Introduction to Mechanobiology
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Week – 08
Lecture – 36
Techniques in Mechanobiology: Hydrogels

Hello and welcome to a today's lecture of Introduction to Mechanobiology. So, this would be our last week of this course and this week we will spend our time discussing some of the tools and techniques that are used in the field of mechanobiology, for studying the effects of physical forces or cell behaviour and studying or measuring properties of cells tissues relevant to mechanobiology.

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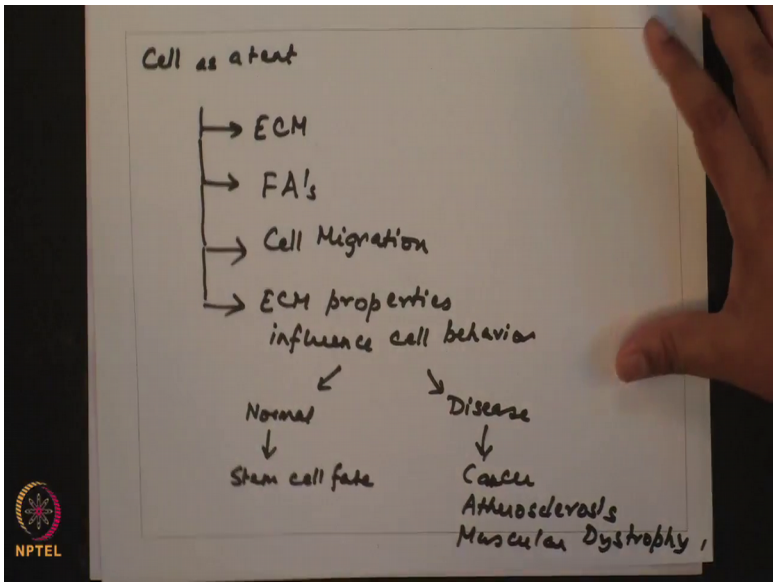

So, once again just to recap what we have covered so far we started off with our analogy of cell as a tent, from there we discussed the extracellular matrix and its properties in great detail we went on to discuss about focal additions and how forces affect focal additions from there we discussed cell migration and the various modes of migration that are possible. After that we started discussing about how ECM properties influence cell behaviour and this was both in the context of normal cell behaviour and in case of disease.

So, in normal we discussed about regulation of stem cell fate by ECM stiffness and via modulation of cell shape in disease we discussed about few cases related to cancer atherosclerosis and muscular dystrophy. So, one of the ideas that I repeatedly stressed on was modulation or change in ECM properties or ECM or tissue properties.

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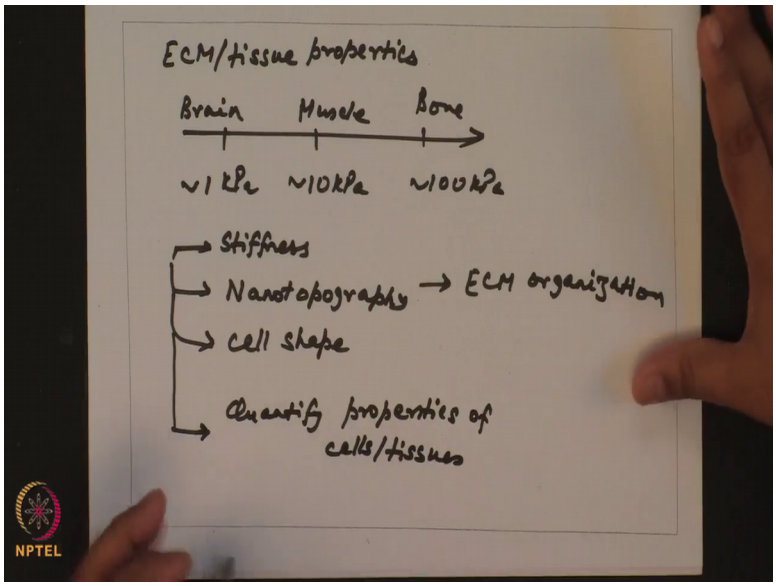

So, within our body we span tissues of multiple stiffness starting from brain which is order 1 kP in stiffness muscle order 10 kPa and bone order 100 kPa.

So, in order to study how cells behave in these different contexts you must have a way of developing substrates where these properties can be regulated. And by properties you can have multiple different things you may want to tune the stiffness while studying the effect of ECM stiffness bulk tissue stiffness and regulating cell behaviour you may want to tune nano topography. So, if you want to see what is the effect of ECM organization you may want to regulate cell shape and you may also want to measure quantify properties of cells and tissues.

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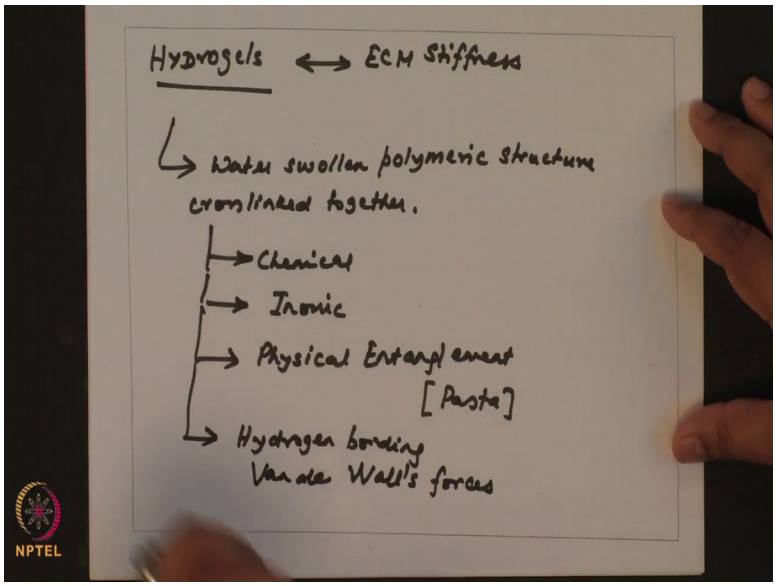

So, as part of the different techniques relevant to mechanobiology today we are going to discuss about hydrogels so what why is hydrogel important again because of its relation to ECM stiffness in order to make substrates which mimic the bulk properties of this of different tissues what you do is you make use of hydrogels. So, that brings us to the question what exactly is the hydrogel. So, hydrogel is essentially a water swollen polymeric structure cross linked together.

So, the source of the crosslink might vary it might be chemical a covalent bonds, it might be ionic, it might have simple physical entanglement physical entanglement, simple example is that of pasta, so given pasta if you want to take out a single strand you require to exert some forces or you might have weak hydrogen bonding or Van de Wall's force mediated forces. So, these are the different sources in which you can make the hydrogen. So, based on the type used you may classify hydrogels, you may classify hydrogens broadly as a homo polymers, copolymers or you can have something called interpenetrating.

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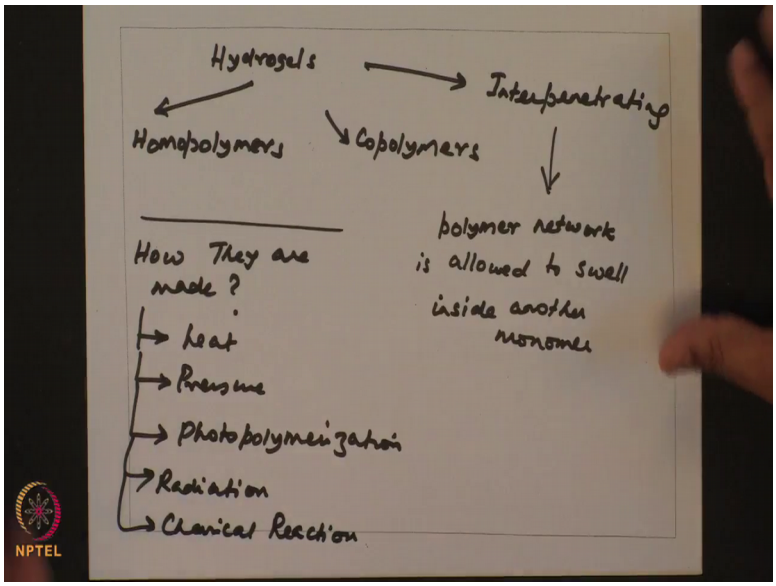

So, homo refers to generally you have a single monomer which is being cross linked together to form a polymer. Copolymer you have two or more entities which are covalently or cross linked. In case of an inter penetrating network, this is an interpretation respect for example, you might have a polymer network if a polymer network is allowed to swell inside another monomer. You can also classify the polymers based on the process by which how they are made, you might have you might modulate heat, pressure, use photo polymerization or radiation like x rays. So, you can have multiple different ways and also I think the most important which I missed is chemical reaction.

So, for making a hydrogel relevant for mechanobiological applications what should be some of the desirable properties, what is desirable for a hydrogel?

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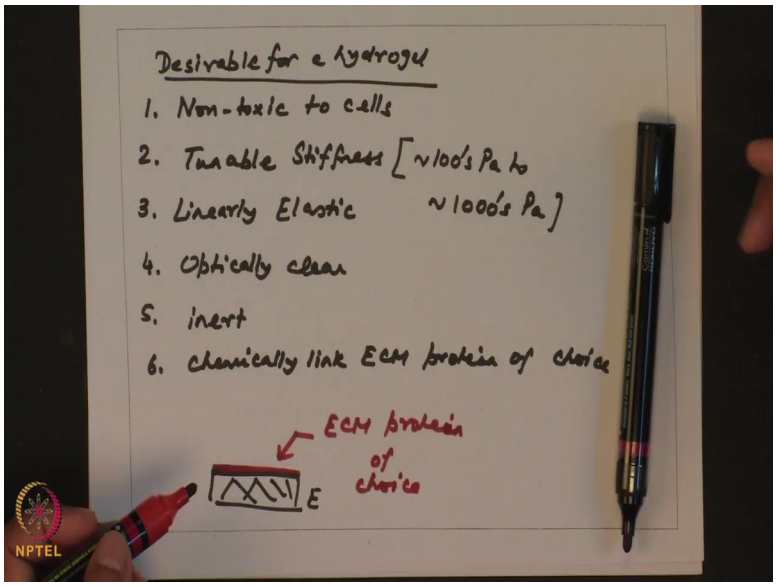

So, first and foremost the gel has to be non toxic to cells. If you are want to you want to conduct studies where you are varying the stiffness over a large range you need a polymer where you can tune the stiffness of the network. So, relevant to in vivo would be 100s of pascals to 1000s of pascals. So, you have a wide range this preferred that the hydrogel is linearly elastic. So, if the material is linearly elastic then in our next class we will discuss how this is this provides us ease in order to quantify the forces that cells can exert, the hydrogel should be optically clear because we will culture cells on them.

So, you if these optically clear then you can image hem for doing phase contrast studies as well as fluorescence studies. And the other thing, most of these hydrogens are generally inert. So, you should be able to chemically link a cross link your ECM protein of choice. So, thus you have a polymer where you can measure you make a material it whose bulk stiffness E you can tune and this gel is typically functionalized this is your ECM protein of choice so that you can make it relevant to different contexts.

For example, if you are working with neuronal cells and you are interested in behaviour of neuronal cells in a brain pneumatic environment this ECM protein might be laminin or in some other case if you are working with smooth muscle cells you might use collagen with E order 10 kilo Pascal so on and so forth. So, there have been several different hydrogen systems that have been used among the most common once and most widely used is polyacrylamide gels, you have another one which is very common poly dimethyl

siloxane in short it is referred to as PDMS you have polyethylene glycol gels you have alginate gels. So, these will briefly, will all fall under probably the synthetic category.

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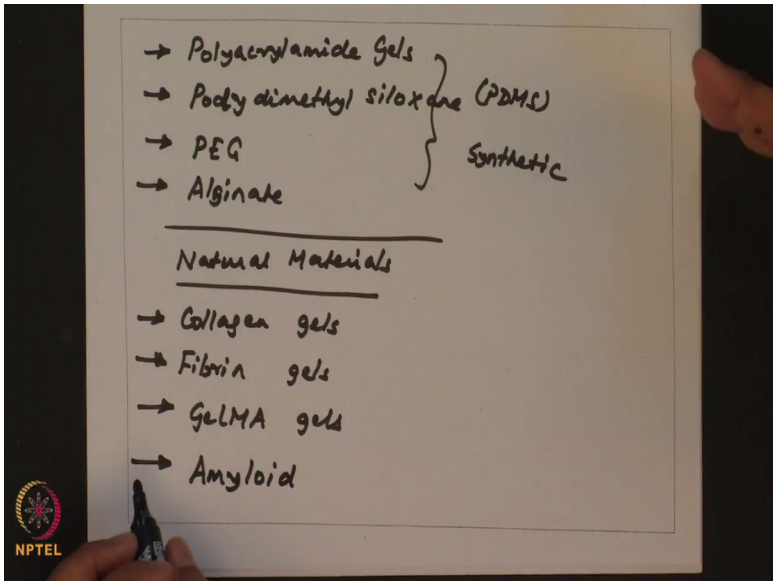

You can also make gels of natural materials, these would even among the most common is collagen gels can make fibrin gels also. So, instead of collagen you might make use of gelatin and make gelatin methylated gels gelatin is not stable at room temperature its solubilizes, but if you methacrylate the gelatin and then crosslinked using UV then you can get gel modules.

Nowadays you also have peptides or materials gels made of peptides and one of them is amyloid amyelod based hydrogels. So, though amyloid has traditionally being associated with disease causing, but there are functional amyloids also and these amyloid gels have recently been used for differentiating stem cells into neuronal precursor cells.

So, I will just discuss as to how you go about making these gels for mechanobiological study I will take the polyacrylamide gel case. So, for PA gels, what you do? You link acrylamide.

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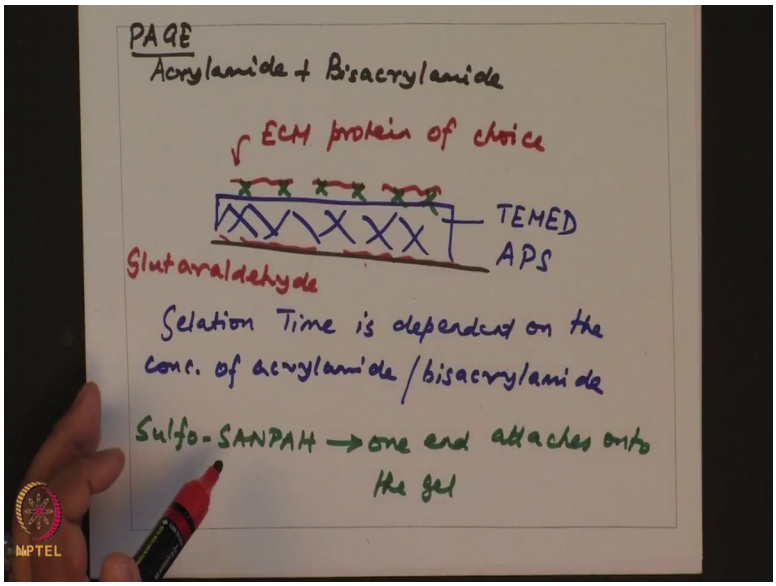

So, you know that you run proteins for western plot on page polyacrylamide gels right. So, in page you have acrylamide which is cross linked with bisacrylamide. So, instead of making the gel in a test tube what you do is you take a glass cover slip you functionalize you treat this with aminopropyltriethoxysilane and then with the glutaraldehyde, on top of which you dump your polymerizing mixture polymerization solution. So, this polymerizing solution has TEMED and APS in it and these allow it to gel ok.

So, if you let it sit this will form a network so the gelation time is dependent the concentration of acrylamide, so higher concentration of acrylamide will typically help our higher concentration of acrylamide or bisacrylamide. So, higher amount of cross linking protein will ensure that this gel gets polymerized much faster.

Now as I mentioned briefly that these gels are inert. So, if you plate cells directly on these gels nothing will stick cells pond stick and your ECM protein amount cancelled. So, what is used is a hetero by functional cross linker. So, this is called sulfo sanpah one of which is linked via UV onto the substrate of the PA gel. So, this one end attaches onto the gel and then once you have done this you know this cross linking then on top you can functionalize with your ECM protein of choice. So, this might be collagen, laminate, vibrating so on and so forth. So, first of all so far as the tuning the properties of the gel is concerned you have two things to control.

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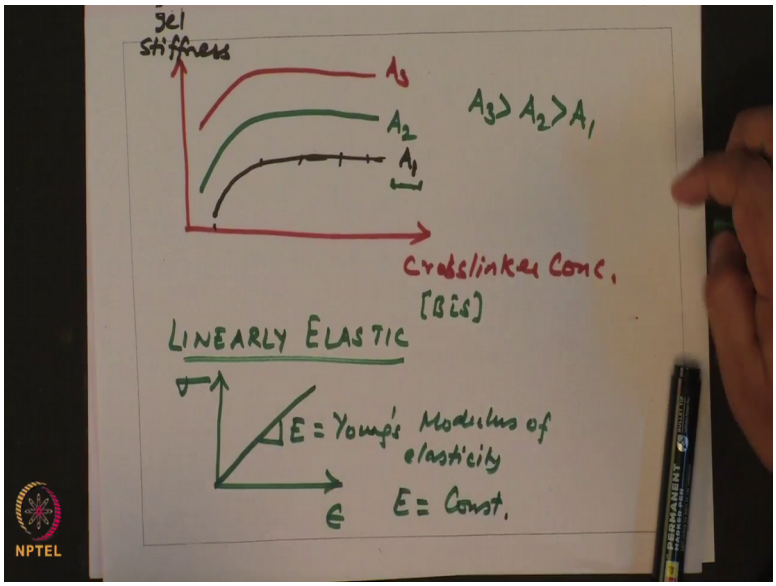

So, let us say this is my cross linker concentration. So, if I use a given concentration of acrylamide and I keep changing the cross linker concentration I will get and let us say my y axis is the stiffness that I will get gel stiffness that you will make.

So, you would see at a given concentration of acrylamide you will get curves like this. So, there is some basal stiffness and then you increase the cross linker then your stiffness saturates the thing the saturation behaviour is because that once all your all your monomers are cross linked then there is no additional cross linking possible no matter how much you add the cross linker. So, this is let say at concentration acrylamide concentration of A 1. If you increase A 1 to A 2 you would get similarly curves which shift upward. So, this is concentration A 3 this is concentration A 2 and we have A 3 greater than A t2wo greater than A 1 ok.

So, you can tweak in order to get a gel of q and stiffness you can change either the monomer concentration which is your acrylamide or the cross linker concentration which is your bisacrylamide. Now the beauty of acrylamide gels is that it has been found that the pa gels are linearly elastic. So, what is the meaning of linearly elastic? So, if you plot if you exert a given amount of strain and measure the stress. So, this is a straight line with a slope being E. So, E is the Young's modulus of elasticity. So, for linearly elastic materials E is constant, for linearly elastic materials E is constant. So, using these systems variety of studies have been performed.

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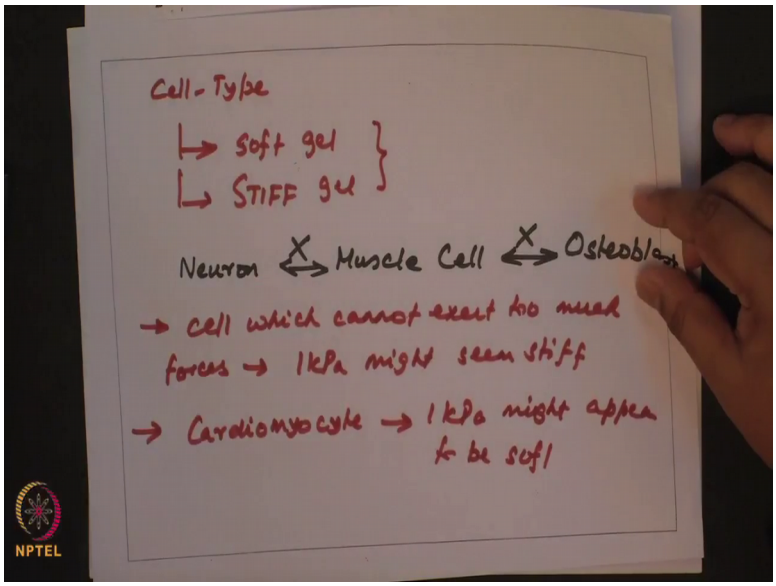

Now one of the question is for a given cell type on what basis do you call a gel a soft gel versus a stiff gel, what might be your basis is for calling us as substrate soft or stiff. So, you would anticipate that what is soft for a neuron is not the same as soft for a muscle cell or not the same for a osteoblast. So, this is not true, and this perhaps depends on the ability to exert forces. So, a cell which cannot exert too much forces. So, even 1 kPa might seem stiff versus the cell let us say a cardio myocyte 1 kPa. So, cardio myocytes are cells which exert lot of contractile forces 1 kPa might appear to be soft.

So, how do you find out given a cell type what is something as soft or what is something as stiff. So, one of the ways of doing it, one of the ways of doing it is actually to measure the cells spread area as a function of stiffness.

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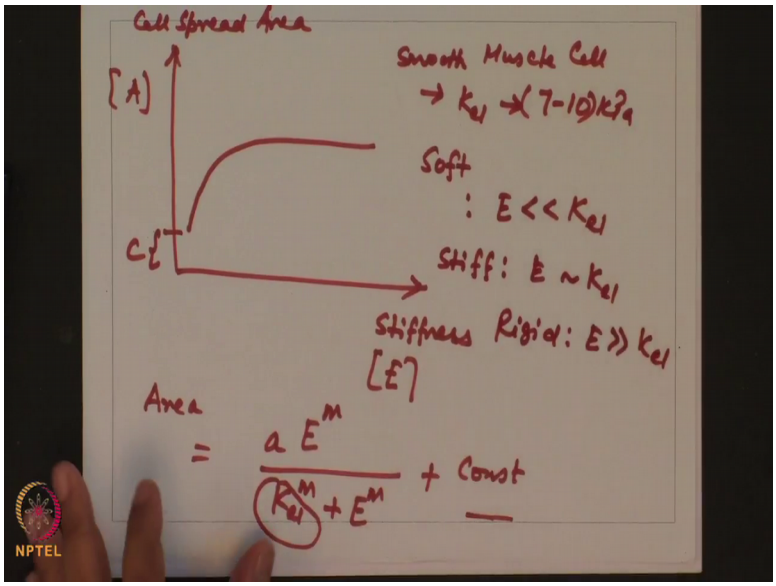

And what you will get you will get these saturating profiles of cells spread area and you can fit if this is my area A and let us say E is a substrate stiffness. So, you can define area. So, this is an example of a hyperbolic curve. So, you can fit it with an expression, so you have a constant this constant is equivalent to this value here this constant and you have this kind of a profile. So, what you backtrack. So, E is the substrate stiffness which is an experimentally measured quantity, area is also a measured quantity. So, you can backtrack this value of K el or some elastic matrix.

So, for a muscle cell for a smooth muscle cell this K elastic constant turns out to be order 7 to 10 kilo Pascal, but this K el provides a way of quantifying whether a substrate is soft or stiffer of given cell. So, you can say that for a cell with a given K el which you determine soft can correspond to when E is significantly less than this value, stiff is with E is comparable to this value and rigid where E is significantly greater than this elastic constant.

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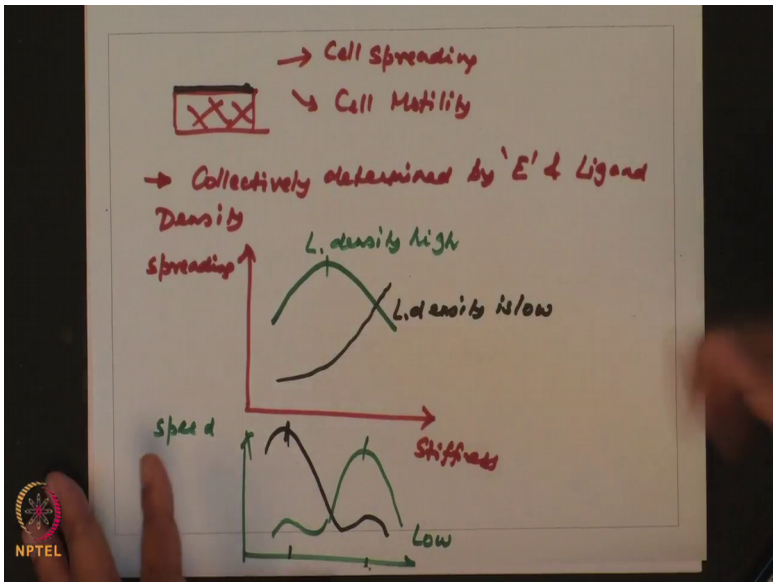

So, one thing I mentioned that given a gel, given a gel you functionalize it with some ligand. So, the measure that, the quantify that you measure either in terms of cell spreading or cell motility is not only dictated by the substrate stiffness, but it is in a collectively determined by substrate stiffness value and the ligand density.

So, if your ligand density is very high if your ligand density is very high you might expect a different response compared to when ligand density is low and these kind of experiments have been performed. So, this is a in a standard example let me say in terms of spreading as a function of stiffness, you might see if your ligand density is low what says when ligand density is very high, in this case what you observe is the maximum spreading is observed at some intermediate stiffness value. While when the ligand density is very low what you find is increasing amount of stiffness is to increase spreading.

So, this shows you this is another important parameter that you must tweak in your experiments. So, depending on the phenomena that you are trying to probe this might be a different value. So, for these particular experiments the authors found that in ligand density low what they observed was a biphasic speed effective speed. So, when they track the speed when it was low you found maximums motility on a stiff surface when it was low, when it was high then the maximum speed was reversed and you got the

maximum speed on a softer surface. So, this is also very important to think off as to what concentration of ligand that you should choose.

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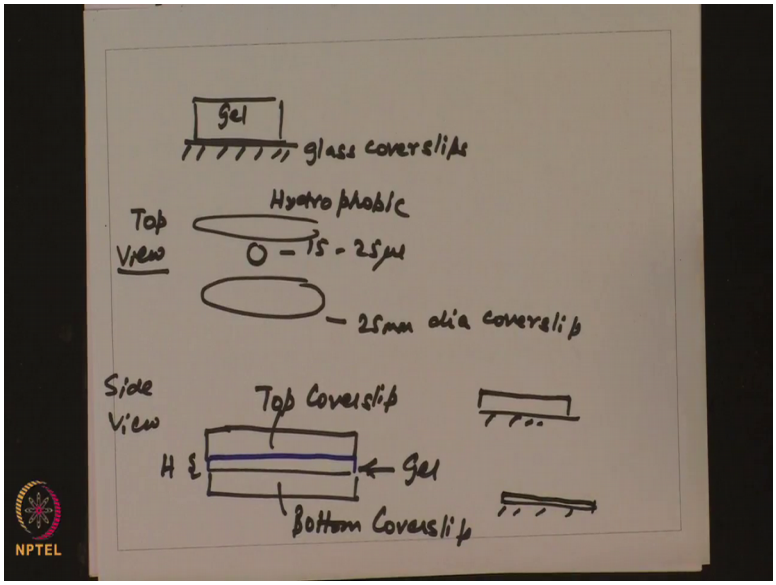

So, another aspect which has to be taking into consideration, so we generally draw the gel like this and these gels are typically cross linked onto glass cowslips which is rigid for the cells and on top of this you have a gel.

So, though I am drawing the gel like this you would imagine for the same cover slip. So, what you do is while making the gel you put a single drop order 15 to 25 microliter drop onto let us say a 25 mm cover slip diameter cover slip and you put another cover slip on top which is hydrophobic this is hydrophobic. So, when you place it on top what you get, so in side with this is top view and in side view this is what you will have you have your bottom cover slip you have your gel which is spread about uniformly and you have the top cover slip. So, this is your bottom cover slip and this is your top core slip and this is the gel that you found.

So, instead of using 15 to 20 microliters if you used 40 microliters or 100 microliters this height will change. So, what depending on how you are doing for the same substrate you might make a gel which is this thick or you might get a gel which is very thin. So, what indirectly you are changing by changing the amount of volume that you are putting is you are changing the height of the gel that you get. So, this substrate, even this gel

resting on a stiff surface, if the gel is thin then it turns out that the cell can actually sense the substrate thickness, substrate stiffness.

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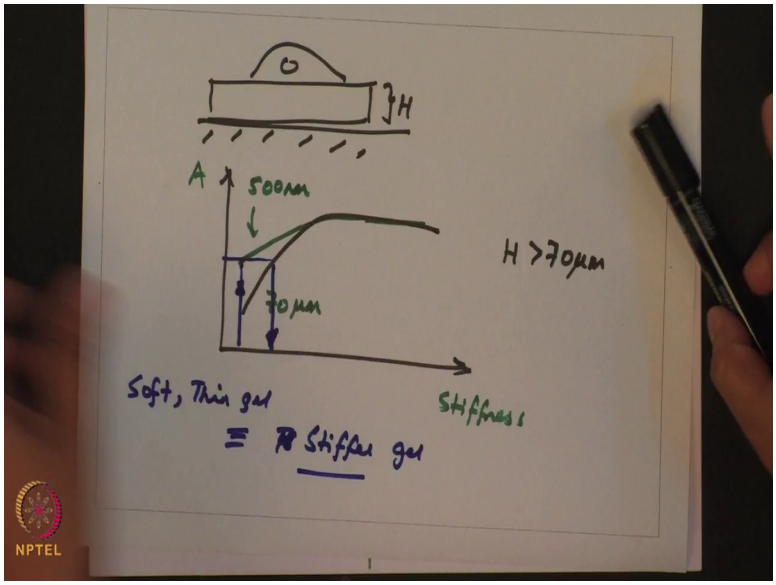

So, if I do this experiment in a controllable manner where I have a PA gel I have a PA gel of height H and on top of which I am plating cells. So, what has been observed is if H is greater than 70 microns and you get a given spreading behaviour if you keep the making the gel thinner and thinner. So, for example, if I plot the spreading response for the 500 nanometer gel what you will observe, this is for 70 microns thick gel this is for 500 nanometer thick gel. So, this is my spreading area this is my stiffness. So, what you observe is for the same value of area a soft and thin gel appears to the cell it appears that a much thicker gel, soft thin gel is equivalent to much thick stiffer gel.

So, you do not want to incorporate the effect of substrate or glass stiffness on what you are measuring. So, for this people have shown that if your height of the gel is greater than 70 microns then you are assured that it will not have any effect of the rigid effect or rigid glass effect on the measure that you are quantifying.

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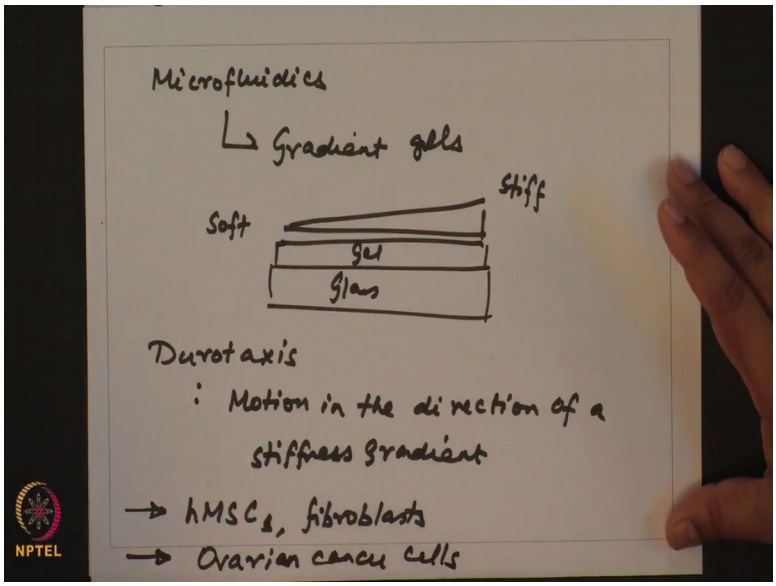

One last thing I wanted to mention, so while these studies these gels are mostly uniform stiffness gels it is possible using microfluidics to generate gradient gels where if this were your substrate you would have and this is your gel you would have a gradient. So, this is your glass, this is your gel you can have a gradient in which one end is soft and one end is stiff, so you can generate a gradient stiffness. And the reason for doing this kind of or making these gels is to demonstrate or to study the effect of durot axis. What is durot axis? Durot axis suggest it is refers to the motion in the direction of a stiffness gradient.

So, it has been demonstrated in HSM camel stem cells, fibroblasts etcetera cells and these cells have a propensity to migrate towards the stiffer end of the gel. Intriguingly very recently it was observed that ovarian cancer cells exhibit reverse durot axis which means that if you plate cells on these gel you would find all of them tend to migrate towards the softered. So, that is it for today I stop here.

So, in summary I have given you an idea of what an hydrogel is, what are some of the properties which you need to keep in mind with gel system we would prefer over some other gels. I did not get a chance to discuss about native gels like collagen or fibrin or methylated, gelatine, where ligand density and stiffness are all related because the same network which is giving you the adhesive ligand that concentration is also dictating the bulk stiffness of that network. And you have that scaling relationship of g prime scaling

as concentration cubed for collagen gels. So, the behaviour of cells in those non-linear elastic materials varies drastically from that observed on materials of linear elastic.

With that I thank you for your attention, I look forward to talking to you again in the next class.