Introduction to Mechanobiology
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Week – 02
Lecture – 07
Rheology

Hello and welcome to our 7 th lecture for the course introduction to mechanobiology. Over the last 2 lectures we have been discussing about properties of ECM networks and the ECM, in particular how ECM regulates cell function through the some of the cues like topography or alignment of these fibers it is bulk properties like stiffness and how chemical So, for example, density of collagen networks can influence it is physical properties, or how do we will measure physical properties of ECM networks. So, over the last 2 lectures we started having some discussion on how to go about quantifying the mechanical properties of biopolymer networks. And in that regard the last class we had some discussion about whether we want to characterize an ECM network as a solid or a liquid ok.

So, for a simple solid like a spring right. So, what distinguishes a solid from a liquid is how they respond when a force is applied on them. So, for a solid you know that as soon as the force is applied you have an instantaneous deformation, and the deformation remains like that till the end or the deviation over which the force is applied. And once the force is removed the solid regains it is original undeformed configuration; however, in terms of track tracking how it deforms or what is the relationship between the stress and the strain we come about by saying a material can be linearly elastic or non-linearly elastic.

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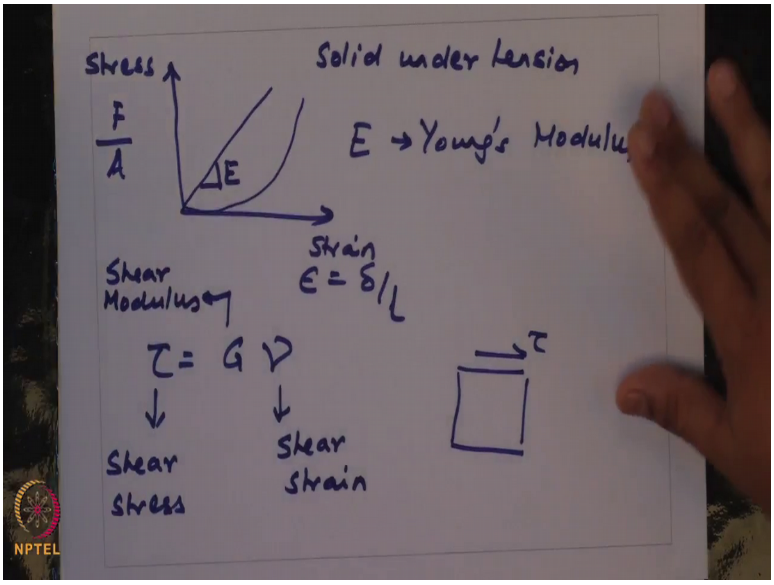

So, what do we mean by that? So, in a stress strain curve in a stress strain curve if this is strain epsilon which is defined by the extension by the original length. And this is my stress this is defined as force per unit area. So, if the slope of this line. So, if your stress versus strain is linear then this slope is the youngs modulus of elasticity of this solid; however, if a curve looks like this then this is non-linear elasticity, where your slope keeps on changing. So, in comparison to a solid which regains it is original configuration instantaneously after the force is removed in case of a liquid. So, when you exact any force it continues to flow number one. And after you have removed the force it will it does not go back to it is original configuration.

So, for a liquid we know that. So, the equation. So, this the way had drawn it here this is for under tension for when of solid is under tension or compression. So, E corresponds to the youngs modulus of elasticity. So, I can write a similar equation of tau is equal to G gamma, where in this case tau is the shear stress that we applied. So, shear stress is applied in a tangential direction. And gamma is the shear strain and G is the shear modulus. So, in the last class I have also told you that for an incompressible material your E, I can write it simply as 3G. So, they are related.

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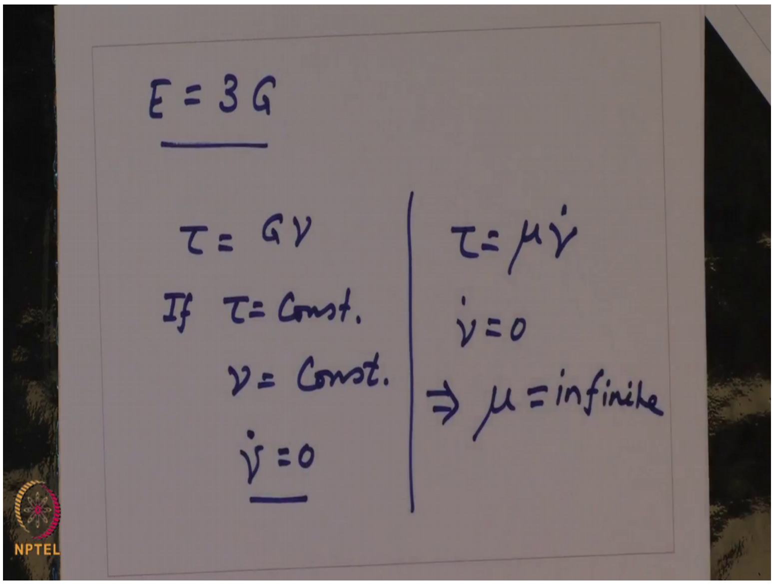

$E = 3G$

$\tau = G\gamma$

If $\tau = \text{Const.}$

$\gamma = \text{Const.}$

$\dot{\gamma} = 0$

$\tau = \mu\dot{\gamma}$

$\dot{\gamma} = 0$

$\Rightarrow \mu = \text{infinite}$

So, coming to the question of whether a biopolymer network is a solid or a liquid. So, it is a it actually exhibit some characteristics of solids and liquids, and hence the term is used as viscoelastic. So, for viscoelastic So, when you apply the force there is some instantaneous response which accounts to the elastic component of the behaviour, and then some time dependent of response which is which accounts for the viscous component of the force. Now when we talk about deformation when any network is exposed to any kind of forces it can deform in 2 way, one in which the volume can change and one in which the shape can change.

So, for most biopolymer networks at the macro molecular level; that means, at the micron scale or 100s of microns range, the volume does not change. This is not true at the micro at the micro scale. So, you can define a macro rheology and a micro rheology. So, at the macro scale the volume does not change, because most of these networks are hydrated; however, the shape does change. So, just to reemphasize. So, for elasticity for tracking elastic behaviour we have the expression tau is equal to G gamma ok.

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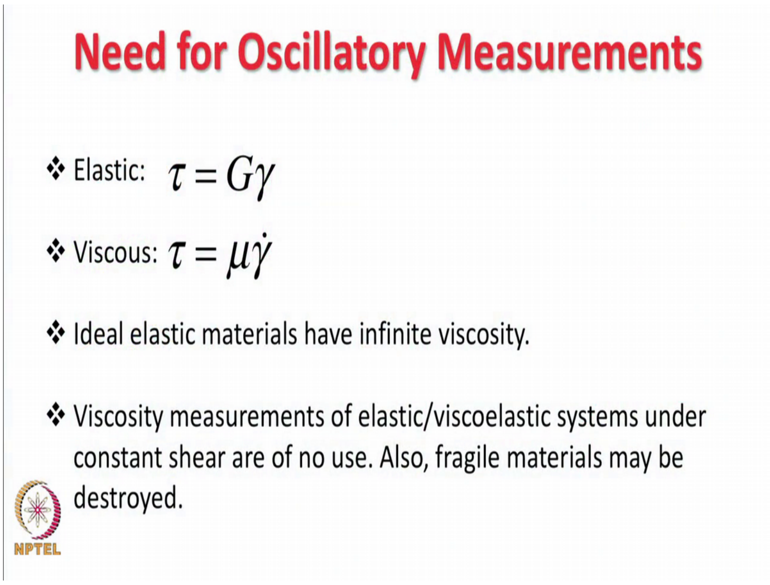

Need for Oscillatory Measurements

- Elastic: $\tau = G\gamma$
- Viscous: $\tau = \mu\dot{\gamma}$
- Ideal elastic materials have infinite viscosity.
- Viscosity measurements of elastic/viscoelastic systems under constant shear are of no use. Also, fragile materials may be destroyed.

So, gamma is a shear strain tau is a shear stress, G is the shear modulus of elasticity. For a liquid you have the equation newtons law of the viscosity which says that tau is equal to mu gamma dot. So, it is not the shear, but the dependencies on shear rate, and mu is the coefficient of viscosity.

So now imagine if I were to consider a elastic entity as a as a liquid. So, if I apply a constant stress if I apply a constant stress. So, that is right So, if I apply a constant stress I know tau is equal to G gamma. So, if tau is constant gamma is also constant. So, this would mean if gamma is constant then gamma dot is 0. So, if I were to consider this equation tau is equal to mu gamma dot. So, if I would to consider a solid as a fluid. So, because gamma dot is 0 this amounts to saying mu is infinite. So, that is to say that ideal elastic materials have infinite viscosity, and this is the reason why when we do viscosity measurements or probe the rheology of systems under constant shear it is not of relevance because if the material is elastic this is infinite. Secondly, if you have fragile materials like ECM networks if you expose them to constant shear they will actually fall apart, then the point of actually characterizing their physical properties no more exists. For this reason what is ideal is to use oscillatory measurements ok.

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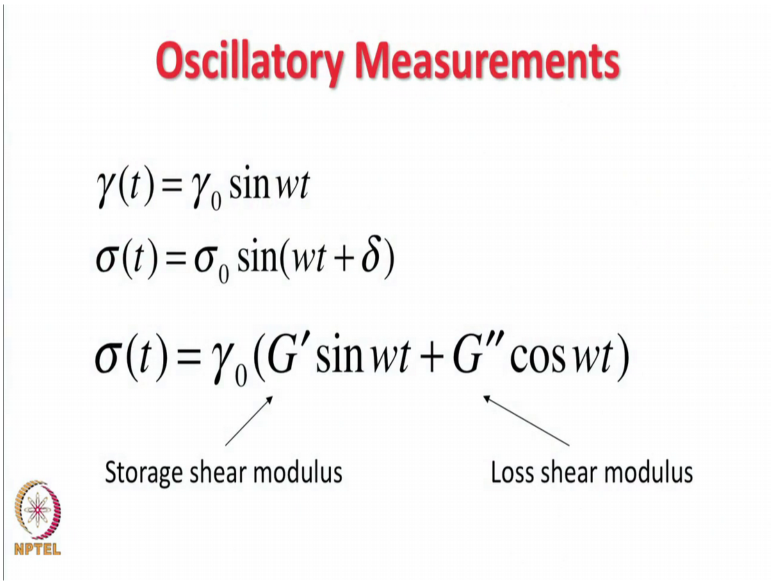

Oscillatory Measurements

$$\gamma(t) = \gamma_0 \sin wt$$

$$\sigma(t) = \sigma_0 \sin(wt + \delta)$$

$$\sigma(t) = \gamma_0 (G' \sin wt + G'' \cos wt)$$

Storage shear modulus

Loss shear modulus

So, as written here and I briefly tested up or test upon this thing yesterday. So, for oscillatory measurements my gamma is not constant, but it is a sin curve. So, you have gamma naught is the amplitude and then you have a sin curve on it with frequency omega. So, the correspondent the. So, if for the material to be viscoelastic, your sigma of t has this dependence where there is a phase shift. So, what is the phase shift?

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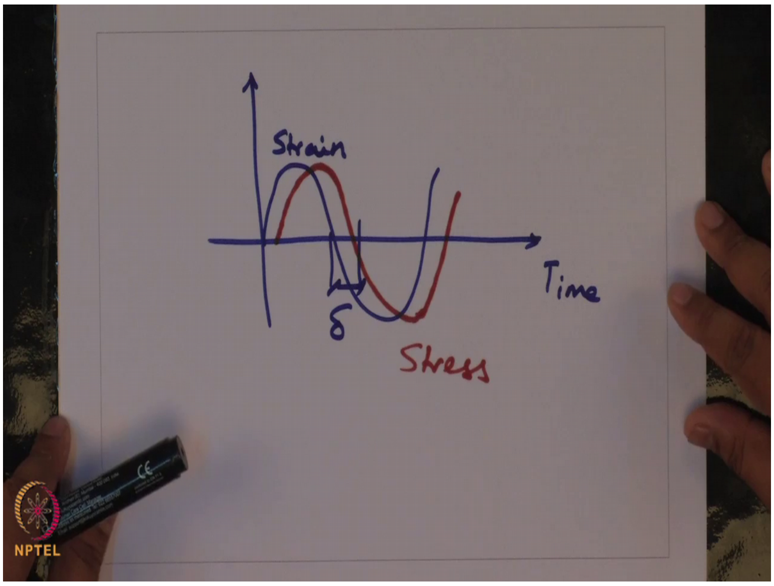

So, if I would to draw if I were to draw if this is my and this is my strain. So, so this would be my stress profile, and this delay this delay is nothing but delta. So, there is a phase shift of delta.

So, if a material was perfectly elastic then delta is 0. If the material is perfectly fluid then delta has somewhere. So, I can break down this particular expression to write and expand it and write sigma of t is over a stress as gamma naught into G prime sin omega t. So, this component is in phase with the strain field, you have gamma naught sin omega t and this is gamma naught G prime sin omega t, and plus G double prime cos omega t. So, because for a fluid when you expose it to stress it flows, but it does not recover. So, that is lost that energy which is pumped into making the fluid flow is lost. This is why this G, g double prime is referred to as a loss modulus actually the loss shear modulus because you are measuring shear experiments. And G prime is the storage shear modulus.

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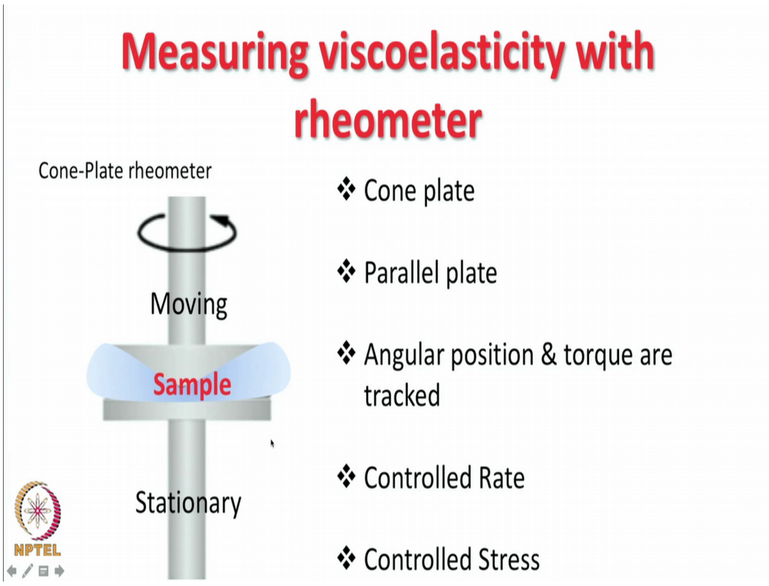

Measuring viscoelasticity with rheometer

- Cone plate
- Parallel plate
- Angular position & torque are tracked
- Controlled Rate
- Controlled Stress

So, for doing oscillatory experiments we make use of a rheometer. And again in last class we had discussed that for a rheometer you can have a cone and plate geometry in which the bottom plate is stationary it is fixed. And there is a top plate which has a conical morphology at the bottom which is in compare in contact with the sample. And this keeps on rotating. So, you actually position the sample between the top moving part and the bottom stationary part. So, instead of a cone plate rheometer you can also have another geometry which is a parallel plate rheometer, in this case instead of having this conical geometry the top will also have a geometry like this, but it will rotate ok.

So, you can have a cone and plate rheometer, but a conical play as well as a parallel plate rheometer. Now a cone and plate rheometer make sure that the impact top surface of the sample is exposed to the same strain. Which is not true for a parallel plate rheometer, where the strain at the center of the sample is 0 and the strain is maximum at the edge. This is one reason why cone plate rheometer is preferred. So, for measuring some for measuring G prime or G double prime, what is actually tracked is the angular position of this. So, you have some reference point which as it rotates you track it is angular position. The second thing that you can track is the torque that is being applied for rotating the sample at that given frequency. You can do 2 different types of experiments with a rheometer one of which is called a controlled rate experiment. And the idea is that you track you rotate it at a given frequency and you track how much torque is required to maintain that frequency ok.

So, in a sense you are controlling the strain you are imposing on the sample. Parallely there can be another mode of operation called a controlled stressed a operation, in which you actually control the amount of torque you are imposing on the sample, and what you track is how much strain does it impose on the sample. So, let us briefly go through solve the measurements that in you can measure using a rheometer.

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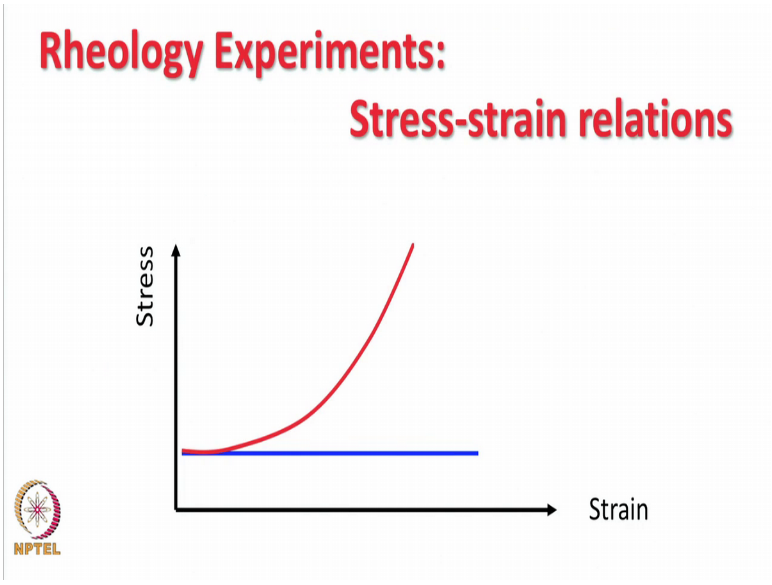

Rheology Experiments:

Stress-strain relations

So, the obvious simplest case is a stress strain relationship if a material is elastic. So, in this case you at this experiment. So, for elastic material you can do this in using either the controlled rate case or using a control stress experiment. So, you can do it in both ways. And I have drawn 2 curves here one a blue one where stress is constant independent of the strain, this is what you will observe. So, the slope of the line. So, this is the slope of the line would give you the modulus ok.

So, what you see this is what you would observe for a linear elastic material. So, no matter how much strain you have it always responds in the same way. The other case is this curve which gives you a non-linear elastic behaviour. So, if the slope of the curve keeps on changing.

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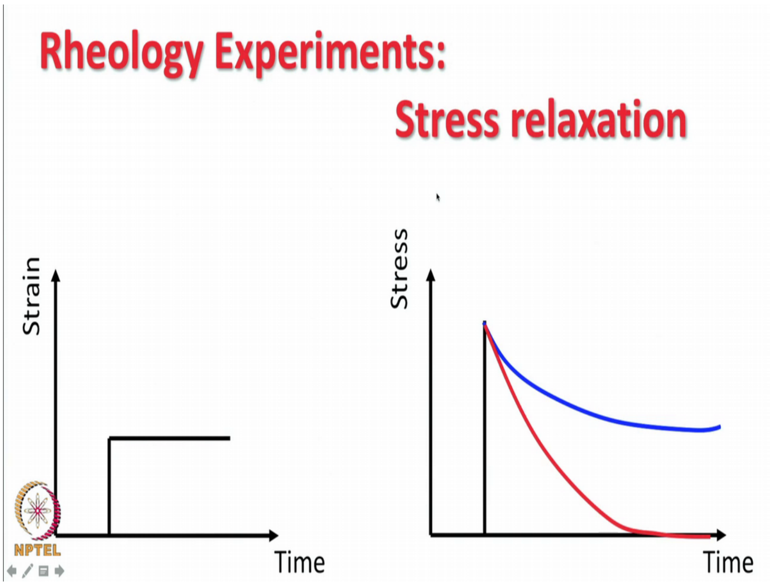

Rheology Experiments:

Stress relaxation

Now, one thing you can also do one type of experiment is called a stress relaxation type of experiment, where your input is the strain So, at a certain point you impose a given amount of strain and you keep it constant, for some extended duration. And you see how the system responds. So, these stress relaxation experiments are performed for viscoelastic samples. So, what you will observed is in terms of stress there is an immediate response, but then slowly these systems the stress will keep on keep coming down. Why does the stress keep coming down? Because this suggests this is indicative of internal rearrangements of the network or relaxation of the respond, hence the term stress relaxation.

So, you can have 2 cases. So, again I have drawn it in 2 ways in which the blue curve when it drops, but it saturates to some value, with the value red curve it drops to complete 0. So, this is what you will observe the red curve is what you will observe for a viscoelastic fluid. At the end this will plateau to zero; however, if it plateaus to some finite value as is the case in this blue curve this is called a So, at the end you are observing the behaviour of a viscoelastic solid. So, you have this is the equilibrium modulus of this network, this is the equilibrium modulus of this network.

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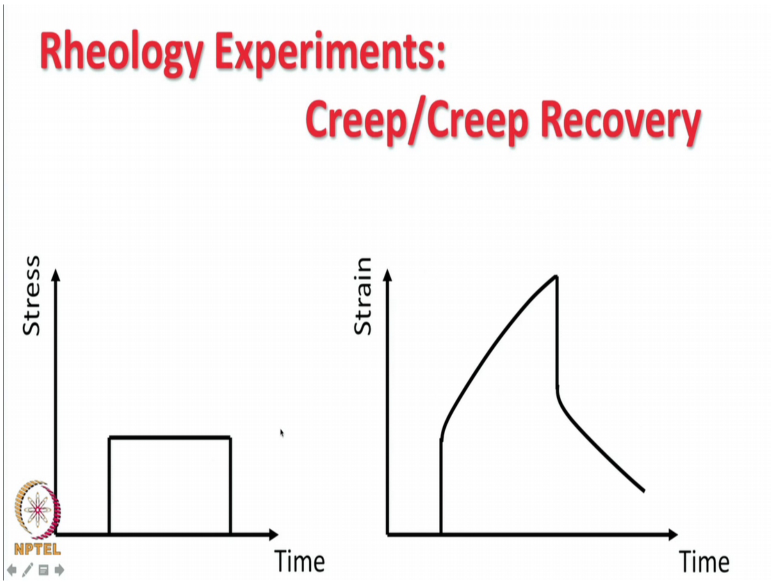

Rheology Experiments:

Creep/Creep Recovery

You can do so, this is the case where you had kept your strain high. So, you impose a deformation very fast that is why it was increasing and then you maintain that level of strain. You can do another set of experiments where you expose it to a force. So, expose it to a certain amount of force for constant amount of time, and see how the system responds to it. So, these kind of experiments are called creep experiments, and these are important for tracking properties of those kind of tissues which are under constant stress. For example, bones in our body are under constant compression. Or even you can have you know blood pressure is one case where the blood vasculature is continuously exposed to blood pressure, blood flow and because of that there is a pressure ok.

So, for doing these experiments you exposed you expose the specimen to a stress constant stress for a extended duration of time, and during that time you track how there is the strain evolved. So, what you find in the strain response is as soon as you impose the stress there is the strain there is an instantaneous strain, and this accounts for the elastic component of the behavior. So, strain is instantaneous and then there is an approximate linear increase in the strain develop which is a maximum. So, this portion of the curves. So, the first 2 portions of the curve is called the creep, the next 2 portion of the curve is after you drop up to the stress again drops to 0 what you get is a similar response, but it is the opposite direction there is an instantaneous drop in the strain, again why because this is relaxation of the elastic component of the network, and then there is some another drop, but this does not drop to 0 it drops to some values if it drops to some value this part is the non recoverable part of the response.

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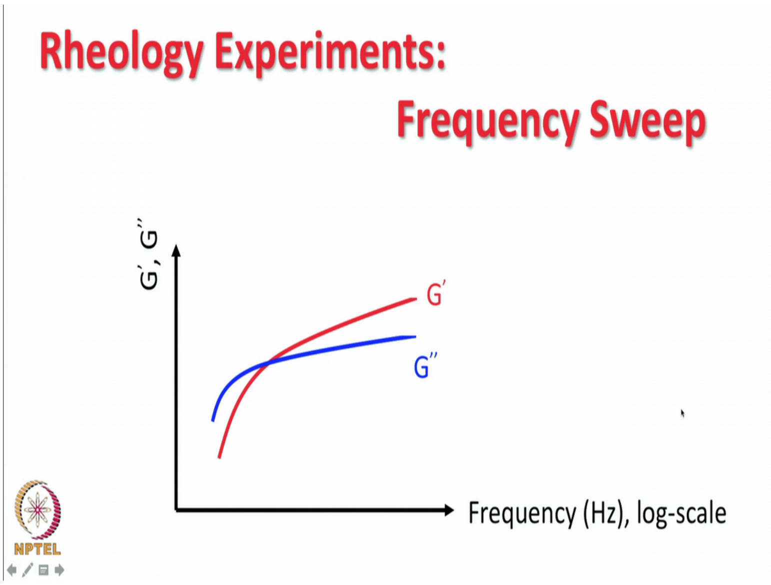

Rheology Experiments:

Frequency Sweep

So, this is attributed to plastic flows. You can also do another set of experiments which are called frequency sweep this is very common, and this is what enables you to track the how the collagen networks would evolve as time progresses and it is exhibit it is exposed to stresses of a certain kind for certain length of time or at a certain rate ok.

So, in this case I have floated G prime and G double prime as a function of frequency. So, the frequency actually follows is varied in log scale in other words you can do 0.001, 0.01, 0.11, 10 100 so on and so forth ok.

So, for large durations large frequency ranges and you track what is the behavior of the network. So, there is one thing that you observed. So, G g double prime the blue curve is actually above the red curve for a certain frequency range. So, initially the network is more viscous in nature. But if you keep So, this actually this is the So, where the viscous component or the last component is more prominent over the storage component. And here because you are deforming the network very slowly. So, so you are deforming the network very slowly you can have these internal arrangements and the system relaxes. But as you increase if you start deforming at a much higher frequency then what you see is G prime increases, and eventually G prime will also saturate. So, G prime increases because the system does not have enough time to relax ok.

So, this crossover point, this crossover point corresponds to the time scale of relaxation of the network. So, this is what you know this is what most ECM networks will exhibit this like hyaluronic acid will exhibit this kind of behavior.

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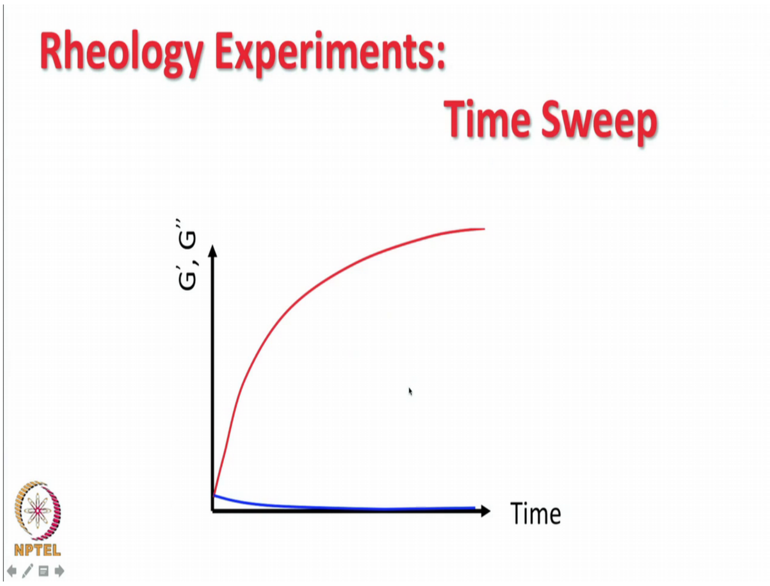

Rheology Experiments:

Time Sweep

You might also be interested in tracking how a system behaves while it gels or in the presence of some enzyme. So, what you can do is you can do a time sweep where the strain and the amplitude is kept constant, and you monitor G prime and G double prime as a function of time. So, once again here what you see is G prime. So, if let us say a gel is polymerizing it is G prime will increase with time and then saturate by G double prime. So, you start off with the very viscous material, which then polymerizes and forms filaments. So, maximum G double prime is at in at the initial time point And then it drops as that network becomes more and more polymerized.

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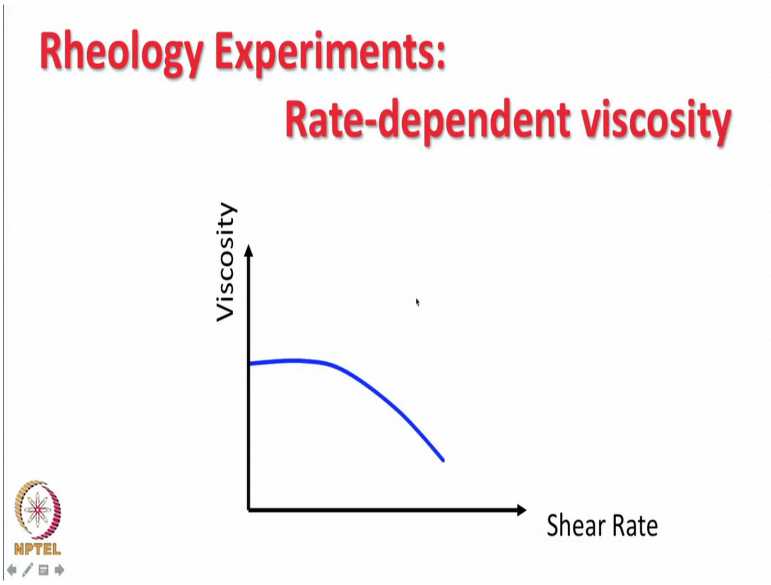

Rheology Experiments:
Rate-dependent viscosity

For probing the behavior of networks like non newtonian networks what you can do you can track the viscosity as a function of shear rate.

So, this is an example where the viscosity is constant of a certain value of shear rate and then drops. So, this kind of fluid is called a shear thinning fluid.

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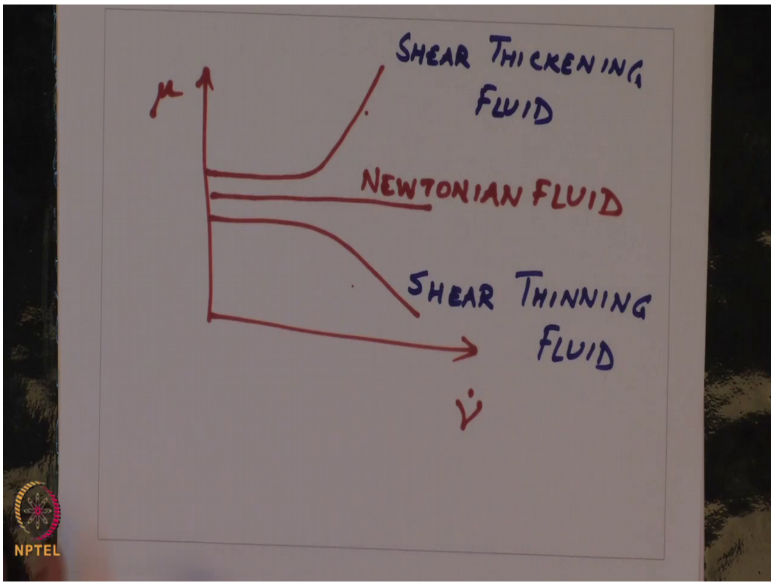

So for, We have gamma dot you have mu or the viscosity. So, you might have 3 different responses. So, this is a newtonian fluid because mu when mu is constant it does not exhibit any dependence on gamma dot you call an newtonian fluid. So, both of these other fluids are non newtonian. So, this is called a shear thinning fluid, this is called a shear thickening fluid. So, that the viscosity changes over time.

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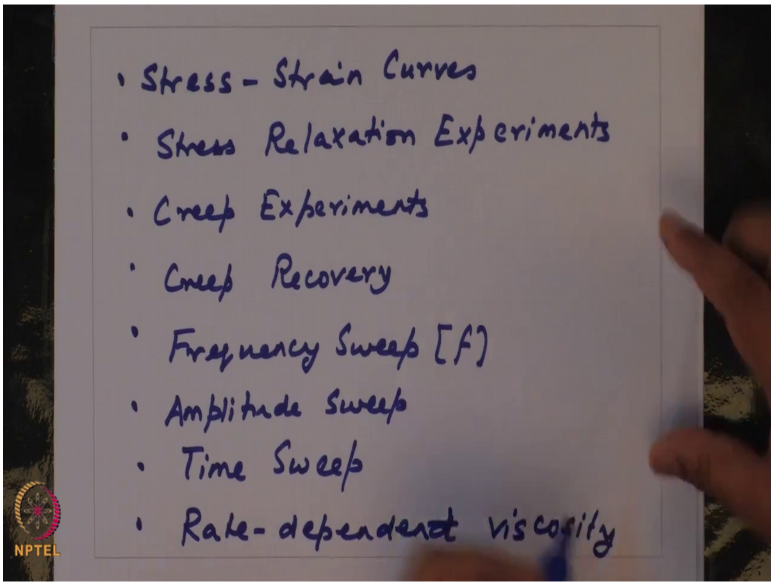

- Stress - Strain Curves
- Stress Relaxation Experiments
- Creep Experiments
- Creep Recovery
- Frequency Sweep [f]
- Amplitude Sweep
- Time Sweep
- Rate-dependent viscosity

So, to summarize So, the number of experiments you can do is quite large, you can do stress strain curves, you can do stress relaxation experiments, even though creep experiments, if a look creep recovery kind of experiments frequency sweep. You can also do something called amplitude sweep. So, in frequency sweep your frequency f changes while you track G prime and G double prime, you can do the same thing in an amplitude sweep where you keep the frequency constant and you monitor G prime and G double prime as a function of the amount of strain that you impose ok.

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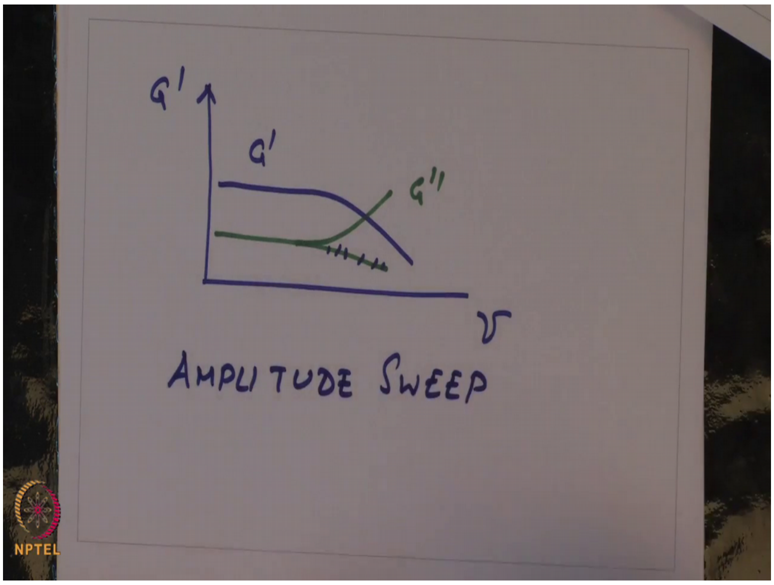

So, a typical case of an amplitude sweep. So, here you have gamma. So, you might have a behaviour like this. So, this that says is G prime and what you see let us see I can also plot G double time on this curve actually sorry. So, this is G prime and this is G double prime. So, this kind of a net this kind of a curve is called an amplitude sweep, and what you see here is beyond a certain strain your G prime decreases. So, G prime is constant in this particular case, and beyond a certain strain it starts to decrease. Versus G double prime which was much lower than G prime, increases beyond a certain value. Why must this happen? Or why? How can this happen? This suggests that beyond a certain strain your network is starting to fall apart as a consequence of which your G prime value is decreasing, and the material is getting more and more viscous because of which digit double prime is.

So, this experiment is called an amplitude sweep experiment. So, you can have amplitude sweep, you can have time sweep right. This is used for tracking the generation kinetics and finally, you can have you can do rate dependent viscosity. So, if I would to summarize So now, in order to do rheology some points need to be taking to taken into consideration for preparing the sample. So, if you are handling solid tissues like let us say bone this is easy to see you should preferably cut them into this shape samples. So, the disk should really match the shape and size of the bottom plate.

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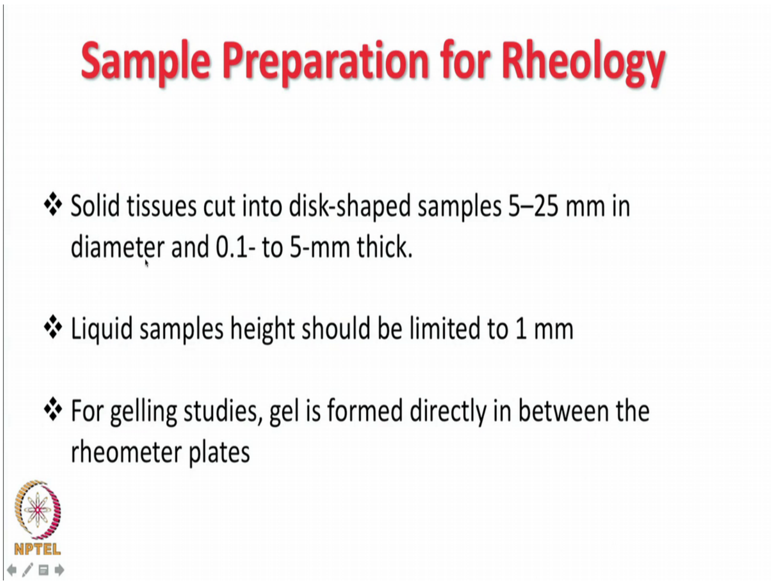

Sample Preparation for Rheology

- Solid tissues cut into disk-shaped samples 5–25 mm in diameter and 0.1- to 5-mm thick.
- Liquid samples height should be limited to 1 mm
- For gelling studies, gel is formed directly in between the rheometer plates

So, it is really anchored properly of samples 5 to 25 millimeter in diameter and it can have varying thickness 0.125 millimeter. Liquid samples are much more difficult to deal with. So, their height should be limited to one millimeter because if you put more amount of liquid it might flow laterally. So, you do not want to do it. For gelling studies for example, when you are doing time sweep experiments the entire gel needs to be initially formed directly between the rheometer plates, between the rheometer plates and initially without putting the top cone plate rheomet cone directly in contact with the gel. So, that the gel forms under an unstressed configuration. And then you slowly push the top plate on to the gel. So, for all these cases you have to exact a little bit of compressive force in order to keep the sample in position, and then you can do your experience.

So, over the last 2 lectures I had spent considerable amount of time in studying the behavior of collagen networks right. So, what are some of the experimental observations that we observed. One was the dependence on concentration right.

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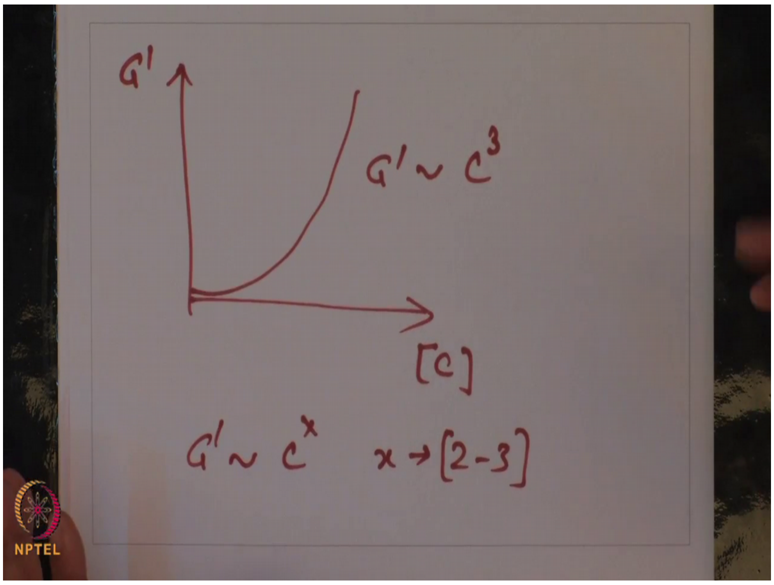

So, I have if I have concentration and I am plotting G prime value I will have values which will scale like this, but G prime roughly scales as concentration cubed. For other biopolymer networks you might have this G prime will still scale as C to the power x where x can have any value between 2 and 3 ok.

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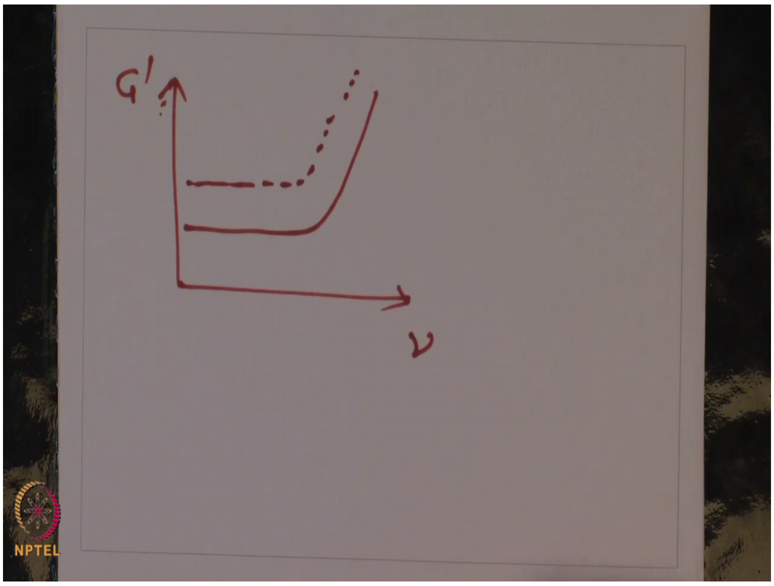

So, you can you observe also observed a strain stiffening behaviour of collagen networks of non cross linked or a non cross linked collagen networks, where G prime value G prime values changed. So, if I plot G prime as a function of strain. Beyond the certain strain you have this increase and then if you were to do it at the higher concentration this curve will just shift upwards. So, this is strain stiffening and as we discussed in great detail this kind of strain stiffening behaviour is induced by alignment of the individual fibrils in the direction in which you are exerting the force.

However so, in this cases once it aligns with the new direction the network will not regain it is original configuration. So, what you need to do to do that to ensure that it is to actually crossing the network. So, you will have deformation and then that deformation will be released when you relax the network it will go back to it is original configuration.

Now, one thing is why do we need non-linear stiffening, why what is the practical relevance of these non-linear stiffening of these networks? The point is in tissues when they are subjected to forces what this does is as the network becomes different stiff; that means, that for the cell also it is difficult to deform. So, this is and a normal process which ensures that the tissue does not get damaged as a consequence of loading, but it regains it is original position. So, non-linear stiffening really protects an instance. With that I stop this lecture in the next class we will see how non-linear stiffening can also be encoded at the level of a single floating.

Thank you for your attention.