INDIAN INSTITUTE OF TECHNOLOGY ROORKEE
NPTEL
NPTEL ONLINE CERTIFICATION COURSE
Biomedical Nanotechnology
Lec- 13
Nanotechnology in Tissues Engineering
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Hello everyone I welcome all to the 13th lecture of this course nano technology in tissue engineering, so in this lecture we are going to learn what is tissue engineering?

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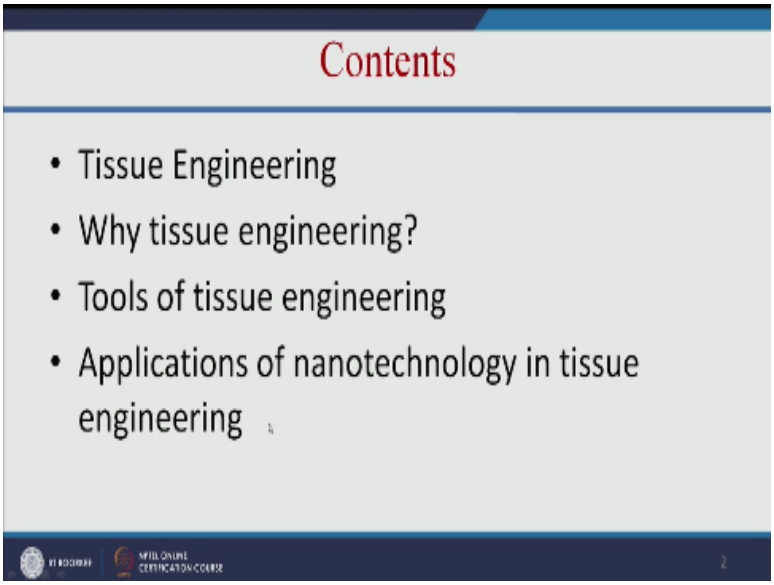

And why we need tissue engineering, and what are the tools required for tissue engineering and also we are going to learn what are the applications of nanotechnology in tissue engineering.

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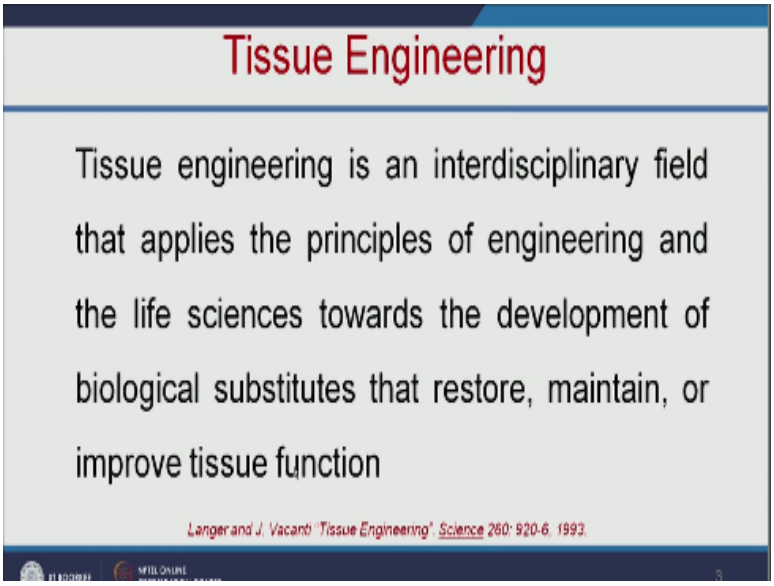

So let us see the definition of tissue engineering, so tissue engineering is an interdisciplinary field that applies the principles of engineering and life science towards the development of biological substitutes that restore maintain or into the tissue function.

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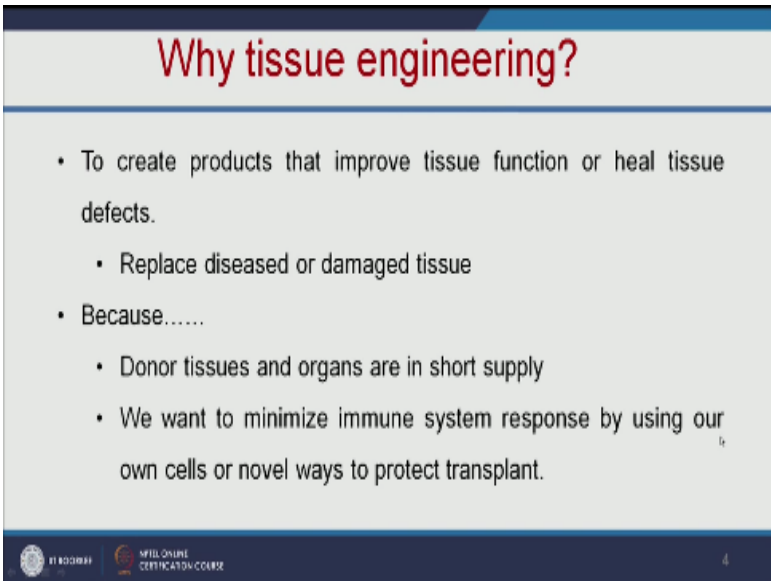

So here why we need tissue engineering to replace the diseased or damage tissue okay, because the donor tissues and organs are I short in supply we do not have sufficient donors for replacing the damage tissues or organs and also if we take the organs from a different person and different animal our immune system will reject it, so to minimize the immune system response we can use our own cells and we can engineer the man made tissues okay.

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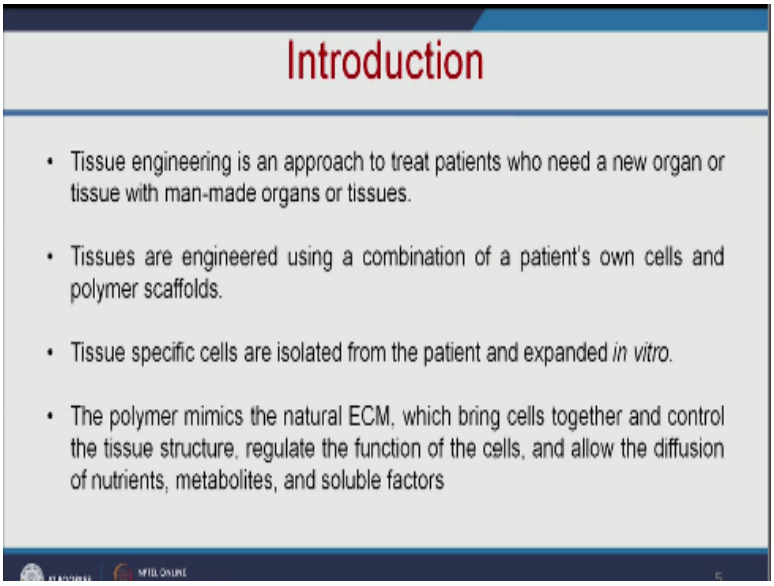

So here tissue engineering is an approach to treat patients who need a new organ or tissue with the man made organ or tissues, so here the tissues are engineered using a combination of patients own cell and polymer scaffolds so here we are going to take the patient's own cell are we are going to add the cells on the polymer scaffolds and we are going to grow that tissue in the lab condition and we are going to implanted the patient. So here the polymer is scaffolds will mimics the natural ECM, that is extra cellar matrix okay, so which brings cells together and control the tissues structure.

And regulate the function of the cells okay, so here the scaffolds act like a support and it will allow the cells to grow okay it will provide a support like a extra cellular matrix ECM and also it allow the nutrients to permit through the pore structure of the extra cellular matrix.

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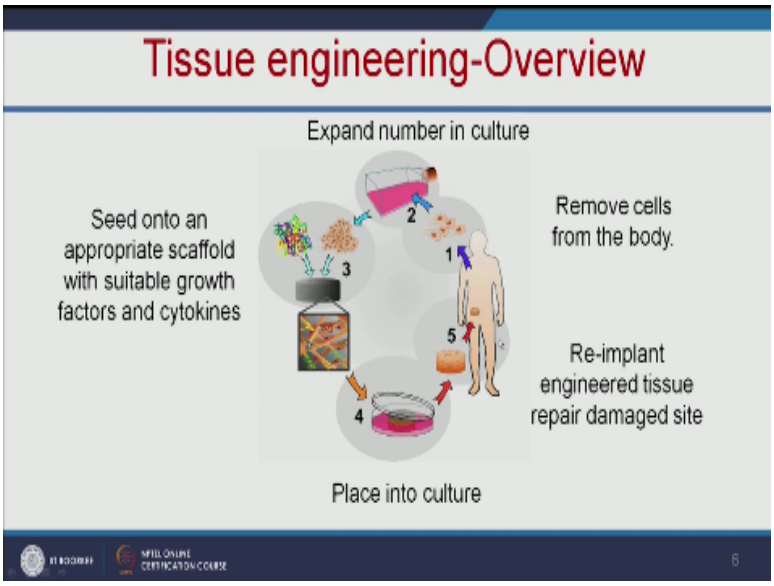

So this is the overview of tissue engineering, so the first step is where going to take the cell from the patient's body and we are going to cells in the lot condition and once we go the cells sufficient number of cells then we can see the cells on the three dimensional scaffold so then the cells grow on the three dimensional scaffold and we can implant the same cells into the repair or damage side okay, so we are re implanting this engineer tissue to the damaged site.

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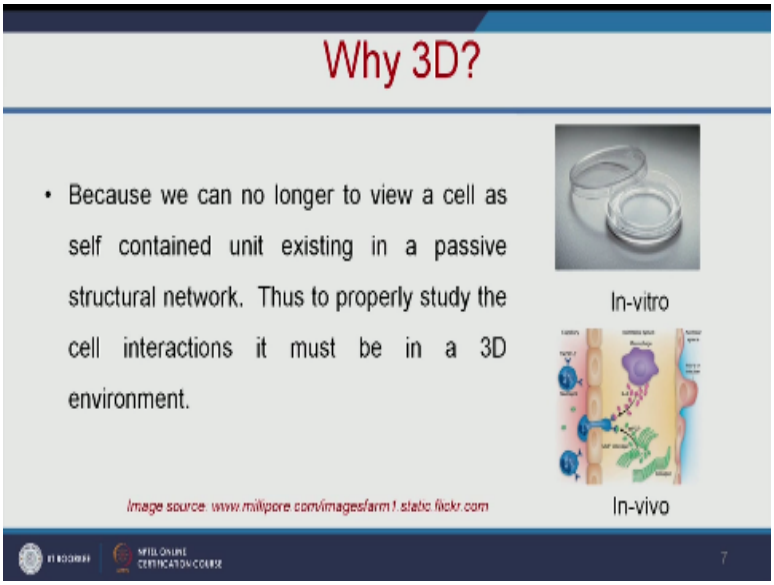

So this is the overview of tissue engineering and why they need three dimensional okay, so in case of two dimensional that own mimics your body condition okay, so inside the body we have all the organs are in three dimensional so we need a three dimensional scaffold so which mimic like your immune condition and also it act like a support to grow the cells and which could be useful for making a artificial tissues organ to replace the damage tissue or organ.

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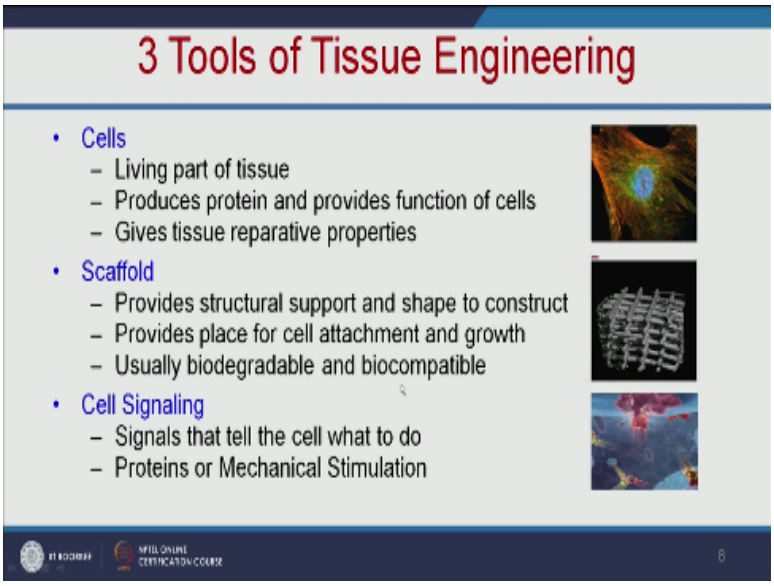

So the tools require for tissue engineering is cells, scaffold and cells signaling the first thing is cells, so that is a living part of tissue okay so that produce the protein or and provides functions of cells okay, and next one is scaffold so this provide just structural support and shape to construct and it should be usefully biodegradable and biocompatible, biocompatible means it should be compatible to the biological system and biodegradable means if we degrade inside the system it should not induce any immune response are toxic effects.

Third one is cell signaling so the cell signaling it may be like a growth factors or hormones okay, so that is going to tell the cell it has to differentiate or not what kind of cell it has to differentiae okay.

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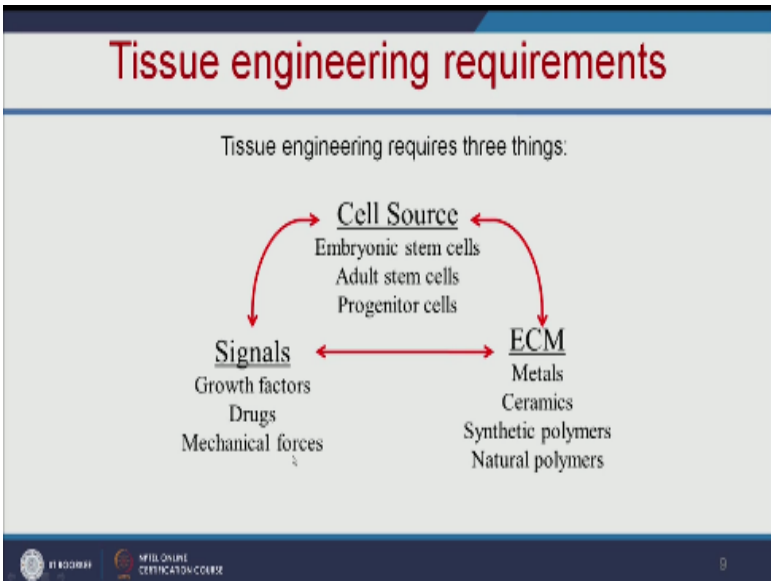

So these are the three things require for your tissue engineering first you need the cells source it can be embryonic stem cells or it can be your adult stem cells okay and next one is your extra cellular matrix that is your support or scaffolds it can be your metal, ceramics, synthetic polymers and natural polymers, so depends on the tissue it want to grow or depends on the organ you want to grow you have to select your scaffold it can be metal for some of the bond replacement and it can be synthetic polymer and natural polymer depends on the application where to select the ECM extra cellular matrix.

And third one is signals are growth factors, so this growth factors it is going to decide what kind of cells it is going to differentiate okay and also some of the tissues we have to apply mechanical force this mechanical force also plays a major role in differentiating the cells.

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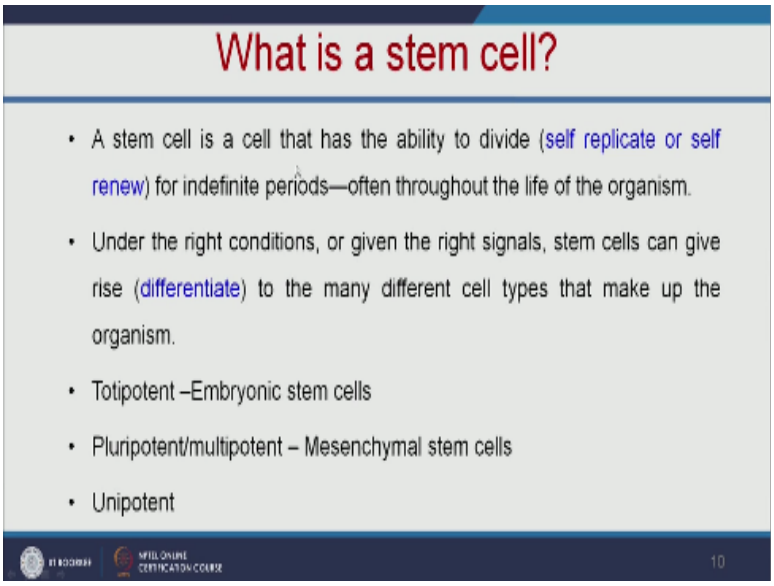

So let us see one by one so what is a stem cell okay, so it is a cell which the ability to divide for indefinite periods and it can be like often throughout the life of the organism okay so stem cell is like your simple term it can assume like it is a seed so from the seed you will get the plat from plan you will get the flower again you will get the fruit and seed okay similarly the stem cells it can differentiate to any kind of cells okay, so under the right condition or given the right signals the stem cells can given raise to many different types of cells okay.

So if you are inducing the stem cells with the suitable growth factors and it will it convert into liver cells or it can be cover into kidney cells okay, so that is called as differentiation so the stem cells again divided into three types Totipotent, pulripotent and unipotent so Totipotent is embryonic stem cells from embryonic stem cell we can get any kind of cells and multipotent cells example is mesenchymal stem cells from here we can get multiple types of cells and unipotent is we can get only single type of cell.

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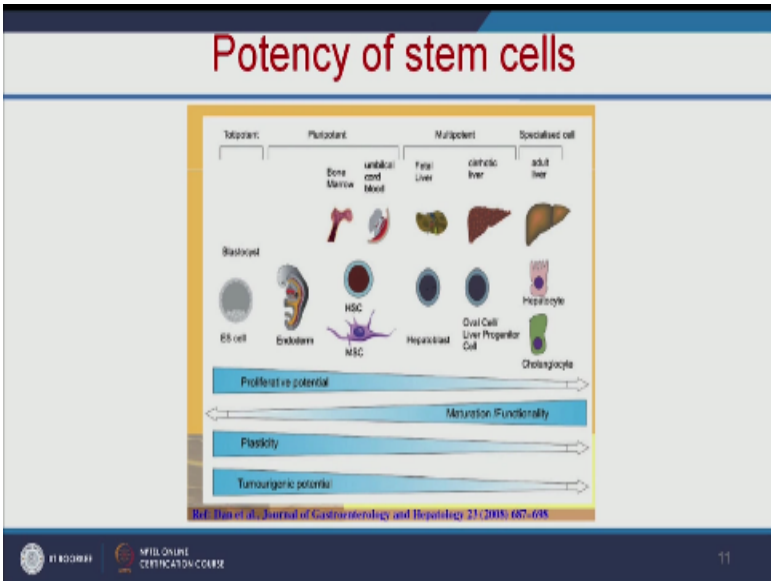

We can see here the Totipotent is a embryonic stem cell we can get any kind of cells and pulripotent are multipotent we can get multiple types of cells and unipotent is your adult liver cell example okay, so we can get the liver stem cells from the liver stem cells you will get the liver cells like cells , so it is only one type of cells we can get it from the unipotent cells so we can use the stem cells and depends on the growth factors okay all the hormones whatever you add the cells will differentiate the stem cells will differentiate into liver cells or we cab differentiated to kidney cells.

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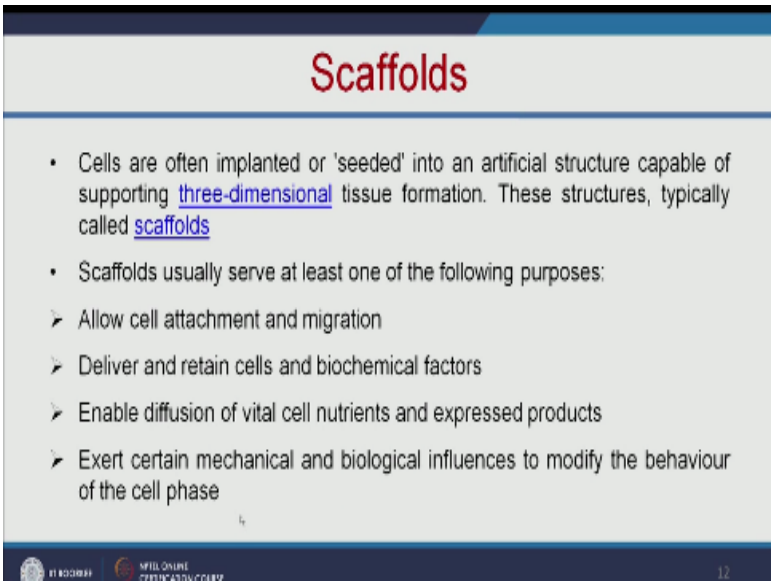

So next one is scaffold so the cells are often implanted or seeded on into a artificial structure and capable of supporting three dimensional tissue formation okay, so these structures typically called as scaffold so the scaffold should serve at least one of the following purposes okay, so you should allow the cells to attach okay and it should deliver and retain the cells and biochemical factors and it should enable the diffusion of vital cell nutrients and also it should exit certain mechanical and biological influences to modify the behavior of the cell fix okay.

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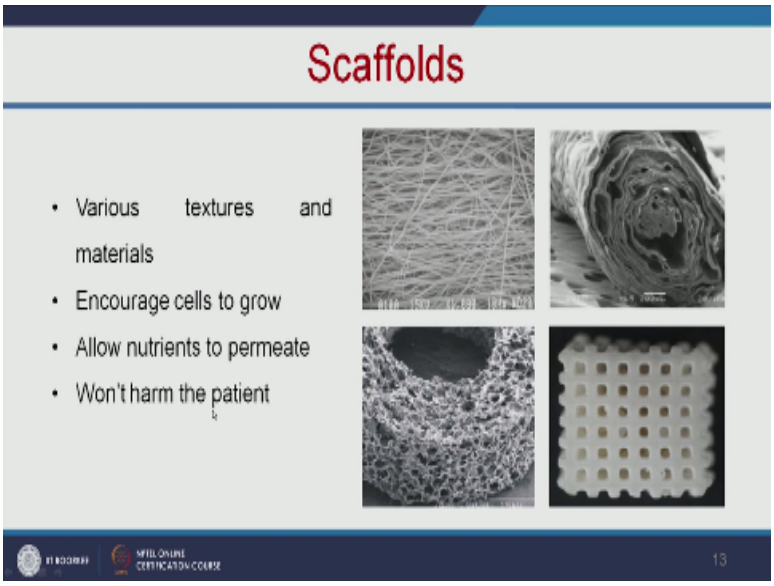

So we can have scaffolds in various structures and materials depends on the tissue we want to grow we have to select the suitable scaffold and the scaffold should encourage the cells to grow okay and we should have sufficient pour as nature, so that it can allow the nutrients to permit and it should be highly biocompatible and biodegradable it should not harm the patient.

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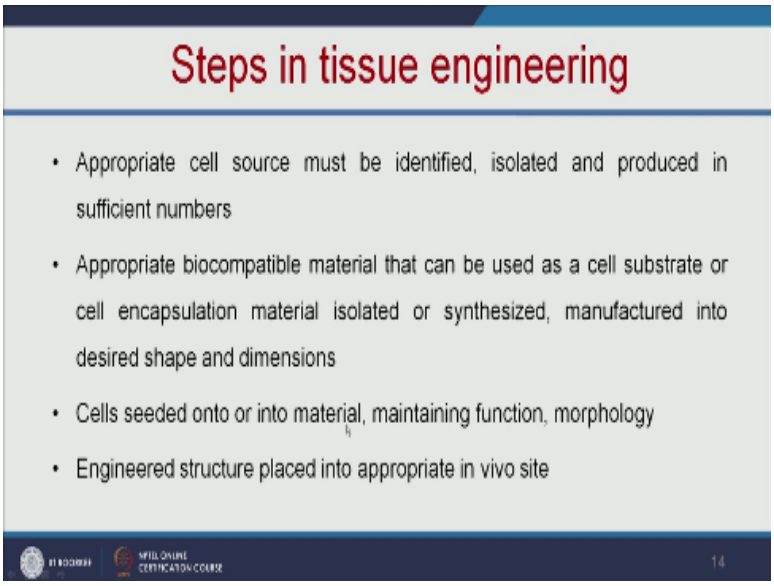

So let us see this steps in the tissue engineering so first thing is we have to select the suitable cells or then we have to select the suitable scaffold then we have to add the cells on the scaffold and grow it okay, so one the cell is ready.

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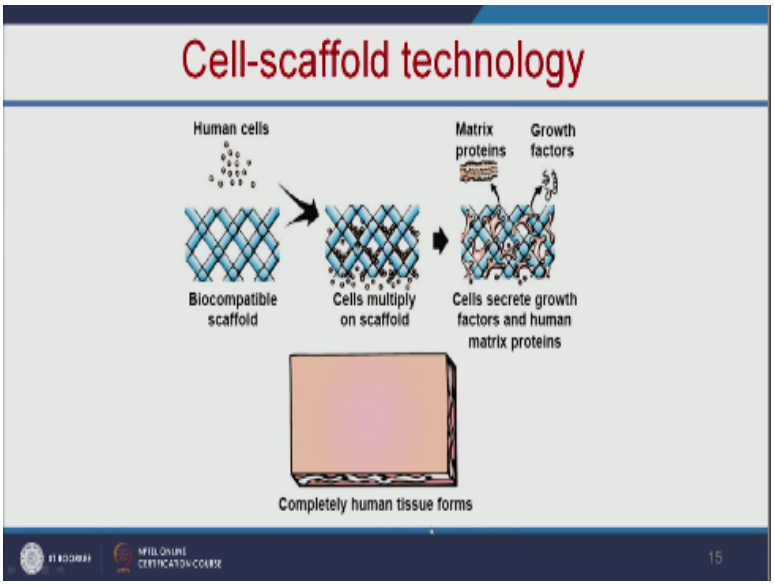

Then we have to transfer into the human body okay, so this is a biocompatible scaffold it can add the human cells and the cells multiply on the scaffold and you can add the growth factors of matrix proteins okay, so then cell secrete growth factors on human matrix proteins and it became complete human tissue.

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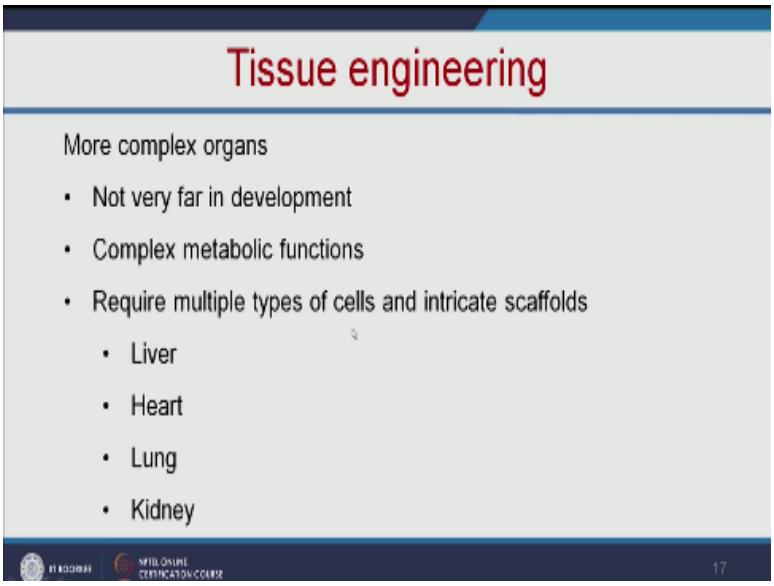

Okay so using the well designs scaffolds and optimized cell growth so these are the tissues these are already successfully engineered okay skin, bone, cartilage this is already successfully engineered but saw the complex organs like liver, heart and lung this is a still under research because it has a complex metabolic function as well as it require multiple types of cells okay. So that is why it is very difficult to make artificial liver or artificial heart, but still lot of research is going on to develop artificial liver or artificial heart and artificial lungs.

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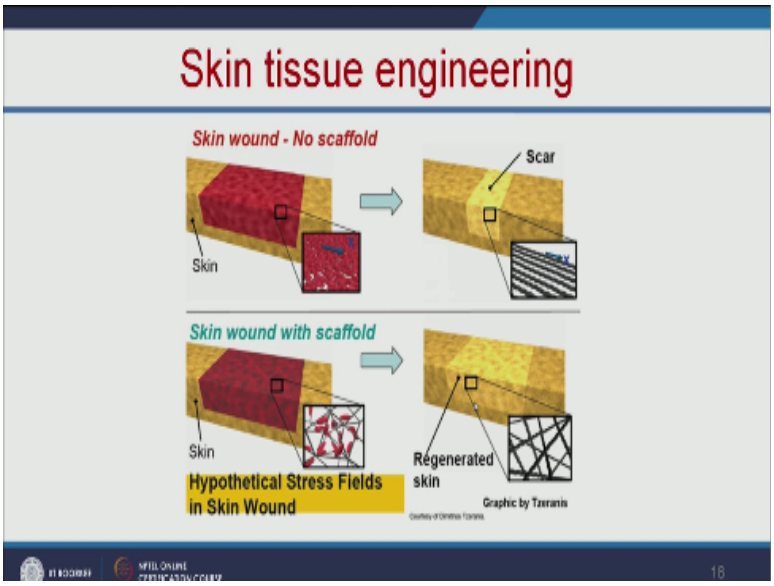

So let us see the example of skin tissue engineering okay, so suppose if we have a own one your skin you are losing some of the cells on the skin and when the skin contract it forms the scar but when you replace the damaged cells with your scaffold with the skin cells it will reach in a scale and there would not be any scar formation.

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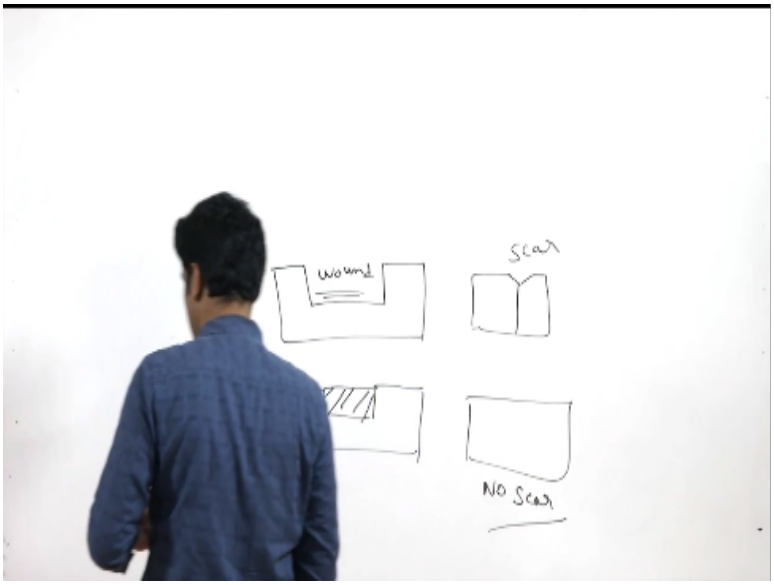

Suppose we were having the damage or own on your skin okay, so you are losing this much amount of cells, so what happen in this when the skin contract there will be formation of scar so but when you replace this damaged cells with the scar fold their own any formation of scar.

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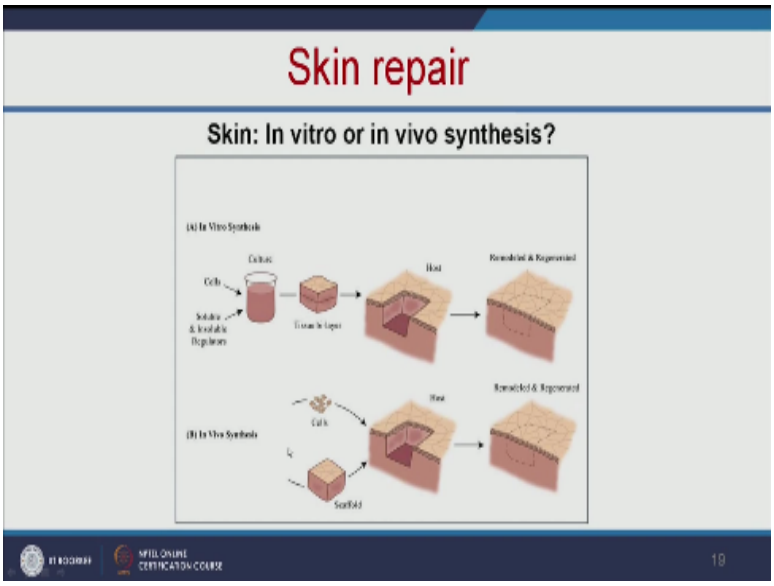

So let us see how to repair the skin so the two approaches one is in vitro and other one is in vivo in the in vitro we will add the cells and other regulators okay to the tissue by layer and will implanted to the holt okay and in, in vivo in synthesis we will put the scaffold into the host and you will add the cells on the host and it will reignited in the host that is called as in vivo synthesis.

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So let us see the example you can see here so this is the wound grafted with the scaffold and if you have the active scaffold so there is no contraction it is blocking the contraction and new tissue formed in skin there is no scar formation but when you not putting the scaffold what happen is there will be a formation of scar due to contraction of the skin.

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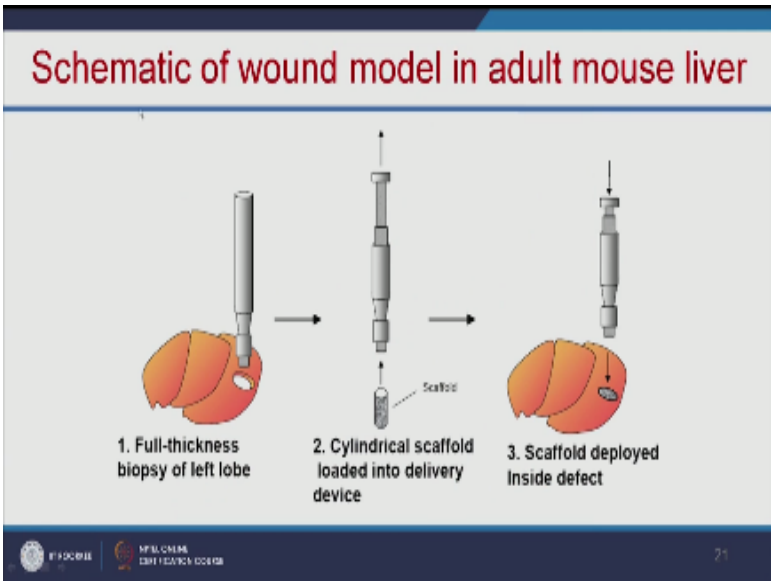

So let us see the another example, here we can see here schematic of wound model in adult mouse liver, so here then making your wound on the adult liver, adult mouse liver and they are replacing with the scaffold loaded with the liver cells and it is.

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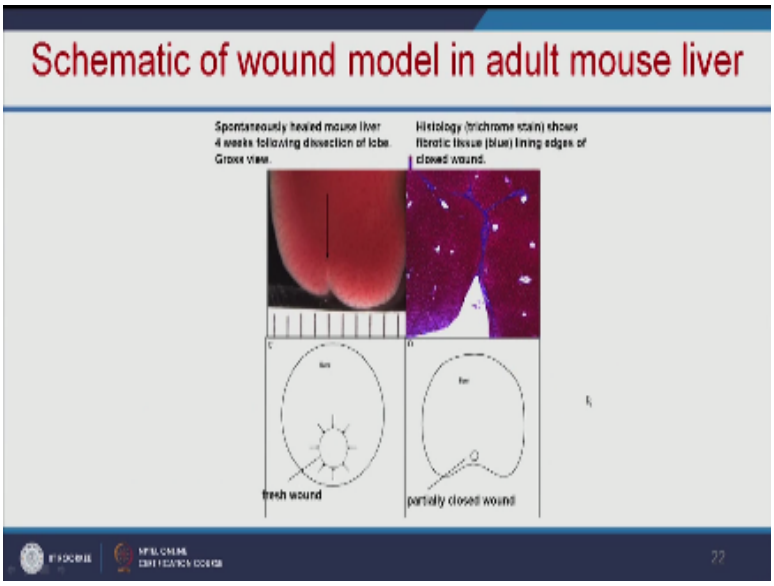

Healing the wound okay, you can see here without scaffold there is no wound healing with this scaffold it is closing the wound.

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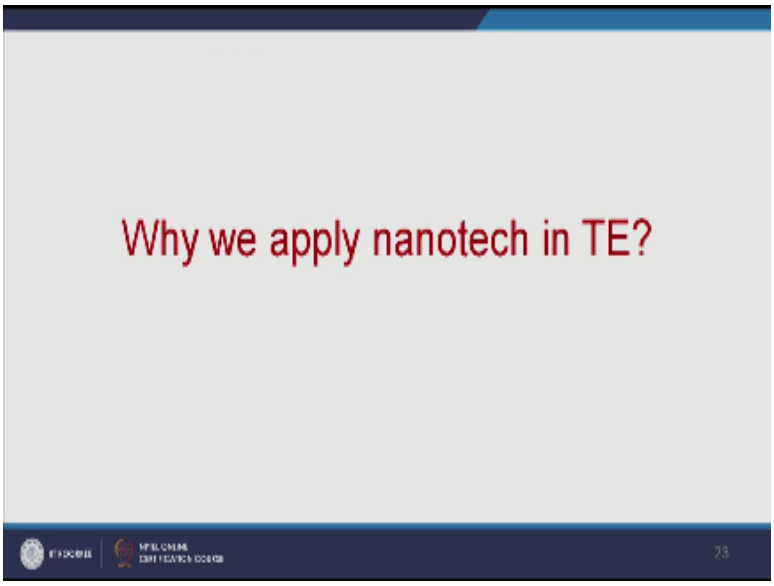

So let us see why we need nano technology in tissue engineering.

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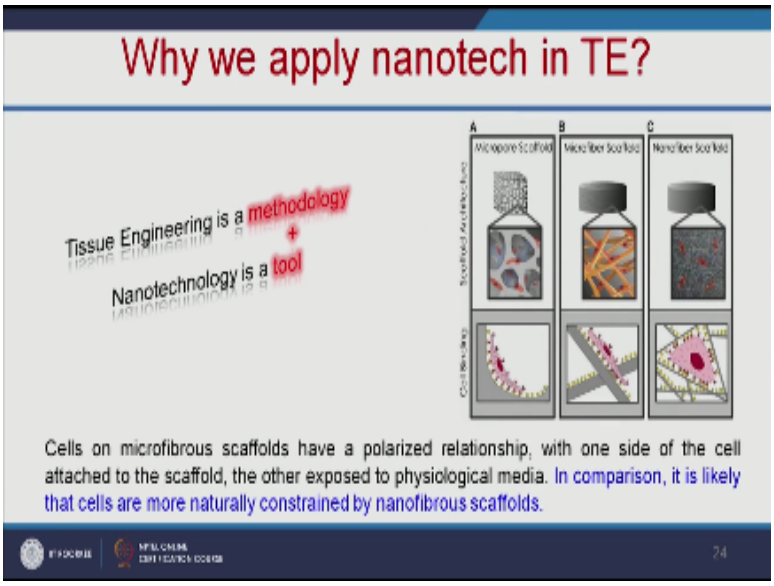

So you can see here, so when you use the microfibrous scaffold the cells are not able to attach properly and at the same case in the microfibrous scaffold also and but when you use then nanofibrous scaffold the each cells it can easily attached to thousands of nano fibrous, so as you know the cytosol cell is in the micro meter okay, when you use the micro pores scaffold or microfibrous scaffold that is not matching with the scale, okay.

But when you use the nanofibrous scaffold a single cell can attached to thousands of nanofibrous that will excitedly mimic like your extracellular matrix ECM, okay. So the cells on microfibrous scaffold have a polarized relationship with one side of the cell attached to the scaffold and the other exposed to physiological media, okay. So in comparison it is likely that the cells are more naturally constrained by nanofibrous scaffolds.

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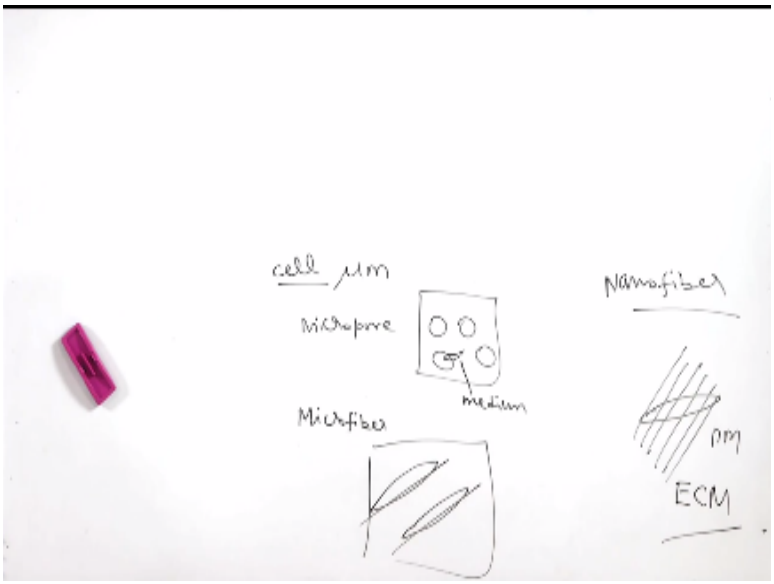

So the cell is in the micro meter size okay, I when I use the micropores scaffold what happens is some cells are partially binding to the support and some are exposed to the medium. When you use the microfibrous scaffold again so when you have the microfiber the cells are growing and the axis of the fiber okay, so again it is not exactly not matching with your ECM, so but when you use the nano fiber.

So here the fiber size is in the range of nano meter and your cell is in the range of micro meter. So each cell can bind and attached to thousands of nano fibers so it exactly mimic like your ECM extracellular matrix. So that is why we need nanofibrous scaffold for tissue engineering application.

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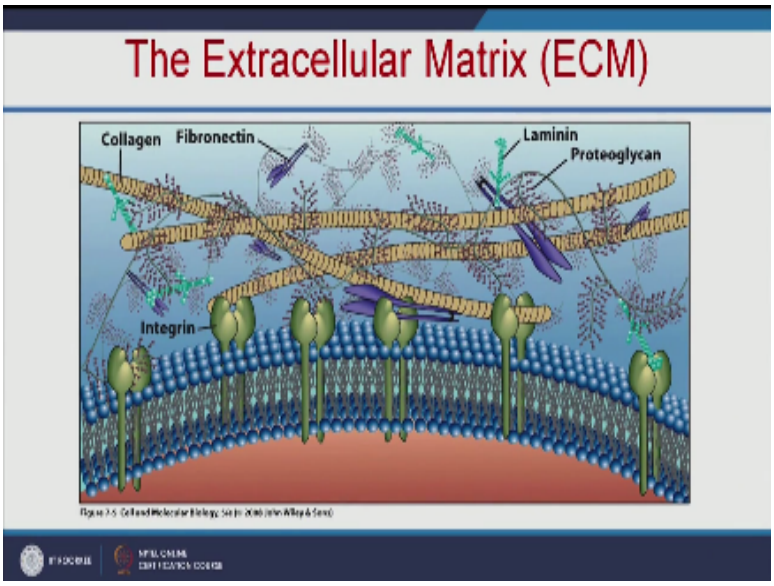

So we can see here this is your extra cellular matrix so each tissues are organ we have this kind of extra cellular matrix on the top of the matrix all over cells are attached and grow and orms the tissue or organ.

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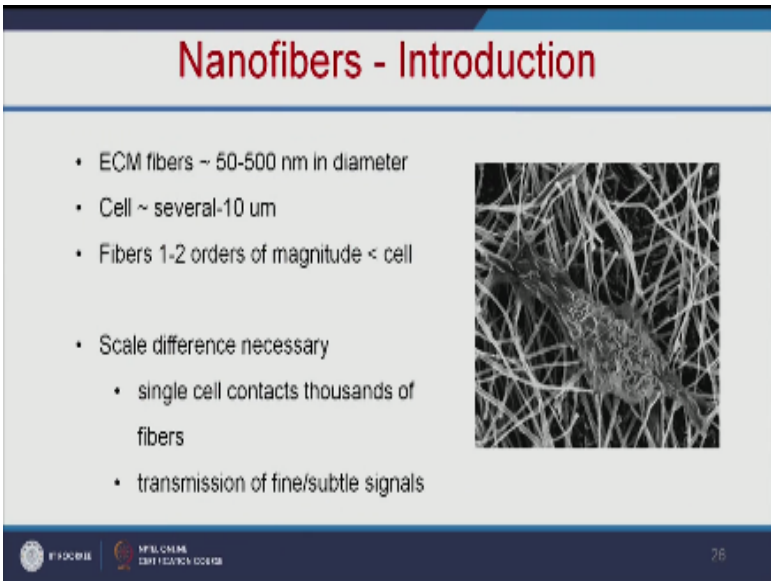

So when you use that nanofibers where this scaffold it exactly mimic like your extra cellular matrix okay, and here you can see here the extra cellular fiber matrix it is in the range of 50 to 500 mm in diameter so the cell size is between 10 to 100 micrometer. So the single cell here you can see it this is the scanning outer microscope picture so the single cell can bind to thousands of nano fibers, so the single cell contact thousands of fibers so the transmission of fine subtle can be easily achieved.

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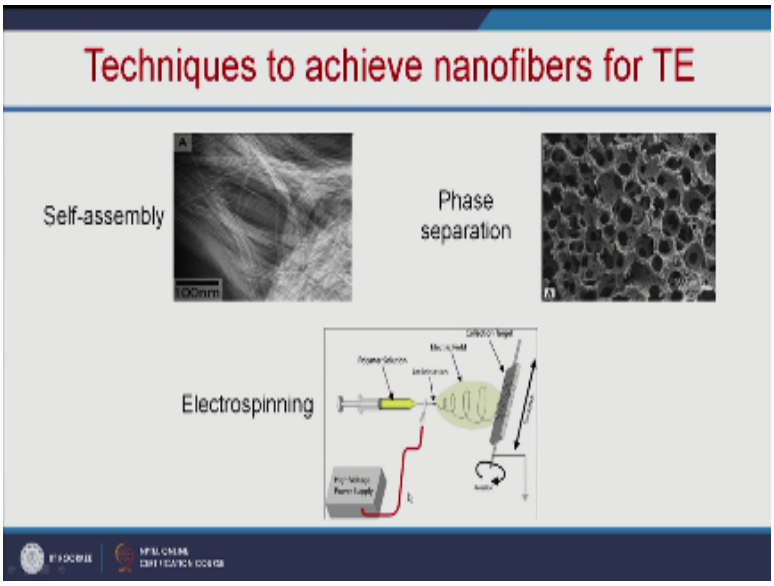

So what are the techniques available to achieve nano fibers for tissue engineering, first one is self assemblies second one is phase separation third one is electro spinning, so we will see one by one.

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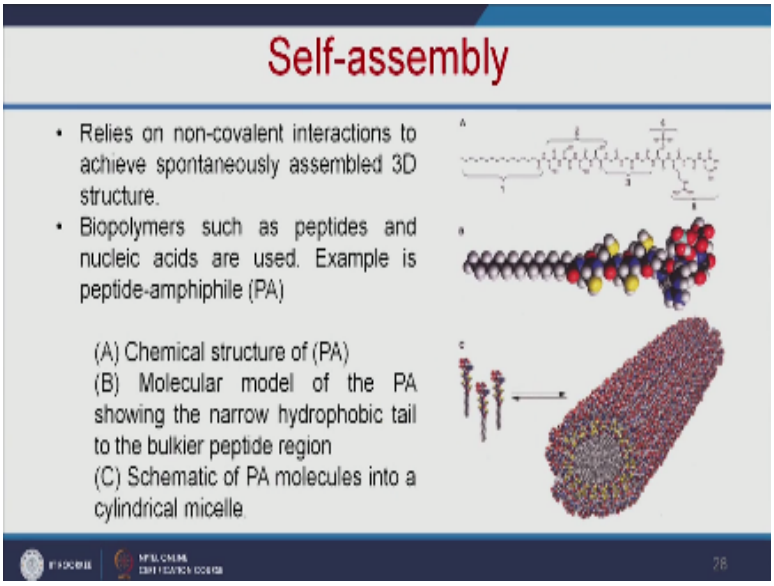

So self assembly which I already explained in the one of the lecture that is protein nano technology, so we can use the peptides with hydrophilic head and hydrophobic tail okay, so it can self assemble and form the three dimensional scaffold.

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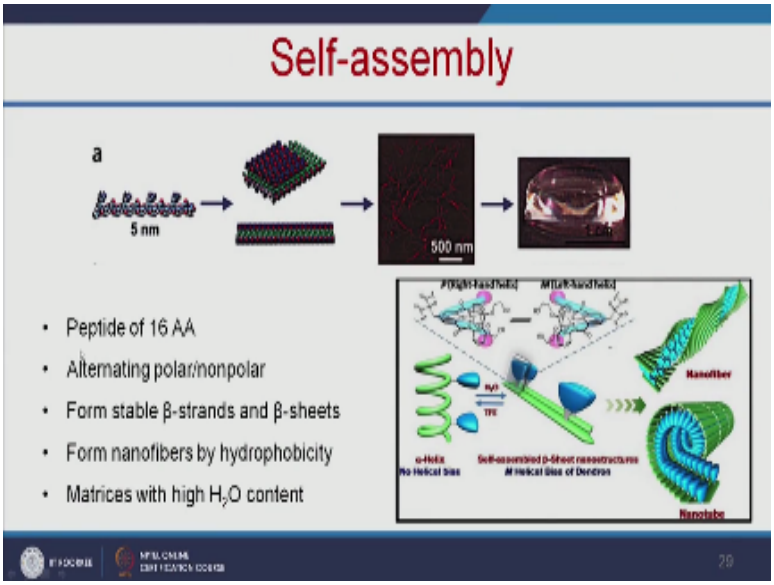

So when you use the peptide of 16 dimensional mode and with alternating polar and non polar okay, it forms the stable β strands and β sheets and forms nano fibers with good hydrophobicity and hydrophilicity okay, so using the self assembly we can make the three dimensional nano fibrous scaffold.

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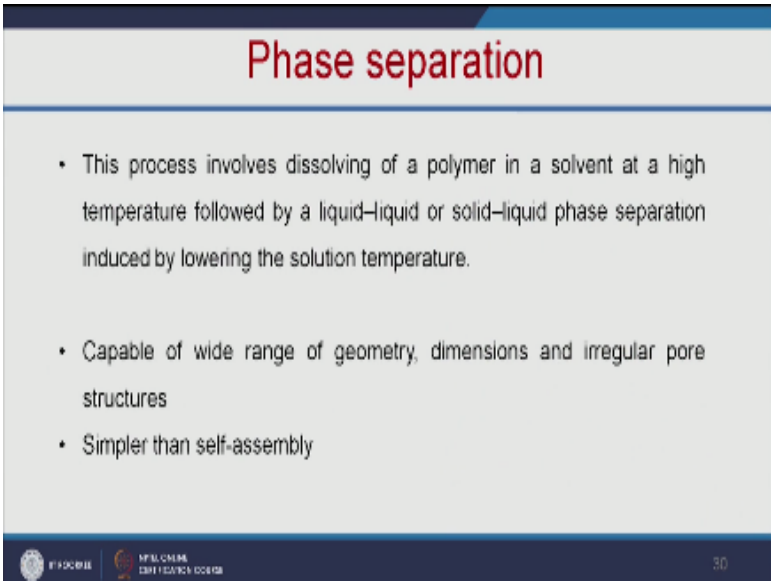

And next one is phase separation, so this is a process involves dissolving a polymer in a solvent at high temperature followed by a liquid, liquid or solid liquid phase separation induced by lowering the solution temperature, okay. So here we can have a wide range of geometry dimensions and irregular pore structures and it is simpler than the self assembly process.

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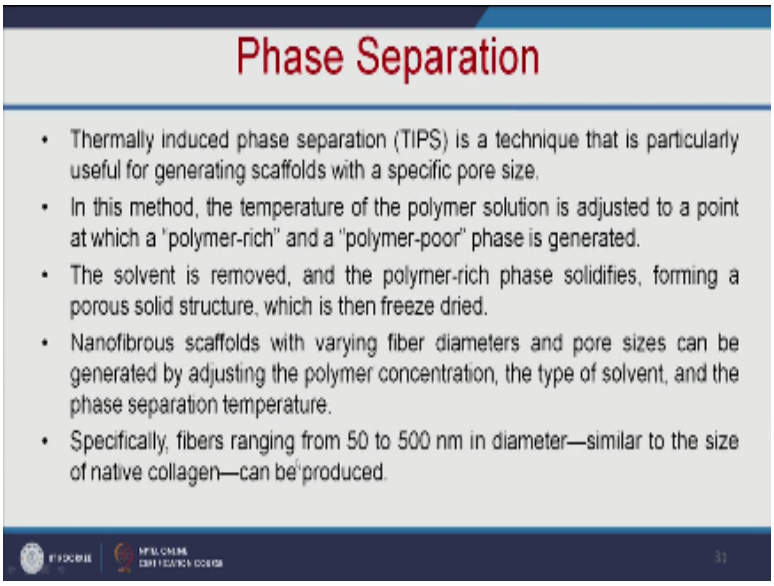

So let us see phase separation in detail okay, so it is a thermally induced phase separation is a technique okay, it is mainly useful for generating scaffold with specific pore size. In this method the temperature of the polymer solution is adjusted to a point at which a polymer rich and polymer pores phase is generated. Then the solvent is removed and the polymer rich phase solidifies forming a porous solid structure which is then freeze dried, okay.

And nanofibrous scaffold with varying fiber diameters and pore size can be generated by adjusting the polymer concentration the type of solvent and the phase separation temperature. By simply adjusting the polymer concentration type of solvent and phase separation temperature we can have a various size, porous size, nanofibrous scaffold. And here the fibers ranging from 50 to 500 nano meter can be easily achieved and which will exactly mimic like your extra cellular matrix.

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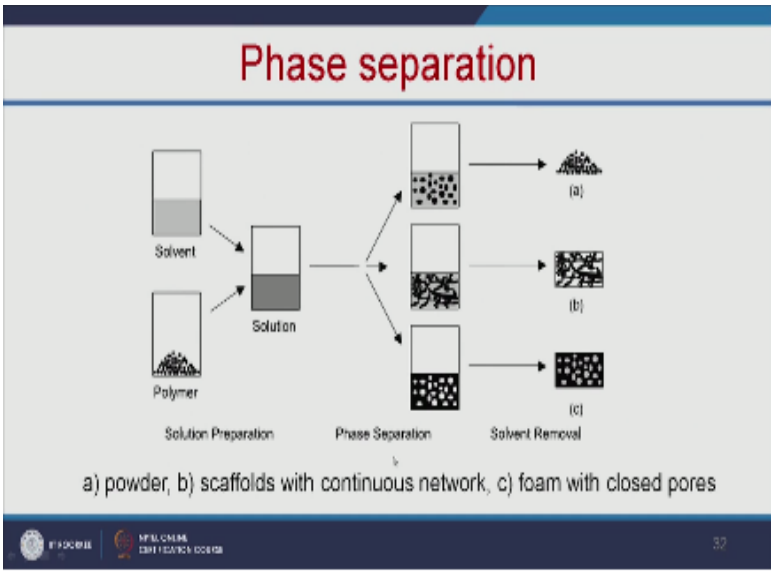

So this is the phase separation, so the solvent and polymer is mixed and form the solution and with respect to temperature we can separate the phase okay, and you can get the different porous and fibrous scaffold.

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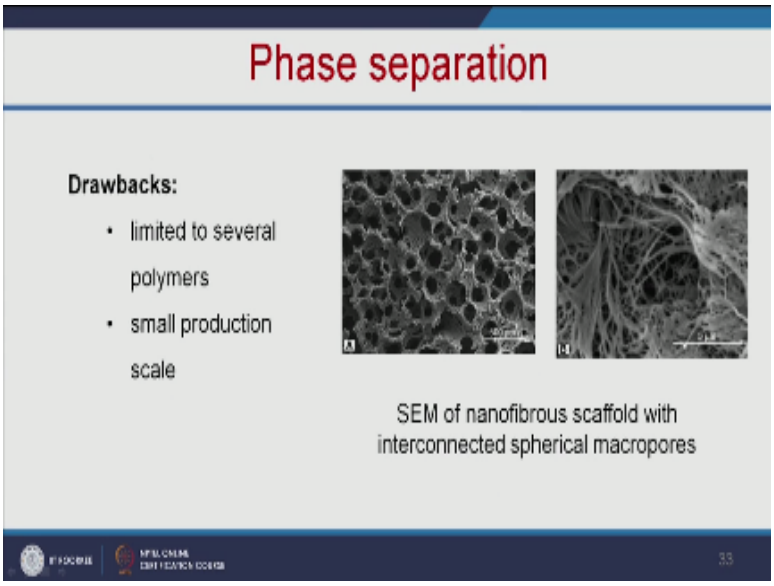

So here the drawbacks are it is limited to only several polymers and it is only small production scale so you can see here this is the scanning ultra microscope picture of nanofibrous scaffold with spherical macropores.

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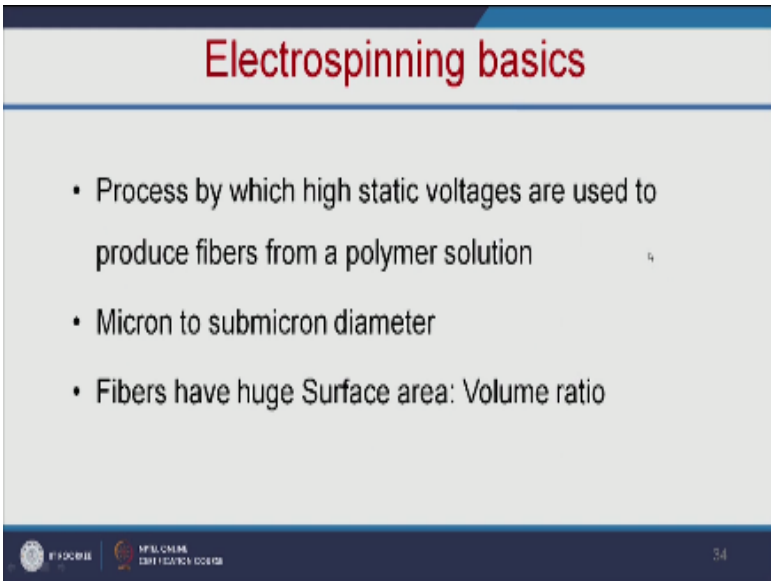

So the next approach is electro spinning technique okay, so this is a process by which high static voltages are used to produce fibers from a polymer solution. Here we can get micron to nano meter size diameter fibers and fibers have huge surface area to volume ratio. So how to make nanofibers using electro spinning let us see a simple example.

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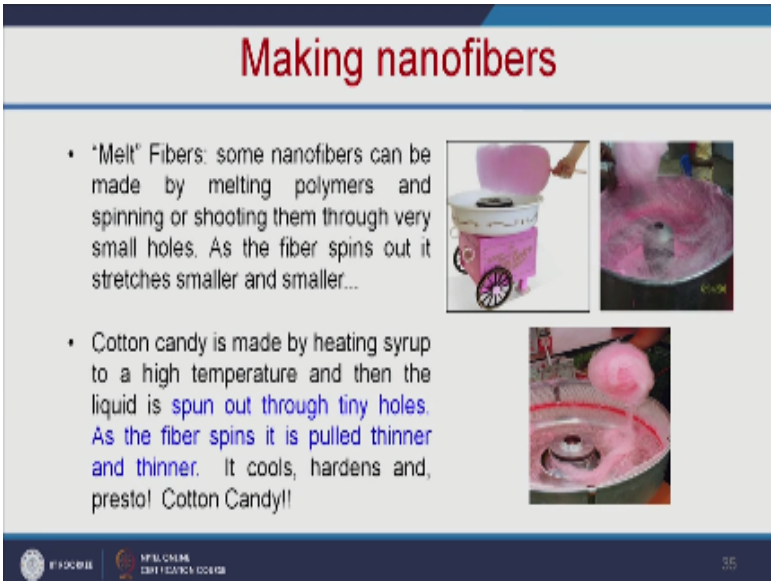

It is similar to your cotton candy, so in cotton candy what is your polymer solution we will be using the sugar solution okay, so here when you use the sugar solution and spun out through tiny holes as the fiber spins it is pulled thinner and thinner okay, so you will get this kind of fibrous structure so the simple example is cotton candy.

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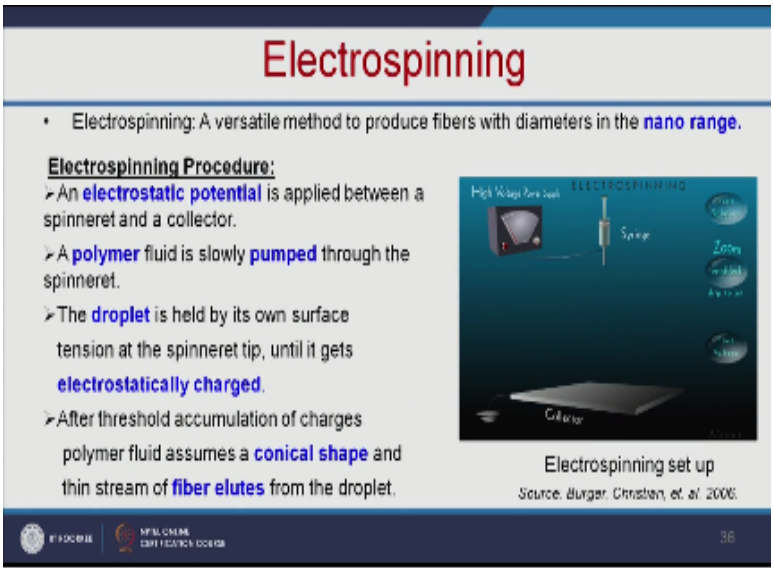

The same principle is for your electro spinning set up also, okay so it is a versatile method to produce fibers with diameter in the range of nano and here will be having a syringe okay, it is connected to high voltage power supply so when you apply the high voltage between the spinner at and collector a polymer fluid okay slowly pumped out through the spinner at and the drop let is held by a own surface tension okay, until it gets electro statically charged.

So after a threshold accumulation of charges this polymer assumes a conical shape this is called as Taylor cone and the thin stream of fiber elude the droplet then it forms the nano fiber.

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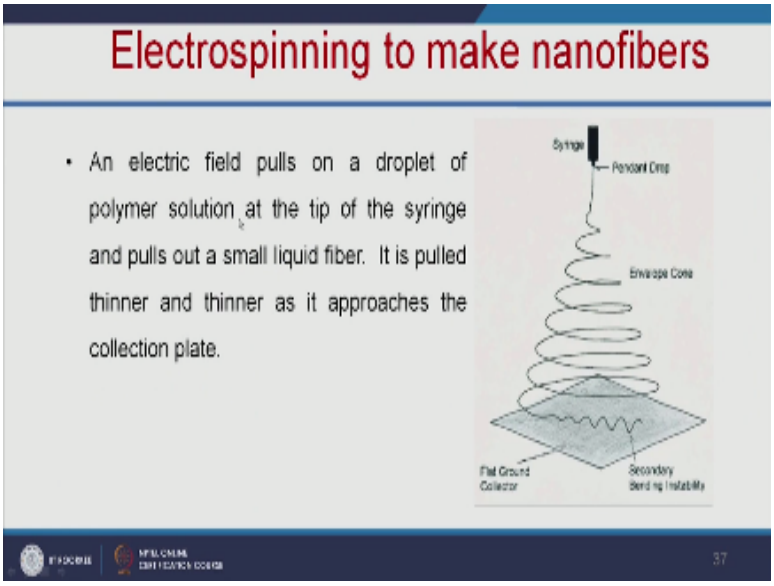

So here the electrical field pulls on a droplet of a polymer solution at the tip of the syringe and pulls out small liquid fiber and it is pulled thinner and thinner as it approaches the collection plate, so here we can easily control the dimension of your nano fiber okay, by simply adjusting the polymer solution viscosity and also the distance between the spinner at and collector.

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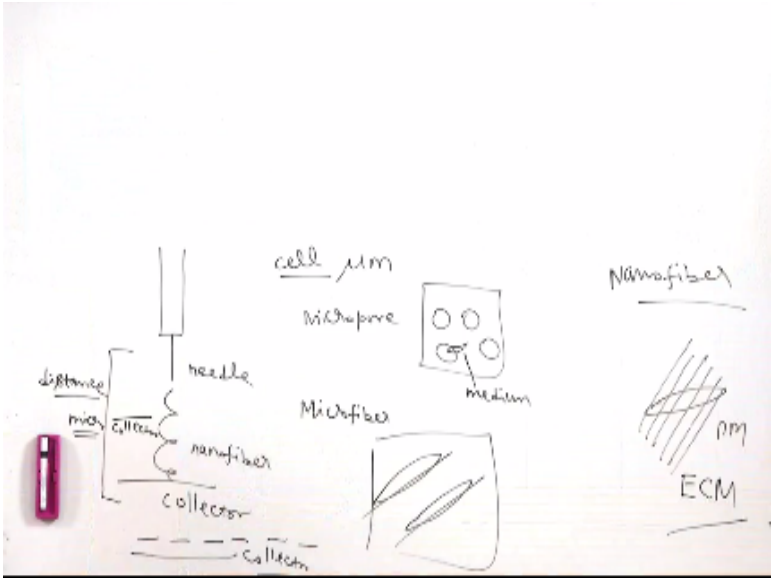

Suppose if I having a mode distance between your spinner at needle and collector so you will get nano fiber, so the distance between this spinner and collector is very important and if you reduce the distance you will get micro size fiber if the collector is moved closer okay, you will get micro size fiber and when you move the collector more far away what happens is you will get the fiber you would not get the continuous fiber you will be having a fiber with break.

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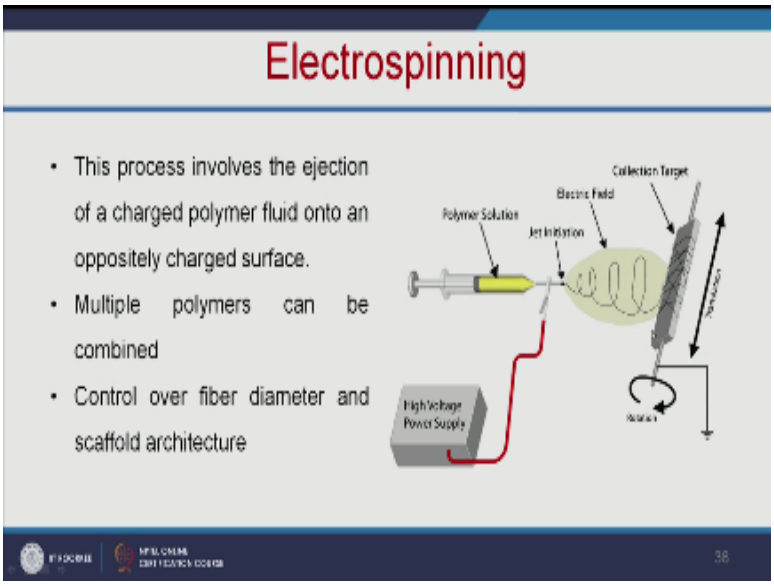

And again this electro spinning available in vertical as well as horizontal and the collector can be static collector and it can be rotating collector.

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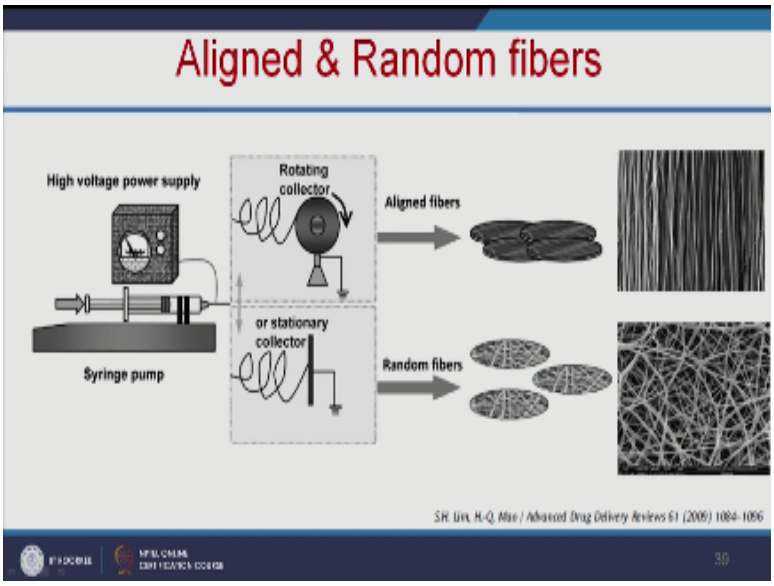

So using the rotating collector and stationery collector you can have the align and random fibers, so when you use the rotating collector what happens is it is similar to your thread bundle okay, so all the fibers are rolled into the rotating collector and you will get the align fibers. But when you use the stationery collector what happens is you will get this kind of random fibers, so when you see under this scanning ultra microscope you will get this kind of beautiful align fibers when you use the rotating collector an you get this kind of random fibers when you use the stationery collector.

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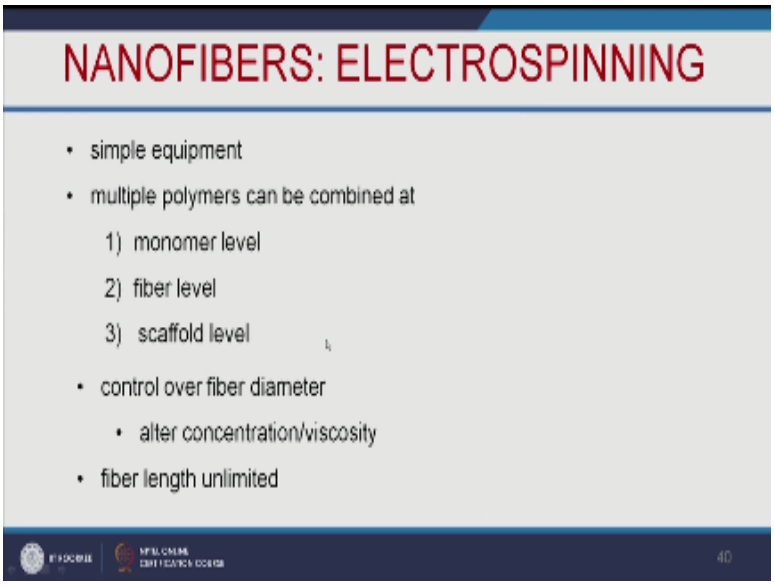

And the main advantage of this nano fiber by electro spinning is it is simple equipment okay, and multiple polymers can be combined at monomer level or fiber level and scaffold level and here we can control the fiber diameter by altering the concentration or viscosity of your polymer and also the fiber length can be unlimited.

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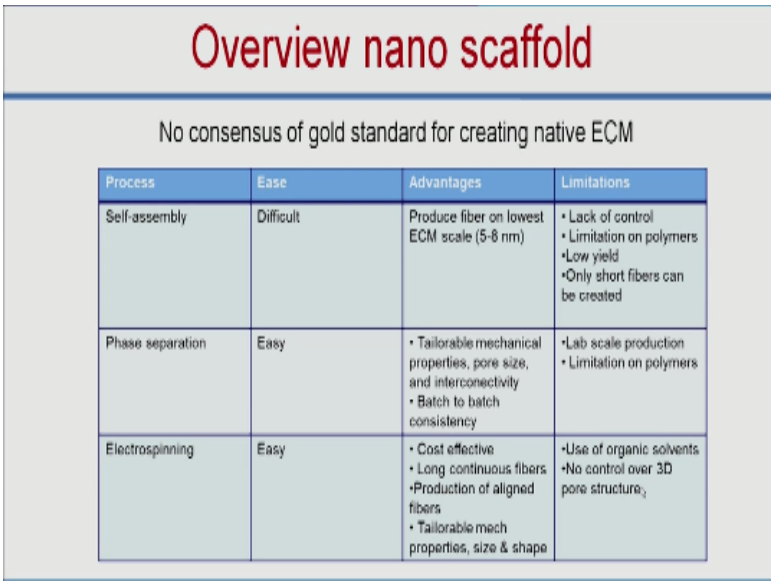

So let us see the overview of nano scaffold and what are the advantages and the disadvantage of each method, so self assembly is very difficult to make but it produces nano scaffold which close in cure matrix, but the draw backs are lack of control and limitation of polymers okay, and also only short fiber can be created and low yield, but when you use the phase separation it is easier when compared to the self assembly and here we can make the tailor able mechanical properties with specific pore size okay.

And here we can make it batch to batch consistency but it is limited to lab scale production and also we use only several type of polymers okay only limitation to particular type of polymers. An electro spinning is very easy, and it is cost effective and we can make a long and continuous fiber and production of aligned fibers is possible, and we can also tailor able the mechanic property size and shape okay.

And the limitations are in some cases we have to use organics solvency and we do not have control over the pre dimensional post structure. So these are the advantages and disadvantages of each method for making nano particles fibers.

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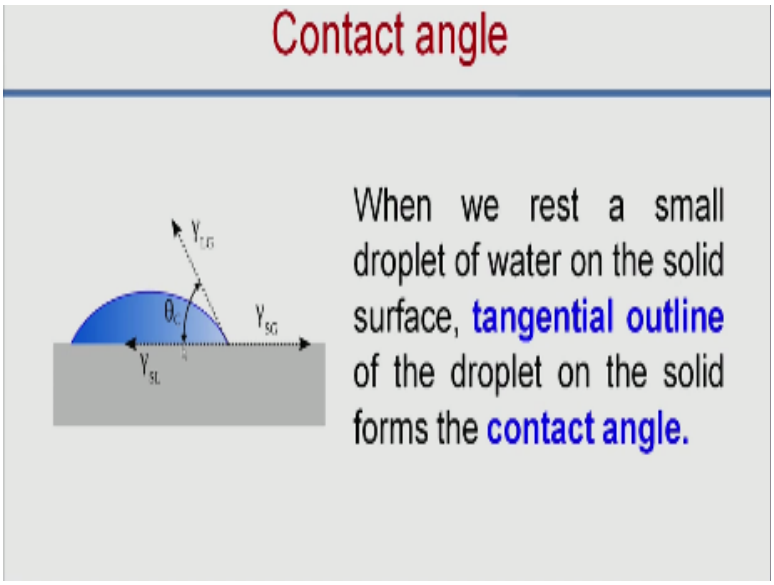

And whenever you make nano fibers or any nano scaffold the first important thing is hydrophilicity of your material, when your material is hydrophilicity so if you allow the cells to attach and grow nicely okay, so how to measure and how to check the hydrophilicity of your material by using contact angle measurements. So here we have to rest a small drop of water on a solid surface okay.

And a tangential outline of the droplet on the solid forms a contact angle, so here you will be adding a drop of water on the nano fiber scaffold, so when you add a drop of water and when you the contact angle measurement set up, you can measure the contact angle.

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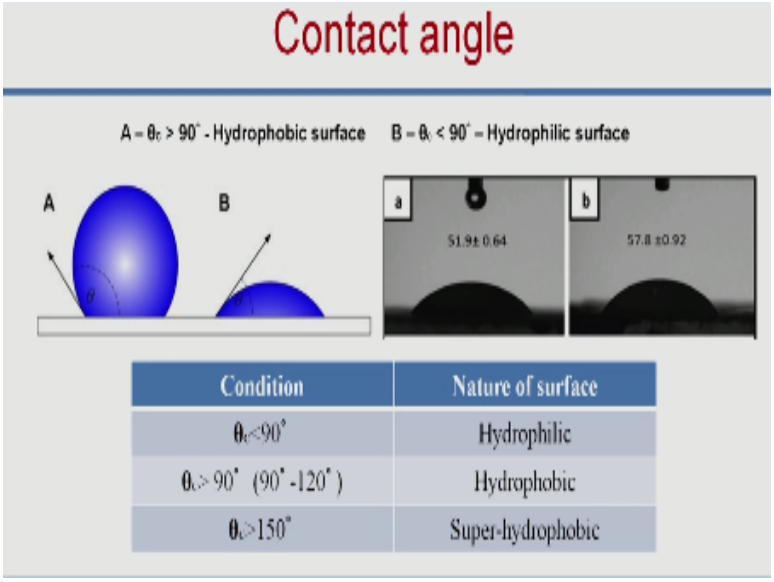

So if the water drop let is giving this kind of contact angle like less than 90 ^{0} that is your material is hydro phallic okay, if you are getting a contact angle more than 90 that means hydro phobic, if your contact angle more than 150 it is materials super hydrophobic okay. So using this simple experiment we can understand whether your nano material or nano scaffold is hydrophilic or not.

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So let us see the applications of nano technology in tissue engineering.

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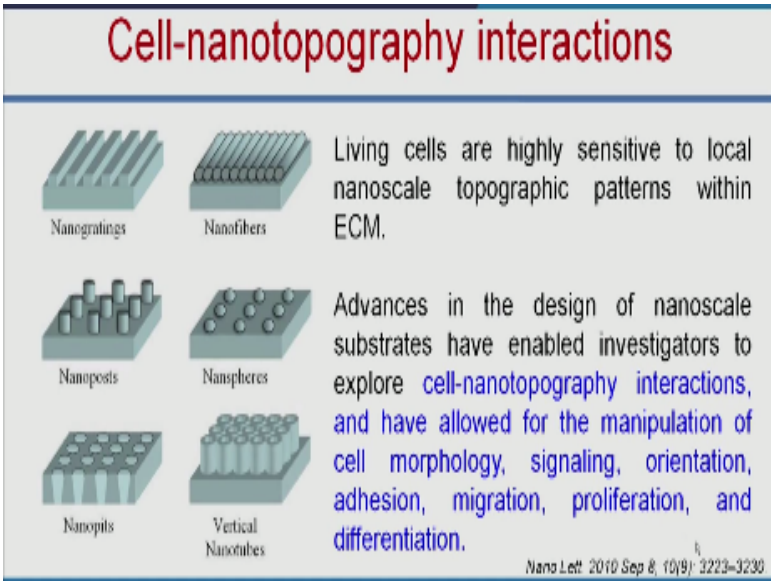

So using nano technology we can make several nano structures nano gratings, nano fibers, nano pits okay. So the living cells are highly sensitive to local nano scale topographic patterns within ECM, and this cell nano topography interaction will decide the cell morphology and also it can induce the differentiation of cells. If you are adding the cell on this kind of structures and nano gratings and it can allow the differentiation. So this nanogratings and nanofibers it can also induce the cell differentiation and also cell signaling.

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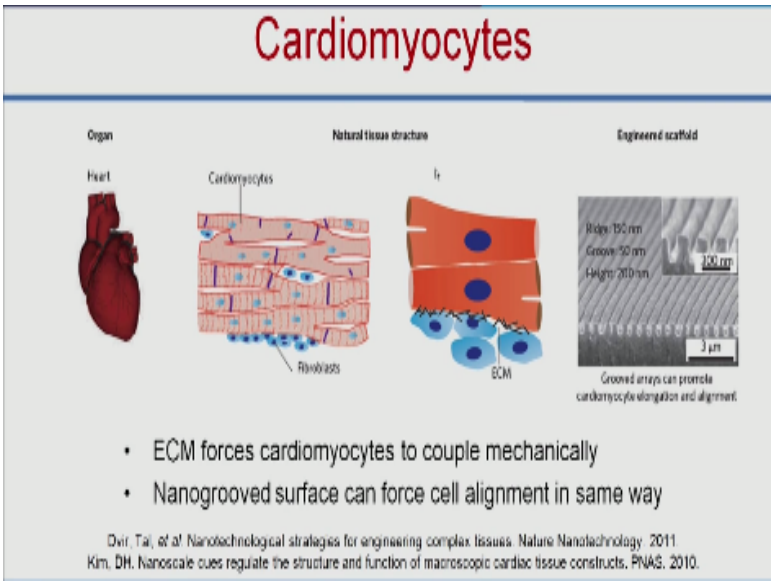

So let us see the example of cardiomyocytes, so this is our organ and this is our natural tissue structure cardiomyocytes okay, so this is the engineered scaffold. Here grooved arrays can promote cardiomyocytes elongation and alignment. So we can have this kind of nanostructure, so when you have this kind of nanostructures the ECM forces cardiomyocytes to couple mechanically and this nanogrooved surface can force cell alignment in same way which mimics the natural tissue structure.

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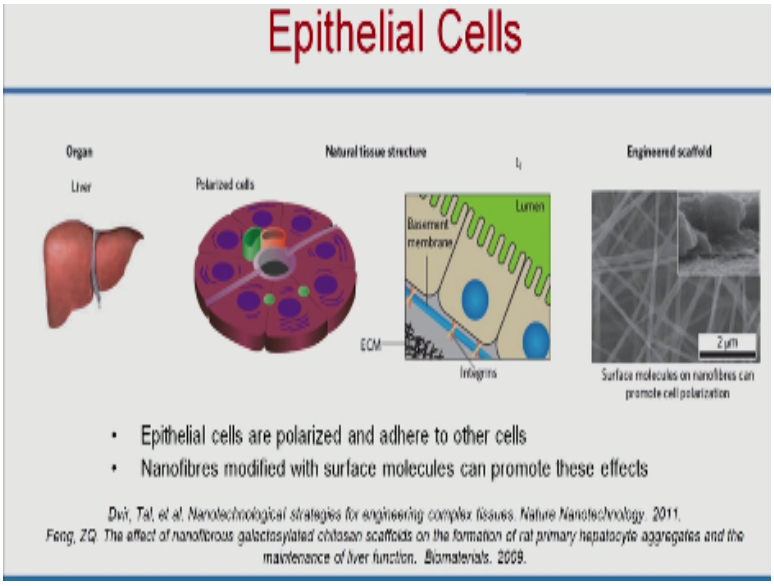

So the next example is epithelial cells okay, so here epithelial cells are polarized and adhere to other cells and when you make the surface nanofibers modified to surface molecule, so that can promote this kind of natural tissue effect okay.

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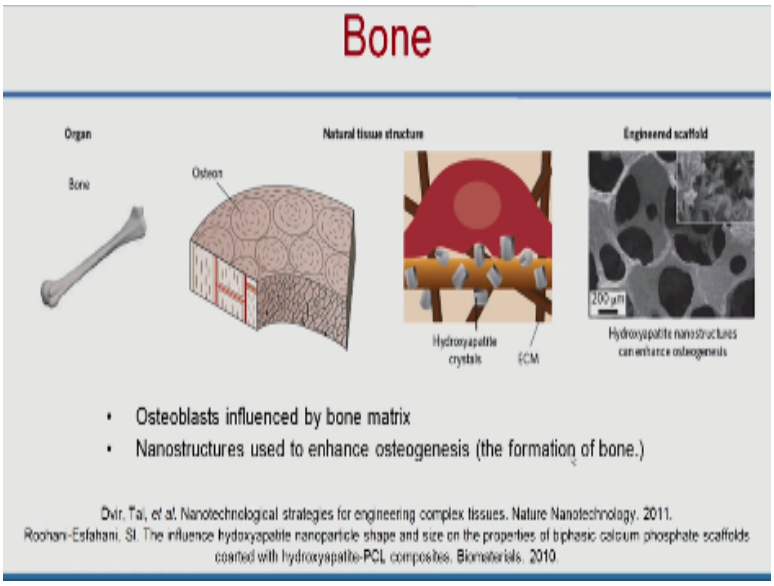

Next example is bone okay, so the osteoblasts influenced by bone matrix and we can make the hydrophilic nanostructure and the pores structure similar to a bone okay, so these nanostructure it can enhance the osteogenesis that is formation of bones.

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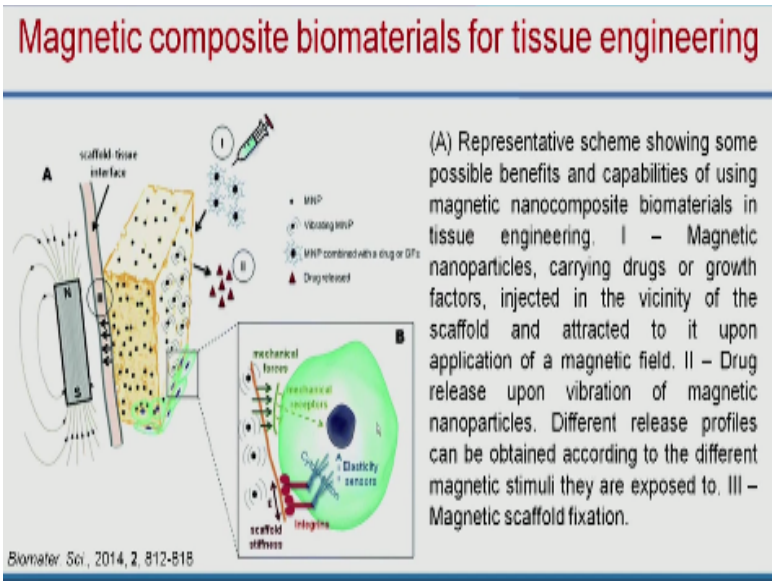

Next example is, we can have this kind of scaffold with a magnetic nano particles and which is caring the growth factor. When you apply the extra magnetic field it will release the growth factor according to the external magnetic field. So when you apply the external magnetic field according to the growth factor, the cells will have different kind of forces mechanical forces. So when you expose to these mechanical forces will induce the expression of various proteins. So that is called as mechanic transaction.

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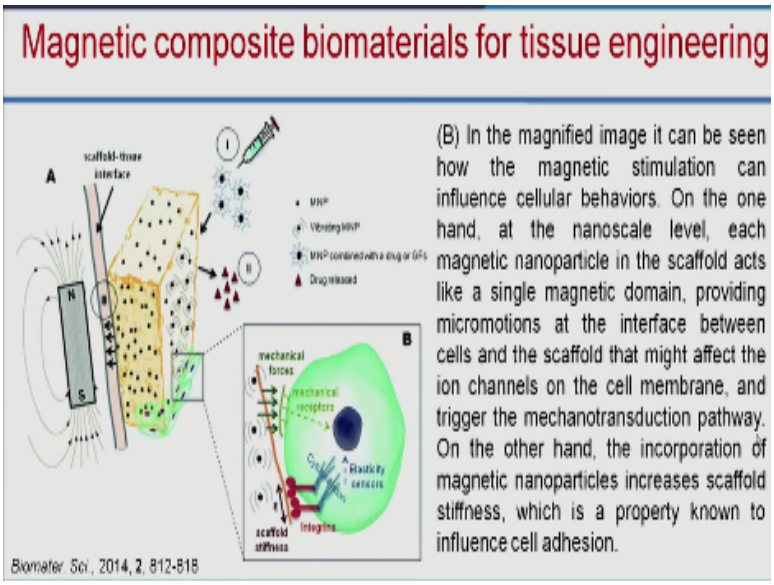

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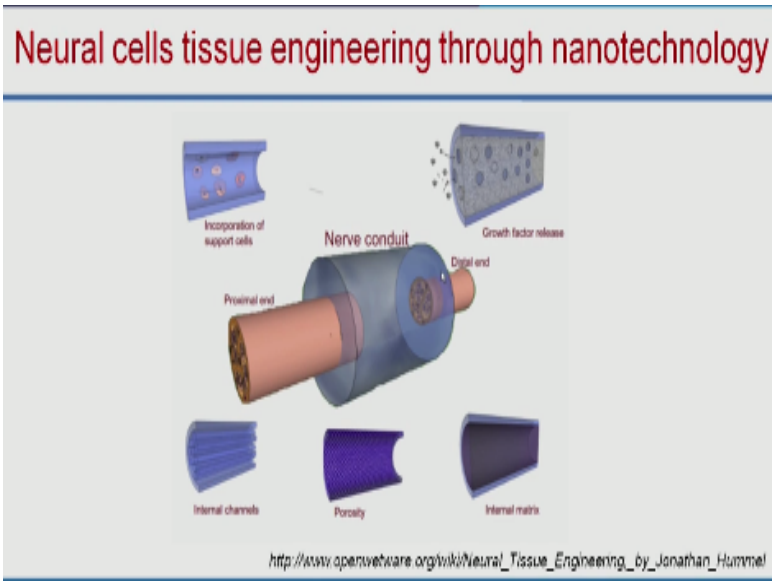

And we can also tissue in the neural cell, so in the nanofibers we can incorporate the supporting cells and we can also incorporate the growth factor and it will be useful for neural cell tissue engineering.

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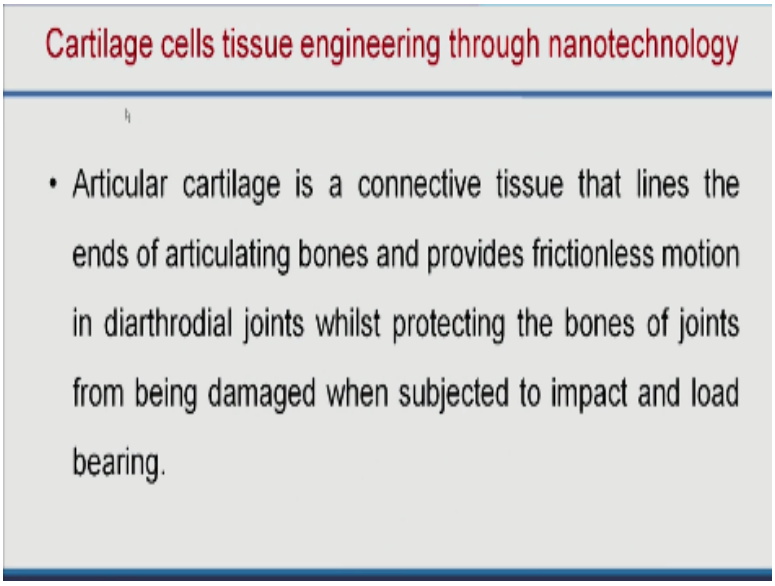

So let us see how does this cartilage cells tissue engineering through nanotechnology, so this articular tissue that lines the end of articulating bones okay, and it provide frictionless motion in diarthrodial joints and it is also producing the joints of bone.

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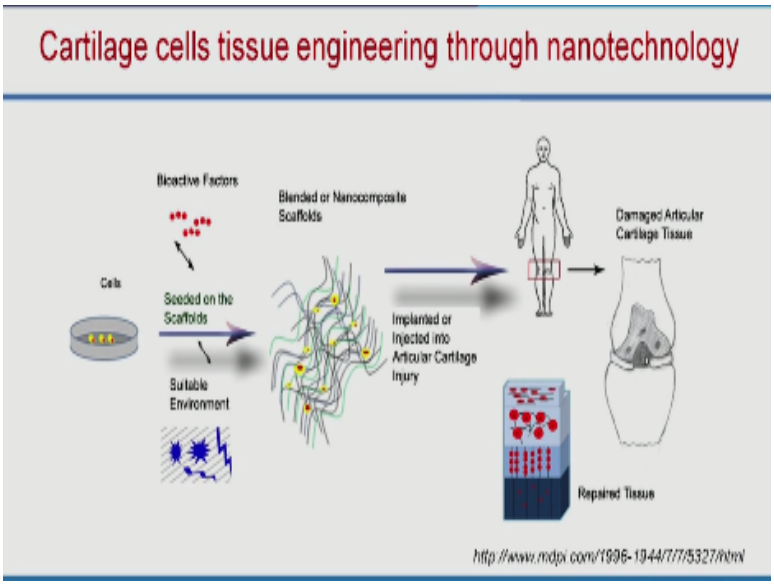

So we can have these cells and seeded the cells on the scaffold and when you add the bio active growth, then the nanocomposite will form the cartilage cells in the artificial scaffold and that could be inject able or that could be implanted into the patient who has the damage articular tissue.

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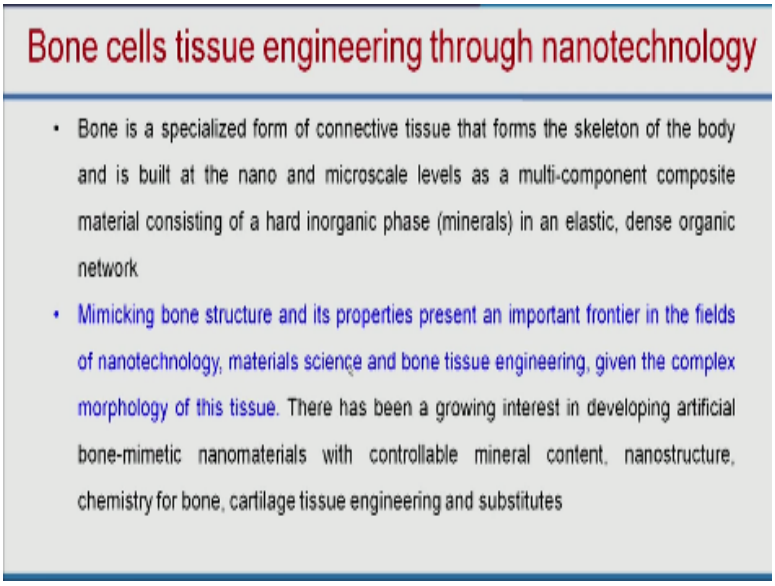

So the next one is also we can injected the bone cells. So here the bone is a specialized form of connective tissue that forms the skeleton of the body okay. So mimicking bone structure and it properties present an important frontier in the fields of nano technology and nano science. So using the help of the nano technology we can engineer the bone.

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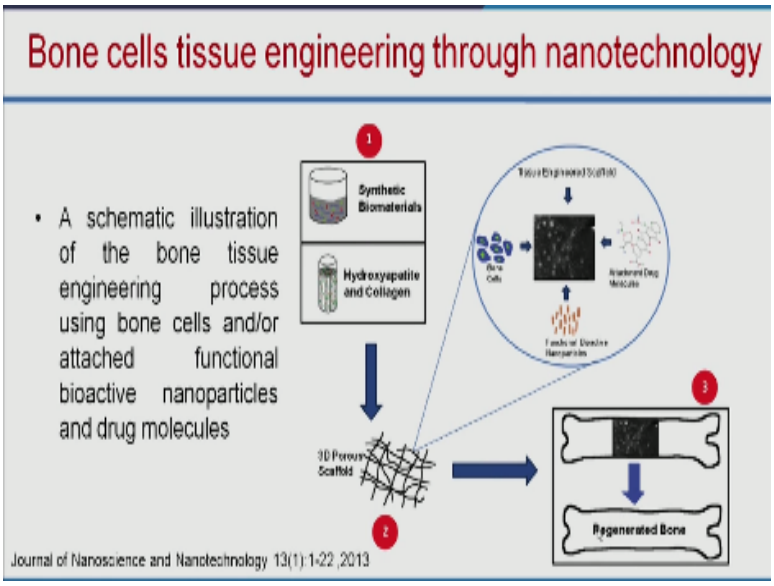

So here we can have the synthetic bio materials which mimic like your bones, and you can add the bone marrow and it can regenerate your bone, damage bone.

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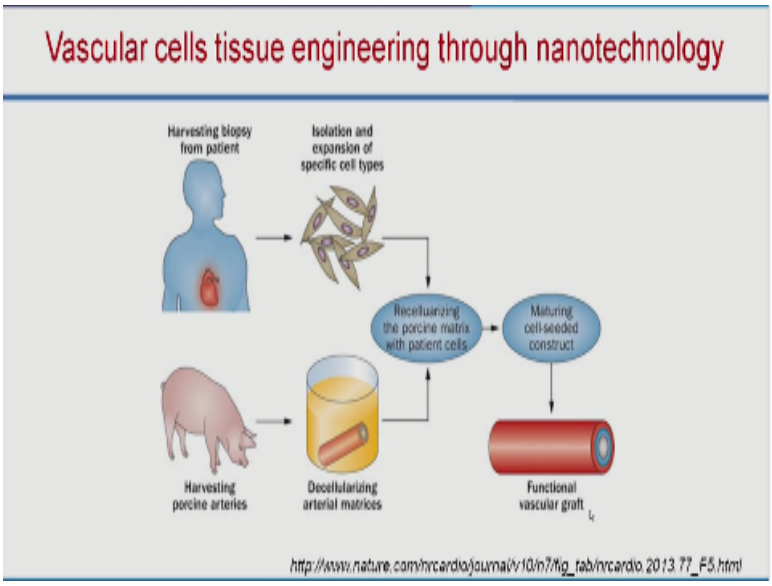

So let us see vascular tissue through nano technology, so here we can harvest the cell from the patient and grow the cells in the lab condition okay, and also you can do the decelerating process to remove all the cells and on the top of the cells recellarising with the patient cells and when it is matured and it will grow and form as functional vascular graft. So here we are using the arteries and then speak and then the cells on human and we are making the vascular graft.

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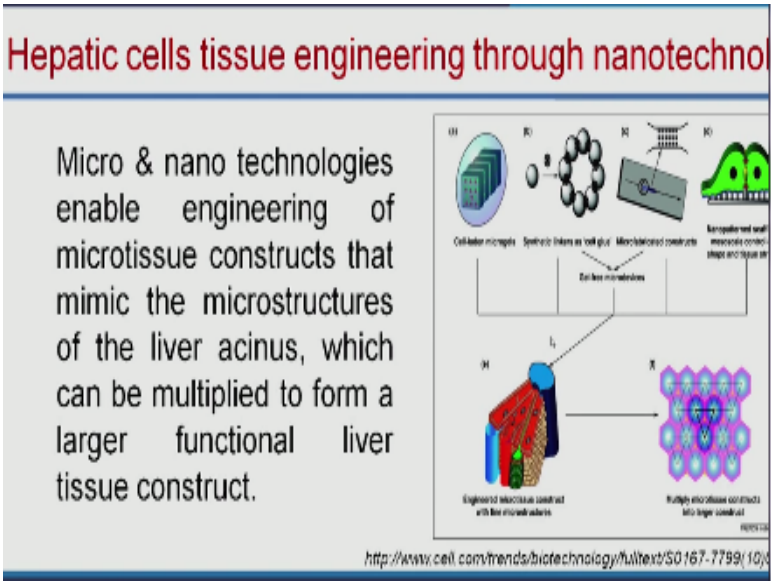

The next example is hepatic cell tissue engineering through nano technology, with the help of micro and nano technology which mimic like your liver and when you add the hepatic cell and it will grow like a liver.

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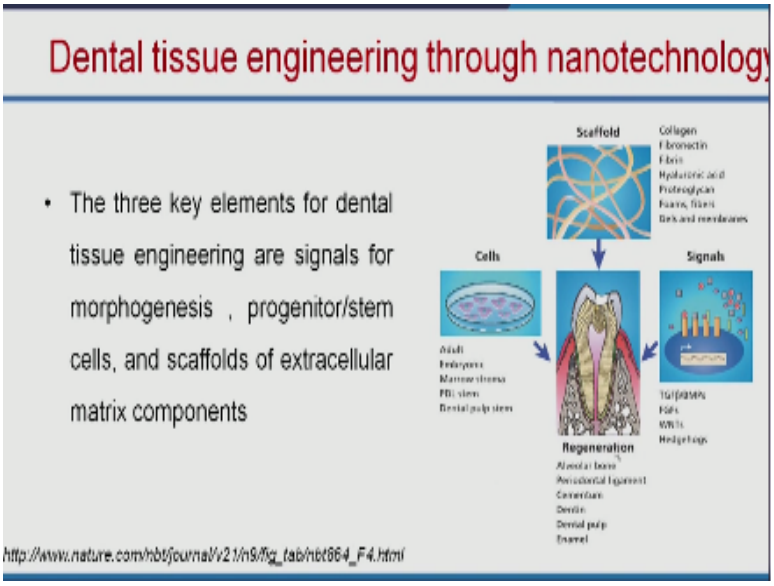

So the next one is we can also use for the dental tissue, so here you can take the dental pulp lentils and scaffold will be, once the cells will grow on the we can implant into the alular bone okay and dental, pulp so it will re grow the dental tissue.

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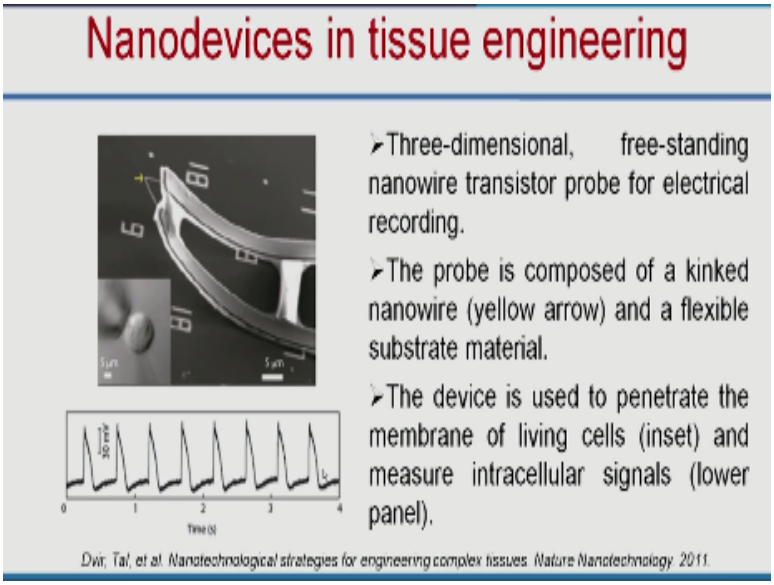

So the next thing is nanodevices in tissue engineering, so here three dimensional free standing electrical probes for recording, so this probe is composed of nanowire and if flexible substrate material, this devices is used to penetrate the membrane of living cells and measure the intracellular signals. You can see here these small nanodevices can penetrate the cells and it can measure the cells.

So the tissue engineering is not only useful for artificial tissues to damaged organ, it can be useful to pupil tissues, that is.

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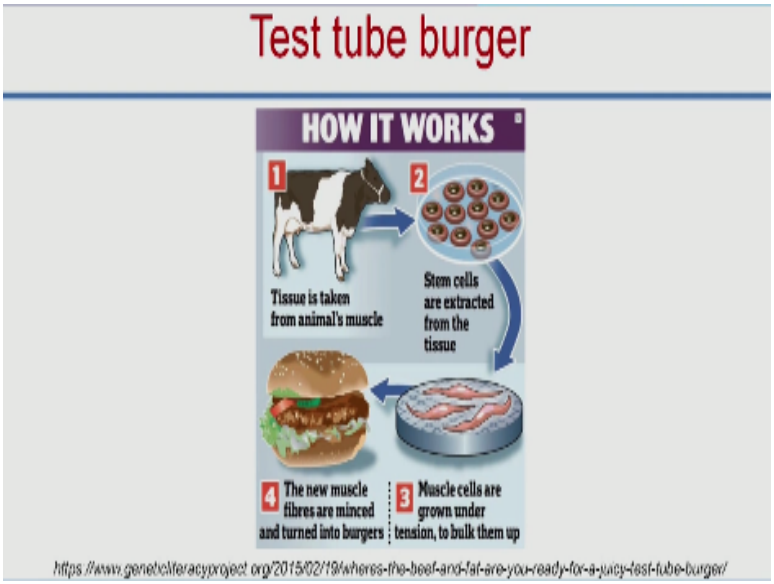

Test tube based burgers, so here you can see that tissue is taken from animals muscle and the stem cells are extracted from the tissue, and the muscle cells are grown under tension, to bulk them up and the new muscles fibers are minced and turned into burgers okay, so this will avoid the floater of house, killing of a lot of animals, so we can have the test tube burgers.

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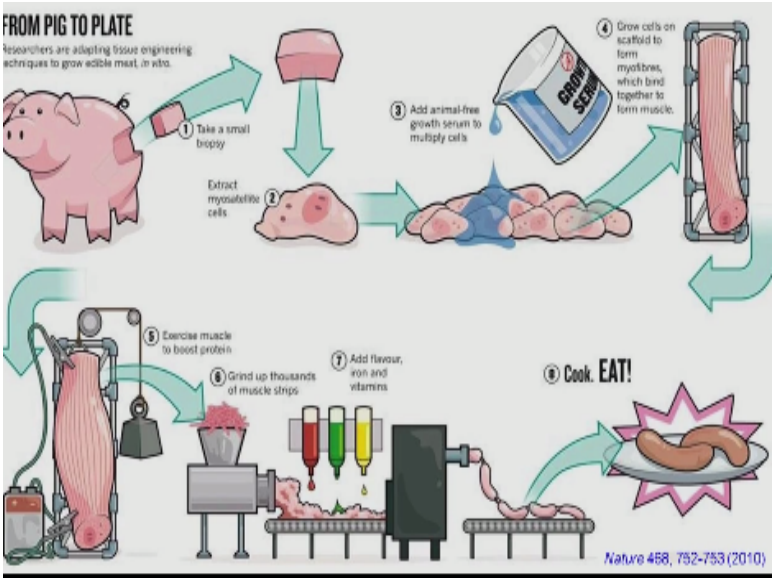

So let us see the example from pig to plate so this artificial in nature, so here the researches are adapting tissue engineering tissue to grow okay, so take a small cell from pig and grow the cells and add animal free grow cerium to multiply the cells okay, then you can grind it up and you can add the vitamin as flavor okay, so that will add flavor to your meat.

And finally you can cook and eat, that means this example you learns like a tissue engineering applications okay. So as a summary of this lecture okay, so in this lecture we have learnt what is tissue engineering? And why we need tissue engineering and also we have learnt what are the tools required of tissue engineering and also we have learnt nano technology tissue engineering. I will end my lecture here, I thank you all for listening to my lecture and I will see you in another interesting lecture.

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