or tensile stress.

This is what has been shown here. That what happens to the cells, you know if you have a nucleus and this is a compression and integrins and shear, so how these cell surface receptors will respond. The question that we have not yet addressed and this is something for the future. that is why I have been addressing it towards the end of this lecture. Of course, it is the magnitude and nature of stress field and its impact on cell viability. what you have shown that experimental results the cell viability is compromised to a moderate extent.

why and how this can be linked to the stresses that the cells are experiencing and what is the implication that is optimal nozzle design and what is the printing parameters. if we develop a quantitative understanding of the stresses that are being developed in the cells

when they are encapsulated in the hydrogel formulation or hydrogel hydrogel based biomaterial ink or bio ink then that will allow us to design this nozzle carefully so that these kind of stresses can be minimized number 1 and that will have a very positive impact on the cell viability. that more number of cells and cells are more viable that means you can essentially get a theoretical cell viability some are the 95 to 96% And then this kind of we will also have a full control on the hydrogel viscoelastic properties and also the nozzle through which the cells are being extruded during the 3D extrusion printing. I stop here and then in the next lecture, I am going to start on the scientific case study to demonstrate that in vivo study outcomes. Thank you.