Introduction to Mechanobiology
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Week - 01
Lecture - 05
Measuring properties of collagen networks

Hello, and welcome to our fifth lecture on Introduction to Mechanobiology. So, over the last few lectures have given you a broad introduction to this field, and given several instances as to how forces are of relevance to biology and how dynamics of cells during processes like migration division so on and so forth, required forces and interactions between cells and their surroundings. I have also started by abstracting the cell as tent I started rationalized in what are some of the basic ingredients, you would need in adherent cells in particular for the cells to have stable shape and interaction and proper function.

So, in particular I started discussing about the extracellular matrix in the last lecture, and what we found was. So, there is a in the examples I had discussed I showed that there is a continuous dynamic crosstalk between cells and the extracellular matrix. So, it is not a fx b or b fx a it is the combination of things. So, there are cells which secrete the extracellular matrix. So, primary among them is cells like fibroblasts in the connective tissue, which secret lot of collagen similarly smooth muscle cells are other epithelial cells ok.

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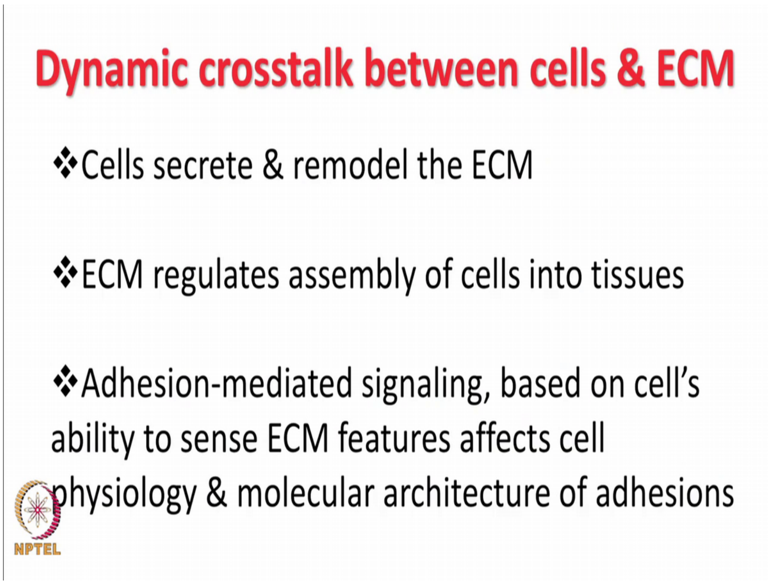

So, these other cells which secrete the accessible matrix, the ECM in return regulates the assembly of cells into functional tissues. So, it holds cells in particular positions and integrates stem into a functional tissue and there are interactions between the cell and the extracellular matrix mediated via additions which regulate cell function. So, what it this requires is for the cell to sense the physical chemical attributes provided by the ECM as distinct cues, which cells might must recognized and accordingly function based on that input. One of the particular ECMs that we discussed was the basement membrane right.

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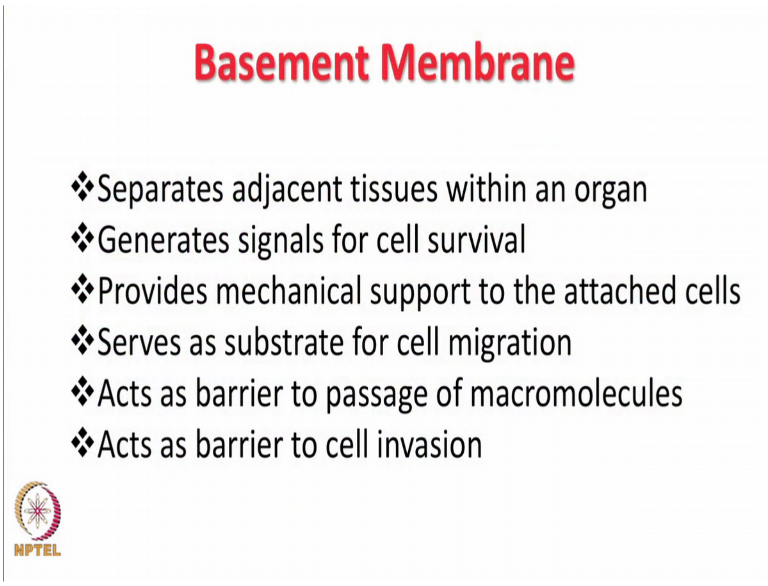

So, basement membrane is only 50 to 200 nanometers in thickness, it is there in almost all the tissues for separating adjacent tissues within an organ. It of course, generates signals for cell survival provides mechanical support to cells and also acts as a substrate for cell migration. Most importantly most of the cells in normal physiology cannot reach the basement membrane there are of course, three exceptions to this one of which is for during the process of development ok immunes surveillance and diseases like cancer.

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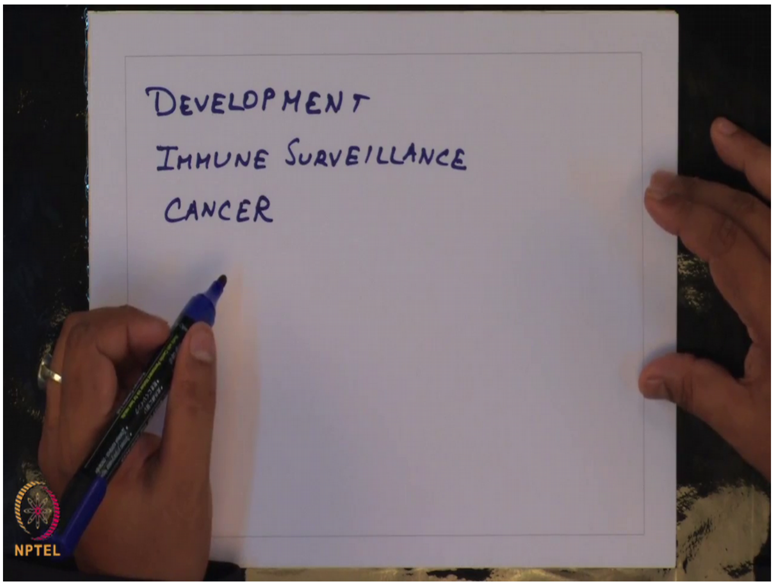

Were cells actually degrade the basement membrane and that represents that degradation of the basement membrane represents the first step in invasion, in initiating the invasion metastasis cascade. So, there are several extracellular matrix proteins and basement membrane in particular has multiple of them arranged together.

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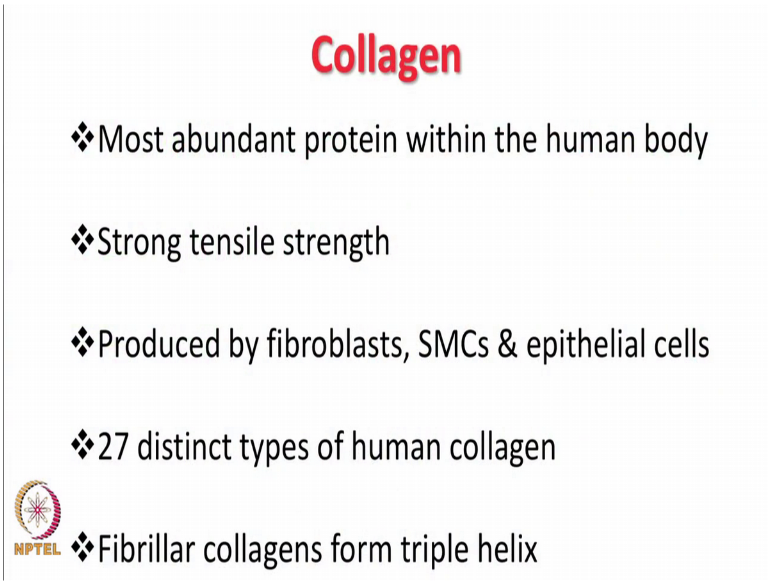

But we discussed about collagen which is the most abundant protein within our body and it provides tensile strength to the tissues, it is again produced by fibroblasts smooth muscle cells than epithelial cells and there are multiple types of human collagen ok.

And what we also know is that fibrillar collagens form this triple helical structure, where you have three chains which wrap around each other giving you a banded pattern of periodicity 67 nanometers. So, now, for collagen let us say collagen in particular. So, individual fibrils will have one particular property, but when you make a network using these collagen fibrils, you will generate a three dimensional structure which has properties which are distinct than the properties of individuals fibril collagens. So, understanding the mechanical properties of ECM networks like that of collagen is of key importance, how do we go about doing this? Think of this you know the spaghetti uncooked spaghetti forms all these rigid rods, and I introduced the concept of persistence which has direct relevance to bending rigidity of those structures.

But the same spaghetti when cooked then what you have is this mishmash of these fibrils which are much more compliant, and when they are held together even if you do not put anything else just together they provide some resistance. If you try to take out a single strand of it you can feel the resistance that the network provides towards deformation of that one strand.

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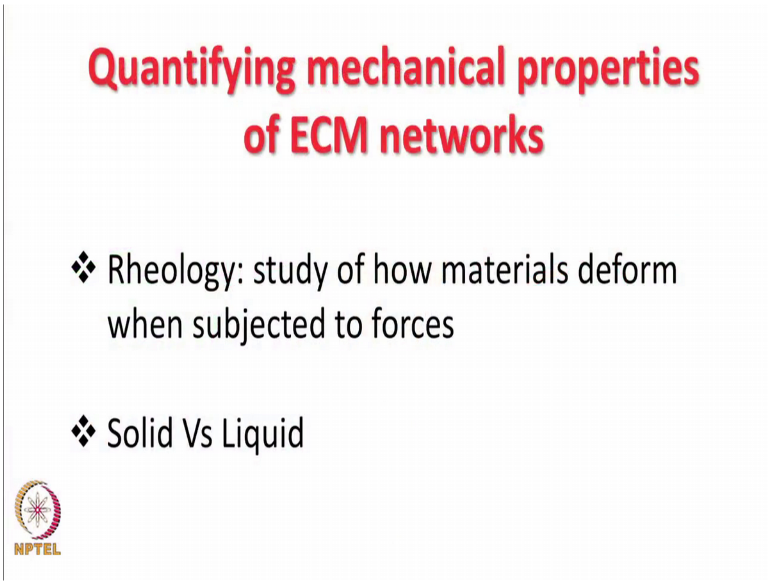

So, the field of radiology in particular has been developed to understand how our probing how materials deforming subjected to forces. So, that of course, brings to our first idea is. So, you know what is the solid, and what is the liquid. So, there must be something which is different in the solid and the liquid, but can we say an ECM network like a collagen gel is a solid or a liquid or something in between.

So, in order to address that we must first try to understand what distinguishes a solid from a liquid. So, if you take a simple thing let us say in case of a solid or if you take a spring let us say. Spring is an example of a li what we call as a linear elastic solid. So, what you have is when you compress a spring or stretch a spring, you observed instantaneous deformation and till the force is being exerted that deformation remains in place. But as soon as you remove the force you see that the spring goes back to its original length or unstretched length. So, this is what we called a solid ok.

So, when there is a deformation there is a when you exert forces there is a deformation of the solid, but when the force is removed it regains its original configuration contrast that with a liquid. So, when you exert any force it continues to deform, for the duration the force is exerted. So, in case I will of. So, note the difference in case of a solid you exert a force there is an instantaneous displacement or deformation, and then that is constant and once you remove the solid regains its original configuration, versus for a liquid you exert the force the liquid will flow and it will continue to flow till the force being exerted ok.

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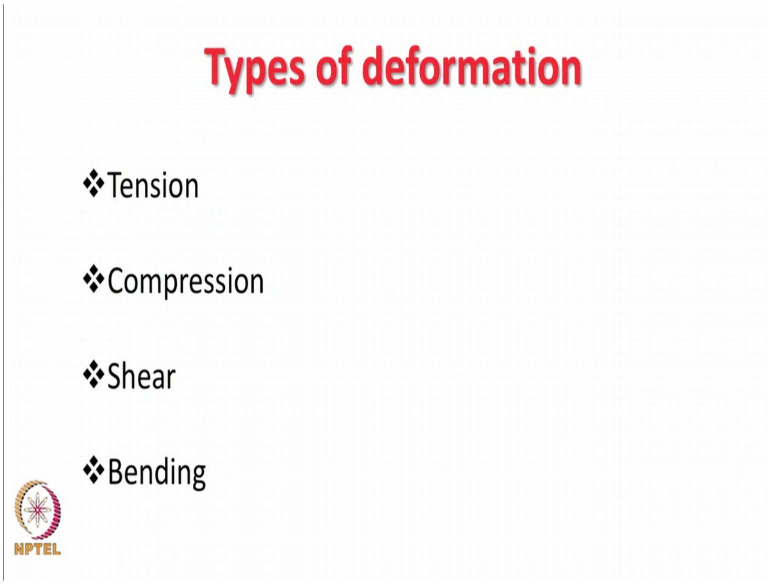

So, what are the different types of deformation that we can subject either a solid or a liquid to. So, of course, taking the example of the spring you can exert something, you can tense it or you can the element can be under tension; that means, you stretch it in in contrast to it you can compress it also. So, for a spring for example, the resistance it provides in tension and in compression is the same, you can share it. So, what do you mean by? So, let me draw what is tension and what is compression is already clear to you.

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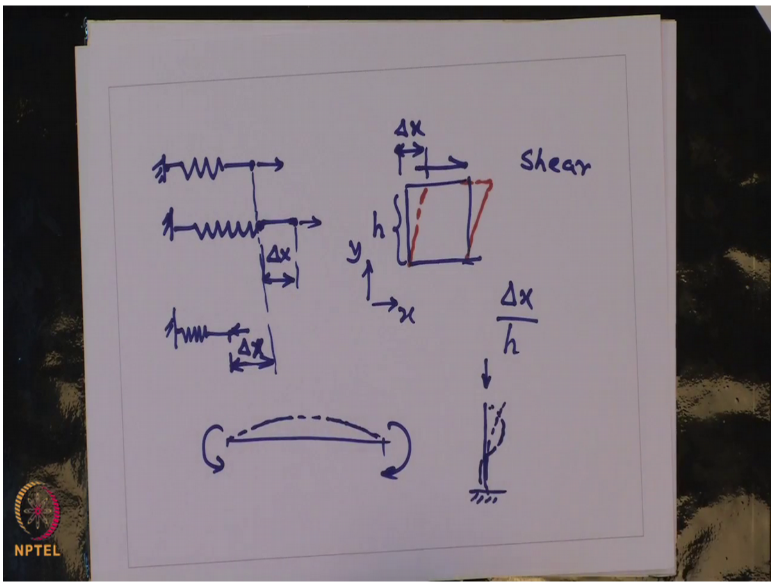

So, you have a spring of certain length. So, let us say this end is fixed and you exert a force.

So, what we will happen this spring, with we will stretch to another configuration. So, this force is there and you see that this length you will have an extension delta x is your stretch we can have the similar case when it is compressed. So, compared to the original configuration this is your delta x what is shear. So, imagine I take a block, and I exert force in this direction. So, let me say this is x and this is y axis. I exert a force in the horizontal direction on the top surface. So, what you will have is a structure where the deformed configuration will looks something like ok.

So, this is called is called as shear and you can have again this displacement at the top which is delta x and let us say this block is of height h. So, you define the shear as delta x by h and you can have something like bending. So, if you take a long object and you tell exert a force which tries to bend it. So, this will exert have a configuration like this. So, this is an example of bending.

So, similarly you can have you know this bending can happen as a consequence of compressive forces also. As an example let us say you have a you know you have a long prismatic beam, which is anchored at one side and you exert a force a compressive force from the top. You can have a geometry where if you have slightly offset this might be one configuration it in which this rod might have or you can have a configuration like this as well ok.

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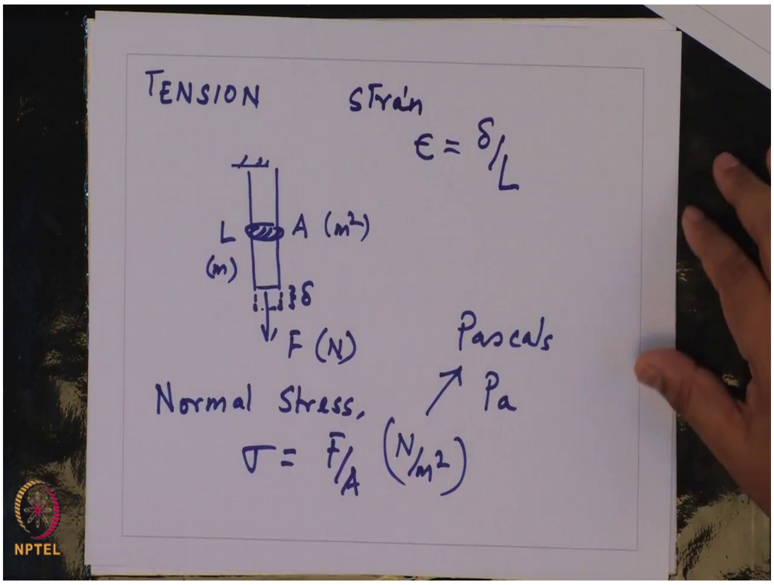

So, these are different bending configurations; now coming back. So, when you exert this forces. So, let us take the simplest case of tension or compression or let us say tension if you take a beam of length L let us say and in exert a force F. Let us assume that the cross sectional area of this beam is A, A is in meter square has units of meter square length has units of meters forces units of Newton's ok.

So, how do you do so? When you stretch it, it will again stretch by a certain distance let us say this extra length is delta. So, you have force. So, you define stress normal stress sigma is given by F by A. So, it has units of newton per meter square newton per meter square is called Pascal's. So, in short it is written as P a capital P and small a. So, unit of stress is Pascal's. So, for example,. So, when you have. So, the unit is Pascal. So, whether it be tension or compression your unit of stress is in Pascal's. Now when you stretch it you so, the percentage elongation the axial elongation epsilon is given by delta by the original length l and this is defined definition of strain ok.

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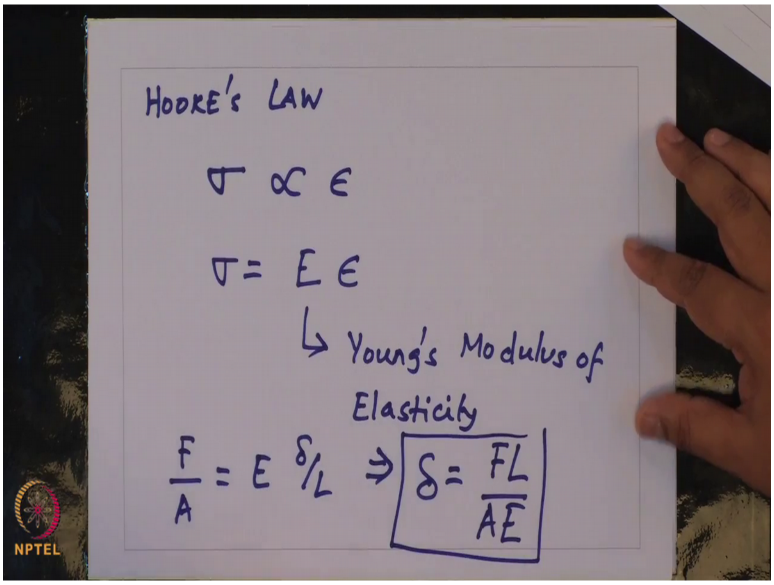

So, you have all heard of Hooke's law and what is the law state that when you stretch it your stress is proportional to the strain. So, sigma is proportional to the strain epsilon, and you can write it as a proportionality constant which is given by E this E is called a Young's modulus of elasticity. So, from here this expression you also know. So, sigma is given by F by A is E times delta by L. So, this expression can be modified to find out how much given the Young's modulus of a material, if you exert a force Ff if I had it has the length of L and a cross sectional area of A how much will it stretch and this will be given by FL by A times E you have delta h al by a fl by a ok.

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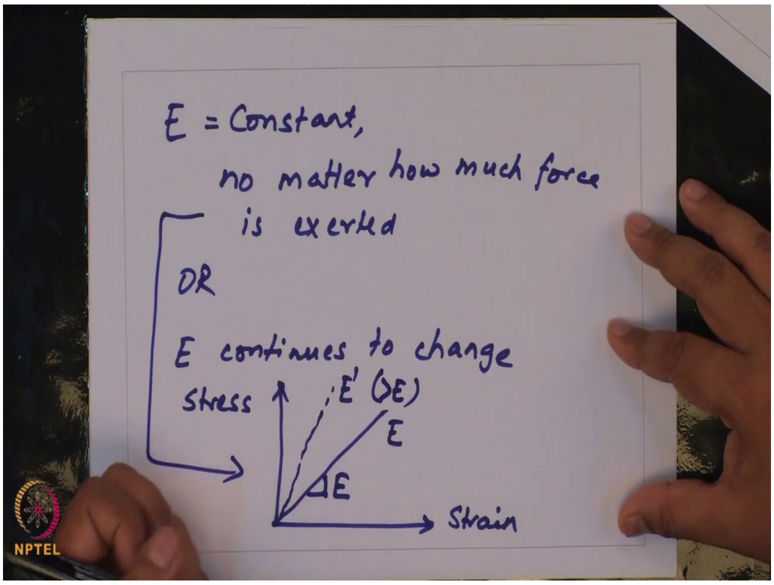

Now, if you consider this Young's modulus E right. So, if you can have two situations in which E is constant no matter how much force is exerted or E continues to change. So, this is an example. So, if you come. So, let us assume E is constant. So, then how will my stress versus strain curve look. So, if I plot stress versus strain, if E is constant then you will have a linear a straight line the slope being E. So, if a material is stiffer then you can have the slope will increase. So, let us say if it is E and this is E prime and E prime is greater than E then the slope of this line will increase.

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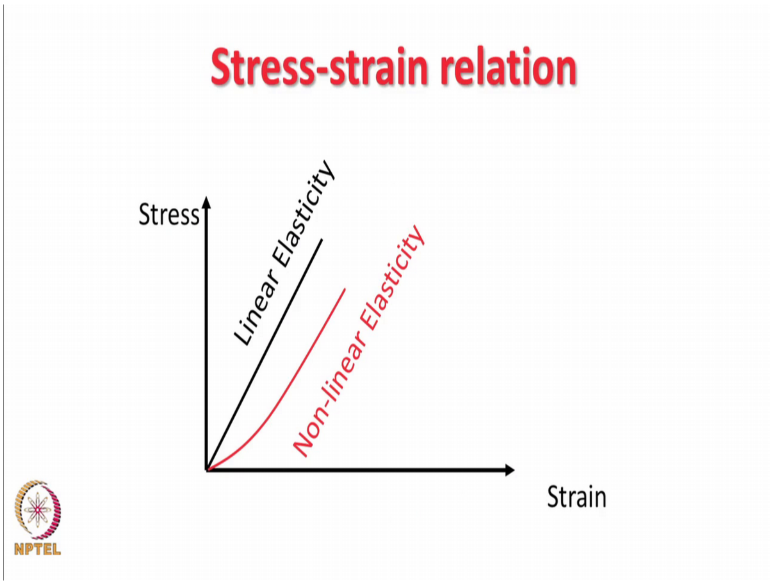

However, if you look at biomaterials or biopolymer networks in many cases instead of this linear elasticity, stress versus strain is straight line; that means E is constant right. So, E is the slope of stress versus strain line. So, this corresponds to linear elasticity, but in red I have drawn this curve where your slope is not constant slope keeps changing. So, this is an example of non-linear elasticity. So, we quickly come and see how elasticity will change ok.

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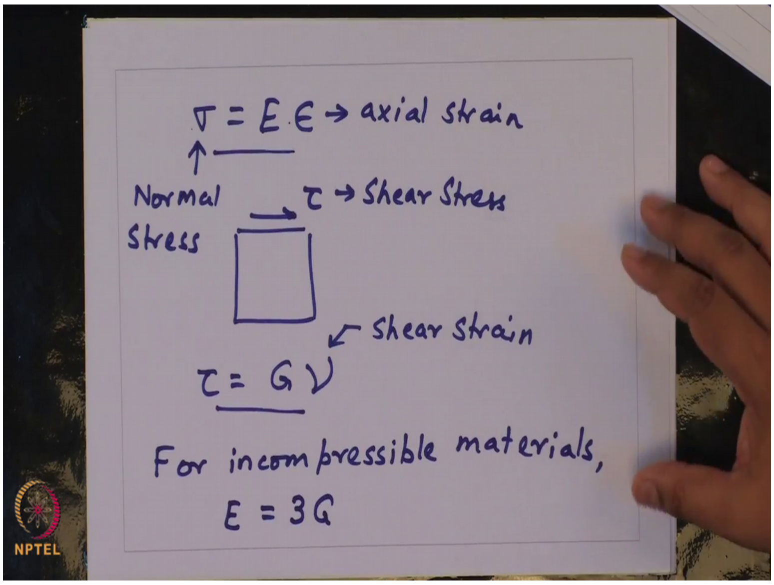

So, for when you exert ensured right. So, I wrote this equation sigma is equal to E times epsilon, if you have a material in which you are exerting shear. So, you can define just like. So, this is normal stress you can define this is a shear stress. So, for sheer stress you can write down the equation sigma is equal to G gamma, where gamma is the shear strain epsilon is our axial strain ok.

So, what you see? The equation is very similar this is normal stress axial strength shear stress shear strain. So, there must be a relationship which connects E and G for incompressible materials. So, this this is another aspect for incompressible materials, it can be shown that E is equal to three times G ok.

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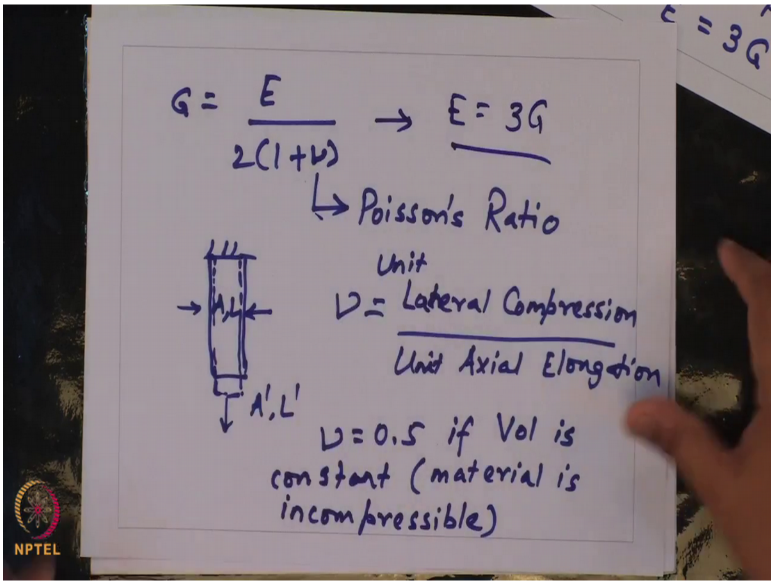

This is because G and E are connected by the equation g is given by E by 2 into 1 plus nu where nu is called the poisson ratio and what is Poisson's ratio? Poisson's ratio is when you for example, if you take this example you had gone from this length area A and length L to let us say area A prime and length L prime ok.

So, poison ratio is given by lateral strain by longitudinal strain. So, when you push pull something along this axis, then it will get compressed in a perpendicular axis. So, Poisson's ratio is that Poisson's ratio is given by lateral unit lateral compression by unit axial elongation. So, it can be shown that nu is equal to 0.5, if volume is constant that is material is incompressible. So, if you put nu is equal to half here then you get back the equation E is equal to 3 times G is what I wrote in the earlier slide.

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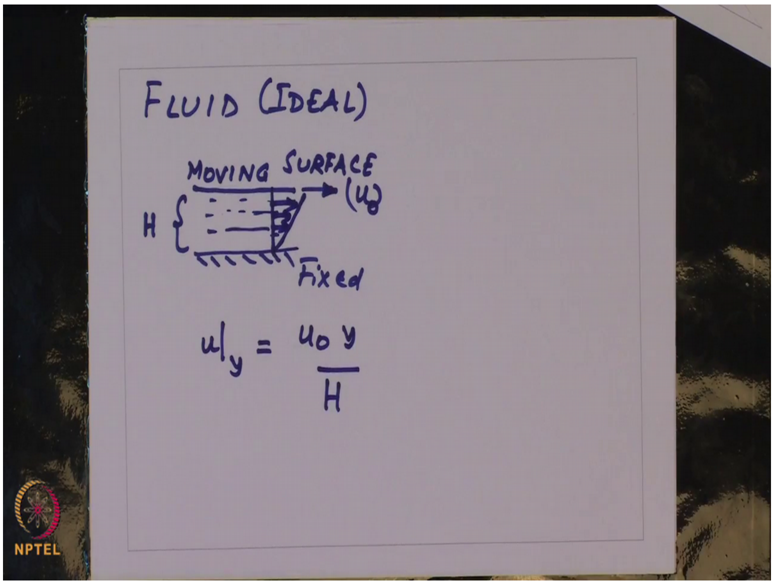

So, this is for a solid now think of a fluid let us assumed. So, thinking of a perfect fluid or an ideal fluid for the case of an ideal fluid. Let us assume you have a surface which is fixed you have fluid on top of it and you put a top surface which you pull. So, this is the top surface this is a moving surface which you pull at a speed of u. So, if this height is h let us say the top speed is u naught, it can be shown that for ideal fluids you will have a velocity profile. So, when you pull the top surface at the speed of u naught, the fluid particles of all the layers will keep on moving and you will get a flow profile which is linearly increasing ok.

So, you have flow profile u at height of y will be given as simply as u naught into y by H ok.

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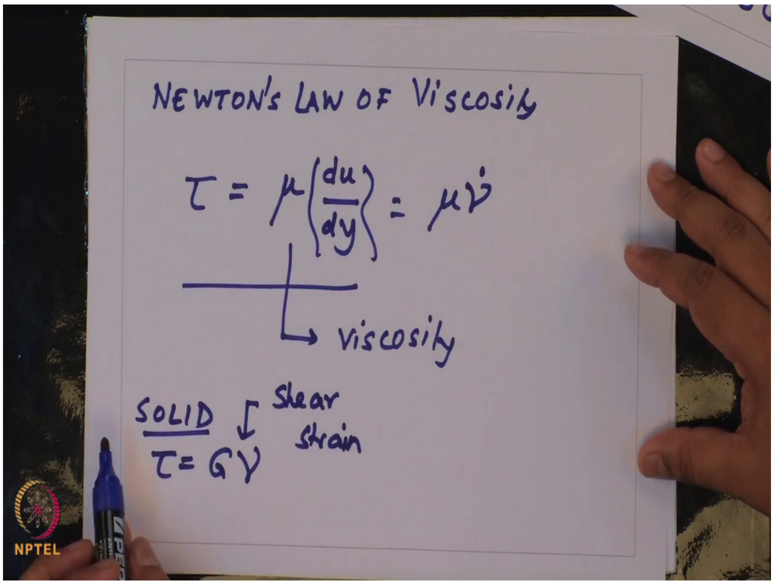

So, this is a flow profile. So, based on this you can have the simple law of viscosity. So, there is Newton's law of viscosity, which says that the shear stress is related to the gradient it says that shear stress is related to shear rate, via this constant of proportionality mu, mu is called the viscosity. So, if you see. So, this can also be written as mu into gamma dot. So, in contrast to the earlier case in contrast to our shear so, for the solid, we had this equation tau is equal to G times gamma.

So, this is my shear strain for a fluid my shear stress is related to not the shear strain, but the shear strain rate which is gamma dot by the constant of proportionality which is mu or the viscosity.

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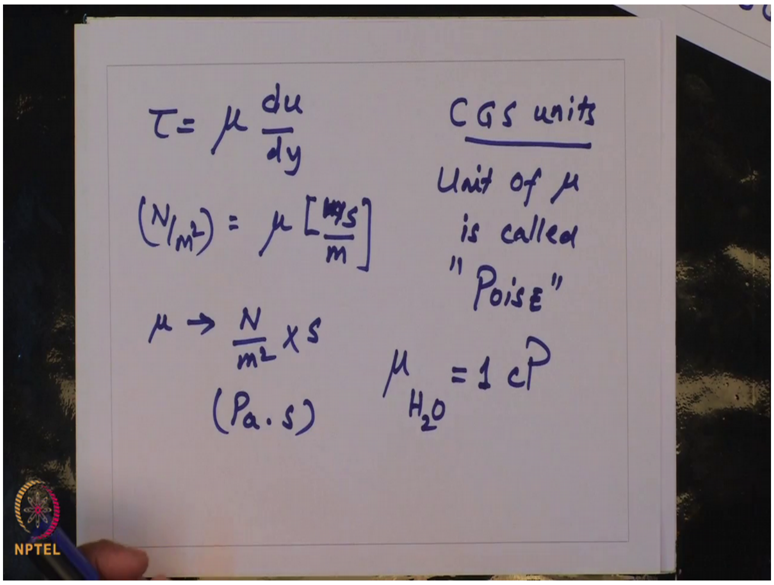

So, what units does mu have? So, tau has units of stress. So, it should be Pascal's. So, I have tau is equal to mu times d u by dy, tau has units of Pascal's or newton per meter square mu times u is meter per second and divided by meter ok.

So, we have units of mu has to be newton per meter square into second or this is called Pascal second you see units of viscosity. So, in CGS units unit of mu is called poise. So, viscosity of water is 1 cP. So, this is written as simple P. So, viscosity of water is 1 centipoises ok.

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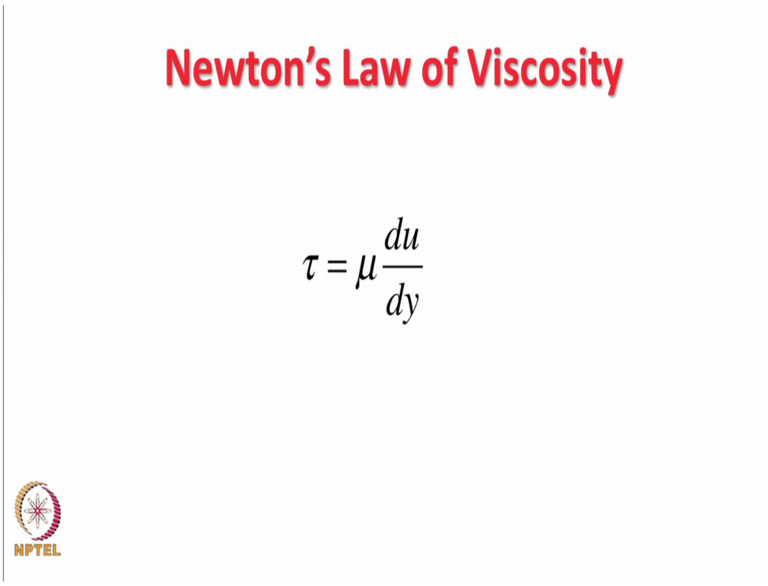

So, this is what Newton's law of viscosity suggests. So, we have gone through the equation now this brings us to the question.

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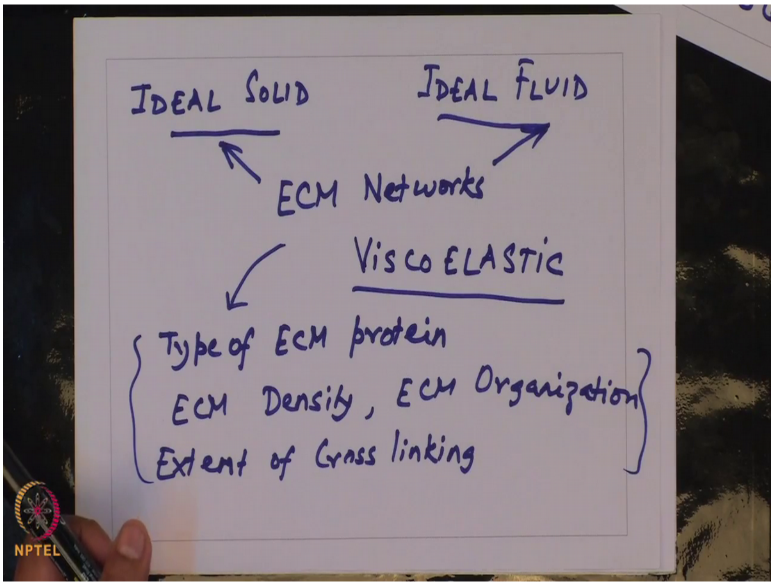

So, we have two extremes an ideal solid and an ideal fluid ok.

So, if you talk of ECM networks. So, where do the lie? Is an ECM network an ideal solid or is ECM return an ideal fluid. So, it depends on various things including the type of protein ECM density extent of cross linking ECM organizations. So, it is not just that it is uniquely determined depending on how you make your ECM network or hydrogen you can have a range of different properties. So, in essential ECM network has some aspect of solid and some aspect of fluid and their hence called. So, ECM networks are generally viscoelastic. ECM networks are inherently viscoelastic depending on how you prepare you might have an solid or a fluid or some property of both.

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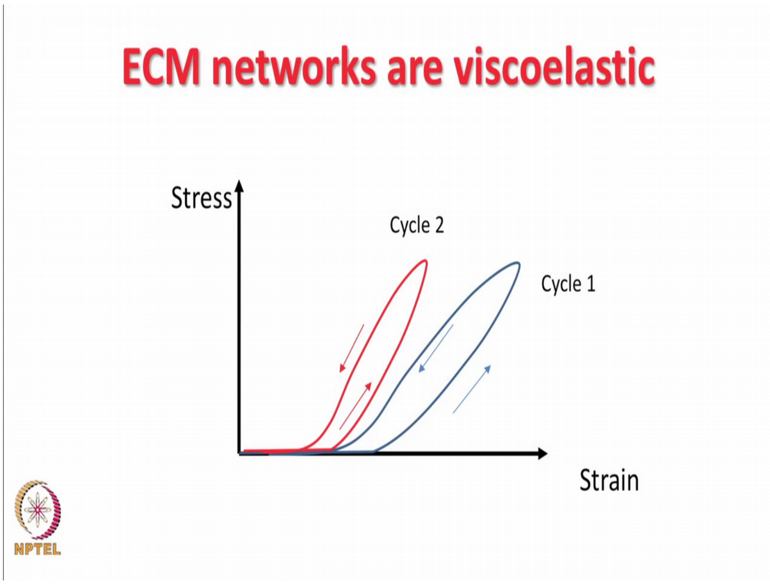

So, this is an example of how a stress strain curve would look for a collagen hydrogen that you fabricate. So, what you see. So, these arrows indicate that at the time. So, what you are doing is using a let us say our stretching device you take a gel. So, you take a gel, this is your hydrogen.

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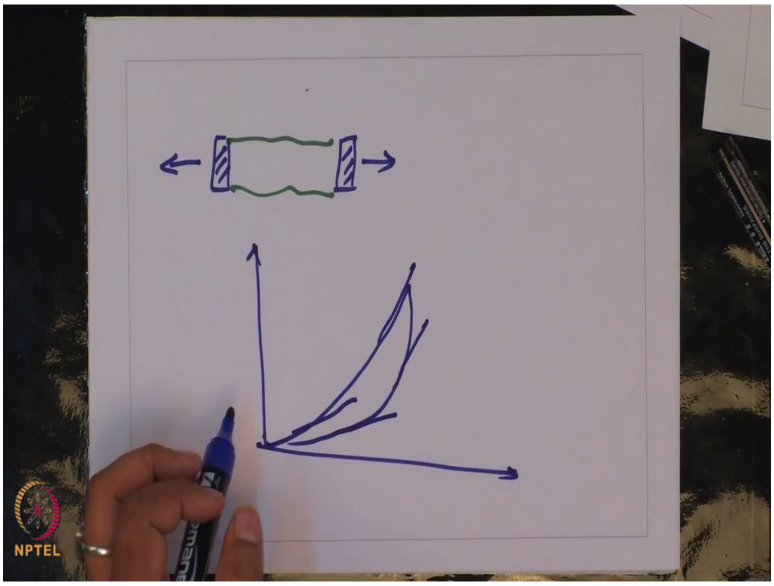

And then you can stretch it back and forth and that is what is referred to as this cycle 1 and cycle 2. So, what you are doing is you are imposing the strain up to a certain point letting it go letting it relax to its original configuration and again stretching it and rinsing it go. So, what you have are these cycles. So, what it shows you that the path the stress strain curve follows at that when you stretch it, is different than the one it follows when you relax. It that is one of the points to be noted the second point is that between cycle 1 and cycle 2 there is a big difference suggesting that something has happened as a consequence of that one cycle of forcing because of which the cycle 2 is different.

So, if I were to approximate them a straight lines if. So, by curves are looking like this ok even if I were to draw the slope of this. So, the slope of this keeps changing, from one cycle to the other. So, suggesting that as you stretch the networking is getting softer and softer. So, if I take the slope this slope is either this slope is less than this slope. So, the network gets is not getting softer. So, it gets it getting difference differ.

So, with that I stop here for this lecture. So, to recap what we have discussed is just a brief background of how to characterize properties of solids and fluids, and with that background we will now try to understand; what are the aspects of a net a net ECM network, which makes it behave like a solid or a fluid or something in between.

Thank you for your attention.