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

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Lecture 57

Welcome to this NPTEL lecture on additive manufacturing. In today's lecture I will be covering one of the most interesting topics. and this topic that I am going to cover is bioprinting in space. this is one of the topic that comes under emerging opportunities which I have promised earlier to cover as part of this course. bioprinting in space has become most relevant as number of countries in the world have begun to explore space, have begun to explore the signature of life in space and also from fundamental scientific experiments on space. A few attempts have already been made in International Space Station as well as by European Space Agencies to explore the idea of space bioprinting and to see whether simple tissue constructs can be manufactured under microgravity conditions.

In this context, I am going to first introduce this topic. This will be followed by what are the scope and opportunities as well as the challenges of bioprinting in space. particularly for the storage of bio-inks in the space microenvironment for longer period of time as well as what are the possibilities of different organs to be printed and some international perspective. Like what are the attempts already being made and also some of the published studies primarily from one of the German group one of my long term collaborator Professor Michael Gelinsky from Technical University at Dresden in Germany.

that some of the papers published by that group also will be discussed with certain with specific details. I will close this particular topic by mentioning the challenges in the microgravity bioprinting this is the typical topics to be covered and what are the keywords that are relevant to classify this kind of topics in the overall context of the course. need for space bioprinting, so human settlements to moon and manned mission to Mars need essentially improved medical treatment because in every manned mission a few astronauts are part of this mission. and it is quite likely under such extreme environment the astronauts can experience space trauma or can experience accidental medical treatment. we need to have facilities on board the spacecrafts or within the extraterrestrial settlements because of the fact that there is no first option to return to earth for any medical treatment, it is not possible.

this kind of space mission should be ideally self-sufficient in terms of providing onboard treatment options to the astronauts in space. So, what are the major concerns? Some of the major concerns I have highlighted here, for example, influence of microgravity, particularly altered gravity conditions. Altered gravity conditions mean on Earth, whenever you do 3D printing, in whatever route then the material and biomaterials and cells always experience 1g conditions this is that under gravity conditions. But when you go microgravity conditions it is not necessarily that you know when the space shuttle or when the spaceship they travel across the different layers. then it is not necessarily that they will be under constant microgravity conditions, let us say 0.

2 g, let us say 0.5 g, it is not possible. even dynamically that gravity conditions will also change with time, right and the way this will move into the space and also cosmic radiation. and this what is the combined influence of microgravity, altered gravity condition, cosmic radiation on cells or tissues in 3D tissue models. Second one is that we not only need to adapt technology but also materials and also maturation to cope with hard space environment with necessary reduction in size, weight of equipments and accessories.

In the space technology, there is something called payload. Payload is something is the total payload of this entire spacecraft or space vehicle that is one of the boundary conditions for this kind of space mission. And then 3D for example, when you talk about space bioprinting, we are essentially talking about the bioprinting machine as well as accessories. the size is a constant, the total weight or weight of this particular bioprinting machine as well as their accessories that also will be one of the boundary conditions. what are the scope and opportunities of the space bioprinting? For example, what is the potential examples? if an astronaut needs skin burns or bone damage.

Then 3D bioprinting in space could offer clinically relevant solutions from skin lesions and bone defects towards complex vascularized tissue constructs including internal organs like kidneys and liver. Then third one is a personalized treatment option. Now personalized treatment option that is quite interesting so prior to the launch if clinicians can isolate cells from a specific astronaut and also take the CT and MRI scan of the whole body of the astronaut and then these two things both the cells as well as the biocompatible hydrogel materials together with the CT and MRI scan can be sent along with the spacecraft. that will allow the need based patient specific scaffold fabrication using 3D space bioprinters. whenever the same astronaut needs that certain scaffolds to be printed using space bioprinter, now you have the CT or MRI scan image available, you also have the patient specific cells also available.

Only thing you need is the synthetic materials which have been tested on the ground facility for its potential biocompatibility as well as the long term stability in human clinical studies. only those kind of materials if you can use it as the biomaterial ink. and then use that astronaut cell as the biological materials to be encapsulated in the hydrogel. You take the CT or MRI scan image of the astronaut and then you consider this particular tissues for example, some bone defects that the astronaut has undergone, has experienced. these bone defects can be essentially printed and then the clinicians on space can utilize it whenever there is a specific need.

let us revisit although as I told you that you know some of the important slides or some of the important schematic diagrams I have been repeating at different time point in this particular course because of couple of reasons. First reason is that in this course is very much very much important not only for researchers in mechanical engineering. but also for researchers in bioengineering field, in material science, and also to some extent chemical engineers. therefore, many of the concepts and ideas are interdisciplinary in nature, it is better to revisit it so that it will refresh your mind in the context of a specific lecture. in the conventional concept of tissue engineering is that you have a tissue engineered scaffolds which have porosity of different sizes and shape.

You have target clinical application specific cells and these cells have been shown either it can be osteoblast or it can be fibroblast and so on. and some of them most of them are single nucleated cells. Then you have a growth factors and bioactive molecules because that will allow essentially the cells to undergo differentiation and in addition you can have functional nanoparticles also. Now this is like carbon based nanoparticles for example. these particular materials when it is combined with the hydrogel based scaffolds and then it will produce certain structure then you can give essentially mechanical, electrical, acoustic simulations.

And when you give this kind of mechanical, electrical, acoustic stimulations, the tissue maturation process can be accelerated. And then subsequently, these scaffolds you can implant in that bone defect model. This is the long bone and in the long bone defect model you can implant this particular defect model. This particular schematic is also has been shown couple of times in this part of this NPTEL course but again I am just showing you. Now the question is now you have a uncross-linked polymer and then this forms a hydrogels and then you can see the cells on that.

Another option is that if you use the bio ink that means these are cells right. These are biological cell you are encapsulating in the hydrogel. Now consider the case of the microgravity conditions and cosmic radiation. one has to essentially understand that how these two factors have a combined effect on the cell and tissues that will be essentially used or developed or matured in the course of 3D bioprinting. the stability of the cells and tissues also over a longer time period before they can be bioprinted and during 3D bioprinting in the space environment under microgravity conditions .

Then, what would be the stability of this total bio ink for example, in the 3D extrusion printing conditions and what will be the maturity, maturation of the some tissues that will be developed. These are like bigger scientific questions that one has to address if the space bioprinting missions need to be successful. differences between short term and long term space missions, for example, short term means that close to earth, for example, 0.16 g to microgravity environment. this mission duration is typically less than 6 months or greater than 6 months is that you know is a long term mission.

Distance to earth is kind of less than 10 to the power 6 kilometer. then crew size can be somewhere 4 to 5 and 10 to 15. logically if you see that if you have to investigate the feasibility of the space microgravity conditions then this is ideally the short term mission with 4 to 5 crew members would be first step to validate any machine or any specific scientific approach to be validated. medical care means medical first aid on site and serious injury like stabilization and return to earth. and then additive manufacturing of casts and medical orthosis and in-situ bioprinting of skin.

this has been quite relevant like in-situ bioprinting of small skin lesions that is something could be very very useful. What is the radiation exposure? Radiation exposure can be less than 400. Whereas, in the long term mission addition exposure can be greater than 400 mSv. Potential mission starts is like 2020s like you know this one and then in the long term mission when it is distance to earth is more than 10 to the power 6 kilometer and 0.37 g to microgravity environment that is a kind of a mission that is a microgravity conditions that this kind of long term mission will have and here the number of crew members can be as high as 15.

in the long-term mission, you can have AM of prostheses and tools for 3D printing, maturation and surgery and bioprinting of all relevant tissues. It can also be internal organs. And here, radiation exposure can be much, much greater than 400 mSv. And it is like, you know, much more futuristic and it is going to be explored somewhere around 2050 or after 25 years or so from this point of time. as I said short term mission most relevant is the bioprinting of skin In the long term mission it is all relevant vascularized tissues that would be of relevance these are the things that I am putting a star which can be relevant for the discussion in this particular lecture.

this shows that what are the possible medical applications of bioprinting in space. preparation wise what you need to prepare on ground and you have to send it along with this space vehicle is the cell isolation from the astronaut, then cell cultivation, then bio ink preparation. Then when it goes to the bioprinting case, you can have cell bioprinting, maturation of tissue and quality control. But third one is the maturation of tissue and recycling of materials that is in the surgery case. what has been mentioned here is that cells must be harvested from the injured astronaut and culture to produce the needed cell numbers.

Otherwise, what is the other approach I have mentioned to you before that cells from a specific astronauts can be isolated prior to the start of the mission itself space mission. And then the bio ink can be prepared and after bioprinting the construct commonly needs further maturation in bioreactor and in case of in-situ bioprinting cells should be printed directly onto or into the wound for example. why bioprinting in space? Because it provides us unique opportunities to print the living tissues under microgravity and cosmic radiation. this is the kind of the environment space vehicle it actually acts as a unique in laboratory like we all work in a research laboratory or we have been working or we have worked in the past in a research laboratory as a researcher to study the effects of microgravity and cosmic radiation on human tissues. in the space, the space vehicle or space shuttle that environment actually is a typical space laboratory you can say.

Then potential for tissue engineering like tissue grafts enabling autonomous medical treatment, then overcoming bioprinting related challenges like cell sedimentation in low viscous in low viscous bio-inks. often cell sedimentation takes place in the bio-inks with low viscosity. Bio-printing as a solution like autonomous medical treatment, clinical medical care by on-site bio-printing. Then, tissue regeneration, bioprinting tissues to address injuries and tissue degeneration. And third one is the improved mission success like enhanced medical capabilities for safety.

Medical challenges in deep space missions like severe health risks, risk for bone fractures and cardiovascular incidents and specific cancers. And second one is the limited medical resources which are available on the space vehicle and impossibility of immediate earth return that we have also mentioned. It is not only long distance but rapid medical evaluation not possible. So what are the tissue chips that perhaps can be available for space biomedicine applications and these tissue chips are mentioned here. One can be brain or neuron, another can be heart or vessel, third one can be kidney, fourth one can be lung, fifth one can be liver, sixth one can be bone or bone marrow.

this schematic essentially shows the bioprinting in space which is just a cartoon just to show you that what is possibly a dream but which can be made possible with the advancement in the technology. what are the challenges? Challenges is the handling liquids throughout the bioprinting steps like cell isolation, bio ink preparation, printing process and tissue maturation like how different kind of liquids can be handled. Now different equipments which are available like clinostats, and rotating wall vessels, these are suitable for cultivating bioprinted constructs but cannot accommodate standard bioprinters. Why they cannot accommodate standard bioprinters? Because size and payload weight limitation, payload weight can be one of the major limitations. What are the critical challenges? Very high cell numbers that are needed for fabrication of volumetric human tissues.

Then relevant tissue concerning the arrangement of different cell types because if you recall in the introduction to biological system I mentioned the definition of tissues. tissue essentially represent the specific arrangement of cells. with either single or multiple different cell types to provide the structure with a certain functionality right. like there is different type of tissues like muscular tissue, cardiovascular tissue and then endothelial tissue and so on. Then mechanical stability of the fabricated constant that is very important and this mechanical stability of the constructs will give biomechanical relevance for this microgravity printed constructs.

Fourth one is the functional and hierarchical arteriovenous systems and that can be microsurgically connected. functional and hierarchical arteriovenous system. Advantages of the microgravity printing, for example, less viscous bioings kind of which might not be possible in the normal gravity 1G conditions become applicable in extrusion-based bioprinting with the sedimentation of cells or bioings with Solid additives like calcium phosphate particles may not be suitable. In contrast, the altered fluid behavior might lead to the stable entrapment of air bubbles and the domination of surface tension forces can impair safe fidelity and lead to unintended deformation during or after the printing process. just to give you some example, NASA in United States successfully printed living tissue using the bioprinted printer made by TechShort and nScrypt on ISS in 2014, Cosmonaut Oleg Kononenko successfully developed human cartilage tissue and a rodent thyroid gland using organ.

aut in a bioprinter by the Russian sector on the International Space Station in 2018, just few years ago. In 2019, NASA planned to send a bioprinter producing beating heart tissue to the International Space Station. these are the few attempts that has been made. Now, as I said in the beginning of this lecture, I will be discussing some of the most relevant research papers published by one of my longtime collaborator, Professor Michael Gelinsky from the Center for Translational Bone, Joint and Soft Tissue Research based in Technical University at Dresden, Germany. what has been mentioned in this particular paper which is published in Biofabrication in 2020.

it is very clear that international space agencies have begun to explore the requirements for human settlements and moon and manned mission tooMars. And this particular, this kind of activities require improved medical treatment on board the spacecraft,   besides the utilisation of bioprinted tissue concept for the treatment of engineering students, it will become available for the three-dimensional tissue models for basic research. there is 3D tissue models for basic for example, concerning the effects of microgravity and cosmic radiation on cells and art. this article has clearly mentioned that European Space Agency sponsored bioprinting activity at TU Dresden. For example, accidental injuries are likely to happen during long-term space exploratory missions, as I mentioned before and extraterrestrial human settlements, right, that also I have mentioned.

liquid cell culture media handling and viscous hydrogel are difficult to handle in microgravity. These are concerns. Upside down bioprinting is the right way because if you have this normal 1G conditions bioprinter, you just put upside down and start showing that whether you can do bioprinting. Third one is the highly restricted loading capacity and limited availability of materials. That material stability, that hydrogel compositions, hydrogel stability or limited availability of materials that is also important.

At least in a semi-automated bioprinting technology and controlling the cell material and cell-cell interactions and controlling the structural integrity like shape fidelity. whenever you are printing a structure using cells, so there is a cell to cell crosstalk. Now, how cell to cell crosstalk happens in the microgravity conditions? Second aspect is that whether cell encapsulations and cell adhesion and cell viability under microgravity conditions, so these are some of the fundamental things in the cell biology aspects whether those are compromised and if they are compromised to what extent they are compromised so that tissue formation and the maturation as a whole also will be compromised, these are the questions to be addressed. approach to develop proof of concept here in this particular paper identify bio ink and their physiological as well as biochemical properties for microgravity. Identify microgravity based bioprinter specification experimental validation and space microgravity and required in-depth case studies and obtain independent confidence on ground surface or ground facility.

what European Space Agency in 2017 actually they proposed and Michael Gelinsky's group actually played an important role in this space bioprinting mission that European Space Agency proposed that plasma could be obtained from the astronaut for whom the tissue constant is being printed. And biopolymers such as alginate, cellulose, gellan gum, etc. can be isolated from algae plants or bacteria which can be cultivated in spaceship or the future extra- terrestrial settlements. essentially this plasma plus alginate or methyl cellulose or gellan gum, these kind of hydrogels constructs can be 3D bioprinted in the space environment. SpaceX was launched in July 2019 and the Falcon 9 rocket about 5000 pounds of bioprinting related accessories were sent to International Space Station.

And this was the fully automated mini refrigerator type bioprinter was developed in nscrypt and Techshot and that actually was accompanied in this particular space shift. And nerve cells and muscle cells was printed along with various protein molecules in international space agencies. This is like a selling bioprinter and this is a gelatin methacrylate printing. This is like UV cure and this is the mechanically robust gel. Now the question is the way all these processes take place under 1G conditions, whether this is possible? Whether this is possible under microgravity conditions.

Now Swedish Space Agency successfully launched a rocket that is master 14 to bioprint this one of the cell types and then to investigate the effect of microgravity on cell maturation process. Now Cellink is a Swedish company and Cellink has been selling this 3D bioprinting machine around the world. and it is a very well-known company. they use the Gelma gelatine methacrylate, methacrylate bio ink system using a selling Bio X printer. And this is the collaboration between Cellink and then Uppsala University in Sweden.

This is Another examples and you can see an astronaut already, this is a crew member, Christina Koch in the Expedition 60. This is organ.aut, this is the Russian developed magnetic bioprinter. This is launched to International Space Station on 3rd December 2018 and this is world's first experiment on printing organ tissue in space using magnetic bioprinter. What it does 2-3 mm human cartilage samples and thyroid gland was developed using scaffold free spheroid based systems under Chondrosphere and Thyreosphere self-assembly method.

This is Expedition 60 by NASA in ISS and biofabrication facility was essentially available to print organ-like tissues and what they have done that fabricated bone tissue was returned to earth surface for further study basically to compare that whether the fabricated bone tissue has structurally and also performance wise similar to that of the bone tissue which is made in the underground facility conditions. I will stop here and then I will continue with this further discussion on the international perspective on the microgravity conditions. And we will also discuss other published studies from Michael Gelinsky's group as well as I will conclude that with my own perception as what are the challenges of these space bioprinting conditions. and then how the challenges can be potentially addressed. Thank you.