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

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Lecture 34

Scientific case study: 3D extrusion printing of Alginate-Gelatin hydrogels

In the last few lectures, I have discussed the process science involved in the 3D extrusion printing of the biomaterial inks as well as 3D bioprinting and 4D bioprinting. From this lecture onwards, I will be showing you some of the experimental results as part of the scientific case study of the 3D extrusion printing of alginate, gelatin, hydrogels. sodium alginate, this is one of the common hydrogel constituents that has been used and gelatin is also another constituent of the hydrogel. as I said that biomaterial ink formulation is at the centre of the 3D printing so therefore people have innovated different approaches to design this biomaterial ink. in this particular case study what you will be seeing, so one of the approach that I have mentioned in the earlier lecture is to use the nano composite bio ink. What it means that you embed essentially nanoparticles in the hydrogel framework.

And here I will be showing you that how nanocellulose has been embedded. this is the generic description of the 3D bioprinting which has been described to you a few times in the last few lectures. one of the main thing whenever you introduce or whenever you want to design these biomaterial inks, one of the primary motivation for you to show that these biomaterial inks can be 3D printed to show safe fidelity compliant structures like the way tubular constructs or cylindrical constructs and so on. And these constructs if you squeeze it and if you release the stress it will come back to its original structure or original position.

in this series what you see is that 3% alginate, 5% gelatin but you can add 1% pure cellulose (CP) or 1% and 2% cellulose nanoparticle (CNC) this CP and CNC cellulose nanoparticles so essentially are added to 3A5G that is the hydrogel constituents. what is the formulation chemistry that was used? you have essentially cellulose, this is the cellulose-mer unit. and you have this alginate gelatin structures. in these alginate gelatin structures if you do concentrated sulphuric acid treatment then what we essentially get is the sulphate ions that will produce negative zeta potential. this slide essentially shows you nano cellulose synthesis.

when you start with the pure cellulose, the pure cellulose with the 10 minutes of hydrolysis, you can see the change in color, . Then within 10 to 20 minutes, this color becomes deep brown. And then after hydrolysis is more than 30 minutes this excess hydrolysis becomes blackish and then within 20 to 30 minutes hydrolysis you can get adequate hydrolysis, it is diluted with large volume of water then you can get nice nano cellulose dispersion at pH 7, so it is a neutral pH. dispersibility of nanocellulose is very important. Why nanocellulose that has been introduced? Because this one if you see this is the pure cellulose dispersion, all the cellulose particles are settled here.

And when you use this nanocellulose particles, this is well distributed in the solution. this dispersibility is one

of the important thing and that has to do is the average size. this is a comparison between different batches of nanocellulose and here you can see this is the around 400-500 nanometer and when this nanocellulose particles you can see that how they are formed, they are dispersed in the scanning electron microscopic image. Crystallite size as we have determined using extra diffraction technique it is around 20 nanometer and so on. when you add this nanocellulose particles , one chain is essentially gelatin.

This another red chains are essentially sodium alginate, these are essentially multi-polymer or bi-polymer or hybrid polymeric hydrogels and then you have cellulose nanoparticles which are embedded in this kind of matrix, that weaker dot essentially indicates this is that weaker bond formation and whereas this alginate and gelatin these chains are intervened. when you produce this kind of hybrid alginate, gelatin and anhydrous hydrogel ink, we have done lot of this spectroscopic study and using this spectroscopic study we have shown how this OH-, hydroxyl ion that particular IR band also evolves and their intensity also changes. And this IR spectra you can very clearly notice here that what are the characteristic IR band that evolves. before I show you more results, I would like to emphasize once again that homogeneous formulation or formation of the hydrogel based ink that is very important. This is the deionized water here and this is the dispersion of nanocellulose in water as you can see.

when this gel is formed So, gel form to solution form you can essentially it is a reversible process even if you once the gel is formed it is so stable you can make simple chemical flask upside down you can see the stability of this gel that is very important. in terms of the biophysical properties, normally one has to measure swelling behavior. As I said that hydrogels, their definition is that it has a unique property of significant water retention. within very few minutes it swells, it swells to maximum extent to 200%. One of the things that you notice here 3A5G that swelling is 200% it drops down to 3A5G2C it is almost 100% swelling.

And enzymatic degradation like how this hydrogel scaffolds it degrades. in 3A5G it degrades quite fast and but it is up to 70 hours or 72 hours like three days it comes down to 0.4. In 3A5G that black one you can see it degrades to almost negligible mass is just above 0.2 normalized mass at the end of 3 days due to this enzymatic degradation in the collagenous solution.

Uni-axial compression in terms of the mechanical properties when these hydrogel scaffolds experience a compression behavior and you can see that when it experience that compression behavior this 3A5G their strength is much low like 0.5 mega Pascal. In 3A5G2C the strength increases to almost 2.5 like 5 times increase. 5 times increase in the strength and then they also show some kind of non-linear behavior after the post yielding or after the yielding.

In 3A5G1C strength increases the modest like 3 times 1.5 megapascal although the strain to failure is little higher like 80% whereas 3A5G2C reduces to around 70% strain to failure. normal urological tissue if you see their strength can go up to 0.2 MPa and this is natural extracellular matrix ECM, their strength can go up to kind of 0.

2 MPa. the stress wise or strength wise this kind of synthetic scaffolds are much better than compared to the natural scaffolds. uniaxial tension of the cross-linked hydrogel based graft. as I said before that it is that compression behaviour. Now, this is the tensile behaviour. you can see in the left hand slide this is the experimental part that how the experiments are conducted.

you make this hydrogel, then you put it in the microtensor. you pull it under tension. when you experience tension, now you can see that how this materials are undergoing tensile deformation, alginate gelatin and this goes up to 600 kilopascal. 600 kilopascal means 0.

6 megapascal. Whereas, in case of 3A5G without nano cells particles it is hardly 50 kilo Pascal. 50 to 600 is almost like 12 times increase. 12 times increase in tensile strength, 5 times increase in compression strength that one can achieve by nano cellulose particle addition. this any times we mention that some nano composite structure is formed whether in case of hydrogels or whether in case of any other materials you need to demonstrate that there is a significant property achievement or significant property advancement that one can realize otherwise you cannot practically call it is a simply nano composite if there is absolutely no property improvement can be recorded. Now viscoelastic property of this synthesized nano hydrogels as I said G prime, G double prime these are the standard storage modulus and loss modulus these are the standard viscoelastic property that one can measure by parallel plate rheometer or chronical plate rheometer but most commonly parallel plate rheometer is used in this kind of property assessment.

This is the tan delta, so tan delta is essentially G double prime by G prime, loss modulus to storage modulus ratio. as expected that for any these kind of hydrogels that with increase in the shear strain the storage both the G prime and G double prime should decrease particularly at high shear strain region and that is the same as the shear stress also it shows that the high shear stress this storage modulus and this modulus also decreases. is tan delta and then loss factor is also is being plotted by shear strain. after the crossover region which is around 100 Pascal, the hydrogel deforms permanently and the viscous part dominates over the elastic part. This results in higher modulus, loss modulus than storage modulus.

And maximum flow induced shear stress in renal tissues typically lies in 0.1 to 1 Pascal. this kind of values are important to remember. As I said before, the two key properties are of importance in the 3D extrusion printing of hydrogel. One is the shear thinning behavior and one is the thixotropic behavior.

in terms of pseudo-plastic behavior like you know shear thinning behavior like when viscosity is plotted against the shear rate, you see that there is a decrease in the viscosity, this 3A5G2C. And also it goes down in case of 3A5G it goes down very very slow and in case of 3A5G2C this it falls from 10 to the power 6 to almost 10 to the power in between 10 to the power 3 to 10 to the power 4 at 100 second inverse. this is the tau shear stress and then you can see that how nanocellulose particles they are contained when the material that deforms. Now, thixotropic behavior, so essentially it drops with time and then up to let us say 60 seconds means 1 minute and then it goes up and then it saturates after 1 minute. this is the typical thixotropic behavior that when viscosity is essentially decreases and it goes through a deep transition like you know it decreases and then it goes to a deep transition before structure recovers .

This recovery of the structure this recovery of the structure is very important of this kind of hydrogen. n (eta) is equal to k gamma dot to the power n-1, that is the fundamental law that defines the shear thinning behavior. this n value that power law constant that one can calculate for different hydrogel composition and it goes as you see with increasing nanocellulose concentration again shear thinning behavior is reduced from 0.

5 to 0.15. what it means that with nanocomposite hydrogel ink formulation, it is not only the mechanical properties like tensile properties or compression properties, they are modulated or they are enhanced but also power law constant like in case of shear thinning behavior that also decreases quite significantly. from 0.5 to

0.15 means at least 3 times decrease , you know it becomes almost like 30% of the original n values that one has measured in the 3A5G hydrogel . In summarizing, this hydrogel composition, they show pseudoplastic behavior as well as thixotropic behavior.

Now, stability of the hydrogel ink with respect to the time. one of the major thing is that suppose you prepare the hydrogel today and tomorrow if you want to do 3D printing, what is the stability of the hydrogel ink? Alternatively, you need to prepare the hydrogel and immediately you need to do 3D extrusion printing. that question also has to be addressed or what is the shelf-life of the hydrogel. shelf-life is very important. with respect to time and oscillatory frequency, so if you see the G prime and G double prime with respect to oscillatory frequency.

this is the different type of compositions of hydrogel formulations that were investigated in our group and then how their frequency dependent storage modulus. normally it shows like a increase and then reaches a steady state but this magnitude essentially that differs. qualitatively they show similar kind of trend but magnitude only differs. Now, storage modulus with respect to time as you see G prime the red one is the G double prime. this is that group of curves these essentially G prime, G double prime.

in both the cases you know G double prime it increases significantly in 3A5G and then 3A5G both G double prime and G prime and G double prime both increases significantly with time like after 2 hours to 5 hours. But there is a modest increase when you add this nanocellulose particles in both the G prime and G double prime at least up to 5 hours of this measurement. Now corresponding to G prime G double prime the tan delta values also show some kind of increase or decrease because tan delta as you know is G double prime by G prime. this loss factor this is the frequency sweep and this is the time sweep you can or the time variation results so one can see that how the stability of the hydrogel ink is maintained or particularly with the addition of the nanocellulose particles. Thermal stability is equally important and as you see viscosity sharply drops at 32 degree Celsius both on 3A5G and 3A5G1C.

here this is the 3A5G1C materials. this particular case we have also measured the G prime and G double prime as a function of temperature and then this with respect to temperature how these at 32 or 34 degree Celsius they show a drastic decreasing trend or they show the initiation of the decreasing trend in this kind of scaffolds. Now, in terms of the biophysical properties, so these biophysical properties are also measured not only thermal stability but also we have used the TGA, thermogravimetric analysis just to see that how weight fraction actually drops down significant to 0. temperature so it has up to 200 to 300 degree Celsius But however in that there is no mass loss in the physiological temperature window like you know 35 to 42 that particular window there is no mass loss. Filament collapse test that I have discussed in the last lecture as well that filament fusion test or filament collapse test FFT or FCT. you have a 3D extrusion printed structure, you have a tubular constructs.

these tubular constructs you can see with 3A5G1C and 3A5G2C, if you stretch it and then you can stretch it and then you can squeeze it, there is absolutely no problem, there is no fracture, so it is very very flexible. Now, in terms of filament fusion test, you have progressively wider and wider pillar separation distance and then you can see that you know how this particularly this hydrogel filaments is stable. Extrudability, you can extrude using 20G, 22G and 25G. You can see that how stable filament and longer and longer filament. It is not only stable filament but also it is a longer filament that is very important that can be extruded without filament failure.

And if you go to the 25G, it can hardly be extruded particularly when you go to 3A5G2C, it forms a kind of a spheroid kind of things. Now if you look at the micro CT based analysis of this kind of scaffolds, this is the pre cross-linked and this is the 3D printed cross-linked scaffolds and you can clearly see that pore volume fraction decreases for both 3A5G and 3A5G1C scaffold and whereas pore tortuosity also increases. Now pore tortuosity like what is the tortuous path of the pores that is very important. If more and more pore tortuosity means that biological transport of the cells and molecules and growth factors and nutrients they can be transported very deep into the hydrogel scaffolds.

that is the major point. Pore diameter analysis, this is all done using microcomputed tomography analysis and you can see that pore equivalent diameter it is kind of 100 to 400 micrometer that has been measured. Now, buildability of alginate gelatin carbon nanofiber scaffolds based hydrogels.

this particularly 0.25 carbon, 0.5 and 0.75 carbon nanofiber based alginate hydrogen scaffolds. you can see how 10 millimeter kind of long cylindrical conduit can be constructed here without any problem. this is the last slide on this. This is how this you do 4D printed scaffold.

you start with this 3D printed sheet. then you put it in the calcium chloride solution and in this kind of calcium chloride solution you can see that this undergoes rolling right. Rolling to spontaneously build this kind of a rolled cylindrical conduits. Now when you stress them under tension, this particular cylindrical things that has been tested here and you can see that under tension that 3A5G 0.75C it goes to the maximum strength of around 2.

5 MPa whereas compressibility is the 3A5G0.75C also it shows the maximum compressibility up to 3 MPa stress. I hope this , you can see that under this compression this how these materials they continuously are getting deformed. i hope in this particular case study I have shown you significant results in terms of the alginate-gelatin based hydrogel scaffolds to show how different structures can be built and then what are the different properties and how the different properties can be modulated can be significantly enhanced with this nano cellulose particle addition. Thank you.