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

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Lecture 33

Process Science: 3D extrusion (Bio)printing/4D Bioprinting

we will continue our discussion on 3D and 4D bioprinting. This is the last slide that I have discussed in the earlier lecture on 4D bioprinting. Let us recapitulate what we learned from this slide. what I mentioned that you have a hydrogel you already know what is hydrogel let us also remember the definitions of a hydrogel. it is a viscoelastic gel with the unique capability of significant water retention and this particular gel also can be cross linked using either chemical cross linker or physical cross linker. this hydrogel which contains so this is a hydrogel and then this is the cells it is extrusion printed in a layer by layer manner and the cells are one of the unique aspects and one of the unique approach that one has to adopt is to retain the cell viability in this scaffold.

Now, how to maintain the cell viability when cells are encapsulated within the scaffold and also under the prevailing stress conditions that the cells will experience during the 3D extrusion printing or other generic 3D printing technique that has to be very carefully considered. because you can get very complex shaped 3D structures of hydrogel but if cells are not alive when they are encapsulated within the scaffold that is no good. therefore, we must maintain the 3D optimal 3D printing parameters so that that cells are live and they also maintain their inherent biological functionality or one can change the functionality the way they desire in case of let us say stem cells. Now in the cell encapsulated scaffolds when they are exposed to physical stimuli like temperature, magnetic field, electric field or light or chemical stimuli or biological stimuli under this exposure to different kind of stimuli if these 3D bio printed scaffolds can change its shape or functions.

Then this particular approach is called 4D bioprinting essentially you are adding one more dimension to 3D bioprinting by providing this external stimulation some of the example, you have this hydrogen scaffolds. Now, if you do dehydration technique, then it shrinks. When it shrinks, it changes its shape. You can see that, this is also case of 4D bioprinting what has been shown. This is like more molecular level or this much more finer description.

You have alginate and you have alginate PDA. this is your top one is your alginate scaffolds. This black one is your alginate PDA scaffolds it is exposed to 808 nanometer radiation and you dehydrate then you also change the scaffold shape and this angle is theta as you can see theta must be more than 280 degree here. it is important that you know in this process of external stimulation. The cell viability is not compromised.

this is the near infrared driven laser assisted 4D bioprinting. essentially this is the laser beam or this is the near infrared 800 nanometer laser beam. it is a bilayered alginate polydopamine PDA cell laden alginate gelma biphasic scaffolds. people have developed independently alginate, people have developed independently gelatin methacrylate. GelMa, so biphasic scaffolds means two phases can be distinguished in the scaffolds and then there is a change in the shape what are the things that one has to remember? There is a shape or functional transformation because of the external stimulation.

strategic design of the bio ink this is one of the important things because this design of the bio ink is at the center of 3D / 4D bioprinting what you see here in the left-hand panel, it is a multi-material bioinks where polymer A and polymer B, they form a copolymer. Then there can be interpenetrating network bio-inks, so you have the network 1 and you have the network 2, this is the interpenetrating network. Third one is a nano composite bio-inks, you have the nanoparticles here, these nanoparticles are embedded within another matrix, so in a subsequent lecture, I will show you that sodium alginate gelatin and nano cellulose. nano cellulose is the nanoparticles which are essentially embedded in the alginate gelatin framework. Fourth generic type is the supramolecular bio-inks, you have a polymer domain and you have a non-covalent bonding domain.

in this polymer domain, this is a backbone chain and this is the non-covalent bonding domain, these are being attached, so they form this supramolecular bio-inks. these are various approaches that bioinks can be designed, they can be formulated using different hydrogel constituents or different polymers and subsequently 3D bioprinting experiments can be pursued. Now cross-linking strategy, so this cross-linking can be done in situ. means during 3D printing itself or ex situ, ex situ means post 3D printing. you have a hydrogel scaffolds you can see this how this hydrogel scaffolds their shape can be deformed and then its shape can be changed.

there is a ionically crosslinked hydrogel. like you know calcium chloride, so Ca plus 2 cations. Then you have a crystallization crosslinked hydrogels. in a specific domain within the hydrogel framework structure, you have the small-small crystalline domain because you recall our discussion in the context of polymer. In the context of polymer cross crystallization essentially means that there is an ordered or localized ordering of atoms in a micro domain within the polymer network structure and those localized domain you can consider is the crystallized domain.

any kind of short range ordering if it evolves during the chemical formulation or processing we can call that the polymer has partly co-crystallized to form crystalline domain. Then you have enzymatic catalyzed or cross-linked hydrogels. Like this is the different type of enzymatic catalysis that which can be added. Example is HRP plus hydrogen peroxide. Then you have a physical cross-link.

this square is the physical cross-links and you have the chemical cross-links. this is a dual cross-linked hydrogen. dual crosslinked hydrogel I will also show you in one of the subsequent lectures like you can use calcium chloride, this is the chemical crosslinking and you can use UV together and then we can see that how dual crosslinked hydrogel they can have better properties. And more traditionally you can have UV cross-linked hydrogel. essentially in the hydrogel structures you need to add photo initiators.

And these photoinitiators is typically EGA cure for example and these photoinitiators when you add and then you expose this hydrogel structure with UV lamp then this crosslinker essentially boosts the properties or it increases the strength. and also makes the structure more compact and as a result you can expect the porosity also decreases. these are the different cross-linking strategy that one can utilize in this hydrogel. Now other aspects in the 3D printing or 3D bioprinting can be one is the single bio ink component. single bio ink component essentially means that you have a hydrogel ink and it will contain the cells.

Now you want this hydrogel scaffold or hydrogel bioinks to be printed in a specific manner like a crisscross kind of situations. this is one layer and this bottom is another layer . then what you see here that the cell laden scaffolds are getting printed in a layer by layer format and Now in order to guide this kind of 3D printed structure formation, you need to have some support ink also. This support ink essentially will print auxiliary layers or additional layers. Within these two layers, this particular layer of 3D bioprinted scaffold will be contained.

this way the supporting essentially are useful. many times the supporting also form a base substrate and on which this particular Bio-Ink 1 or Bio-Ink 2 can be essentially built. third one is the cross linker. you can utilize the let us say this is the one of the bio ink. this bio ink essentially is getting printed on a tissue culture plastics for example and you have a cross linker solution.

synchronously this cross linker solution is also now getting printed and then as a result you can have cross link 3D printed structure. Fourth one can be you have a bio ink and you have a sacrificial ink. Now this at the end of the 3D printing process you need to remove the sacrificial ink so that you can get a unique pattern and this unique pattern will have one layer of the bio ink 1 B1 and then top layer can be bio ink 2. what you learnt here from this particular slide is that you can design this entire 3D bioprinting experiments by involving the use of support layers as well as sacrificial layers. And this is the multi nozzle extrusion bioprinting like more than one nozzle you can change the nozzle during the 3D printing experiments the way you desire as a result you can essentially complete these experiments.

What are the different process parameters involved in extrusion bioprinting? this is the generic description of the 3D printing. You can see that this is the different layers, layer by layer it has been printed. Now, you apply the pressure from the top. And under the pressure your hydrogel will be forced to flow through this particular nozzle. the geological properties are important.

For example, viscosity, gel strength, yield properties, shear thinning and post-printing recover is important. You have the hydrogel based bio ink. And then once it is extrude, then extrudability is also important. Extrudability means like what is the pressure that is required, what is the flow rate, what is the nozzle type, nozzle size, let us say 22 g, 22 gauge, 24 gauge, 26 gauge and so on. And whether this nozzle is plastic nozzle or steel or metallic nozzle.

Then, you have the printing temperature or print bed temperature, you have the nozzle height, printing time, filament stability. All these things are important that will define the printability or extrudability. Then, here comes buildability. Buildability essentially means how many layers of this particular scaffold one can print without any shape distortion and so there must be a limit to which let us say 40 layers, 50 layers. This kind of limit should be there because once you get a very stable shape fidelity structure without any distortion, shape distortion that is what will define the buildability.

And this buildability is to be with respect to the CAD design, what is the infill pattern, what is the cross linking mechanism, layer height, line width, line spacing and platform temperature. Now this is the generic description of 3D printing or biomaterial printing. Then there is also 3D bioprinting and in the 3D bioprinting if you remember in one of the earlier lectures I have defined that what is the difference between biomaterial ink and bio ink. Let me recapitulate those two definitions. Biomaterial ink essentially means for example your hydrogels.

without any cells. we called it as a biomaterial ink. The moment when we use hydrogel in the Hydrogel ink with cells and growth factors we call it as a bio ink. this is the distinction between the biomaterial ink and bio ink. you have a biomaterial ink, you have a viable cell, so these cells when they will under stress like the way it has been shown this is the velocity profile. at the center velocity would be more, at the edge velocity will be less and there will be lot of friction because of the friction between this nozzle wall as well as the hydrogel.

cell suddenly will experience stress. What is predicted here? And perhaps one has to computationally find out that what is the stress profile? What is the stress level? What kind of stress? Is it tensile stress? shear stress. For example, here the cells will experience more like a shear. The moment it goes through this nozzle here it is more tensile or extensional stresses that gel will experience. Now, if you look at these cells, so as I said multiple times in last two lectures, it is important for us to maintain the cell viability.

If the cells under extreme stress in their cellular microenvironment, cell will undergo apoptosis. We do not want the apoptosis to take place. We want the cells to maintain viability and their inherent functionality. So, one has to find out one has to also determine the self-fade processes in the post printing like viability, functionality and tissue maturation. These aspects are also discussed in the last lecture as well.

Now if we go through this particular triangle where bio ink formulation at the centre of the triangle and these three vertices are cellular functionality, biophysical properties and scaffold printability. in one side you want scaffold to be printed to a safe fidelity compliant I use I write this phrases safe fidelity compliant scaffold structure. At the other side, you also want their biophysical properties like mechanical properties, degree of hydration, scaffold degradation also to be uncompromised. And third one is a cellular functionality like cell adhesion, proliferation, differentiation, cytocompatibility, all these attributes are also to be compromised.

also to be maintained. with the description of this slide once again you are in a position to understand that what is the different process parameters and how the process science plays an important role in the 3D extrusion printing of hydrogels. what are the different stress states the cells experience? As I mentioned earlier very briefly, so this is the shear flow dominated behavior and here there is a slipping of the layer. this is the layer, this is the region where you can expect The cells will experience more shear stress and then the way you can see this is a cell this is a normal shell shape and then when it is when it will undergo shear it will change its shape right that has been mentioned. Now when biomaterial inks will flow through the nozzle then there will be extensional flow. and this extensional flow region you can see the cells also will experience more like a compressive force and that has so some part it is a compressive stresses like you know that it is the same force into same directions and also in the extensional flow cells will be experiencing tension so it will be stretched so this region.

we are essentially seeing that. a cell population depending on their position or depending on the space at which they are residing at any instance during the 3D extrusion printing they will experience the compression of shear stress and also tensile forces. Question is that what is the magnitude and nature of this stress field? and its impact on cell viability. Remember that magnitude and nature of stress also would depend on viscoelastic properties of gel and also printing parameters like extrusion pressure for example. What would be the implication? Implication would be that optimal nozzle design and printing parameters. this will allow once you know that what are the kind of stress that cells will experience that will definitely allow you to design your nozzle much more effectively.

interplay of rheological properties, so what we learnt in one of the earlier lectures of the fundamentals of rheology, you know G prime, G double prime and tan delta is equal to G double prime by G prime. viscosity and shear thinning behavior. shear thinning behavior essentially means that if you plot viscosity n (eta) as a function of shear rate, so it should follow that decreasing trend and it can be essentially expressed by this equation n (eta) is equal to k gamma dot to the power n-1. n is essentially shear thinning coefficient. Now there is also another property that I have emphasized while emphasizing the rheological properties is thixotropic behavior.

Now thixotropic essentially how G prime or G double prime is essentially goes down or goes down with that of the time, so thixotropic behavior. you have the shear thinning behavior, so for any hydrogel printing both shear thinning I will put a star and thixotropic behavior is mostly desired. And viscoelastic property has also been kind of emphasized, so shear stress is tau, shear rate is gamma dot and then gamma dot so you have Newtonian fluid which is more or less straight line then you have a pseudo plasticity or dilatant fluid which has been just mentioned on this particular plot. if you plot this G prime and G double prime as a function of shear stress then you can essentially find out that what is the yield point of this viscoelastic fluid and what is the flow point. You have seen that in case of metal 3D printing we are more interested to see that you know how the yield strength or yield stress as well as ultimate tensile strength remain uncompromised.

But here what you see that in the hydrogel is more like viscoelastic properties and also either temperature or shear rate dependent viscosity and G prime and G double prime these are the two properties which play an important role. what are the different critical parameters which will influence the printability? One is the extrusion pressure. if you look at the extrusion pressure this is actually data from published studies and if you look at this extrusion pressure. These are the different kind of stress level 10 kilopascal, 25 kilopascal or 40 kilopascal. This is the different stresses that the hydrogels will experience and under this pressure what you see that this is the pressure here and what is the distance from the separating location.

that when the filaments are being extruded at what distance this single strand filament is broken or in other words what is the length of the stable filament once it is extruded. And if you see that around 30 to 40 kilopascal level their length is almost like 80 to 100 millimetre, so that is very good. Now in terms of printing angle, if this hydrogel is getting printed in a like oblique angle or perpendicular or vertical or in the acute angle, how the hydrogel will be experiencing the stress and shear. What is the printing speed? typically the stability zone has been shown here. this is the line width as a function of nozzle feed rate and that you can see here line width essentially decreases with respect to the nozzle feed rate but this region is the stability region.

Layer height, so line width versus printing distance and you can see this is the sharp corner and this is the more curved corner. this is essentially layer height keeps on increasing with respect to the printing height. Infill patterns are also important. infill pattern like 1 millimeter to 2 millimeter to 4 millimeter infill pattern and you can see how the hydrogels are being printed. evaluation of the printability, first one is the extrudability.

you do not want drop like pattern, You want the swollen or stretched or consistent pattern. The consistent pattern is most important, this high quality filament. This high quality filament structure is the key to the good 3D printability. you can also define that what is the filament diffusion by considering the geometric parameters DL that is the infill pattern length, you have the filament width W and if you consider the psi value here. psi is nothing but A_{rt} by A_{re} divided by A_{rt} into 100% and A_{rt} is defined that is area.

A_{re} is also defined this is smaller area and then W is the width. Filament collapse test perhaps is the most widely used in the academic institutions for the 3D printability evaluation. what you do you have a progressively wider strips. Now, hydrogel is kind of printed on this particular area. FCT tester then you can find out that what is the collapse factor.

CF is nothing but Aac by Atc into 100% what is Aac that has been mentioned and what is Atc that is also being mentioned here. the way this distance between the two pillars that has been mentioned like progressively increasing distance. Then another one is a printability factor. this printability factor also you can see this is the proper gelation of the hydrogel. you get perfect strands In case of overgelation, you can get more like this kind of pattern and this is the undergelation.

I will come back to you with the more examples of the 3D printing and 3D bioprinting when I will also show you that how artificial intelligence and machine learning can be introduced to the field of 3D printing and 3D bioprinting. This is one of the review paper and in this review paper we have essentially discussed the process science as well as current status of the field of Gelma based biomaterial inks. Thank you.