Oral Biology
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Module - 03
Scaffolds for Oral Tissue Engineering
Lecture - 28
Immune response to biomaterials

Welcome to today's session of Oral Biology. Today we will be learning about Scaffolds for Oral Tissue Engineering. So, in recent years we have seen an upsurge in regenerative medicine. And if we see in dentistry there has been either a partial or total loss of one of the other tissue, which may be a dental tissue or a oral mucosal tissue.

And these has been conventionally addressed by means of either a filler material which may be an inert or bioactive material or by dental bridges or partial or total prosthetics or using implant materials. But restoration or growth of new tissue has not been used in clinics yet. Until now lot of advancements has been carried out in oral tissue regeneration.

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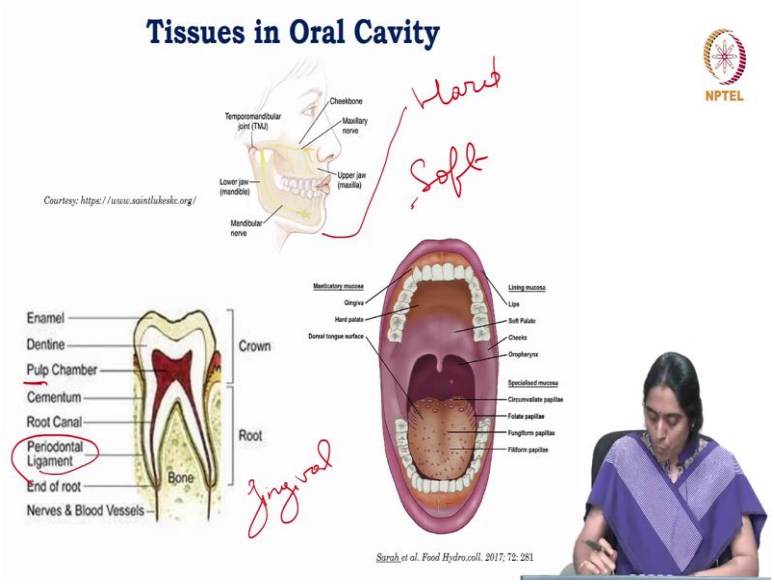

Let us know, what are that? So, before entering into the regeneration of oral tissues and to know about the various scaffolds used in the in that technique we need to know the different types of tissues in the oral cavity. So, oral cavity though it is a small space

compared to the different organs present inside the body, human body; it has different types of tissues in it. Broadly speaking they have hard tissue and soft tissue.

So, when we take into account the hard tissue it includes the orofacial jaw, the bones consisting of the mandible and the temporomandibular joints and when we consider also if we take the whole tooth structure the crown and the alveolar bone which constitute the dental tissue are also hard tissues.

Then the soft coming to the soft tissue; the gingival tissue, which is not present here; gingival tissue which is the base connecting the mandibular bone and the maxillofacial bone with the tooth is the gingival tissue the supporting tissues of the tooth that is the periodontal ligament and the pulp are all soft tissues. Not only that the whole lining of the oral cavity is by oral epithelial mucosal cells and these are again soft tissues.

We are not going into the different types of mucosal cells basically they are made up of epithelial cells and combination of fibroblast in different arrangements. So, the damage can occur in any part of this these tissues; either there can be a total loss or partial loss due to pathological conditions or due to trauma or birth anomalies.

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So, what are the different conditions where regeneration of oral tissues or dental tissues might help? So, if we look commonly the most population in the world today is elderly population. Each one will be facing one or the other dental issues. Either they may be

partial loss of the tooth or there may be total loss of the tooth requiring entire replacement and in case of others there is I do not think there will be any individual who might not have one or the other dental issues.

Caries has become a common disorder. So, it all starts with the caries right the caries is what it starts it is a characteristic where there is a imbalance between demineralization and remineralization of the enamel tissue due to the formation of biofilms; that is the plaque formation high plaque formation. So, this high biofilm accumulation causes different biochemistry right, leading to high acidity. And will erode the enamel tissue layer there and further causes cavity leading to loss of subsurface mineral structures.

And when not addressed if that is not treated even at that stage then it can lead to further cavity formation irreversible damage leading to permanent cavity formation and that needs to be cavity formation, which exposes the pulp. So, the pulp is the vital tissue of the teeth. So, if you consider the enamel has the hardest tissue which helps in mastication, but apart from that it protects the enamel along with a dentin tissue protects the pulp; it chambers the pulp tissue dental pulp issue.

So, when this is not addressed. So, further it will cause decay of the pulp. So, the bacteria and other pathogens will enter into the pulp and will cause decay of the pulp. So, this is the entire thing is caused due to caries the other damage which can be caused in tooth is the periodontitis. This is the inflammation occurring to the periodontal ligament at the interface between the periodontal ligament cementum and the alveolar bone.

So, that can also loosen the tooth and there maybe even total loss of the tooth because of this and due to trauma there can be dental fracture and there can be total loss right. So, apart from this due to birth anomalies also there can be these kind of issues. So, this is regarding the diseases and disorders related to dental tissues.

Now, if you take the orofacial bones right there can be again birth anomaly related issues or there can be fracture due to trauma ok or accidents or there may be loss of bone due to tumor. So, these all needs either replacement with fresh tissue new tissue, but as of now it has been addressed with grafts artificial grafts or implants using biomaterials ok.

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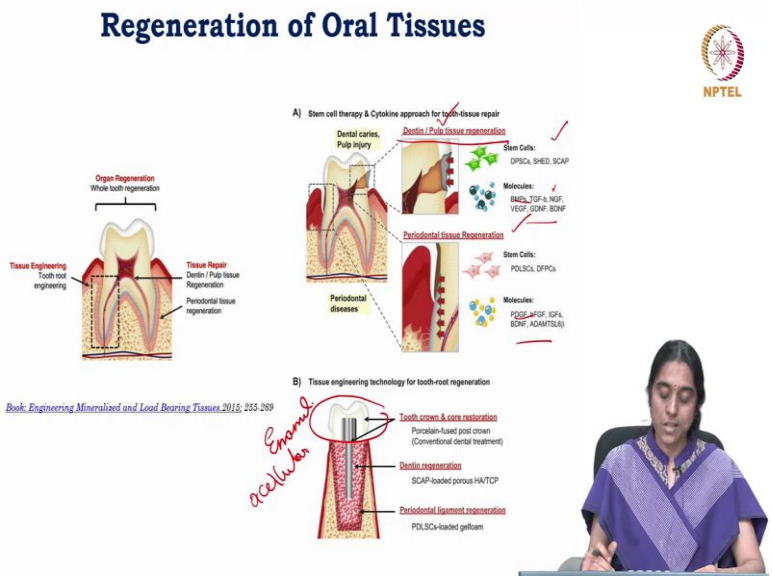

So, we saw that these are the areas where regenerative dentistry can address and can change the future dentistry right. So, how far it has advanced? So, generally you can see that for any tissue regeneration we need three important ingredients; one is the cell of the tissue cell type of cell required for that particular tissue then the biomolecules which will help in its growth proliferation differentiation right etcetera.

And a substratum a support medium which will guide in the formation of that particular tissue which is nothing but the matrix; so, then these are the three important ingredients for any tissue engineering. So, it also goes true for oral tissue engineering. So, with this these things they have in the literature you can find that they have successfully done dentin tissue regeneration, pulp tissue regeneration, periodontal tissue regeneration.

So, according to the tissue type, the stem cell you can see that it is varying the molecules are different right. So, according to the type of the tissue which has to be regenerated the stimulus varies likewise the crown region. So, there is one thing special about the crown region we saw that in the crown region there are three layers; one is the enamel, next comes the dentin where is you have layers of dentin and then the pulp region is it not that is (Refer Time: 10:45).

Now, this enamel is very unique in its structure, why so? It is a acellular region it does one of the hardest tissue in the whole human body. So, this enamel is formed in the developmental stage itself during embryogenesis using the cells called as amyloplast. So,

if we see by composition enamel consist of 90 percent of hydroxyapatite crystals embedded with some organic material which includes collagen, water content and some part of you know the proteins which were involved in its formation.

So, these amyloplast they secrete certain proteins called as amelogenin. So, this amelogenin along with other proteins called as enamelin and (Refer Time: 11:52) they aid in the nucleation growth and then elongation of the crystal structure ok of this enamel. It has a very unique crystal structure rendering at a high mechanical strength.

So, this enamelin once formed will not have cells. So, they are acellular which means in situ once it is damaged in the body it cannot be regenerated in situ. For example, other tissues if we are giving the required cues the stem cells will be able to regenerate that particular tissue, but enamel is not so.

But people have tried in lab they have tried to take the stem self differentiate it into myeloblast and then they have formed the tooth butt and the enamel was successfully generated then it was placed in animal model that much has been tried.

So, as of now we can say that the whole tooth structure in theory in lab is possible, but for all this we have to keep in mind that the particular the characteristic of the tissue the required extracellular matrix and the required stimuli in from either bio biological cues in the form of other cells or from biochemicals as in the case of using growth factors or using cytokines or using certain you can see the BDNF and BMPs or it can be through the extracellular matrix whose surface topography can also mediate this.

So, all these factors has to be kept and finally, addressed if we have to reach towards successful regeneration of the tissue. So, if you see here there is so, though it seems like a small tissue. The complexity the different architectural hierarchies in this structure makes the regeneration of oral tissue still difficult. Probably it may take a decade to see a successful regeneration of human tooth and in its translation in human patience ok.

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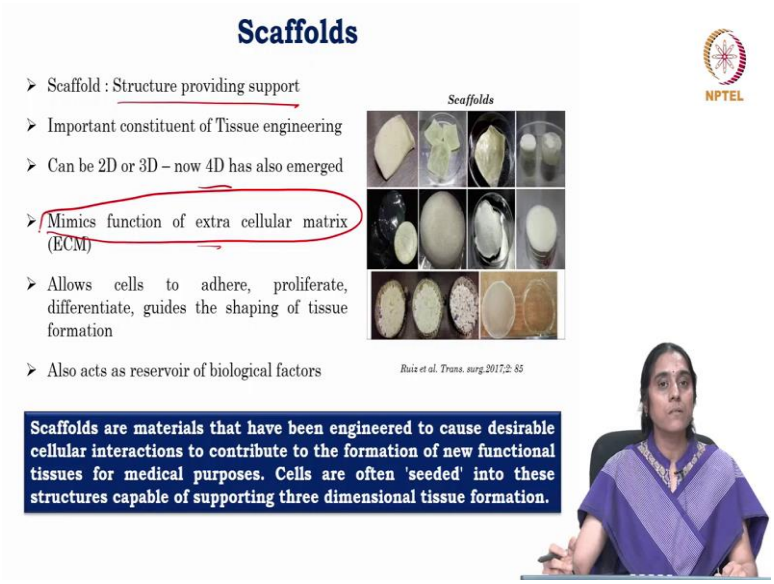

So, now let us come into the topic about the scaffolds. So, what are scaffolds? They are structures providing support an important constituent of tissue engineering. So, they can be either two dimensional or three dimensional in shape and structure. Now there are also four dimensional scaffolds what it means this they have shape memory.

So, they can be injected in a instead of being implanted a solid scaffold, they can be injected and when it reaches it is site of regeneration it regains its shape there assembles there and tries to regenerate. So, this minimizes invasiveness. So, that is what is 4D about. So, then as we have already seen they have to mimic this is what is more important they have to mimic the function of extracellular matrix.

So, what does extracellular matrix do? They will help the cells to adhere, proliferate, differentiate and guide the shaping of tissue formation and also should act as a reservoir for various biological factors. So, here in this picture you can see the artificial lead may fabricated scaffold which is in different shapes structure and prepared from different material, but I think this is made from a single material in the chitosan which is carbohydrate derived from insect.

So, by definition scaffolds or material that have been engineered to cause the desirable cellular interaction to contribute to the formation of new functional tissues for medical purpose and in these material cells or seeder which will which are capable of supporting 3-dimensional tissue formation.

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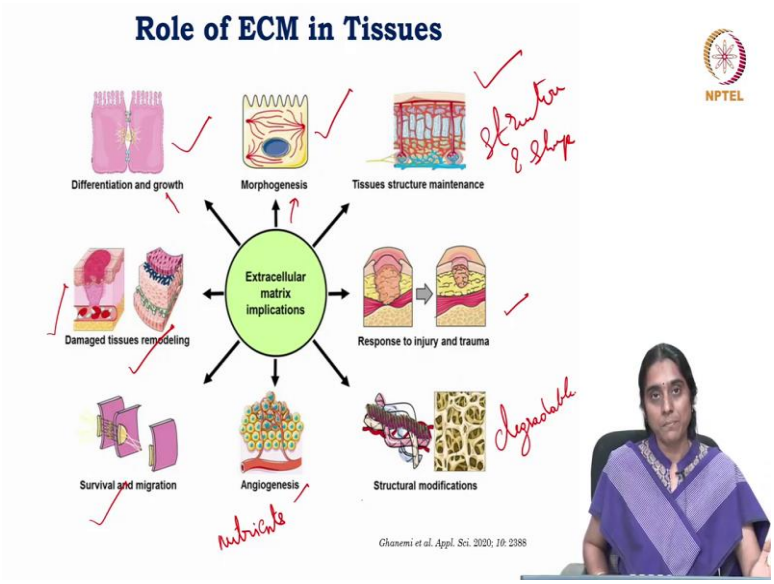

So, we have to know particularly what are the different functions of ECM so that can be mimicked in the scaffolds. So, basically it has to help in the differentiation and growth hide in the division or proliferation of the cells maintains the structure and shape right. So, for survival and migration survival it needs nutrients right. So, where does nutrients come? Through blood supply.

So, angiogenesis should also be supported by is also supported by the extracellular matrix blood vessel formation and they also help in the movement of cells a extracellular matrix and a tissue should allow the moment of cells, why? Because at any given condition if there is any damage or if there is any invasion of pathogen the there is a need for other cells to come or there is a need for the cells there present to move away from that place.

So, if for example, there is a infection happening at that place, there will be at I mean you know accumulation of immune cells. So, immune cells has to move into that space into that tissue so, migration is allowed. And similarly if there is any wound caused then there should be a migration of stem cells which are residing in the nearby place to come there and differentiate into the particular type of cells. Or fibroblast may come and help in formation or production of more collagen in that space.

So, this is where there is damaged tissue remodeling will happen with the help of extracellular matrix in response to injury and trauma and in case of you know any

physiological situations; there will be structural modifications happening inside the body which is also mediated by these extra cellular matrix.

If not all of these functions the scaffolds for tissue engineering should give structure to the tissue to be regenerated help in the cell division and proliferation, differentiation and growth of the tissues of the cells if there is any damage it should be able to remodel help in the survival and migration of cells and also should allow angiogenesis to happen.

So, we need at least these if not structural modification. In addition, it should be degradable over a period of time. Once the new tissue is formed it should be the scaffolds should be out from the place either it has to be resolved or should be physically or enzymatically degraded from that place.

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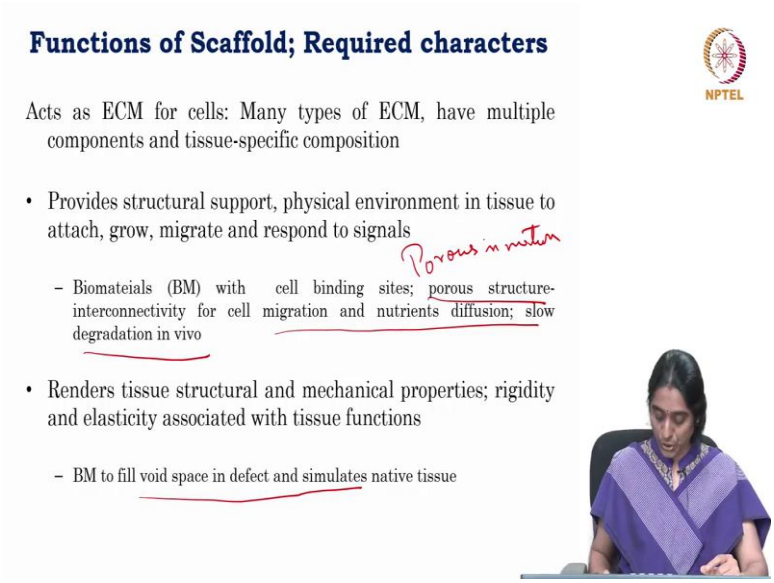

So, that is what is being discussed in these slides. So, what are the functions of the scaffold how we get those required characters. So, they have to provide the structural support, physical environment in tissue to attach, grow, migrate and responds to signals. So, this can be attained by having a porous structure in the biomaterial which is used for those scaffold formation.

And this these porous structure should be interconnected which will help in easy migration of the cells, nutrient diffusion and slow degradation in vivo. So, all these characters of the extra cellular matric is just met if it is porous in nature right. The next

important aspect is having a structural and mechanical property similar to that of the tissue to be regenerated.

So, the rigidity and elasticity should be associated with the tissue functions. So, how that is going to be met? The biomaterial has to fill the void space in defect and stimulate the native tissue so that it reaches that kind of mechanical property. So, if we take the any tissues in the body it has a particular mechanical strength right.

The stress strain and the tensile strength all these things are unique for each tissue. So, the biomaterial which we are going to choose for the scaffold formation and the scaffold form should at least have a near similarity of mechanical properties of that regenerated tissue.

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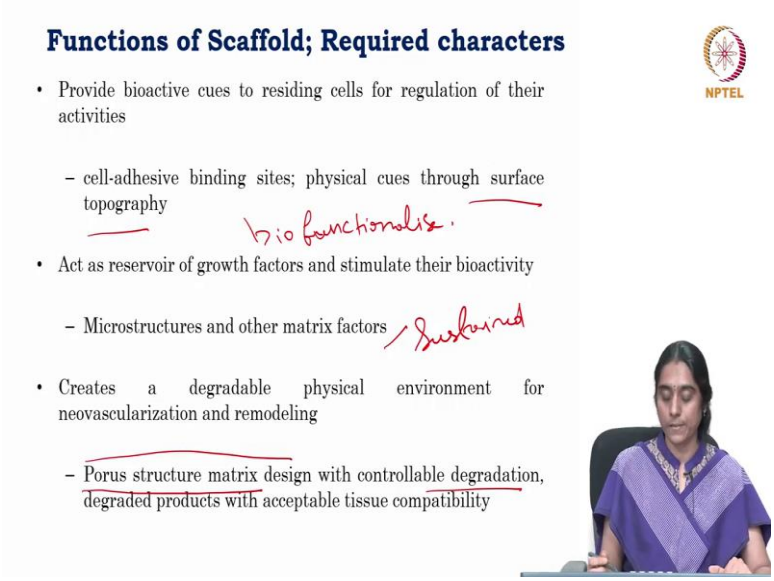

Then as we saw earlier it should provide bioactive cues for the cells to differentiate and to regulate their activities. Now that can be rendered by either the surface topography the roughness of the surface it can provide that is by the physical cues of the scaffold. So, which can be you know designed based on our fabrication process or we can bio functionalise the surface with the proteins present on the extracellular matrix, such as; collagen or the RGD peptides or proteins extracellular proteins such as integrins ok.

So, that can be functionalized on the surface; that is another way. And the next feature is that it should act as a reservoir in certain cases for growth factors of that particular cell to

stimulate its bioactivity. So, the scaffolds should have enough microstructures to hold these growth factors and should be able to release it in sustained manner ok that is another feature, sustained release, not a burst of release.

Then finally, it should have a degradable it should create a degradable physical environment for new vascular tissue formation and also for remodeling of the entire tissue. So, that can again be rendered by the porous structure of the matrix which can be controlled which and the design of the porous structure can control the degradation process.

Also the chemistry of the scaffold can determine the degradation rate. Not only that the degraded product of the scaffold should be biocompatible, it should not cause immune reactions; it should be safely either resorted or it should be eliminated from the body without causing unnecessary reactions. So, tissue compatibility for the scaffold as well as its degradation product is very important.

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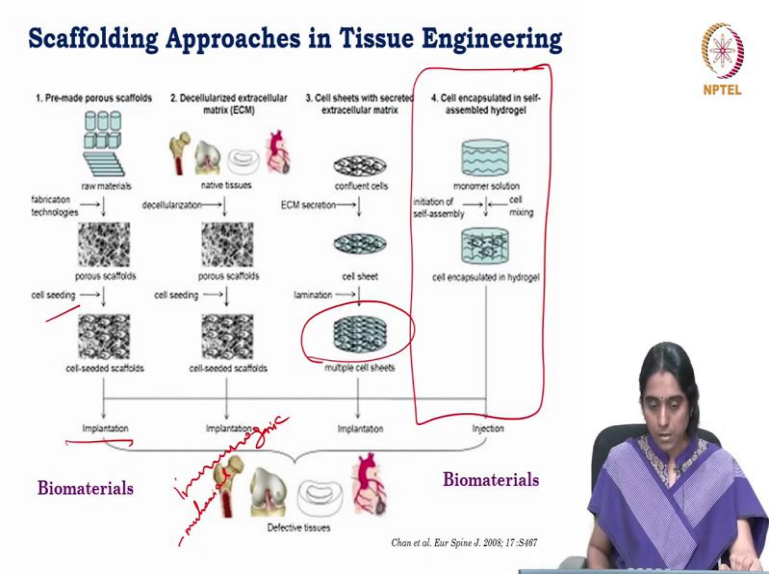

So, usually what are the approaches scaffolding approaches in tissue engineering if you say these are broadly there are four the broadly four different kinds of scaffolding approaches for any tissue engineering not only for oral tissue engineering for any tissue engineering there are four different types of scaffolding approaches. One is pre made porous scaffold wherein either a polymer material or an inorganic or organic material of

a natural or synthetic origin is fabricated into a porous structure solid porous structure and the cell seeder.

Next step; these cell seeded scaffold are implanted. So, these are the steps involved in that. The second one is a very conventional one and off let it is becoming very popular also de-cellularized exist extracellular matrix. So, either allogenic or xenogeneic tissues organ for example, the heart of a person is removed and the cellular part of the heart is removed either by physical method or enzymatic degradation all the cells are removed which will just leave behind the extracellular matrix.

But so that is going to be a wonderful scaffold because it knows it will have all the required cues reservoir growth factors and the shape shaping of the tissue everything is going to be perfect, but one important issue is that if the cells are not removed properly it may cause immunogenicity ok.

So, that is one issue with this. So, immunogenicity immunogenic and it may also cause if the cells are removed, but due to adverse processing condition if the processing or the removal condition is not optimized the bio the you know extras extracellular matric chemistry biochemistry can be disturbed.

So, that may also happen. So, there should be very careful optimization of removal of cells. But even though it has been carefully done the mechanical property is not maintained is not retained. So, that is an issue with decellularized extracellular matrix. Then there is this cell sheets which is secreted by the cells itself extracellular matrix is secreted by the cells it is not from external sources.

So, the cells are grown in a you know thermo labile polymers to confluency and the cells are allowed to secrete their own extracellular matrix and by change in temperature the extras this polymer is removed and we are left with the cell sheets. What they will do is over and over they grow more cell sheets and they form a multiple cell sheet called as laminated sheet which are then implanted. This has been successfully done for cardiac tissues even decellularized extracellular matrix has been shown to be successful for cardiac tissues.

The last one is very popular for almost all cells even for oral tissue engineering this is what people have mostly used and shown it to be successful that is use of self assembling

hydrogels. So, monomeric solution is used which are allowed to self assemble at a given set of conditions which are encapsulated with sea cells of interest then it is either placed as a scaffold solid scaffolds or it is injected at the particular site. So, this is what is being more popular these days.

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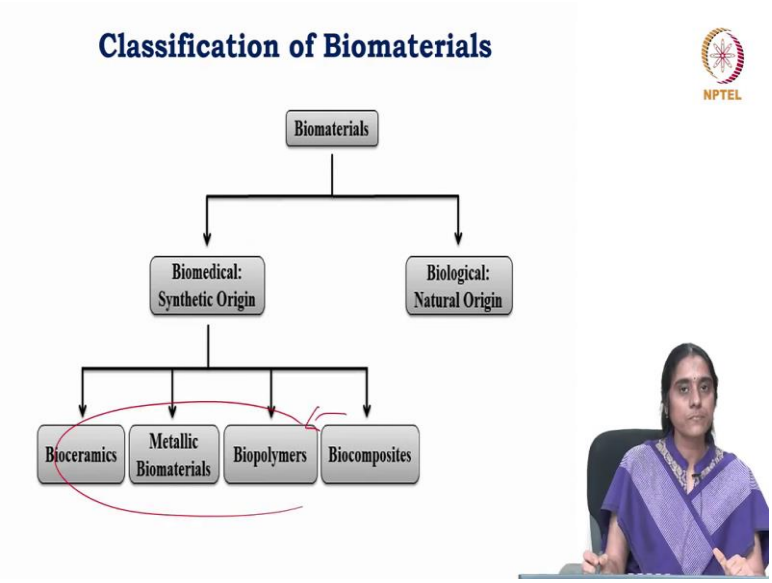

So, we have been seeing throughout that for the fabrication of scaffold biomaterial is required. So, generally by in the previous module and previous sessions you might have known about biomaterials. So, biomaterials can be of either synthetic or natural origin and in synthetic origin we have ceramics, metallic, biomaterial, biopolymers and composites, which may be combination of all these right.

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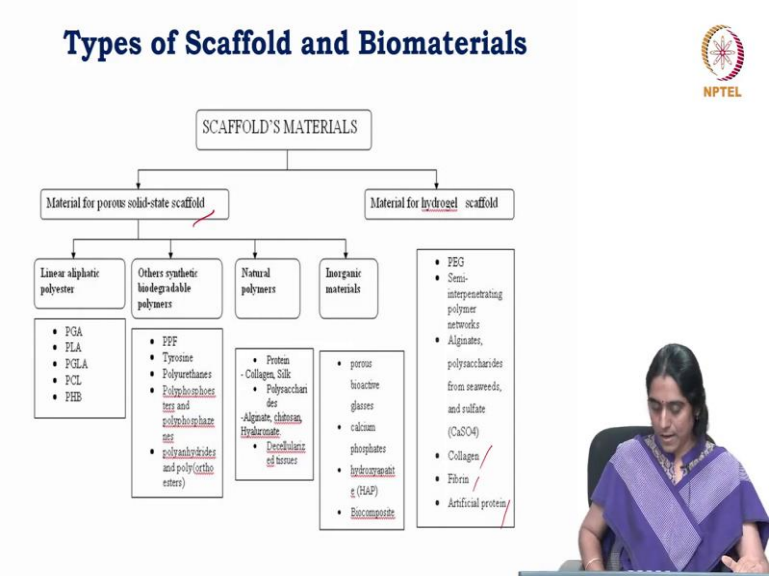

So, if we take the different type of scaffolds made out of these biomaterial so, scaffolds are broadly classified into two types; porous solid state scaffold and hydrogel scaffold ok. So, the porous solid state scaffolds is made up of can be fabricated either by aliphatic polyester such as; polylactic acid and then poly glycolic acid etcetera or it can be fabricated by synthetic biodegradable polymers such as polyurethanes polyphosphoesters phosphazenes etcetera.

Or it can also be fabricated from natural polymers which is very compatible and similar to that of more similar to that of the extracellular matrix. Example is the proteins such as collagen, silk and polysaccharides such as chitosan, alginate and as we saw earlier decellularized tissues.

Inorganic materials are more popularly used for porous solid state scaffold fabrications. And for this purpose hydroxyapatite bioactive glasses porous bioactive gases and we also find in the literature that mesoporous silica material MSMs are more widely used in these applications and the mixture of the protein and inorganic material bio composites are also tried.

Now, when we see hydrogel scaffold, it is mostly using natural or synthetic polymers; polyethylene glycol has been commonly employed for this and natural proteins like collagen, fibrin and artificial proteins are also used. So, here they are there is a use of

chemical cross linker and then certain methodology is used to create a porous structure in these hydrogels.

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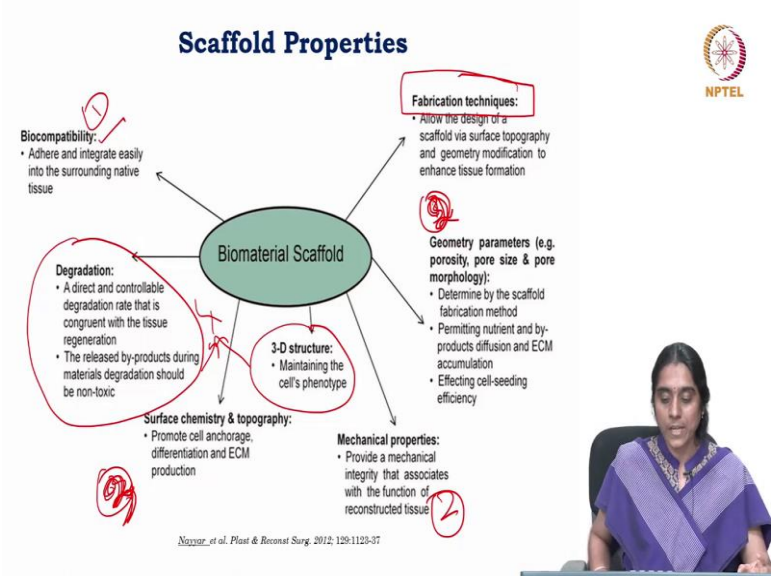

We will see how it has been done ok. So, how now until now we saw the functions and how we can modulated by in the scaffolds. So, to summarize all that this slide shows what are the required properties of a scaffold. So, the first and foremost property is biocompatibility without which it is not going to allow the cells to adhere and proliferate. So, that is that should be there, the next important thing is the mechanical property which should be similar to that of the tissue to be regenerated.

So, that will provide the in mechanical integrity and the associated function for that particular tissue otherwise it may give a different issue in the forthcoming slides I will show you how the difference in mechanical strength leads to different tissue formation.

The 3rd important parameter is the surface chemistry and I can I will give this also 2nd ranking when we say about these scaffold properties that needs to be thoroughly considered surface chemistry and topography, because that will determine how far a cell is going to be anchored on the scaffold.

Then the important aspect the other important aspect is the geometric properties. The pore size, pore morphology right so, because this will determine the diffusion of nutrients migration and movement of cells inside the scaffold rearrangement of the cells

inside the tissues. So, and also this will determine how efficiently the cells are going to be seeded, how homogeneously the cells are going to spread and aid in the regeneration of the particular tissue.

So, this is also important I we should say that this has to be considered as the 2nd most important and then this should be considered as the 3rd important. So, the 1st important one is the biocompatibility, mechanical property the 2nd and the and as well as the geometrical parameters then comes the surface chemistry and the topography. Maintenance of 3D structure is also important and the degradation property. So, this, these together can be the 4th property that has to be considered. because as I told you the release of degradation product should not cause adverse effect right.

So, the geometry and modification geometrical modification you know this pore size porosity they can be controlled by the fabrication technique that is going to be employed. So, the technique in the technique itself by controlling various parameters we can control the pore size its interconnectivity and then pore morphology etcetera. So, we will see in the next slides how what are the different types of fabrication.

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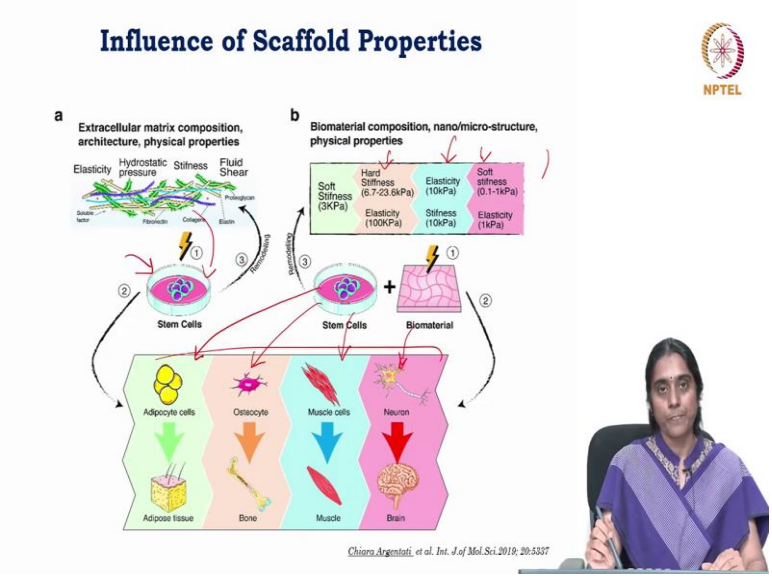

So, I we saw that the scaffold property is going to influence the tissue regeneration. So, as an example here you can see that the composition architecture and physical properties are influencing the tissue formation. Here you can see there is high when there is high stiffness ok.

For example, in case of tendons there is a regular arrangement of collagen which gives its high tensile strength the tendon tissue ok. So, if such an arrangement is given the stem cells tend to differentiate in to tendon or you know chondrocyte kind of cells. Whereas, if we take skin cells. Where again the extracellular matrix is made up of collagen, but there is random arrangement there is not a regular arrangement.

So, that gives it flexibility as well as toughness. So, the composition is important, the arrangement is important, the strength is important. So, do you understand now how it is going to influence a particular type of cell? So, here the stem cell can be a can differentiated to any cell right.

So, depending on the stiffness it can differentiate into either of these cells. So, by just varying the elasticity the physical properties hydrostatic pressure stiffness and fluid shear and without changing any chemical cues biochemical cues we can modulate the differentiation of the cell ok. Similarly here you can see that the biomaterial composition which is going to again influence the structure and physical property.

The elasticity can change stiffness can change hardness can change right. So, when there is when the stiffness is 3 kilopascal the same stem cell is differentiating into adipocytes. When the hardness that is hard stiffness in the biomaterial it leads to formation of bone cells osteocytes.

When the stiffness is 10 kilopascals ok it leads to the formation of muscle cells elasticity is high ok with elasticity stiffness is 10 kilopascal. And when there is soft stiffness which means the pressures is very low 0.1 to 1 kilopascal; it mostly leads the formation of helps in the formation of neural cells. So, if you want to regenerate a bone tissue.

And if you are going to keep the stiffness at 1 kilopascal definitely the cells are not going to differentiate into bone cells like probably they will, but there no there may not be optimal differentiation. So, this not only defines the properties not only defines the structure of the tissue to be formed, but also influences the differentiation of the stem cells right.

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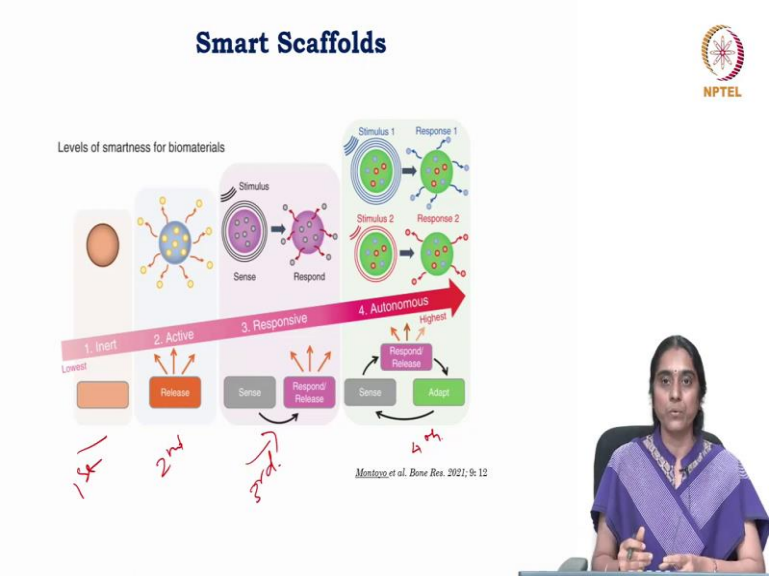

So, this we have to understand ok. Over the years there has been different generations of scaffolds form. So, at the very start of tissue engineering which started in early 19 I mean 80s they were using inert scaffolds which means it did not react to the cells they just acted as a substratum for the cells to proliferate.

And to give them a shape that is it. So, that was the 1st generation. The next generation scaffolds or active scaffolds which were releasing certain factors. So, people were loading these scaffold with required factors which when implanted in the site or which when grown used in-vitro condition also was releasing the factors.

But the release was not sustain which was active a burst of release. So, that was the 2nd generation. The 3rd generation scaffolds or as a response of scaffolds which means they can sense the particulars with a stimuli and towards that stimuli, they will be able to respond. So, when we are loading the scaffold with a factor it is not just like that going to release.

It will sense the when the required condition is there then it will release. And when there is say for example, overcrowding of cells cell has proliferated, it will stop the release of the factors. So, it may be sensitive to temperature or it may be sensitive to space right. So, all that is the 3rd generation responsive scaffold now the 4th generation scaffolds are autonomous scaffolds are we can say artificially intelligent scaffolds which are capable of learning.

So, here it can be any response that will it is just going to release, but in the case of autonomous scaffolds, it if it the you can load more than one or two factors and this scaffold will recognize two different stimuli and it has the intelligence to partially release that particular required stimulus or response or factor it is not going to release all the factors contained in it and it will know when to release. So, that kind of creating a intelligence kind of machine learning technique is also being mimicked for scaffolds.

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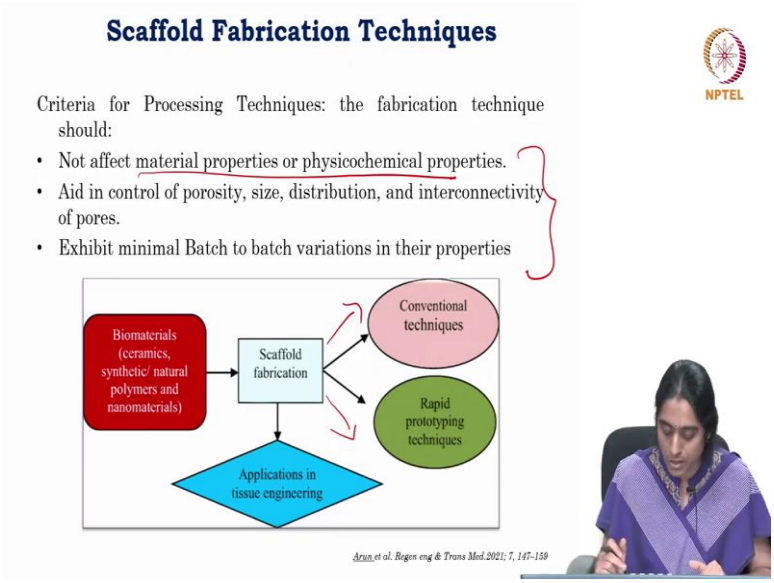

So, that was about the different properties and how the different types of what are the material employed in scaffold you know formation fabrication. Now, let us see the fabrication techniques. If we have to see broadly we can classify the fabrication process into conventional technique and rapid prototyping technique.

Before seeing what are those conventional and rapid prototyping techniques we need to understand this whatever may be the criteria for the fabrication any process you choose, but we need to keep these three points in mind. The fabrication process should not affect the material properties or physicochemical properties it should aid in the control of porosity size distribution and interconnectivity of the pores.

And foremost important thing is there should be minimal batch to batch variation in their properties as we scale up there is a tendency for batch to batch variation right. So, that has to be minimal we cannot say that it is entirely there is going to be no error, but there should be minimal error minimal variation that has to be kept in mind when we choose

or when we subject the scaffold for or produce the scaffold through a particular fabrication process.

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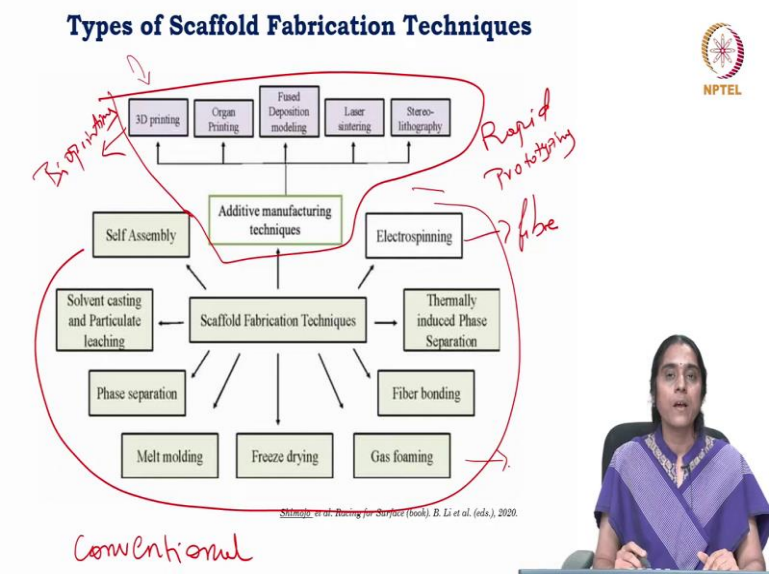

So, conventional these are the conventional methods these are the conventional methods and this is the prototypic additive manufacturing techniques which are now termed as rapid prototyping and these are conventional methods. So, in case of this conventional method we have the commonly known electrospinning technique, gas foaming technique, freeze drying, melt molding phase separation, solvent casting and particulate leaching right and self assembly.

The scope of this course is not to go into each and every fabrication technique for that there are other NPTEL courses there is a separate course on biomaterials and tissue engineering course where you can learn in detail about all these just know these are the methods and these are all in electrospinning it forms fiber like structures using electric field they use the principle of electric field is applied to the material and fiber like nanofibers are formed.

And in case of gas foaming again organic solvent is solubilized in solvent and carbon dioxide gas is subject foam is just pressurized on it to create pores on it. Likewise in case of freeze drying lyophilization it is a kind of lyophilization process. So, two different solvents are mixed together emulsions is formed and it is frozen and later on using

lyophilization through the process of sublimation the water is all removed and that causes the formation of pores.

So, likewise you can go in detail about the other techniques, but if we see about the additive manufacturing techniques what is unique in that is they use computational technique along with automated designing. So, there it is more precise easy to personalize and customizes customize it according to the patient needs, but in conventional method that is not.

So, if you see the additive manufacturing techniques we have again different types starting from stereolithography laser centering; these were the old techniques fusion deposition modeling and latest is the 3D printing. And now we have bio printing, which is advancement of the 3D printing, organ printing is also an advancement.

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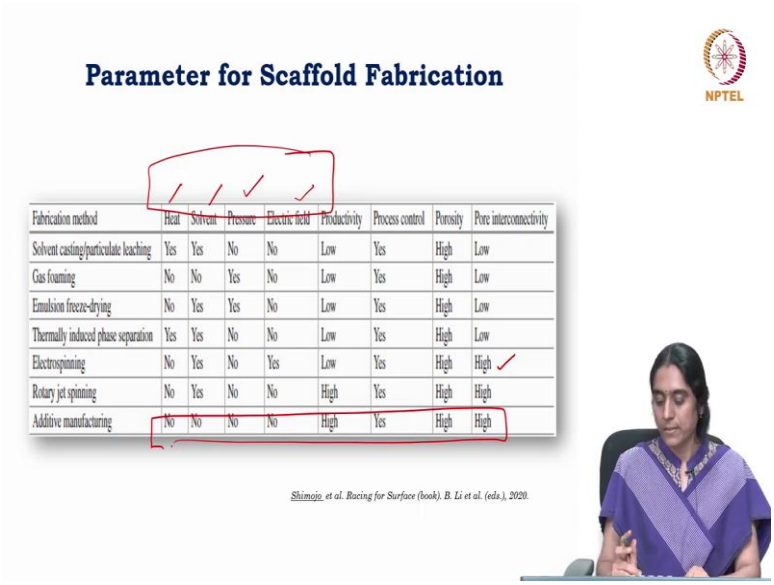

Fabrication method	Heat	Solvent	Pressure	Electric field	Productivity	Process control	Porosity	Pore interconnectivity
Solvent casting/particulate leaching	Yes	Yes	No	No	Low	Yes	High	Low
Gas foaming	No	No	Yes	No	Low	Yes	High	Low
Emulsion freeze-drying	No	Yes	Yes	No	Low	Yes	High	Low
Thermally induced phase separation	Yes	Yes	No	No	Low	Yes	High	Low
Electrospinning	No	Yes	No	Yes	Low	Yes	High	High
Rotary jet spinning	No	Yes	No	No	High	Yes	High	High
Additive manufacturing	No	No	No	No	High	Yes	High	High

So, what needs to be considered when we choose a particular fabrication process? So, these are the parameters which can influence the scaffold character especially the porosity and pore interconnectivity. Because that is what is going to determine the cell proliferation and you know the structure mechanical property everything is dependent on that. So, heat, the solvent used pressure, electric field and the process control.

So, these are all going to especially the physical properties are going to influence porosity. Out of all these if you see the best one seems to be additive manufacturing as of

now. And in conventional method electrospinning is seems to be comparatively good right. Because the pore interconnectivity is very high with electrospinnings compared to the other techniques.

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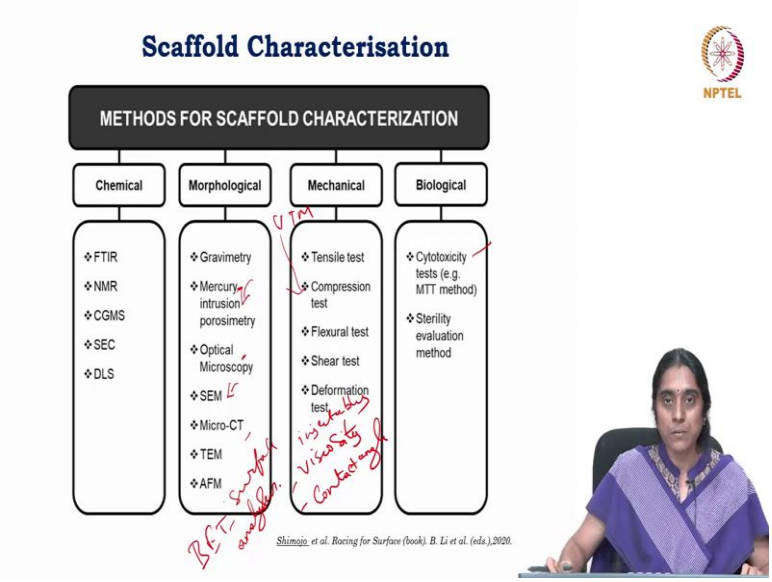

So, now, that we have seen how to fabricate scaffold what are the different techniques applied how they are characterized because if we have to know particularly how the tissue or the cell behaves we need to know the chemical, morphological, mechanical and biological character of the scaffold.

So, for chemical characterization, for the composition chemical composition the functional groups and the side chains or on the surface for all that we can they use FTIR which is the kind of spectrophotometry Fourier Transfer Infrared Spectrophotometry NMR ok. Then NMR is also used which is nuclear magnetic resonance spectrometry CGMS is gas chromatography with coupled with mass spectrometry and DLS is these are all used for characterizing the chemical composition, the functional groups etcetera.

But DLS is used to see the molecular size of the material of the scaffold. So, that is dynamic light scattering. So, if we have to study the morphological features these are the techniques, which are used the gravimetry mercury intrusion porosimetry this is a very costlier technique and moreover they use mercury which is poisonous. One of the cheapest method is optical microscopy, but beyond the level we cannot analyze it.

So, the other higher techniques which can be employed to assist the scaffolds or scanning electron microscopy which will give more features or information on the morphology; micro CT is the best one, but it is still costlier because there is no need for preparation of the sample. Whereas, in SEM we need to prepare the sample even in TEM we need to do some preparation for the sample before it is being analyzed.

Micro CT is also non invasive we can place the sample analyze it and again use it back, but still it is a costliest thing we can see the three dimensional morphology of the scaffold itself by micro CT which is microcomputer tomography, atomic force microscopy again gives the surface roughness and morphology of the scaffolds.

And again when we come to the mechanical property, how to test it. Usually they go for UTM Universal Testing Machine which will give the mechanical properties of the scaffold and if it is going to be a injectable scaffold we need to know the viscosity. So, that should also be tested and compression test which is done by UTM flexural test is done, shear test is done and deformation test is done.

And apart from that one more thing which I wanted to add here mercury intrusion porosimetry is used for morphological analysis right which will give the an idea about porous pore size and inter thickness of the interconnecting walls and all. There is another method which was used called as bet. So, this bet will gill the pore size and it will also give the thickness of the wall pore walls ok.

You can just see bet surface analyzer and if you want to know more about it you can. So, here and if this is the case with injectable scaffolds we need to know viscosity I told and one more thing which need to be known is the contact angle which will give an idea about the hydrophobicity and hydrophilicity of the fabricated scaffold that is also should be known. Then of course, we should know the biological characteristics after all we are going to use it for biomedical application.

So, how is the cytotoxicity against if it is a synthetic material definitely we should test for cytotoxicity very preliminary and sterility evaluation methods is as important because if it is not sterile it may cause infection leading to inflammation and failure and cause other further adverse effects. So, apart from this there are various other histopathological evaluation genotoxicity evaluation, it is not the scope of this lecture, but as a most of you must be from biology background may maybe knowing about that ok.

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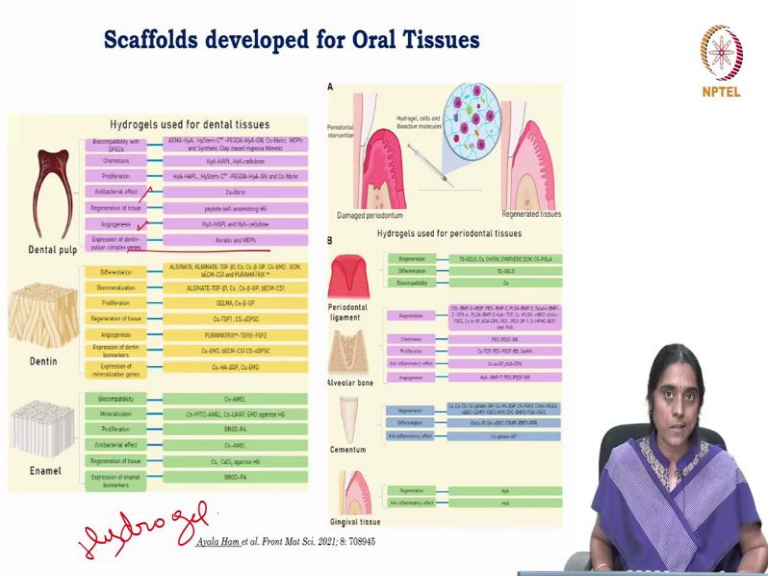

Now, coming to the scaffolds developed for oral tissues. Now, as we saw these are the different dental pulp, dentin enamel, periodontal ligament alveolar cementum gingival tissue everything has been regenerated in lab. So, in most of the cases what are the scaffold that has been used it is the hydro gels sorry. So, here you can see in this table they have given this is from one of the review articles they have given that these tissue have been regenerated.

They have either used employed synthetic hydrogel or natural hydrogel like alginate, chitosan or collagen in combination with inorganic material such as; hydroxyapatite bio glass and other biological factors are also incorporated. So, all the tissues have been successfully regenerated, but you if you carefully look into these it depends that on what we want to regenerate depending on that the kind of hydrogel would have been chosen.

And see for example, in case of dental pulp, there is high chance of infection. So, for that antibacterial effect has been incorporated angiogenesis important. So, that is been addressed by having a highly porous structure and expression how do we know that particular cell has been sorry tissue has been regenerated, you need to have a marker for that the keratin or the MDPH that is being analyzed right.

Likewise if we see dentin for that there is a marker for dentin express which is expressed by the dentin, like beta GP and EMD. So, you if you see enamel has a particular marker called as BRGD PA which is the protein ok. And what are the material used for

mineralization of enamel agarose has been found to be a best material enhancing enamel formation.

And here if we see the periodontal ligament mostly it is chiton then they have used even PGLA synthetic polymers. And if we see alveolar bone in combination with various factors like BMP and FGF 2 collagen has been used as scaffold material. And if we have to look at the anti inflammatory effect has also been incorporated while regenerating alveolar bone.

In case of cementum they have used chiton see they the it is as of now there is we cannot say that chiton is the one for cementum or collagen is the one for alveolar bone. They have been trying different combination keeping in mind the requirement of the tissue and the characteristic of the tissue. So, some are working and some are successful all these have been done in laboratory. But when we are going to translate how far these are going to be successful we are not sure. So, we see that almost all the cells have been tried.

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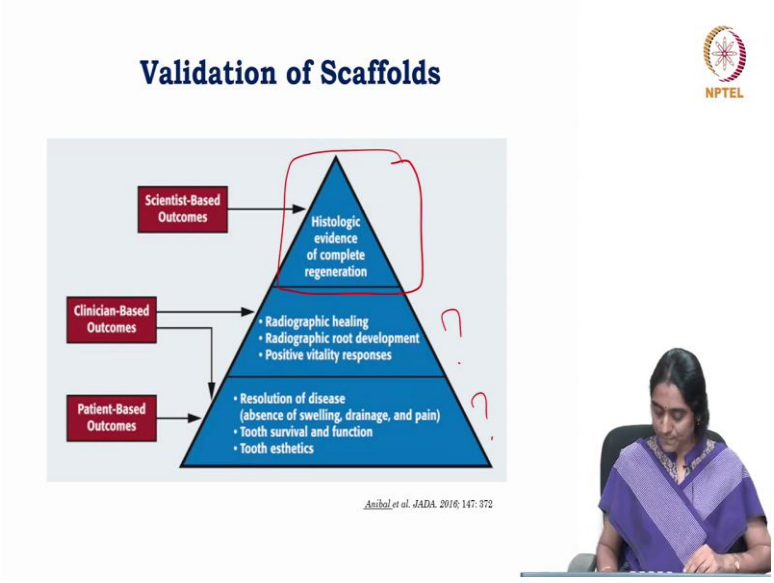

So, that is about we saw that all the cells has been regenerated, but how far it is valid. Scientific validation has only been done in animal models. So, histological evidence of complete regeneration has been reported until now. But clinician based outcome is still a question mark. It is being tried maybe in a decade we will have answer for that.

So, how do we check if we are going to test these fabricated scaffolds? So, radiographic healing radiographic root development positive vitality responses see I should mention one thing in case of periodontal ligament regeneration some of the scaffold has been tried in humans also like fish collagen bioactive glass based membranes this has been already in marker and it has been tried.

So, they are being analyzed clinically by these healing properties and patient based outcome is what will really tell whether the scaffold is successful or a failure. So, the resolution how far the disease has been cured the cell absence of the symptoms and pain should be there then the tooth survival hand reversibility of the function should be absorbed if there is loss of anesthetic tooth anesthetic should also be restored. So, these are the parameters which we have to look into when we see scaffold for clinically successful oral tissue for oral tissue regeneration.

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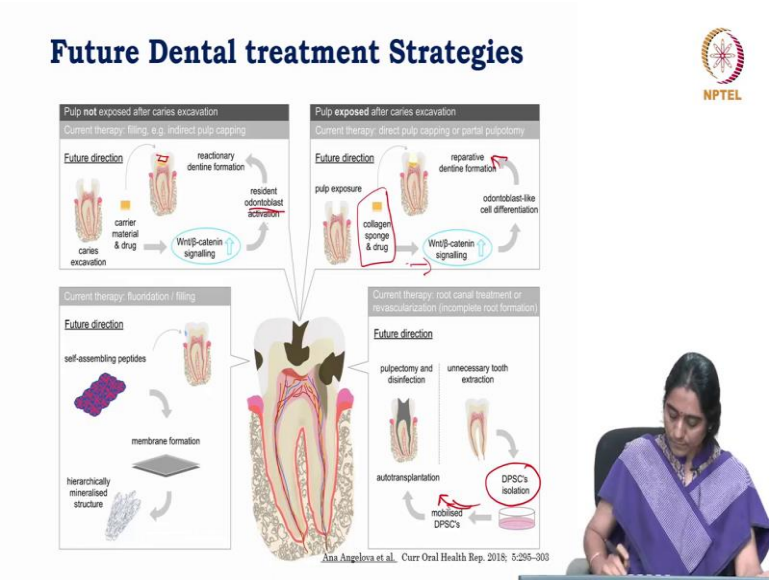

So, in future as I have been telling that maybe in a 10 years of time we may have a different strategies for dental and oral tissue treatments or diseases. For example, in this case this has been proposed in one of the reviews recent reviews. When there is pulp exposure either we can use material carrier material which is going to have a drug that will signal the catenin signaling WNT beta catenin signaling causing reactionary dentin formation.

This will activate the odontoblast and the reactionary dentin formation will be activated and that will close the or help in the formation of the mineral structure over here. Or other case if there is total or partial the pulps are exposed after carry caries excavation now currently what they do the pulp is capped or partially pulpectomy is done.

But in future they may use collagen sponge with a drug which is going to again cause the WNT beta catenin signaling causing osteoblast like cell differentiation which will enhance the reparative dentin formation and that will be restored ok. So, the host repair mechanism is going to be exploited.

If we take in like say there is partial loss of tooth structure due to fracture or something what maybe done is, self assembling peptides as we saw in case of in the previous modules you might have learnt about the tooth structure formation and enamel formation.

So, mostly proteins are involved in the nucleation and then crystal formation. So, similarly they will try to mimic that we may have a self assembling peptide helping in the mineralization of that portion enamel formation. So, there might be a membrane formation and which will enable the hierarchically mineralized structure all these are speculations ok. Then if there is going to be infection and lot of loss of dentine structure.

Then the current therapy is RCT right, but in future what they may do is they may totally remove pulpectomy and it will be disinfected when there is a unnecessary tooth inside the patients oral cavity that will be extracted the dental pulp stem cells will be removed, proliferated and then transplanted auto transplanted. This is the realization of an expectation of tissue engineering.

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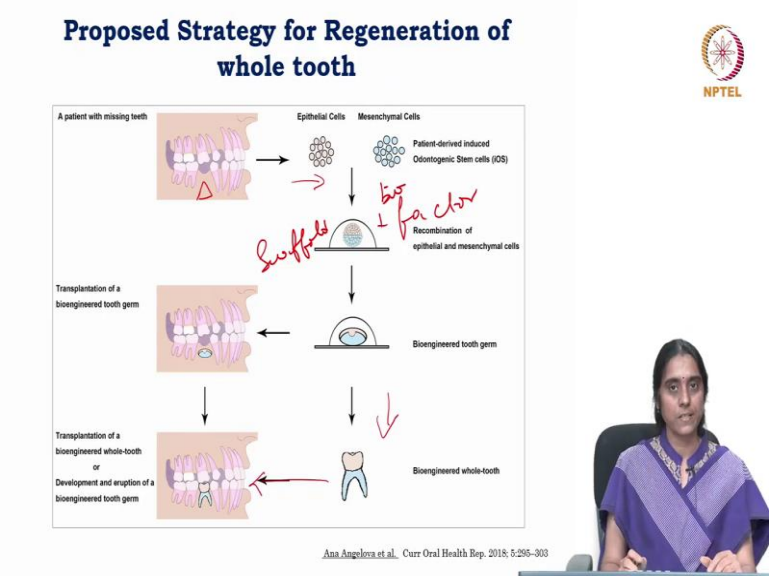

So, this is the model proposed for whole tissue whole tooth regeneration. So, from if there is a missing tooth what we do?

Presently either a denture or an implant or a crown is bridge is placed there, but what people will be trying to do in future is epithelial cells and mesenchymal cells derived from patients, odontogenic stem cells from the patient will be taken out and in recombination with various factors and scaffolds, scaffold plus factors bio-factor ok it will help in the formation of tooth germ and this tooth germ will be allowed to grow as a whole tooth bioengineer tooth which will be placed.

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So, the we are somewhere near to that ok. So, coming to the final slide of this session what we saw today. So, we saw the different types of regeneration strategies for oral tissues, the role of and function of scaffold based on the functions of the extracellular matrix.

And we saw these different scaffolding approaches the biomaterials employed for scaffold fabrication then there was a brief overview which we discussed about the fabrication and characterization techniques of scaffold. So, lastly we saw the proposals in future how the oral tissue engineering might change the dental and oral disease treatment. So, there are lot of scope and innovation in development of scaffold for oral and dental tissue with this we will conclude this session.

Thank you.