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

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Welcome back to this lecture and let me continue with this challenges and opportunities in additive manufacturing. In particular, I will be discussing on the 3D printing related challenges. And in the last lecture, I have mentioned about one of the new techniques called melt electro-writing there are challenges which are involved in some of the lesser explored 3D printing technique as well as there are also challenges involved how to maintain the shape fidelity of the structures and so on. let us spend some time in this particular slide and try to understand that what are the implications of the information that are contained in this particular slide. if you look at that classical biological structure of let us say neuron, you have a long axon. You have a cell body with a nucleus, you have dendrites and so on.

you have a blood capillary. in the blood capillary, so blood is a tissue, blood is a tissue and it contains different type of cells like RBC, WBC platelets which are very important because we need to maintain a particular level, particular number of platelets like 1.5 to 2.5 lakhs in every human patients.

There is a low platelet is kind of indication of the poor maintenance of the physiological health status and then you have a spheroid. So, you have blood vessels and so on. this is a biological scales and these biological scales if you look at the structure It goes for the minimum feature size like you know around 10 micron for examples. this animal cells you know like 10-15 micron at this space, then if you go to blood capillary, the spheroids it can grow up to 100 micrometer and so on. And then blood capillary and their sizes can go up to somewhere between 10 to 100 micron.

Now, you have the different kind of 3D printing technique which you have seen in previous lectures. One can be DLP, digital light processing or stereolithography. One is melt electrowriting, MEW or MPL, the multi-photon lithography. Then you have an extrusion bioprinting, then you have an electro-writing and then you have a volumetric printing. out of that volumetric printing essentially means that you are able to create large volume structures but there should be a trade-off between buildability.

and then resolution of the structure. This has been mentioned to you before, but this slide essentially reemphasizes that point once again. you have this is that MPL, this points belongs to MPL, that multi-photon lithography, where the volume throughput can be as low as 10 to the power minus 5 millimeter cube per hour with the minimum feature size is 1 micron to maximum up to 10 microns. Then, you go to this electrospinning or melt electro-writing This is the melt electro-

writing. You have very less data points because this technique is not that well explored.

these are melt electrowriting. EBP is the extrusion bioprinting. In extrusion bioprinting, the feature sizes, it goes from 100 micron to 1000 micron, almost like a millimeter. And there are other techniques which is that volumetric printing here. This is one data points in the volumetric printing.

As the name suggests, volumetric printing essentially allows like 10 to the power plus 6 millimetre cube per hour. that is the level that structure can be built or this particular technique can handle or has this kind of ability to produce structures at that rate like you know 10 to the power 6 millimeter cube will be printed per hour and which is a lot actually. what you normally notice that ideal situation or which encompasses DLP primarily, digital light processing and also to some extent MPL, multi-photon lithography. This is one group of data points. Another group of data points is this one which is again essentially DLP, but you know that it goes for the very high throughput here.

Extrusion bioprinting is this feature size is higher, this is the extrusion bioprinting. Feature size is higher between 100 and 1000 micron and then buildability also is the moderate like 10 to the power 3, 10 to the power 4 millimeter cube per hour. what you notice that if you take a mean average then the way I am doing this kind of a dotted line. buildability, volume throughput and then also minimum feature size, they are kind of linearly related. you can build the structure, large volume structures or large volume throughput only with larger feature size.

you cannot build large structures with a very small feature size like what DLP can do. this trade-off needs to be learnt from this particular data points or these are from the summary of several experimental studies related to some of the techniques which are moderately well explored or relatively less explored like TLP or multi-photon lithography or extrusion bioprinting or melt electro-writing volumetric printing. what is the other challenges that what are the cell numbers per construct that you can print? in the last slide what you learnt is that volumetric printing. That is one. and one is that minimum feature size.

Now, if you have a single cell biology or spheroids or microencapsulations, then you can essentially encapsulate kind of 10 to 1000 kind of cells. In the hydrogels or bioprinted scaffolds, what are the limitations? Limitations is that maximum 10 to the power 5 to 10 to the power 7 number of cells can be encapsulated in this hydrogel scaffolds. And recellularized or organ scaffold like cell seeded scaffolds or recell organ scaffolds, then you can go up to 10 to the power 7 to 10 to the power 8 cell numbers per second. the challenge remains how to encapsulate large number of cells with uncompromised viability or functionality. this is the threshold for solid organs.

in the solid organs you have 10 to the power 99 to 10 to the power 10 number of cells are there. we are somewhere very near to that with the use of the hydrogel bioprinted scaffolds. the message that you learnt, hydrogel bioprinted scaffolds you can essentially encapsulate 10 to the power 5 to 10 to the power 7 number of cells. this is one such examples that I am showing you that biodegradable scaffolds for urethra tissue engineering based on 3D printing. This is published in ACS Applied Biomaterials.

here people have used 2 different polymers polyglycolic acid and polycaprolactone. what is the use? Use is that PLGA is highly biocompatible but it is not stable mechanically. addition of PCL makes the scaffold biodegradable. make more harder and good biocompatibility and then TEC content in actually significantly improve the elongation and break. you need to have strength ductility combination.

Elongation and break is essentially strength ductility combination. this is the mass ratio of PLGA to PCL like you know you can go from 100 is to 10 to 50 is to 50 that kind of ratio and this is the Bad compatibility. if it is a good compatibility like reactive compatibilization that means both the polymers they are compatibilized using one of the linker that is the TEC that they have used. And how this is processed like you have a sacrificial template and where they have made particular solution with a particular ratio of PLGA, PCL and TEC, you have water, you have a PLGA, PCL and TEC and you have a sacrificial template, then when you get this kind of structures essentially you get this kind of a structures here like a tubular kind of structures. And then tubular kind of structures you can compress, you can squeeze, you can stretch.

then the moment you remove the forces essentially the structure goes back to its original shape and size. this is kind of a uniqueness of this kind of a structure as it is been shown here. This kind of different mechanical actions essentially allow the structure to be deformed in this particular case like stretching or compressing of the scaffolds. I repeat this is the case for the PLGA, PCL and TEC and the role of TEC is to compatibilize that PLG and PCL which is otherwise non-compatible and then they have used that sacrificial template technique. for fabricating the artificial urethra.

This is another one is this case for the 3D bioprint of urethra of PCL-PLCL blend and they fabricated PCL and PLCL blend base spiral scaffold as outer layer and cell laden fibrin hydrogel as the inner layer. this is to mimic that natural urethra of rabbits and with the help of 3D bioprinting tissue engineered urethra for large number of patients could be produced and their diameter was bioprinted urethra 2 centimeter with outer diameter 4.7 millimeter and lumen diameter 3.2 millimeter. And long biodegradation time and poor elasticity was overcome by adding PLCL.

this is like how researchers have utilized CT scan image processing for the 3D bioprinting of urethra. A to C is like a CT scanning image, CT scan image of the urethra. then from D to F is the CT scan image of the urethra field with contrast agent. And then you have a G that is 2D slicing, this is the G is the DEFG this is essentially A, B, C, D, E, F, G, H. So, G is your 2D slicing of urethral design made by West Forest Institute of Regenerative Medicine, United States, their printing coding program.

And then 3D rendering which was given in the urethral design approach that is in age. this is the work that was published by Anthony Atala's group from Wake Forest Institute of Regenerative Medicine. Now, they have used the 3D bioprinting by using polymer and cell laden hydrogel and they have crosslinked the hydrogel and this crosslinking, you can see that after crosslinking, this is the crosslinked hydrogel. And then, bioprinted urethra, they have put it in the culture medium. that is in C, this is the culture medium and 3D bioprinter with one polymer syringe and two hydrogel

pumps and then this is in the D and E and then 3D bioprinter scaffold part with polymer nozzle.

this is the one that they have used. this is the structure that they have made in the 3D different design structures and you can see this is a simple cylindrical structures but they have also utilized a different corrugated or certain porous or lattice structure they have introduced or this is the kind of a different design with spiral design or columnar design or spiral design or native rabbit urethra. this is the rabbit model. this is how one can do this 3D printing from lab to clinical or preclinical translation at least. you started with different type of scaffolds like let us say Gelma, Pegda or different urologic cells and then also you can utilize either separately polyurethane nanoparticle, gelatin and SMCs based bio-wings.

two different kind of bio-wings you can print it or coaxial printing, then you can essentially construct either urethral graft. or bladder patch and then that you can put for bladder patch for the urethral augmentation purpose or urethral graft for this particular graft regeneration reconstruction purpose and then you can put it in the rat model for the preclinical studies and so on. Now, this slide is very important and this slide is mostly related to the extrusion 3D bioprinting related things. I will spend lot of time on this particular slide because I want to go slow so that you understand that what are the key challenges and why there is so much scientific activity, large number of research papers are being published in the field of 3D extrusion printing, in the 3D printing but I am not aware of any hospital in this world which uses extensively 3D bioprinting for the patient care treatment and they are essentially taking the CT scan image of the patient. In the same hospital complex, they have 3D printers.

They print the structures for the soft tissue reconstruction or regeneration and they are utilizing that one instead of autograft. therefore that one of the major challenges is the translational research for the patient care. this is the center of the slide you can see this is the standard 3D extrusion printing, this is the schematic illustration essentially you can grow different type of structures depending on the properties of this particular hydrogel or depending on what is the kind of 3D printing parameters that you are utilizing in this particular case. The first and foremost is that bio ink development, now bio ink development there is that material properties. let me explain these things in a much more with more examples.

there is a batch to batch variability, earlier you have learnt that you have a gelatin and then you transform gelatin into gelatin methacrylate, Gelma. And this gelatin sources can be either porcine or bovine or fish cartilage. Now whatever sources that you do when you synthesize the gelatin. and you essentially make some 20 microliter or 50 microliter or 100 microliter batch. Then you do this gelatin to gelma because gelatin you hardly can use it for extrusion bioprinting, you have to use a gelma and why gelma that has been also emphasized in the earlier lectures that it has RGD sequence which serves as a cell attachment sites if you use gelma as a matrix for many tissue engineering scaffolds.

that is all understood. But what I am trying to point out here that for example, one batch you produce 100 microliter of a gelma. Then another researcher is kind of following the identical protocol to produce 100 microliter gelma from same source. Let us say we are talking about the

porcine source. from some same porcine source, porcelain cartilage, when you are making this Gelma, there is a problem of the reproducibility or variability, reproducibility of the either degree of substitution like how much is the degree of substitution when you produce Gelma or the Gelma when you produce what are the viscoelastic properties of Gelma, what is the G prime, G double prime values, how the temperature sensitive are those properties because all those things are important for the 3D extrusion printing. Now, in so many large number of research papers people use.

different type of hydrogels like Gelma let us say we have I have shown you that Gelma, Pegda these are like secondary addition for viscosity modifier Gelma, Pegda and sometimes people have used hydroxyapatite or carbonaceous filler like carbon nanofibers and so on to make it is a electroconductive hydrogel ink and so on. the point that I am trying to mention, the moment you make multi-component hydrogel , this particular batch to batch variability would be even more challenging. you essentially end up experiencing more challenges in terms of reproducing the properties or in terms of reproducing the different viscoelastic properties and the gel properties of this Gelma based hydrogel ink. And that actually leads you to the difficulties in producing 3D printing compliant viscoelastic gels and 3D printing biocompatibility, 3D printing and shape fidelity compliant structures. you have learnt that you know in some of the earlier lectures there is something called shape fidelity compliant.

shape fidelity compliant structures. the point that I am trying to make let me summarize now that even for the basic Gelma when you scale it up, when you scale up from 10 microlitre to let us say 100 microlitre to let us say 500 microlitre batch. Reproducibility is a problem. Reproducibility is a problem for the multicomponent hydrogel. therefore, when you go for the patient care, it is not that you just prepare one scaffold in the most regulatory compliant manner and then you are successful in the use of this scaffold in for example, for one particular patient, but when you try to produce the same scaffold in a multiple numbers with unique set of properties or the identical set of properties you will experience challenges and it needs to be done in a GMP compliant facilities. for research publication point of view, it is all nice because you are making 5 samples or 10 samples and then these 10 samples can be test samples.

It does not need to be clinically relevant structures that you are essentially implanting in the human patients. And then it does not need to work on the physiologically complex micro and physiologically complex environment like inside in human patients and so on. therefore, for the simple laboratory testing, in vitro model, even preclinical models, these are all fine. But the major challenge when you go to scale up.

cell viability. In the last slide, I think I mentioned about, last to last slide I mentioned about these cell numbers with common bioengineering, biofabrication methods. And here I have mentioned that typically 10 to the power 5 to 10 to the power 7 number of cells can be encapsulated using some of the specific hydrogels to make the bioprinted scaffolds. Now, these 10 to the 5 to 10 to the 7 number of cells can be put and then what I am saying maintaining high cell viability and functionality post printing. And that actually is very challenge because this extrusion essentially introduce this 3D printing extrusion by the very process here it will introduce different kind of a

stress field. And then cell viability is compromised because cell viability is compromised because of the different stress fields, suppose you are able to load 10 to the power 5 to 10 to the power 7 number of cells in a hydrogel scaffold.

What would be the guarantee that it is more than 90% cells are viable? And, once viable, they can perform their intended physiological functions. For example, if it is osteoblast, then if it is OBs, then it would be leading to bone formation, for example. this is also important. Then comes resolution and precision. Now this resolution and precision and this one if I go back to one of the earlier slide, this resolution and precision depends on the minimum feature size like you know what is the minimum feature size here 1 to 10 micron, 100 micron, 1000 micron.

What we have learnt from this particular case that you can essentially build large volume structures but with coarser feature size. coarser feature size is like 100 to 1000 micron and so on. I will come back to this in the next class because this will take long time in this particular case like resolution precision and large volume 3D to 4D bioprinting here and that we will have more time for this explanation. Thank you.