Tissue Engineering
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Lecture - 23
Signaling and biomolecule delivery in Tissue Engineering

Hi everyone. Today, we will be speaking about biomolecule delivery for cell signaling in Tissue Engineering.

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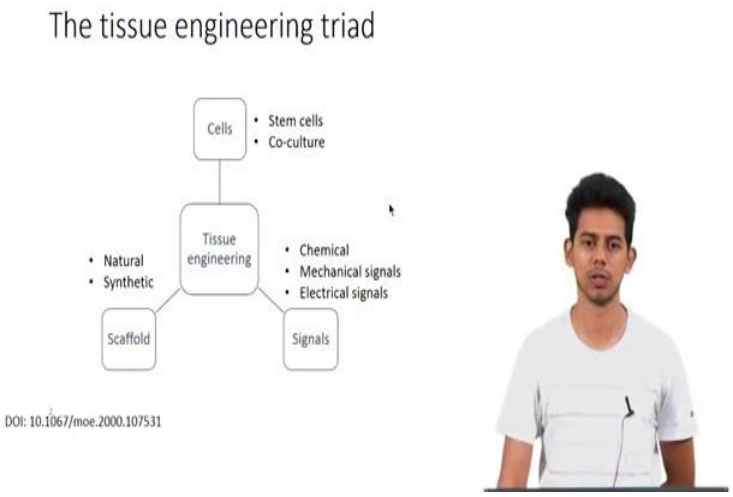

We know that tissue engineering triad is composed of cells, scaffolds, and signals. Signals will be the aspect we will be focusing on today.

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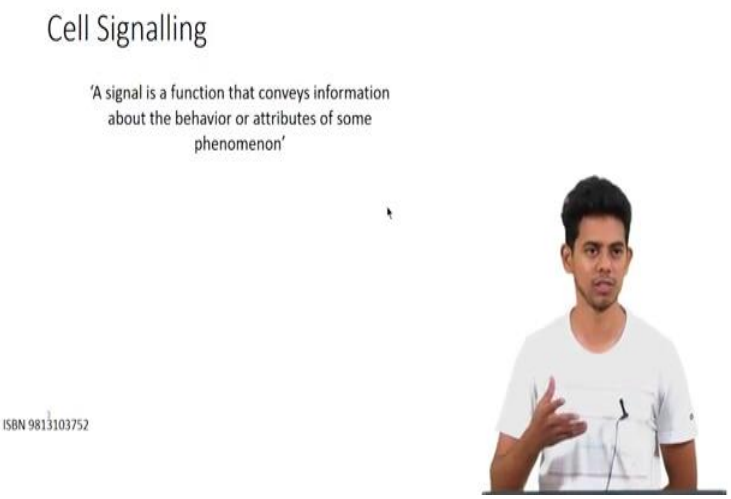

So, what are signals? Signal is a function that conveys information. In a cell, every activity and function is controlled by signals.

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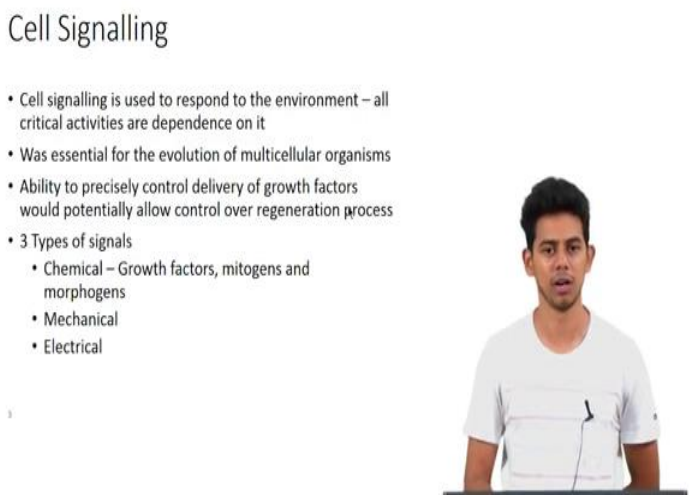

The signaling is used to communicate with other cells and also from the environment the cell takes in signals. This was essential for multicellular organisms to evolve. So, it is a very crucial aspect, especially in tissue engineering. The ability to control the signals would entirely give us complete control over making a tissue grow in the way that we want because that is what is naturally being carried out by the organism itself.

The three kinds of signals which are present are chemical, mechanical, and electrical. When it comes to chemical signals, there are growth factors, mitogens, and morphogens.

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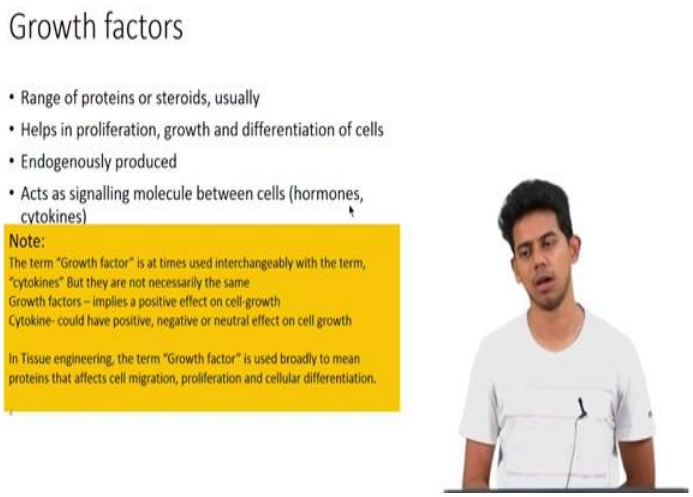

Growth factors are small soluble proteins that are produced by the cells itself, and they act as signaling molecules between cells like hormones and cytokines. They help in proliferation, growth, and differentiation of cells.

Something to note here would be that growth factor is a term that is frequently confused with cytokines. What is important to notice that growth factors usually always imply a positive effect on cell growth, whereas cytokines can have a neutral or positive or negative effect on cell growth. The reason for both of them to have an overlap in terminologies because cytokines derived from the immunology field, whereas growth factors derived from the developmental biology field.

So, there were many proteins that are found to be having the same function in both fields. In tissue engineering, we would be referring to growth factors to refer to all the proteins which affect cell growth, migration, proliferation.

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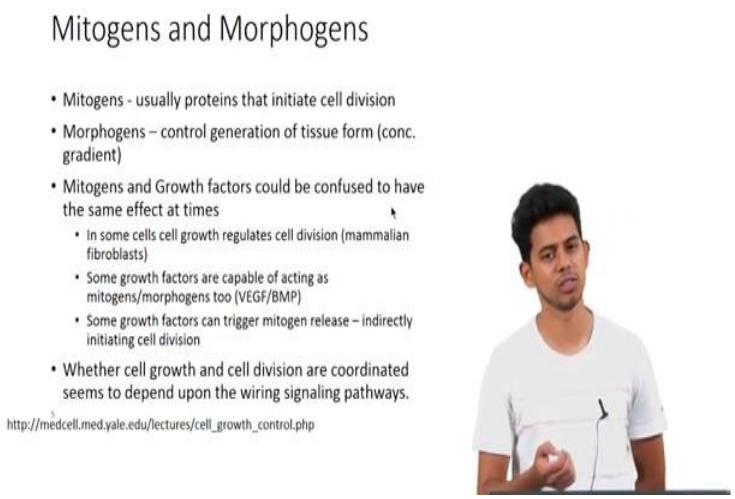

So, even mitogens and morphogens, we would broadly call them as growth factors. Mitogens are usually proteins that initiate cell division, whereas morphogens control the generation of tissue form; that is the structure that it takes. So, it ends up having a concentration gradient. Based on the concentration gradient, the tissue ends up taking its form.

Mitogens and growth factors could be confused to have the same effect too at times because sometimes growth and cell division are interlinked. In mammalian fibroblasts, for example, cell growth regulates the cell division. Also, at times, the growth factors would activate the mitogens, thereby indirectly initiating cell division. Sometimes growth factors can be a mitogen or morphogen like VEGF and BMP.

There is some level of overlapping between them. So, it all depends on how the cells are wired. Based on that, the growth and proliferation could be interlinked or to an extent or not.

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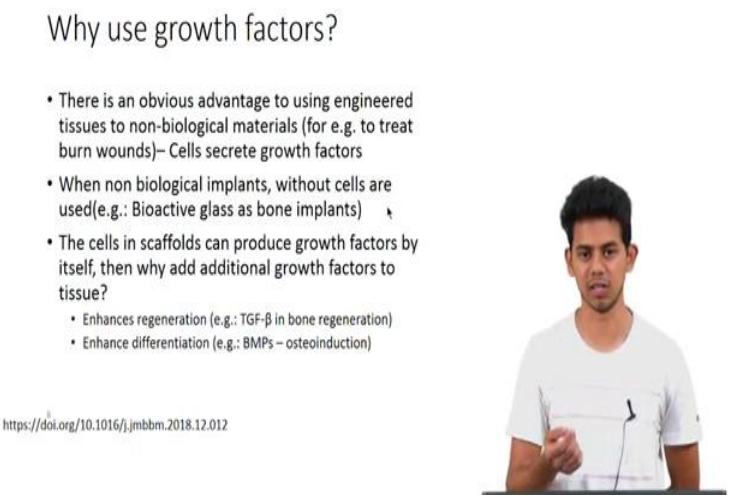

Now the question is, why do we use growth factors? We can always see in tissue engineering that there is an advantage to using biological materials like a cell for treating a disease. Having a cell in the scaffold ends up meaning that the cells are able to produce these growth factors and proliferate the cells and improving the healing time. There are also constructs in which we do not use cells. Even here, the growth factors are important because you want the cells to migrate to the site of the wound and end up healing the area.

Now, a question would be. If we can use cells, then why use growth factors separately? When we are using cells, it will be useful to add growth factors because it can enhance the regeneration and also the differentiation. An example is TGF-β in bone regeneration and BMPs in osteoinduction.

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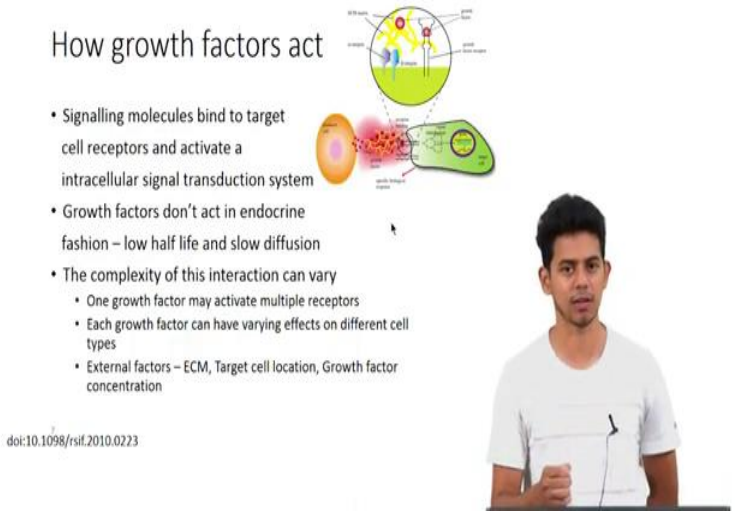

Now on how these growth factors act. Like all signaling molecules, these growth factors bind to specific receptors on the cell surface, and they activate the intracellular signal transduction system and thereby attacks. The growth factors do not act in an endocrine fashion because it has a very low half-life usually. So, it's not usually released into the bloodstream to act on far off organs or tissues. So, its usually in a local area.

Now, the complexity of this growth factor interaction can vary with the receptor. One growth factor might activate multiple receptors at times, and also it can activate multiple receptors on different cells and end up having a different effect on each of these cells. And something which adds to this complexity level is the fact that the ECM, the target cell location, the concentration of the growth factor, all these play a huge role in how the growth factors act.

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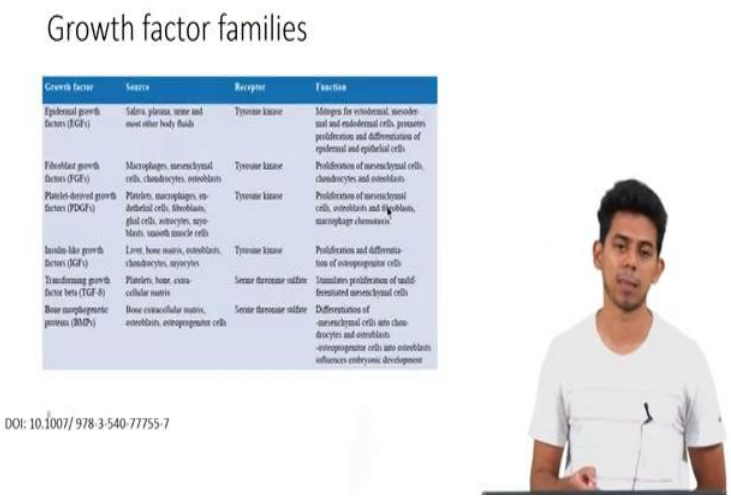

Now, this is an image of the common growth factor families that show the receptor and the function. Here, you can see that the first growth factor, for example, the epidermal growth factors, it acts as a mitogen at the same time for ectodermal and mesodermal cells and also promotes proliferation. This is for epithelial cells.

So, this goes to show how growth factors can actually have a lot in common with mitogens and morphogens and also at the same time have a different effect on different cell types.

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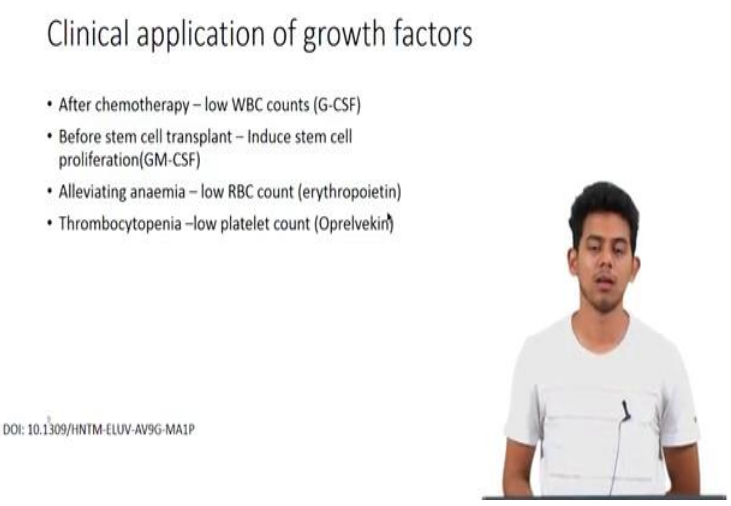

Now, in clinical applications, growth factors are not limited to just tissue engineering. So, we will take a look at what the other applications commonly have been used for. After chemotherapy, when there is a low white blood cell count, the granulocyte colony-stimulating factors are given to increase the WBC proliferation and get back the count to normal.

And even before stem cell transplant usually, those stem cells are taken from the bone marrow. In that case, it's a painful process to take it from the bone marrow. So, an improved technique is actually to give growth factor which induces the stem cells to proliferate and thereby come out of the bone marrow into the bloodstream.

And from the bloodstream, it can be easily collected and transplanted to the same patient or another patient. Then in alleviating anaemia, low RBC count, or low platelet count, all these aspects, growth factors are being used.

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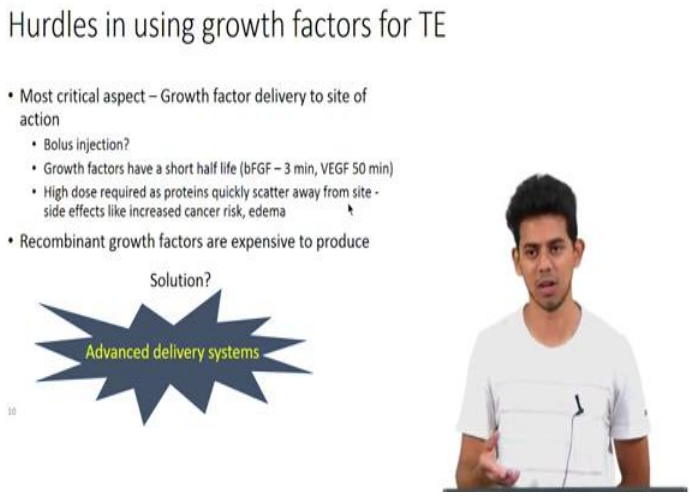

Now the hurdles in using growth factors in tissue engineering. The most critical aspects of using growth factors in tissue engineering are its delivery to the right site. We can always think of injecting these growth factors to the site that we need, as a bolus injection. Now, the problem there is the half-life of these growth factors is very short. So, bFGF has just 3 minutes, and VEGF has just 50 minutes; these are very short actually.

We would have to actually inject a large dose of these growth factors, so that we have any considerable amount of growth factors which can act on the tissue and bring about the effect. So, this high dose, in turn, ends up leading to other side effects like edema or an increased risk of cancer. This is something which you would like to avoid, and also growth factors are quite expensive to produce. So, the solution would be to use advanced drug delivery systems.

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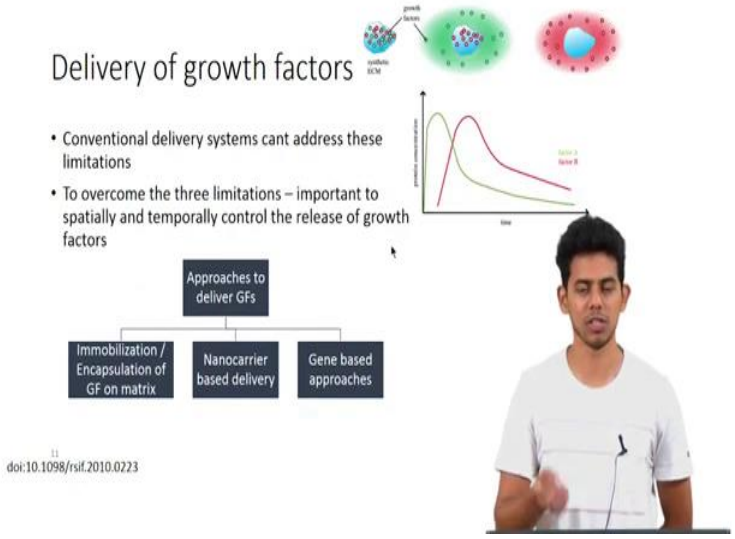

What is the advantage of using these systems is that they are able to give a temporarily and spatially controlled release profile. So, they can give a sustained release of these growth factors from these delivery systems for a prolonged period. Conventional delivery systems usually cannot do this. A large dose of growth factors is not required when you use a delivery system.

Also, it is able to go to the right site and act in the right environment alone, thereby reducing the side effect too. And also, it is not exposing the growth factors usually to the biological environment, thereby reduces the rate of its degradation by the system. The approaches to deliver growth factors mainly include immobilization or encapsulation of these growth factors onto scaffolds or matrix, else using nanoparticles for delivery of these growth factors or else gene-based approaches using nucleic acids.

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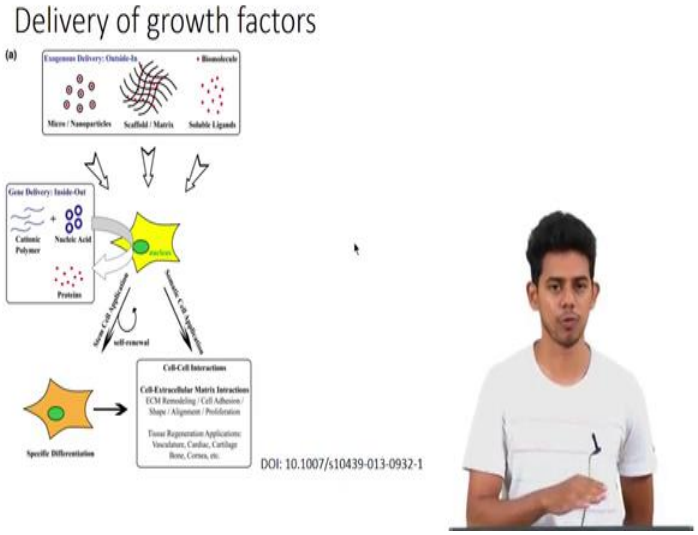

This shows an overview of all these three approaches. There is the delivery of growth factors by using nanoparticles or immobilizing them on a scaffold or a matrix or else gene delivery using nucleic acids into the cells.

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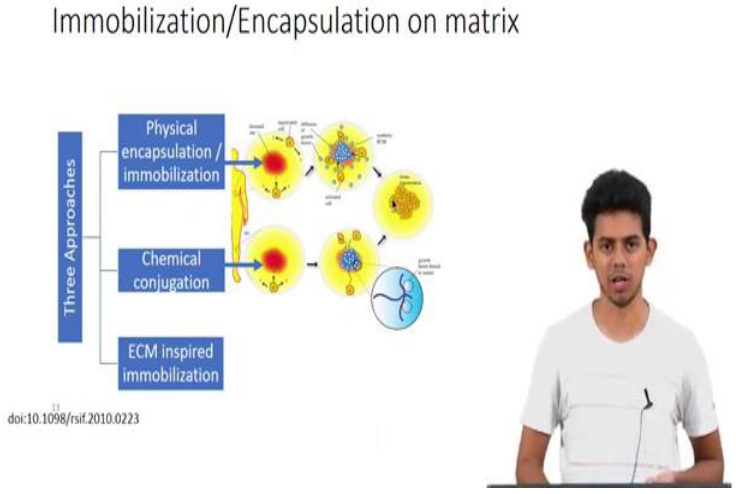

When it comes to immobilization or encapsulation of growth factors on to a matrix, there are the three approaches again there. One is physical encapsulation, another one is chemical conjugation, or there are approaches wherein its ECM inspired from the extracellular matrix of the body itself.

In physical approaches, what we do is we end up physically encapsulating these growth factors into a scaffold or a polymer. You put it into the site of the disease, and the growth factor slowly diffuses out, thereby causing tissue regeneration and healing of the wound site. In chemical conjugation, the growth factors are conjugated onto a matrix chemically, and then it is again implanted into the tissue site, and it stays there and ends up helping in the proliferation of the tissue and tissue regeneration in the end. First, we will look at physical encapsulation or immobilization techniques.

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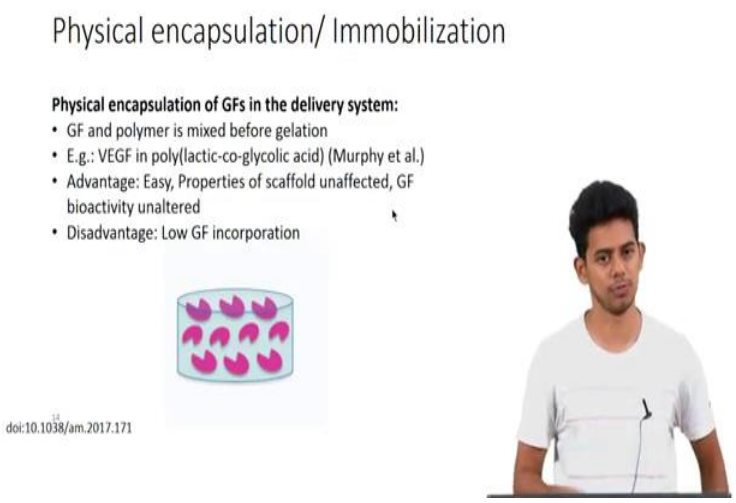

The first one is the physical encapsulation of growth factors by mixing the growth factor in a polymer before its gelation. So, the growth factors are trapped inside the polymer, and you can transplant it. The advantage of this is, its quite easy to make, and also the properties of the scaffold and also the growth factors are not affected much. But the disadvantage is the loading capacity of growth factors into these polymers would be quiet low.

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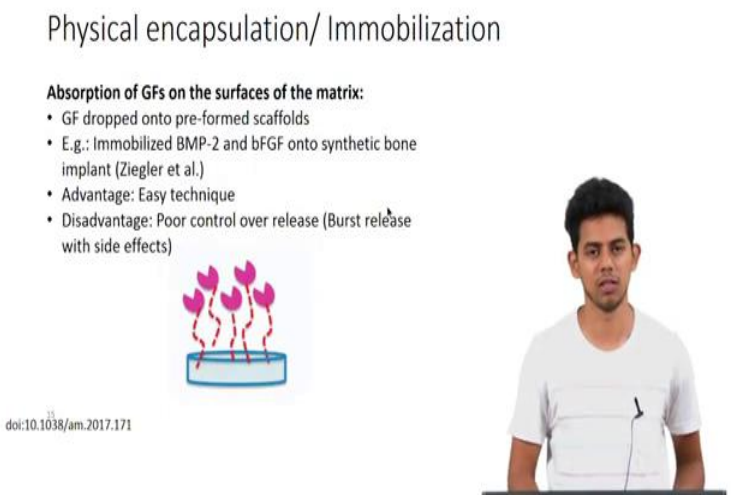

The second physical encapsulation technique is the absorption of growth factors onto the surface of the matrix. Here, a pre-formed scaffold is taken, and the growth factor is actually just dropped onto the surface. Here it just gets absorbed onto the scaffold, and it stays there. Scaffold with the absorbed growth factor is implanted in, and then it releases over time. But here, the issue is since it's just absorbed on to the surface, there is this possibility of burst release happening, which is not preferable. Because it can cause side effects by spiking the concentration of growth factors in the body to a higher dosage than required. The advantage here again is that its an easier technique.

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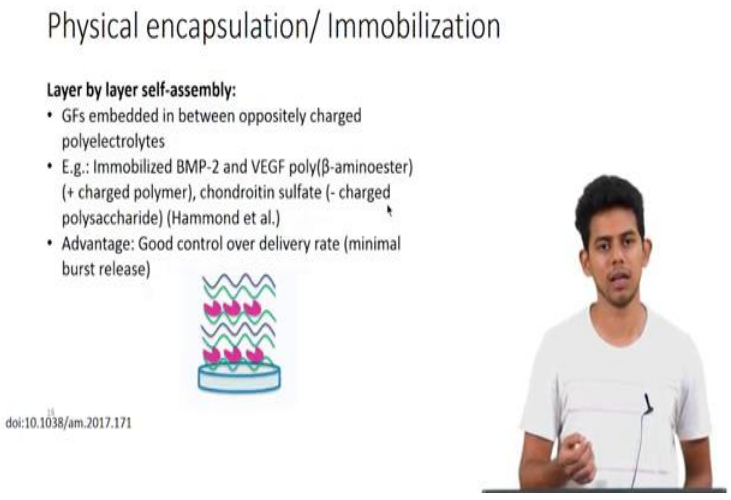

Then the third physical encapsulation technique is layer by layer self-assembly. Here, what we use is a polyelectrolyte. Polyelectrolytes are layered, and the growth factors are sandwiched in between. Polyelectrolytes have different charges; they can be polycations or polyanions, positive or negative.

The growth factors based on that charge too can be sandwiched in between, and we can go for multiple layers, as shown in this picture. There can be multiple layers of growth factors sandwiched in between these polyelectrolytes. Here, what the advantages are that we have very good control over the delivery rate. So, there is no issue of a burst release here.

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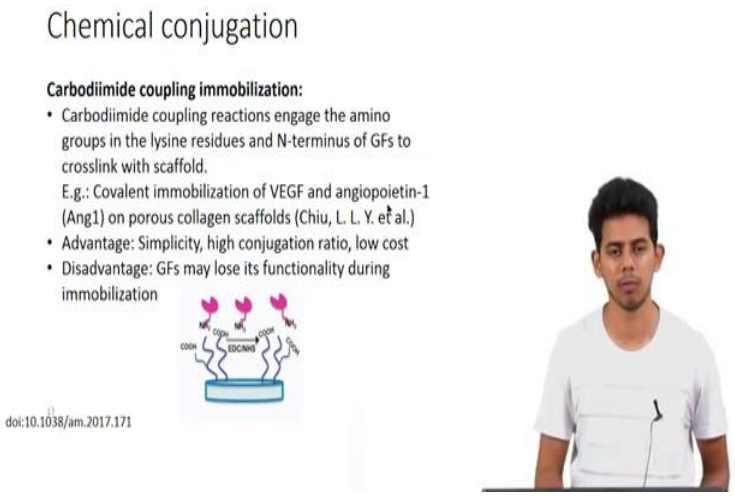

Then coming to chemical conjugation, one of the most commonly used techniques is carbodiimide coupling immobilization. Carbodiimide coupling involves crosslinking the growth factor to the scaffold, usually using a crosslinking agent. The example here is a covalent immobilization of VEGF and angiopoietin to growth factors on a porous collagen scaffold.

The advantages are, it is quite simple, and you get a very high conjugation ratio and a low cost. But the disadvantage is during this coupling, when you crosslink the growth factors onto the scaffold, it can lose its functionality if its active sites are altered.

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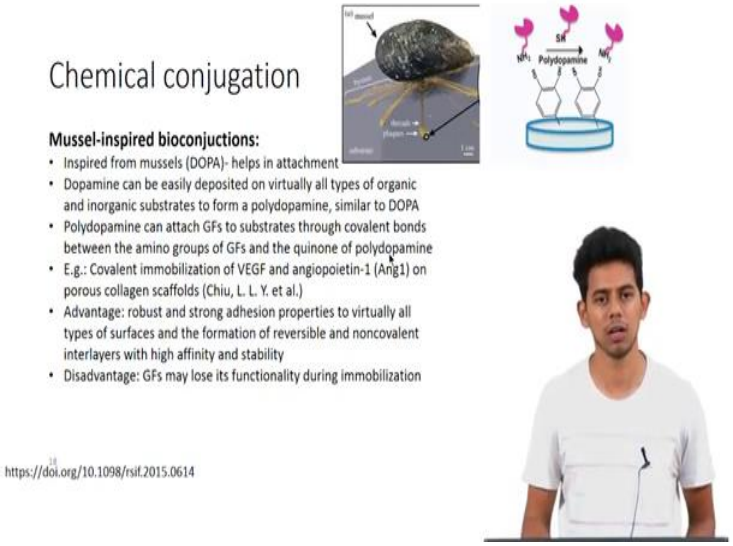

Now, coming to the second chemical conjugation technique, its mussel-inspired bioconjugation. Here, mussels are these organisms that grow on the beach on the rocks; you would find them attached near the sea. They use a chemical called DOPA, which can very strongly attach to almost all surfaces.

Similar to that, what is been developed is polydopamine using dopamine, which has a similar property to DOPA. This DOPA can be coated on almost any scaffold surface, and the growth factors can be made to conjugate onto it. The advantage that it is a strong adhesion. So, you would get a high affinity and also stability. There would not be a burst release or uncontrolled release pattern. The disadvantages again as previous chemical conjugation technique, the growth factor can lose its functionality during the immobilization technique.

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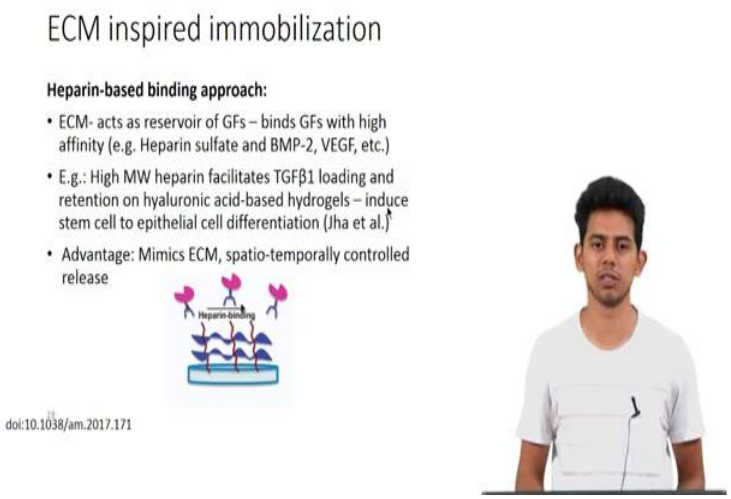

Now the ECM inspired immobilization approaches. In the ECM, we find a lot of proteins or molecules which can control the release of growth factors. They act as reservoirs by having a very high affinity towards these growth factors and thereby altering how the growth factors are distributed in the environment.

One such molecule is heparin sulfate. It has a very high affinity to growth factors like BMP 2 and VEGF. You can use these molecules to coat the surface of your scaffolds and ensure that the growth factors like the ones which have an affinity towards heparin sulfate end up getting immobilized on the surface. The advantages are it mimics the ECM. It is a much more natural environment that we are simulating here, and we get very good spatial and temporal control.

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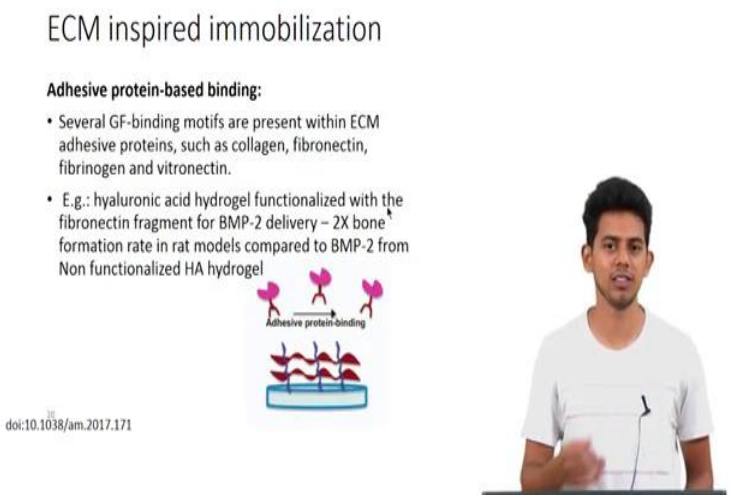

Another ECM based immobilization approach is adhesive protein-based binding. Just like heparin sulfate, there are proteins too like, collagen and fibronectin, which have an affinity to certain growth factors. Based on that, we can even coat the surface of your scaffold with these proteins like fibronectin or fibrinogen. The example given here is BMP 2, which has a higher affinity to fibronectin fragments. We can do that and ensure that the growth factors remain attached stably onto the scaffold.

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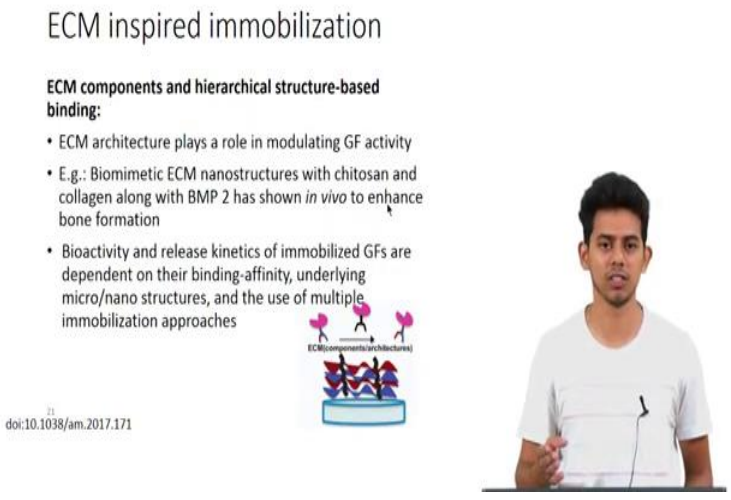

Another approach inspired by ECM is using the structure that the ECM provides. So, biomimetic ECM nanostructures can be achieved using your scaffold itself. This too can improve the growth factor affinity towards the molecules.

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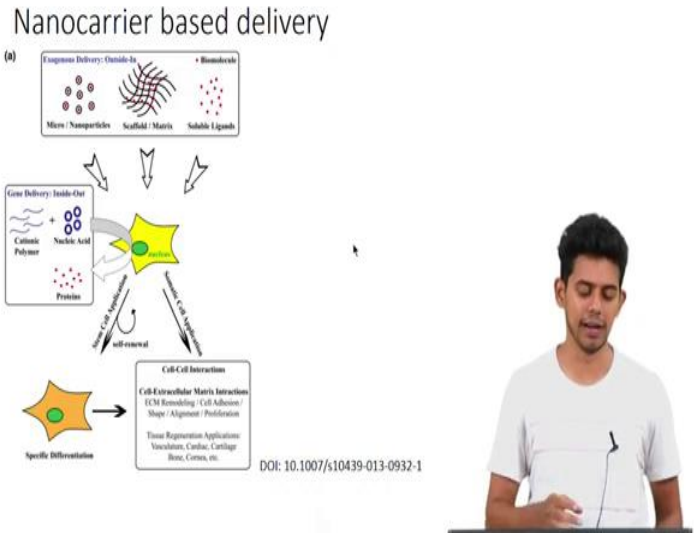

The next approach in delivering growth factors we are going to look at is nanocarrier-based approach wherein you use a nanoparticle to deliver these growth factors.

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The advantage of this is nanocarrier-based delivery has already been studied quite well because it is commonly used in drug delivery systems. It can have a very high loading

efficiency, and also it can quickly respond to environmental stimuli such as temperature or pH.

So, spatially, it would be easy to control. It can go to the target site and only then release the growth factors, which is a great advantage. Also, they protect the growth factors from the bio-environment, thereby preventing the growth factors from being degraded easily by the physiological system.

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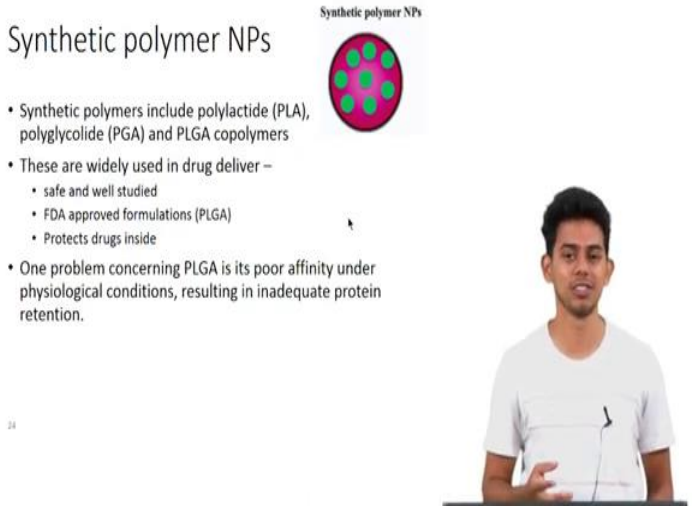

The first nanoparticle-based system we will be looking at is synthetic polymers. Synthetic polymers, like PLA, PGA, and PLGA, are widely studied in drug delivery. PLGA is FDA approved, and also it protects the drug inside, and its well studied. One problem when it comes to PLGA is its poor affinity to the proteins when it comes to physiological conditions. So, protein retention might be low. The proteins can actually leach out in the physiological environment.

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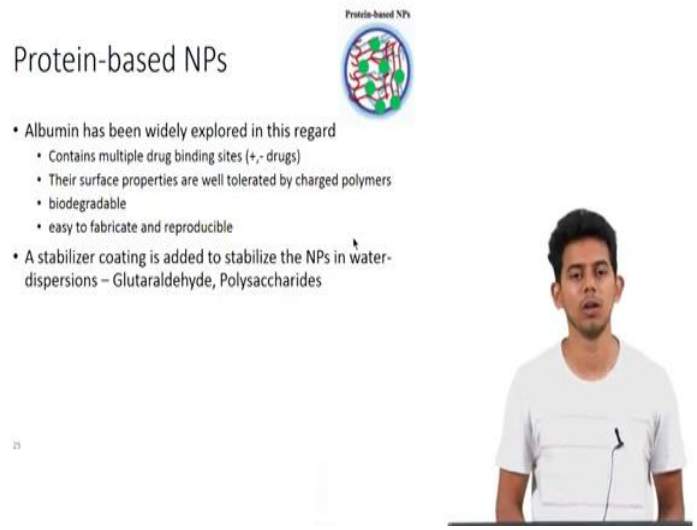

Protein-based nanoparticles. Albumin is a protein that has been widely studied for this application. It has multiple drug binding sites. So, you can conjugate the growth factors quite easily, and also, their surface properties are well tolerated by charge polymers. They are biodegradable, and also, they are easy to fabricate, and they are reproducible. Usually, a stabilizer coating is given to these nanoparticles, usually of glutaraldehyde or other polysaccharides; this is done to prevent aggregation.

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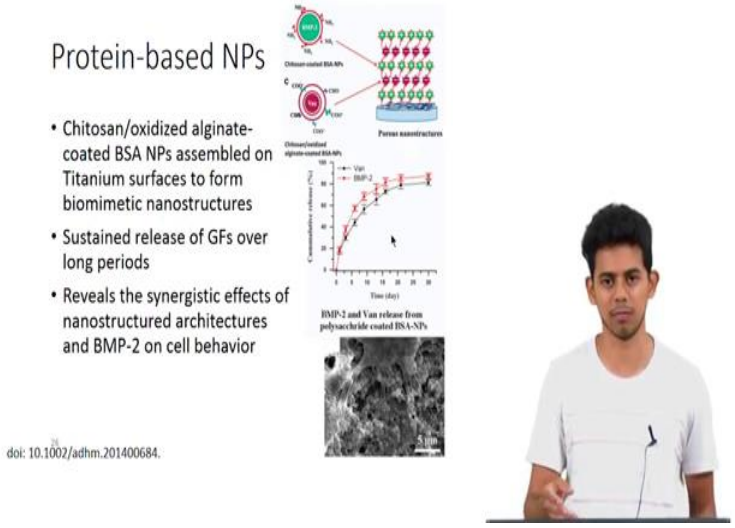

An example of this protein-based nanoparticle approach, which has been integrated with the microarchitecture approach, which is previously mentioned, is this study. Here, chitosan alginate coated bovine serum albumin nanoparticles were used. Two different growth factors, as you can see has been incorporated into these nanoparticles, and they have been arranged based on the charge in a biomimetic nanostructure. It has shown that it has a very sustained release.

So, you can see the two growth factors releasing in a very controlled fashion in an overall sustained period. So, it's releasing for around 30 days. Also, the microarchitecture has been shown to have a synergistic effect along with these growth factors in improving cell behavior.

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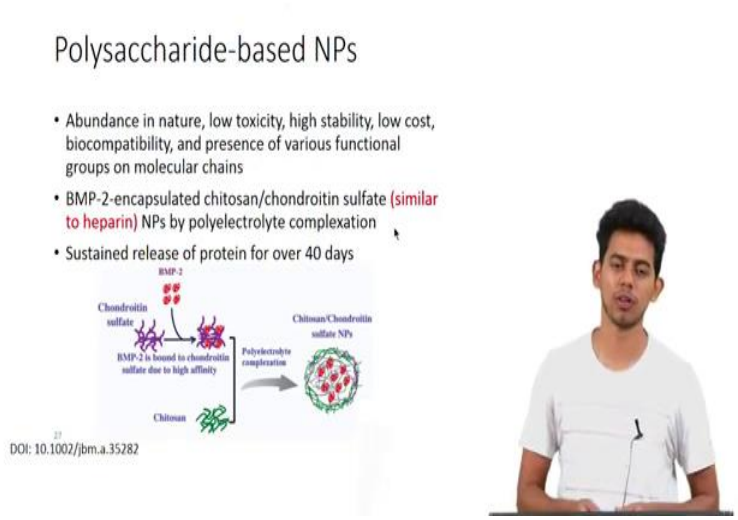

The example shown here is BMP-2, which is been encapsulated in a polysaccharide-based nanoparticle, chitosan base. And also, it is arranged in a polyelectrolyte complexation fashion, which is similar to the layer by layer approach that we saw.

So, chondroitin sulfate is similar to heparin is able to easily attach to BMP-2 because of its high affinity. It forms a complex, and chitosan ends up complexing with this due to polyelectrolyte because of the charges between them, and they form a nanoparticle.

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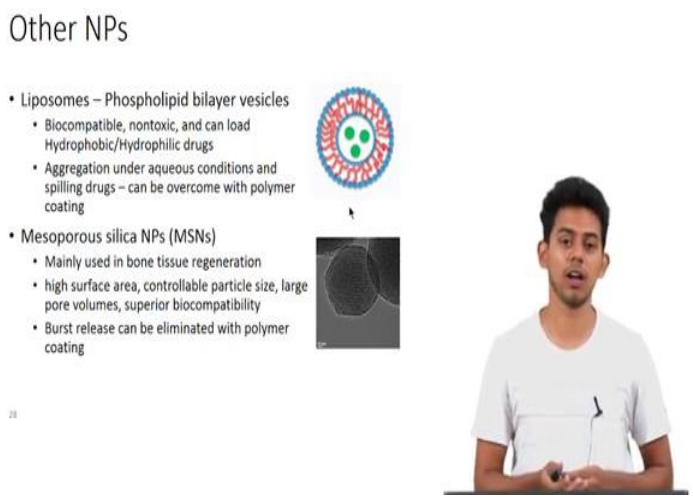

Other nanoparticles that are worth mentioning are liposomes, which have been widely studied for drug delivery applications. It is a phospholipid bilayer vesicle which has hydrophobic and a hydrophilic region. Based on the molecule of interest, you can incorporate it in the hydrophobic or the hydrophilic region, which is an advantage when it comes to delivering nanoparticles. But aggregation is one of the issues in liposomes. This can be overcome just like previously discussed by giving a polymer or a polysaccharide coating.

Another nanoparticle worth mentioning is mesoporous silica. It is a silica nanoparticle with a lot of a very porous structure. It has a very high surface area, and the particle size can be easily controlled and has good biocompatibility. Even the liposomes have good biocompatibility. So, burst release can be eliminated by giving a polymer coating.

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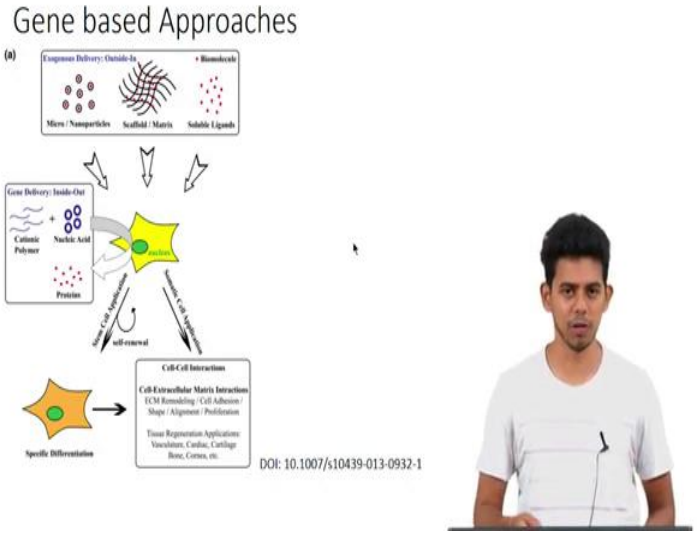

Now, coming to the third approach that we will be talking about is the gene-based approach.

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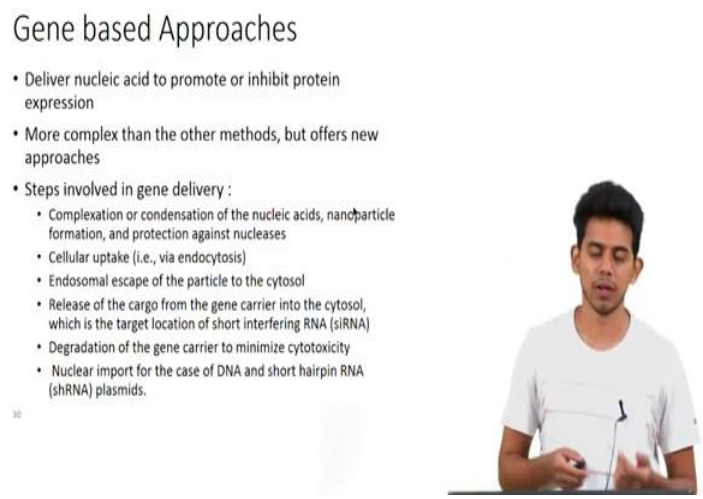

This is a little more complex method, but it offers us a lot more approaches wherein you can activate or deactivate genes that are already present in the cell, or else if you want, you can even introduce new genes that are not present in the cell. So, that is something that this approach uniquely offers. The steps mainly involved in delivering a gene into the cell involve; first, a complexation or condensation of this nucleic acid and a

nanoparticle formation, and then this nanoparticle is taken into the cell via endocytosis. So, it forms an endosome bilayer around this nanoparticle.

Now the payload has to break out of this bilayer, which is the release of the cargo into the cytosol. If the payload is a small interfering RNA, which can go and bind into mRNA and stop translation into proteins, it will take its action in the cytoplasm itself. If it is a DNA that needs to be carried into the nucleus, it is further taken into the nucleus wherein it can incorporate with the host DNA or else stay as plasmids.

Then degradation of the delivery system that you used inside the cytoplasm; that is very important because it can lead to cytotoxicity. So, that is something which we will look at avoiding.

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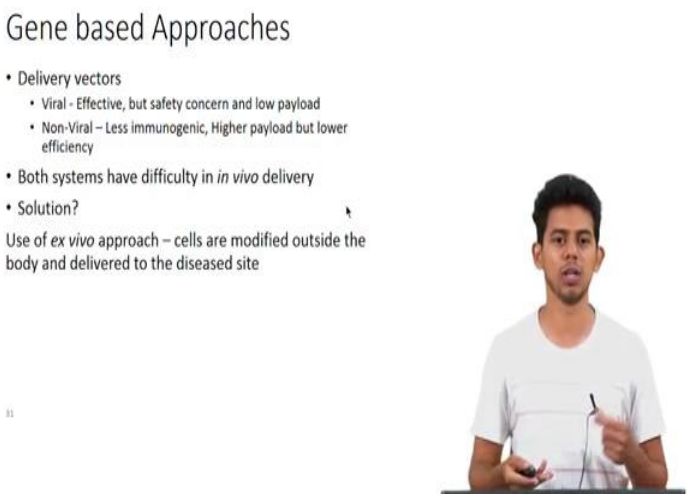

The delivery vectors usually used for delivering nucleic acids are viral or non-viral. In the viral vectors, the advantage is it has a very high efficiency of delivering these genes, but at the same time, safety is a concern and also low payload. The safety concern is that although the viral in part of this viral-nanoparticles are deleted out, it can always mutate back into being virulent; that is the disadvantage of viral vectors.

When it comes to non-viral vectors, they are less immunogenic. So, they are much safer when compared to viral vectors, and also, there is a higher payload, but its efficiency is quite low. Both of them have difficulty in delivering genes in the in vivo delivery. The

solution to this is to take the cells out into an ex vivo environment. Then, transfect these cells with the gene of interest that you want, and then reintroduce them into the body.

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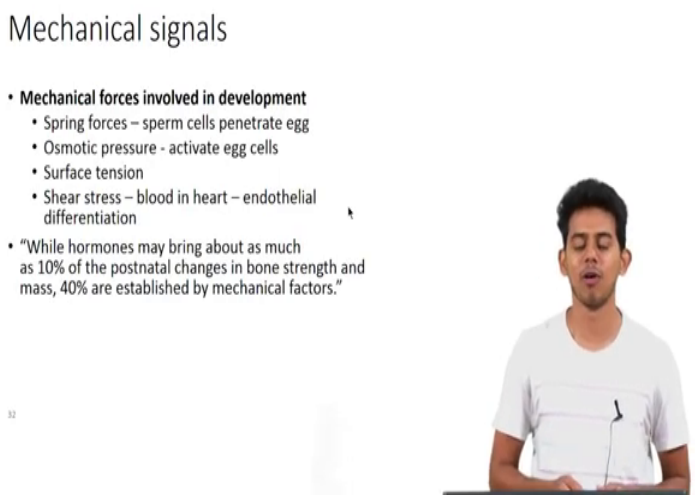

Now, after looking at the chemical signals, we move onto mechanical signals that are used for tissue engineering. Mechanical signals are normally used by the cell to grow, proliferate, and so on. The normal development of most tissues requires some sort of mechanical force is to be involved. These are few of the mechanical forces which are in the development process of tissue.

Spring forces, as you can see, it's similar to a spring wherein if its compressed its tends to relax. So, this is what drives the sperm cells to penetrate the eggs. Osmotic pressure is something that activates egg cells. Shear stress, which is found in the heart wherein the blood, is pumped by the heart, and it experiences shear stress on it, and this also actually affects the endothelial cell differentiation. It's quite important for the development of these tissues.

Something interesting to observe is that only about 10 percent of the postnatal changes in bone strength is influenced by hormones, whereas the rest, like around 40 percent, is just by mechanical factors.

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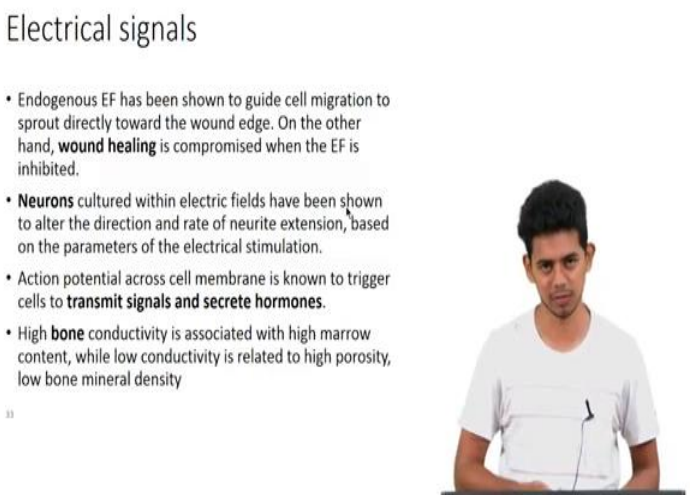

Now, moving onto electrical signals, these are also very vital for the development of the tissues. It has been shown that endogenous electrical fields are usually formed at wound sites to direct cells towards this wound site, thereby helping in wound healing. When these electrical fields are not present in wound sites, it ends up comprising how the wound healing takes place. Neurons cultured in an electrical field have been shown to alter its direction and rate of neurite extension based on the parameter of electrical stimulation.

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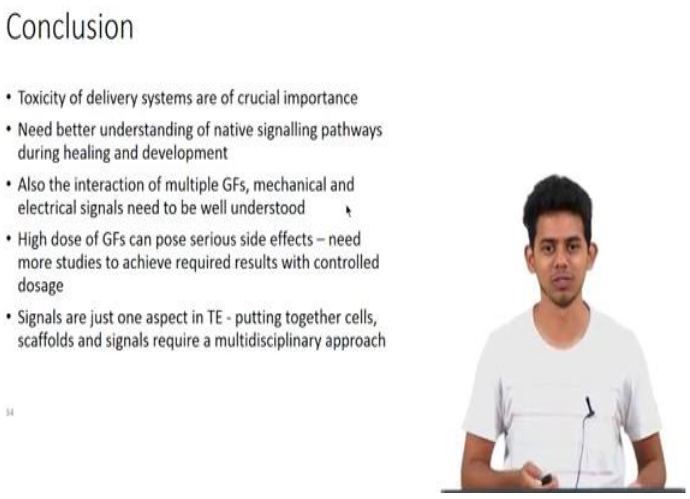

High doses of growth factors can pose serious side effects. So, we need to study and make sure that the controlled release of these growth factors ends up giving only the concentration that is required for that. Cells are just one of the aspects of tissue engineering, and we need to put the cells, scaffolds, and signals together, which requires a multidisciplinary approach.

Thank you.