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

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Lec 40 Labscale demonstration of 3D extrusion printing of hydrogels

welcome to the Laboratory for Biomaterial Science and Translational Research at Materials Research Center, Indian Institute of Science, Bangalore. this is the Laboratory for 3D Bioprinting and Cell Culture Lab. as part of this NPTEL course, I have already explained to you in sufficient details about the 3D printing of hydrogel scaffolds. And in today's lecture, I'm going to give you, I'm going to demonstrate to you the 3D printing process of soft hydrogel. this demonstration is composed of a few parts. In the first part, I'll be showing you how to design a particular scaffold using engineering design software.

Second part essentially involves the demonstration of the soft hydrogel preparation. Third step involves the description of the key components of the 3D extrusion printing setup that is available at our laboratory. In the fourth step, I'll show you the 3D extrusion printing of hydrogel after setting the required printing parameters. Subsequently, I'll show you the cross-linking mechanism of the 3D printed scaffolds.

And when these 3D printed scaffolds are imaged using microcomputer tomography, how the 3D microstructure can be visualized and quantified using Avizo software. it is expected that this particular lab-scale demonstration will provide you much more practical understanding of the 3D printing process as a whole and also different stages of important stages of the 3D extrusion printing of hydrogels in particular. if you look at this particular gel, you will see this gel is transparent? And you can see that if I just invert this gel, it is quite stable. gel stability is important. That means gel must have sufficient strength so that these viscoelastic gels remains very strong.

And this is the quality of the hydrogel or this is the quality of the gel that we need to ensure before this can be 3D printed. what I'm showing with my hands is the alginate gelatin gel. Now this alginate gelatin gel I have also covered in my NPTEL lectures in much more details while I have shown you several case studies related to the alginate gelatin with different additives. One of the additives I have shown you and this is this is the additives is called nano cellulose. when you add this nano cellulose certainly you can see the quite transparency of this alginate gelatin is lost.

this is the right hand, it is alginate gelatin, left hand it is alginate gelatin with nanocellulose. the addition of this nanocellulose as an additive to make this alginate gelatin gel more multifunctional essentially also changes its not only color to some extent but also its transparency. Now this is the gel you can see this is the black color gel. from the appearance of the black color you can immediately see that this must contain carbon nanofibers. this was the original alginate gelatin gel.

Now the moment you see when you add even small percentage of the carbon nanofibers you can make the gel black if this gel appears in black in color. this small amount of nanofibers can be 0.1 percent nanofiber, can be 0.2 percent nanofiber. Even in this NPTEL lecture, I have explained to you that what is the role of these carbon nanofibers to make it electroconductive or electroactive biomaterial ink, so that it can be used when it is successfully bioprinted with neuro 2S cells or neural cells, it can be used for the nerve conduit applications.

this is what I have described of the gel. Now what you are going to see in this demonstration, that how this gel you can make it 3D printed to this kind of structures. Now if you look at the structures clearly, this is a kind of typical cylindrical matrix type of structures and you can see that there are specific holes. It has very good resolution. This is the 3D extrusion printed scaffold.

This is not 3D bioprinted scaffold, but this is 3D extrusion printing of the biomaterial inks. this is alginate gelatin with the nanocellulose. You can also see this kind of tubular structures. If you see these tubes very clearly, you can see from end to end, this hole can be extended? this kind of large cylindrical structure is very much required for nerve conduit or for peripheral nerve injury applications, as I've explained to you all during these NPTEL lectures. this is another type of scaffolds.

The other things is that when this black colored alginate gelatin with nanofiber hydrogel is 3D printed, you can see even after 3D extrusion printing, these scaffolds can be printed very nicely. This is the alginate gelatin with the carbon nanofiber. You can see that I'm trying to squeeze it. It remains quite structurally stable. if I can twist it, I can bend it, this scaffold remains quite good.

And this is what you can see earlier, this scaffold, this white color scaffold, this alginate gelatin nanocellular scaffolds, again I can twist it, I can bend it in different directions, I can squeeze it, it can regain its particular shape and size. for synthesizing 3D printed hydrogel based scaffold or 3D printed implant, 3D printed hydrogel based implant, the starting material is some biopolymeric or natural resources powder or protein. in our study, what we use the gelatin based powder. The gelatin is basically a product which is extracted from naturally available protein collagen. Similarly we use cellulose.

Cellulose is also a naturally available polysaccharide. Now to improve the mechanical property of the 3D printed hydrogel based graft we modify it with certain material called carbon nanofiber. So most of the starting material for the hydrogel based scaffold fabrication technique is basically powder. you have to dissolve the powder in water to make it soluble and finally when you will get the homogeneous solution that I will show in the next video and then you can make it 3D printable. the hydrogel based formulation for the 3D printing are basically made by the sequential mixing of powder in magnetic stirrer or certain mixing condition at certain temperature and RBM.

here you can see this is pure alginate gelatin based hydrogel solution and you can see it is homogeneously mixed. now it is in the solution form and that's what you can see when I will tilt it this solution is moving. that means it is now not in a gel solution. we have to cool down it at a certain temperature to make it gel. this is the pure alginate gelatin solution and now I will show what about after incorporating the nanoparticle in the hydrogel solution.

when we incorporate the carbon nanofiber in the hydrogel solution. You can see the carbon nanofiber is homogeneously mixed in that pure alginate gelatin and here you can see like this is now in the solution form. when we cool down this gel formulation at 4 degree Celsius it will form the gel. Similarly, this is you can see this homogeneous white color solution. this is basically after incorporating the nano cellulose in the hydrogel mastic.

And you can see the nano cellulose is highly homogeneously mixed in the solution. this homogeneity of the hydrogel based solution is very important for doing the 3D printing. after doing the 3D printing, the hydrogel based graft should be highly stable. Now here I will show after 3D printing what is the stability, flexibility of the hydrogel based graft. Now here you can see this is a 3D printed hydrogel based scaffold.

And this is nanocellulose reinforced hydrogel based scaffold. And here you can see you can clearly hold it. Although it is a hydrogel it is highly sensitive material. But you can hold it properly and you can squeeze it. that means it is highly mechanically stable.

and similarly you can see all the filaments and internal hole are properly distinguished similarly i will show without incorporation of the nano cellulose you can see here also the structure is highly stable and you can squeeze it you can fold it so which means the viscoelastic property of the hydrogel based graft is highly maintained after the 3D printing. Not only with that nano cellulose, you can see this is a black colored graph, black colored 3D printed graph basically made by carbon nanofiber. And here you can see the carbon nanofiber is highly homogeneously mixed. that's what we obtained this kind of mechanical property. And not only that, this hydrogel based graph is highly stable.

It is not brittle. not only different kind of matrix shaped graft we can print different kind of hollow tubular graft and here you can see this is carbon nanofiber reinforced hollow tubular graft and this you can see the lumen patency is clearly maintained which is very much important when you use the hydrogel based graft for reconstruction of biological tissues which require this kind of hollow lumen structure. you can see by using certain 3D printing technologies you can make this kind of hollow tubular graft and lumen patency is properly maintained. Not only this kind of shape you can also make the hydrogel based graft of this kind of square shape. And also here you can see you can squeeze it, you can press it which means it's good mechanical integrity. after that I will show like 3D printed hydrogel based graft you can make this kind of film kind of structure.

this kind of film kind of shape is very much important when we want to reconstruct some wound, when we want to heal some wound basically to make a patch. this kind of patch shape morphology also we can obtain from 3D printing. for 3D printing of any hydrogel, you have to fill the hydrogel for extrusion 3D printing in a printing cartridge. how you can do that? Suppose this is a homogeneously formed hydrogel solution. and so you have to fill this hydrogel solution inside this 3d printing cartridge so this is essentially a 5 ml printing cartridge so you have to fill the hydrogel solution inside this cartridge , how you will do that, for that we will use this kind of adapter so this adapter will help to connect the printing cartridge as well as this syringe.

we will extract the hydrogel solution from the syringe and we will transfer it through the adapter to the printing cartridge. this is basically acting as a transferring agent, the hydrogel solution from the syringe to the printing cartridge. And now I will show how to do that. this is the syringe, you can see. before that you have to check this syringe whether it is not creating any obstruction inside that.

Because if it creates any obstruction inside the syringe due to air and all, that air will create the bubble inside the hydrogel solution. And due to the formation of the bubble, your homogeneity of the solution will be compromised. And the mechanical property of the ultimate graft will compromise. now I will show how to do that. first you have to extract the gel through this syringe.

Very carefully. now I will connect this adapter on the top. another side will be connected to the printing cartridge. this is the setup. You can see.

This is the printing cartridge. This is the adapter. And this is the hydrogel filled syringe. now what I have to do. I have to gradually push this hydrogel inside the syringe. you can see the hydrogel is gradually filling inside the syringe.

printing cartridge. again I will extract and then again we can fill it like this. We will put it like this and gradually we have to insert these hydrogel solutions inside. why you want to inject very gradually? The reason is not to insert much bubble due to the air. you can see this cartridge is almost filled.

now we will get directly this cartridge. now you have to keep it in 4 degrees Celsius for half an hour or one hour to make this solution to gel. Because for gelatin the solution to gel transformation happened at comparatively low temperature and the complete gelation happens at 4 degrees Celsius. after forming the gel you can see this is a homogeneously formed nanocellulose incorporated alginate gelatin based hydrogel ink. we have to do the 3D printing with this hydrogel based ink. And in this session I will just try to show what are the different kind of structure can be 3D printed with this kind of ink.

when you are doing the 3D extrusion printing, it is very important to select the proper nozzle dimension to get the accurate resolution. in our lab research, primarily we use one is called this kind of 22G nozzle, blue nozzle and another one is called 25G nozzle. based on the hydrogel based composition, based on the viscosity of the hydrogel, you have to select what kind of nozzle you are going to use. as we move from 22G to 25G, that essentially meaning that the finer filament will be extruded. As from 22G to 25G, the internal diameter of nozzle is gradually decreasing.

that's what more fine filament supposed to be extruded. But what happens when your hydrogel viscosity is higher? your hydrogel viscosity should be synchronized with that nozzle dimension to get the accurate printing resolutions. That means the hydrogel with a little lesser viscosity can be extruded from a very fine nozzle to get a good printing resolution whereas the hydrogel with higher viscosity can be extruded through some different nozzle to get some proper resolution that means the hydrogel with higher viscosity should be extruded from a nozzle having comparatively higher internal diameter so then i will come how we can fit this hydrogel filled cartridges to the 3d printing machine now I'll explain you the working functionality or working principle of commercial software which normally is available with every 3D printing machine. Now, this particular 3D extrusion printing machine is procured from an Indian startup company based in Bangalore, that is Avayi Biosciences Private Limited. Now, this is the company's most advanced generation of 3D extrusion printing machine, and it is known as Mitoplus.

the software that I'm going to show you now is the Mito Plus software, which is a versatile design platform where you can design the way you want to design any scaffold structure. for the purpose of this lab demonstration, I am going to show you only two type of structures. The first one is that matrix structures with designed porosity. Second one, I'll be showing you these hollow cylindrical structures. typical matrix structures is used for different type of biomedical applications and hollow cylindrical structure is used mostly from the purpose of the nerve conduit applications.

this is the first landing page of this particular structure. this second window that is in front of you, you can see several parameters in this particular window. One is the infill patterns. Infill patterns can be either linear or concentric or honeycomb type of structure. Let's first start, let's first see that what will happen if we use that linear infill pattern.

Infill angle is 90 degree. You can change the infill density. Now for the purpose of demonstration here, let us take 25% of the infill density. We have two extruders, extruder 1 and extruder 2. We'll be checking with only one extruder. you can also the option of the curing like either you can cure it in situ during the process of printing or you can cure it after the printing is over.

Now what you see in this particular window, you can change the layer height and layer width. here, for example, I can change it to 0.5 and I can also change the layer height as 0.5.

there is certain machine related limitations is there. One cannot go to a very high layer height and layer width? We have to restrict that. now is the perimeter is one print speed is six millimeter per second and then print move moving speed is 60 millimeter per second like you know how the print head will go up and down that will be 60 millimeter per second z speed is 10 millimeter second material density has been kept as One gram per cc extrusion delay is 100 milliseconds, and post print height is 5 millimeter. That is what our target. Now, if I say save profile, then it will essentially save all these parameters. once that is done, now you can start the slice the object.

if you do the slice the object, you will be able to Preparing so it is now preparing so it will take around 10 minutes so after 10 minutes you will be seeing that is this scaffold structure is being designed and then it you can see different views of the design scaffold now . This particular design scaffold, then you can load the G-code file that will be generated and G-code file can be loaded in this particular software that is called METO+. Then E1 and E2, essentially extruder 1, extruder 2, you can set that what is the temperature, what is the pressure, what is the bed temperature. Now here I want to mention that this specific temperature of the print head or print bed has to be determined based on the viscoelastic or rheological properties of the hydrogel that you want to print. And this I have explained to you in much more details during the lecture that when this parallel spectrometer is used to characterize the rheological properties of hydrogel, then you get frequency sweep, you get temperature sweep of the G prime and G double prime.

G prime is your storage modulus, G double prime your loss modulus. And also the other properties which are of relevance for this 3D extrusion printing of hydrogel is that gel must have a shear thinning properties. What is shear thinning properties? If you measure the viscosity as a function of shear rate, then viscosity will decrease with increasing the shear rate. This has been emphasized time and again during this different lecture of this NPTEL course. Another important property of relevance for this hydrogel is the thixotropic property.

this shear thinning property, thixotropic property has been emphasized to a large extent multiple times during this NPTEL course. and here you can see that what is the extruded print head temperature and print bed temperature that you have to define currently and then we'll see this how this scaffold design is constructed , now let us see that another design option that is the cylindrical nozzle ,thi s is the hollow conduit of 8 millimeter of outer diameter, 6 millimeter inner diameter, and around 2 centimeter is high, that means 20 millimeter in height. Now why these numbers are important? Because for the peripheral nerve injury applications, when a neurosurgeon wants to use a nerve graft, he or she has two options. One is the autograft, like cut the nerve from other anatomical locations of the human body and put it at the injured site for the treatment of the peripheral nerve injury. Another option is to use the synthetic hydrogel scaffolds with cylindrical conduit structure which can be placed and which should have sufficient electrical conductivity, and that electrical conductivity is required for the nerve regeneration.

Now, if you look at these particular scaffolds, the cylindrical structure, you can see it is in through and through hole. some of the important thing that you need to notice here that this particular through and through hole that inner surface roughness is also equally important. also the diameter should be the concern that diameter should be uniformly maintained after the 3D extrusion printing and after the cross linking operation is over. So I repeat, outer diameter, inner diameter, and then total height of the cylindrical conduit structure is all the three dimensions are important. Now you see that what is that 40 layers , this software has automatically assumed that this cylindrical conduit structure will be composed of 40 layers and each layer time that will take layer by layer extrusion printing that will take 8 seconds and overall material it is required as 0.

44 gram that also depends on what is the density and total volume and layer each layer this around 11 milligram will be used to print one layer. this is that again extrusion head, the print head that is one and two and then one has to set the temperature and bed temperature before anybody can start the printing operations. Another thing you notice is that in the left hand side bottom panel it is the UV window. UV window essentially there are a couple of unique numbers you can see that what is the second so you can essentially select UV on for 5 seconds or 4 seconds or 3 seconds and what is the intensity and what is the timing. if you want to do the in-situ UV cross-linking operations it is possible.

What many people do is that they add igacure which is used as a photo cross linker along with the hydrogel and this gel they print it. After they print it they essentially use the UV cross linking operations. Or they can use the chemical cross-linking operations by using calcium chloride. And this calcium chloride cross-linking is also another useful route for the cross-linking of the hydrogel scaffolds. In one of the NPTEL lectures, I have shown you that we have also developed.

.. GGMA scaffolds where we have used dual crosslinking operations like UV crosslinking plus chemical crosslinking operations and we have shown that dual crosslinking indeed is useful to enhance the mechanical properties as well as the biophysical properties compared to the single crosslinking operations. I emphasize again that cross-linking is very important. The scaffold properties depend significantly on the cross-linking operations as well. Now, Sulob actually will show you this printing operations as well as the loading of the hydrogels into the machine. And then after that, he will be showing you that how different structures has been printed, what are the different materials have been printed, hydrogen materials have been printed so far in our laboratory.

this is already I have shown that this is a hydrogel filled printing cartridge. Now so far for 3D printing what I have to do I will fit first this nozzle. This is called nozzle. This is 22 nozzle. this nozzle I have to carefully insert this and then I will put it in the machine.

this is the 3D printing machine. as Professor Basu explained that this is extruder one, there is two extruder, extruder one and extruder two and this is extruder one, this is extruder two. when you will fill the hydrogel ink in the printing cartridge, so you can see this is the homogeneously formed nanocellulose based hydrogel ink. what I will do, I will insert the nozzle. this is called 22G nozzle and from this nozzle the ink will extrude. Similarly, like a syringe and the needle, but it is called printing cartridge and printing nozzle.

this is 22G nozzle. And what is this? This is basically the print bed. there should be certain substrate on which the 3D printed hydrogel will deposit. And from this study, we will take this kind of petri dish. as a printing bed. when the hydrogel will extrude, it will extrude on the surface. Similarly, we can take the well plate also, 24 well plate, 96 well plate, when we want to print the 3D printed graph directly for doing the in vitro cell culture experiments.

now i will show how to fit this printing cartridge along with the nozzle to the extrusion 3d window so this is the extruder one i told you so what i will do i will just fix it like this and this is the pressure pipe from which the compressor will generate the pressure for extruding this hydrogel-based ink in our extrusion 3d printer the maximum pressure can go up to the 4 bar and you have to based on the hydrogel viscosity you have to select or change you have to adjust the pressure to get the optimal sized filament so and basically this is the pressure pipe from which the pressure comes now i will fit it like this and then I will fit like nozzle like this and then I will fix it. Now as I told before starting the printing we have to check the pressure. Similar thing I will do it here. how to check the pressure? in this machine you can see there are two dial.

two dial is there. this is the pressure what is showing in extruder 1. And this is the pressure what is showing in extruder 2. now I will check the pressure , you can see the printing filament is excluding that means your pressure is now optimized. Now we directly go to the printing. here you can see like how the 3D printing happens.

we I have given the certain printing design in the software. And here the 3D printing has been started, which is basically a disk shaped structure having 20 percent infield density. this structure basically consists of the 30 mm diameter and 20 mm, that means 2 cm in height. gradually you will be able to see how a 3D printed construct will build with respect to Hydrogel just now you are seeing that the printing video where the nanocellulose reinforced alginate gelatin based hydrogel was printing. at the end you can see you will get this kind of structure. And Professor Basu was explaining that hydrogel thixotropic behavior is very important.

That means the recovery of the viscosity. you can see due to the good thixotropic behavior of the hydrogel, the ultimate graph is extremely sustainable. on the substrate. Although the hydrogel based graft is stable after the printing but to give the final structural stability of this hydrogel based graft and you can see we have to crosslink it through some calcium chloride based crosslinker solution. this is a liquid based crosslinker and immediately we can crosslink it through this crosslinker solution and we have to keep it for certain time and after that when we will take it out , we will get exactly the hydrogel based scaffold like this. Similarly, when you will fill the hydrogel printing cartridge with this kind of carbon nanofiber based hydrogel, so that time you will directly get the structure like this.

the procedure is same, you have to play around with different different material, you have to play around different material viscosity and as well as other printing parameters you can get a different kind of structural construct having very wide range of structural fidelity and as well as different morphology. this way I will conclude that this is the overall state of the art of using 3D printing of hydrogel based scaffold for making versatile biomaterial implants. after doing the 3D printing of any hydrogel based scaffolds, the three dimensional porosity has to be analyzed. the main technique to analyze the three dimensional porosity is by micro CT once you do the micro city of a 3D printed hydrogel based scaffolds, you will get that the morphology like this. here you can see like when you are observing the scaffold in 3D micro CT, it is a 3D volumetric interconnectivity and porosity you can see.

just now I have shown the 3D volumetric porosity analysis of the 3D printed scaffold. before the 3D printing of the scaffold, there is a hydrogel ink that we fill in the printing cartridge. what is the morphology of that hydrogel based ink? what is the volumetric porosity in the hydrogel ink so that thing also can be analyzed through micro CT, so if you see the micro city analysis of the hydrogel based ink you can see this kind of 3d volumetric porosity and you can see the pores inside this entire network is highly interconnected so which means your hydrogel base ink is highly homogeneously dispersed after doing the 3D printing of the hydrogel-based scaffold, so just now I have shown from the micro CT, how you can see the 3D volumetric porosity. Now, when you have obtained the 3D volumetric porosity from the micro city, you have to analyze. the analyzing software is Amira and Avizo software. from this software you can analyze by making certain recipe and you can see this kind of 3D pore interconnected modeling by using different methodologies.

you can see the formed 3D printed hydrogel based scaffolds is highly interconnected and here you can see it by some sphere fitting model. to conclude this lab video demonstrations, what we have shown you that how to prepare the hydrogel, how to characterize the hydrogel using different parameter, different techniques that I have already discussed in the lectures and how to operate the 3D extrusion printing machine, how to use the slicing software and how to load the file as well as the printing operations in details. as the end of all this, as an outcome of all this exercise, what you see essentially, you can get either this kind of a matrix kind of a scaffolds with this kind of design pore structures, and then you can squeeze this matrix type of scaffolds, and then you can essentially see the recovery of the shape also of the structure. one of the things that I have mentioned in this NPTEL course, that safe fidelity of this hydrogel scaffold is equally important. Safe fidelity means if you twist it, if you bend it, if you stretch it, these scaffolds remain same, right? You can see that all this kind of a different kind of operations.

this is that alginate gelatin with nanocellulose. I have also shown you that this is the kind of another cylindrical kind of a structure. In the cylindrical structures, it is made up of the alginate gelatin with the carbon nanofiber. And here, this structure is made first as a sheet. Then it was essentially put it in the calcium chloride crosslinker solution then it is self rolling, it takes place and then it is rolled into cylindrical structures.

And this particular structure preparation you can call it is a 3.5D or pseudo 4D kind of a printing because without any additional, without any external stimulation simply by chemical crosslinking this structure is rolled into a cylindrical conduit structure. and this is the alginate gelatin carbon nanofiber structure. I hope together with the academic learning as well as this kind of lab demonstrations, you have developed sufficient understanding of 3D extrusion printing of hydrogels in general and gelatin-based biomaterial ink printing in particular. But this kind of knowledge that you have acquired through these classroom lectures as well as lab-scale demonstrations will be useful for you to design new biomaterial ink formulation, setting the 3D extrusion printing parameters as well as printing and cross-linking these biomaterial scaffolds in future. Thank you.