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

Prof. Bikramjit Basu

Materials Research Center, Indian Institute of Science, Bangalore

Lecture 58

I will continue in this lecture on this one of the emerging opportunity in the field of additive manufacturing and that is the bioprinting in space. I have already introduced you to the subject in the previous lecture. I have also discussed to some extent international perspectives and now I will start with the storage of bio-inks and also challenges in the microgravity conditions. Before I do that I will just mention this, this is one of the attempts made by Netherlands based 3D printing company that is called Felix printers organization. they have actually manufactured Felix bio printer. And they have essentially manufactured bioprinted they have manufactured the artificial cartilage.

that was possible by simultaneous deposition of two cell laden materials into any desirable tissue structure. It was funded by European Union EU Horizon 2020. And it was with a collaboration with Technical University Denmark, DTU. And low payload and capable of space bioprinting offered by ESA.

Now if you look at the other attempts like NASA's BFF biofabrication facility that is mission CRS-18, CRS-19 and CRS-20, this is the NASA's launch program and they have used, this is the TechShot, right, this company I have mentioned before. This is a microdispensing bioprinter, they have developed and they are essentially trying to see that whether bioprinting of soft blood vessels or muscle tissue can be printed And in fact, they demonstrated that these tissues of length 30 millimetre length, 20 millimetre width and 12.6 metre height, heart muscle tissue was printed using this BFF facility. And after printing and tissue culture for 45 days, it was returned to earth using a SpaceX cargo Dragon capsule. So essentially they have shown that long term tissue maturation was experiments that was conducted for more than 6 weeks like 45 days right and this is supposed to be very very significant experiments in space and then after that long term experiments they sent the mature tissue to ground facility to see that whether the tissue will have similar kind of tissue architecture and functionality like the same if it was grown on the under 1G conditions.

Now this is that again as I mentioned that TU Dresden that is a ESA recognized European Space Agency recognized center for bioprinting facility and they have used that ESA bioprinted skin and bone samples and as I mentioned before European Space Agency was actually interested to see whether the human blood plasma can be used as a nutrient rich bio ink. and then calcium phosphate also used as a bone cement material for the structure supporting material and methyl cellulose MC and alginate. This was one of the hydrogel materials which was widely investigated for the space bioprinting purpose by that Michael Gelinsky's group and they have essentially used that upside down approach and then upside down approach you essentially approximate 0.38 times g that is the microgravity 0.38g microgravity conditions that you can simulate in this upside down bioprinting facility.

And these are close up of the 3D printed growing tissue, bone tissue that you have seen before. this essentially allows one to explore new opportunities in space like semi-automated fabrication of 3D cell and tissue constructs and less crew time is required. Then bioprinted tissue constructs whether that mimic the real tissues

better than conventional 2D culture and easy combination of several cell types. several cell types or materials for manufacturing of complex tissues and organ models. Then printing with cell aggregates and organoids is also possible.

Microgravity might facilitate the printing of hollow objects and should allow the utilization of low viscous bioinks. This is also another possibility that I have to use. Applications like tissue models for research, treatment of engineered astronauts and life support system like whether algae and plant cells can be grown in the hydrogels and they can essentially be used to create oxygen rich environment in the space shuttle or in the space vehicles. this is like 3D bioprinting, this is from one of the papers published in Biofabrication 2020 and again European Space Agency supported the TU Dresden for the microgravity things. What you see here that is the 3D bioprinting for example, micro extrusion or inkjet, this is the most feasible and bioprinting facility that can be utilized in the close to earth.

Now as the distance increases, duration also increases, radiation also increases and risk also increases. what it means that from earth as the distance increases, so all the other factors like space duration, the cosmic radiation exposure and risk also increases. what I was talking about ISS, International Space Station, this actually is operated here and then you are operating in the lunar vicinity that is proving ground in 2020s and after 2030s leaving Earth-Moon system and reaching Mars orbit. This is how this entire plan has been done in a very ambitious manner and what are the things that we can do is that you can essentially manufacture internal organs, bone and cartilage and skin. Skin is the first thing that people tried.

Emergency return is not possible in a very very long mission but short mission it is possible and whether the surgery in situ can be done you know on board so that using that all the surgical tools. In the 3D printing case whether one can have this cast and spleens and then other kind of implant 3D printed products that are available for certain medical treatment. This is 3D biofabrication facility as I mentioned before in July 2019 it was launched in International Space Station. What you can see that astronauts on board and these astronauts this is August 13, 2019 and this is a 3D bioprinter of human tissue now available for research on board and International Space Station National Laboratory. This is another paper on the 3D printing biofabrication in microgravity by Michael Gelinsky along with several of his co-workers.

One of them is Marcy Zenobi Wong from ETH Zurich. And in this paper what they are trying to see it is a more state of that review paper like opportunities, challenges and possible applications in space. here what you see bioprinting is capable of producing clinical tissue models. whether it can support the autonomous medical treatment options for astronauts in future long term and far distance space missions. This article discusses opportunities but also challenges of operating different types of bioprinters under space conditions mainly in microgravity.

And also this environment can help overcome problems such as cell sedimentation in low viscous bio-inks. This is the aspect that I have also mentioned before. this particular slide which has been shown to you before. Now why I am showing this specific slide here just to refresh your memory that these are some of the printing process parameters which are involved in extrusion bioprinting for example. What are the parameters like pressure, hydrogel based bio ink composition, the rheological properties.

The rheological properties is one of the properties that are bound to be influenced under microgravity conditions. Rheological properties means, viscosity, yield strength, yield properties, shear thinning behavior and

post-printing recovery. Here, two aspects I must mention. One is the shear thinning. and one is the thixotropic properties.

Now these two properties I have essentially shear thinning and thixotropic properties. These were emphasized in earlier lectures in this NPTEL course but in the context of extrusion printing of soft hydrogels and where I have mentioned categorically that any bioprintable hydrogel must have shear thinning properties and thixotropic properties, Sheer thinning properties like how viscosity is decreasing with time and Thixotropic properties essentially also the similar things whether with time you know how the viscosity also changes. Then extrudability that is important but these particular properties also influence the extrudability like what is the pressure, flow rate, nozzle type, temperature, printing time, filament stability. Another important thing is the buildability with respect to CAD design. Buildability depends on what is the cross-linking parameters, what is the layer height, line width, line spacing and subsequently the key things in the microgravity conditions is the self-fit in the post-processing.

As I mentioned to you in one of of the specific slides or this kind of cartoon that when a cells you are encapsulating within the hydrogels and then you are applying pressure then what is very important under stress whether the cells will experience large amount of stress so that cell viability can be compromised or not. And this is like cellular functionality, biophysical properties of the bio ink and scaffold printability. Whether all these parameters, how microgravity conditions and cosmic radiations can have a significant influence on these properties. For example, it is already expected that for in case of inkjet bioprinting. Differences are expected due to microgravity at B1 like you know sedimentation of cells and then cell B2 droplet and then B3 is the construct.

So, B3 is the construct. this is the layer by layer fabrication of hydrogels with cells and you can see that is much less monolayer that is kind of much less layers which can be built under the microgravity conditions. I have told you the viscosity of hydrogels here is important, viscosity of hydrogels is important in this kind of inkjet bioprinting experiments. Now you need standard cell culture for example 1G conditions like 5 minutes cells are floating and in the 5 hours cells can be sedimented In the standard cell culture in microgravity we cannot expect much of the cell sedimentation. this is the 3D cell culture under earth gravity.

This is inherent tendency of cell sedimentation with time affect the uniform cell maturation process. Under microgravity, easier tissue culture without cell sedimentation and better cell-cell and cell-material interaction, mostly gel-based media encapsulating cells. Now, what are the potential advantages like less shear and turbulence? Cell viability remain uncompromised. Then retention of design dimensions with high resolution and accuracy and homogeneous cell setting and controlled proliferation. And bioprinting disadvantages, extremely expensive technology and maintain enough blood supply during the surgical operations, this is the different integrated vials and perfusion system involving terrestrial procedures.

For example, in the terrestrial procedures what you do is that you take the cells and matrix and what we call bioresins. bioresins is the term essentially cover cells and matrix together. Then, you have a vial for tomographic printing. It is like you open the one-way valves and then you get the air extracted and bioresin is filled in. Then vial for xolography and flight, now you have a perforated caps on the top.

Then you have a perforated caps on the top and then you have a one-way valves and then air is extracted and refrigerated or cryopreserved until used. this is the case for the terrestrial procedures. In the extraterrestrial

procedures, this resin constitutions to be stored under normal refrigeration 4 degree Celsius or cryopreserved and printing vial preparation for tomographic projection, xoography and flight fabrication in the extraterrestrial procedures. what you see in the tomographic projections with vial rotations, let us say this is human heart. you can essentially slice, then after the slices the filament light projection system you can use and then use a resin removal and media perfusion.

You can see this is the heart model and then use the gas exchanger incubator 37 degree Celsius and CO_2 5% CO_2 atmosphere. But the question is that you can essentially create the miniatureless heart as an organ under microgravity but the thing one has to very clearly see that what would be the functionality of the hearts being printed under the microgravity conditions. Because under the ground conditions still 3D bioprinted hearts have ever been implanted in the human patients during the heart surgery. it will be most interesting and very important for us to explore the microgravity printed heart, whether it can be used or whether it can be implanted during heart surgery for astronauts on space. The second one is that in-situ data mining for intelligent bioprinting.

What is the objectives? Objective is the first time right bioprinting, automatic check of the defects, improved cell viability, process optimization. Now, if you have the sensing platform with the layer height analysis can be done and one can monitor extrusion pressure, velocity, temperature and standoff distance. This is for example let us say 3D bioprinter like typical model printed by cellink. And layer wise analysis you can do monitoring geometry. monitoring thermal signature and monitoring cells related information and monitor cell fidelity.

what it shows the layer by layer fabrication means you start with layer 1, layer 2, layer 3, layer 4 and go to layer n. So, you can have a data mining platform like a feedback control, then you can have a digital twin like some of the AIML approaches and then you can have data on map of the defects of the 3D printed scaffolds, deviation from normal trajectory and thermal deviations as well. Post printing maturation and tissue characterization to space. this is a multi-step process, it is a large process footprint. First, you have to do the bioprinting.

Now, 3D printed tissues can be transferred to the 3D bioreactor in the feeding vessel. Then, it can be given this manual transfer to histology and cytometry. tissue bioprinting, tissue cultivation and post-culture analysis. In contrast, in the International Space Station and in the confined printing conditions, in the 3D bioprinting in space, you can have everything together in one integrated setup.

and that is much more challenging. You can see, you can have a bioprinting at the top here, you can have a instrumented vessel and you have a perfusion bioreactor for tissue maturation. That is what it is called all-in-one bioprinting cultivation and characterization device. And non-destructive analysis can be done in an online monitoring purpose like spectroscopy or MRI and ecography can be done to see the tissue structure characterization. This is another paper from Michael Gelinsky's group that is on the bio-inks for space mission and this paper is very important for bio-ink storage. In the bio ink storage, they have done these experiments using alginate methyl cellulose hydrogel blends and these blends with either green microalgae of the species chlorella vulgaris with different human cell lines.

And what is the human cell lines? One is the human mesenchymal stem cells that is HMSE, SaOS-2, HepG2 and primary human dental pulp stem cells. And these bio-inks are filled into printing cartridges and stored at 4 degree Celsius up to 4 weeks. What are the major conclusions? They observed the microalgae survived the

storage period very well and there is absolutely no loss of growth of the microalgae or the functionality. However, significant decrease is visible for human cells varying between the different cell types that what are the 3 or 4 cell types they have used. If this has to do something with that what are the challenges in space bioprinting because space flight has limited capacities for bringing payload into space that is one thing.

It also involves significant cost to each mission with the bioprinter and difficult in bringing sensitive or fragile components into space. What is the biggest problem? It is sending live cells whether encapsulated or cultured media to space. And is cryopreservation of the cells is the answer because it certainly creates a necessity of several manual process steps after arrival before such cells could be further used. And what is the solution? That experimental results by Michael Gelinsky's group has shown that long term storability of premixed bioinks is technologically feasible and it also allows to store such cell laden inks for several weeks at 5 degree Celsius without complete loss of cell viability. This is the experimental setup that you know that you have a control is without storage like room temperature 30 minutes and you have the bio-ink as I said that alginate methyl cellulose bio-inks with cells and what are the cells either SaOS-2 that is the microalgae or one type of human mesenchymal stem cells, HepG2 is the hepatocellular carcinoma cell lines, DPSC, dental pulp stem cells,SaOS-2 like the sarcoma osteogenic cells line and this is like a immortal, these are like immortalized human cells, those are used.

bioprinting is done, then cultivation is up to 4 weeks in culture and then analysed like in terms of cell viability, DNA content and cell activity. And entire bio-ink storage was done at 4 degree Celsius. As I said that microalgae laden bio-inks is most relevant for potential applications such as a live support system. Visibility, viability, cell growth and photosynthetic efficiency of these kind of bio-inks, this was carried out with triplicate samples and also cell growth was done at 1, 3 and 7 days of cultivation and photosynthetic efficiency. essentially chlorophyll content, photosynthetic efficiency.

Now, if you see day 3 to day 7, there is an increase like the more the storage or cultivated samples see vulgaris, then it shows the more photosynthetic activity. Then comes that C, this is the C part, this is the vulgaris laden algin methylcellulose were used as a bioprinting immediately after preparation at different time for the biowings storage. The biowings are brought to room temperature before bioprinting. And this is like bioprinted scaffolds after week 4 of the storage what are the different structures and if you look at this all these structures at week 0 certainly this is much more resolution is much better. Week 1, week 2, week 3, week 4 is almost like comparable that is not significant difference at the week 2, week 3, week 4 compared to week 1 scaffolds.

Now, challenges in the extrusion bioprinting, extrusion-based bioprinting microgravity. essentially what is the critical role of microgravity? It is expected high Ohnesorge number. Now, what is Ohnesorge number? It is one of the important dimensionless number which is used in fluid dynamics and I would like to remind you that you know that this is the one that I have mentioned in the context of binder jet printing. You remember in the binder jet printing where I have given you 2 scientific case studies, one for titanium 6 aluminum 4 vanadium using 2 different type of binders. One is a starch based binder and one is a in situ cross linkable binder and these two binders what we have done we have developed more quantitative understanding of the process science and first step is that we essentially characterize the ink flight.

in terms of the dimensionless number and these dimensionless numbers are Reynolds number, Weber number and Onnes-Schorcher number. And then subsequently we have extended our quantitative modeling efforts to see what is the capillary infiltration and then using Washburn model we have quantitatively analyzed. And third

one is the in-situ polymerization that is DWS approach. Ohnesorge number was defined as the viscosity divided by square root of surface tension multiplied by density multiplied by nozzle diameters. if you remember this particular shadowgraphy experiments and then this is that ink is being deposited or it is on its flight then you can calculate that what is the diameter in the droplet diameter distribution like 50 micron and all.

And then you can use this is the different softwares and one of the softwares we use a lavision particle master analyzing software. Reynolds number is V rho A by eta and Weber number is V square rho A by gamma. in the Reynolds number surface tension was not involved but Ohnesorge number surface tension is certainly viscosity is certainly involved. viscosity and surface tension both are involved. if you go back to this particular slides and let me spend some more time on this particular slide that what are the challenges.

second one is so, enhanced printing accuracy like optimising wettability is crucial for precise 3D bioprinting. Then process parameter optimisation like force analysis understanding the effect of printing speed, layer height, nozzle size or net force acting on sample. is essential. Simulation tools like advanced bioprinting simulations can predict optimal process parameters and printability windows. Then, fluid delivery system like system selection, the superiority of volumetric feed control systems like syringe pumps over pressure driven flow systems in microgravity.

Now, optimizing inkjet or drop and demand printing in microgravity. Now, gravity on earth still limits the inkjet printing capabilities with well-known drawbacks and that could be somehow leveraged with microgravity. For example, material viscosity. Low viscosity leads to cell sedimentation whereas microgravity prevents cell sedimentation. Enabling higher cell densities and then you remember that a typical number of cells that can be bioprinted without compromising the cell viability is one of the important challenges or presents one of the important challenges.

Homogeneous bioink distribution. Now, static yield stress like low static yield stress restricts the height of the printed structures. buildability. Microgravity reduces gravitational forces and taller structures with lower viscosity materials. Surface tension like larger droplets extruded in microgravity increase surface tension forces and potential for larger structures. Printing process with microgravity like working distance, droplet behavior is very unpredictable and also it requires new experimental parameters and increase cell viability is very important.

Liquid control, droplet management and new hardware, printing speed, slower liquid motion in microgravity, reduced droplet ejection frequency, droplet shape, spherical droplets in microgravity and surface coverage compared to earth. Third one cell viability, cell behavior microgravity like cell adhesion, absence of buoyancy microgravity and challenges in achieving uniform cell distribution within printed constructs. this brings me to the end of this lecture on the microgravity or space bioprinting. what we learnt that one has to carefully understand scientifically the influence of microgravity and cosmic radiation on the hydrogels rheological and viscoelastic properties as well as the combined effect on the cell and tissue behaviour and their maturation. Second challenge is that to design and develop payload compliant bioprinting setup.

with considerable reduction in size and weight along with accessories. And third one is that whether one can develop ground based automated system so that from the ground scientists can monitor the microgravity printing processes and also this can be tuned with data mining approaches so that AIML also can be adopted for futuristic predictions or projections of the space microgravity. There are many challenges but the most

satisfying promise is that space bioprinting can potentially allow the culture of the microalgae and that is very interesting for the life support system for the astronauts. Space bioprinting has already demonstrated its success to some extent on printing cartilage tissues, on printing beating hearts or skin lesions or skin tissues.

which can be used for astronauts. There are different potential approaches can be explored in the future space missions where astronauts can carry their own isolated cell samples along the CT MRI scan and if needed whether those CT MRI scan can be used to print the astronaut specific tissues which can be implanted without significant medical interventions All in all this particular topic holds a great promise from both scientific research perspective as well as medical healthcare perspective for the astronauts on space shuttle or spacecraft. Thank you.