

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

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Lecture 32

Process Science: 3D extrusion (Bio)printing/4D Bioprinting

Welcome back to this NPTEL course. In this lecture, I am going to discuss the process science involved in the 3D extrusion printing of biomaterial inks or bio inks and accordingly it is also known as 3D bioprinting as well as 4D bioprinting. I will emphasize more on 3D printing and then I will show also one or two slides just to describe the concepts of 4D bioprinting. This 3D printing and 3D bioprinting although it is being covered under this additive manufacturing course. These topics are also covered in other courses named as bio fabrication course. bio fabrication essentially means this manufacturing or layer by layer manufacturing of materials containing the key components of the biological systems.

key components of the biological systems means mostly it is cells of different types or proteins. these cells can be either bone cells or muscle cells or cardiac cells or nerve cells, neural cells and so on. I think quite few lectures back we have seen this slide and I thought that it is important for me to remind you fundamental definition of 3D printing and how it is different from conventional manufacturing. conventional manufacturing is best described as subtractive manufacturing processes, whereas 3D printing belongs to a generic manufacturing class called additive manufacturing processes.

What do you mean by subtractive manufacturing processes? You start with a cube, cube shaped material Then you try to drill it to make holes and these holes are of kind of similar size but in all the faces of the cube. A cube has 6 faces, on the 6th face, you would like to make 6 holes. what you have to do, you drill hole in one face. Then you turn the cube to the other side to make the other face towards the drilling machine. Then you start drilling.

Then you turn it, then you turn to the other sides and so on so forth. you get the 6 drills. And in the process you get lot of waste of the materials because it is being drilled. in the additive manufacturing process you start with the powders. instead of this cube and then powders you take one of the generic manufacturing processes then you make the cube 3D objects and you can see when the process is under progress.

This AM process under progress then essentially you see that how the structure is being built. Additive manufacturing or rapid prototyping or 3D printing essentially represents a range of techniques that involve computer controlled manufacturing with layer by layer deposition of material, based on a computer design model or digital model to fabricate physical objects or assemblies in three dimensions and often with complex geometry or topology. here the waste is much less in additive manufacturing. This 3D bioprinting or 4D bioprinting essentially revolutionized this particular scientific domain that is called tissue engineering. Now what is tissue engineering? It is more belonging to biomedical engineering or bioengineering.

It is an emerging multidisciplinary research area at the interface of biology, engineering and medicine with

immense potential to request our healthcare and essentially in terms of regenerative medicine for maintenance, restoration, amelioration of damaged tissues and hence organ functions. what are the three major components of tissue engineering? Major components in tissue engineering, one is scaffolding materials, scaffolds, one is cells and one is biological cues like growth factors, proteins and so on. if you make the scaffold by 3D extrusion printing, using biomaterial ink then we call this a 3D printing. But when you add cells to it then we call it as a 3D bioprinting. Add cells to it means you encapsulate cells within the scaffold.

And cell encapsulated scaffolds, you are essentially 3D printing to a complex structure. that complex structure also contains live cells. That means cells are alive and cells have certain biological functionality. And if you are able to manufacture that kind of structure using a generic additive manufacturing process, then you can call this technique as a 3D bioprinting. And many scientists have shown that it is possible to regenerate muscle tissue or nerve tissue or connective tissue or epithelial tissue by 3D bioprinted structures to a reasonable extent.

I must emphasize the phrase reasonable extent because There is no ideal synthetic tissue engineered scaffolds available in this world which can perfectly mimic either of this muscle tissue, nerve tissue, connective tissue and epithelial tissue in physiological context and in the clinically most acceptable manner. this is very important to emphasize this part. tissue engineering you know that there is a clinical treatment approach which clinicians or surgeons are using for decades or following these particular things in decades, autograft. Like you take some part of the tissue from other parts of the body, you place it in the other part of the body for the same patient. allograft means human to human, from one human to another human.

if you transfer the tissue from one human to another human and that is used for the regenerate the certain tissues. Xenografts means from higher animal like sheep or pig this kind of model. Very large animal whose physiological complexity is very similar to that of the human, homo sapiens and therefore you take the tissue structures of the sheep or pig model then you put it there in the human model in the human subjects and then you can reconstruct you can regenerate this tissue. you have synthetic materials it has physical properties and live cells and then you can combine them you can do this. This particular slide perhaps is a summary of what you have seen before.

You have allografts, you have autografts, you have isografts and you have genografts. And these are like different tissues like heart, cardiac tissues, then you have a nerve tissues, you have a liver, you have a kidney, so all these different tissues, artificial tissues that one can essentially manufacture this functional artificial tissues and can transplant in the human patients to restore the original functionality of the same organs. The way I am describing this process is extremely difficult and clinical validation takes lot of time and then you have to recruit the patients and you have to get strict test regulatory approval because this kind of tissue engineering approach in any country has to pass through strictest regulatory approval before it can be used in human patients. this is another slide just to I am showing you different slides because many of the viewers and many of the students of this course. may not be necessarily from biological sciences background.

Therefore, it is important for me to substantiate this concept of tissue engineering not only in one slides but also different slides so that I am trying to explain you from multiple directions for you to observe this concept in a much better manner. what I have said before that this is your scaffold. As you can see these scaffolds appear to be of very complex designs. We use different types of cells. Then you can use growth factors or bioactive molecules or functional nanoparticles.

I can show you that how these nanoparticles can be introduced to it. And once you make these scaffolds using a 3D printing or 3D bioprinting, so you can do both the approach. One is that you 3D print these particular structures and then you put it in the bioreactor system containing these cells. Then you can allow the cells to be infiltrated into the structure and then you can essentially allow the maturation to take place then this is 3D printing parcels. please listen to me carefully when you 3D print these particular structures you do not print scaffolds only.

You allow the cells and other bioactive growth factors and bioactive molecules to be added into the scaffolding gel or hydrogel or viscoelastic gel. Then you do layer by layer fabrication process to make this structure so that you not only get this kind of design porous structure but also you get the structure which contains this kind of cells. And these cells one of the major challenge is to maintain the viability of these cells when they are being encapsulated within the scaffolds. The way I am saying it is very challenging because when you do this 3D printing, extrusion printing which I am going to explain to you more in details, you will see the cells will essentially experience different type of stresses either shear stress or extensional stresses. under the stresses cell viability can be compromised.

if you allow the cells to grow freely on a cell culture plate and if you allow the cells to be encapsulated and to be printed together with the scaffolds, it is completely different cellular microenvironment that cells will experience and therefore it is quite likely that the viability is compromised and that is indeed reported in multiple experimental research papers in this area. Coming back to that, that when cells are contained within the scaffolds and then you are doing 3D printing, this process is called 3D bioprinting. Then you allow the maturation to take place, this 3D bioprinted scaffolds. in a bioreactor system. Now when the tissue is matured then you allow this mechanical or electrical stimuli or acoustic stimulation to this printed scaffolds and then you implanted into this bone for example.

this is the perfect example of the bone. this process is considered as 4D bioprinting. you adding extra dimension by allowing mechanical, electrical or acoustic stimulations to be provided into the 3D bioprinted scaffolds. 3D printing when you say bio that means you are essentially printing scaffolds with cells On the 3D printed scaffolds, you give electrical stimulation, mechanical stimulation, acoustic stimulation, you call it as a 4D bioprinting. I hope that you are able to distinguish between 3D bioprinting versus 4D printing or 3D bioprinting versus 4D bioprinting.

you start with the biometrial ink. This is uncross-linked polymer. Then you go to the 3D bioprinting machine or 3D extrusion printing machine. After the printing is over, these 3D printed constructs, this is the for example, hydrogel, it is the standard material with different composition has been investigated. very very extensively and you can just give the keywords you get hundreds of papers on hydrogels particular hydrogels 3D printing.

Maybe with different composition with different cross-linking approach with different type of printers. with different type of stimulations that they experienced during 4D printing. as I said before that these hydrogels when it is cross-linked at the chemical crossing calcium chloride or UV cross-linking, physical cross-linking, then it becomes little stiffer. Then you grow cells onto it, seeding cells into cross-linked hydrogel scaffolds. Then you go in vitro culture, then you allow the cells to expand, proliferate.

ECM is synthesized, extracellular matrix is synthesized and then you get this tissue like structure. The another approach as I said that it has cells, this is like cells. Cells containing hydrogel, you do 3D bioprinting,

bioprinting machine and once this embedded hydrogel, then you can do chemical cross-linking or physical cross-linking and then you do tissue maturation. this part is also called tissue maturation. now if you want to essentially construct a patient specific tissue, so you take the MRI or CT scan data of the patients.

Now you design that particular organ, let us say human heart, so you want to construct that human heart, so you take the design, now with that design you formulate the bio ink, this bio inks functionality should be commensurate with the natural heart functionality. that means if the cells are cardiomyocyte cells, if you contain the cardiomyocyte cells within the hydrogel framework, the cardiomyocyte cells should have beating functions. and then gels that should hold this cardiomyocytes cell must allow this beating functions to take place. And then this cardiomyocytes cells the way it is very difficult to culture so it has to be contained within the hydrogel and then if you go to the after the scaffold printing is over you can go to bioreactor maturation that means tissue maturation and then you get this tissue CAD model then you start printing either inkjet, stereolithography, laser based and extrusion printing then you get final 3D printed structures. this is the key thing you start with the bio ink, you see the scale scaffold materials, then you go to the actual 3D printing and then you get the human heart shaped constructs. what is hydrogel? A gel is a specific gel composed of one or more hydrophilic polymeric components that are suspended in water to form a 3D network.

something related to water, gel. gel is not a solid materials but it is more like a viscoelastic materials. It is not a fluid like water but it is viscous fluid but at the same time it has significant water retention capacity. this is water molecule, it is a dry polymeric network, once you put in the water molecule, it retains this water molecule, it gets swollen, its swelling properties, that is very important in hydrogen. it has a hydrophilic polymeric component, this is very important. what are the classification of hydrogels? You can classify the hydrogels in terms of the structures, components, physical property or cross-linking properties as I am going to show you in the next couple of slides.

Classification based on the components, it is homopolymer like n is equal to 1. For example, n -poly- n -isopropylacrylamide. A copolymer like N is equal to 2 as the name suggest copolymer means it is a combination of 2 -mer units. And these -mer units either can be styrene copolymer or 2-hydroxymethacrylate copolymer. N is equal to more than 3 or greater than equal to 3 is multipolymer. You can have polyacrylamide, polyvinyl alcohol, chitosan. That means this is the polyacrylamide gel with PVA and chitosan.

According to cross-linking, cross-linking is a chemical process that involves the formation of covalent or non-covalent interactions among the polymer chains. cross-linking can be different type, physical cross-linking or can be of chemical cross-linking. Physical cross-linking like you make some situation where you can do physical entanglement or electrostatic interaction or hydrogen bonding like alginate, gelatin, hydrogen. Or it can be covalent cross-linking like polyvinyl alcohol hydrogel or gelatin methacrylate hydrogel or ionic cross-linking that is alginate trial with calcium. Hydrogels classification based on the network structures, so network structure can be macromolecular network structure and it can be polyethylene glycol like you expose into UV, you already added some photocross linkable materials into it like irgacure and you get functional groups and radicals and macromolecular network.

Interpenetrating network that IPN they call interpenetrating network like multiple polymer networks coexist within a single polymer and polymer hydrogel. this is one type of polymer, this is another type of polymer, so you get the interpenetrating network structures, IPN structures. this is one of the examples very complex molecular structures, this is the interpenetrating network structures. there are some authoritative review papers

which are published on the gelatin methacrylate as well as the gelma hydrogels particularly for different applications which you can essentially refer to.

let us move on to the more process aspect. this is more like introduction to hydrogel, introduction to 3D printing, examples of 3D bioprinting and what are the classification of hydrogels, not only definition but also classification. As I said that cells, Scaffolds and growth factors, these are the 3 major key components of the tissue engineering. cells can be different type of cells like muscle cells, osteoblast like bone cells, neuron cells, stem cells, macrophages which are also important cells when in the material is placed in an animal body and you have T cells and cells can be agglomerated to make a cell spheroid also. Materials are important, what are the different synthetic polymeric hydrogels, hybrid polymers, nanoparticles can be added, acellular matrix can be added, natural polymer can be added. there are synthetic polymers, there are natural polymers these are like biomaterials.

as I said these two things can be added, this is plus sign that means cells and materials together it can be added. And you have a biomolecules like DNA, growth factor, enzymes, plasmid and so on that can be added further. this is all the story of the 3D printing. As I said that in case of 4D printing, you must add some external stimulations either magnetic stimulation or electric stimulation or certain UV radiations and so on.

that materials change its shape. this is very important what is 4D printing. One of the things that I have highlighted and I have emphasized that when you go from 3D printing to 3D bioprinting, one of the things that you have to be very much conscious about is the cell viability or biocompatibility. And you have printability and shape fidelity, shape fidelity means suppose you want to use 3D print a heart structure that means it should mimic the natural heart structure to the closest extent possible and what is the fidelity of that shape that you are printing This is the traditional biofabrication window and there is a ideal biofabrication window using advanced bio-inks. you have different type of 3D printing techniques where we can use hydrogels, you can use laser engineered, laser induced forward transfer, you can use inkjet printing that I have shown you before, thermal and piezoelectric, vat photopolymerization and robotic dispensing.

this is the advanced extrusion 3D bioprinting. this is like 3 type of very advanced 3D extrusion bioprinting techniques that which I have shown you. It is called coaxial bioprinting. what you see here, you have a container filled with calcium chloride. Calcium chloride is your cross linker, chemical cross linker. this is a scaffold you are preparing here and this is the coaxial nozzle.

this is the jet shaped platform and attached to the jet stage and coaxial means delivery of 2 different type of solutions. One is a calcium chloride and one is let us say alginate and which contains cells. you are coaxially printing and delivering at the same time in a synchronous manner and it has been shown here. You are essentially sending the alginate and cell and this is your red one is calcium chloride flow, this is your alginate flow, this is calcium diffusion and this is like your gelled alginate and un-gelled alginate that is formed. There is another technique called additive lathe bioprinting.

essentially you use the functioning of lathe machines and here you can use one inner layer material and outer layer material and in the inner layer you can use some bone marrow derived stem cells and also you can essentially expose them to UV. and then see that how bone marrow derived stem cells can be incorporated in GelMa hydrogels. And whether PC12 cells can be incorporated that is nerve cells in nerve conduit inner surface. this is a rotatory rod. these rods will be rotating and these rods are being placed here.

These rods are placed here and on the top of it continuously you will be depositing the nerve cells as well as the materials. Other one is the chaotic bioprinting. as the name suggests, in the 3D printing nozzle, we will essentially create chaotic motions of the cells and the gels together. And this you can see that it is not typical regular motion, it is like a chaotic motion and it goes and then how multilayered architecture can be formed in this chaotic bioprinting conditions. I have mentioned at the beginning of this lecture that I will be also showing you some slides related to the 4D bioprinting which has been described with some details.

Here you can see little bit more details. you get 3D hydrogels. you essentially print hydrogels plus cells in 3D bioprinting. cell encapsulated scaffolds when these are exposed to physical stimuli like temperature or magnetic field or electric field or light. or chemical stimuli that is pH just pH changes or biological stimuli that is enzymes. Then you essentially get into the domain of the 4D bioprinting.

Now if you look at this that there is a transformation either in shape or functional, functional means the way this hydrogel cell will function that will be very different from 3D bioprinting. You cannot call it as a 4D bioprinted structure unless you demonstrate that there is a distinctive shape changes which you can quantify A or B that by giving the stimulations that you are essentially introducing the functional changes in this 4D bioprinted structure. This is very important. I will stop here and then I will continue with this process science of this 3D and 4D bioprinting in the next lecture. Thank you.