Introduction to Mechanobiology
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Week – 08
Lecture – 39
Techniques in Mechanobiology: Microfabrication

Hello and welcome to today's lecture of introduction to mechanobiology. So, what the last few lectures, I have started discussing about some of the tools which are relevant for mechanabiological studies.

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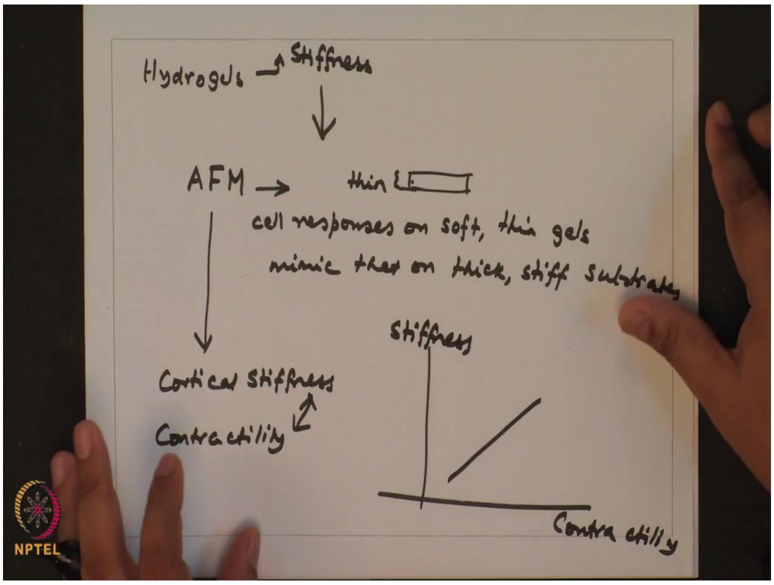

In that regard I started off by discussing about hydrogels where in you can tune the stiffness of these hydrogels by changing the cross linker or monomer concentration, and gave examples of how different kind of hydrogels can be generated and by a different techniques. So, for characterizing the stiffness of cells or gels in that regard, I introduced AFM atomic force microscopy and showed how AFM can be used for proving the properties of hydrogels, cells and in an tissues. And in that regard I also gave a few instances of how geometry of the cell or boundary conditions might influence the properties of cells or what you are measuring. So, for example, if a gel is very thin then cell responses on soft thin gels mimic that on thick and stiff substrates.

So, while using the AFM I discussed how you can measure cortical stiffness of cells, and in the last class I introduced the techniques decided how you can go about quantifying contractility of cells and there is for most adherent cell types there is a very close relationship between the two, in that if you increase contractility your cortical stiffness or stiffness of cells is going to increase you have a linear correlation.

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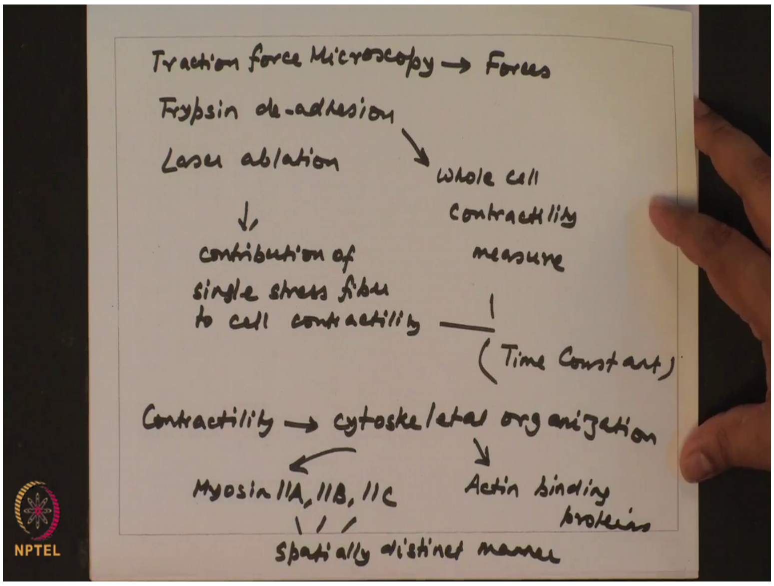

However a more direct approach of measuring contractility in that regard I introduced three techniques traction force microscopy, trypsin de adhesion and laser ablation. While laser ablation gives us information about contribution of single stress fiber to contractility this is cell contractility trypsin de adhesion gives us a whole cell contractility measure, and traction force gives us forces it main measures forces exerted by cells. So, even in both these techniques you measured something called time constant of relaxation. So, this is an indirect measure of contractility, while traction force microscopy gives us forces exerted by cells at distinct focal condition.

Now, if you talk about contractility, contractility is dictated by cytoskeletal organization and when I say cytoskeletal organization I am talking about myosin motors, in non-muscle cells you have 2 or 3 different isoforms of myosin 2 A, 2 B, 2 C these localized in specially distinct manner. Cytoskeletal organization is also dictated by actin binding proteins by actin binding proteins these would include cross linking actin cross linking proteins like filamin, alpha actinin, formin etcetera ok.

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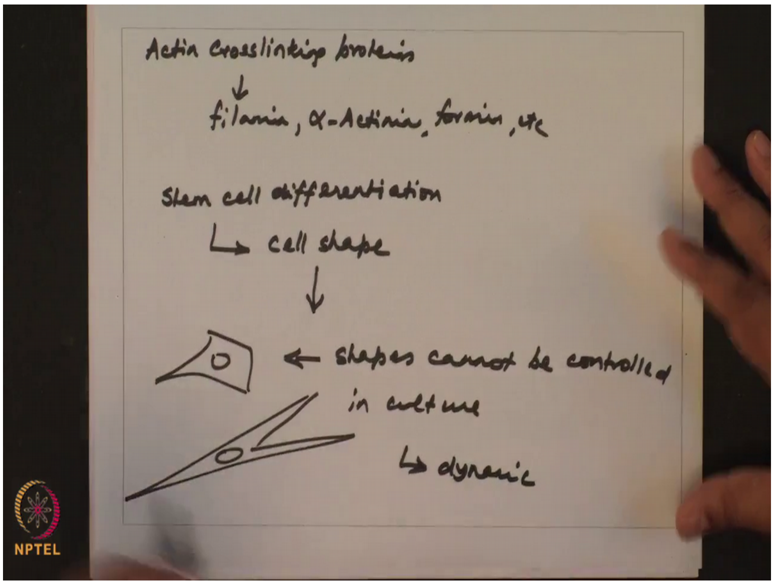

But these are all internal controls or internal molecules which will alter cytoskeletal organization, but in the context of stem cell differentiation has said that cell shape is one of the important regulators. So, the question I want to raise todays, how do you go about controlling cell shape? So, if you plate cells in culture most of the times the cells will have shapes like this. So, this might be a standard shape of a fibroblast or this might be another cell so, but this you cannot control. So, these kinds of shapes cannot be controlled in culture instead the shape is of course, will dynamic in nature.

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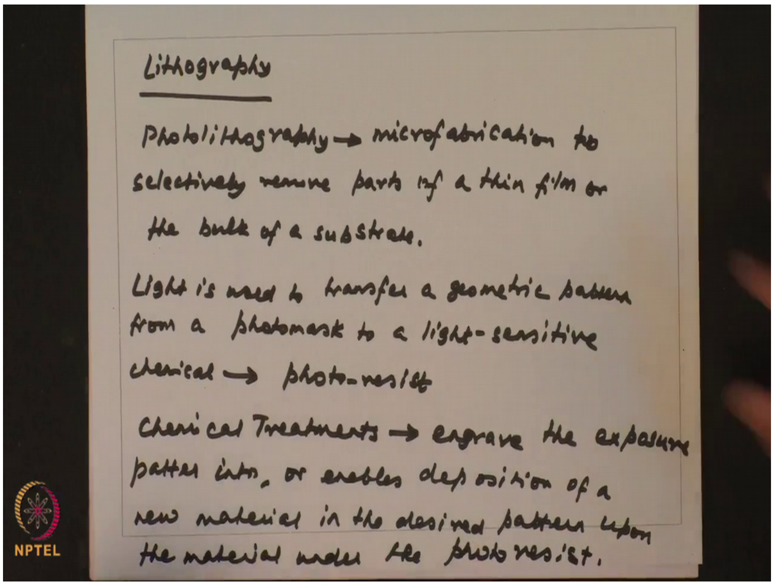

So, how do you go about controlling cell shape in a controlled manner, and in that regard I want to talk about this technique of lithography ok.

So, photolithography this is the process used in micro fabrication which or to selectively remove parts of a thin film or the bulk of a substrate. So, in this technique light is used to transfer a geometric pattern from a photo mask to a light sensitive chemical, this chemical is called photoresist. So, you then have a series of chemical treatments. So, then you have various chemical treatments, which either engrave the exposure pattern into or enables deposition of a new material in the desired pattern upon the material under the photo resist ok.

So, how do you go about doing this? So, you use typically a cleanroom. So, your process workflow is you take a substrate ok.

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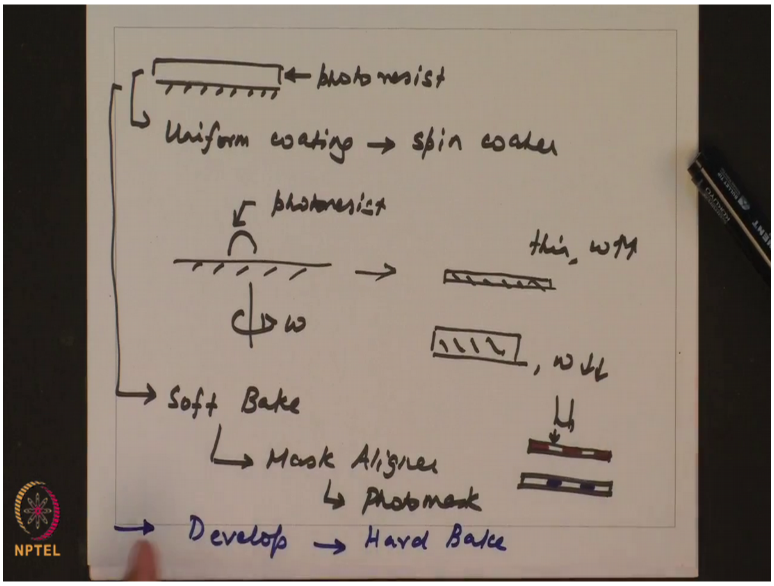

This might be silicon or glass any substrate; you coat it uniformly with your photoresist. So, this it is important to have a uniform coating and to achieve this uniform coating you use something called a spin coater. The idea of a skin spin coater is very simple if this is your substrate this is mounted on a motor which rotates you put a drop. So, this is your spin coater you put a drop of photoresist, and you spin at a given rpm depending on the omega how fast your spinning, it you might get a very thin layer when your omega is high or a much thicker layer when omega is low.

So, after you have done your photoresist, you do a step called soft bake and then using this material you use something called a mask aligner and you shine light onto your material on substrate through a photo mask. So, if this is your spin coated substrate you have your mask aligner and here is your mask. So, the mask has selective zones through which light might be able to pass and selective zones which blocks of the light. So, as a consequence, so, if this is your photoresist, in this zone is getting an exposed to the light and these free zones are not getting any light exposure. So, after you do this after doing this you go ahead and do the next step after photomask you do again a develop you do what is called a develop, where you put this in a development solution and then finally, you do a hard bake which is an optional step ok.

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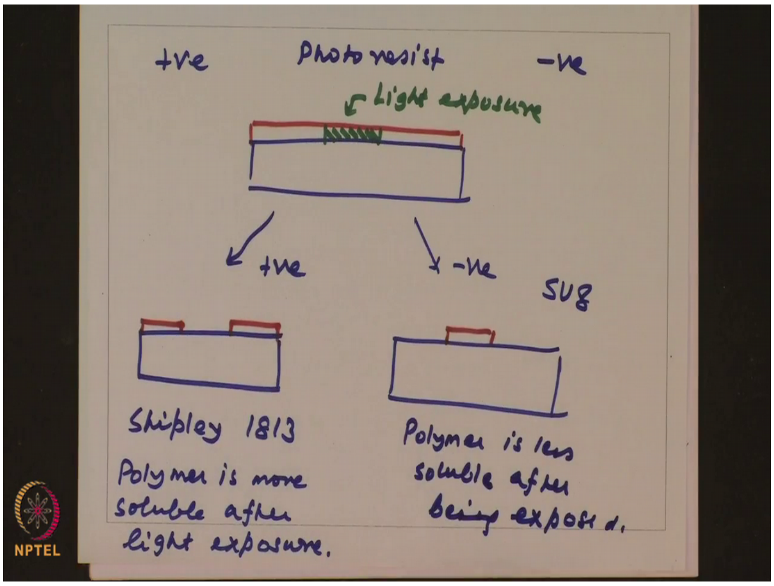

So, in this context there are two different types of photoresist which are possible. One is the positive photoresist and other is the negative photoresist. So, if this is your substrate and imagine you have this is your entire photoresist of which you have exposed this zone to light this is light exposure. So, if this was a positive photoresist if you develop the material then eventually you will have the following thing on the substrate. So, this is an example of positive photoresist and example is shipley 1813 is an example of positive photoresist. So, in this case the polymer or the photoresist is more soluble after light exposure. In contrast you can have the other situation this is called negative photoresist. So, the polymer example is SU 8 then the polymer is less soluble after being exposed ok.

So, in order to have positive and negative photoresist you also will have two different types of photomask. So, as I said that onto your substrate this is my substrate and this is the uniform photoresist which is being exposed.

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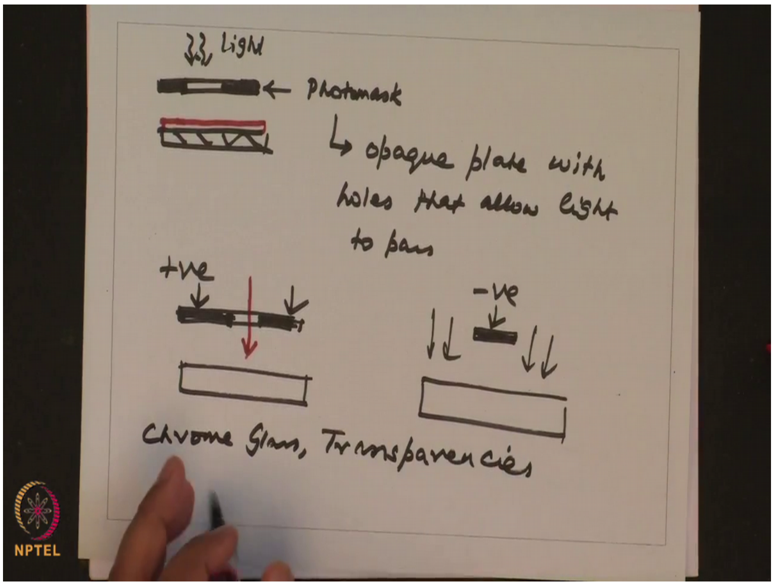

So, this gets passed and then this is your light coming, this object is called the photomask. So, this is an opaque plate with holes that allow light to pass. So, for a positive photomask see if you go back to the example earlier, if this is your positive photomask then your eventual product at the end of it your light is going to pass through this. So, this is your positive photomask and in case of your negative photomask you only have the centered zone and the surroundings will allow light to pass. So, again this is your substrate this is your substrate. So, light will pass through here through here, but it would not pass through the middle really get structure in this case it gets stuck in this two points ok.

So, you can have photomask of different material example is chrome glass or even transparencies now. So, this is broadly lithography what is soft lithography? Soft lithography is what is used for biology applications ok.

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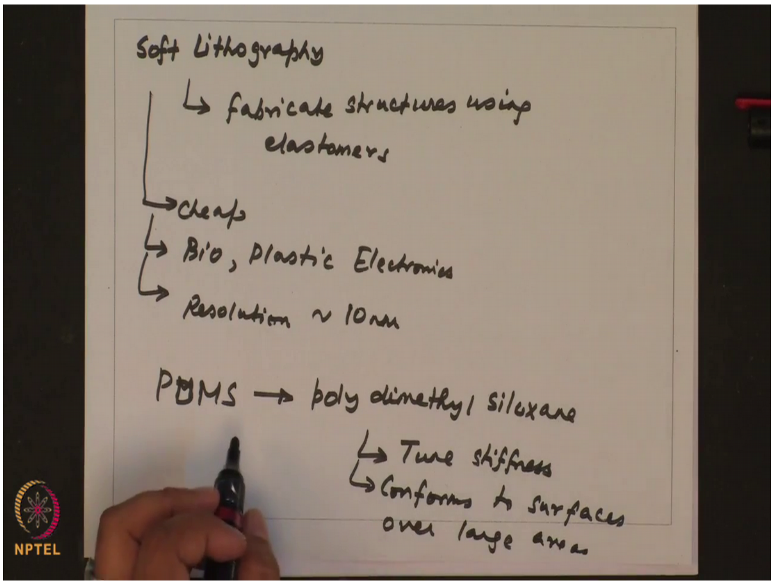

So, this it refers to family of techniques for fabricating structures using. So, you want to fabricate structures using elastomers. So, this technique is cheap and it is well suited for bio applications, also is applications in plastic electronics and you can reach resolution up to order 10 nanometers ok.

So, for soft lithography the region that we use is PDMS. So, this was PDMS was also used for making some of the gels hydrogels, but in this case PDMS is used for making the masks not the you know the patterns. PDMS is full form is poly dimethyl siluxane. So, once again advantages you can tune the stiffness and it conforms to surfaces over large areas. So, after you make something from PDMS, you know you can functionalize something to PDMS using plasma oxidization ok.

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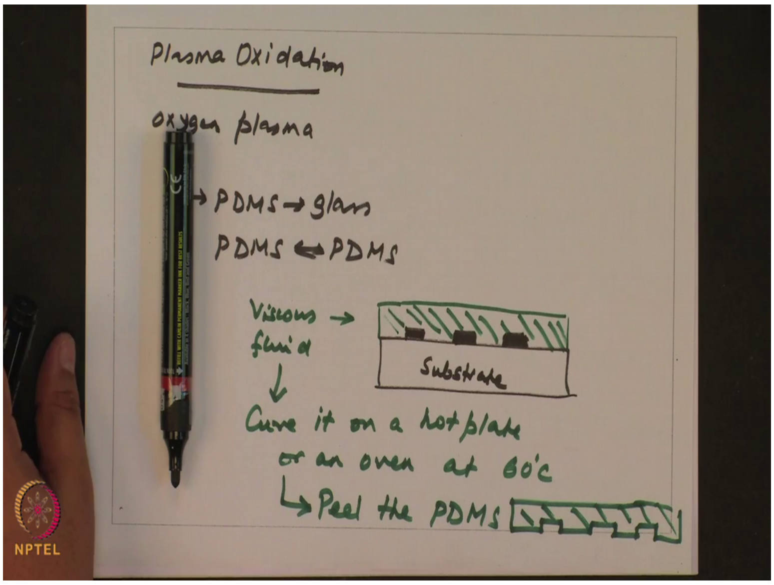

So, where you expose this to oxygen plasma and using this you can have PDMS stuck to glass or PDMS sticking to other PDMS. The problem is this process is not reversible because covalent bonds are made.

So, how do we go about making a PDMS material? So, what you do you begin with the same thing you have using lithography, what you have achieved is you have transferred a given topography of your photoresist on the substrate. So, this is a photoresist. So, what you do? You first dump PDMS on top of this you dump PDMS. So, this is initially a viscous fluid initially a viscous fluid and after you dumped it what you do is you cure it. So, curing it on a hot plate or an oven at 60 degree Celsius, what you will have is this material will then polymerize. Once it has polymerized you can actually peel the PDMS. So, what your with your left with is something like this is what with your (Refer Time: 19:11) ok.

Now, once you have done this. So, of course, what you can tweak is these geometrical features; however, you would intuitively expect for example if I am making this kind of a pattern, but these are very tall these features you might have defects where these actually collapse onto each other.

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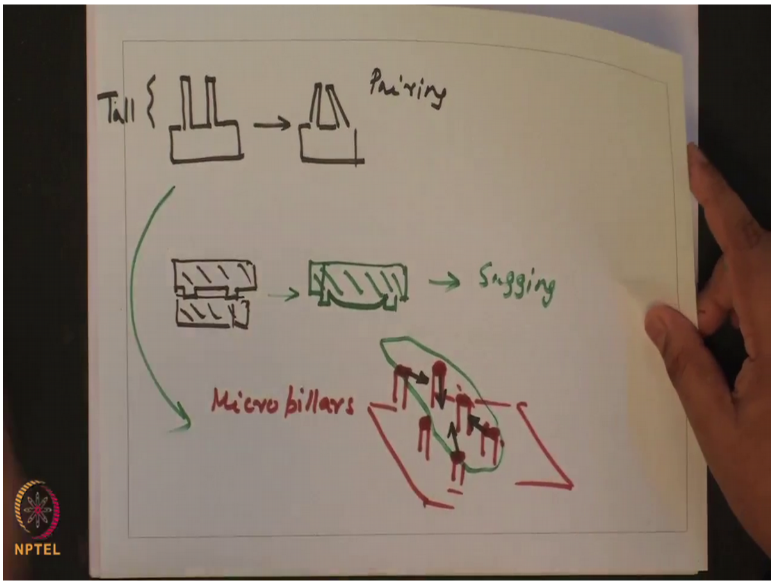

So, this is an example of a defect and this is called as pairing in addition on top of the substrate let us say if this is your substrate. So, after you have done the PDMS what you want to do is on a new substrate you want to invert your PDMS, after coating it on a given with a given ligand. But in this case you might have the PDMS might sag. So, you might this kind of a geometry which is. So, this is a sagging issue.

So, using PDMS people have optimized the composition of the PDMS that the use and like this you can use this very well you can use this for getting micro pillars. So, in other words if I were to draw it in a two d fashion, this is what you will have. Similarly you will have this kind of patterns on a 2D plane. So, if this was true then you can plate a cell on top of this and you can have cells which. So, if this was ligand coated. So, the top if you had coated it with some ECM ligand then the cell can actually spread and then exert. So, you will have a cell assemble on this, an exert forces which are inward direction which are directed inward.

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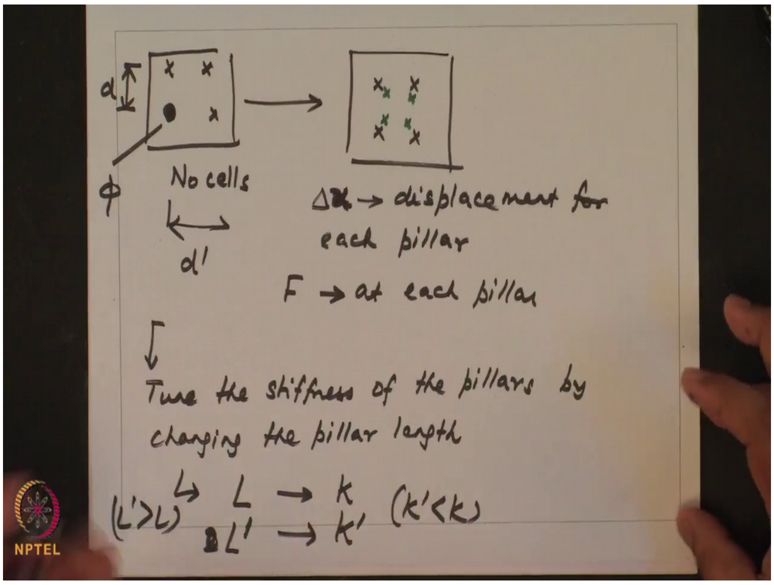

So, as a cause so, if I were to look at the same thing on from the top, what I will get is so, let us say I just write down the I am drawing the center point in top view, you have this four type if we grid when no cells are there. So, you can design in such a way this d is known this d prime is also known, and the diameter of each of these pellets phi is known ok.

So, when you put the cell you can track the position. So, let us say if this for the original positions. So, if the cell you are exerting contractile forces, then the tops would be displaced inward you can have this and for each of these pillar. You can find out what is the displacement delta d delta x is the displacement for each pillar. So, because you know the pillar displacement and if you know the stiffness material of this material then you can I exactly determine the force at each pillar. So, the advantage of this technique is first of all you can tune the stiffness of the pillars, by changing the pillar length. So, if the pillar is L and let us say its stiffness is K, if you have 2L if you have L prime by L prime is greater than L then K prime is going to be less than K. So, if L prime is greater than L simply because if you make the pillar tall and tall it becomes more compliant. So, it is easy to deform it ok.

So, this is one of the big advantages of using micro pillars because you do not need linear elasticity theory to solve the inverse problem, for finding out the forces exerted by cells here once you know the displacement you can exactly map out the force. So, this is one example where microfabrication has been used, the other example of controlling cell shape is using micro contact printing.

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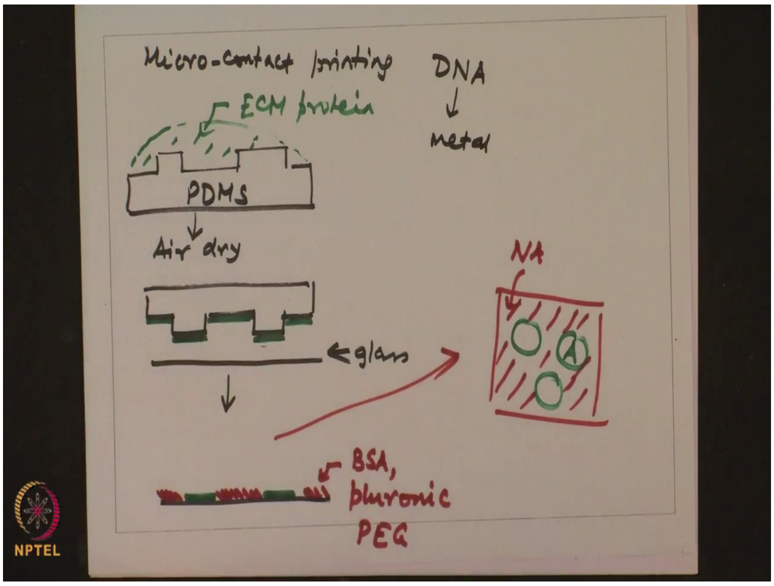

So, in micro contact printing what you do is let us say this is your PDMS mold, mold you incubate it with some ECM protein of your choice. So, you incubated with your ECM protein of choice let it fit for some time and then what you do? You take a substrate let us say this is glass you invert the PDMS. So, after doing this you let it air dry ok.

So, as a consequence if you let it air dry only these points all these points will be coated with your ECM protein, but when you stamp it then what you will be left with is the ECM protein only at these ex protrusions will get transferred. So, at this point your ECM protein is getting transferred and after you have done this, you can block the remaining zones with BSA or Pluronic or any other made or peg any non adherent material. So, if you look at it in top view what you will have is as follows ok.

So, let us assume. So, geometry is were circles and the remaining zones. So, red is non adherent and green is adherent. So, that is how you can control cell shape, but what you have to optimize is to make sure that your coating of pluronic or peg is uniform if this does not work then your micro contractibility would not work. So, and instead of coating just your ECM proteins you can also micro print DNA on it or any other metal on it. So, you are not limited to type of thing in which you are with which your working. So, I just discussed one last study. So, what was shown is the link the first one of the first studies what it showed was the linkage between cells fading and apoptosis ok.

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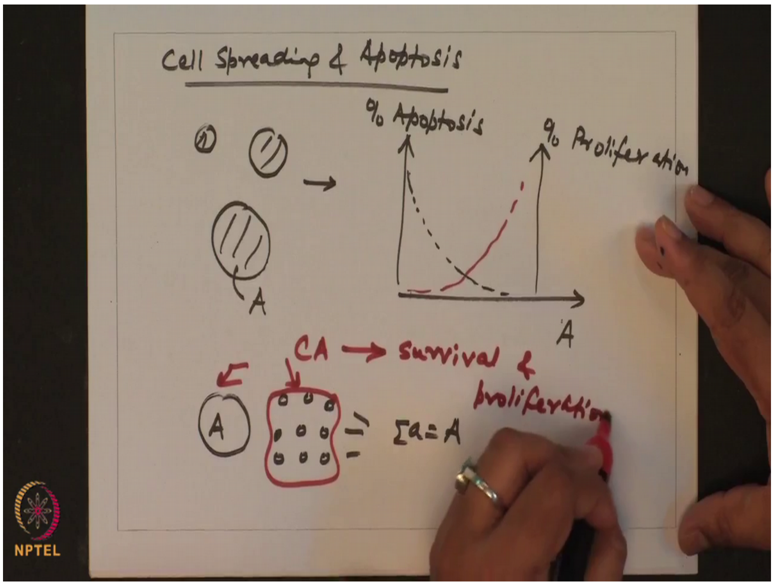

So, what was done was to make these islands of different sizes. So, the surface area keeps on increasing you plate cells on this, and you track what is the percentage apoptosis. So, x axis is your spread area. So, this area A. So, what was found was the apoptosis was very high when you are spread area was less, but it dropped off as soon as your area kept increasing. So, this suggested that you need an optimal amount of size for a cell to be to survive, and as they was a dropping apoptosis if you plot the percentage proliferation, you would see a corresponding increase in the proliferation rate ok.

So, this was the first study to demonstrate the relationship between idle size and islands and apoptosis and what it was also shown. So, because increasing spreading area is associated with increase in focal addition area. So, they did generated different areas where you have one circular eye, in other case this same area you distribute across multiple points at equal spacing. So, that summation of area is same as A and in this case what is happening, is the overall cell spread area. So, if a cell spreads like this this cell spreading area is much greater than this area and what it was found that it is the cell spreading area which regulates survival and proliferation.

So, with that I stop my lectures here and I to summarize I have discussed how you can make use of soft lithography or microfiberzation, for tuning generating structures like micro pillars for your traction force microscopy studies or using micro contact printing to generate islands of different sizes and shapes, and study cell behaviors beat spreading survival proliferation differentiation so on and so forth.

Thank you for your attention.