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

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Lecture 60

In this penultimate lecture, I will be discussing some of the key points that I have covered over last 28 to 29 lecture hours in this NPTEL course. So, the topics or the concepts that I will cover or I will recapitulate and I will summarize from my earlier lectures include introduction to biological system and 3D bioprinting, some of the illustrative case study that I have covered on 3D extrusion printed hydrogels. And more importantly, I will be covering or I will be revisiting some of the concepts that I have taught in this course as part of my lectures on emerging topics which include artificial intelligence machine learning and space bioprinting and I will be Closing this lecture with key challenges that this particular 3D extrusion, 3D printing in general experience as far as the translational aspect is concerned. one of the things that I have emphasized time and again in this course. So, this is indeed a multidisciplinary course and it cuts across the different disciplines which include material science, manufacturing science, mechanical engineering, design, biological sciences and medicine. So, I have explained some of the key definitions and key concepts as relevant to this course at different point of time.

So, what I have covered earlier is that concept of cytocompatibility, biocompatibility and clinical validation. that structural organization in the human body if you see that this is a eukaryotic cells now different type of cells they come together they form a tissue and tissue has a specific functions on its own. Then next level of hierarchy comes organs and then organ system. And then different organ systems they function in a cooperative manner and that is essential for life of any organism that is the full organism.

So like say human body like humans or any lower order animals like monkeys and then rats and then rabbits and mouse and so on. So, whenever there is life in an organism, it must be due to the cooperative functioning of the different organ systems as a whole. So, this concept is very, very important that we must bear in mind that it comes with cells, tissues, organ, organ system and then organism. Then other things that I have taught that is more importantly from scientific perspective that whenever any material whether it is an implant or whether it is scaffold which is implanted into the organism that is bound to interact with the key components of the living system. And then key components of the living system is protein, then cells, then blood.

and then bacteria but we do not want biomaterials to support colonization of the bacteria that means it must have anti-infective property, it must have antibacterial property. At the same time, many of the blood components like platelets and so on, we do not want to get adhered or absorb to the biometer surface because all these things can lead to thrombosis. We do not want that RBC to be damaged. RBC has a very characteristic doughnut shape. We do not want RBC shape to be damaged.

We want proteins to get absorbed, we want cells to adhere and then cells to function on a synthetic biomaterial surface. When I mean by biomaterial, it is the synthetic material which may be of non-living origin and which is always fabricated, synthesized or manufactured outside the human body and for the purpose of the reconstruction and regeneration of the tissues. As tissues in the human body it is essentially placed inside an organism. So therefore the compatibility with the living system like which we call it is a biocompatibility like also compatibility with tissues that which we consider as biocompatibility that is very important. Typically, we grow one single type of cells, let us say bone cells, let us say fibroblasts, connective tissue cells, let us say cardiac cells and so on and biomaterials to assess that whether those cells are able to adhere, those cells are viable and those cells are able to perform that desired functions while adhering on the particular substrate.

So this is called monoculture. In today's world many research groups around the world they do co-culture of cells that means 2 or 3 different type of cells are being cultured at the same time. So, their you know culture media composition plays an important role. What is the ratio of the different type of cells which will grow on the biomaterial substrate that also play an important role. all those things are important.

Third concept is a biocompatible animal model, what we call in vivo study. In vitro study is a typical cell culture or protein adjacent study like the study or experiments which are conducted using glassware and also under simulated physiological context, those experiments are called in vitro study. But studies which are conducted in the full organism like in animal body or animal model or experimental animals, those are called in vivo study. So, I have categorically stated while taking this kind of this specific lecture. as part of this NPTEL course that if you do only cell culture on any hydrogel scaffolds or any synthetic biomaterials which is 3D printed.

not fair to mention the word biocompatibility, one can use the word cytocompatibility, cyto means cells, cell compatibility. If you do the test in an whole organism or animal model and then understand the compatibility not only with tissue but also with blood components and so on like serum biochemistry analysis and so on, so then you can call that you know that that particular materials have been tested for its biocompatibility. Third one is the clinical validation in human subjects. This is an example like in an implants are being placed in the jaw of a human patient and then we are seeing that you know whether this implant essentially encourages osteointegration right, integration in the jaw or maxillofacial cavity then it is called clinically tested. So, these entire things actually explains that what is the role of the study or what is the importance or relevance of the study of any synthetic biomaterial or any synthetic scaffolds or implants.

with the components of the biological system. Now let us move on to the 3D extrusion printing. This has been taught in the last lecture as part of the summary of this NPTEL course. I would like to revisit you to bring to your attention that you know this kind of soft gel, soft hydrogel when they undergo 3D printing, rheological properties are very important. And the definition of hydrogel is that it is a 3D network of hydrophilic polymers which can be cross-linked and which must have the ability to retain significant amount of water molecules.

So therefore swelling and biodegradation are very important for hydrogels that we have essentially considered as part of this course or in general hydrogel must have the swelling and biodegradation properties. Now in the context of the bio printing so essentially when you encapsulate the cells these are like different type of cells. So these cells are encapsulated or loaded into the hydrogen scaffolds and when they will experience this pressure one of the major challenges is that what would be the fraction of the dead cells. This fraction of the dead cells must be minimized and we want more and more viable cells. So I am put the double tick so that you know.

These interactions under the extrusion pressure should not compromise the cell viability. And once the cells are viable, it is important to know, important to see whether the cells are able to function as they are desired or what cells are able to express their biological functionality, that is also important. Other things that we have discussed categorically in the last lecture is that when we optimize the 3D extrusion printing parameters for any biomaterial inks, our aim would be to use those optimized printing parameters to see that whether this biomaterial ink containing cells or in other words bio ink can be printed into a complex structures to validate their buildability at the same time to validate that cell viability is not compromised while getting 3D extrusion printed. So this was the whole challenge in the field of 3D bioprinting community as such. So, just to see an example that we have as part of one of the case study, I have shown that when cells are being printed, when it is loaded into the 3D biomaterial inks or 3D bio inks and then in this particular case, I have shown HMSCs, human mesenchymal stem cells and live cells are stained in the green.

And, you can see that these cells along with the bio-wings were printed at 21 degree Celsius and extrusion pressure was varied 0.8 to 1.3 bar depending on what is the kind of composition of the hydrogel that we have taken. Cell density was 2 into 10 to the power 6. And depending on this is the 5G means 5% gelma, so you may recall that one of the major objective of all to demonstrate to you that we are able to print very low concentration of the gelatin methacrylal gelma compositions in the biometrial ink and bio ink formulations and in order to make this 3D printable we have used viscosity modifier, we have used secondary cross linker and so on and so forth.

So one of the things that I have shown carboxymethyl cellulose we have modified it and then we have incorporated into the 5% gel matrix in order to see that whether this biomaterial ink can act as a heart tissue replacement scaffold so we have also added you know 1% of hydroxyapatite very small amount of hydroxyapatite. We have also seen that in this particular class of hydrogel, we have used 5% Gelma with 5% Pegda. So what is the role of Pegda? What is the role of carboxymethylcellulose? Those things were essentially emphasized when I have covered in an earlier lecture. So what you see immediately right after printing, you can see there is a lot of green stain regions. So these are like live cells.

At day 3 and at day 7, The cell viability varies between 85 to 92%. So in the cell biology community or as such in the biometrics community, any cell viability is more than 80 to 85% of control that are considered as a good number to show that cells are largely viable. And when particularly in this particular case when the cells are encapsulated in the biometrial ink and they are being extruded and then this kind of numbers are quite good. This is another example of the Neuro 2A. This is the Neuro 2A cells.

This is for neural cells and then how these neural cells are viable and here viability can range between 80 to 96 percent. Printing temperature is again 21 degree Celsius. Extrusion pressure is varied 1 to 1.

5 gp bar. So, 1 to 1.5 bar that is the extrusion pressure and then these are like different gelma concentrations or gelma scaffolds like 7.5% gelma, 7.5% gelma with 0.1% carbon nanofiber, 7.

5% gelma, 7.5% PEGDA. and with that 0.1% carbon nanofibre and some of the biometrics scaffold we have considered is 7.5% Chelma, 7.

5% Pegda, 0.5% Zylengum and 0.1% carbon nanofibre. So each of these components has its own role in terms of Modulating the viscoelastic properties in terms of modulating the rheological properties to have the best 3D printability and buildability to enable us to obtain the clinically relevant desired structures. For example, either freestanding cylindrical conduit structure of 2 to 4 millimeter internal diameter and around 20 millimeter in length of the cylinder. So, one of the things that you notice after growing these 3D printing, 3D bioprinting and incubating them in a CO2 incubator, when we have imaged under the fluorescence microscope, at day 2 and day 5, you can see large number of green-stained live cells, that is the neuro 2S cells, that means cells are surviving or cells survive quite well in this kind of 3D bioprinted scaffolds. This is some another examples that I have also shown and discussed.

This is that 3A5G type of 3A5G scaffold that 3% sodium alginate, 5% gelatin. So, alginate gelatin scaffolds is another type of scaffolds that I have also discussed at length as part of this NPTEL course. And there we have used nanocellulose and cellulose particles. So, nanocellulose and cellulose particles when you have used like 1% of cellulose particles, the extrusion pressure is varied between 0.

2 to 1.4 bar and nozzle dimension is 22g. We get the layer height of 0.38 nanometer and so on and then you can see that how the filament test which is one of the test for this 3D printability. That gives the most acceptable results in the case of the 3A, 5G, 2C because the sac things are not there.

So, it is almost straight. So, that shows that these materials or these biomaterial inks can indeed be 3D extruded or 3D biobased, 3D printed. So, I have highlighted 22G that is the best nozzle size for good extrudability of the hydrogel scaffolds. Another thing that I have also emphasized time and again in this particular NPTEL course is that particularly in the context of 3D extrusion printing, safe fidelity is important. How you can this materials with different type of matrix structure, with different type of cylindrical structure or cuboidal structure and whether if you can compress it and release it whether this structure can be retained. So, you can see that this is the kind of very nice structures that essentially you can build with this 3A5G1C or 3A5G2C and you can see very clearly.

the inner diameter, right, inner diameter. And one can do microcomputed tomography analysis to essentially see that what is the three-dimensional structure of the 3D printed scaffolds. What I have also shown you that biocompatibility study particularly particularly when the scaffolds are placed. You can see this is the scaffolds, these are being placed into the intraperitoneal cavity in the subcutaneous region of these experimental animals and then we have seen and we have We have regularly monitored the health as well as the vital status of this animal up to 30 days and after 30 days these animals are sacrificed and you can see that full hair has been grown at the place of the implantation. And then I have shown you that what are the different things, how this body weight actually regained, body weight is increased with time and this is an uneventful animal study with the 3D printed scaffolds.

So, as I said some times ago that serum biochemistry analysis is total blood count and serum biochemistry analysis including bond, the blood urea nitrogen. Those things were also being investigated as part of the acute stage implantation and sub-acute stage inflammation at 7 days and 30 days in culture. And we have seen that many of the parameters like monocytes, neutrophil, platelet count, WBC, these all shows through a maxima at the 7 days of the study. So, foreign body response were carefully analyzed by studying that both that H&E stained histology images or MTA stained images and presence of foreign body giant cells for example and granulation tissue in terms of macrophages, fibroblasts and capillaries were investigated both at 7 days and 30 days. Wherever you can see MNGC, these are multinucleated giant cells, wherever you see So, that you know these macrophages and fibroblasts they are being also being indicated.

So, BV essentially stands for blood vessels. We have seen in many kind of places these are the kind of blood vessels. So, this is the kind of a blood capillary. It also shows signatures of angiogenesis in this kind of a histology sections. For both these, 3A5G and 3A5G-1C scaffold at 7 and 30 days demonstrated gradual healing response.

So, initially inflammation is there. Inflammation you cannot rule out whenever you put any synthetic materials or synthetic scaffolds in an animal model. One of the things that I have also mentioned very emphatically that these are the biodegradable scaffolds. What it means that these scaffolds will degrade over time particularly when you put it in that very complex physiological environment in any experimental animals. And this degradation can be essentially faster compared to what you study in the in vitro because you know there is a dynamic physiological changes that happens after the implantation with these scaffolds in an animal model and therefore we have carefully taken histology slices from kidney, brain, heart, liver, lungs, intestine, adrenal spleen, stomach and testes. So these are some of the vital organs that we have taken the tissue sections and see whether the original tissue architecture is retained and whether there is any abnormal see that we can observe in this tissue architecture and b that whether this particular tissue architecture shows kind of very acute level of toxicity because of the degradation products.

We did not see any abnormality, so therefore all tissues demonstrated healthy morphology without any noticeable alterations. The other things that we have carefully considered is the TNF alpha, tumor necrosis factor, alpha expression and this is a normal physiological reaction of the vascularized living tissue to trauma and invasion of the scaffolds. And then we have essentially shown you this is the particular region which we have essentially zoomed to show that what is the TNF alpha expressed region. in the 7 days and 30 days and then this is the 3A5G, this is the 30 days, this is the 30 days region and this is the 7 days. And then we have seen in both the cases for the 3A5G and 3A5G1C this kind of scaffolds they show inflammatory response but which is gradually reduced from 7 days to 30 days because if you compare for any scaffold This is the extent of this inflammation at 7 days and when you compare that intensity of this TNF alpha expressed region it certainly decreased, it certainly reduced.

So, essentially inflammation due to trauma and invasion of the scaffolds that is clearly reduced and that has been shown by TNF alpha. Angiogenesis, blood capillary formation, blood capillary, evidences of blood capillary formation, so that is, that was analyzed by CD31 expression and then here again 3A5G and 3A5G1C, you can see 30 days and 7 day scaffolds, how these different blue stained region that is increased with 30 days of the post implantation. And you can see that compared to 3A5G, 3A5G1C certainly has more CD31 expression that is essentially indicates vascularization density. Now, buildability of this alginate gelatin scaffolds and carbon nanofiber scaffolds, that was studied by essentially demonstrating the different type of matrix structures, not a very simple matrix structures, but these matrix structures have good shape fidelity. You can see you can compress it, you can press it, you can squeeze it and it can get back its original shape and size.

One of the things briefly I have mentioned that is that pseudo 4D printed conduits. So what you see here it is a 3D printed sheet. And this 3D printed sheet when you put it in the calcium chloride solution then you can see. When you can put in calcium chloride solution, then you can see it gets a cylindrical shape. And particularly the cylindrical graft it is retained and you can see this microcomputed tomography images.

This is very smooth wall and then also it is a through and through cylinder that one can construct in this pseudo 4D printed constructs. Now stress versus strain plots essentially show this is a tensile stress strain and you can see this tensile stress strain essentially the strength of the materials increased by having this 0.75% carbon nanofibers in the 3A 5G scaffolds. In the compressibility of these materials again this green one if you see it can be compressed up to 3 mega Pascal and up to 100% strain and then you can see that how the compression experiments were conducted in the microtester facility here. Now, what is the working principle of microcomputed tomography? We use X-ray source.

This is like, you know, up to 70 to 80 kilovolt, much more than that of the X-ray diffraction, that X-ray source that is used. And then, this is your sample which is rotated in 3 dimensions. You get, every time it rotates, you get these 2D slices. And these 2D slices, they are vertically stacked together, then they will form a 3D reconstructed volume.

And that has been clearly detected by Avijo software. So, these are the things that we have learned as part of the materials characterization also. This is another part of this animal study, we have shown carbon nanofiber based scaffolds when they are being implanted into these experimental animals, what are the survivability? All the animals survive, there is no mortality. The other things that why we have taken it so seriously carbon nanofibers? Because there is a widespread concern about the carbon nanofiber that it may cause toxicity Although we have used this carbon nanofibre as 0.

5% or 0.75% carbon nanofibre into this scaffolds. And we have carefully analysed the health status as well as these vital statistics or vital stats of these animals at day 1, day 7, day 15 and day 30. And you can see after this day 30, these animals this complete here. So, this complete hair recovery and also animals are able to move fairly well. So, these are the things that we have seen. So, full recovery of the hair growth and wound closure that we have observed.

So, like the earlier animal study case, here also when you have measured carefully at different time interval, the weight essentially shows a regular increase and it goes up to 450 gram of this particular animal. Moving on to some of the emerging topics, what we have learnt as per the emerging topics, I think I have spent lot of time in introducing these topics because I personally think that many of the students or many of the candidates who are taking this NPTEL course, they may not be having sufficient knowledge and understanding of the key concepts of data science. Again, as I said that it is a multidisciplinary subject, it cuts across various disciplines up to the computer science particularly data science aspects. So, therefore, it is not expected that everybody will come from the required knowledge or required domain knowledge when they will take this NPTEL course. It was my responsibility to introduce those concepts to a sufficient extent so that they can follow some of the case study that I have presented as part of this NPTEL course.

So, artificial intelligence, it is the science that uses algorithms and techniques leveraging computer to mimic the human intelligence. And machine learning is a subset of artificial intelligence whose performance improves over time as they are exposed to more data. So, more the quantum of data, better is the performance of any specific machine learning algorithms, right. So, this is the thing that we have emphasized, I have emphasized and we have learned. So under that machine learning, we have supervised learning, trained and level data.

For the unlevel data, we have unsupervised learning, datasets without level and then we have reinforcement learning. The goal is to take action to change the state so that you can receive awards, for example. Deep learning, here you use more quantum of data so that wherein multilayer neural networks learn from a vast amount of data. So, why machine learning is important in this particular artificial 3D printing. Now, what I have shown you in some of the case studies and what you have learnt as part of the science of additive manufacturing, like when you use certain set of 3D printing parameters, let us take the examples of the laser based 3D printing parameters.

You vary the laser power, you vary the layer height, you vary the powder feed rate or some of the other parameters and as a result of that what you see as an output that you know how the part quality or additive manufactured part quality that evolves for example whether that has some defects, whether they have some porosity, whether they have some cracks and so on and so forth. So, there we have seen that you know based on this particular set of additive manufacturing test parameters you get certain output. But this output to input this relationship is not governed by any empirical relationship not governed by any known scientific principle as such and there you have to learn the input data sets to see that how it can be correlated with this some of the output. So, therefore, it is most relevant artificial machine learning in the absence of any established relationship between dependent and independent variables. That is the message that I have explained in this particular NPTEL course time and again because I believe that one should know what is the relevance of artificial machine learning.

What are the basic workflow when now I have moved to the next slide on the machine learning model generic description. So you have the raw data. So you have essentially extract the features and output so in an unbiased manner. In essentially you distribute this raw data, datasets in the training and testing datasets and typically training to testing it is done is 80 to 20 ratio that has been mentioned time and again as part of this emerging topics discussion. So, you take the train, you train the different machine learning algorithms and different machine learning algorithms can be tree based algorithms, it can be different linear regression that is the most simplistic that people have been used for ages now.

But there are many new algorithms that are being developed every day around the world and these new algorithms can be tested, can be trained using the training set. And then you do this hyperparameter selection and validation, then you develop a model. Then you use the test datasets to estimate. And then you can also use in some cases validating dataset to tune the model. Then you have get the new instances, you can get the final model and predicted output.

So, first phase is extraction, second phase is training phase and third phase is prediction. So, this ultimately you get this is the predicted model based on the dataset strain, based on the how this you are training this, how you are training the different machine learning algorithms. The performance of machine learning algorithms depend on a nature and quantum of data sets. For the same nature and quantum of data sets, if the quantum of data sets increase, the different type of algorithms may perform better. Now the question is that what are the different performance matrices? The first performance matrices that what we have learnt is R square.

That is coefficient of determination. is equal to R square is equal to 1 minus RSS by TSS that sum of squares of residuals divided by total sum of squares. So, you have typically you get this Y versus X plot and there is a mean line, right. So, that it is possible and in reality it It always takes place these data are essentially distributed around the mean line in a random manner. So what is the difference between that value of the y value with the value of that particular x point or x location from the mean line.

That one is important. So, if R square is 0, that means there is absolutely no correlation between Y and X, this is the case. If R square is equal to 1, this is a perfect correlation, all the data points will be ideally located on this particular 45 degree line. If R square is 0.8 or 0.9, that means they are very closely distributed along both the sides of this particular line.

Another performance matrix is RMSE root mean square error which has been defined here. Then third one is the residual of errors. Residual of errors what we learnt it should show a Gaussian distribution. The maximum value should be close to 0 and you can do the residual analysis both for the training datasets as well as for the testing datasets and you should show then both the cases the residual the maximum density should be close to 0. So, for the good residual plot, it should be high density of points close to origin and symmetric about origin.

Best performance, R square should be close to 1, RMSE should be close to 0. So, these are the three things that we have learned to analyze the performance of this machine learning algorithms. Now, some of the case study that I have covered in this particular course and that I am summarizing. So, as I said that directed energy deposition case, what are the three parameters of relevance or importance in laser power, scan speed and powder feed rate, then you get the melt pool. So, I have shown you that when you use the 240 datasets, generated datasets and you can analyze what is the clad area, what is the penetration area, what is the height of the area, what is the depth of the area, what is the width of the area. Then you see that some of the tree based regression algorithms we have used like gradient boosting for example in 3 cases it shows it is a perfect combination of R square and RMSE which is the best.

But for the penetration depth for the same set of datasets it is penetration depth I mean I am talking about the penetration depth dataset it is the XG boost algorithm which performs the best like R square in terms of the combination of R square and RMSE values. So, it is a gradient boosting and XG boost, these are the two different type of algorithms which work best in case of this DED data sets that I have covered. In case of classification analysis like defect classifications, whether there is a lack of fusion or whether the intratrack porosity, same laser power, powder feed rate. scan speed and overlap percentage that has been shown and we have used the train confusion matrix this is some of the classification test confusion matrix. So, train AUCROC curve and test AUCROC curve this kind of analysis to show how this parameter how this data set can be used to predict that whether For an unknown or unseen data sets or for an unknown or unseen parameter combination like unknown or unseen combination of laser power or scan speed one can essentially predict that whether that will give the best quality DED track without any defect like lack of fusion intra-track porosity.

So now I think with these two examples you are able to see what are the relevance of this machine learning algorithms and how artificial machine learning can be used to minimize total number of experiments to minimize the cost at the same time develop our predictive capability to see whether this particular combination of parameters will give the best output either in terms of the good quality of the products or in terms of the desired combination of the track width or track depth or 3D printed track width or track depth and so on. Now, another topic that I have covered also to a reasonable extent is the space bioprinting. What is the space bioprinting? Like as you know that, for example, Indian Space Research Organization has been very successful in recent years, right, all of you are aware of that. So, at some point of time in near future, we expect human settlements in moon or manned missions to Mars that need improved medical treatment like space trauma or accidental medical treatment of the astronauts for facilities on board the spacecrafts or within the extraterrestrial settlements. This is because there is no first option to return to earth that is not possible, right.

It is no first option is possible. Now, what is the major concerns for the space bioprinting? What is the influence of microgravity or altered gravity conditions or dynamic changes in the G conditions and microgravity conditions or cosmic radiation on cells or tissues in the 3D tissue models? And also how one can adapt the technology and materials and maturation to cope with hard space environment and also to comply with the minimum payload and the necessary reduction in size, weight and equipments with accessories. What are the potential examples for the space bioprinting? What is the clinical relevance like treatment of skin burns or bone damages? 3D bioprints could offer clinically relevant solutions from skin lesions and bone defects towards complex vascular tissue constructs. And personalized treatment option like isolate the cells from the specific astronauts to create surrogate structures which can travel with the astronaut in space together with the CT and MRI scans of the astronaut, allowing the need-based patient-based scaffold fabrication using the 3D space bioprinter. So, if we use the astronaut's own cells and use it in a synthetic scaffold, that acceptability in that astronaut would be much more compared to the cells from other patients or other donors. So, here again we use the same principles, we learn that we have to first start with the cell isolation, cell cultivation and bioing preparation, then comes bioprinting and then comes maturation of the tissue and then comes the surgery.

So, these are the three things that we learned there. And why bioprinting in space? This is unique opportunities in space not only from scientific standpoint but also from the treatment of the astronauts like microgravity and cosmic radiation because space environment will provide unique laboratory to study the effects on human tissues. Potential for tissue engineering like tissue grafts, enabling astronauts, enabling autonomous medical treatment, overcoming bioprinting challenges like issues like cell sedimentation in low viscous bioings. And bioprinting as a solution like autonomous medical treatment, tissue degeneration and improved mission success. medical challenges in deep space missions like severe health issues, limited medical resources on board and impossibility of immediate Earth return, right. So, there is some of the many research groups around the world, they are working and then I have essentially I have shown some of the work being done by one of my collaborator, Professor Michael Galinsky's group at TU Dresden, who has a European Space Agency approved the laboratory at TU Dresden.

And some of the things that I have also mentioned that there is a possibility that we can develop tissue on chips. like brain or neurons or heart or vessels or kidneys or lung or liver or bone marrow that can be sent to the along with the astronauts and which can be used in case of emergency for the astronauts medical treatment. What are the challenges? Challenges include handling liquids throughout the bioprinting steps such as cell isolation, bio ink preparation. Clinostats and rotating wall vessels, they are suitable to simulate this kind of space environment but cannot accommodate standard bioprinter size and payload limitations. And there are other critical clinical challenges, very high cell numbers that are required, relevant tissue constructs, mechanical stability and functional and hierarchical arteriovenous system.

These are some of the clinical challenges that has to be addressed in the years to come. So, one of the other things that I have shown that in-situ data mining approaches for intelligent bioprinting like what is the layer height analysis using that significant amount of data that can be generated and what is the layer wise analysis under the microgravity. These are some of the things, these are for future research and data mining platform can be used particularly the digital twin, here artificial machine learning can play an important role. So, this is the final slide on the challenge in space bioprinting and then that is essentially what it means the biggest problem is sending live cells encapsulated in culture medium. And also we have learned that what is the relevance of the storage, what is the influence of the storage time on the quality and properties of the bio ink that we can send it to the space that is also another thing. And experimental results with the long-term storage at a bit of the pre-mixed bio-wings possible to store the cell-laden bio-wings for several weeks at 5 degree Celsius without complete loss of the cell viability.

That is the work that I have presented. This is the Galinsky's work, Michael Galinsky's work from TU Dresden. Now, these are the challenges in the extrusion bioprinting in microgravity in terms of weightability of the print bed. This is the process parameter optimization like what is the force analysis elimination tools and also fluid delivery system. These are the things that has been very significantly discussed as part of this emerging topics course. Now, this is the last slide of the summary lectures and what I have mentioned that some of the key challenges for the 3D extrusion printing is more on the translational research.

And some of the basic things that I have discussed at length actually these key challenges part I have taken almost like 4 to 5 lectures. One is the bio ink development. The second one is the resolution and precision of the existing printers and what is our ability to develop complex tissue architectures that we have to enhance our ability and post-printing maturation like tissue maturation, integration with the host tissue that we have to do animal study and so on. So, vascularization aspect has been taught in the context of animal studies, multicellular architecture. Placing the cells inside the scaffolds during the bioprinting, this is perhaps one of the biggest challenges and also large volume 3D and 4D bioprinting.

So, scalability is important because scalability and clinical studies there I have mentioned scalable production. So, we normally synthesize in the milliliter level biomaterial ink at laboratory. So, when you go to milliliter level to much much higher level in the liter level bio ink, whether the properties, rheological properties, viscoelastic properties are compromised or not, whether we can develop several batches of this hydrogel and still do not compromise the properties and still all the biomaterial ink formulations they have significantly they do not have any significant difference in terms of their printability and buildability. These are the questions need to be addressed. Otherwise, we will keep on publishing papers but then ultimately when it comes to the it is for the patient care the 3D extrusion printing for patient care we will still be far away from that reality.

Other things that I have mentioned the industry engagement and clinical study on the human of the bio-wings. So, many papers are coming up in this field where people develop the bio-wings, they develop different chemistry based approaches, they do preclinical study, they do animal study but But going or moving from animal studies to human clinical study, there we are still not going for many of the biomaterial links. For example, one of the biomaterial links perhaps is most widely investigated in this world is the Jelma. But still, many of the Jelma-based products are not available in clinics for the human treatment, human patient care, right, or patient care. So, another thing is also what is the roadblocks or what are the challenges, the regulatory challenges.

Now, today you are developing titanium based implants. These titanium based implants are being developed, are being manufactured by conventional manufacturing unit like machining. Now, you are using for the 3D printing as long as the 3D printed titanium implants will have similar properties and performance, clinically relevant performance and then you can demonstrate that we are not compromising the performance in the 3D printed structure compared to the machined structures or machined component then you can get the regulatory clearance that if there is a then you can use that this is a predicate device manufacturing process is different but structure and properties and performance remains identical to that of the existing device. But there is no such examples in the case of the 3D printed hydrogels because these hydrogels or 3D printed structures are not or were never used by the clinicians. So first of all the acceptance by clinicians, second one there should be several evidences that this is very safe and used in humans and third one we need to also do large large animal study like you know in the larger sized animals like pig study and so on so where we can show that these hydrogels they perform very they perform well in the large sized animals as well. And then accessibility like what is the high cost and technological complexity these are like another challenges.

So, I hope in this NPTEL lecture, I have covered significant amount of the concepts that is required for you to follow the course content. I have also covered not only metals and ceramics but also soft hydrogels that have received very significant attention in the global scientific community. I have also covered to a large extent on two emerging topics, one is artificial engineering and machine learning and one is space bioprinting. I have also discussed to a large extent of the key challenges that this field experience, so that it will open up future avenues for doing more translational research and with the expectation that this many of the additive manufacturing techniques will be an important role for human healthcare and other societally relevant applications. I close this NPTEL lecture by wishing you all the best and then I am sure you will be able to use the learnings from this course in your professional life, in your research career and wish you all the best.