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

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I am continuing my lecture in introduction to biological system in the context of sufficient details that are to be understood to appreciate the manufacturing process called bio fabrication which is nothing but 3D printing of materials with key components of biological system. in the last lecture I have already covered that hierarchical structure of the human body and biological hierarchy of human body as well as concept of cytocompatibility, biocompatibility and clinical validation. let me now focus more on proteins. proteins are essentially available in abundance. in human body. From the very basic chemistry, you have two types of amino acids, amino acid 1 and amino acid 2.

When they react together, then what will happen, this dotted one OH and H, they form a water molecule. they release the water molecule and what you see here that CO and this N these particular things is called peptide bond. this reaction essentially illustrates that how peptide bond is formed and the way the protein structure is to be retained is that your N terminal should be always on the left and your C terminal can always in the right. And the two amino acids how they are different? They are different because of this R and R prime.

This is the two organic radicals and these organic radicals are different. therefore protein amino acid structure is different. If the two amino acids are different, contain different R, R prime, R double prime and so on. essentially all these amino acids when they react together then they will make a peptide structure and then if you put all these different amino acids then it can contain AA1, AA2 up to AAN. AAN is the nth amino acids in a large polypeptide chain.

in the biological context, proteins are defined as a biological heteropolymers made up of 20 genetically coded amino acids arranged in a linear manner forming polypeptide chain. This is a textbook type of definitions which essentially needs to be followed. You cannot make your own definition as far as this some of the key terms are concerned. it is a hetero polymer, it has at least 20 genetically coded amino acids which are arranged in a linear manner and making the polypeptide change. And amino acid sequence of protein is determined by the information found in the cellular genetic code and typically the sequence is written always with N terminal on the left and C terminal on the right.

this is the very basic introduction to the protein, if you look without going into very much details, so this what I have essentially described in the last slide, this is content in the primary structure of the protein, the primary polypeptide. Then in the secondary structure, so you have a alpha helix kind of a structure. or beta platelet structure of protein. in the alpha helix type of structure if you see that this is a helical pattern and this helical pattern that the different amino acids are also joined together by hydrogen bonds. Anytime in the cell biology book or you know different chemistry and biochemistry or biology book if you see dotted type of bonds, these are essentially weak bonds, hydrogen bonds or van der Waals bonds.

you have the secondary structure. this is the alpha helical structure or beta platelet structure. Then you have a tertiary structure. tertiary structure essentially it makes more coiled form and this is. essentially if you look at these things, the complexity of the protein structure.

complexity increases as you go from primary structure to secondary structure to tertiary structure to the quaternary structure. And if you go to quaternary structure, you can consider this is one particular domain, you consider this another domain, these two coiled domains are there and then you have other type of domains, these are also there. you can have alpha polypeptide or beta polypeptide, this kind of different polypeptide structures, then they make a more globular kind of a form. These are like more globular forms, globular protein structures. protein adsorption is the first key step.

what you see here, this is the fibronectin. part of the fibronectin protein, let us say type III domain of the fibronectin protein which was used in one of our computational study that is called molecular dynamic simulation study. And I told you in the last lecture that protein adsorption is important, I just wanted to show you this particular slide for you to understand that how this protein adsorption study is conducted using computational platforms like MD simulation, molecular dynamics simulation without going into details. if you see this is the type 3 domain of the fibronectin protein. why fibronectin protein? This is one of the protein which plays an important role in the cell material interactions.

it is widely investigated. And fibronectin protein if you take one structure the fibronectin protein it is a solvation cell. it is a aqua solvation cell outside the fibronectin protein and this is your biomaterial substrate let us say hydroxyapatite. Why hydroxyapatite? Hydroxyapatite is one of the most common inorganic component or it is the inorganic component of the human bone or human tissue. this is the hydroxyapatite surface, you have a solvation cell and you have a protein.

adsorption means whether the distance between the protein and the materials decrease over time that means the proteins are getting more and more attracted towards material surface and then getting absorbed and also that what is that? different point to point distance of the protein structure and what is the more number of contacts positions or contact sites. more the number of contact sites on the biomaterial surface, the more is the adsorption of the proteins on the biomaterial surface. Now, coming back to that key definitions of this biological system, for example, organ. Organ is a part of a body adapted and specialized for the performance of a particular physiological function.

Tissue. Tissue is the level of organization Tissue is a level of organization in multicellular organisms. consisting of a group of structurally and functionally similar cells and their intercellular material. That means tissue always contains structurally and functionally similar cells. Tissue engineering means cells, biomaterials and suitable molecular physical factors. I will explain tissue engineering in more details in some of the subsequent slides.

Alone or in combination. The cells, biomaterials and suitable molecules, if they are being used alone or in combination to repair or replace tissue, either it can be used for repair or you can regenerate tissues to improve clinical outcomes. Osseointegration, the ability of a biomaterial implant to establish a stable contact with neighboring osseous system with adequate vascularization. this is the osseointegration. what you see this is a natural tooth right in the jaw with a crown and all natural tooth.

Now if in the neighboring place if the tooth is replaced when you put this dental implants so you see that it is threads. And these threads you can see that you know that only dental implant without the process is crowned and if it is osseointegration there is a very nice contact. you can see that contact of the natural tooth with the jawbone, contact of the dental implant with the jawbone is almost qualitatively similar as you can see in the X-ray radiograph. Or many times you can see the CBCT image CBCT means cone beam computed tomography images CBCT images then you can see this implant and then that will be good to show that whether the any synthetic implants are being integrated or not. now once you know little bit sufficient details of the proteins, let me start with the other cells.

this is the biological structure of a bacterial cells. this is also called prokaryotic cells. What is prokaryotic? Prokaryotic is that primitive type of cells. it is unicellular organisms, let us say 1 to 2 micron in size, have neither a well-defined compartmentalized nucleus. you can see this DNA is essentially dispersed in the entire cytoplasm Ribosomes are the sites of the protein synthesis that I have mentioned earlier.

Now, you can see these are like pili. These are like small hair like structures. These are like pili or fimbriae are cellular appendages for the bacterial attachment to the material or tissue surface and this flagella they actually is like you know that monkey has a tail. Similarly, this flagella help in the motility or migration of the bacterium over distances on any synthetic surface. this is essentially very brief description of bacterial cell I said it is called prokaryotic cells that is primitive cells like it does not have all the cellular organelles in the most matured shape and morphology.

again in school biology you have read about the gram positive bacteria and gram negative bacteria. these two are example of gram positive bacteria. these are essentially based on the gram staining and this is essentially gram negative bacteria. clearly the morphology is different gram negative bacteria is that like E. coli this is a more rod shaped bacteria whereas gram positive bacteria is more like a spherical bacteria right spherical shaped bacteria.

these are like two different type of bacteria and now we will move on to eukaryotic cells. Eukaryotic essentially means it is truly nucleated cells. textbook type of definition is it is a self-contained structural and functional unit of living organism with the capability of replicating itself in proper biological microenvironment. replicating itself means that fundamental property of cells if it is alive is that cells must divide that means cells must proliferate. when appropriate cellular microenvironment exists around the cell under question in general, truly nucleated cells, they are enclosed by a lipid bilayer.

This is the lipid bilayer, this is the cell membrane structure. The way it has been shown the cell membrane structure, in reality, it is much more complex if you view the cell membrane structure. lipid bilayer and contain the necessary genetic material needed to direct the continued propagation of the cells. That means that is continuous division of the cells and these are the genetic material which is contained in an organelle called nucleus you have a DNA deoxyribonucleic acid. And as I told earlier that it is the nucleus where DNA undergoes transformation to messenger RNA that is mRNA in the process called transcription process and through this hole or through this pores this mRNA goes out to the cytoplasm then it will be transformed to protein at the organelles called ribosomes that is a protein synthesis sites and this process called translation.

Transcription inside the nucleus, translation outside the nucleus in cytoplasm and this is called central dogma of molecular biology. you have the truly nucleated cells. if you look at this particular cell membrane, it also has

voltage-gated calcium channel and also sodium-potassium channels. that means these cell membranes have some transmembrane proteins. transmembrane proteins means proteins which will go inside to outside transmembrane proteins and they also have a small channels and voltage-gated calcium channel essentially will allow calcium diffusion.

Sodium potassium channel will allow the entry of either sodium or potassium. These are like very selective channels. This nucleus, nucleus you have a nuclear pore complex. What it means that there are pores which are contained in the nucleus and these pores will essentially allow the transport of the biological macromolecules like you know when the DNA goes to mRNA, mRNA can be transported to the nuclear pore complex. Each of the organelles in the eukaryotic cells, whether it is a nucleus, whether it is a mitochondria, whether it is a ribosome, all these organelles have a very characteristic double layer structure.

these organelles are much more matured, well developed compared to the prokaryotic cells. and eukaryotic cell their division process like cell division process also much slower than compared to prokaryotic cells. Prokaryotic cells they divide very fast like their division time is roughly 30 to 40 minutes. Now tissue functions. As you have seen a few slides back I have defined tissue that tissue is the self-organized unit or a level of a hierarchical structure of an organism which contains structurally and functionally cells, similar type of cells.

tissue function means homeostasis. This is the biological term. Tissue repair for example wound healing, tissue formation is morphogenesis and developmental biology. these are like more details is out of the scope of this course. what are the things a cell can perform or cell must perform in the context of overall tissue functional homeostasis that is the cell replication, proliferation, division, these are all synonymous. Cell differentiation, cell can undergo death like any human being, cell motion, motility.

cell motion, cell migration, cell motility all are synonymous. Cell adhesion like cells when it will adhere. number (5) means this is adhesion, right. cell replication is to like you know one cell goes to daughter cell, two daughter cells this is cell replication. Cell differentiation means it undergoes transformation to functionally different type of cells like stem cells in the context of stem cells I will describe that.

Cell death essentially means cells are no more alive. that actually many times assessed by mitochondrial functions because mitochondria is the energy powerhouse of the cells. And then some of the biological assays those are used to find out that whether the cells are alive or not, then when you will see that when mitochondrially active cell numbers increase over time. Cell motion like number 4 point like cells can also crawl like you know a baby crawls on the surface. cells also crawl on the surface but at a much much lower speed than a baby which starts crawling on a floor at the initial stage of the life.

other things as mentioned that cells also communicate and coordinate their efforts through cell fate processes. whenever I say cell fate, the cell fate essentially means that this is the 5 different functions that a cell is supposed to perform. one is the cell replication or proliferation, differentiation, cell motion, cell adhesion, cell death. And cells communicate means what I call it as a cellular crosstalk. Cellular crosstalk means two cells can communicate to each other and then coordinate certain functions, two human beings they coordinate, talk to each other, similarly two cells they talk to each other and that is possible by cell signalling processes.

Now, cell cycle, again, it is a fundamental in the cell biology. this cell cycle, again, I am pretty sure you have seen this kind of diagrams in your basic biology course. cell cycle means cells will undergo a complete cycle

during the process of replication or proliferation. it starts from G1. And then you can see this is a resting phase G0 and this is called checkpoint.

Checkpoint means when a cell goes from one phase to another phase. Certain aspects are being checked on the cell status and these aspects are whether the cell size is maintained, whether there is a constant supply of nutrients that means cellular microenvironment, growth factors and DNA is not damaged. If DNA is not damaged that means cross, if growth factors are there, nutrients to the cell size is increased, maintained, then the cell will go to the S phase. and S phase is DNA synthesis phase. Then again there is a checkpoint G2 checkpoint then it will go to the G2 phase and this checkpoint is that cell size is maintained or increased and DNA is duplications.

if there is a small n number of DNA it must be forming 2n number of DNA because when a mother cell goes to 2 daughter cells then equally nn DNA should be also transferred to daughter cells. this is in the most simplistic manner I am trying to explain all these many cell biology aspects because I believe that many of you who are taking this course or who are the viewers of this course, they are coming from multiple different disciplines and some of the viewers or some of the students of this course may not have necessary background or may have forgotten some of the very fundamental details of the cell biology or biological system in general, again in these particular things, these checkpoints are very important. Why these checkpoints are important? Suppose a cell is not qualified to move from G1 to S phase and it is stuck at the G1 phase at the checkpoint because some of the criteria is not fulfilled let us say is the cell big enough if it is the cells have not increase in size or environment is unfavorable or DNA is damaged. It will stuck in the G1 phase and after some time when it is not able to go to Gs phase, then it will initiate its own suicidal mechanism that means it will undergo apoptosis. I will explain to you in the subsequent slide what is apoptosis, the programmed cell death.

the two things you remember that this process is irreversible process like going from one phase to another phase G1 to S phase again S to G phase again G2 phase again G2 to M phase these are reversible phases. And what is the meaning of this phase? G1 means gap 1 phase, S phase means synthesis phase, G2 means gap 2 phase, M phase means mitosis phase this as I said G1 to S, S2, G2, G2 to M, these are all irreversible process. Second one is that if a cell is stuck in a particular phase of this cell cycle, then what would happen? That cell would initiate its own suicidal mechanism and cell will undergo apoptosis programmed cell death. These are two things that I thought I must mention before I move on to the next slide. as I mentioned sometimes back that you know cell signaling processes that means this particular cell if this cell wants to remain alive.

or if this cell wants to sustain its physiological function, it has to survive that means this cell must receive that different signaling molecules supply all the time during its life that is A, B, C. Now if this mother cell as I said that this is N to 2N like if this is when it goes to 2N DNA then N and N will be equally distributed DNA and if cell is going to two daughter cells then it must receive additional signaling process molecules like D and E. If it goes to differentiate whenever it goes to differentiate the nucleus must be marked differently or must be shown different color or different size or different shape differentiation. And when a cell die like apoptotic cell, this cell undergoes death. And this particular when cell undergoes death then that means all the signaling processes are removed all the signal molecules are removed that means cellular microenvironment is unfavorable for the cells to remain alive and cell will die.

It is like when a patient is on the ventilation at the final stage of his or her life and then when the ventilation process is removed the patient will essentially die. similarly for any biological cell when all the possible

signaling molecules which are necessary for its own survival if they are removed then the cell will die and this is called apoptosis or necrosis and this I will explain to you soon. this is the necrosis, necrosis is a pathway of cell death resulting from the environmental perturbations. For example, a person is experiencing an injury due to accident. this is the accidental cell death and uncontrolled release of inflammatory cellular constituents, then it is called necrosis.

Apoptosis is as I said before it is a programmed cell death that avoids cell membrane rupture and initiation of an inflammatory response. Without initiating an inflammatory response and without cell membrane being ruptured, this is the programmed cell death that is why it is called apoptosis. Apoptosis is a Greek word it means that falling tree leaves like from a tree leaves in a specific season you know that tree leaves will fall. Similarly, in the case of apoptosis also this is how the Greek word is used or phrase is used to explain the cell death process. up to this part, I think I will come back to you to discuss this particular part of this lecture, particularly introduction to biological system in the context of more other cell fate processes like cell differentiation and then move on from there. Thank you.