Tissue Engineering
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Lecture - 03
Introduction to Tissue Engineering - Part 3

Today, we will continue our discussion on the Introduction to Tissue Engineering. We looked at the two arms of the tissue engineering triad; we looked at what are biomaterials and how they can be used. We also looked at cells and what are the different sources and types which we can use.

Today we will talk about signaling molecules; signals basically, not just signaling molecules. We will first start with the signaling molecules, and I will also briefly introduce other signals. Our focus here is just an introduction. So, we will go into greater details in the later part of the semester, ok.

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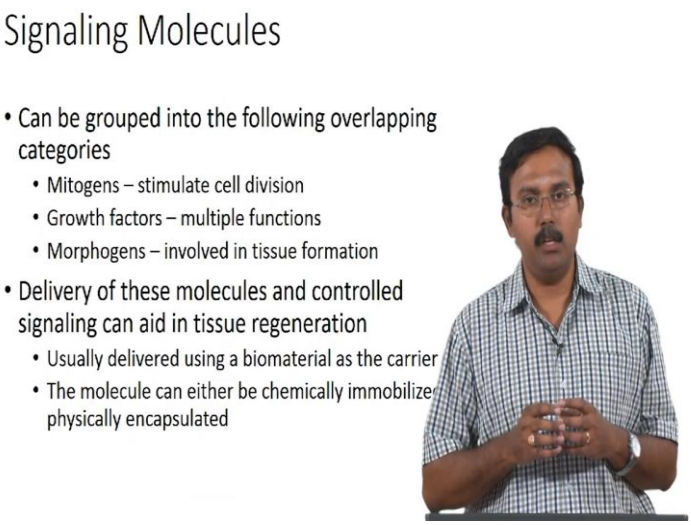

Signaling molecules themselves can actually be grouped into three major categories, but there are overlapping category; some of the molecules will act as both as a mitogen and a growth factor or a morphogen and a growth factor and so on. These are mitogens, growth factors, and morphogens. Classically, mitogens are the ones which will simulate cell division, growth factor was initially identified to be the molecules that help in cell proliferation, and it was later identified that it actually can have multiple functions.

The major challenge with respect to signaling molecules is how you deliver these molecules. You need to have a controlled delivery with maybe spatiotemporal release; so that there can actually be proper control over signaling, which will aid in tissue regeneration. Usually, this is delivered using a biomaterial as the carrier, and the molecules can be chemically mobilized or physically encapsulated to provide some kind of a controlled release. So, this is what is currently being looked at.

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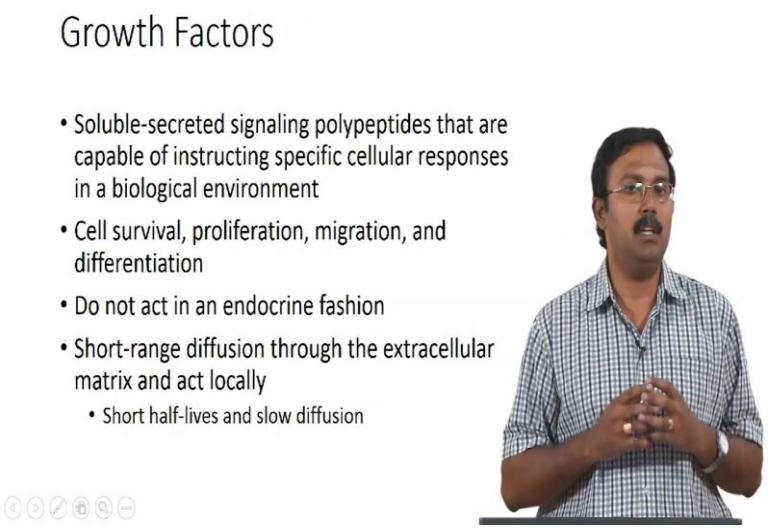

So, what are growth factors? These are soluble secreted signaling polypeptides that are capable of instructing specific cellular responses in biological environments. Can you identify some growth factors which you already know?

Student: BMP.

BMP ok. So, that is a Bone Morphogenetic Protein, that is a growth factor.

Student: VEGF.

VEGF which is?

Student: Vascular Endothelial Growth Factor.

Vascular endothelial growth factor, you know what is the role for that?

Student: It helps in blood vessel formation.

Ok, It is part of angiogenesis. So, there are many other growth factors we will look at some examples which are commonly used in tissue engineering applications. Actually, growth factors can help in so many different cellular responses from cell survival to proliferation to migration to differentiation and even with tissue formation.

It has a wide range of applications, and it is seen that they do not act in an endocrine fashion. It's not that like the growth factors can circulate in your bloodstream and reach different places. That is primarily because they have short half-lives, and because of this, they only go through diffusion. But these are actually proteins; these are reasonably large molecules.

So, they are not going to diffuse very fast; so, they have very short-range diffusion, and this diffusion happens through the extracellular matrix, which is present in the tissue, and they will act locally. So, it will not have a large, like a systemic effect.

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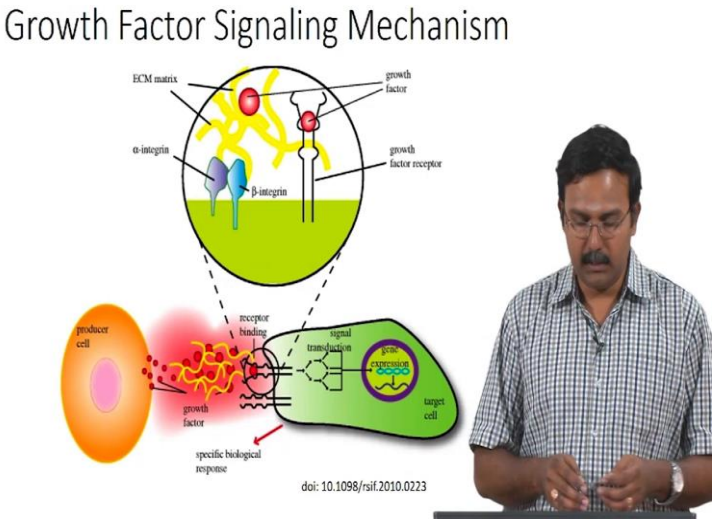

This is a general growth factor signaling mechanism. So, this is not for any specific growth factor. What you have is a producer cell, which would be producing some growth factors. All the growth factors are secreted by some cells.

So, different cells will produce different growth factors. And these will diffuse through the ECM and come in contact with the receptor on a cell surface. There will be some single transduction cascade, which will elicit the response you are looking for. And this

is a general phenomenon which is commonly observed, what you need to look at carefully here is what is zoomed here.

What you see is the ECM? You actually can have the molecule delivered to the site, to the cell in a spatiotemporal fashion; it is not like all of them go and reach the cell directly. So, some of them reach the cell in certain regions; some of them reach at different time points, and this actually matters. This kind of spatiotemporal releases was one of the major roles of ECM other than just supporting the cells to grow. This helps in providing the proper signal for the cell to behave the way it should.

That is why people have studied that if you just implant stem cells into a tissue, they usually tend to differentiate to that particular cell line because there are signaling molecules and ECM microarchitectures and niches which help in that kind of differentiation.

Also, ECM plays an active role in signaling. This is one major aspect which needs to be accounted for when we design scaffolds. If you are looking to design bioactive scaffolds, mimicking the ECM becomes very crucial.

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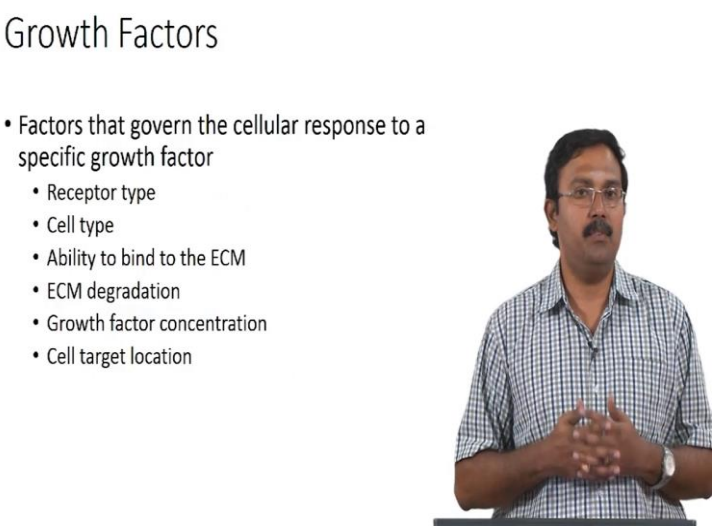

Different factors govern the cellular response for a growth factor. It is not that you have one growth factor which will always cause the same effect at the same levels; it does not

work that way. There are many factors which actually govern what response you observe for a growth factor.

Depending on the receptor type to which the growth factor attaches, there can be a different response. And depending on the cell type again, there can be a different response. Some of these things have effects on multiple cell types; it is not that the growth factor can act only on one type of cells.

So, when there are multiple cells which can be acted upon by the growth factor, it depends on the cell itself. Because the intracellular machinery where the signaling cascade you are going to observe, is going to be different for different cells. So that means, the cellular response can also be significantly different. And the ability of the growth factor to bind to the ECM will also matter. If a growth factor can bind to the ECM, then the diffusion is going to be much lower, it's going to a get stuck in the ECM for a while.

So, that is going to have a role and also ECM degradation. If you have the growth factors encapsulated in the ECM, then looking at the ECM degradation will control the release profiles and the release pattern of the biomolecule, and this will play an important role. Concentration and cell target location can also play major roles when it comes to what you observe for a cellular response for a growth factor.

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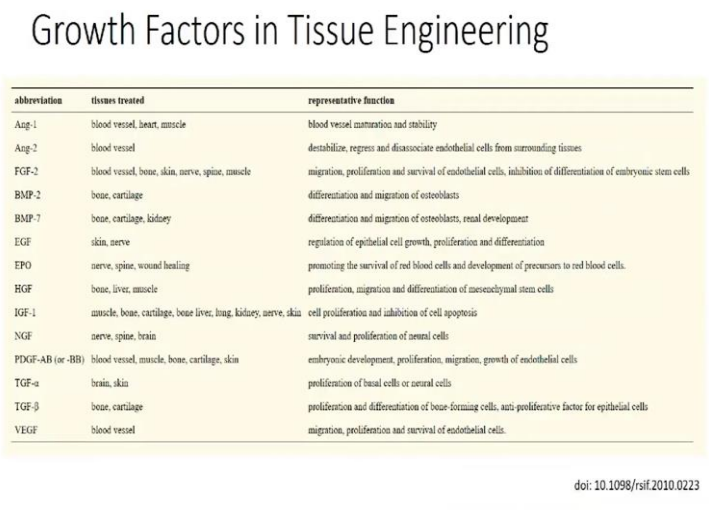

So, this is a bunch of growth factors that have actually been tried out for different tissue engineering applications. So, I am not sure if you can read it from there, but I can read it from here. So, I will read it out for you, ok. So, ANG-1 is angiopoietin, and ANG-2 is also angiopoietin 2.

So, these have been used for treating blood vessels and heart and muscle tissues. ANG-1 affects blood vessel maturation and stability, whereas ANG-2 can destabilize, regress, and disassociate endothelial cells from the surrounding tissues. And FGF-2 is used in a blood vessel, bone, skin, nerve, spine, and muscles. So, this helps in migration, proliferation, and survival of endothelial cells; it also inhibits the differentiation of embryonic stem cells.

FGF has multiple roles, depending on which cell is being targeted. BMP-2 and BMP-7 are bone morphogenetic proteins, see many of these growth factors you would always have some associated number with because there are many variations. So, BMP is a class of bone morphogenetic proteins.

BMP-2 and BMP-7 have been extensively studied because they take part in differentiation and migration of osteoblasts. BMP-7 has shown to affect renal development. So, that is why it has been used in kidney tissue engineering as well.

EGF is Epidermal Growth Factor, EPO is erythropoietin. EGF has been used in skin and nerve, whereas EPO has been used in nerve, spine, and even wound healing. So, all these molecules have a significant effect on different cellular responses and based on understanding what cellular response they have; you can use it for the appropriate application.

These are all the bunch of other things which you can look up. VEGF is another thing which is commonly studied because it helps in migration, proliferation, and survival of endothelial cells. So, if you need angiogenesis, you can actually use VEGF and hopefully get a vascularized tissue. That is something which people have been exploring for a while now.

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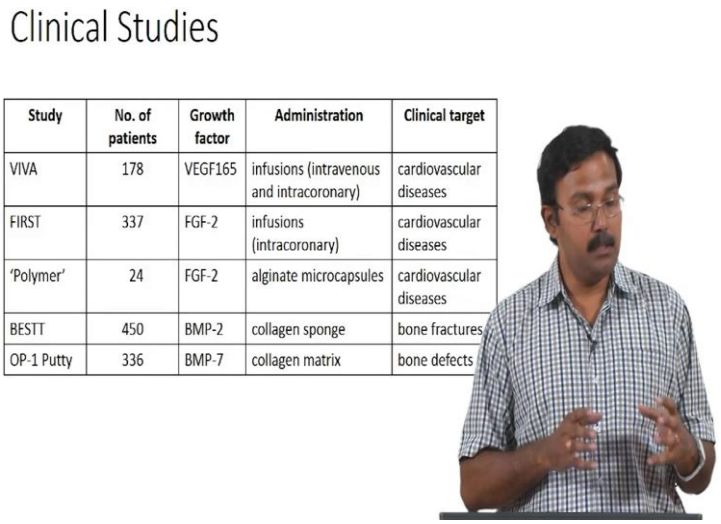

By using this signaling molecule, people have actually taken some products to a higher level. They can take it even up to clinical studies. So, these are the clinical studies, and you have VIVA, where 178 patients have been tested with VEGF165. Here they have just done infusions, where they gave intravenous and intracoronary injections to deliver this molecule, and this was done to treat cardiovascular diseases. However, the results were not very promising; the results were negative; I think in phase 2 probably.

So, clinical trials have multiple phases. You have phase 0 where you test in on other animals then you have phase 1, phase 2, phase 3 and finally, phase 4, where it is in the market, and you get market feedback. So, except for the last two (BESTT and OP-1 Putty), the other ones had not gotten past phase 2. These two have been commercialized. But as I was looking up OP-1 Putty today morning, and looks like it is taken off the market, I am not very sure as to how it was. So, it was initially brought in by striker, and it was purchased by Olympus and 4 years back it was dumped.

I do not know if somebody has taken it up because there has been a lot of controversy with respect to the side effects of it. I was just reading up a little bit on that and saw there was controversy associated. But the other one BESTT, that is basically a product which is currently also available. It is infused by Medtronic; we will talk about that product a little bit later.

So, they have tried different growth factors and what you see is the first two things are just simple infusions, where you just deliver using some kind of injection, intravenous or intracoronary and they have actually failed, and people also tried alginate microcapsules, and that did not work either.

Collagen sponges and collagen matrices have actually shown reasonable promise when it comes to delivering these molecules, and it is understandable, right? Collagen is what your ECM is made off. So, you are going to have a better chance of mimicking the ECM when you use that.

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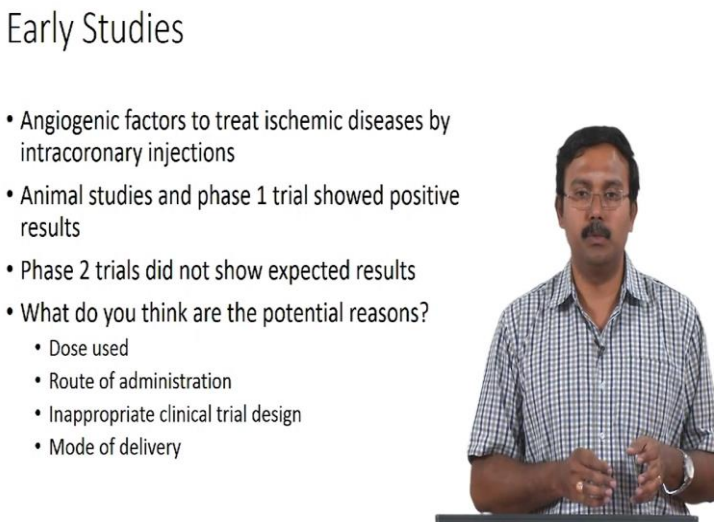

As you see, early studies were focused on treating ischemic diseases; that is why you saw the first few examples of cardiovascular diseases. You want to treat ischemic diseases using intracoronary or intravenous injections of angiogenic factors. It was a simple and straightforward approach. People just thought; ok, I know this molecule can trigger angiogenesis; I know this tissue is not getting blood supply because it does not have enough blood vessels.

So, you put this growth factor there, you should have new blood vessels formed, but it did not turn out to be as simple. Because the results were promising from the animal studies and phase 1 trials, but phase 2 trials did not show expected results. Why do you think it failed? What could be the potential reasons? Think of all possible reasons from a

clinical trial standpoint has to, why something can fail? And then going to specifics of why this probably failed?

Student: Side effects.

Ok Side effects; so, why do you think side effects would come?

Student: Because it is not targeted enough.

Ok, it's not targeted enough.

Ok, You have a question?

Student: What is the difference between phase 1 and phase 2 trials?

Ok, So phase 1 - you are supposed to give sub-therapeutic levels of sub-therapeutic dosage, and it is given for a smaller number of patients, and in phase 2 - you are supposed to deliver therapeutic levels to a larger patient sample size.

Student: Sub-therapeutic? What is different between both of them therapeutically?

Ok for any drug, you have something called the therapeutic index right. You have to have a minimum effective concentration and a maximum toxic concentration. If the drug or any molecule is at a concentration higher than the toxicity levels, it is going to have serious side effects, if it is less than the minimum effective concentration you are not going to have any effect. So, within this window is called the therapeutic concentration.

So, you initially you give something which is sub-therapeutic, which is lower than the therapeutic concentrations because you want to know that concentration. You would have studied and identified that at this level, this concentration, this dosage, will have the desired effect, but you do not know if that dosage will cause negative effects, ok.

So, what you do is; you start with a very low effect, knowing that it will not do any good, but you still want to know if there is any harm which is being done ok. So, that is phase 1. In phase 2, what you do is you do both; you basically group patients into control groups and other groups, and you put them as one group with sub-therapeutic and one group with therapeutic levels.

If the sub-therapeutic levels do not show any negative effects then you go to phase 2; then you compare sub-therapeutic with therapeutic effects as phase 2 trial and then phase 3 you do all therapeutic levels for a much larger sample size.

The sample sizes will depend on what type of drug we are dealing with and whether you are looking at specificity. For example, if you are looking for a cancer drug then you might want groups for different types of cancers and see how the drug would affect different cancers, you might say that it works for oral cancer, but it does not work for some of the colon cancer right.

So, there could always be differences. So, those kinds of things you need to look at; That is what the clinical trials are. What do you think could be the potential reasons? Side effects are the serious cause, but what do you think causes the side effect?

Student: Immune response like the immune system there is a slight variation from person to person.

Ok. So, that is a more of personalized medicine which you were talking about. You would not have if you are using one general thing, there can always be a problem. But here chances of an immune rejection would be much lesser because you are only looking at a protein which is already present in your body and you are just supplying it as an intravenous injection or an intracoronary injection.

Student: Then maybe the drug delivery aspect of it.

How you deliver it, the mode of delivery could be a problem; yeah.

Student: Because in vaccination like I read that it is better to inject the vaccination in the muscle rather than directly in the bloodstream because muscle or skin surfaces would. So, what he is talking about is just a mode of delivery related to whole ideas for vaccination.

Yeah. See, mode of delivery can have a role to play here, but not really about how it is intravenous or intramuscular or intracoronary. So, it could have an effect with respect to whether it reaches the site or not.

When it comes to the vaccine, its different mechanism, here you are looking for it to reach the site. If I give an injection; intravenous injection, it is probably not going to reach the ischemic tissue, which is probably close to my heart right. So, intracoronary would probably be better, because it delivers directly to the site. That mode of delivery could have a role, but not exactly the way you say, but in a different approach.

Student: Sir, because it worked in animal studies and did not work with people basically. I mean, you mentioned some factors which determine the response of ECM whether it is encapsulated the ECM and affinity to the ECM and cell type and its receptors, it might be something to do with that.

Yeah. So, it also depends on that actually, how it is delivered, not just the mode of delivery we were looking at, whether it is encapsulated in a molecule. Because you need to know how it interacts with the organism. so, it would interact differently in a smaller species, like a rat or a rabbit and when you take it to larger animals or humans it is going to have a slightly different effect, quite possibly can have different effects. Usually, not more than one-third of the animal studies are reproducible in humans. So, there can always be some issues with that, Ok anything else? So.

Student: When we say animal studies, do we even consider monkeys in that?

So, monkeys are animals so, we would consider.

Student: They are very close to human. So, is there.

See not really; technically speaking apes are the closest to humans, monkeys are not, and even amongst apes its actually quite difficult to do very large-scale studies. So, if I want to perform studies with maybe a hundred chimpanzees, it is not really going to happen. So, there are other statistical issues associated with how the data is interpreted, and you cannot completely take this forward. In some cases, it will be easy to extrapolate; it depends on how the complexity of the tissue itself, and it also depends on how that particular organ functions compared to the human organ.

For example, if you were to take pancreas, a pig's pancreas is closer to a human pancreas than an ape's pancreas. Because of the insulin response, the sugar response, and insulin

release are quite similar. So, it just depends on all that; So, you cannot just say doing it in ape will be the best thing.

For that particular tissue, what would be the best thing? Again, for thrombotic effects, if you can work with lambs or pigs, they would have a much more aggressive thrombus formation compared to what you would see in humans. So, then you are now going to have a very different set of results. There is always going to be variations which you have to account for.

So, what I had here was basically some of the things which you already said, the side effect is primarily because of the dose used. When you use sub-therapeutic levels, you are basically using very low concentrations. See, if you take low enough of a concentration of anything, it is not going to cause any effects. Even cyanide cannot kill you if you take it in a low enough concentration right.

So, when it is in a sub-therapeutic level, it is not going to cause any effect, and that is ok, but once you take it to therapeutic levels, that is when you are actually doing at a level which actually has any meaning, and if that causes problems, it basically throws away your molecule. And the route of administration or mode of administration; so, what I called route of administration and mode of delivery are basically talking about whether it is encapsulated or whether it is in solution and so on.

I already said that ECM plays a role in presenting the growth factor to the cell; it is not just about the growth factor reaching the cell right. The ECM has a way to present it in a spatiotemporal fashion, that actually controls the precise way the cellular responses happen. There are factors, and there can always be inappropriate clinical trial designs. So, which could also lead to such results, which do not really give you conclusive proofs.

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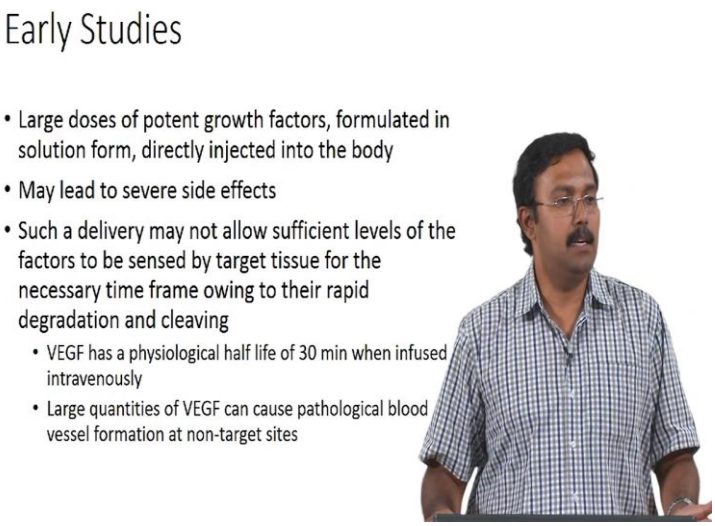

Basically, what was done in the early studies was large doses of potent growth factors which were formulated in the solution were directly injected into the body. The bad thing about this is; it can lead to severe side effects because you have to give a very large quantity of the molecule for it to have any therapeutic effect because I already said they have very short half-life right.

They can degrade very quickly, and if you are going to give it an intravenous injection, it is not going to reach the site of action for a long time. It will not survive for that. So, for you to make sure that at least the therapeutic level reaches the site of action, you have to load a lot of the growth factors into the body which will be a very bad thing to do.

Again, the other side of it is; when you the deliver using intravenous procedure, even if you load very high concentration, it still not going to reach the target tissue in the necessary time frame within which it degrades right. So, then what happens is you are only observing side effects, and your desired effects are not even seen. These are serious problems when you try to give an intravenous injection or just supply it as a solution.

So, one example would be VEGF. VEGF has a physiological half-life of about 30 minutes when infused intravenously. See as I already said none these growth factors operate in endocrine mechanism right. So, they do not actually flow through the bloodstream.

Hence, when you put it in an intravenous procedure, they are going to degrade very fast and 30 minutes is probably a very short time for VEGF to reach the site of action. To ensure something at least reach as a site of action, you are going to have a huge concentration of VEGF loaded to the with the injection. And when you do that, you are going to have pathological blood vessel formation, like what you would see in cancer right. In cancer, VEGF is actually overexpressed. So, there are therapies cancer drugs which basically just block VEGF.

When you have very high concentrations of VEGF, you are going to have some kind of a dormant tumor, which is going to be formed and that is not really something you want to do. So, people need to have a better understanding of it, how to deliver it; that was the major lacuna which had to be addressed eventually.

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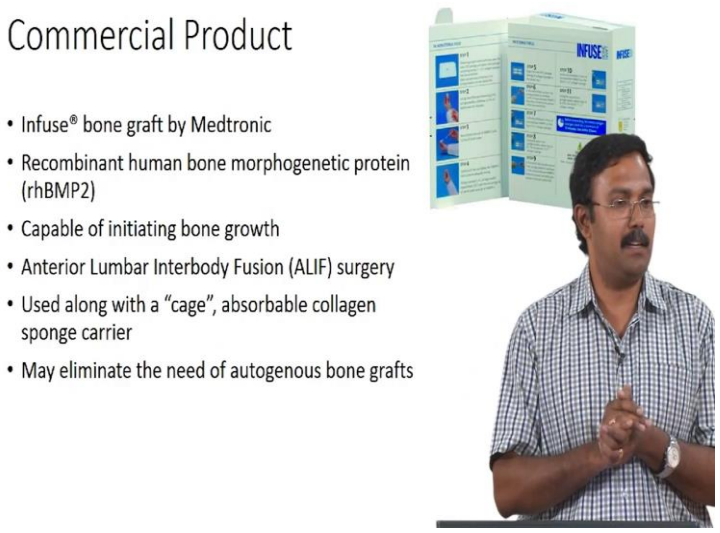

So, what people did was to put it in some kind of a matrix and then deliver it. So, one thing which was reasonably successful is the commercial product - Infuse. So, this was, this is being marketed by Medtronic. This is used in anterior lumbar interbody fusion surgery. Basically, spinal fusion surgeries and what they use is recombinant human bone morphogenetic protein. This is just produced using fundamental molecular biology techniques, BMP is overexpressed in an organism and produces a recombinant protein.

So, these bone morphogenetic proteins have the ability to initiate bone growth. Because of this, the material Infuse is highly osteoinductive, and this helps in the regeneration of

the bone tissues. This is used along with a cage and an absorbable cotton collagen sponge carrier. They call it ACS carriers. This can potentially eliminate the need for autogenous bone grafts. That is what they claim to do, but again, there are still limitations about the size of it and how you can replace like for in some cases it can actually be a substitute for autogenous bone grafts.

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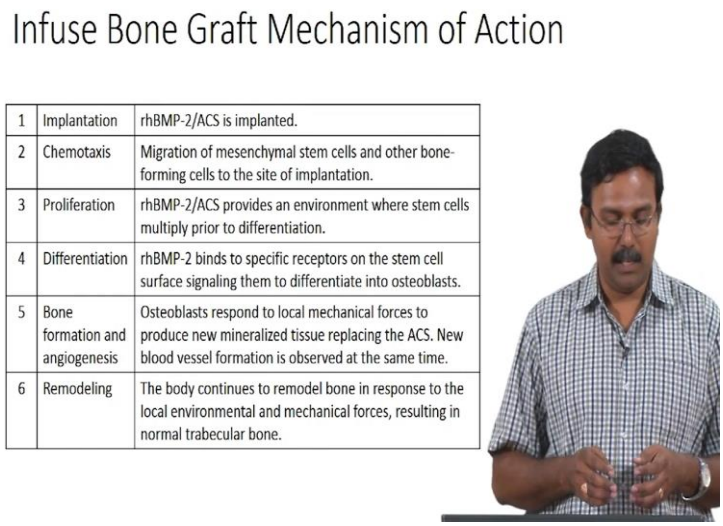

This is how it works. So, what you have is the first step is implantation. You basically implant this combination of BMP-2 with the collagen sponge, and then you would see chemotaxis, where there is the migration of mesenchymal stem cells and other bone-forming cells to the site of implantation.

Because you have the bone morphogenetic proteins, there is going to be signals sent to the nearby bone cells and bone-forming cells to come to this site. So, they start migrating towards it and then there will be proliferation, where this particular material provides the environment where stem cells can multiply before differentiation because you have a collagen matrix on which they can attach and start multiplying. And the differentiation happens because you have the BMP-2, it binds to the receptors on the stem cell surface, and then this helps in the differentiation of stem cells into osteoblasts, and then you have bone formation and angiogenesis.

The osteoblast will respond to the local mechanical forces to produce a new mineralized tissue which replaces the collagen matrix which they had used, and new blood vessel

formation is also observed at the same time. So, this is not truly triggered by this molecule itself, but there have been studies which say that BMP and VEGF can actually have a dialogue basically. So, they can interact and create vascularization. There are some studies which suggest that.

Finally, you have remodeling, where the body continues to remodel the bone in response to the environment and the mechanical forces. So, this will finally form the trabecular bone. This is the mechanism of action. This is directly from their website, and this is what they claim to have been the mechanism of action for Infuse.

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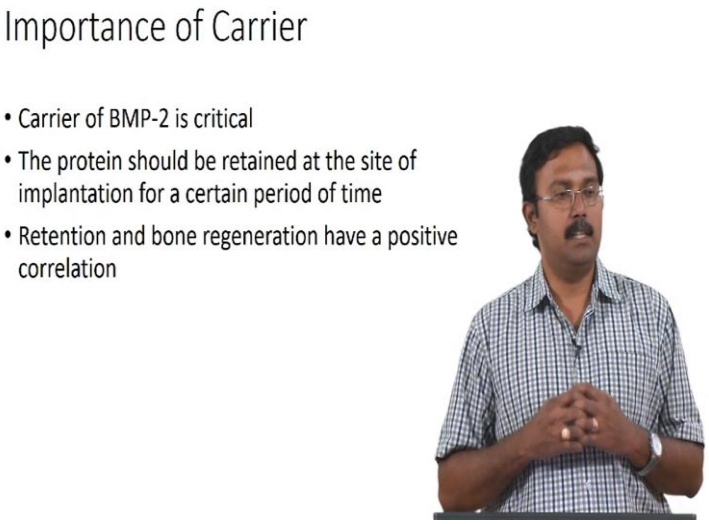

Another important thing which we need to look at is the importance of the carrier. So, whenever we are looking at signaling molecule delivery, again, the material we use as a carrier comes into play. That is why biomaterial is a very crucial component when it comes to tissue engineering; It is not just about providing a scaffold and support. It is also about delivering the molecules and presenting the molecules in the right way.

So, the protein should be retained at the site of implantation for a certain period of time; only then it can have an effect. If it is going to get diffused away right in a very short time, you are not going to have enough time for the cells to migrate towards the site or to differentiate or proliferate and so on.

So, people have shown that retention and bone regeneration have a positive correlation. If you can retain this molecule for a longer period of time, you are going to have better bone regeneration. That is why people try to look at the controlled release of these molecules. People wanted it to come out in a very slow and sustained fashion.

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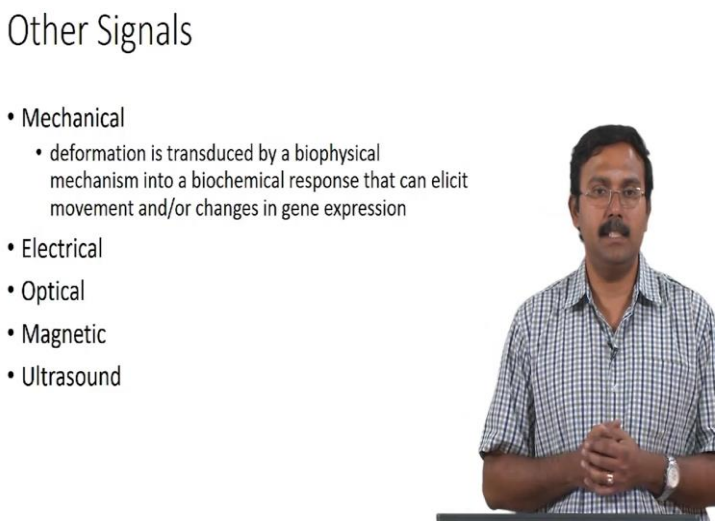

There are many other signals as well, which I will not go into great detail, which can be used for stimulating cellular responses. Recently mechanical stimulation has been explored extensively, and there are other things like electrical, optical, magnetic and ultrasound signals as well, which can be used for stimulating cells either in vitro or in some cases even in vivo.

Mechanical simulation is basically deformation, which is transduced by a biophysical mechanism to create a biochemical response, and this will result in either the cell migration or gene expression and so on.

People have shown that the mechanical property of a material such as elasticity or strength of the material, and all those things can affect how the stem cell differentiates. Cells which are cultured on these can differentiate even without the presence of other growth factors. So, these factors also to be considered while you are developing any tissue-engineered product.

If you are going to develop a reactor, you would want to provide these kinds of signals as well. So, the idea is, reactors for tissue engineering are slightly different from the reactors you would have studied as part of your bioprocess training.

These reactors are here to provide signals as well as maintain the controlled environment. In case of a bioprocess you want a controlled environment, and maybe do large scale production, but here this can also provide signals, and usually, the reactors are designed in a way that you can deliver desired signals; either it could be mechanical or electrical or whether its perfusion and so on. So, there are so many factors which should be looked at. We will talk about how reactors can be designed later in the semester.

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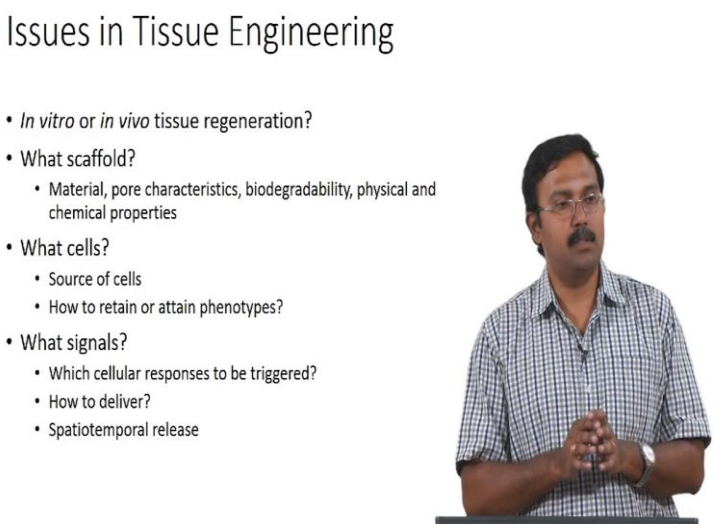

So, now that we have looked at the basic introduction. This is the summary of the issues in tissue engineering. When we talk about tissue engineering, the first question we need to have an answer is, do we want an in vitro tissue to be engineered or in vivo tissue regeneration right. There are two terms, tissue engineering, and regenerative medicine right. These are used interchangeably, but technically speaking, they are slightly different.

Tissue engineering is engineering tissue in vitro and then implanting it in the human body, whereas regenerative medicine is providing the support environment, which can help in regeneration of the tissue in vivo. So, that is the difference between the two; however, it is very commonly used in an interchangeable fashion. There are journals and

association which are just called tissue engineering and regenerative medicine because they kind of come together when it is discussed for research ok.

Once we know what we want to do there, then we need to identify how we take the three questions; the three arms of the triad like what scaffold to use, what cells to use and what signals do you want to deliver. So, what scaffolds? It is not just about what material you use; it is also about how you fabricate it, what should be the pore characteristics, what should be the surface characteristics, what should be the physical and mechanical properties and the chemical properties of it.

Whether it is biodegradable or if it should be degradable, then at what rate should be degradable because depending on the tissue, you are going to have different rates of the tissue regeneration itself, bone tissue can take maybe up to 16 to 18 weeks for it regenerate. So, I would not want my scaffold to degrade in 2 weeks whereas a skin or wound replacement might actually regenerate in 2 to 4 weeks. So, I would not want the material to be remaining for 6 months right. Depending on how I want to tailor the degradation, and I would have to look at how I would crosslink it, what type of materials I can use, there could be something like PCL which has a very slow degradation; you can use something like PEG which actually just gets dissolved in a much faster way.

Depending on all these things, we can actually tailor the material to provide these desired properties. And you can also functionalize these materials to get desired surface properties like hydrophilicity, hydrophobicity, and electrical properties and so on — all these things you can do with respect to the material.

So that you need to decide based on the tissue and based on what you can accomplish, you should not try to over-engineer stuff, in the sense that if we are trying to design something which is a very simple tissue, do not go for the most high-end technology just because it is readily available and accessible. Use something which would make scientific sense; look at what would be the best thing to do for this particular application and try to use that, and then the next thing is to identify cells.

Here, you could use primary cells of which are differentiated or stem cells and how to retain or attain phenotypes. Because while using stem cells, you need to differentiate them to get the desired phenotype. If you are looking at differentiated cells, then you need to make sure they maintain their phenotypes, because once you put them in a

different environment, they might dedifferentiate or redifferentiate and they can actually become something else.

So, there are always problems associated with those things as well, and the cells are not just that to maintain the phenotype, they should finally deliver with the functions as well right. It is not just about maintaining and expressing the receptors, and so on. It needs to make sure that it can do the functions at the final stage. Final aspects are to identify the signals as we discussed, there are like so many different molecules which you can work with, but you need to identify which cellular response you are targeting.

Whether you want bone regeneration or blood vessel formation or cell migration, depending on that, you first identify the molecules you want to work with and then how you deliver these molecules, whether you directly load them to some material using physical crosslinking or do you chemically crosslink, so that the cells can migrate towards it.

If you are going for a chemical crosslink, will the molecules still provide the same signals because it may not leach out? And the covalent bonds should not affect the molecule itself. So, there are so many factors which you have to account for when you do that, and the last aspect is the spatiotemporal release. So, this is a major challenge.

Your ECM actually tells the cells, how will they should behave, right through different molecules. Everybody knows VEGF does this in your body. VEGF is controlled in a way that it creates a healthy vessel. So, first VEGF acts and then PDGF acts and then you have other growth factors like angiopoietin acting to make sure that you get healthy blood vessels which are not leaky; whereas when we engineer this; we cannot actually control, we cannot load three different growth factors and control when which one gets released and then store the rest of it.

If I start releasing VEGF, even if I load 3 different growth factors, all of them are going to a come out at the same time. So, how do I then control, which comes out at first and then followed by the other one, and what is the trigger for the next thing to follow, right. How do I know that has been enough effect of VEGF and now it is time for PDGF to act, and if I know that there is enough effect of VEGF, how do I stop VEGF from releasing?

So, these are serious questions, and people try to address it. There are different things which people try to do; we will look at those things, we will share papers which you guys will be presenting at the end.

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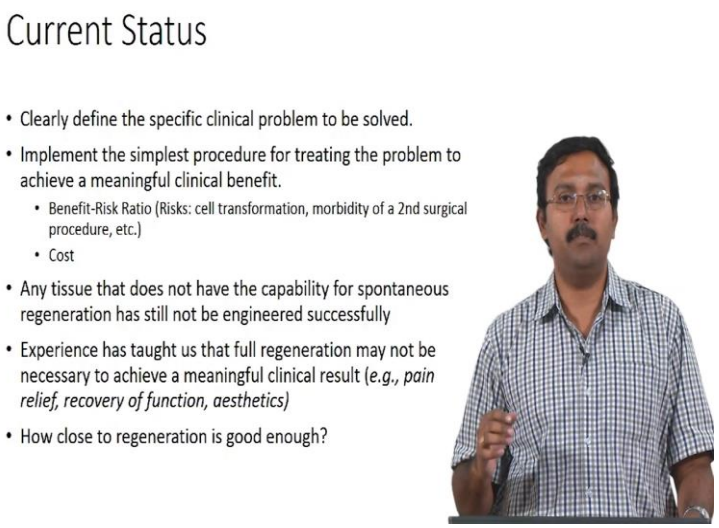

So, the current status of the field of tissue engineering has clearly defined the specific clinical problem to be solved. Implement; try to implement the simplest procedure to treat the problem to achieve the meaningful clinical benefit, do not go for the most advanced technology for something too trivial.

This is not the sensible things to do, ok. Because you need to look at cost and benefit-risk ratio. You need to make sure that the procedure should not have too much an adverse effect. Any tissue that does not have the capability for spontaneous regeneration has still not been engineered successfully.

That is one major challenge which we need to address, and experience has taught us that full regeneration may not be necessary to achieve meaningful clinical results. There could be like some aspects which would provide satisfactory results right, which is what use you start with. Most of the implants are not the best-case scenario, but you still use them because you know that gives you enough of a result for meaningful clinical improvements.

Because this is the case, you need to identify how close to good, is good enough, how much regeneration is required. You would have to address that and as you work on the field. So, this is what the overall state of tissue engineering can be given as and we need to focus on different applications; you see where exactly we stand, how exactly it can be taken forward.

Thank you, guys.