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

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Welcome back to this NPTEL course on additive manufacturing. A major part of this course will also focus on the bio fabrication aspect. Now, bio fabrication essentially means additive manufacturing of materials involving key components of the biological system. what I mentioned here bio fabrication. And this biofabrication approach essentially will involve 3D printing or additive manufacturing of materials containing materials with key components of biological system. In some of the subsequent lectures, you will see how 3D printing or 3D bioprinting or 4D bioprinting can be pursued using clinically relevant biomaterial scaffold systems.

So far you have got sufficient depth of the principles of the additive manufacturing process in general and few classes of AM processes like binder jet 3D printing, SLM, DED like metal 3D printing techniques also in particular. Before I start that 3D extrusion printing of hydrogel systems more from the perspective of the bio fabrication aspects I thought it is instructive to introduce you to the biological system and also biomaterials in general. Please remember that this course is neither on biomaterials and implants nor on introduction to biology. therefore, my lecture would more focus on the only on the relevant aspects of the biological system.

which according to me would be necessary for you to appreciate how the synthetic material system containing the key components of the biological system can be printed in a layer by layer manner following the generic principles of the additive manufacturing process. with this introductory remark, let me start this lecture on this introduction to biological system. This will be the topics that will be covered in this particular lecture, hierarchical organization of human body, key definition of relevance to AM course. And, structure and properties of key components of biological systems like cells, bacteria, protein, tissue, cell fate like how biological cells have different characteristics or different attributes which are essentially important in the physiological context and biomaterials and biofabrications. Some of the major keywords are also mentioned.

Now, if you look at that hierarchical organization of a human body, let us look at this is that human skeleton. human skeleton one of the major important organ is heart. and all of

you have taken biology course at some point of time in you as part of your academic curriculum at the school right. you remember heart is a primary organ which is responsible for our life in general. if heart stops its functioning that is the end of our life.

in view of its significant performance of the functioning of the human body. Let me give let me take heart as an example of an important organ at the highest level. hierarchical system means it is a different level of its structure right. in the heart you know that you know heart essentially pumps the blood. And this blood is essentially spread across the entire human body and then blood carries oxygen.

therefore, that blood circulation through the different parts of the body is very important right. if now this let us say fully functional heart, it is composed of cardiac tissue. essentially organ is at the highest level. Then if you go down the hierarchy, then you have tissue. you have the cardiac tissue.

In biology or in medicine, anything related to cardiac or cardio means it must be related to the heart. at the cell level, so tissue is what? Tissue is the self-organized structure of cells having unique function so that every tissue is supposed to perform specific physiological function. Then these cells what you are seeing here, these are called cardiomyocyte cells. cardiomyocytes have the very particular very specific function as you know that heartbeat like 72 per minute right. therefore, cardiomyocytes must have that beating performance.

it beats in synchronous with the heart beating right or these cardomyocytes they have a beating performance and that leads to essentially overall this heart pumping at a specific rate. Now, this is this cardomyocytes and also they have smooth muscle cells in the heart. this is essentially what you see at the system level this as I said at the system level heart actually pumps the blood to different parts of the body so there are vein and aorta and then as you know that when somebody takes blood from you for the blood testing so they just take it from that. And then you have the cardiomyocyte cells that I have mentioned. Now, if you go down further in the cells, now then you have this molecule, so different protein molecules, so these cells, then you have the abundance of the protein molecules in the biological system.

And then if you go down further in the cells itself you have a DNA deoxyribonucleic acids which undergoes you know transcription and translation process to form that RNA and that is like messenger RNA so inside the cell. inside the nucleus then it goes to DNA to RNA, mRNA and then mRNA goes out of the nucleus then at the ribosome the proteins are synthesized. I will highlight some of the things here which I am going to explain you in the next few slides. what you learn from this very specific first slide on

this introduction to biological system. that hierarchy in the human body right and that I have mentioned this cardiovascular system.

here we have learned for the cardiovascular system very briefly. cardiovascular system important component is the heart and then heart contains cardiac tissue, then cardiac tissue it contains cardiomyocyte cells and then I have mentioned about DNA, protein and so on. then you have atoms. let us move on to the next one. now if you look back, you know what you see is that you have atom, then atom they come together, they make a molecule and these molecules, so there is in the biological context we always call macromolecular structure.

for example, protein is one of such macromolecular structure. Then cells contain these molecules, macromolecules, biological macromolecules, cells form tissues. Now tissues can have very, very different structures. And then I will explain, I will show you that how four or five different type of tissues They are contained in the human system. Then comes tissues and from tissues it goes to organs.

these organs essentially function certain organs have specific physiological function. some of the terminology that I am introducing that is very important kind of. this terminology is that physiological context or physiological functionality of the organ. and that has these organs they perform specific functions right. For example cardiovascular system their functionalities are blood supply right.

For example kidney that is for the urine related neurological structures. So these are like you know different kind of functions they have. Now let us see that whatever I have discussed so far, this has been also mentioned at the more hierarchy level from cells go down. what you see it is a very hypothetical shape of a cell. It is a eukaryotic cell, eukaryotic cells means truly nucleated cell.

this is the nucleus. if you go to the nucleus, if you zoom it, what you see this is the nucleus and inside the nucleus you have chromosome and then you have a double helix structure of the DNA. double helical structure of the DNA this is already you learnt in your school biology and then you have a gene that is the segment of DNA which actually contains the genetic information or genetic codes that is why it is called gene that is a segment of DNA and this you remember from your basic school biology it is A T G C that is the adenine. these are like ATGC code is there, so that is in your DNA. Now, I just briefly mentioned in the last slide, so you have this ATGC that is very clearly shown here in the DNA. in the eukaryotic cell, so DNA to RNA this conversion essentially takes place inside the nucleus of the cell.

Outside the nucleus in the cytoplasm that RNA to protein that translation process takes place. these are the two terms that you learn , one is the transcription process and one is the translation process. And this transcription and translation process in the cell biology they call central dogma of molecular biology. I repeat DNA to RNA this is the called transcription process, RNA to protein this is called translation process and these proteins are essentially synthesized in ribosomes inside the cell and you may recall that Venki Ramakrishnan from Cambridge University UK actually he got the Nobel Prize for discovering the structure of ribosomes along with two other international scientists a few years back. Now, coming back to this particular lecture what you see here that this particular part I want your attention to and this particular part of the lecture what you see that this is the kind of a typical tissue structure.

this tissue structure if you see I am just kind of drawing your attention to these particular things . this tissue structure what you see that is epithelial cells. Epithelial cells means skins that what is outer cells epithelial tissue and these particular cells have a very characteristic structure which you can very clearly see that is that closely spaced cells so that means cell membrane to membrane there is a direct contact which is a typical characteristic of epithelial cells. below the cells you have the basement membrane and then you can see different type of cells A tissue can contain same type of cells or different type of cells and they have different cell morphology. what you see epithelial cell morphology which is more or less like a cuboid or cube shaped morphology and then other cells this is like fibroblast cells or it can be there is a different type of cells and some of the cells are called fibroblasts like connective tissue cells.

This is the connective tissue cells. Then some of the cells can be osteoblast, osteo means bone, blast means formation, so this is the bone forming cells. Now these cells are essentially dispersed rather randomly in the tissue below the epithelial tissue. this matrix are essentially called extracellular matrix in short they call ECM. And this ECM essentially provides mechanical support.

it is like holding. ECM is essentially the matrix which holds the cells different type of cells together. That means it must provide mechanical support or you know mechanical or physical support to the cells so that the cells can stay there on the extracellular matrix. At the same time if you go into very much depth into biology like two human beings they talk to each other like two cells also they talk to each other they call cross talk of the cells and this cross talk of the cells are essentially facilitated by transferring the signaling molecules from one cell to another cell. I will very briefly mention the cell signaling processes which are important for every functions a cell perform in physiological context essentially extracellular matrix has a very important role. I will come back to more on the extracellular matrix in the subsequent lectures.

But to complete the discussion in this particular slide, I must highlight that ECM actually plays an important role and ECM essentially provides mechanical support and also in the biological context ECM also supports the cell signaling transport between different type of cells that are contained in a tissue. There is also other things that you can see in this particular region that these two different type of cells, one you can see spindle shaped cells, this is mostly the fibroblast cells. You can see that more flat shaped cells, these are like mostly the osteoblast cells. often you know that they are essentially abbreviated as OB like osteoblast or FB is fibroblast cell sometimes in the scientific community. And these cells they must adhere to the extracellular matrix to some anchoring points and this is called cell adhesion.

In the more mechanical terminology ECM extracellular matrix must have good elastic stiffness to support this kind of cellular activity at a much broader scale. this is like more discussion on the level of structural just to recapitulate level of structural organization in human body. cell is the basic functional unit of the tissue. tissue is the more self-organized structure containing cells to perform a specific physiological functions, then organ is at the next level. if you look at the different level L1, L2 is the tissue, L3 is the organ, organ system is L4 and then entire organism like you know total human structure we call this organism.

similarly if you go to lower order animals like if you go to pig, if you go to sheep, if you go to dog, if you go to rat or mouse, you see that this structure that different organ size. and their functionality can go down in size that size also go down because their overall weight of that different animals or overall physiological complexity also will decrease as you go lower and lower order animals. I will explain to you later that whenever we develop any kind of synthetic materials which are processed outside the human body for example by using 3D printing or bio fabrication processes. It must go through animal study and one of the animal model that people use most extensively is the mouse animal or rat animal. Then after that animal study, they go one step higher, maybe rabbit, maybe dog model, then go to even higher.

for some of the life-saving drugs for cardiac drugs, cardiovascular drugs and other drugs or neurological drugs they can go to even higher order animal. before it gets clearance for the human clinical study. So all this animal study must be completed according to the nature and performance of this particular biomaterials to be used for healthcare and then the main idea is that you cannot take a drug or take a material direct to the human clinical trials without knowing their performance, without knowing their safety and performance at the lower animals as I have explained for last few minutes. What is the compatibility of the living system? For example, any biomaterials that you develop outside the human

body, when you place it inside the human body, it is expected to interact with the key components of the human body. And what are the key components? For example, protein.

protein, once you put it in the human body, protein actually needs to get absorbed on the biomaterial surface. And protein absorption is the first key step before a cell essentially starts interacting with the biomaterial surface. Then next step is compatibility with cells. This particular word is known as cyto-compatibility. Cyto means cell, cyto-compatibility means cell compatibility.

And you can see that a biomaterial structure must facilitate protein adsorption or must allow protein adsorption to take place very fast. Then it allows that cell adhesion and same functionality to take place. Then comes compatibility with blood like blood compatibility and blood has different components and then you have this antibacterial property like bacterial adhesion, bacterial colonization. all this bacterial adhesion, bacterial colonization and more colony forming, unit formation that can lead to infection. essentially a biomaterial should not facilitate this bacterial colonization.

that is why it is said no, no to any of the bacteria. What is the bacteria it is shown here? It is a standard E. coli bacteria which is responsible for unit tract infection and in general many other infection in the human body which you have surely come across this particular bacteria. the cells that can be cultured in different manner because typically in most of the research laboratories around the world most extensively people do monoculture of the cells. For example, they grow osteoblast like bone forming cells for any bone implant applications or they grow fibroblast cells that is the fastest growing cells but in very less number to the monoculture of cells, co-culture of cells that means that is more than one type of cells or two or three different type of cells they are being cultured together.

that is called co-culture of cells and that has been shown by different colored cells here as you see in this particular slide. In vitro essentially means that experiments conducted using glass wares to simulate physiological context that means, in vitro experiments are always done at 37 degree Celsius because the 37 degree Celsius is the body core temperature. I am mentioning 37 degree Celsius, pH 7.4 that is the blood pH. these are some of the conditions that are maintained when you grow or incubate cells on a synthetic biomaterial surface and then you allow the cells to grow on the biomaterial surface and then you compare for example the same cells if it is grown on a cell culture flask.

What is the growth kinetics of the cells like at different point of time how the cell

number increase and with respect to that if you grow on a particular synthetic material substrate what is the growth pattern of the cells. then you kind of compare between these two then you say okay these particular materials it can support the cell growth, cell proliferation. it is a kind of a sign of a good cytocompatibility of the materials. But another thing that I must emphasize here that whenever you mention cell compatibility or cytocompatibility, one should not use the word biocompatibility because biological system as I have explained to you so far is much, much extend beyond simple testing with biological cells. It has proteins in it, it has blood components in it, it is also you know if it is a non-infective part of the human body then it will certainly not have bacteria but then if there is some infections take place in some part of the human body then also you can expect different type of bacterial population.

and also you have tissue. unless you investigate the compatibility of any biomaterial with all the key components the way I have explained one should not call it as a biomaterial because it does not you are not sure about his interactions with the different key components of an organism now let us move on to the other part of this particular slide. as I said biocompatibility. whenever you use that animal model, we call it is a in vivo. In vivo essentially means experiments or study conducted in a full organism. whenever experiments or study is conducted in a full organism then only you call it as a in vivo study.

as I said that in vivo study typically in an university ecosystem always conducted in the small animals like. go to this mouse, rat, rabbit, then go to medium sized, then go to higher animal and all, rabbit and then go to this dog animal and all. You know that there are some of the national labs for example in India in Hyderabad DBT has started animal husbandry There you have the dog model and even larger animals and this kind of large animal facility is there only at the some of the specific institutes like Indian veterinary research institutes IVRI. This IVRI, it is there at Izatnagar in Uttar Pradesh, IVRI also is there in Bangalore and some of the specific locations, but there they maintain the large animal study. Typically, very large animals are not kept in the university in different academic university campuses, however, I must mention that some of the monkey models which are used extensively for neurophysiological functionality, simulation and also study those kind of monkey models are also available in an academic university like Indian Institute of Science.

Now, once this animal model study like you know study or test conducted in the full organism are completed and then a biomaterials or a drug or a tissue engineer scaffolds or 3D printed scaffolds, their safety and efficacy are proven at the small animal, then you essentially go to the human clinical study. one of the things that I must emphasize again that each time that you do some of the biological experiments, for example, in vitro test,

so you have to have institute biosafety cabinets, institute biosafety committees approval that your lab has that IBSC approval. Then if you do stem cell study, any time you do stem cell study, then you have that committee called stem cell and regenerative technology committees approval. And then you have to declare that you have used the source of the stem cells, whether you are genetically modifying the stem cells before you do this test. Then, once this in vitro studies are completed, those in vitro results need to be put together.

And if you want to continue for the in vivo study, animal study, then you have to apply for the Animal Ethical Committee approval. And this Animal Ethical Committee approval, their approval forms or the proposal form or protocol forms. must have sufficient evidence that your material has gone through the in vitro studies and your material did not cause any toxicity to the cells in the IBSC approved cell culture facilities in your research group. And then you have to show those results before you get animal ethical committee approval. And if you go to the larger animal like sheep model and all then you must show that smaller animal ethical committee approval and then safety study being completed in the smaller animals.

that will constitute the data to be shown as an evidence for the safety of that particular tissue engineer scaffolds formoving to the large animal study. And then once large animal study is done then you can do for human clinical study. But if your material is a predicate device that means already similar material is being used for the similar type of implants in human body you are not changing the material you are only changing the design of the materials and then if you complete the rabbit animal study then you can essentially apply for the human clinical study and depending on the human clinical studies verdict or whether institutional ethical committees verdict then you can start doing this human clinical study. Here again I must mention this approval process one has to be really one has to do follow it in the strictest sense in the both word and spirit okay. So human ethical committee approval like every institutions wherever human clinical study is done they must have that institute human ethical committee.

And once this institute human ethical committee approves then it goes to the hospitals or the clinics their ethical committee board and there you have to start the human clinical study and after you get this approval for the hospital then both the approval you have to submit to the clinical trial registry of India and that is as per the India's regulatory norms. I must mention that every country has its own regulatory norms which must be followed in this entire translational research like from the lab to the human clinical study. And this what I have discussed in last few minutes, it is mostly relevant to the Indian system. this is just the case for the placing the dental implants in a human patient during the pilot clinical study. I am sure that I think I have explained you within sufficient details the

hierarchical structure of the human body, what are the different level of the structures and very briefly what is the cell compatibility, in vivo biocompatibility and so on. I will continue this with the next lecture. Thank you.