Tissue Engineering
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Lecture – 21
Cell Adhesion

Until now, we have been talking about the different aspects of the cell when it comes to tissue homeostasis, so we talked about cell growth, death, differentiation. There are just two more concepts when it comes to cells, the first is adhesion, and the next one is migration. So we will talk about cell adhesion today. Understanding cell adhesion is crucial when it comes to tissue engineering because you need to know how you can design your scaffolds in a way that you will be able to help cells to adhere.

So, you need to identify what type of cell you are going to work with, try to understand how those cells interact with each other and with the matrix and how they adhere to the surfaces. Based on that, you can functionalize your material to get desirable adhesion properties.

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When you talk about cell adhesion, there are aspects that you need to understand are the concept of the surface receptors. So, each type of cell could have different surface receptors which help in cell adhesion. These receptors usually have an extracellular domain, which is the one that interacts with the surface or other cells. And you have a

transcellular domain and an intracellular domain, so through which signals and other cascades can be triggered.

You also have a cell to cell adhesion where cells of similar kind or different kind adhere to each other using some of these receptors and form different junctions. You could also have cell to matrix adhesions. So, it is important to know the difference between the two, and they usually are uniquely different; some types of junctions are more commonly seen in the cell to cell interactions, whereas others are more commonly seen in the cell to matrix interactions.

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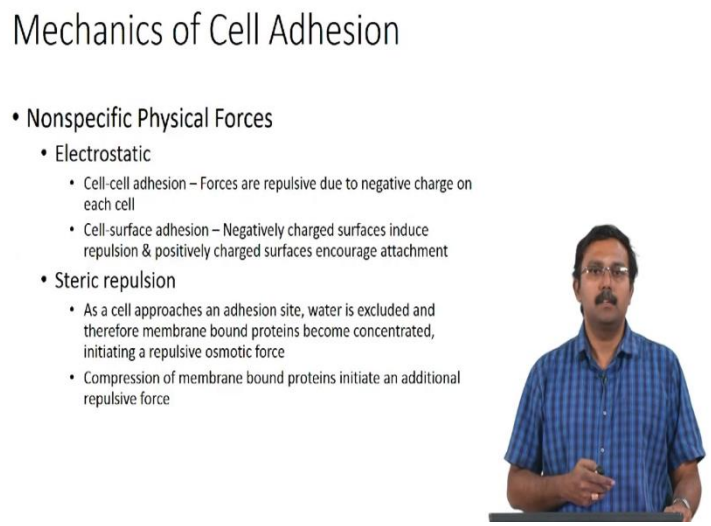

When you are talking about the mechanics of cell adhesion itself, there can be nonspecific physical forces such as electrostatic or steric repulsion or Van der Waals forces, which could play a role when it comes to the interaction of cells. When we are talking about electrostatic forces, the cell-cell interaction can have a negative effect. There would be a negative charge on the cell surface on both the cells, which should have repulsive effects when it comes to electrostatic forces.

Whereas when you are talking about cell-surface interaction, you can try to use this to your advantage. If you have a negatively charged surface, then you can induce repulsion of the cells. Whereas if you have a positively charged surface, you could attract the cells toward the surface.

Student: Sir, if naturally two cells, both have negative charges and repel each other and how do they steric.

It depends on how far away they are and which forces have the biggest impact. So, it is not going to be one force that is acting. So, you have electrostatic forces, steric repulsion, Van der Waals forces, and then you have the specific interaction because of the ligand-receptor interactions. So, based on the distance at which they are present and based on the affinity of these, like the dissociation constants or the affinity constants of these forces, you would actually have interactions.

So, we will talk about that, each of them would have their own properties. Steric repulsion is seen when a cell approaches an adhesion site; what happens is water is excluded. Therefore, membrane-bound proteins become concentrated, which causes a repulsive osmotic force. So, the compression of the membrane-bound proteins initiates an additional repulsive force, which is seen as the steric repulsion.

Again, all these forces are more active when the distance between the two surfaces which are interacting. It could be both cell surfaces or a matrix surface, and the cell surface is at different levels; at some particular distances, you would have an electrostatic effect having a bigger role, whereas, at some other distance, it will be steric repulsion.

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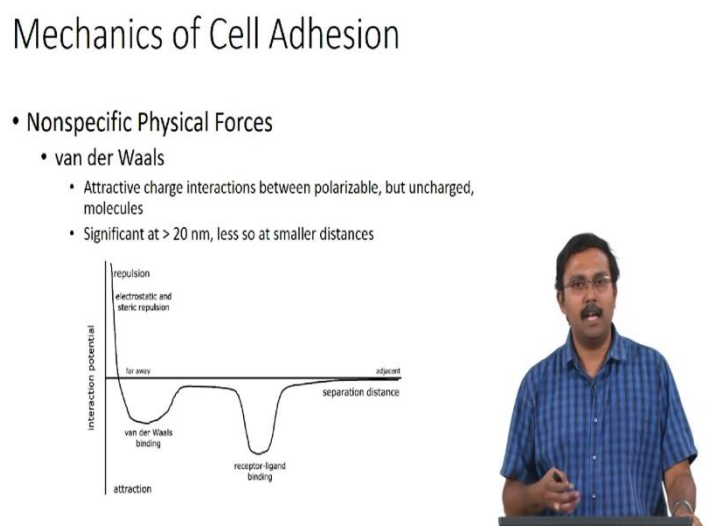

The last nonspecific physical force is Van der Waals forces. Attractive charge interactions between polarizable but uncharged molecules are called Van der Waals interactions. This is significant when the distance is greater than 20 nanometers, and it is less important when it is very close because the other factors play a bigger role.

The graph you observe here, the interaction potential versus separation distance. This graph shows you how the overall effects of all these three are, so this is not individual effects; this is the cumulative effect of these three nonspecific physical forces. Electrostatic and steric repulsions are seen when the cells are farther away, and you have the receptor-ligand binding, which can have a significant impact, and Van der Waals binding, which would have a lesser impact.

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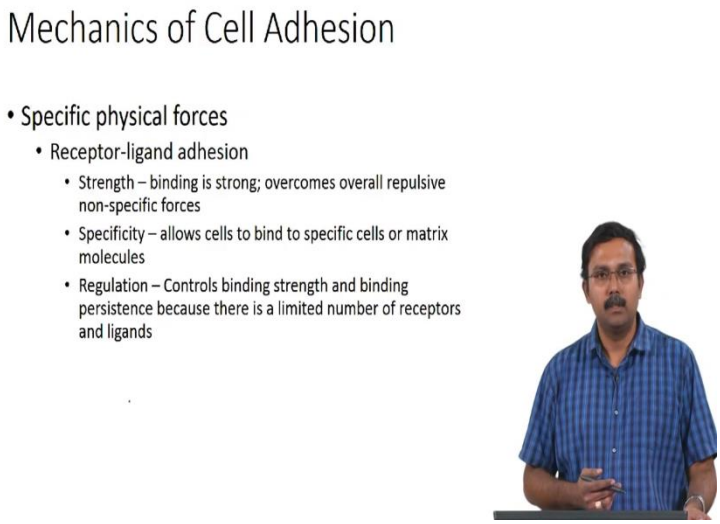

The specific physical forces, unlike the nonspecific ones, are the receptor-ligand adhesions. This depends on what surface and what are the ligands present and what cell and what are the receptors present. So, the strength is much higher when it is compared to other non-physical forces. Again, this is not a chemical bond that is being formed; it is still a physical interaction.

However, the binding is very strong; it overcomes all the other repulsive nonspecific forces when this happens. It is much much stronger than all the other forces put together. The specificity also provides ways to bind specific cells to the matrix. So, you could have some cells adhere to a surface, whereas other cells might not actually like this surface as much.

Depending on the receptors which are expressed, you can tailor your material in a way that the specific cell types adhere to the surface.

Student: Sir.

Yeah?

Student: Sir, these interactions are with respect to tissue-engineered cells or like.

No, in general, even in nature, if you have an extracellular matrix, the interaction is going to be there still, right.

Student: It is like why are we talking about like distance between the cells like a natural tissue is like, is there a distance between two cells?

Yeah, because it is not that the cells are always present there, and they are proliferating right. So, cells would have to migrate from the stem cell niche and come to a specific site. There will be factors that will play a role. So, it would have an impact even in natural conditions, and it will be crucial to understand that to exploit it in tissue engineering applications, ok.

This receptor-ligand binding controls the binding strength and the binding persistence because there are limited number of receptors and ligands on the surface. Based on that, you can regulate how strong it is, where it actually binds, and so on.

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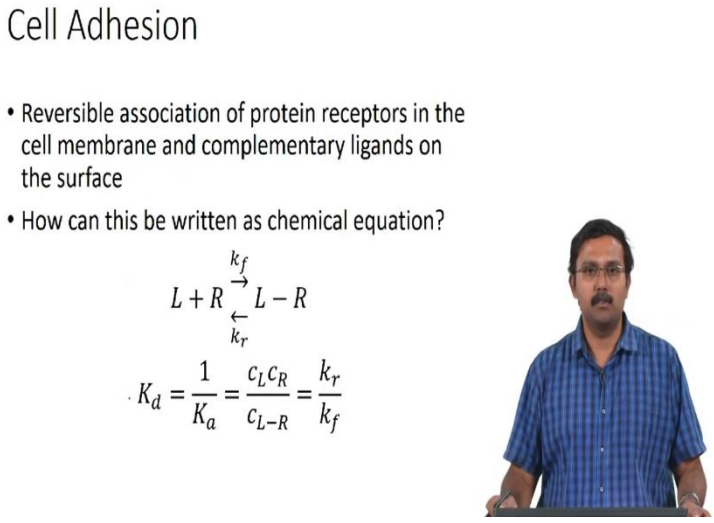

When we talk about cell adhesion in general, we usually talking about this ligand-receptor binding. This reversible association of protein receptors in the membrane to the complementary ligands, which could be present on the surface or on another cell, is what we talk about when we talk about cell adhesion. So, this is what you would have studied when you studied cell biology. So, I hope you were taught cell adhesion as part of cell biology, so it is an important aspect when it comes to cells.

When we talk about that from a biological perspective, this is what we are talking about. If we are looking at it from a chemical standpoint, it is again similar to the ligand-receptor binding, which we looked at when we talked about growth factors earlier.

So, what would happen is, the ligand and receptor forming a complex. And this would be obviously, a reversible reaction because of the specific physical force, but it is a reversible process. So, there would be a dissociation constant and association constant. If the dissociation constant is very high, then the affinity it is lesser. If the dissociation constant is low, the affinity is much better. So, the interaction would be much stronger.

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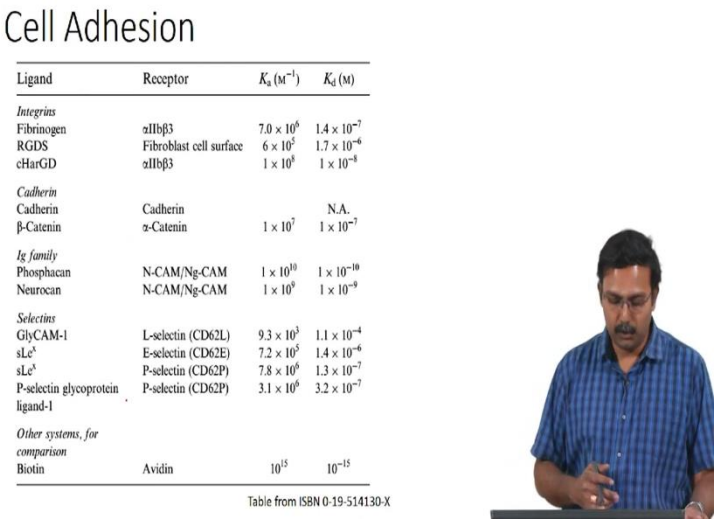

This shows you the association and dissociation constants for different-ligand receptor complexes. These are classified based on the type of junctions or adhesion, which would be done. Integrin is one type under which you see fibrinogen interacting with the αIIbβ3 receptor, and you see that the dissociation constant is really really small.

So, that shows that there is a significantly strong interaction. There would be some which are not that strong. So, if you were to take selectins where GlyCAM-1 and L-selectin, its dissociation constant is not that small, it is orders of magnitude higher, indicating that it is much weaker. There are other things that are as close to even covalent bonds like the biotin-avidin complex.

Biotin-avidin complex is as strong as a covalent bond. The dissociation constant is about 10^{-15}. Understanding these things can help, because specific cell types will express the specific surface receptor. So, fibroblast cell surface will have receptors that will bind with RGD, which is known.

So, if you can tailor your material to have RGD, then your fibroblast can be recruited. Similarly, you would have to figure out what would be the suitable ligand for different cells that you are working with. If you have a bone tissue engineering in your mind, we need to figure out what receptor osteoblast would like and try to modify your surfaces with that. Hopefully, that will help in making sure that more bone cells are attached to the surface.

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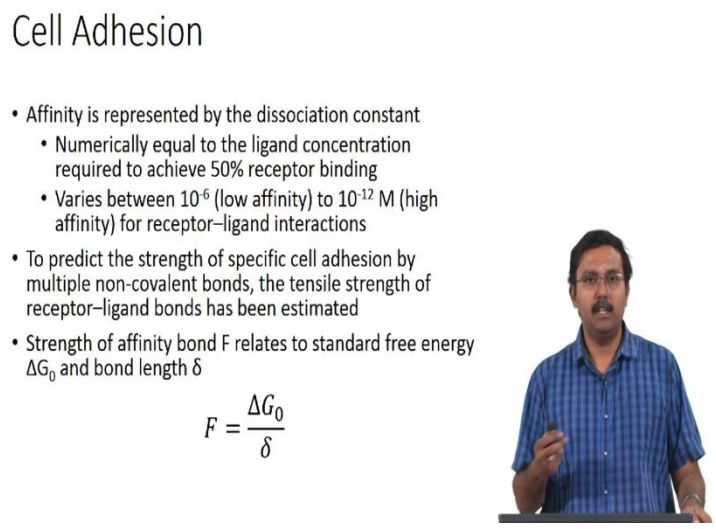

As I already mentioned, affinity is represented by the dissociation constant; dissociation constant is numerically equal to the ligand concentration required to achieve 50 percent receptor binding. So, this is like your Michaelis-Menten constant. So, that is the 50 percent of your reaction rate is achieved at a particular substrate concentration; that is you Km.

Similarly, the numerical value at which you can get 50 percent receptor binding the ligand concentration is the dissociation constant.

This varies between 10^{-6} to 10^{-12} M. So, 10^{-6} is considered to be low affinity versus 10^{-12} is considered to be much higher affinity. To predict the strength of specific cell adhesion by multiple noncovalent bonds, the tensile strength of the receptor-ligand bond has to be estimated. Basically, to see how strong this binding is, there are experiments to do this; there are many studies. I will give you a reference which you can go through.

So, that discusses some of the experiments that can be done to study the receptor-ligand binding strength. The strength of the affinity bond relates to the standard free energy, which is ΔG_0 and the bond length. So, what does this ΔG, what is Gibbs free energy?

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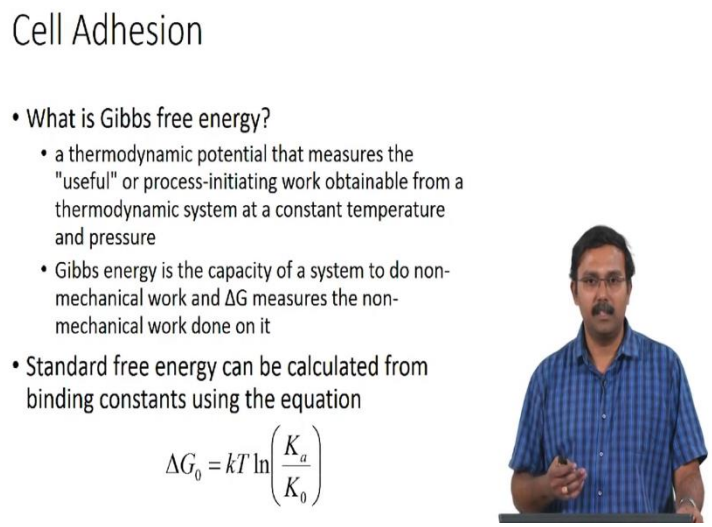

At least the ones who are studying thermodynamics in this semester.

Student: The spontaneity of the process.

That is the outcome.

So if your Gibbs free energy is negative, then the reaction will happen spontaneously; that is fine. But what is Gibbs free energy? Your quiz 2 would have gotten over just now, right? I am pretty sure you would cover Gibbs free energy.

Student: Already.

What is Gibbs free energy? You studied Gibbs free energy right.

Student: Yes.

So what is it?

Student: Nature of feasibility of thermodynamic energy or any reaction.

So that is one aspect, so in general, it is a thermodynamic potential. Basically, it is a thermodynamic potential, and it measures the useful or process initiating work obtainable from a thermodynamic system. So, it could be a reaction or any other process. Reaction is what we commonly look at when you talk about Gibbs free energy. So, we always try to associate it with that, but it is not necessary that it needs to be for that.

Gibbs free energy is the capacity of the system to do non-mechanical work, and ΔG basically measures this non-mechanical work done on the system. Anyways so, the standard free energy can be calculated from the binding constants so that you would get

$$\Delta G_0 = kT \ln\left(\frac{K_a}{K_0}\right)$$

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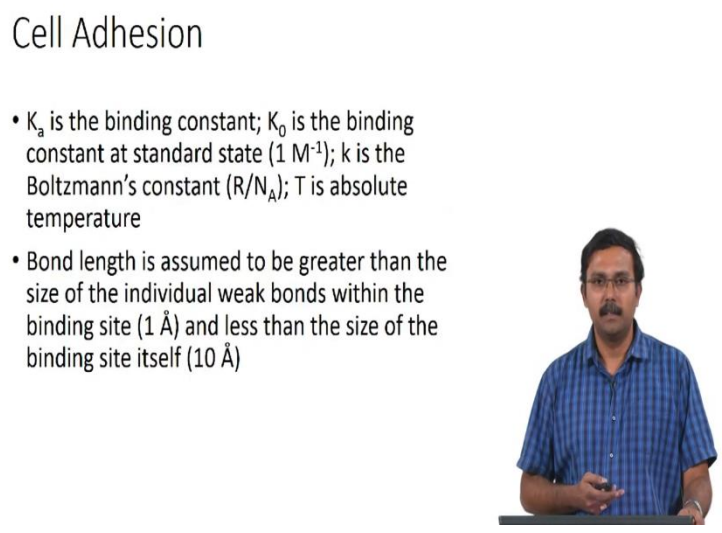

So, K_a here is the binding constant, and K_0 is the binding constant at a standard condition usually 1 M^{-1}, and k is the Boltzmann's constant, and T is the absolute temperature. So, the bond length is assumed to be greater than the size of the individual weak bonds within

the binding site and less than the size of the binding site itself. So, it will be somewhere in between; using this, we can calculate the free energy from which you will have an understanding of what would be the strength of the bond affinity.

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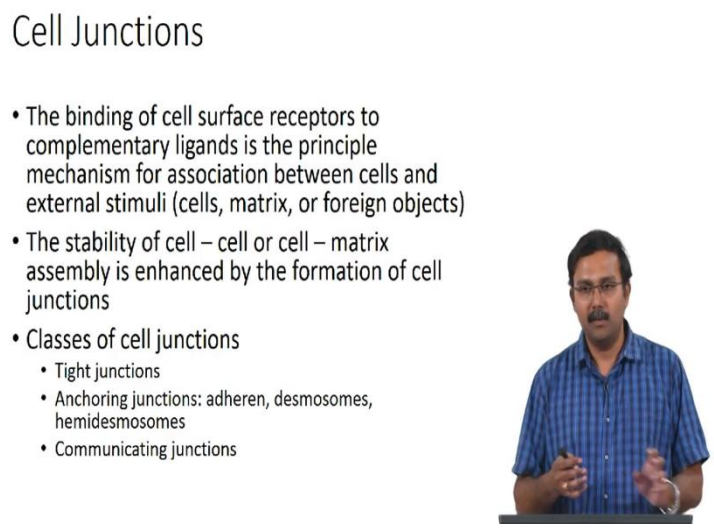

Coming back to the biology aspect of this, so cell junctions are created when we talk about cell adhesions. The binding of cell surface receptors to complementary ligands is the process in which there is an association between the cell and external stimuli. External stimuli could be cells, matrix, or foreign objects. So our scaffold would be technically a foreign object.

We would have to figure out how the interaction happens. The stability of these cell-cell or cell-matrix assembly can be enhanced by the formation of these cell junctions. Instead of just having nonspecific physical forces, if you can have these specific junctions which are formed, then you are going to have much tighter and much stronger interactions. There are different classes of cell junctions. The first type is called a tight junction; you also have anchoring junctions and communicating junctions. We will quickly go through what these are.

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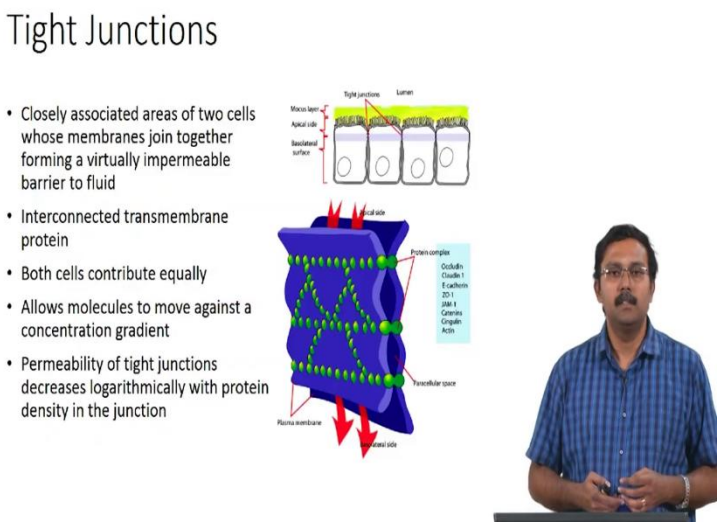

The tight junction is something where closely associated areas of two cells whose membranes are joined together, form a virtually impermeable barrier to the fluid. This is a very tight interconnected junction, so the transmembrane proteins interact with each other to form a very strong protein complex.

Here, both the cells are contributing equally, so you would have the receptors and ligands interacting from both cells at an equal level. This allows molecules to move against a concentration gradient; that is the importance of having these type junctions. It is not just diffusion through which it is going; it can be active transport of molecules is possible through this tight junction.

The permeability of tight junction decreases logarithmically with protein density in the junction. Because it is dependent on the concentration of the proteins, it will be the first-order mechanism. So, you will have an exponential increase and logarithmic decrease, and so on.

You would see that, as the protein density at the junction increases, you are going to have a significant reduction in permeability. But it will become much stronger interaction, thereby helping in crossing the barrier like helping in transfer from lower concentration to a higher concentration.

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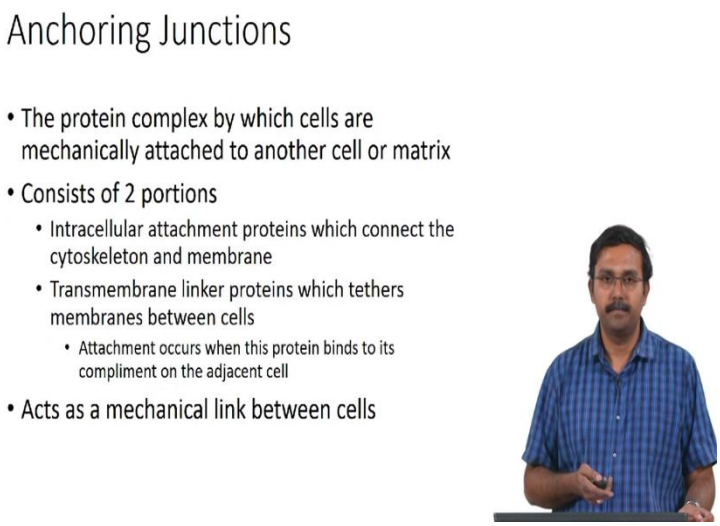

Anchoring junctions are the ones where the protein complex is formed where the cells are mechanically attached to another cell or matrix. This usually consists of two portions; the first one is the intracellular attachment of proteins, which connect the cytoskeleton to the membrane, and then there is transmembrane linker protein which tethers the membranes between the cells. The attachment occurs when this transmembrane protein binds to its complementary protein in the adjacent cell or the matrix. So, this acts as a mechanical link, like a hook that has been connected. This is now connected to the transmembrane protein, and then there is an intracellular attachment protein that connects it to the cytoskeleton.

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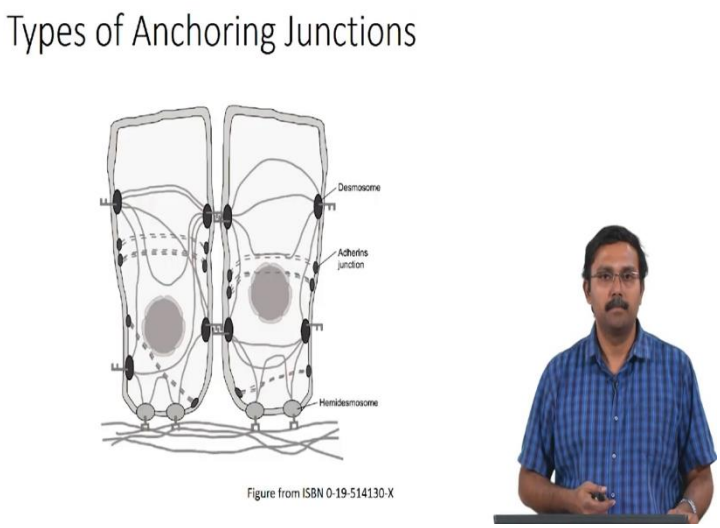

So, these are some of the anchoring junctions. As you can see, you have the desmosomes, you have adherins, and you have the hemidesmosomes. So, these are the different anchoring junctions that can be formed.

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Anchoring Junctions – An Overview

Junction Type	Transmembrane Protein	Extracellular Ligand	Intracellular Linkage (Accessory Proteins)
Adherens	Cadherin	Cadherin	Actin filaments (catenins)
Desmosomes	Cadherin	Cadherin	Intermediate filaments (desmoplakin, plakoglobin)
Hemidesmosomes	$\alpha_6\beta_4$ Integrin	ECM protein	Intermediate filaments
Focal contact	Integrin	ECM protein	Actin filaments (α-actinin, talin, vinculin)

Table from ISBN 0-19-514130-X

This is an overview of the anchoring junctions. If you are talking about adherens, their transmembrane protein involved is cadherin, and the extracellular ligand is also a cadherin. The intracellular linkage, which is the intracellular accessory protein, which is present, would be actin fibers.

In the case of desmosomes, you would have cadherin-cadherin interaction; however, the intracellular linkages are intermediary filaments which are present in the cell. Hemidesmosomes are not cadherin dependent; they form interactions based on the integrins and the ECM protein.

And what you can identify from that is adherens and desmosomes are usually used in cell-cell interactions, whereas hemidesmosome is the type of anchoring junctions that are formed between a cell and a matrix. Again, intermediate filaments are involved in the intracellular linkage. Focal contact is an integrin-ECM protein junction in which actin filaments act as the intracellular linkages.

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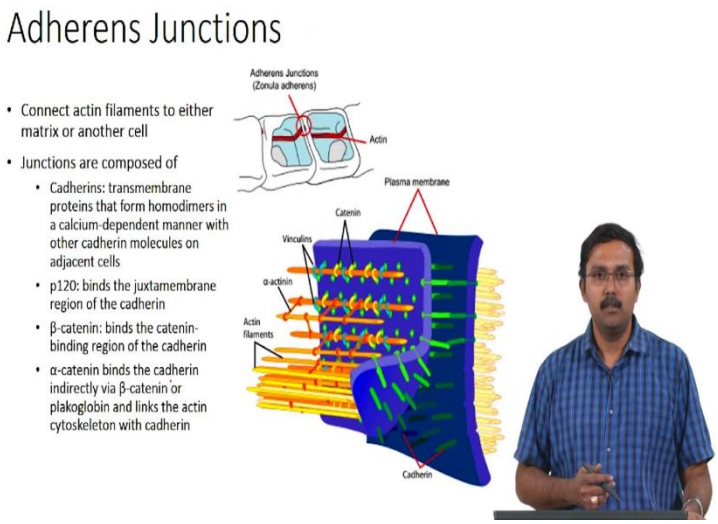

Integrins and cadherins are usually involved in these types of anchoring junctions. Adherens junctions are the types of junctions that connect actin filaments to either the matrix or the cell. And the junctions usually are composed of cadherins, which are transmembrane proteins that form homodimers in a calcium-dependent manner. Cadherins require calcium to form this junction to be active.

p120 binds the membrane region of the cadherin. And β-catenin binds to the cadherin binding region of the cadherin. And α-cadherin binds the cadherin indirectly via the β-catenin or to the plakoglobin, which should be the intermediary filaments and links it to the actin with the cadherin.

So, these are just the structures; the understanding of these details of this is probably not very crucial for tissue engineering applications. But what is more important is to understand how these are formed or like what are the receptors and ligands which are involved in these things, which can be applied to tissue engineering applications.

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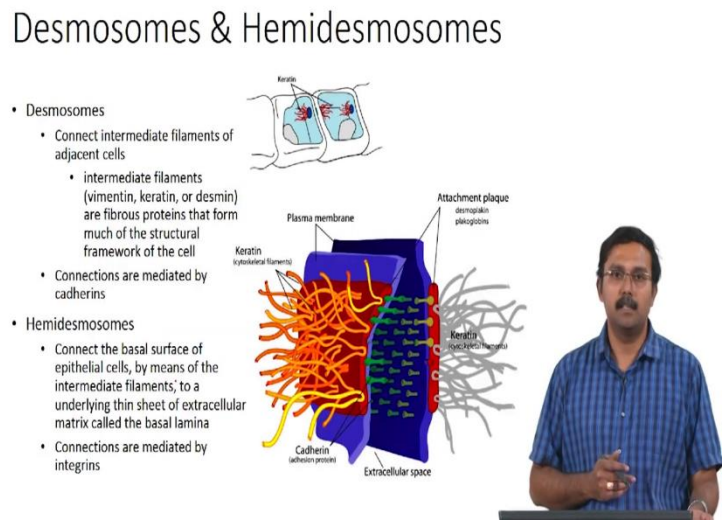

Desmosomes and hemidesmosomes are also anchoring junctions. In the case of desmosomes, what you see is a connection between the intermediate filaments to the adjacent cells. The intermediate filaments are usually made of a fibrous protein that forms much of the structural framework of the cells.

Some of these would be keratin, or vimentin, or desmin, so these are just fibrous proteins that are present to create this structural framework. The connections here again, are mediated by cadherins. These two things, the adherens, and desmosomes are cadherin-dependent anchoring junctions; the hemidesmosomes are integrin-dependent anchoring junctions.

Here what happens is there is a connection between the basal surface of the cells by means of intermediary filaments. These interact with the extracellular matrix to form the junctions. These are some of the representations of how these interactions happen.

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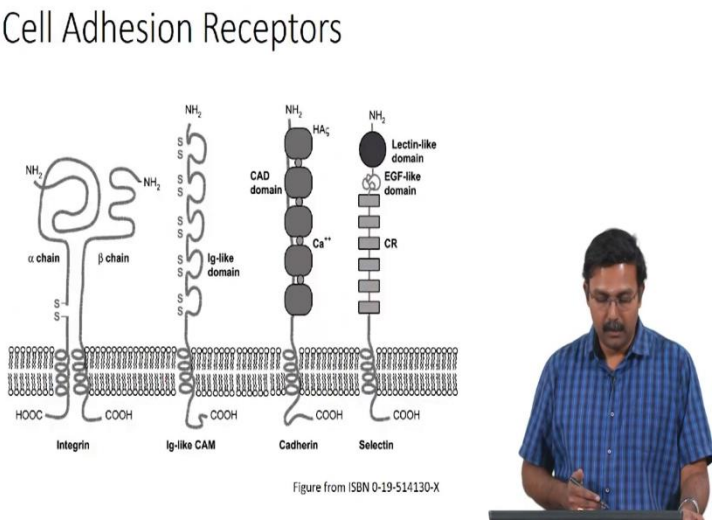

These are the receptors; so if you look at the major cell adhesion receptors which are exposed, they can be categorized into four major groups. Integrins, Ig-like CAM, or cadherins or selectins. These have different structures, and depending on which cell type you are working with, one or more of them can be overexpressed. So you can design your scaffolds appropriately.

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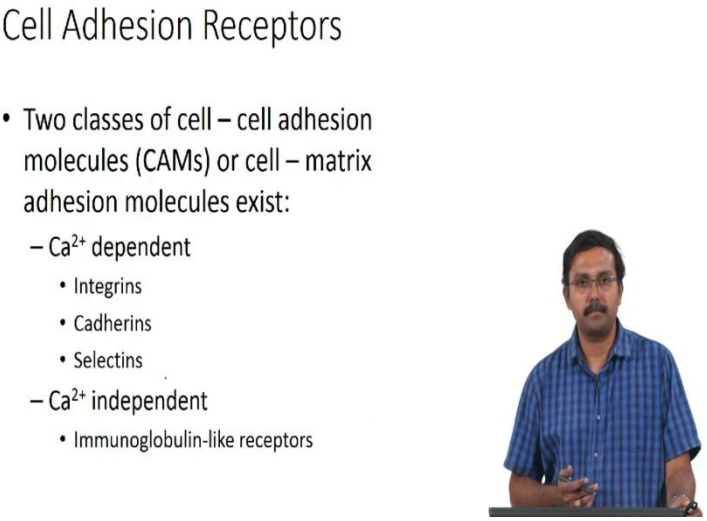

Basically, these four can be again grouped as two major things; one is the calcium-dependent, and the other is the independent calcium receptors. The cell adhesion molecules

which come under calcium-dependent would be the integrins, cadherins, and selectins. Immunoglobulin-like receptors or Ig-CAM receptors is calcium-independent.

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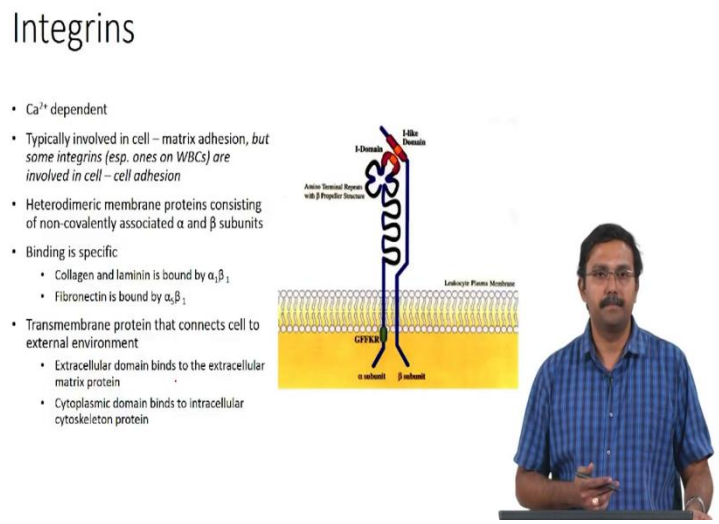

Integrins are calcium-dependent proteins that are involved in cell-matrix adhesion, but some can be involved in cell-cell adhesion as well. So, what happens here is there is a heterodimeric membrane protein, which consists of non covalently associated alpha and beta subunits. This is the structure of the integrin, and the binding is very specific. It binds to collagen and laminin through the α1β1 integrin, and whereas to fibronectin through α5β1 integrin. Depending on the two subunits, you can have different specificity for attachment.

If you are talking about this transmembrane protein, this connects the cell to the external environment. The extracellular domain binds to the ECM protein, and the cytoplasmic domain binds to the cytoskeleton in the cell. This way, you have proper interaction within the cell, with the matrix.

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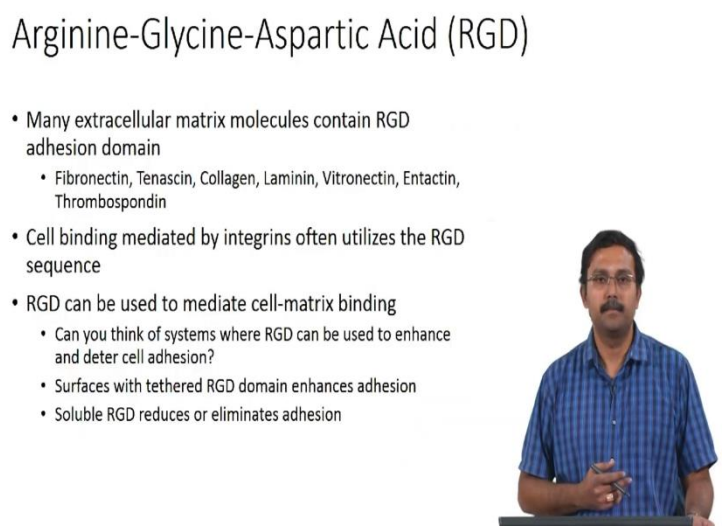

RGD is one of the ligands which interacts with the integrin. Many ECM molecules contain RGD domains. This is very popular; fibronectin contains a lot of RGDs, so does collagen and laminin and even keratins, and so on. Cell binding through this RGD domain is often mediated through integrins. This was one of the early peptides which were used for improving cell adhesion.

When you are looking at modifying material surfaces, people are trying to attach RGD domains or incorporate RGD domains as part of this matrix. They also try to use molecules that would have these RGD domains, usually, proteins should have RGD domains to prepare these scaffolds, so that way was trying to improve cell adhesion. It can also be used to reduce cell adhesion; if you were to use RGD as a soluble RGD in the media.

If you want just to move it away from the scaffold, that might be required if you are looking for some kind of cell separation. So, where you say that cells that do not express receptors for RGD ligands should adhere, whereas the cells which express these receptors should be in suspension. So, if you want to do something like that, then you would use RGD in solution, thereby preventing adhesion of the cells.

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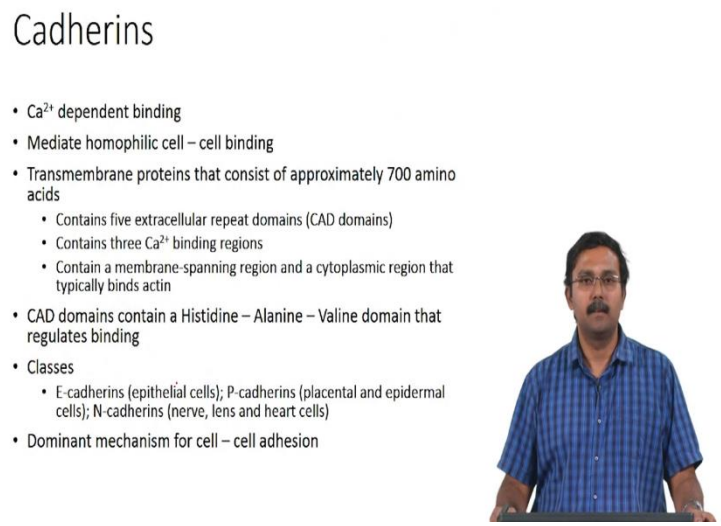

Cadherins are again calcium-dependent binding.

Student: Would you give an example of why that would be required using RGD to prevent.

So, you remember we talked about cell isolation. During that time, I was saying one of the things which can be done is to selective adhesion to surfaces or selective separation. So, this would be one of those applications.

Student: Sir, I have heard that RGD mutagens are widely used in bone tissue engineering.

Student: Is it how is it actually.

So, all types of fibroblasts have receptors that will interact with RGD; osteoblast can have similar like surfaces; they are more like fibroblasts present in bone tissue. Because of that reason, they adhere in nicely to RGD. So, this can be seen with chondrocytes; many of these types of cells have integrins which can bind to RGD, so that is why people try to use that.

These cells are advantageous because they can also secrete their own matrix; like fibroblasts, and osteoblasts, or chondrocytes, they secrete their own matrix. So, if you adhere to them, as the scaffold you used gets degraded over a period of time, you would have a new matrix formed because these cells are depositing their matrix. So, that is why they try to optimize it that way.

If you are talking about cadherins, these are mostly involved in cell-cell binding, unlike integrins. Integrins are mainly involved in cell-matrix bindings. These are transmembrane proteins, which consist of about 700 amino acids; they contain 5 extracellular repeat domains, which are called the CAD domains and the 3 calcium binding regions. They contain a membrane-spanning region, which is the transmembrane region. And then a cytoplasmic region that typically binds to the actin filaments in your cell. The CAD domain consists of histidine, alanine, valine domain, which regulates the binding.

Just like how RGD is one of the ligands, here the domain itself contains this histidine, alanine, valine domain. There are different classes, you have E-cadherins, which are the epithelial cadherins; P-cadherins which are placental and epidermal cadherins, and you have N-cadherins which are seen in nerve cells, lenses, and heart cells. This is the most dominant mechanism for cell-cell interactions.

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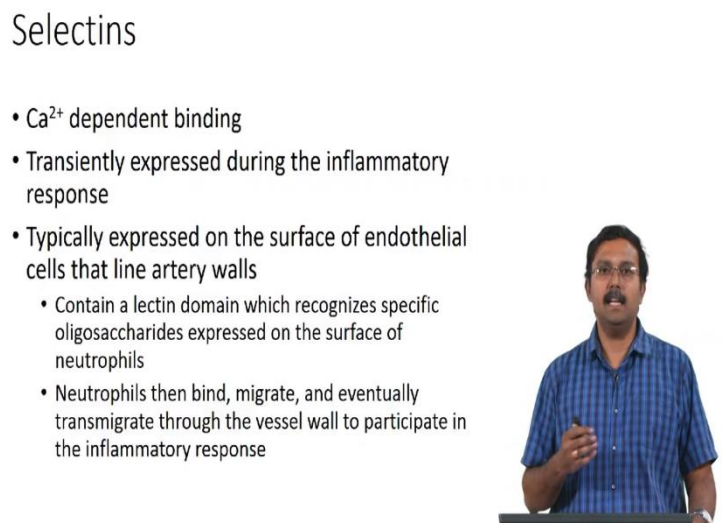

Selectins are again calcium-dependent bindings; these are usually expressed in a transient way; these are not very involved in regular cell adhesion processes. These are typically expressed on the surface of endothelial cells that line the artery walls. They contain a lectin domain that recognizes the specific oligosaccharides expressed on the surface of the neutrophils. Thereby, they can attract neutrophils when there is inflammation, so this is overexpressed only at that point. The neutrophils will bind, migrate, and eventually

transmigrate through the vessel wall to participate in the inflammatory response. So, that is where selectins play a crucial role.

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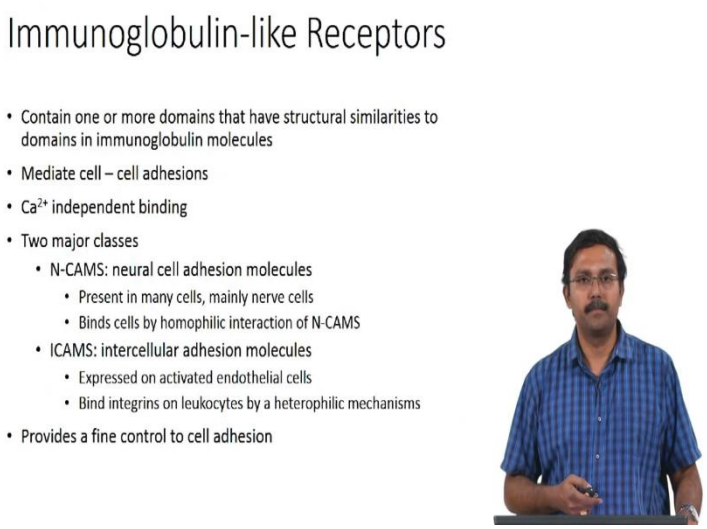

The Ig-CAMS or the immunoglobulin-like receptors contain one or more domains that have structural similarity to the immunoglobulin molecules. This again mediates cell-cell interaction; these are calcium-independent binding. There are two major classes; there are N-CAMS and I-CAMS. N-CAMS is where involved in neural cell adhesion, whereas I-CAMS are involved in other intercellular adhesion molecules.

N-CAMS are present in many cells; however, they are primarily seen in nerve cells. These bind cells by using homophilic interactions of N-CAMS. Whereas I-CAMs are expressed in activated endothelial cells, and these bind integrins on the leukocytes by heterophilic mechanisms. They provide very fine control of cell adhesion. Depending on the structure of the immunoglobulin, which is present, they can selectively attach cells and so on.

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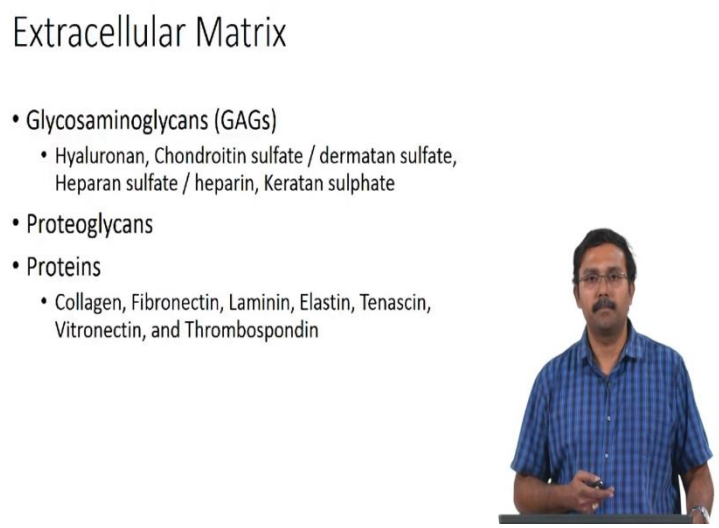

We looked at what the cells do; so the other side of it is what are the ligands which are present on the matrices; you try to understand that to emulate cell adhesion in tissue engineering. So, we have already looked at what ECM is, ECM is made up of different things proteins, proteoglycans, and glycosaminoglycans. Glycosaminoglycans could be Hyaluronan, Chondroitin sulfate, dermatan sulfate, Heparan sulfate, Keratan sulfate, and so on. You could have proteoglycans and proteins such as Collagen, Fibronectin, Laminin, and Elastin, and Tenascin, Vitronectin, and Thrombospondin and many other things.

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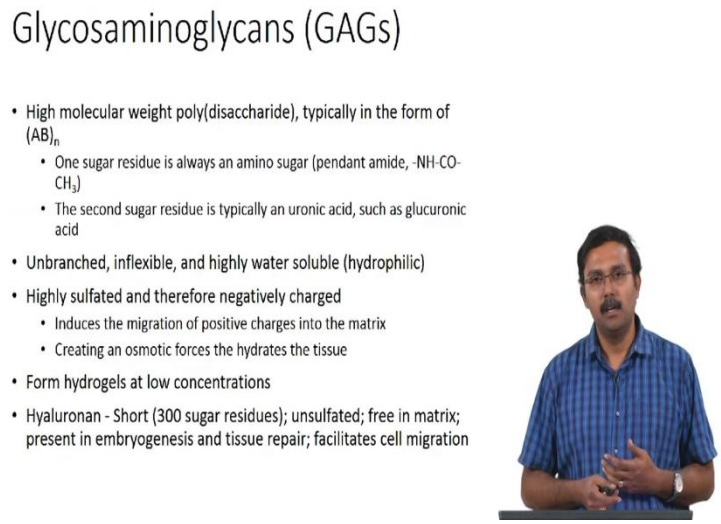

Depending on what is present, each of them would have different ligands, which will help in the attachment of cells. The glycosaminoglycans are usually high molecular weight polysaccharides, which typically form $(AB)_n$; it is a poly disaccharide. Your hyaluronic acid is poly disaccharide, and so on.

One sugar residue is always an amino sugar, and the second residue is typically a uronic sugar; usually a uronic acid such as glucuronic acid and so on. These are unbranched, inflexible, and highly water-soluble, they are very hydrophilic, and they are highly sulfated as well; therefore, they have a lot of negative charges.

This induces the migration of positive charges into the matrix creating osmotic forces that ensure that the tissue is hydrated. These are the molecules that are used to form hydrogels even at very low concentrations. So, hyaluronic acid is one example for that, it has been shown to help in embryogenesis, tissue repair, and it also facilitates cell migration.

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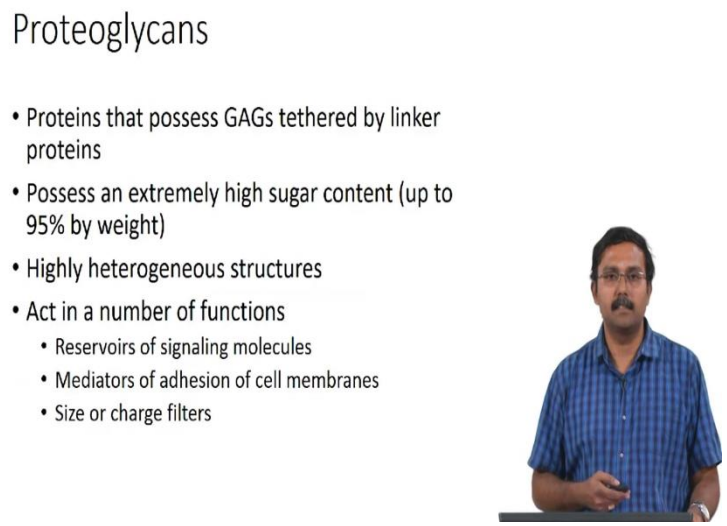

Proteoglycans are proteins that possess glycosaminoglycans linked, using some kind of a linker protein. They possess an extremely high sugar content which could be about 95 percent of their weight because the glycosaminoglycans are very large polymers which can be attached to these proteins. They are very highly heterogeneous structures and take part in many functions; they can act as reservoirs for signaling molecules, or mediators for adhesion of cell membranes, or size or charge filters for the molecules which are crossing.

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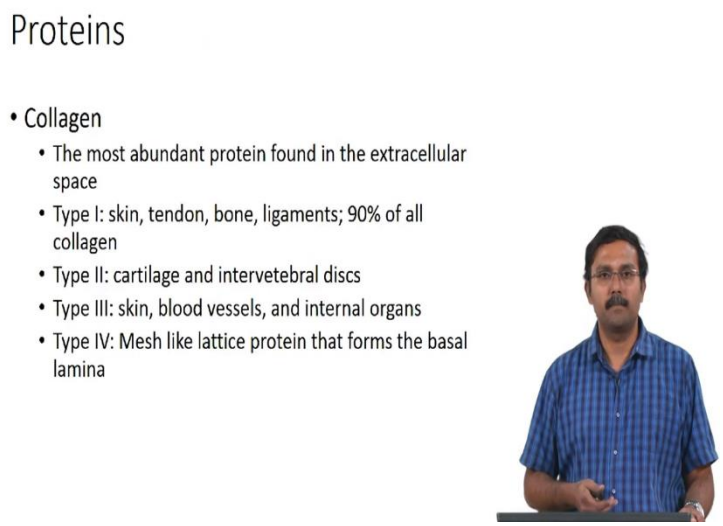

Proteins, as I have mentioned collagen, which is the most abundant protein in ECM, there are different types of collagen, and they are present in different regions.

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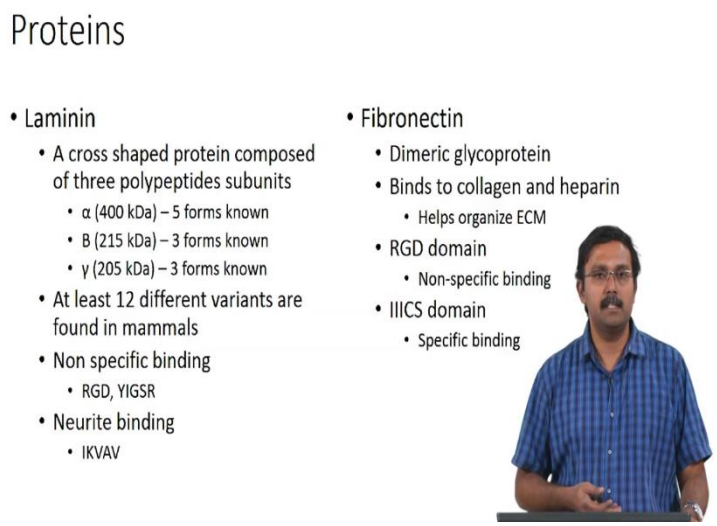

You have laminin; laminin has nonspecific binding; laminin is one of the proteins which has a very high amount of RGD domain. Fibronectin also has RGD domains that help in integrin-mediated cell adhesion.

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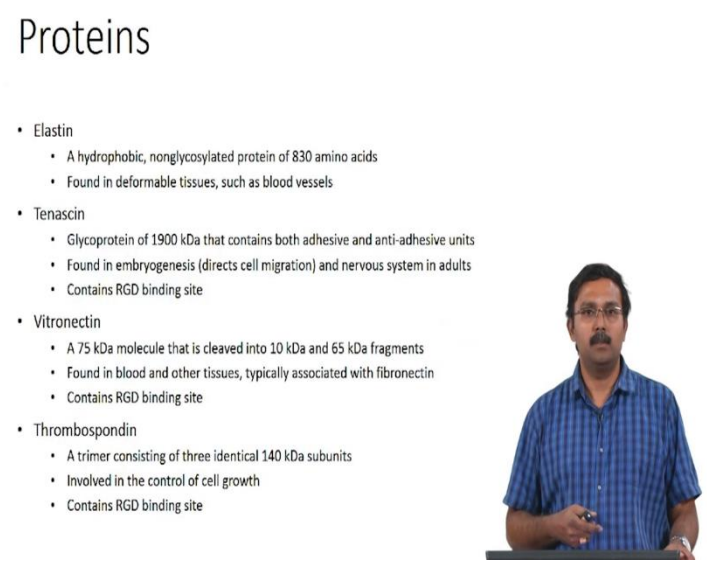

Other things are elastin, tenascin, vitronectin, and thrombospondin. All of these are involved in different aspects, and many of them have RGD binding sites. As you see, RGD is a very ubiquitous ligand seen in many of the matrix components, so that is why it has been extensively studied. If you were to look up strategies to improve cell adhesion are attaching RGD peptide would be one of the most common strategies.