Drug Delivery Principles and Engineering
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Lecture – 32
Drug Delivery in Tissue Engineering-II

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles. I am Rachit and we are talking about some of the tissue engineering strategies and how drug delivery is involved in some of these strategies. So, just a quick recap of what we learned in the last class.

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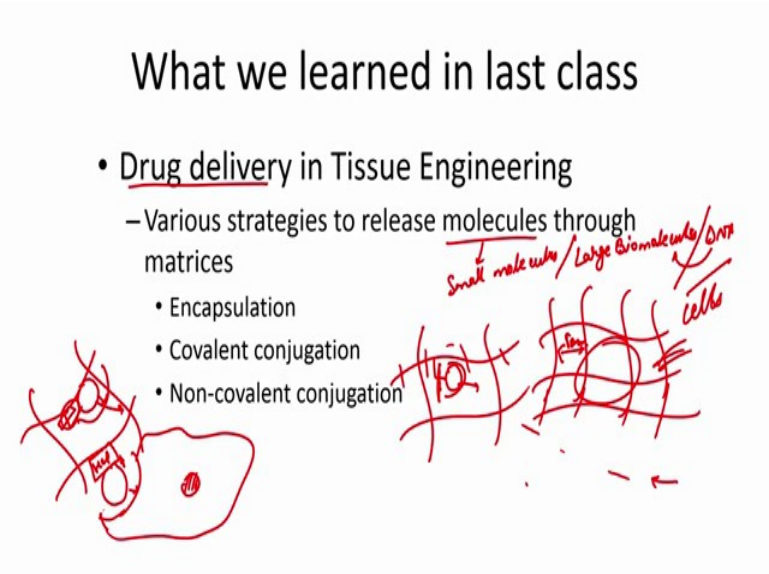

So, what we learned is essentially what are the drug delivery aspects in tissue engineering. So, before last class we were talking about tissue engineering and giving some basic concept. Now, we are moving towards learning how drug delivery is used for tissue engineering. And so in that, what we discussed is first of all various strategies to release molecules through matrices and these molecules can be small molecules, these could be large bio-molecules and then these could be even DNA which is essentially a part of large bio molecule or this could be even cells.

So, just basically depends on what is the application, but you can use various strategies to release all kinds of things from your matrices for tissue engineering application. And so, we talked about one simple thing is you can use encapsulation. So, you essentially

use size and, we have talked about this several times in the hydrogel class, in other polymer matrices class is; let us say you have a mesh network which has a pore size of 5 nanometre.

And if your drug itself is 10 nanometre, then it is going to be staying there and you can slowly degrade these matrices to release this drug over time. The other is the covalent conjugation. So, mostly we discussed this in terms of growth factors. So, let us say, if I have a polymer matrix chain and I want the growth factors to be there for quite a bit of time, what I can do is, I can link the growth factors.

So, let us say this is growth factor to my polymer chain through some covalent bond. One of the disadvantages here is that if the cells want to internalize this or the cells want to see a gradient of this coming through, it will be hard. So, to alleviate that concern what you can do is, you can have these polymers chains, which are degradable. So, if these are degradable, what you will have is, let us say if this is a matrix zoomed out.

So, as you go further away from it, you will have less and less growth factor being there, because the growth factors can be released and going into a sink in the system. So, that will give a gradient for the cells to come in. So, that is one way, but what if the cells really want to come in and also still feel the growth factor there then, what you can also do is you can have something which is a bond that cells can cleave.

So, if that is the case, then cells will bind to it they can then cleave this bond and essentially use this growth factor to then either differentiate or whatever they are planning to do. And then finally, we talked about non-covalent conjugation. Quite successful for such kinds of applications here and. So, all its saying is rather than having and the cells to do all the work here, what you can do is you can sort of immobilize your a growth factors with some affinity. So, the growth factors can have direct affinity to your polymer chains.

And so, in that way the growth factor will stick to the polymer chain with some affinity and when the cell comes in, it may bind with the larger affinity and take this growth factor away. The other option you can do is, you can bind the growth factor to a molecule that has affinity to your ECM. And so, in that way then the growth factor, which is bound to this molecule will attach to ECM and the cells can still pull it away along with this molecule that may decrease some of the activity of the growth factor.

But, it does still be accessible or what you can do is, you can bind heparin to your ECM chains and then, if you put growth factor heparin as we talked in the last class as quite a bit of affinity for lots and lots of different growth factors. So, in that way, the cells will essentially be able to interact with this growth factor and be able to even take it away, if they need to because this will have a higher affinity than the heparin growth factor affinity.

So, these are some of the strategies we discussed; we again discussed some examples used in the literature. And so, how these systems can result in lot better improvement in cell function and than let us say a covalently conjugated or just encapsulated system.

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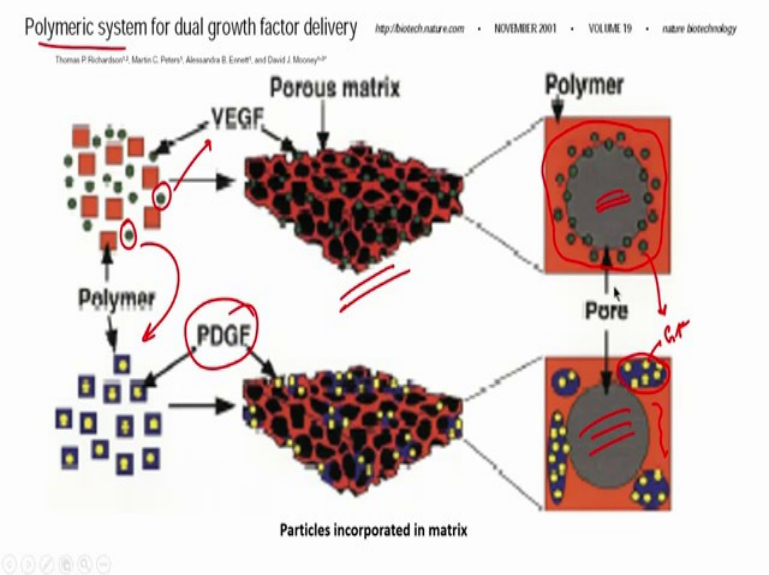

So, let us continue our discussion further. So, here is another example again from the literature, we will continue to give lots and lots of examples. So, that you guys are getting accustomed to some of the literature as well and learn their application.

So, what is being done here is they have used a polymeric system and they have used dual growth factor delivery. So, in this case what they have done is, they have created a porous matrix and in that porous a matrix what they have done is, they have either encapsulated their VEGF which is a growth factor they are using directly into the matrix. And, alternative to that what they have also done is, they have encapsulated this VEGF into a polymer and then, they have used a PGDF which was directly encapsulated here.

So, in such a system what do you typically see and then; obviously, there are lots of pores around. So, what do you typically see is this - essentially you have a system which has pores and the polymer around it contains the growth factor that you are trying to deliver, whereas, in this case you have pores, you have polymer around it and that polymer is encapsulating another polymer that contains your growth factor. So, these are the two systems that they have created.

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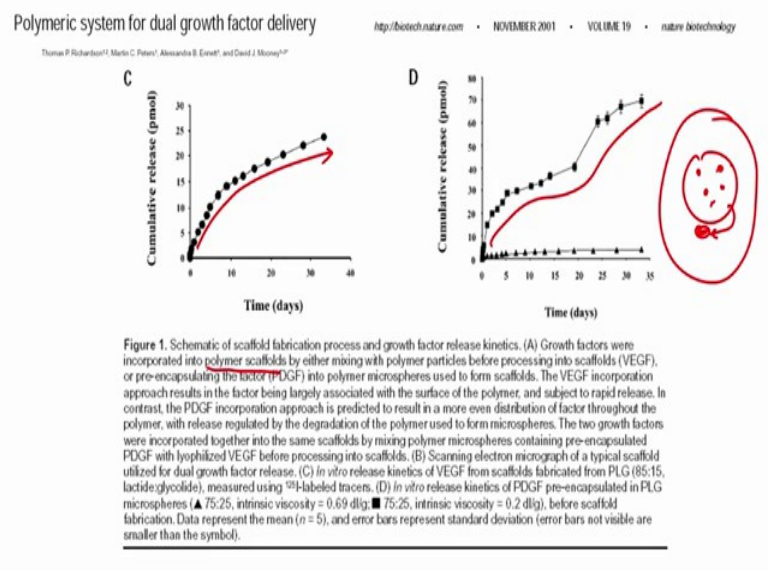

Figure 1. Schematic of scaffold fabrication process and growth factor release kinetics. (A) Growth factors were incorporated into polymer scaffolds by either mixing with polymer particles before processing into scaffolds (VEGF), or pre-encapsulating the factor (PDGF) into polymer microspheres used to form scaffolds. The VEGF incorporation approach results in the factor being largely associated with the surface of the polymer, and subject to rapid release. In contrast, the PDGF incorporation approach is predicted to result in a more even distribution of factor throughout the polymer, with release regulated by the degradation of the polymer used to form microspheres. The two growth factors were incorporated together into the same scaffolds by mixing polymer microspheres containing pre-encapsulated PDGF with lyophilized VEGF before processing into scaffolds. (B) Scanning electron micrograph of a typical scaffold utilized for dual growth factor release. (C) *In vitro* release kinetics of VEGF from scaffolds fabricated from PLG (85:15, lactide:glycolide), measured using ^{125}I-labeled tracers. (D) *In vitro* release kinetics of PDGF pre-encapsulated in PLG microspheres (▲ 75:25, intrinsic viscosity = 0.69 dl/g; ■ 75:25, intrinsic viscosity = 0.2 dl/g), before scaffold fabrication. Data represent the mean (n = 5), and error bars represent standard deviation (error bars not visible are smaller than the symbol).

And let us see what they have done with this? So, in this case, now they are doing a cumulative release from this polymer of that growth factor. So, what they are seeing is; if you encapsulate the growth factors into the polymer scaffold. So, you get a certain type of release. So, here you are seeing that as the polymer is degrading or the molecules are diffusing, you get a certain release whereas, in the other case you can where in the case D, what they have done is they have pre-encapsulated this growth factor into another polymer.

So, this is the same case as we see here. There is another polymer which is encapsulating these growth factors and when they have done that then this whole polymer then gets encapsulated into another polymer which contains pores through which these can diffuse out. So, now the growth factor first has to wait for this matrix to degrade and then they can start to diffuse out. So, you see a much longer release and sort of a biphasic release

in this particular example. So, this is just some of the ways these authors have tried to change the release of their molecule through these matrices.

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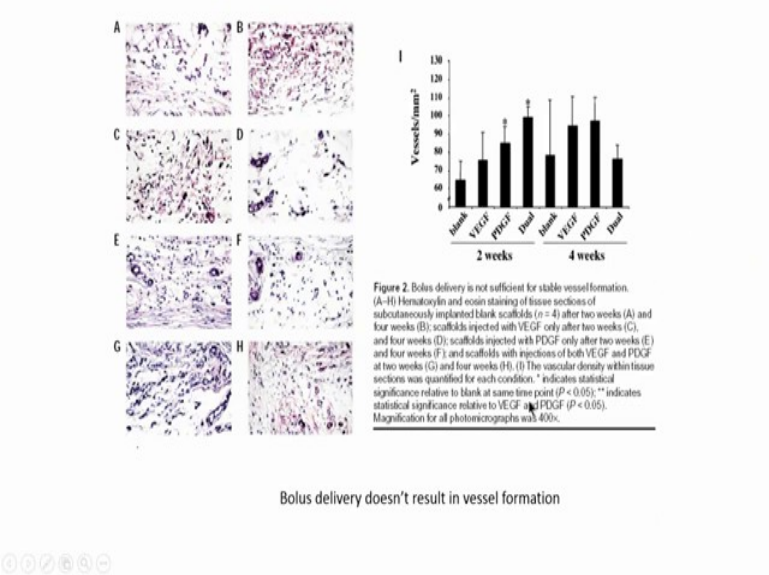

And so, here is just another example. So, what they have done is in this case, they have done bolus delivery. So, essentially no matrix is used and they are trying to see whether they can generate some blood vessels and they use (Refer Time: 08:21) some staining to see, if there are any blood vessels being generated. So, what they are looking at here is an implant and seeing how many blood vessels have grown into that implant.

So, what they see, if they just have a blank implant, there is a certain amount of blood vessel density that you find; if they give a bolus injection of VEGF or PDGF, which are again two of the growth factors that we said are involved, they get certain response. So that, response goes up from 65 to let us say at max up to 100 and this is both at 2 weeks and 4 weeks.

So, it is still a marginal improvement, but does not really result in a whole lot of blood vessels being formed, which is also pictorially you can cannot really see many blood vessels I mean this is a blood vessel here, but not a whole lot of density.

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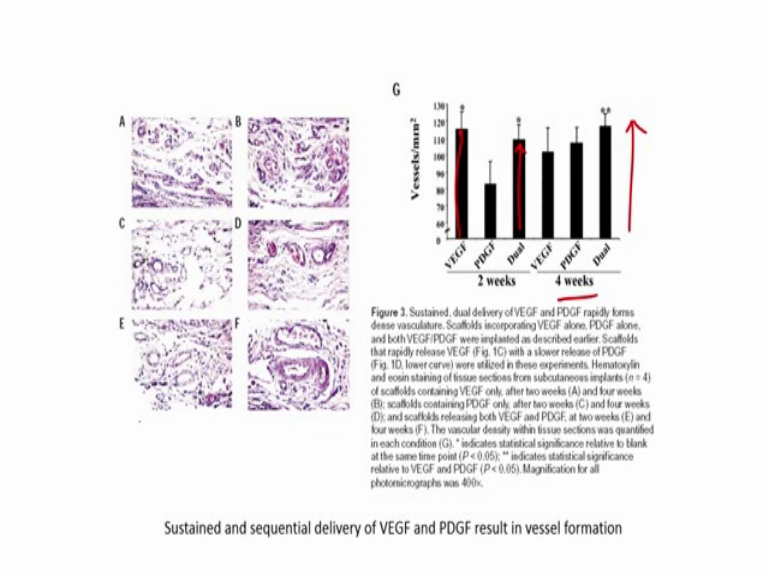

Whereas if you do a sustained release, so, remember how you are getting these values to be about 70-80. Where in this case now what they have done is they have sustained and a sequential delivery is being done.

So, what they see now is if they only lives one it goes up to a certain value in 2 weeks and if they do dual it also go again goes up to certain value and then at 4 weeks they also get good enhancement with both these deliveries. So, again the numbers are much higher. So, it is a better system rather than just giving a bolus injection; so, just one way of how you can cause the blood vessels to grow into your tissue.

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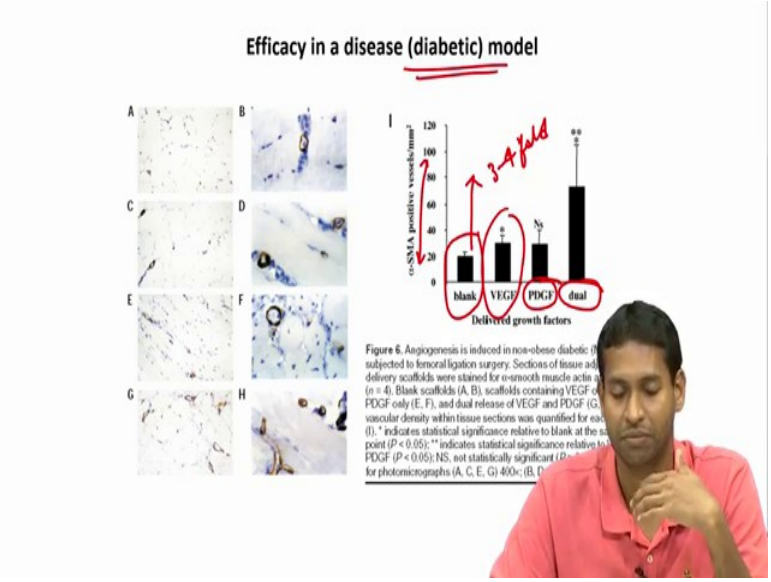

And then they further went ahead in a disease models. So, again in disease case the body's intrinsic capacity to heal is much lower. So, this is a diabetic model that they have used and what they see again is if they use only a matrix they get very little vessel cells. But, if they start releasing VEGF they get certain enhancement, but still fairly marginal with PDGF they do not really see much enhancement, but if they release both of them they see quite a long jump; almost 3 to 4 fold, higher number of vessel cells being in the vicinity.

So, this is again showing that if you do a sustained release you give the enzymes and cells enough time to experience the growth factor in the matrix and move towards it; you get a much better response. Say, in compared to the bolus delivery, what will happen if you do a bolus delivery? These growth factors are small they will quickly clear away from the body.

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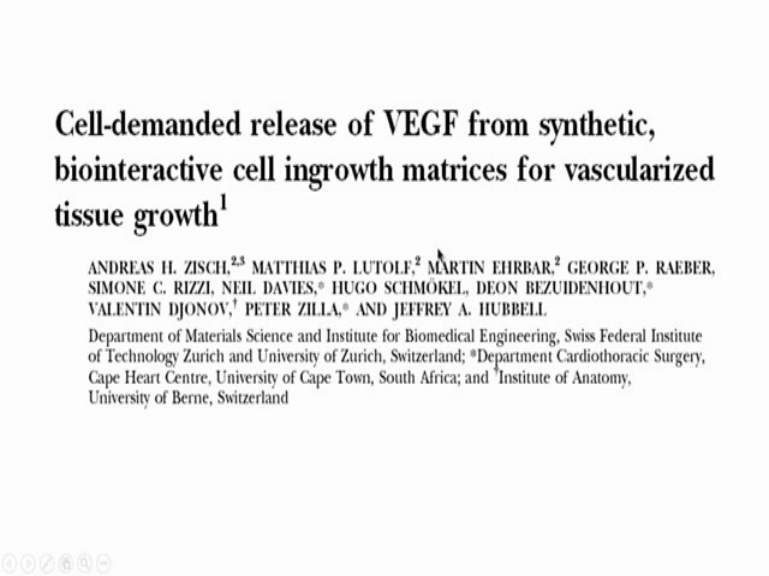

Cell-demanded release of VEGF from synthetic, biointeractive cell ingrowth matrices for vascularized tissue growth[1]

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So, here is another paper that talked about cell demanded release of VEGF from synthetic bio interactive cell in growth matrices.

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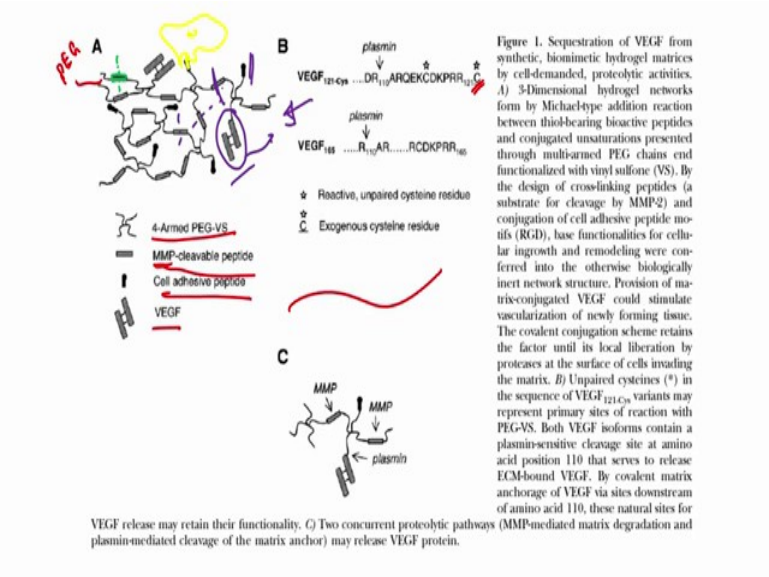

Figure 1. Sequestration of VEGF from synthetic, biomimetic hydrogel matrices by cell-demanded, proteolytic activities. *A)* 3-Dimensional hydrogel networks form by Michael-type addition reaction between thiol-bearing bioactive peptides and conjugated unsaturations presented through multi-armed PEG chains end functionalized with vinyl sulfone (VS). By the design of cross-linking peptides (a substrate for cleavage by MMP-2) and conjugation of cell adhesive peptide motifs (RGD), base functionalities for cellular ingrowth and remodeling were conferred into the otherwise biologically inert network structure. Provision of matrix-conjugated VEGF could stimulate vascularization of newly forming tissue. The covalent conjugation scheme retains the factor until its local liberation by proteases at the surface of cells invading the matrix. *B)* Unpaired cysteines (*) in the sequence of $VEGF_{121\text{-}Cys}$ variants may represent primary sites of reaction with PEG-VS. Both VEGF isoforms contain a plasmin-sensitive cleavage site at amino acid position 110 that serves to release ECM-bound VEGF. By covalent matrix anchorage of VEGF via sites downstream of amino acid 110, these natural sites for VEGF release may retain their functionality. *C)* Two concurrent proteolytic pathways (MMP-mediated matrix degradation and plasmin-mediated cleavage of the matrix anchor) may release VEGF protein.

And so again what they have done here is this is a synthetic polymer that they have used. So, they have used a 4 armed PEG vinyl sulfone. So, what this does is this will cross link any peptide which contains a cysteine or any thioles. So, they have created a matrix out of that and in that matrix what they have also done is they have incorporated peptides which are first of all protease cleavable (MMPs) and they have also used peptides that

are cell adhesive and finally, they have incorporated VEGF which also has a cysteine. So, that also gets bond to the network.

So, this is what you see here. So, in the system you are seeing. So, this is a polymeric chain, so the PEG chain. This right here is the cleavable site. So, the cells can actually break it. This is a cell adhesive peptide. So, the cells can actually bind to it and then finally, this right here is the VEGF which this cells can come and interact with as well as the VEGF can release over time as more and more of these bonds are cleaved and cause more cells to migrate in.

So, this is basically the whole system that they have designed here; very mimicking of what you see in the tissue where the cells can move around, the cells have growth factors to bind to, the cells have adhesive ligand through which they can attach to the surface as well as they can if they want they can cleave the surface and further move around.

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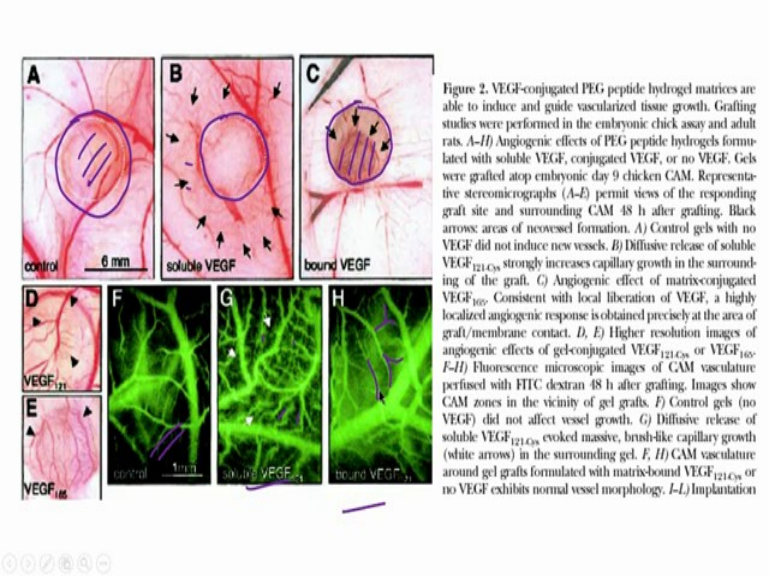

Figure 2. VEGF-conjugated PEG peptide hydrogel matrices are able to induce and guide vascularized tissue growth. Grafting studies were performed in the embryonic chick assay and adult rats. *A–H)* Angiogenic effects of PEG peptide hydrogels formulated with soluble VEGF, conjugated VEGF, or no VEGF. Gels were grafted atop embryonic day 9 chicken CAM. Representative stereomicrographs (*A–E*) permit views of the responding graft site and surrounding CAM 48 h after grafting. Black arrows: areas of neovessel formation. *A)* Control gels with no VEGF did not induce new vessels. *B)* Diffusive release of soluble $VEGF_{121\text{-}Cys}$ strongly increases capillary growth in the surrounding of the graft. *C)* Angiogenic effect of matrix-conjugated $VEGF_{165}$. Consistent with local liberation of VEGF, a highly localized angiogenic response is obtained precisely at the area of graft/membrane contact. *D, E)* Higher resolution images of angiogenic effects of gel-conjugated $VEGF_{121\text{-}Cys}$ or $VEGF_{165}$. *F–H)* Fluorescence microscopic images of CAM vasculature perfused with FITC dextran 48 h after grafting. Images show CAM zones in the vicinity of gel grafts. *F)* Control gels (no VEGF) did not affect vessel growth. *G)* Diffusive release of soluble $VEGF_{121\text{-}Cys}$ evoked massive, brush-like capillary growth (white arrows) in the surrounding gel. *F, H)* CAM vasculature around gel grafts formulated with matrix-bound $VEGF_{121\text{-}Cys}$ or no VEGF exhibits normal vessel morphology. *I–L)* Implantation

And so, they went ahead with the system and what they see here is this is a control gel. So, this no VEGF here and they see a certain density of the blood vessels in this site. So, this is where the implant was put in. Then they have put in the implant and given a soluble VEGF in the in the implant and what they see is some of the blood vessels have started to grow in, but very little because obviously this VEGF will get cleared very quickly. And, then what they have done is the method that we described in the previous slide where they have essentially put the VEGF into the implant and what you start

seeing is quite a lot of blood vessels in the implant suggesting that this strategy is much better and this is again pictorially represented.

So, this is your control zoomed in image in this area and so you see certain density of blood vessel, this increases with soluble VEGF. So, you start seeing these small branches coming out from some of these larger vessels. In the bound VEGF we actually see these branches being fairly mature and in a much higher number then compared to the soluble VEGF. In fact, the whole area looks quite of green, green is the staining for the blood cells.

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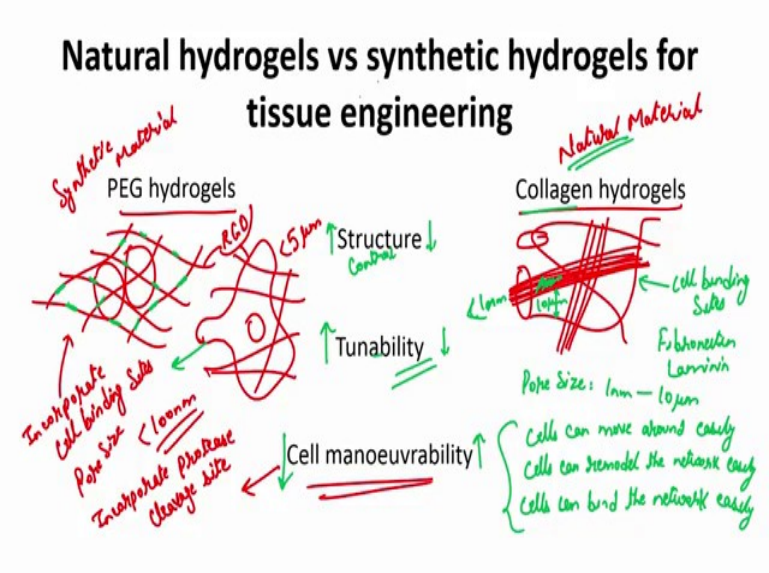

So, one other thing I want to quickly touch upon is some of the differences between natural hydrogels and synthetic hydrogels for tissue engineering. Again, we have talked quite a bit about both of these and you guys have seen different examples of them, but I also want to talk about further as to what can be done with these natural and synthetic hydrogels and what to use in what case and how do cells interact with them.

So, let us see. So, in this case I will take two examples; one is a PEG hydrogel, where we are looking at a synthetic material and another is the collagen hydrogel which in this case is a natural material, collagen is of course, one of the most abundant protein in our body. So, how do these hydrogels differ?

So, let us see. Let us talk about the structure in general. So, this goes back to what we already discussed with the PEG hydrogels. Typically if you have a PEG hydrogel, you will get chains which are sort of cross-linked either physically or chemically depending on the functional group that are present on these PEG, mostly chemically, and then there will be a random orientation of them, there will be individual chains units binding to another change chain unit and you get a certain pore size which will be heterogeneous, but not as heterogeneous. Its somewhere hovering around the averages plus minus few microns and nanometres depending on what is the average and you can encapsulate things in that you can release it over time, things can migrate in if its degradable or the pore size is big enough. Whereas, the collagen hydrogels first if you use the same number of chains of collagen as you used for PEG.

Collagen actually is known to form fibrils. So, most ECM proteins that we find in a body actually forming thick fibres and then the way they form thick fibres is essentially several single chains will come together and essentially cause a thick fibre to form. And, then you can have another thick fibre going through that and then similarly you may have few thinner fibres also crisscrossing all of this and so, this is a type of hydrogel that with that collagen hydrogel.

And so, as you can see it is a very different system between this hydrogel and this hydrogel. So, that is that is in terms of this structure. What about the tunability? So, again the tunability is present in both of them, synthetic matrices definitely have a lot more control since you can define each single chain. So, each of them will have a certain orientation, each of them will have a control over what is the stiffness (Refer Time: 18:03) They will be fairly uniform throughout, because these are all random chains will be fairly uniform. And you can change the polymer density you can get a lot more chains in there.

So, you can decrease the pore size, you can increase the mechanical properties of your system. Whereas, in cases of collagen hydrogels what you will find is the control is there, but it is not as much right because these fibrils, first of all, have really changed the stiffness in the local area. So, if I measure the stiffness of this particular gel or this particular fibre it is going to be much stronger than these individual fibres. And the other thing is since they are natural materials its sort of hard to modify them and its sort of self

assemble into these structures, so, the control is much lesser. And what about the cell manoeuvrability?

So, that is another good example here. So, now, these PEG hydrogels they do not have any cell binding site. So, first of all the cells may not want to interact with it. So, what do you have to do is you have to incorporate cell binding sites, and what I mean by that is that if the cell comes in and wants to interact with this, at this point the PEG chains do not have any ligands. So, unless I put a ligand lets say RGD that we talked about in the previous case and this cell is not able to bind to this. Only if there is RGD or some other sequence the cells can actually bind to these matrices. So, that is one.

The other is what if the cell wants to move around? Now, the way the system is set up is these pore sizes are a fairly uniform because these are single chains and what you will find is typically to form a hydrogel you need quite a bit of chain. So, these pore sizes are typically always less than 100 nanometre.

Now, when we talk about cells we are talking about at least mammalian cells we are talking about mammalian cells which are typically always greater than 5 microns. So, now, if I am saying the pore size is 100 nanometre which is fairly uniform plus minus let us say 10 nanometre or 20 nanometre, these cells cannot really move through that. They are sort of logged into all kinds of polymer chains crisscrossing them and they cannot move anywhere if you have encapsulated these cells inside.

So, if you want cell manoeuvrability what you have to do is you have to also incorporate the protease cleavage size, which basically means that, now if I change this sequence to let us say these green regions which are degradable by certain enzymes that cells have on their membranes or can secrete, only then the cells can actually start degrading this.

So, they will break it, essentially breaking the whole chain and then they will be able to start to move out. And, so that is one of the issues with these synthetic hydrogels because you have to take care of all of these things before it becomes cell compatible; because essentially if you want the cells to be fairly happy in that system, you want some manoeuvrability of the cells in that system.

So, you will have to do all of that. And even then it may not be enough because let us say a cell wants to move at a much faster rate. But, it first has to wait for the cell to degrade

the protease cleavage site which could be at a much slower rate or vice versa, maybe degrades too much and now suddenly the cell has nothing to bind to its completely in our pore it is looking for an active site and everything is getting cleaved.

So, all of these issues start to come in when you are talking about synthetic materials and you have to do a lot of research to sort of figure out what is the rate at which you want the these peptide sequences to cleave and that will vary with cell to cell type. And, not only that it will vary from application to application and then not have a very control on that system in terms of this cell happiness or the cell compatibility with that system.

Now, let us compare to what happens with the collagen hydrogels. So, we already talked about the structure and tunability, structure is described here tunability you get a better response from synthetic hydrogels. But when it comes to the cell manoeuvrability all of these themselves have cell binding sites, most ECM proteins will have a certain cell binding site. So, this could be collagen as is written here or this could be fibronectin, could be laminin, could be something else all of these have cell binding sites.

So, these will bind to the cells. So, that is not required. What about the movement of the cell? So now, what you have done here is because these fibres have sort of self assembled into big fibrils the pore sizes are very different. So, what you will find is typically the pore size in such a gel can be easily changed from let us say few nanometres, let us say 1 nanometre to all the way to 10s of microns.

So, certain regions will have large pores certain regions will be fairly compact as you are seeing here. So, this you are talking about less than 1 nanometre gaps and this you are talking about huge gaps of let us say 10 micron and because of that you do not really have to worry about cell manoeuvrability. So, when the cell wants to move around in the gel, it does not have to wait for some sort of degradation kinetics to happen and move around.

So, this can happen whenever the cell wants to move around and not only that, these are natural materials as we just talked about. So, the cells have capability to actually go ahead and degrade the collagen fibrils if they really want to or deposit more. So, the cells can actually come and deposit more collagen and that collagen will then interact with this collagen that is already present and so, the cells can move around easily, cells can remodel the network easily, and then cells can also bind the network easily.

So, you can see all of these are sort of advantages of a natural system that you get because you do not have to worry about now making another component that as cell binding site, you do not have to worry about making another component that as cell cleavage site that are all inherently present. At the same time there is obviously, drawback that the tunability is fairly low.

So, this is cell maneouvrability is very high, in this case it is low. Tunability is low in this case, high in synthetic gel and the structure is obviously, different and the control on the structure is high here, lower here. So, these are some of advantages and disadvantages of both synthetic and natural hydrogel systems when you are looking at the cell level and how does the cell interact with it.

So, just something to keep in mind and something to consider when you are choosing a material for any application that you want to use for in vivo or in research. So, it is important to consider these factors. So, we will stop here and we will continue further in the next class, see you then.

Thank you.