Introduction to Mechanobiology
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Week - 02
Lecture - 06
Properties of collagen networks

Hello and welcome to today's lecture. So, in the last class we were discussing how to go about quantifying or characterizing the properties of ECM networks and in that regard we had the you know introduced the terms of rheology which is the field of that study is how materials deform when subjected to force. We had also discussed for the different types of deformation we can impose on a material.

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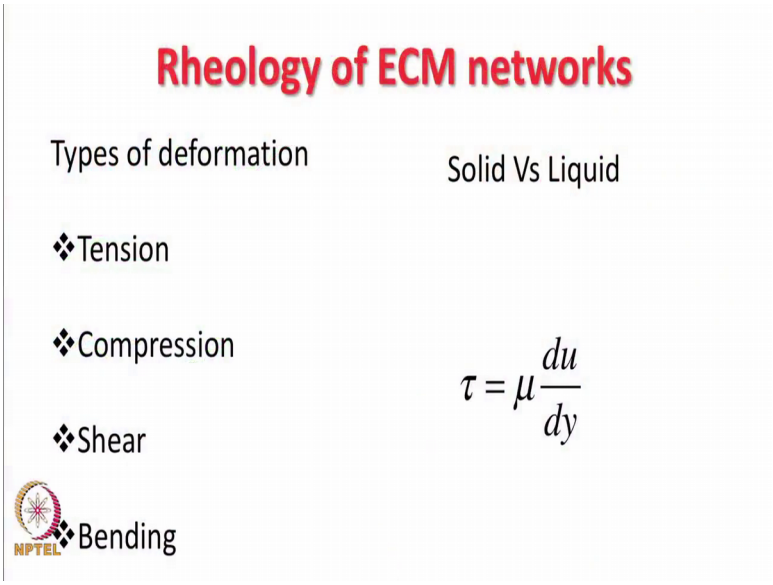

So, these include tension compression shear and bending. So, tension compression is very clear bending is where instead of something straight you exert forces so that the final configuration is something like this.

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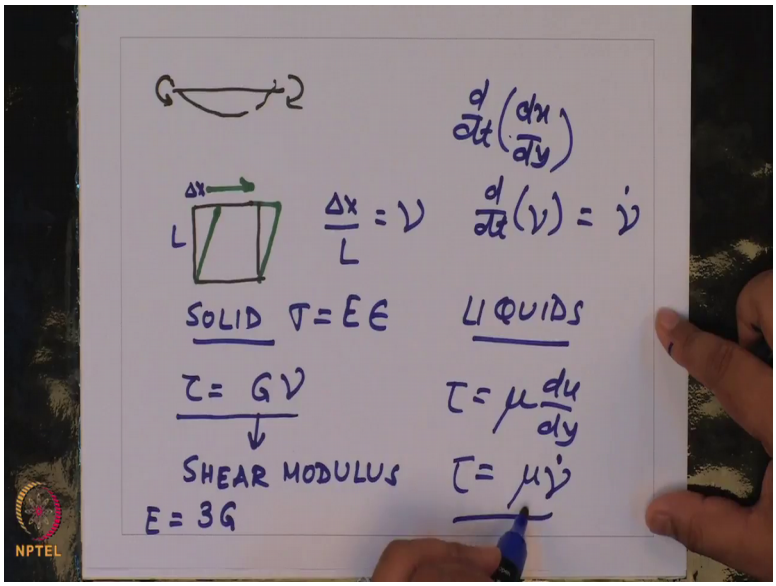

I am sure we had discussed in great details where if you have a block you insert tangential forces on the top surface as a consequence of which the block deforms to a shape like this, and this delta x, so delta x by l this is my length l and this is delta x this is what is given by your shear.

Now, we also said that how what is the difference between behavior of a solid idea perfect solid versus a perfectly and the main difference is for a solid you write down this expression well shear stress is given by G times gamma G is called the shear modulus and G. So, for incompressible materials we have shown that Young's modulus if elasticity. So, this is shear you can write sigma is equal to E epsilon for normal stresses. So, E is given by 3 times G for incompressible materials.

So, tau is equal to G gamma for solids and for liquids you have Newton's law of viscosity this tau is equal to mu into du dy and what is du dy. So, du dy is nothing, but d dt of dx by dy. So, dx by dy is nothing, but the definition of shear which is gamma. So, this is nothing, but d dt of gamma this is equal to gamma dot. So, this equation is same as writing tau is equal to mu gamma dot. So, the main difference is in a solid tau is proportional to shear for a liquid tau is proportional to shear rate the constant of proportionality here is the shear modulus the constant of proportionality here mu is viscosity right.

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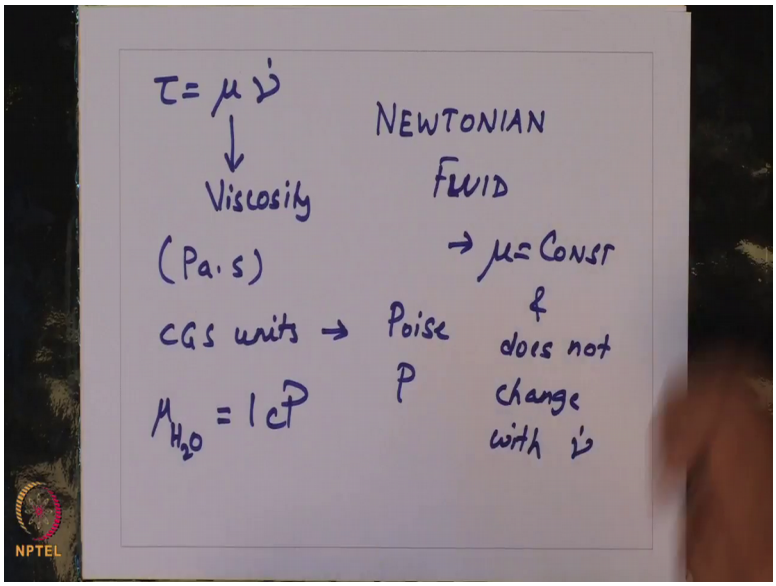

So, for tau is equal to mu gamma dot, mu is viscosity and we showed so units of mu is Pascal second in mks units and in CGS units we call it as poise or simple P we write it as simple P ok. So, mu of water is one centipoise at room temperature. So, also important to say that mu has a dependency on the temperature. Now for materials where mu is constant independent of shear rate this is called a Newtonian fluid, mu is constant and does not change with gamma dot.

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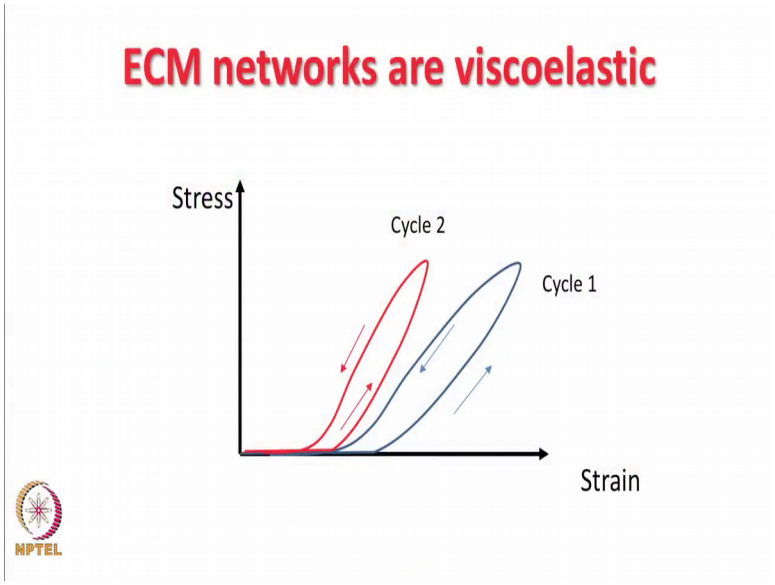

So, I had shown this slide in last class saying that ECM networks are viscoelastic.

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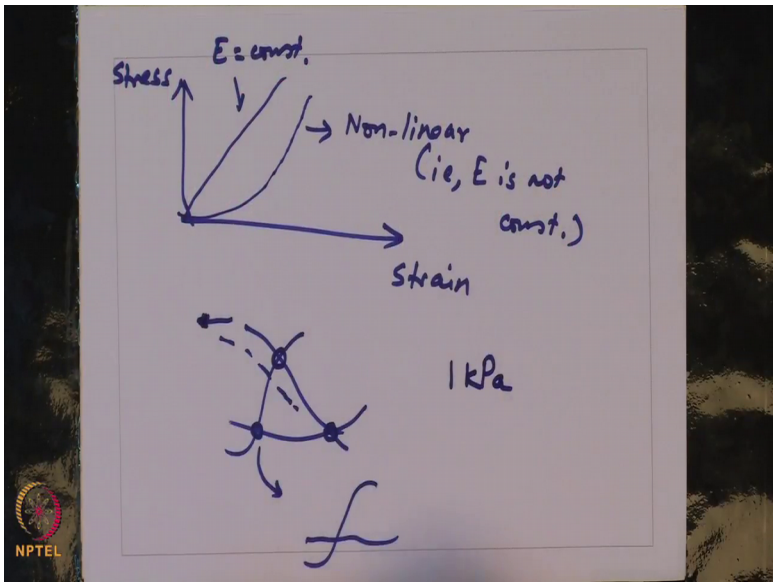

So, as opposed to let us say this is my stress strain curve for linear elastic materials my stress strain is linear, for non-linear elastic materials, so this is non-linear that is E is not constant and here E is constant why because the slope of the line is E. For viscoelastic materials like ECM networks, this is a simple example of how the stress strain curve would look if you make a collagen gel and put it in a uniaxial tester which kind of keeps on pulling it repeatedly. So, what you see is so the repetition is corresponds to the cycle number and what you see is the path by which it follows in the stress strain curve when you exert when you stretch it and when you relax it is different and also the average slopes of these lines keeps on increasing with the increasing passage of cycle number. Suggesting that there are some dynamic rearrangements with happen.

So, this is what we want to focus now as to what are those attributes of a ECM network which dictates is functional properties. And you would remember this picture I drew from last class saying that the scaffold that you create in terms of its organization here I have drawn one as random and one has aligned these have robust effects on how cells process these cues.

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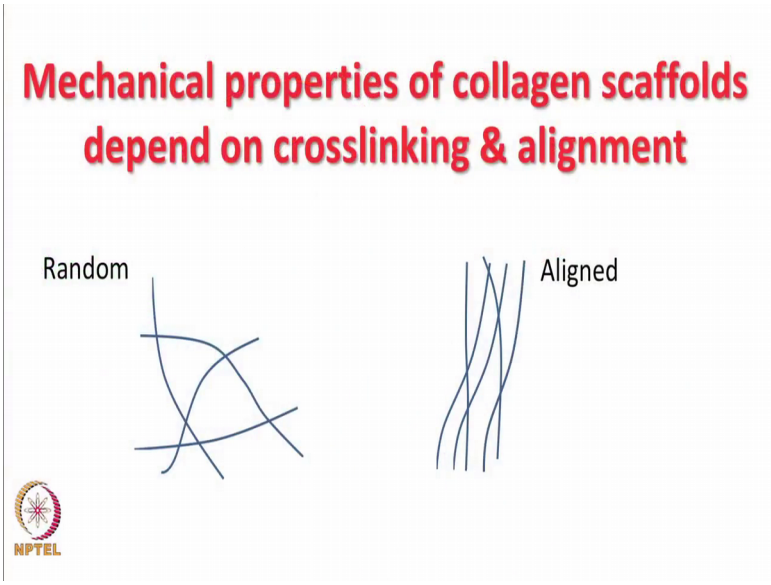

So, for example, on a random matrix cells will tend to migrate in a random fashion without any persistent motion. While on an align matrix like that the cells will not only stretch itself along the alignment direction of these fibers, but also tend to migrate along these aligned fibers taking them as a directional cue. So, how do we characterize the properties of these random versus align matrices? And what I suggest is the properties of these collagen scaffolds depends on two things cross linking and alignment. So, alignment is very clear in this, but for the same network. So, imagine I had raised this analogy of cooked chowmein right. So, when you try to extract one strand of noodles from the entire bunch, so this does not come out so easily even though there is no chemical glue which is bonding them together there is relative friction ok.

So, even a random network will exhibit some resistance, which means it has some bulk stiffness. But if I fought this random network let us say I had this random network in one case if this network is just that these fibers are put together and I exert force on this fiber. So, I will lead to a configuration, but this might slowly come out of this network leaving the remaining two fibers like this. So, in this case the properties of the network will change dynamically versus if you had fixed all these points together in other words you had cross linked the network then when you exert a force here this gets transmitted to the entire network. So, the network resists that force as a whole. So, the response that you would observed for such a network is drastically different if it was not cross linked purses it was cross linked similarly, the case of alignment.

So, what would be an equipment with which we might be able to probe these properties note that these networks are super soft. So, typically a collagen network might have a stiffness order of one kPa. So, this is super soft. So, you cannot use traditional instrument used in material science like indentures which can only probe the material properties of stiff materials. So, that brings us to what we called a rheometer. So, what is the rheometer?

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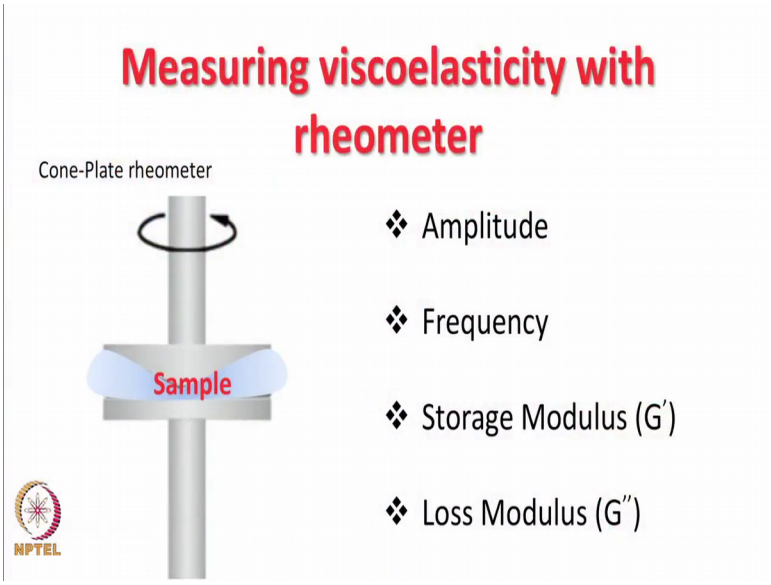

This is a picture of a rheometer where you have a sample which is placed between two things one portion of switch the bottom portion of switch is fixed and the top portion of switch is rotated. So, imagine a mortar and pestle in which the top thing you keep on rotating. So, if you put a sample in between when you exert this rotation the sample is essentially being sheared. So, you can essentially probe its shear properties shear modulus and if you knew the poisons ratio of the material you can backtrack what is the Young's modulus of elasticity.

So, several things I wish to say about the rheometer. So, you have what you can do. So, you can do two things first is you can change.

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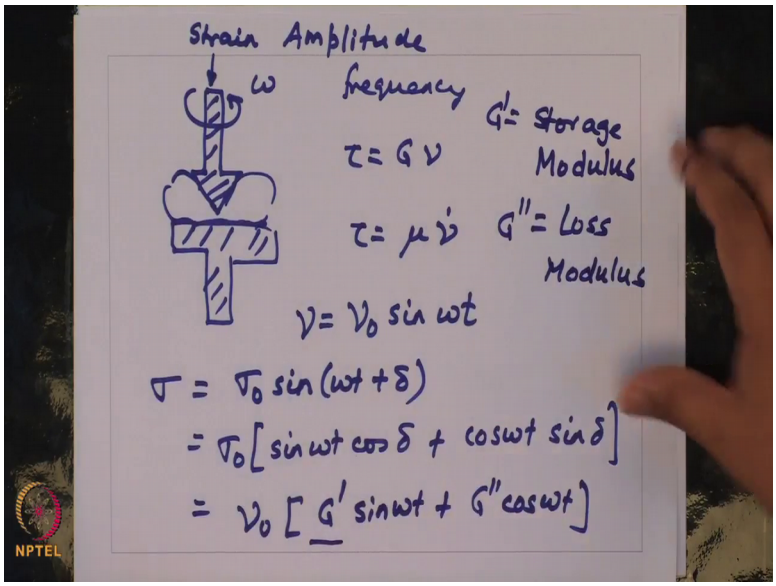

So, you have this set up where you have a sample. So, this one you are rotating. So, you have control over the rate at which you are rotating you have also control over how much you can indent you can push into the sample. So, this is called, so this is your imposing a strain with how much your pushing the sample and your controlling the amplitude of rotation the frequency of rotation. So, strain amplitude is one thing we are controlling and we are controlling the frequency. So, again for a pure solid we have T is equal to G gamma for pure fluid we have T is equal to mu gamma dot.

So, in general if I were to apply a oscillatory fluid in other words I can write if I were to write gamma as gamma naught sin of omega T if you exert an oscillatory strain then your stress field can be written as sigma 0 sin of omega T plus delta. So, if you expand this equation, this sigma is then written as gamma dot into; so it has two components G prime sin omega 2 and G double prime cos omega T this term G prime is called the storage modulus. So, G prime and G double prime is called the loss modulus.

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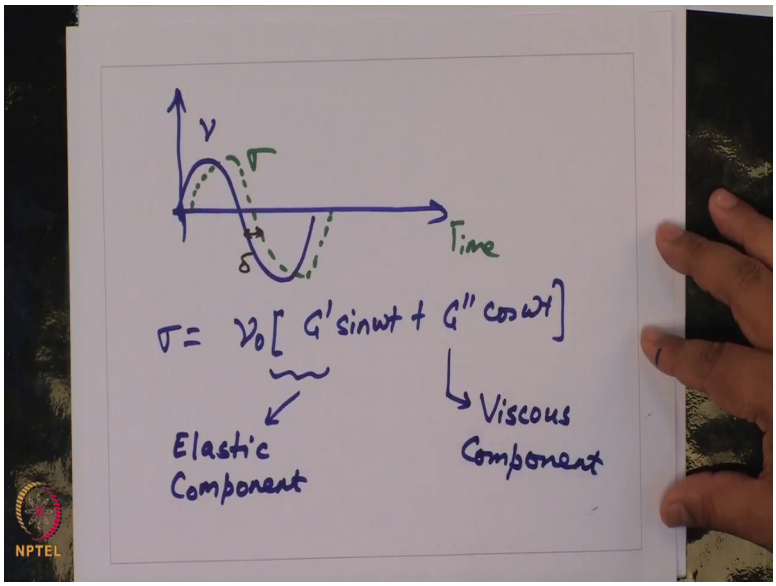

So, if I were to pictorially depicted you would have a curve like this. If this is your gamma function you have a phase shift this is what delta is, this is gamma and this is sigma this is time. So, you have one component, so you have sigma is equal to gamma dot into G prime sin omega T plus G double prime cos omega T. So, you have this component which is in phase with the applied strain field which means that as soon as you exert the strain immediately the material deforms. So, G prime or the storage modulus corresponds to the elastic component this corresponds to the elastic component and it is called the storage modulus because this energy can be retrieved when you release it you again go back to the original configuration. The G double prime is the viscous component and hence it is called the loss modulus because this is dissipated, this is dissipated.

So, how, as I said that when I make this ECM network what can I say about G prime and G double prime. What is known in the literature and has been shown by many groups that if you make gels of various concentrations.

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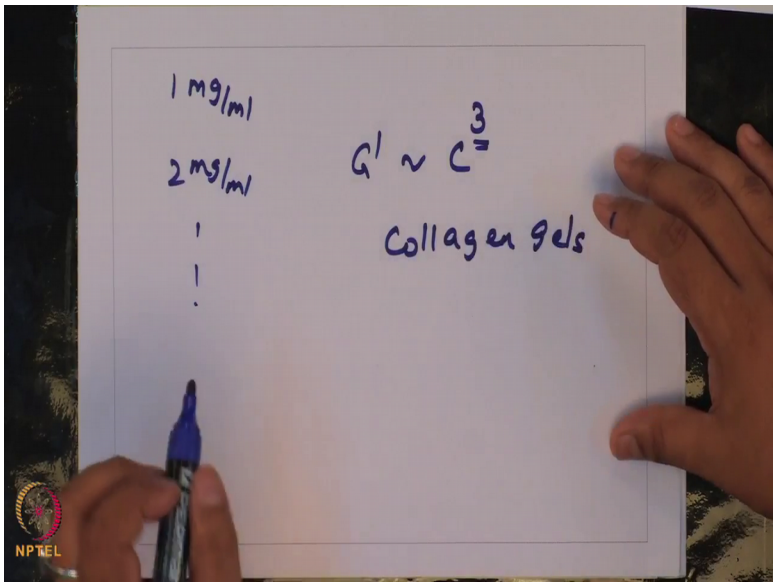

It means that if you vary the concentration that say 1 mig per mil 2 mig per mil etcetera. So, your G prime will scale as concentration cubed x is roughly 3 for collagen gels, for collagen gels G prime will scale as concentration cubed which means that in order to stiffen a matrix you can increase its consecration; however, since cells directly bind to this material when your changing the concentration your changing the bulk stiffness, but at the same time you are changing ligand density. So, this is one problem of working with collagen gels if you want to probe the independent effect of ligand density keeping stiffness constant that is why people tend to use synthetic substrates where they can control its stiffness and then independently functionalized ECM proteins like collagen onto those substrates.

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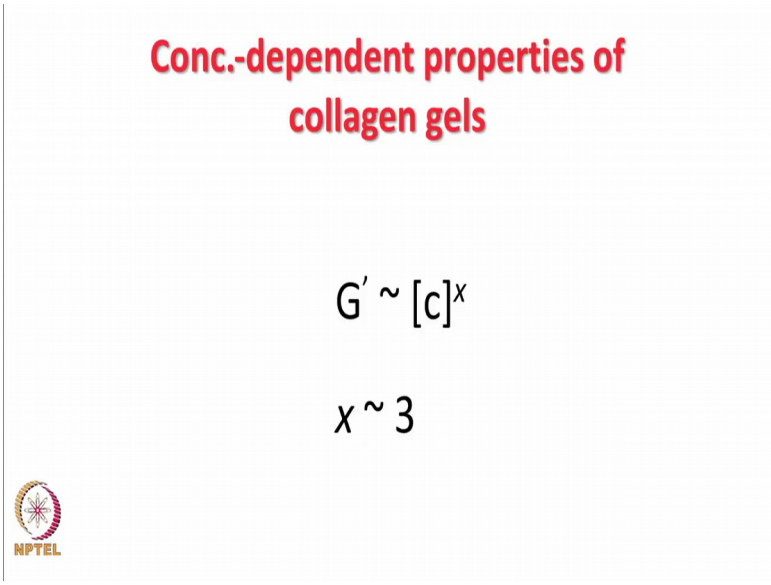

So, this is one aspect, other aspect again coming back to the linear versus you know non-linear materials.

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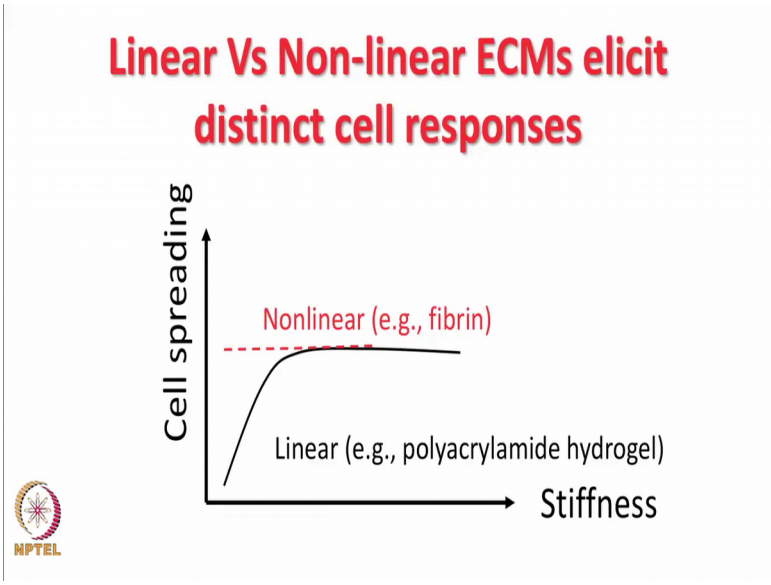

So, what I have shown here is how cells would spread on materials of different stiffnesses if it was a linear material linear elastic material case of polyacrylamide hydrogel or a non-linear material. So, what you see of cells spreading in linear materials is that when you increase the stiffness cells spread more and more and beyond a certain threshold there is no change in cells spreading it saturates. But if you take a non-linear

material no matter what concentration you start from whatever is this initial stiffness you see that the cell spreading does not have any dependents on stiffness. So, suggesting again that there are these rearrangements which can happen which can drive this increase in stiffening.

So, this is saying that whatever be the starting stiffness when you put cells on it which pull on these matrices the matrices deform and becomes differ in the process. So, this is called strain stiffening, this is called strain stiffening and for fibrin similar to fibrin collagen gels also have been shown to exhibit this profile. So, if I plot the storage modulus as a function of strain what you find is at a given concentration first your storage modulus is constant and beyond a certain strain beyond a certain strain it starts to increase. So, it becomes non-linear in nature ok. So, this phenomena is called strain stiffening.

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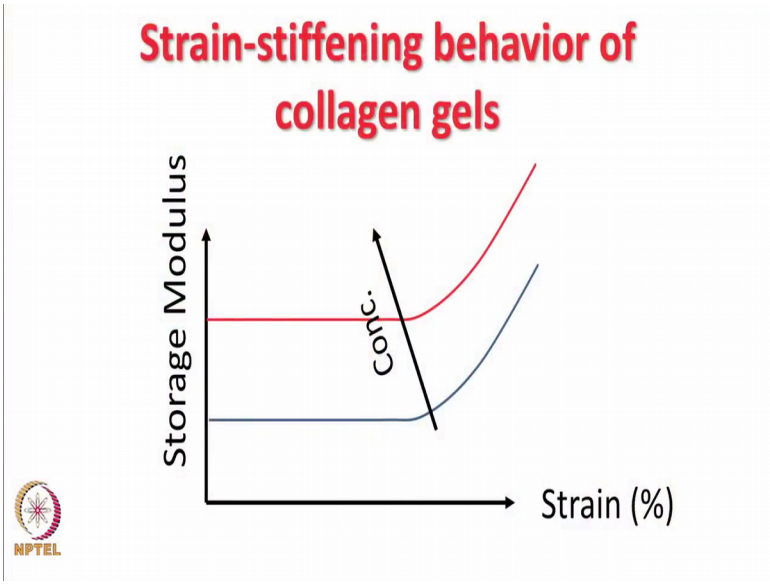

And then the red line, you have another variable because we are called just by the concentration alone, but playing with the concentration alone you can get more and more stiffening also. So, that is why the red curve is above initial again flat and then it rises. So, you see that there are two independent ways in which you can in which cells can different collagen gels.

So, first is for a given ligand density just by exerting forces the gels becomes different stiffer and the other is that just by playing with the concentration alone you can generate

materials of difference differences. So, this brings us to the question that what is driving this behavior of strains stiffening and what it turns out is as I was drawing before when you have increase in strain then individual fibers tend to along along the direction of strain. So, as oppose to having a network like this when everything gets aligned then the fibers then the effective stiffness increases in that direction. So, an isotropic thus stiffness is an isotropic invention in one direction it is very stiff and it can be experimentally probed by shaking the orientation of fibers within a gel.

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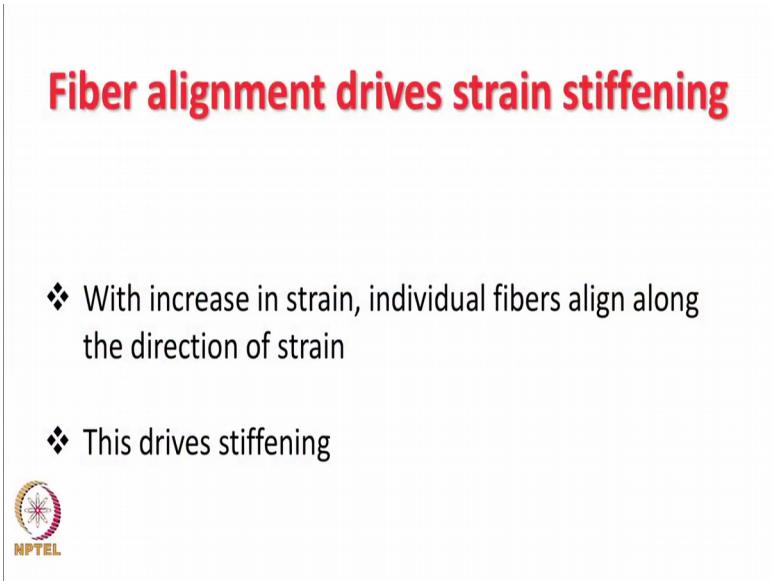

So if I take a collagen gel let us say where you have fibers in all directions and I align them actually I pull this matrix along the long axis.

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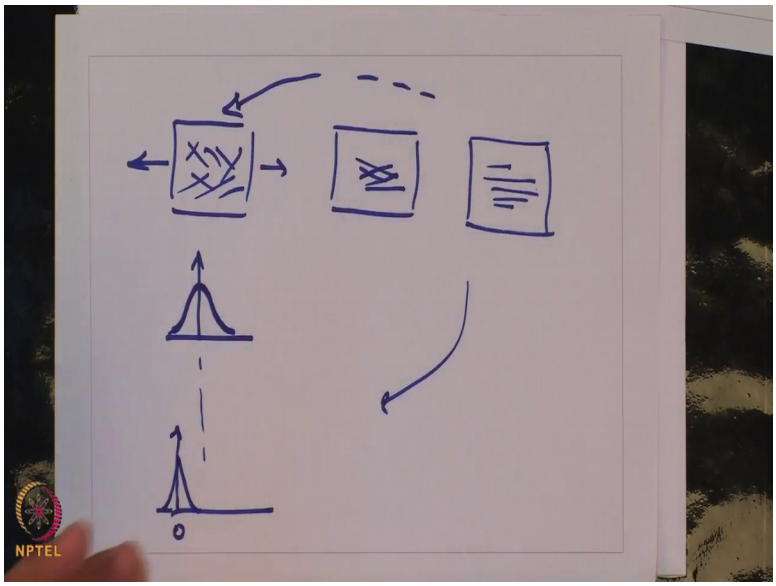

So, if I draw the average orientation as a function every orientation of fibers what I will find is I will find a uniform distribution a normal distribution, which suggests that there is equal propensity to find fibers which are oriented in one direction or the other direction, but as I align more and more. So, as I align more and more you will see they will generate this kind of geometries eventually where everything is aligned in one direction. So, your average axis, your average angle will change and from here in this particular case you will see that everything will align along 0 this is 0 close to 0 you will see everything will align along 0. So, this is what drives differing.

However, one of the things about this network is if it was not cross linked, if it was not cross linked when you remove the force when you remove the force the alignment of these fibers remains in the final configuration. So, from here you do not go back to this configuration.

So, how would you do that and the way to do that is essentially cross linking. So, the way to do this is to cross link it. So, when you have two fibers if they are cross linked at this point when you tend to force along this direction they will align, but as soon as you remove the force they will come back to its original configuration. So, this suggests that you can do these experiments in which you can track the angle distribution as a function of pulling and as a function of cross linking. So, you will see that if it was an uncrossing gel that you made your angle evolves and then stays put as the final configuration, but if

you take a network which was cross linked wanting to see that if it was 100 percent cross linked first it will even its basics basils stiffness will be increasing the other thing is it will keep on returning to the same position.

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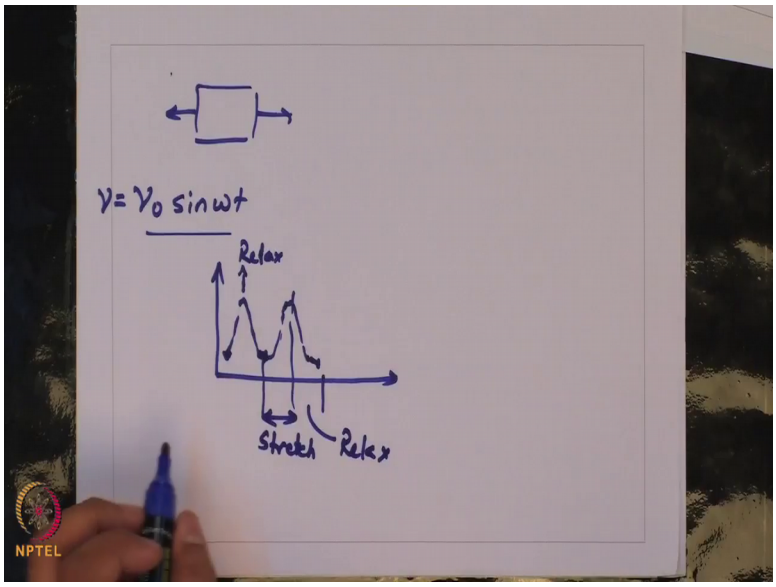

So, if you do it in an oscillator you way, let us say what I am doing is I am doing it in a sin wt fashion. So, my strain is some gamma naught into sin wt along the accesses. So, what you will see is the angle distribution will keep on changing like. So, let us say your angle increases and when you really release at this point you release relax the angle will keep on coming to the same position again you increase. So, this in this stage you stretch here you relax.

So, your angle will keep coming back to the same configuration after you remove. So, that is about it for our discussion of collagen and their material properties.

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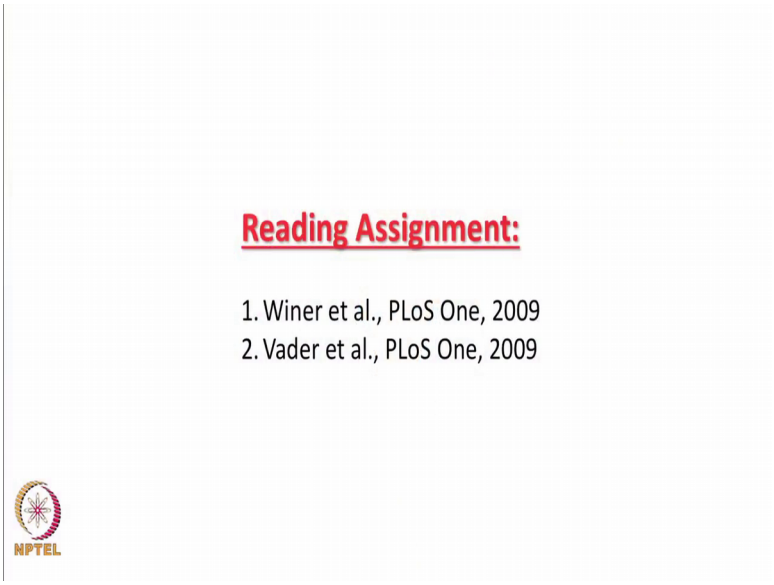

I would ask you to read these two papers - one on which and how cells can sense non-linear stiffness and how they change their properties in response to non-linear stiffness and the other one this paper, the other paper is about this orientation and effect of cross linking and alignment on these fibers. So, both these papers are in; I have been published in plus one which is an open access journal which means that you can download these papers anywhere you do not need any special access to be able to download these papers.

So, in this course I will give you reading assignments in which I would recommend you to read certain papers. So, it is mandatory if you want to follow, if you want to follow up of what we are doing in class then it is mandatory that you read these papers even the quiz questions that will be asked in the course will be posed from these particular papers and the content which has been covered in the class. So, that brings us to close on our discussion about collagen we would discuss about one more protein, so I do not have much time today I will just introduce this protein is fibronectin.

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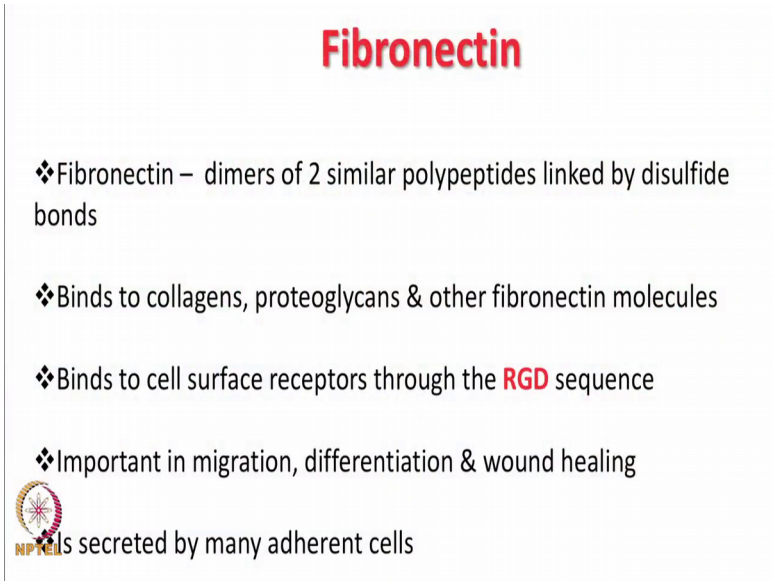

So, fibronectin is a protein which has dimers of 2 similar polypeptides which are linked by disulfide points disulfide bonds. And fibronectin has that RGD sequence which is critical for cell addition, and that has led to peptides addition peptides of RGD being alone used for sustaining cell spreading or cell addition.

So, fibronectin is known to bind to polygene (Refer Time: 24:22) and other things is plays very important role in migration and differentiation wound healing and it is secreted by many adherent cells.

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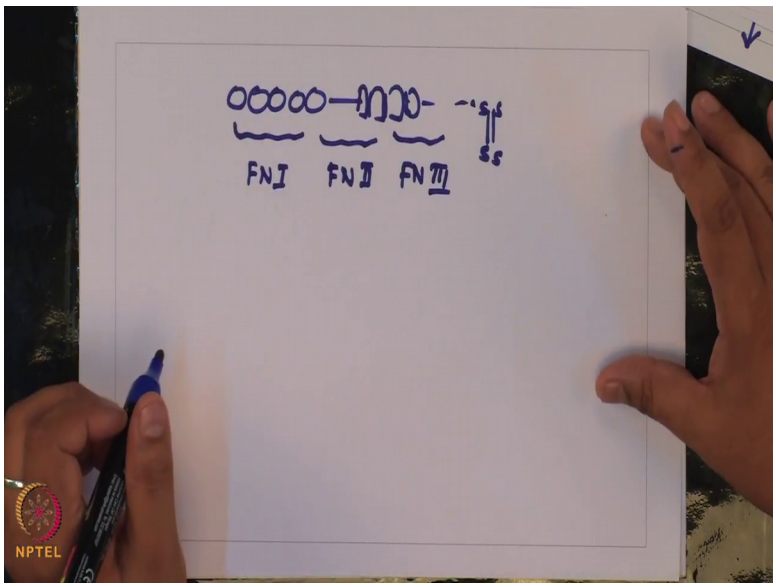

So, if I were to draw the structure of fibronectin so you will have multiple units like this and at the end you have these disulfide bonds. So, these individual domains are referred to as FN 1, FN 2 and FN 3 domains. So, you know for any protein which has multiple domains for its function it folds into one particular structure.

So, the question that I am going to ask is how does folding of the protein participate in regulating signaling and how does forces influence the folding kinetics of this protein and its role in subsequent function. So, this is just to give you an intro to fabricate. We will start discussing in detail about fibronectin in next class.

Thank you.