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

Prof. Bikramjit Basu

Materials Research Center, Indian Institute of Science, Bangalore

Lecture 35

3D extrusion printing of Gelatin glycidyl methacrylate/alginate/ nanocellulose-based hydrogel

In this lecture I will continue with the another scientific case study related to 3D extrusion printing of hydrogel scaffolds. And here I will be introducing you to the new hydrogel formulation gelatin glycidyl methacrylate in combination with alginate and to develop this nano composite hydrogel scaffold we have used nano cellulose particles which are embedded in this particular hydrogel scaffolds. this work was done in my research group by a master student Garga Mondal in collaboration with one of the senior PhD students in my group Sulob Roy Chowdhury if I recall that bio ink formulation is at the center of this 3D bioprinting or 3D printing of the hydrogen scaffolds. there are different approaches one can adapt in the bioengineering design. One of the approaches that I have been showing you in last scientific case study as well as this one. is this nanoparticles are embedded in this particularly hybrid hydrogel scaffolds.

Why hybrid? You are using two different type of polymer network structure here. One is that GGMA and Algenate. Algenate is another one. this is the main thing in this strategic biomaterial ink formulation.

Another thing that I will be showing you this particular. part of this slide that is the dual cross-linking approach. dual cross-linking approach means chemical cross-linking followed by UV cross-linking and in this dual cross-linking approach I will show you that how this specific gelatin glycidyl methacrylate, alginate based scaffolds can be cross-linked to provide better combination of properties Then one can achieve only with the use of either UV crosslinking or chemical crosslinking approach. gelatin glyceryl methacrylate it is an in-situ cross linkable biomaterial with cell adhesion sequences and nano cellulose as you have seen in the last lecture that it significantly improves the rheological properties and mechanical properties like compression strength or tensile strength. Alginate is a viscosity modifier that undergoes ionic cross linking.

by addition of alginate you make the hydrogel ionic cross linkable like chemically cross linkable and also it has the cell adhesion sequence like RGD. motivation is to quantitatively analyze the impact of alginate and dual cross linking on the mechanical and biophysical properties of GGMA based composite scaffolds. What are the research questions that were addressed in this work and then that will be also shown in the experimental results in next 20-25 minutes or so are these what are the possible interactions in the 3D network of hydrogel, how do the microstructures vary with dual cross-linking, also that how do the biophysical properties correlate with the microstructure and is this specific hydrogel compatible with the 3D extrusion printing. you have the gelatin structures and you have the GMA structure, glycidal methaacrylate. this is the mer unit of the glycidal methaacrylate.

you have the gelatin is NH2 group and you have this specific functional group in the GMA. you crosslink that in the nucleophilic substitution reactions, SN^2 type of reactions this is very commonly used. the organic chemistry and then you essentially synthesize gelatin glycidal methacrylate structure. you started with this neutral medium synthesis of the GGMA like you started with the PBS buffer, then deionized water, you do dialysis, lyophilization and gelatin glycidal methacrylate form. There are important signatures of this particular GGMA which were recorded using FTIR, Fourier transform infrared spectroscopy, particularly CH stretching band.

CC stretching band and CO stretching band as well as NC stretching then CC bending. both the bending and stretching vibration bands related IR bands were recorded very very clearly on this particular IR results. We have also done this TGA analysis just to show how this materials degrade. if you see gelatin that degrades more or less at significant degradation starts at 300 degree Celsius whereas GGMA this degradation is around 150 degree Celsius as well. apart from FTIR.

One of the most powerful technique that biomaterial scientists use is that NMR, nuclear magnetron resonance spectroscopy and here you can see at 5.5 and 6 this is H_a and Hb these particular peaks are due to the methylene protons from the double bond of glycidal methacrylate residue, this is for the gelatin and this is for the gelatin glycidal methacrylate. You can see it is completely absent gelatin and this thing appears in this one ^{1}HNMR. You have Ha and Hb and around 2, it is much more pronounced here, Hc. from this you can also calculate that what is the degree of substitution.

In this particular case, we have determined the degree of substitution is around 57 percent. Now, when you start with this GGMA, now you start with that photocurable hydrogel using GGMA. you started with Irgacure. Irgacure is a radical initiator or photocross linker, you add it in specific amount. to this GGMA scaffolds, then you can

do photo crosslinking and this photo crosslinking can take place at 365 nanometer wavelength and then also there is a specific intensity.

you have these functional groups radicals and then you have this macromolecular network structure. Now modification of pure gelatin glycidal methacrylate, now this particular GGMA if you change the concentration like 6%, 7.5% and 10% you can see independent of the composition all of them they show shear thinning behavior. shear thinning is n(eta) is equal to k gamma dot to the power n-1. this is your gamma dot, this is your eta.

viscosity decreases with increasing the shear rate and which confirms the shear thinning behavior. and increasing the amount of GGMA the initial viscosity improved significantly. if you go from 6 to 10% that viscosity suddenly increases this is 6 this is 10% GGMA. And now when you add this nano cellulose particles to it, let us say we have taken the medium concentration, let us say 7.

5. this is the concentration and then you add this 0.5% nano cellulose particles to 1 to 2 to 3, you do not see any significant change in the behavior, it almost stresses at the same manner. essentially what you see here that shear thinning behavior is maintained and there is no quantitative variation in terms of the viscosity modification or viscosity changes at very high shear rate. Now, modification of hydrogel with alginate, so independently we have studied that nanocellulose addition to GGMA, nanocellulose addition together with alginate. For example, we maintain the 7.

5% GGMA, so this G stands for GGMA, which we have mentioned before. And what is the alginate percentage that has been added 1 and 2% so 7.5% GGMA 1% alginate 0.5% nano cellulose particles and 2% alginate 1.5% nano cellulose particle.

What you see with respect to 7.5% gelatin GGMA structures the viscosity essentially improves or viscosity improves like viscosity is higher in case of this alginate addition. the role of alginate is a viscosity modifier that has been established using these experimental data that eta is a function of shear rate that has been very clearly mentioned. you have this n value that is shear thinning power law exponent and this n value is kind of 0.

1, 0.21, 0.25, 0.26. rheological properties as I have mentioned a few times that parallel plate rheometer is the main facility that was utilized for measuring the G prime storage modulus, G double prime loss modulus or tan delta that is the loss factor and tan delta is the ratio of the G double prime to G prime. Now what you see here essentially how this G prime and G double prime that varies with shear strain, shear stress or angular frequency.

if you look at this kind of this G prime data, so this is like G double prime. green is also G double prime and this is also G double prime. these datasets are essentially G double prime that is the loss modulus.

These datasets are more storage modulus and you can see storage modulus beyond a shear strain of 1, it decreases,. Whereas the G double prime that actually goes through an increase and then subsequently decrease. The same thing is available here it is the G double prime data and these sets of data is a G prime data. This is G double prime data and these sets of G prime data and you can see that as per the shear stress is concerned up to 1 kilopascal it shows from 0.1 to 1 kilopascal it either maintains at very high shear stress like several hundred of Pascal it shows a decrease, modulus shows a steep decrease.

Now temperature wise you can find out that what is the gel stability window and gel stability window is where the loss factor remains constant over a broad range of temperature. that means what is the gel stability? This should be the optimal window for 3D printing when you do 3D extrusion printing, how this print head temperature is to be maintained that should not be more than 23 degree Celsius and that can vary from as low as 15 to 23 degree Celsius. it is not only this extensive measurement of this viscoelastic properties is important, but also how these viscoelastic properties will be utilized or will be exploited to determine the optimal 3D printing window and also to understand that how the shear frequency and temperature that will have an influence on the viscoelastic properties G prime, G double prime and tan delta. rheological properties by far play an important role on the 3D printability, buildability of these hydrogel based scaffold that I must emphasize at different point while I am showing you different scientific case study related to the hydrogel 3D printing. signatures of different constituents in the hydrogel formulation were obtained again using FTIR analysis.

And you can see that IR bands as 1030 it is that single carbon-oxygen-carbon, at 1405 it is the symmetric stretching of the double bond, so C-O bonds. These two bonds are essentially confirm the presence of alginate which is used as a viscosity modifier in this kind of materials. As I said that dual cross-linking approach has been utilized in this particular case. UV cross-linking, so you use the Irgacure, then you expose the hydrogel at UV 365 nanometer wavelength Then you can also do this after the UV cross-linking then you get this cross-linked structures. Now when you add this alginate, alginate also provides you the ability of the hydrogels to be chemically cross-linkable.

without alginate you cannot use the chemical cross-linking and then if you immerse this in calcium chloride solution, this calcium ions essentially will be incorporated to the structure, alginate containing structures and what is classically described as like a egg

box kind of model. you can see here. You can see here if you go from 7.5G tube to alginate 0.5% nanocellulose addition with dual cross linkable, the stability of the scaffolds is very very good.

in terms of the microstructures like you know this is that porous microstructures of the GGMA composite scaffolds and what you see here in this, this is all 7.5% GGMA, this is that UV crosslinked structures. different magnification just to show how the porous microstructure evolves. These are dual cross-linked structures up to high magnifications that magnification micron bar is also mentioned at the below. you do see there is some changes in the microstructure particularly in case of the dual cross-linking with respect to single cross-linking you can see the structure is relatively less porous structure.

pore area as I mentioned, UV to dual crosslinking it is decreases and as well as a pore diameter also decreases with dual crosslinking. whenever I use this any sample or hydrogel sample ends with DC that means dual cross linking UV is only UV cross linking. It is very clear from 7.5G to A.5C this dual cross linkable structure that compression properties is very high.

compared to that 7.5G only which is roughly around 400, this is 1400 kilopascal, so it is like 3.5 times. Compression modulus also here in the chemically cross-linked and the UV cross-linked that is kind of this with respect to UV chemically cross-linked structures have higher compressive modulus. And toughness that is the area under the stress-strain curve that also increases in case of dual cross-linked structure quite significantly. Now biophysical properties, biophysical properties as I mentioned before that is very important particularly swelling analysis and in case of 7.

5G any times you modify this kind of hydrogels with secondary additions you notice that swelling decreases for example here it decreases from almost like 500% to 200-225% by addition of nano cellulose and alginate and after the dual cross linking or UV cross linking. this is essentially the way I mentioned that is that particular statement has also been shown here. we not only measure the swelling data but also we carefully analyze the kinetics analysis following that Q is equal to Qe plus Ae to the power minus t by k. t and k at the swelling rate constant and if you see the value of k, it decreases from 5.

4 to 1.98. quite significant decrease in the k value as we keep on modifying that 7.5% GGMA hydrogels not only with hydrogel composition but also UV or dual cross-linking approach. from many of these swelling analysis, one can also do more studies particularly when you consider the diffusion control swelling behavior and you can use simple mathematical expression like F is equal to k to the power n, this one is a diffusion coefficient, F is the swelling function and k is the characteristic constant. And, from there

you can get the diffusion coefficient or diffusivity that capital D is equal to pi r square k by 4 to the power 1 by n. And, this n value what we see here in this particular case it is almost like half like 0.

31 for 7.5% GGMA and after dual cross linking it is reduced to 0.16. Diffusivity value , the d prime is equal to d by pi r square in 10 to the power minus 3 that also reduced almost like 4 times 3 to 2.7. diffusion in this materials or this scaffolds will be much slower compared to that of the 7.

5% GGMA. when you do this cross-linked hydrogel scaffolds, we also are interested to know that how this biodegradation or enzymatic biodegradation happens and this is essentially just to show the schematic that you have a cross-linked hydrogel solution, you add with cells and there is an ECM secretion and scaffold degradation has been monitored and experimentally recorded. this is the way I am stressing this is the 7.5% GGMA. It goes to zero mass at the end of let us say 48 hours or so.

this is at the end of 2 days. Modified hydrogel scaffolds they can still degrade but at a much much less aggressively and at the end of 3 days 72 hours still around 0.25 of the original mass remains. Now the question is that what is the overall impact of the dual crosslinking you have a UV and you have a C^{2+} this is the DC dual crosslinking approach and you can see that swelling in the UV the swelling is almost like 500% close to in dual it is reduced to 200%. We have investigated both the extrudability as well as the buildability of this new hydrogel scaffolds. in the GGMA and nanocellular scaffolds with alginate, This particular case we can see that this is the 3D printed scaffolds and with hydrogel modifications you do see there is a change in the filament extrusion or filament extrudability.

And this is also build up printability, this is called filament collapse state or filament fusion test. this kind of FCT and FFT was essentially measured experimentally. couple of parameters were changed initially during experiments that is infill pattern and also we have carefully analyzed the layer integrity. once these filaments are extruded, that extrusion width is important and stability of the extruded filaments.

for example, this is no good. This entire thing is no good 20g. here again it is good 7.5% GGMA like 20g but if you see the 22g this is okay but if you go to 27g that is not okay.

This is also not okay. this is also not okay. what I am trying to emphasize that depending on the composition, hydrogel composition you need to use different nozzle diameters and to see which can give rise to good printability of the structures. as I said in the last slide that infill density was essentially changed from 10% to 40% and also

honeycomb structures were also 3D printed using this kind of scaffolds and And this essentially indicates that 7.5% GGMA, 2% alginate, 0.25% nano cellulose particles, you can essentially print them into different structures. We have this particular slide essentially shows you that how the GGMA based hydrogel scaffolds are being 3D extrusion printed in a laboratory setup and this has been also being printed in a Bangalore based startup company.

And, this particular startup company is also taking this particular biomaterial forward for commercialization. we have used the combination of the 3D printing parameters, particularly layer width of 0.22 millimeter, print speed of 0.8 millimeter per second. and jet speed of 9 millimetre per second and curing interval is 60 seconds.

every 60 seconds, this scaffold is exposed to UV and then we have found out and then we have continued the cross linking. the printing pressure for the rectangular grid it is like 1.2 bar particularly when you modify this scaffolds to 7.5G2A0.5C rectangular grid printing pressure was increased up to 1.

73. In case of cylindrical printing also it is increased to 1.6 bar compared to the 1 bar in case of the 7.5% GGMA. Print head temperature also increased to 0.22 degree Celsius but you remember that in one of the earlier slides I have shown there point that stability window is somewhere around 16 to 23 degree Celsius.

within that window the print bed temperature needs to be kept and this the print bed temperature it was cooled down to below room temperature is 12 degree Celsius that was maintained. And in all these cases largely we have used 22G that has been mentioned here. This is the 27G for 7.5% GGMA. plastic and in case of the other 2 scaffolds, it is the 22g that is good.

all these filament printability, filament extrudability and then 3D printability, experimental evidences were very useful to find out that what are the optimal parameters that one can use in the real life experiments. And all these videos are also useful for you to see how this 3D extrusion printing of this kind of different hydrogel combinations were carried out not only in research laboratory but also in one of the startup company. thank you very much and then I will come back to you with more scientific case study in the next few lectures. Thank you.