Drug Delivery Principles and Engineering
Prof. Rachit Agarwal
Department of BioSystems Science and Engineering
Indian Institute of Science, Bengaluru

Lecture – 31
Drug Delivery in Tissue Engineering-I

Hello everyone, welcome to another lecture for Drug Delivery Principles and Engineering. Let us talk about Tissue Engineering that as we have been discussing for the past few lectures.

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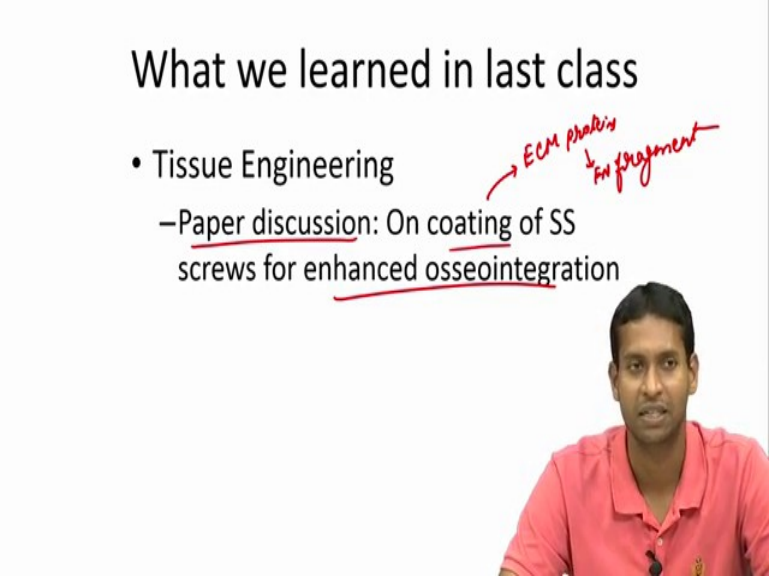

So, just a quick recap of what we learned in the last class. So, again as I said we are talking about tissue engineering in this particular module and in the last class we basically focused on a paper which had actually done coatings of ECM proteins.

So, in this particular case this was fibronectin fragment and what we showed there is, you can use protein adsorption to coat your protein of interest. And in this particular case, what the author showed is if you coat a particular protein which can bind to integrins alpha 5 beta 1, this fibronectin fragment will essentially start signalling through this alpha 5 beta 1 and will produce more bone on these surfaces. And then the authors further went ahead and used a rat model and showed that in a rat model this leads to enhancement in the osseointegration

So, such coatings can be then useful for treating osteoporotic patients if they get fracture. Because a bigger problem that they face is that once they are implanted with bone and bone screws, over a period of few months, these screws start to become loose and that causes a lot of pain and they cannot put weight on it.

So, they have to go back for the surgery and at that point they have also lost more bone because this screw needs to be drilled into the hole into the bone as well as the bone is resorbing around the plate and the screw. So, some of these strategies can be used. So, this is just one of the ways tissue engineering to support function can actually further enhance the function than when it was after the fracture.

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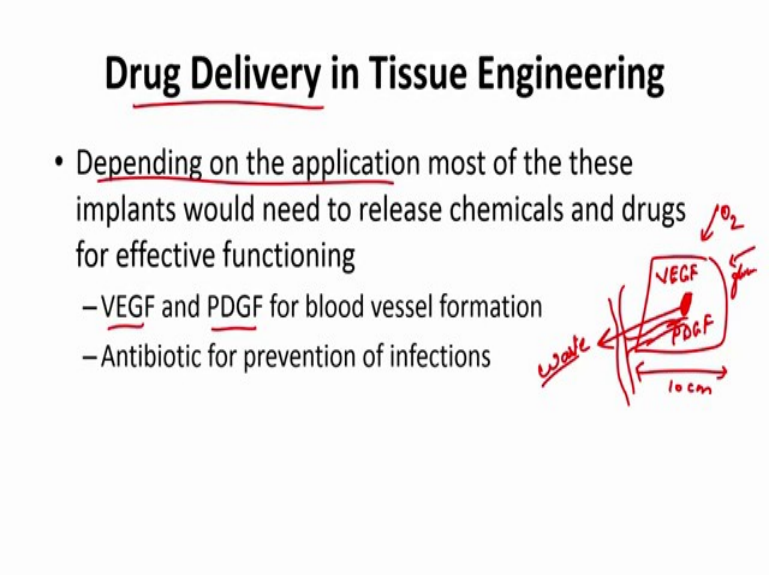

So, we will continue further on this discussion. Let us now focus, I mean this course is drug delivery, let us now focus more on drug delivery in tissue engineering. And what we are saying here is essentially figuring out why is drug delivery important in tissue engineering. So, having got some base in tissue engineering; we will move forward with this and say that it depends mostly on application. And this is something that you will find right throughout the course where most of the things will depend on what application you are using it for.

And then nearly all application of tissue engineering will require some sort of release of either chemicals or of the biologics, drugs, sometimes even cells. So, all of that becomes important in tissue engineering and that is why it is an integral part of a drug delivery

course. And so in the today's class and next couple of classes will learn how drug delivery is being used and how we can essentially modulate that to get better tissue response.

So, just to give some examples before we go further in depth. Protein such as VEGF and PDGF are required for blood vessel formation. So, let us say if I put an implant into the body and this implant is fairly thick, let us say this is 10 centimetres, then the tissues and the cells inside will not survive because the oxygen, the glucose as well as the waste transport is severely limited since there are no blood vessels. When I put this implant and let us say if there are cells in it, the cells have no way to survive unless they can get oxygen, they can get glucose, they can remove the waste from the surrounding.

So, what is the major problem? It is that there are no blood vessels and so what people have done in the literature is to encapsulate molecule such as VEGF and PDGF, which are growth factors and signals that causes the blood vessels to form. And so what can happen is let us say if I implanted this and there was a blood vessel going near this, it can induce neovascularisation, new blood vessels to form and penetrate this and essentially provide these nutrients to the cells in these implants; so that is important.

Another example is to use antibiotics to prevent infections. So, a lot of the time it is being seen; that if you have an implant, it can get infected. Since this is an external implant maybe at the time of surgery things were not pure.

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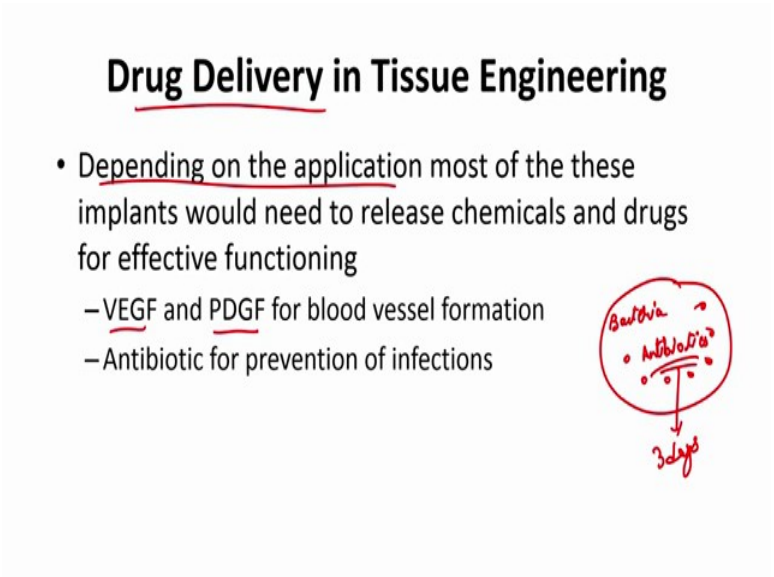

So, let us say if I have an implant which is susceptible to start having colonization of bacteria. So, what you can do to prevent that is, you can always encapsulate few antibiotics. So this is sort of a prevention based method where you have encapsulated antibiotics in an anticipation that maybe after the surgery the there might be few bacteria around. And this antibiotic does not need to release for a long duration maybe it is only going to release for let us say 3 days, but that might be enough to kill of the surrounding bacteria and ensure that the implant does not get infected.

Because again the implant does get infected and then instead of helping the person with any comfort, it is going to make the life worse with lot of inflammation, lot of pus formation and the healing will not happen. In fact, the tissue will get more damaged and the only solution then most of the times becomes is to remove the implant completely.

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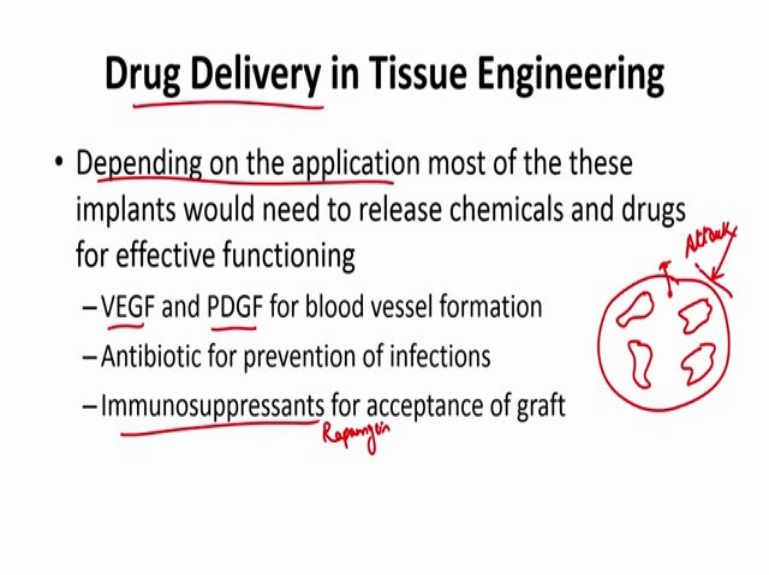

Lot of the times, as we talked about in the organ donation, you may be putting in something that is foreign. And if you do put in something that is foreign, again the immune system is going to recognize it.

Let us say if these contains cells that are derived from pigs or some other human for that matter, then my immune system, my antibodies, the T cells, B cells, they will recognize this as foreign and essentially they start to attack this implant. And of course, if they start doing that the implant cells will die and not only that, again it will cause a lot of

inflammation causing lot of sickness to the patient. And so, what is done here is pre-emptively you will release molecules that are anti inflammatory or immunosuppressants.

So, molecules like rapamycin are typically used for this or other drugs basically and these will come out. These make sure that this attack is blocked. And so that way the implant can survive longer. And again you may want this release of immunosuppressants to be over a period of quite a long duration, I mean as long as these cells are here you may want this immunosuppression to keep happening. And so that is why the drug delivery as well as the controlled release becomes important in this scenario.

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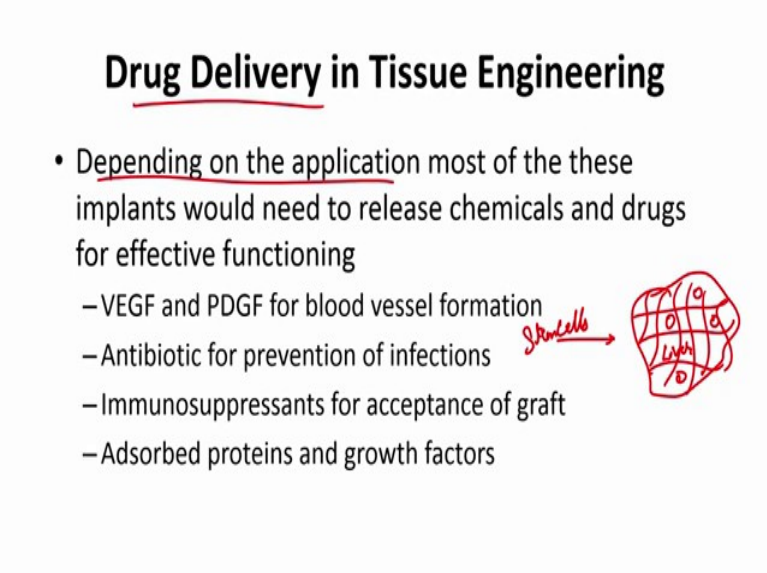

And then of course, as we discussed in few cases maybe sometimes you want the cells that are coming in to your implant to get signalling in a certain manner. So, that let us say this implant is to regenerate liver tissue; let us say you are putting an implant that contains matrix essentially.

And what you want is the cells to actually come in and use this matrix to sort of may build up the lost tissue. But then the cells alone may not be sufficient; the cells may require certain signals and at that point you may want to release some growth factors from here.

Let us say these growth factors cause the cells, these are stem cells let us say, and let us say these growth factors cause the stem cells to differentiate into liver cells. So, in that

case what we will find is these growth factors; if you slowly release them and ask the surrounding stem cells to come in to the liver, this will have a better therapy for your tissue engineering.

So, again these are just few cases I am giving, there are several of them. You can pretty much pick up any tissue engineering paper and you will find that there are release of molecules that is happening throughout this process or adsorbing proteins or absorbing factors, releasing drugs; all of this is it is a continuous part of tissue engineering and we will discuss some of these strategies as we go along.

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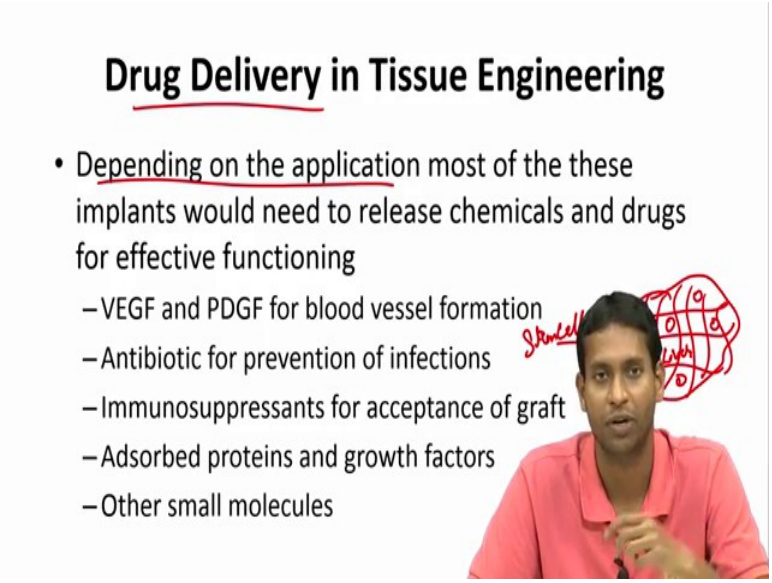

And then it is not limited to these molecules it could be any other molecule; could be a painkiller, it could be something else just depends on the application and what you are trying to cure.

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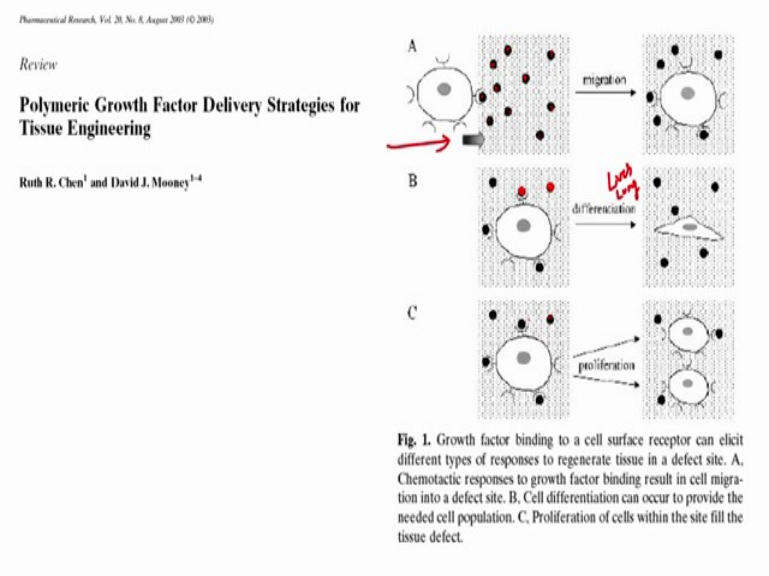

So, here is just a review paper talking about using polymeric growth factor delivery strategies for tissue engineering. So, what you are seeing here is essentially three different cases. In one case what you have done is very similar example what I just gave previously.

You want cells to migrate in and if you do want cells to migrate in you are basically causing a gradient of these black dots inside your scaffold; which then we will slowly come out and these cells get attracted by these black dots, let us say. And so these cells will come in and start to migrate into this implant because of the gradient of these molecules that you have encapsulated.

Another example here is maybe you want to use stem cells and want them to differentiate. So, again there could be drugs that are encapsulated in a matrix that will cause these cells to come in and then differentiate into the type of cells you want. Maybe if it is for liver or whether its for lung, just again depends on what is the organ that you are trying to treat and that will cause the tissue engineering applications. Or this could be just to increase the number of cells.

So, maybe you are putting in cells with the implant itself, but you want to increase the cell density so that more and more of them are there and the function is getting restored more and more; so that you can also do using these proliferative agents or these small drugs that will help in proliferation of the cell.

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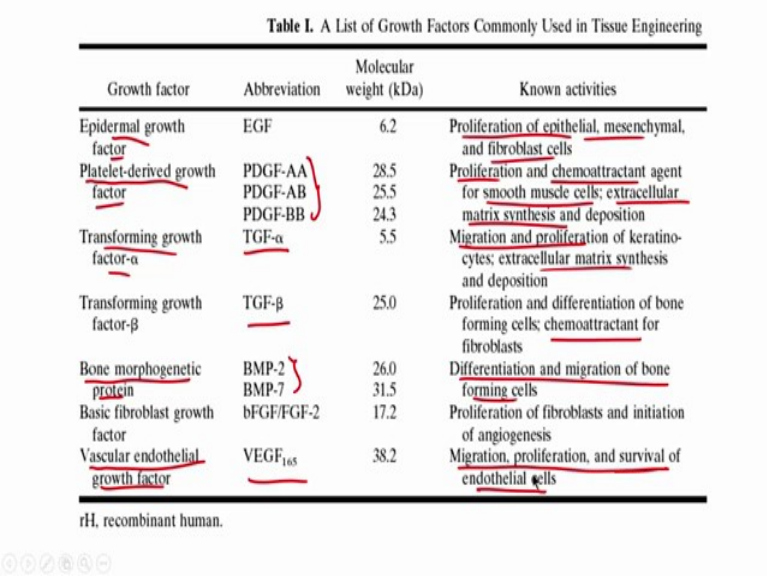

Table I. A List of Growth Factors Commonly Used in Tissue Engineering

Growth factor	Abbreviation	Molecular weight (kDa)	Known activities
Epidermal growth factor	EGF	6.2	Proliferation of epithelial, mesenchymal, and fibroblast cells
Platelet-derived growth factor	PDGF-AA	28.5	Proliferation and chemoattractant agent for smooth muscle cells; extracellular matrix synthesis and deposition
	PDGF-AB	25.5	
	PDGF-BB	24.3	
Transforming growth factor-α	TGF-α	5.5	Migration and proliferation of keratinocytes; extracellular matrix synthesis and deposition
Transforming growth factor-β	TGF-β	25.0	Proliferation and differentiation of bone forming cells; chemoattractant for fibroblasts
Bone morphogenetic protein	BMP-2	26.0	Differentiation and migration of bone forming cells
	BMP-7	31.5	
Basic fibroblast growth factor	bFGF/FGF-2	17.2	Proliferation of fibroblasts and initiation of angiogenesis
Vascular endothelial growth factor	$VEGF_{165}$	38.2	Migration, proliferation, and survival of endothelial cells

rH, recombinant human.

Here is a list of some of the growth factors that are commonly used, very widely used actually and growth factors in tissue engineering. And here are some of the major ones. One is EGF, Epidermal Growth Factor, again very widely used. Its major function is to cause proliferation of epithelial, mesenchymal and fibroblast cells. So, in the previous case it was an example c that we saw that you want these cells to proliferate and may be due to some accident you may be lost 20 percent of the cells.

So, in that case you may want to deliver EGF in a scaffold in that area so that not only the cells do migrate in, but you still have to make up for the 20 percent lost cells and so that way they can start to differentiate and proliferate. Then we talked about the PDGF; the Platelet Derived Growth Factor. And there are three types of them and as I told you before that this is to mature the blood vessels.

And so, what you get is you get proliferation as well as chemoattraction of smooth muscle cells which are essentially the cells that surround the blood vessels. And that causes the maturation to happen. They also cause ECM synthesis and deposition. So, if you want the cell environment to improve, you want more ECM to be there some of these growth factors are used.

Another one is Transforming Growth Factor alpha; so also known as TGF alpha and this is used for migration and proliferation of the cells again, very similar to EGF and also extracellular matrix synthesis. Then there are several of them then there is TGF beta also

acts as chemoattractant, you have BMP; one of the most important proteins in a body and have several applications in different types of cells. They called bone morphogenetic, but essentially they have applications and other cells as well, but essentially differentiation migration of the bone forming cells. Then you have VEGF, we talked about, again very widely used, VEGF is vascular endothelial growth factor. And that will cause the migration, proliferation, survival of endothelial cells which are the cells that line up blood vessels.

So, if you want the new vessels to grow in; you want to have some VEGF in that area and that will attract these endothelial cells, they will cause the migration, they will proliferate as well as they will survive and form new blood vessels at those sites. Again, so you do not really have to remember the functions for the part of this course. But it is still good to know because lot of these you will read in papers and lot of these will be maybe you will use in your own research. So, this is something just for your information.

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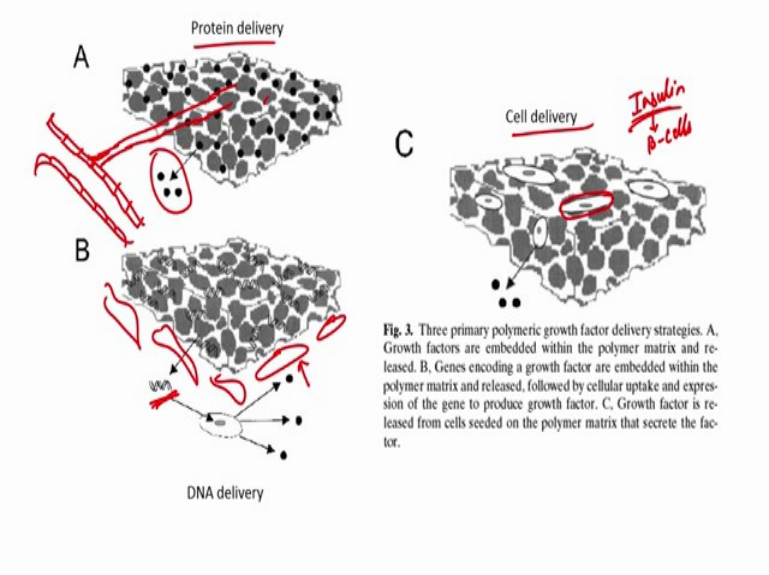

Fig. 3. Three primary polymeric growth factor delivery strategies. A, Growth factors are embedded within the polymer matrix and released. B, Genes encoding a growth factor are embedded within the polymer matrix and released, followed by cellular uptake and expression of the gene to produce growth factor. C, Growth factor is released from cells seeded on the polymer matrix that secrete the factor.

So, again using a scaffold you can primarily deliver three things and again not only these things you can deliver some of that is small molecules as well, but majorly you are looking at protein delivery. So, let us say if I am delivering some VEGF or some PDGF to cause the blood vessels to form in these scaffolds. So, then I can use protein delivery, I can release let us say VEGF; it is going to go and signal on these endothelial cells that are lining the blood vessels and essentially it will cause sprouting of new blood vessels.

So, these cells will now get attracted and they'll want to move in this area. So, they will start forming a sprout, that is now going into this and further proliferating as it goes. And that way you can have good oxygen and glucose presence within the scaffold as well. The other thing you can do is, you can instead of delivering a protein; you can deliver the DNA that codes for that protein.

And that DNA slowly releases out whatever cells are in the vicinity take up this DNA and then they produce the protein of interest. So, that way you can have more sustained release because the DNA is going to be there for a lot longer duration. Because once the cell gets transfected maybe it remains transfected with that DNA for a period of over days to months and that way you can have release over a period of months. Or the third example here is a cell delivery where essentially you can just have cells encapsulated in them. And these cells may be performing certain function maybe you if you are lacking insulin.

So, you can produce insulin through these cells; through pancreatic beta cells or if let us say you are lacking a certain enzyme, these cells are known to produce those enzymes, you can encapsulate those cells. And that way they remain in the site where you want these proteins to be present; as well as they are happy because they are in a matrix surrounded by them.

Sometimes you want to protect them from the immune system, also for that use matrix as well. And we will discuss some of these cases through this course as we go along.

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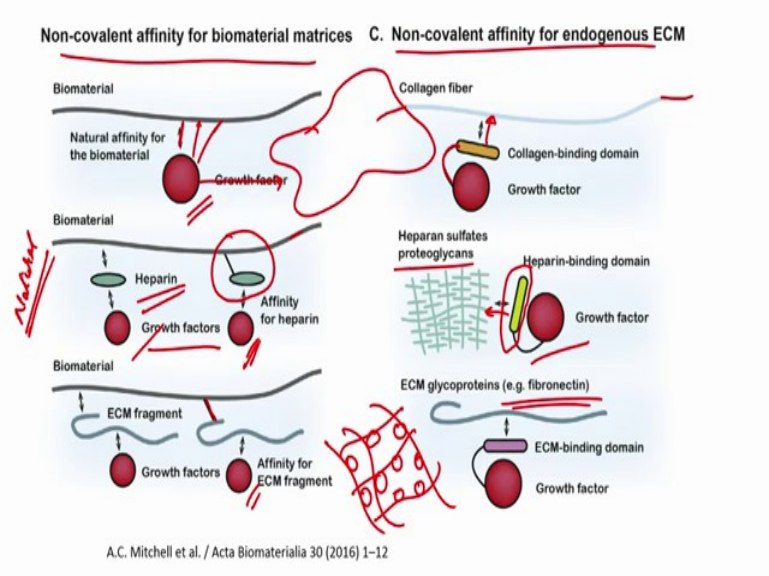

So, here are some more sort of zoomed in images of a matrix. So, let us talk about non covalent affinity first for biomaterial matrices. So, you have a biomaterial, this could have a natural affinity for growth factor. That means that the growth factors will itself go and interact with it and if I make the biomaterial completely out of these chains; then what will happen is these growth factors are since they are interacting; they are binding to these chains with some affinity. And then they will slowly release in the media as the time goes on or as the material degrades itself.

The other case you can take is you can use a molecule called heparin. So, this is derived from the literature where you will find that heparin has quite a lot of affinity for growth factors and it has several binding sites to these different kinds of growth factors. So, what you can do is; you can conjugate your heparin to your polymeric chains and what that will do is that will create an affinity for growth factors by itself. So, you do not really have to cross link the growth factor and that way you can essentially ensure that these growth factors are there.

So, important point to note here is that the natural ECM, so if you take natural growth factor, they have all these binding sites for heparin and your growth factors. So, those can also be used, but this is if you want a certain class of polymer for its particular property; you can still modify these polymers to be able to release these growth factors.

And obviously, you can always directly conjugate it as well it; there is no problem with that. And then the other thing you can do is you can use some of these ECM fragments themselves; that I just said has natural affinity for these growth factors, you can conjugate the ECM fragment and then they will automatically start to bind your growth factors.

So, that is one way to sort of get your things delivered as well as bound; what it ensures is that in none of these cases, you are actually covalently binding a growth factor to your polymeric chain. And that would ensure that your growth factors are not losing their activity because if you covalently link the molecule; then those molecules will tend to have lower activity compared to the non covalently linked.

And then there are other strategies is to use non covalent affinity for endogenous ECM. So, in this case this is not a problem at all. So, you have a natural material called; let us say here for example, collagen and what you can do is you can bind your growth factor with a collagen binding domain. So, this domain since its collagen binding it is going to go bind you the collagen chains; essentially linking your growth factor to your chains.

The other example is heparan sulphate; so the same thing you can do is you can again have binding affinity conjugated to your growth factor with the heparin and then the heparin binding domain will go and bind to your heparin matrices. And again very similar to this you can make your ECM or incorporate other ECM components into your things and you can again put your ECM binding domain.

So, these non covalent affinity are actually very important because see what happens is; let us say if a cell comes and the cell wants to take this growth factor up. So, let us say there is a large cell that has binding site for this growth factor. If these growth factors are covalently linked to your material then the cells cannot really take them up because the cells will try to take them up because, but the material is huge compared to the cell size.

So, the cells are not able to take this. In this case what you are doing is if the cell affinity is higher than this natural affinity or any of these affinities. Then the cells can come they can take up the growth factors at the rate that they want to dig this up and that will ensure that the cells have lot more control which typically always leads to better healing.

So, that is some of the advantages here for using non covalent affinity. Obviously, you can always do the covalent affinity and bank on the polymer to degrade or maybe it is just a cell surface receptor the cell does not need to internalize it. In those cases those systems will also work, but typically what has been seen with the research is these systems work a lot better than the covalent binding ones.

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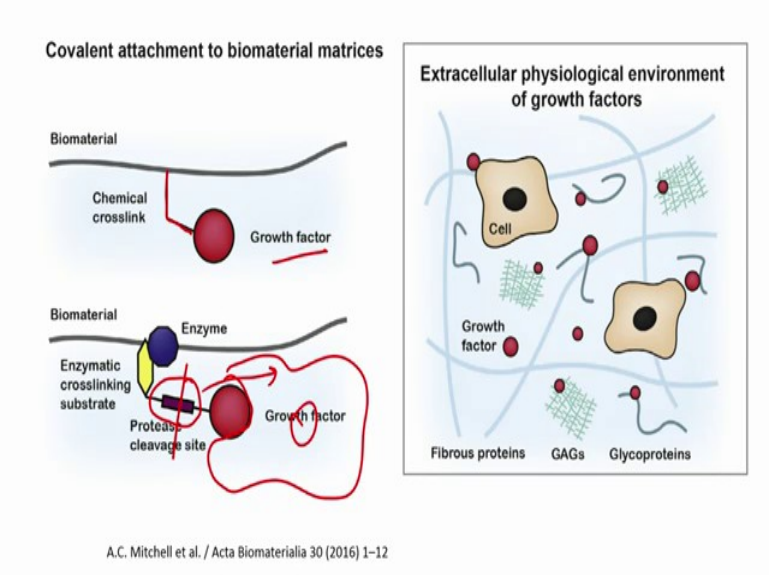

And again as we were just discussing; so you can have covalent binding; so you can actually take a chemical moiety and cross link your growth factor or you can have it linked such that there is a protease cleavage site. So, what that does is even though its covalently linked there is a cleavage site; the cells can cleave the cells all have proteases both secreted as well as on the membranes. So, if you do that then these will essentially be cleaved and the cells can come and bind to this growth factor; whenever they need to and take it up.

So, here is a typical example of how this looks like; so an extracellular physiological environment of growth factors. So, you can have growth factors linked either to your chains or just encapsulated, the cells will come in; they will interact with your ECM as well as these growth factors and essentially that can help them perform or enhance their function in quite a good manner.

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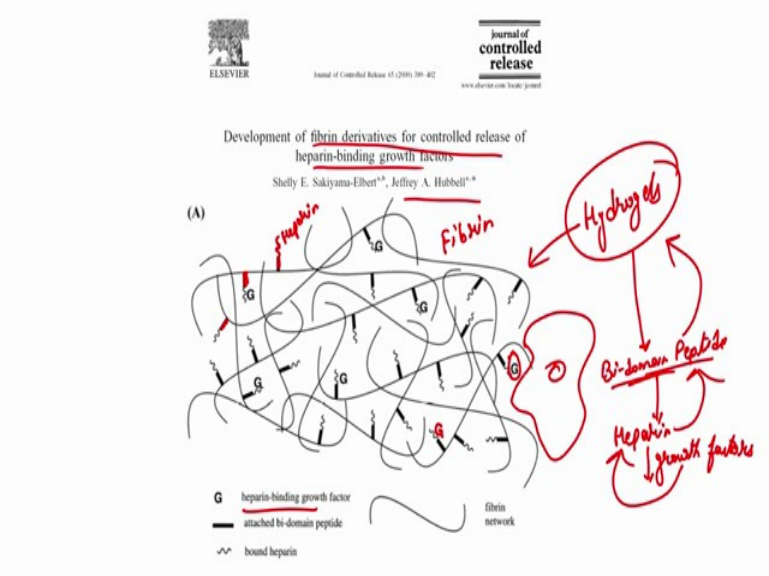

Here is another example of this; this is a group Jeffrey Hubbell's, group where they have developed fibrin derivatives for control release of heparin binding growth factors. And so what these authors have done is they have made gels or you can also call them hydro gels since fibrin derivatives are fairly hydrophilic.

So, they have made these hydrogels and in that what they have done; they have attached some peptides. So, this is a bi domain peptide where one domain binds to your fibrin and another domain binds to let us say heparin. So, if it is a bi domain one domain binds to this, one domains binds to the heparin, and so this is your heparin.

So, all you have to do is you have to make the hydrogel; you have to link your bi domain peptide. Then all you have to do is just incubate this with heparin and once you incubate this what will happen is first of all this bi domain peptide will go and bind your hydro gel chains; which are made out of fibrin.

And once you put the heparin there you can wash it in the middle if you want, if you have putting this in excess. This heparin is going to go and bind to this bi domain peptide. And then all you have to do is just put your growth factors and as I described in the previous slide that heparin is fairly promiscuous in binding lots and lots of different types of growth factors and those will automatically go and bind. So, essentially this G here represents the growth factor which binds to heparin and there are several of them so,

that way you can achieve; so again this G is now available right I mean if a cell comes and it wants to interact with this growth factor.

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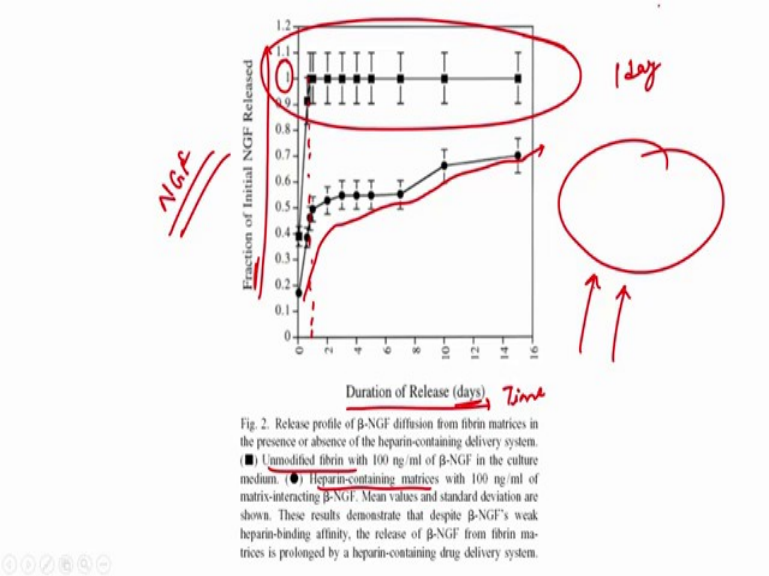

Fig. 2. Release profile of β-NGF diffusion from fibrin matrices in the presence or absence of the heparin-containing delivery system. (■) Unmodified fibrin with 100 ng/ml of β-NGF in the culture medium. (●) Heparin-containing matrices with 100 ng/ml of matrix-interacting β-NGF. Mean values and standard deviation are shown. These results demonstrate that despite β-NGF's weak heparin-binding affinity, the release of β-NGF from fibrin matrices is prolonged by a heparin-containing drug delivery system.

All it has to do is just have more affinity and compared to the heparin which typically most surface receptors on the cells do. And so that way it will be able to take up this growth factor. So, here is some example; so what you are seeing here and this is a gel that is being formed and on the x axis, you have time which is in days. And on the y axis you have how much of the growth factor in this case a growth factor which is used is NGF is being released and essentially in fraction. So, fraction of one basically means all of the growth factor was released.

So, if you have unmodified fibrin which is not conjugated to your bi domain peptide and the heparin what you see is pretty much within less than a day, almost all of it is released. Whereas, if you put in a heparin containing matrix, since we use the bi domain and bind it to the heparin and then put the growth factor in there, you see more continuous release and some of it is has not been released even at the end of 2 weeks, but the cells can of course, come in and take whatever is remaining

So, such systems will give you a lot more sustained effect, more and more cells will continue to move into your hydrogel. Because they are continuously sensing this growth factor being released from it; whereas, in this case the cells will move in for 1 day. But

once that is over the cells have really no incentive to move in or even stay there; so that is one example.

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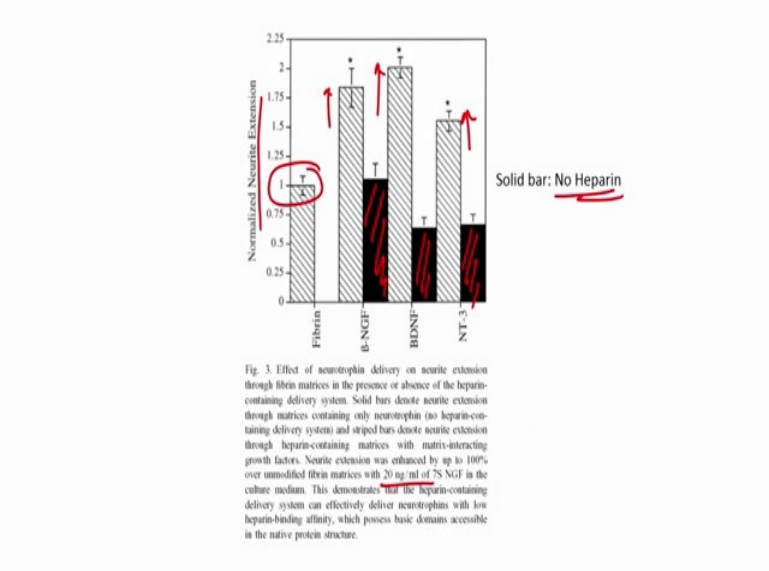

Fig. 3. Effect of neurotrophin delivery on neurite extension through fibrin matrices in the presence or absence of the heparin-containing delivery system. Solid bars denote neurite extension through matrices containing only neurotrophin (no heparin-containing delivery system) and striped bars denote neurite extension through heparin-containing matrices with matrix-interacting growth factors. Neurite extension was enhanced by up to 100% over unmodified fibrin matrices with 20 ng/ml of 7S NGF in the culture medium. This demonstrates that the heparin-containing delivery system can effectively deliver neurotrophins with low heparin-binding affinity, which possess basic domains accessible in the native protein structure.

And then further the authors went ahead and showed, so these gels then they started using I mean NGF is a Neural Growth Factor. So, these cells and they used for a neurite extension experiment; where if they only have fibrin, as its being shown here, they get a certain extension which they have normalized to 1. But then as you put these growth factors and use the system, you see a lot more pronounced effect you can start seeing; so solid bar is essentially no heparin.

So, you see that if you have no heparin and you are releasing growth factors; you do not really get much response as was fairly clear that these growth factors are getting released within a day also. But if you do put your heparin in there you see a lot more extension of these neuronic cells just because there is a sustained release of growth factor over time.

So, essentially that is what is described here the concentration they used here was 20 nanogram. But again this is more a conceptual thing, if you have a continuous release using some heparin or continuous retention of this growth factor, the cells are liking it much mor. I think we will stop here and we will continue further in the next class; see you then.

Thank you.