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

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Lecture 39

let me continue the scientific case study which we started in the last lecture. that is on the GelMa hydrogels for the neural tissue regeneration. In the last lecture I have provided you sufficient clinical relevance and clinical background to the peripheral nerve regeneration as well as a low concentration GelMa and high concentration GelMa how their properties are different. where we stopped is that our discussion on the nerve guidance conduit, we continue from there and what we have shown you that typically critical size nerve defects will have length of greater than 10 millimeter. And depending on anatomical location whether it is ulnar nerve then it is the average diameter is 2.6 to 4.2 millimeter. If it is a radial nerve then it is 1.4 to 5.6 millimeter.

If it is sciatic nerve that sciatic nerve their diameter is 16 to 20 millimeter. And in many of these animal studies you know reported in literature I have seen the researchers they essentially cut the part of the sciatic nerve because it is the largest diameter like 16 to 20 millimeter diameter and that 16 to 20 millimeter diameter if you can regenerate the nerves in that. then the same hydrogels if that can be printed to much smaller diameter like let peroneal nerve like 1 to 2.6 mm perhaps that is the smallest diameter nerves. if that can be printed to this kind of small diameter nerve conduit structures then certainly they will allow more axonal regeneration To summarize that when you develop these kind of hydrogels for 3D extrusion printing or 3D extrusion bioprinting, our objective should be to manufacture these constructs into the length of 20 millimeter that is as I said is critical size defects.

and different diameters like you know outer diameter, inner diameter , outer inner 10, 8, 6, 4 and according inner diameter 8, 6, 4, 2 millimeter respectively. You can also use cells, neural cells like neuro 2a cells for example, neuro 2a cells you will see some of the results soon. or bioactive molecules which will help in the differentiation process. Cylindrical nerve conduit structures 20 millimeter in length and some are around 4 to 10 millimeter in outer diameter and consequently inner diameter is essentially around 2 millimeter less from the outer diameter. If that kind of constructs can be manufactured in a reproducible manner from a particular 3D bioprintable hydrogels that would be ideal for the treatment of neural tissue regeneration.

So in this particular case study what we have used, you have this hydro gel, gelatine methacryloyl, so this is GelMa, so which is a RGD motif And this RGD motif essentially in one case you have N-terminal and C-terminal and this particular area has been expanded, you can see what is the molecular architecture here. We have used photoinitiator like LAP and this LAP is one of the photoinitiator which is also used alternative to Irgacure. you have used secondary crosslinker. photoinitiator this primary crosslinker, secondary crosslinker we use PEGDA, polyethylene glycol diacrylate PEGDA and viscosity modifier we have used gellan gum. their molecular structure is also being mentioned here.

Now to introduce electroactivity, so this is like electroactive filler, so this electroactive filler we have used carbon nanofibers. this is that overall gelatin methacrylate, this is synthesis process, gelma synthesis process,

you start with gelatin type A and then you use methacrylic anhydride in PBS phosphate buffer saline at pH 7.4, 50 degree Celsius, 3 hour treatment. what you expect you essentially expect the transformation of gelatine type A to gelatine methacrylyol by addition of this particular functional groups here as I am circling. And the byproduct or the residue would be methacrylic acid.

Then I said that you know that we have used 2 type of crosslinking, one using lap and this lap is used as alternative to irga cure, it has 2 benzene as you can see here. when you use this UV crosslinking, essentially this is the crosslinked product that you get. Then you have also chemical cross-linking that is secondary cross-linked by PEGDA. addition of PEGDA essentially facilitates by secondary cross-linking to you get GelMa PEGDA conjugate here and chemical cross-linking of PEGDA that is also possible that is you get PEGDA PEGDA conjugate. we have this GelMa, we have the PEGDA, you have the carbon nanofibre and you have a gellan gum and this gellan gum also is a viscosity modifier, then as I said before that you can use Neuro 2SS in the neural tissue engineering applications.

when you start with the gelatin to gelma that you know that this conversion process is monitored or their signature of this conversion can be recorded using that NMR and you can see there is something called Ha-Hb-Hc. I think I have mentioned before Ha-Hb-Hc and this chemical shift you can clearly see this particular NMR band appears only in the GelMa. And this is like X-ray diffraction patterns and this weight loss as a function of temperature, this one can calculate by TGA and this FTIR, FTIR perhaps is more important you can see certain IR bands which are characteristic or that for the particularly for different amides or other phases in this particular case. Now this data has been sufficiently mentioned before or this kind of data has been sufficiently shown to you before like what is the impact of this hydrogel modifications on the mechanical properties like whether the stress strain response like compression stress strain response, then what is the strength in kPa, modulus in kPa and strain in percent. Now if you see lower percentage gelma or intermediate percentage of gelma like 7.

5% gelma when we start modifying by adding xylangam by adding PEGDA. in this hydrogel compositions whenever they are indicated P stands for PEGDA. G essentially stands for Gellen gum and C essentially stands for carbon nanofibers,. if you see these groups of data, for example, this group, this group and this group, one thing must be clear to you that if you add this carbon nanofibre in a very small amount let us say 0.1% carbon nanofibre just in the 7.

5% gelatin gelma the strength improvement is not that significant. But when you add the 7.5% gelma with 7.5% PEGDA the strength is much high and then even that strength is maintained but modulus is significantly increased with the addition of the carbon nanofibres. you can get the combination of modulus almost like 1 megapascal 1000 kilopascal strength properties of almost at 600 kilopascal and there is differences essentially strain to failure is reduced.

elastic stiffness, the major message here, elastic stiffness and strength can be improved by blending with PEGDA as the secondary cross linker and interpreting gellengum showed a synergistic effect on mechanical properties in the presence of PEGDA only. Now what happens to this microstructure or morphology of the cross-linked pores? you will agree with me certainly when you start modifying this hydrogel by adding PEGDA, by adding carbon nanoparticulate, by adding gellen gum. average pore radius essentially decreases but when you add this particularly carbon nanofibre it actually increases and this wall thickness of the pores that shows a regular increase with this hydrogel modifications. This is the scanning electron microscopic image and

this scale bar is essentially 200 micron. Now rheological analysis of the viscoelastic properties, you have the storage modulus I repeat your tan delta loss factor is G double prime by G prime and then what you can see here that viscosity as a function of temperature that also goes through a very rapid decrease.

n (eta) is equal to k gamma dot to the power n-1 and as I told you that this slope is essentially n-1 because it is plotted in the log-log scale, this was important and you have to also note that what is the temperature window that this particular gel is to be printed. It should be somewhere between 20-25 degree Celsius because it is the 20-25 degree Celsius you can see. Viscosity also drops very significantly with the hydrogel modifications. Now, in terms of the printability or extrudability, we have used different nozzle diameter and we have used both the plastic nozzle as well as the metallic nozzle, this is the P stands for plastic nozzle. Now, you can clearly see that with the modifications of this 7.

5% Gelma, their extrudability is almost very good when you use 22G size nozzle and then not the plastic one but the metallic one. And you can notice that in the case of the unreinforced GelMa this different nozzle sizes does not give a very very stable filament length and filament shape. as required for establishing good printability of the hydrogel scaffolds. Now, if you go back to the different sizes that I have mentioned a few slides back, then you will remember that we are essentially interested to see that whether 20 millimeter long nerve guidance conduit structures with a diameter ranging from 4 to 10 millimeter outer diameter can be constructed using 3D extrusion printing Now, what you see here that indeed with this particular modification of GelMa and then by printing them in the narrow wind of 20 to 22 degree Celsius or 20 to 23 degree Celsius under the pressure of 1.

2 to 1.8 gigapascal is the most complex or most multifunctional biomaterial ink. one can essentially go to the diameter of good printability of 6 millimeter to 8 millimeter to 10 millimeter diameter and this length is almost like 20 millimeter length. you can see that 7.

5-gelma, 7.5-PEGDA, 0.5% gellan gum 0.1% carbon nanofiber also gives a very stable nerve conduit cylindrical conduit structures. But that was not the case when you use for example 7.5% gelma with 4 millimeter diameter outer diameter. So the red one is not acceptable, green one is only what is acceptable.

Now what is the shape fidelity compliance and how this structure which is printed, if you compress it and if the pressure is released, can it come back to the original shape and size? That was the question. What is the flexibility of the 3D extrusion printed constructs? This is the 3D extrusion printed constructs. what you see here? when you compress it you can compress and when you release it you can release it like you know then you can get this structure you can see the complete through and through length and the same thing you can see here this is that as 3D printed this is compressed and then what the pressure is released you can see that through and through length of the cylindrical conduit. What are the compressive modulus? The compressive modulus here it is around 30 to 1.

03 kilopascal. This is the present work and as you can see this varies from 3.4 to 3.5 megapascal in some of the earlier GelMa concentration. At the beginning of the last lecture I have mentioned that most of the gelma scaffolds which are used for nerve tissue engineering applications, they were essentially fabricated largely from the higher concentration gelma like 10 percent, 20 percent, 30 percent gelma with very very less kind of data are available with the lower concentration gelma and that actually was the motivation of our work to see whether low concentration gelma also can be explored for nerve tissue engineering applications and how their

properties and performance will be different from that of the high concentration gelma. The purpose of adding the carbon nanofiber is to enhance the electrical conductivity of the materials or to make the hydrogel electroactive.

therefore electrical conductivity measurements was an important aspect but the way I am saying the electrical conductivity measurements of the soft hydrogel was not a cakewalk it was quite difficult for that the student who has done his Ph.D. at Indian Institute of science. Soumitra Das has essentially designed and fabricated a customized setup at Centre for Nanoscience and Engineering at IISC and then we have used 4 probe method to measure this conductivity and then we see that with increasing addition of this carbon nanofibre like and particularly hydrogel modifications you get is like 0.6 millisiemens per centimetre conductivity when we have used the 4 point conductivity, 4 point measurements.

Now, cyto-compatibility evaluation, there are 3 different ways we have grown cells on this scaffolds. Simply, we have essentially 3D extrusion printed biomaterial inks without cells, then we have grown the Neuro 2a cells. and then we have allowed them to grow for day 2 and day 5, then we have done this WST-1 for live data analysis and fluorescence microscopy observations. 3D encapsulation bioprinting, what it means that you encapsulate or you use cell laden hydrogel and then you do 3D printing, then you do UV curing in situ during the 3D extrusion printing process. and then see what is the live or dead of the cell viability.

In the third approach, you do 3D extrusion printing but without in-situ UV curing but you do post printing UV curing. And this is like in situ UV curing, post printing UV curing and then you grow and then you see the cell live dead staining images, live dead analysis to see how much fraction of the cells are alive. standard growth media is used like DMEM based media, but we have also used differentiation media like DMEM supplemented with 10% FBS, 20 micromolar retinoic acid and 1% antibiotics in the differentiation media. this is cell viability of the seeded N2a. this is the first approach, the 2D seeding.

Now you see the cell results. you can see day 2 and day 5, you can see small, small clusters of these Neuro 2a cells that grows at day 5. The scale bar is 200 micron. In the growth media, this is the morphology at day 2 and day 5 and then if you grow in the differentiation media, the morphology at day 2 and day 5 is little different and you can see at day 5. more number of cells and also a very closer look you can see that is neurite like outgrowth also has taken place. And you can see this neurite like outgrowth these are like thin axonal growth and which is spreading at the 7.

5% GelMa, 7.5% Pegda, 0.5% gellen gum and 0.1% carbon nanofibers you can see and this is the length of the neurite like outgrowth and number of branches and length of the branch length how it has been used. When this encapsulated cells in cell laden hydrogels or the cells are encapsulated in the 3D extrusion printed hydrogels and then see viability of the neural cells you can see this lot of green spots which essentially shows you the live cells and viability and proliferation of N2a. In the biofabricated scaffolds were also measured in the growth media live data assay. Then proliferation and viability encapsulated in the differentiation media also over 2 to 5 days that has been done.

Now you remember I have shown you the cylindrical scaffolds a few slides back. Now on the cylindrical scaffolds when we have printed the cylindrical scaffolds with cells incorporated that is N2a, Neuro 2a cell learning scaffolds. Then at day 2 and day 5, we have just used that live cells and you can see that most of the cells are alive. And this particular work was done by one of my PhD students Soumitra Das. And this work was

published in ACS Biometerials Science and Engineering, so one of the major conclusions was that this conductive carbon nanofiber addition resulted in 4 fold improvement in the electrical conductivity and then strength and modulus was enhanced by 3 and 8 fold.

Conventionally cast scaffolds can support the differentiation of neuro 2a cells and the most important result has been excellent cell viability neural cells in 3D encapsulated structures. this work is recently published in 2024 and you can see the more details in this published study if you are interested to know more details. with this I believe I have completed this particular scientific case study on gelma based hydrogels for neural tissue regenerations. I have given you sufficient background on what are the different sizes in terms of the length and diameter critical sizes nerve grafts that are required to be 3D bioprinted. I have only shown you the in vitro results and then also the results of the 3D bioprinting essentially we are able to maintain the cell viability of these Neuro 2a cells and then also Neuro 2a cells when they are grown in the differentiation media it does so axonal regeneration and then also that how carbon nanofiber addition makes the scaffolds more electroactive and also combination of metal strength and modulus can be obtained by modifying this hydrogel compositions.

And here I must mention that we have used 7.5% gelatin methacrylon which was not extensively investigated for neural tissue in generation. That was the motivation to show that how lower or intermediate concentration of GelMa can be modified or that GelMa based bio ink can be developed which can be 3D extrusion printed to make the safe fidelity compliant structures. Thank you.