Introduction to Biomicrofluidics
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Lecture – 04
Fluidics in living systems and mechanobiology

Building blocks of living or nonliving things are attracted by that physical force. This physical force and mechanics are paramount importance for their shape as well as that biological outcome. So, far in the biological area all the biological outcomes are explained in terms of reaction diffusion mechanism.

But, in some cases deduct mechanical forces involved to move the molecule ourselves for the complex biological outcome. So, mechanotransduction is the process which converts that mechanical force to all the biological event and that convert the mechanical force to chemical signals by which the cells can do all of it is manifestation, like say growth maintenance differences and functioning and last of all that apoptosis.

So, in this lecture I shall concentrate on the what are the forces mechanical forces particularly say fluid flow and other interstitial flow and how these forces are sensed by that cells and they process it or convert it to that chemical signals and do all the jobs. And to understand all phenomenon how that fluidics platform is essential to mimic, the biological means confinement or biological reactions by which we can understand that that in vivo biological phenomenon more or less in details.

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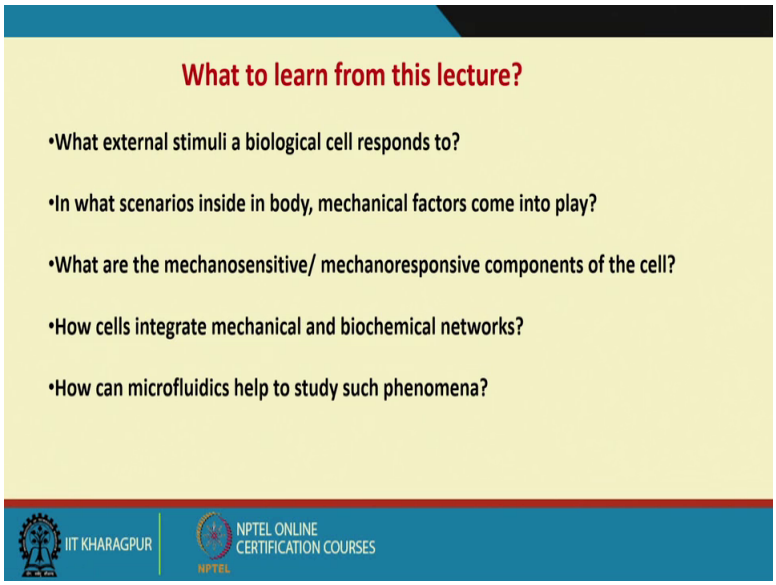

So, what we learned from this lecture? What extend the stimuli of biological respond to; in what scenario inside in the body mechanical factors come into play? Then what are the mechanosensitive or mechanoresponsive components of the cell? How cells integrate mechanical and biochemical networks? And how can biomicrofluidic cells to study such phenomenon.

Now, if you see that cell that is in these units of all living system is a viscoelastic material if we look for that physics point of view and it is surrounded by that.

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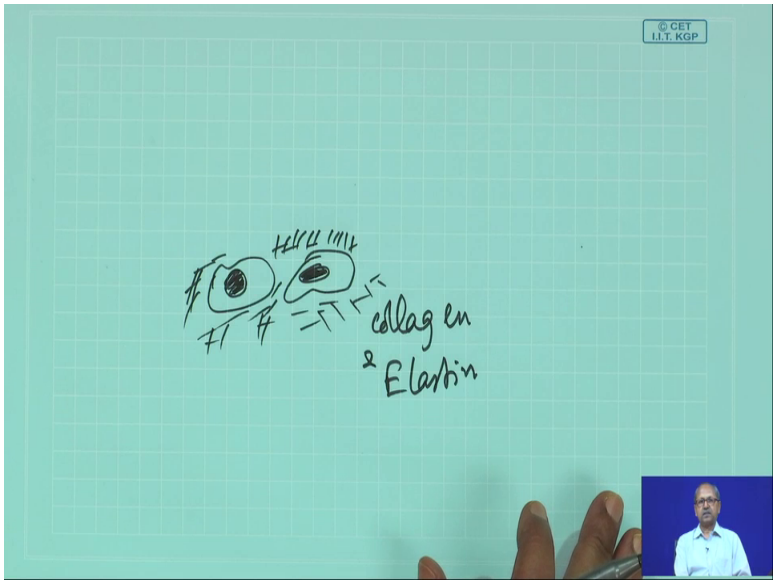

It is surrounded by that extracellular matrix protein like say these are the 2 cells and this is the surrounded by that extracellular matrix proteins. These extracellular matrix proteins provide the surface or provide that support to sustain that cells and these ECMs extracellular matrix it contains mainly that protein collagen and elastin.

Collagen provides the tensile strength stiffness as well as it hinders that your strain due to compaction, that cyclic strain by which that cell can sustained. So, now we can understand that cell is a viscoelastic material and extracellular matrix also has a mechanical properties. So, the cells and that extracellular matrix they give the signal to each other by which they can manifest their programs or you can tell that outcomes.

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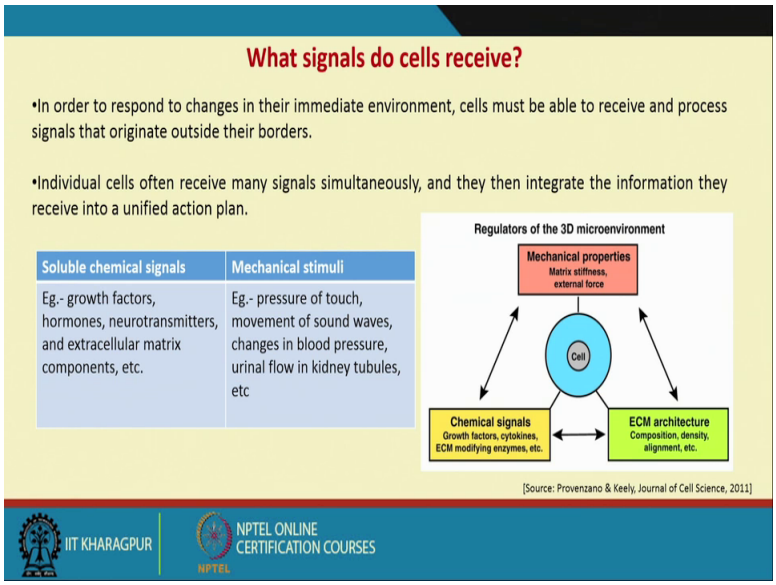

So, if you can see that here that cells it is acting it is own mechanical properties and ECM architecture at the same time chemical signals. Means cell can integrate all the physical forces together then convert to that is your chemical signals and do all that biological outcomes. Now, what are the chemical signals? Chemical signals are growth factors, hormones, neurotransmitter and etcetera and mechanical stimulation likes say pressure touch movement of sounds waves and etcetera.

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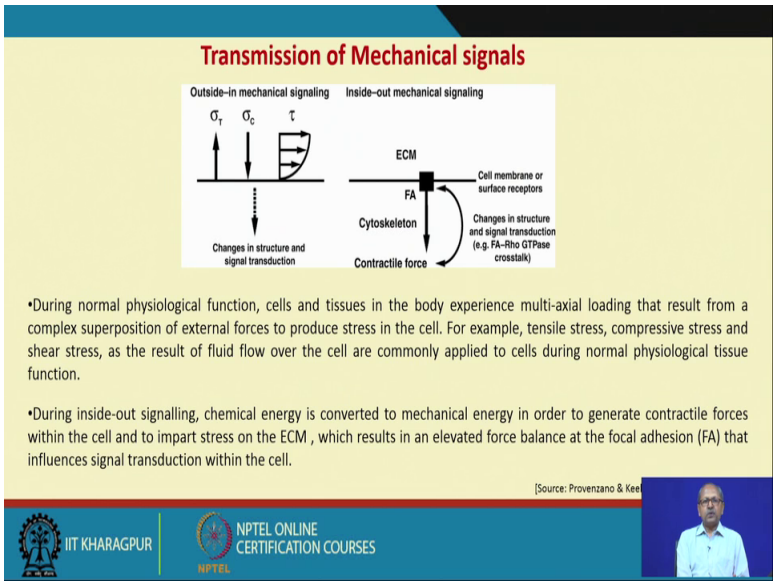

If we look for that extracellular matrix point of view, they get the signal from outside to in means that stiffness of that extracellular matrix provides that your fluid stress then tensional force then compaction force. And on the contrary cell will provide from their chemical signal process to mechanical signal through focal adhesion site gives the transmit the force so this is signal inside to out. So, signal outside in and inside out they feedback each other and do the biological outcomes.

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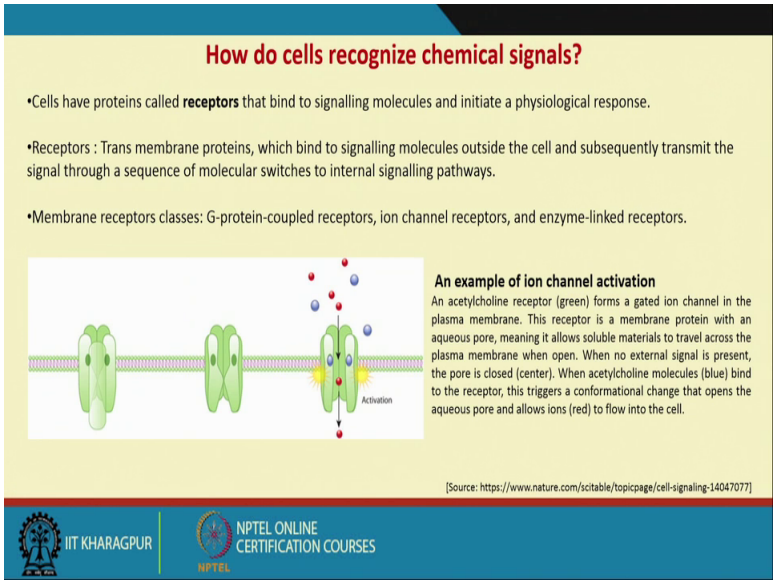

So, look for that what are the means your chemical signals, in our biological fields we know that all the chemical reactions are governed by your reaction diffusion. So, here that chemical signals are recognized by that cell surface receptors basically they acts as a antenna like things.

So, these receptors will recognize that outside chemical signals they transmit that signals to second messenger molecules then so and so forth, ultimately go to the nucleus and they process that message. So, if you look for that this diagram here that ion channel receptors are there in presence of particular molecules acetylcholine, that ion channels is opened off they need open the channel for passing that all the ions from outside doing.

So, if you can classify that receptors are of 3 types basically one is your membrane receptors class another is G protein coupled receptors and ion channel receptors.

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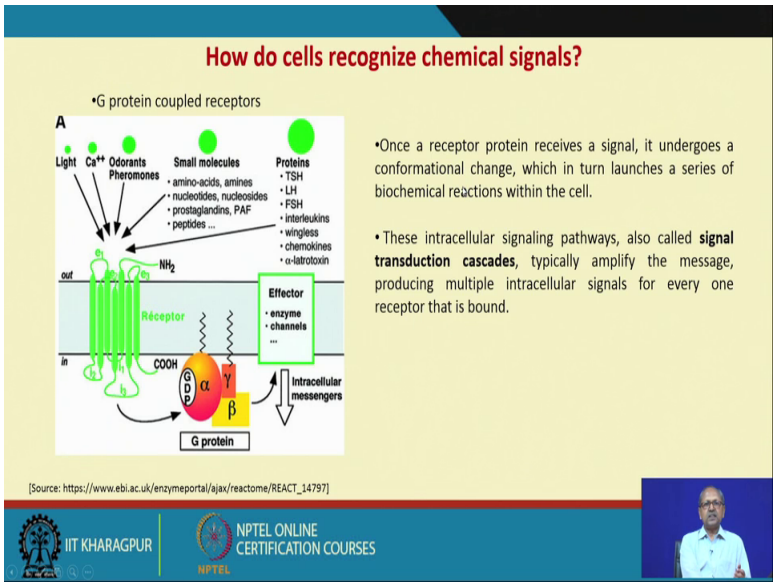

So, just now we discuss that ion channel receptors and what are the G; G protein coupled receptors there are a variety of signals like say light ions and your different types of hormones and etcetera they bind to their specific receptors like say these are the specific receptor then the pasta signal to G protein. Now, the G protein will trigger that membrane round some enzymes or say ion channels to give the second messenger molecules.

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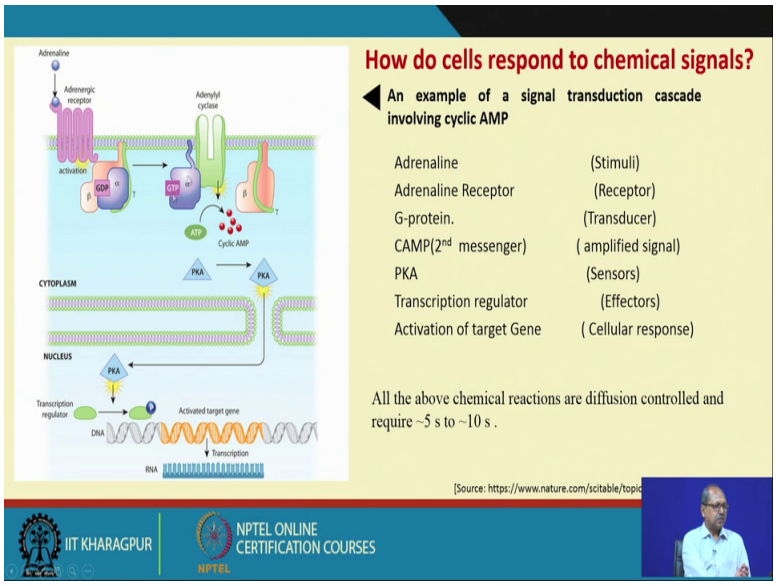

Like say if we take an example of say adrenaline, it is a stimuli that adrenaline receptor in that membrane it acts as a receptor, then G protein acts as a transducer, cyclic amp produced from that adenine cyclists that is amplified signal. Then this amplified signal activates the protein Kinase A that is acts as a sensor, then it is going to the nucleus then effectors molecule is transcription regulator.

Then gene activated means outside signal is recognized the receptors, then they pass that signals, and these signals are diffused in the cytoplasm, then they go to the nucleus. Then they give the form of messenger or an a transcription. Hold the process it takes around 5 second to 10 seconds.

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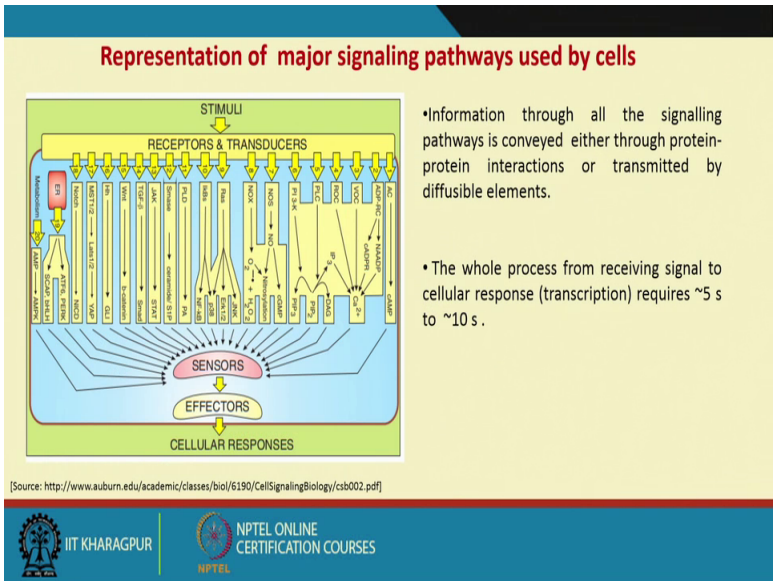

So, if we summarize all that your chemical signal transduction pathways basically there are around say several pathways and near about 16 or 17 pathways and there are some pathways which are signals are generated inside that cytoplasm. These metabolites and other signals work by that either protein interactions or second messenger molecules. And these signaling pathways more or less they always crossed talk with each other and for a specific biological outcome.

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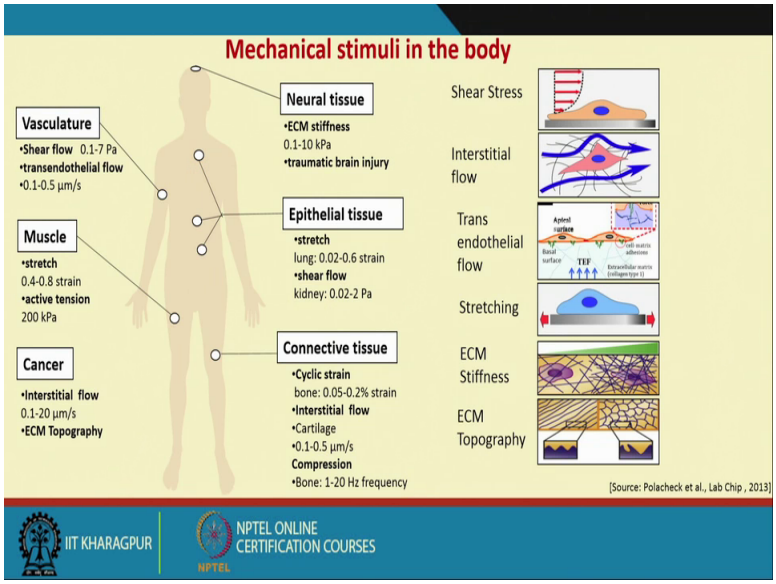

On the other hand if we look for that in our body what mechanical stimuli we face we face different type of mechanical stimuli in the different parts of the body, say shear stress interstitial flow transendothelial flow stretching. These are if we go through that diagram actually we can see that in vasculature we can see that shear flow interstitial flow in the muscle. We can see that stretch active tension in case of neutralities. So, we can tell that ECM stiffness then epithelial tissue, we can look for the stretch shear flow and a connective this is cyclic strain and interstitial flow and compaction.

Moreover that, ECM extracellular matrix which provide the steepness of the tissues, it has also a architecture like say topography in the Nano scale topography which is very much important for functioning the cells. And from this diagram we have a little bit idea what types of mechanical forces are important by that fluid flow or compaction.

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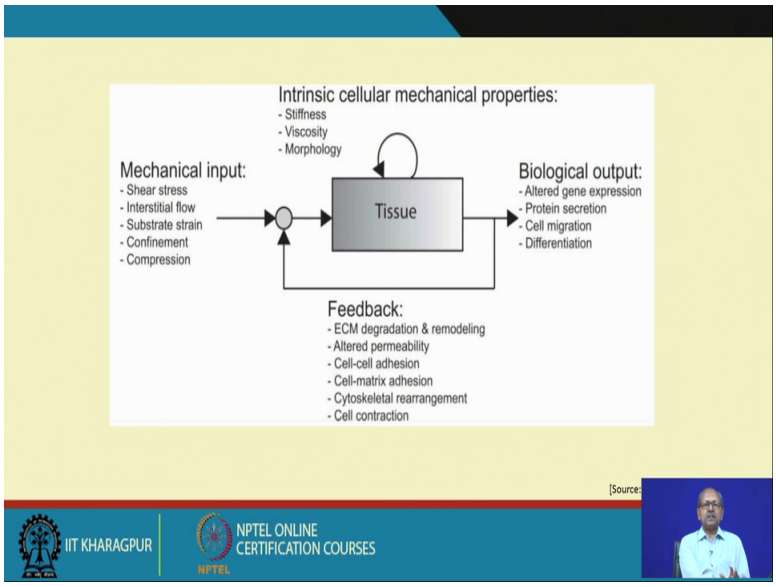

So, this is an means how mechanical inputs like say shear stress interstitial flow is going to that tissues is going to tissues, then tissue with it is intrinsic mechanical properties like say stiffness viscosity. It is converted to biological outputs like say your altered gene expression protein secretion cell migration differentiation. And then this goes the feedback to mechanical input by which tissue can modulate itself to do that biological output.

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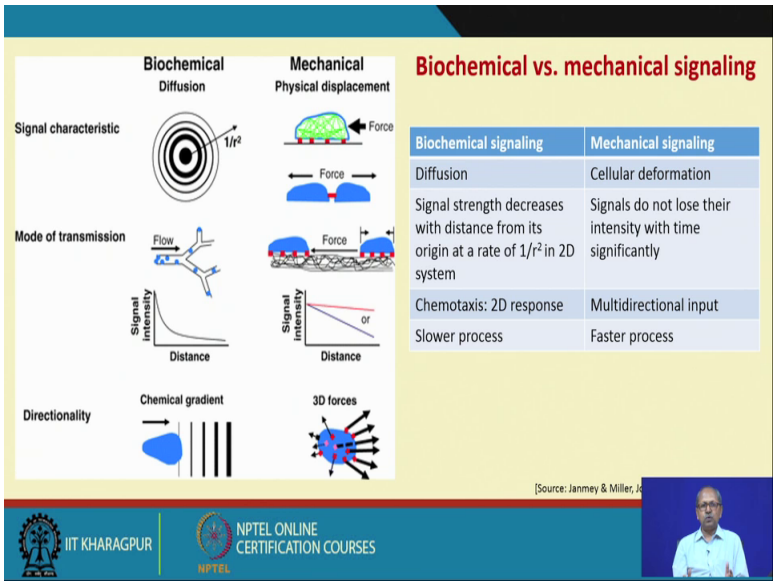

Now, how these 2 signals means mechanical signal on the other hand chemical signal differs basically. So, biochemical signals or chemical signals these are of diffusive in nature, whereas mechanical or a physical year force it is a displacement like (Refer Time: 12:28) is a basically it makes a deformation of the cells. Otherwise they are transmit that force by cell interactions or through extracellular matrix proteins.

Next is that chemical signals are transported or transmitted by your symbol wise diffusion that is why it takes long time. Whereas, mechanical signals they transmit by your compaction, so there is a not much loss during the transmission may be due to some damping.

But that not too much basically that is why your mechanical signal transmits within a say 5 microseconds, whereas chemical signal transmits around say 5 to 10 seconds. And last difference is that cells sense that your chemical signal in a 2 dimensional way like say chemotaxis, it is 2 dimensional and it sense that your chemical signal 2 dimensional. Whereas, in the mechanical signal they sensing totally 3 dimensional way that is that 3D force the sense.

So, we can have an idea how that mechanical signal differs from biochemical signals, but at certain point of time that mechanical signal will be converted to chemical signal, that is why that mechanotransductions is coming to that scenario.

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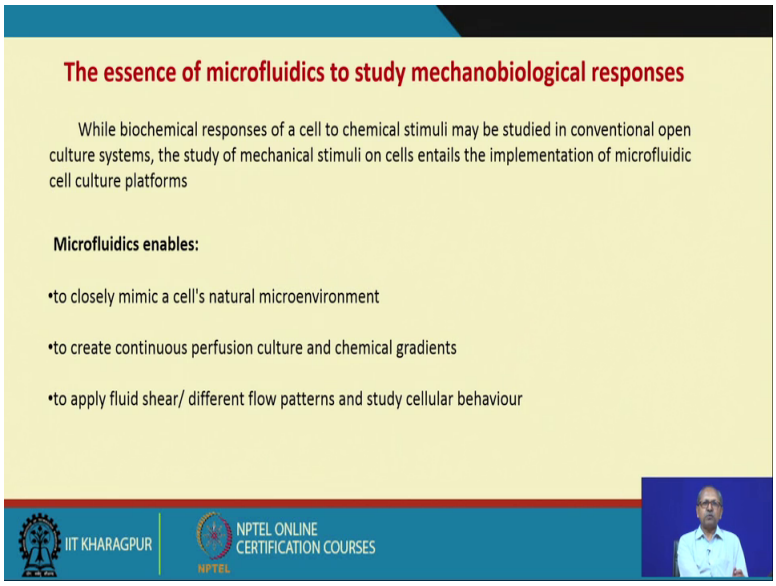

So, a sense of bio micro fluidics so study the mechanobiological responses are that to closely mimic that cell natural micro environment means, which environment or which confinement cells exist along with that mechanical forces and to create a continuous perfusion system. And, chemical gradients or you can tell that mechanical cues gradient to apply fluids shear or different flow patterns to study the cellular behavior. Like say in our body we have pulsatile flow but you know in all the cases pulsatile flow may not exist, basically particularly in the venice situation it is a steady flow.

So, we have to create different types of flow to study that cell behavior what is occurring in vivo, if we can mimic they are in vitro to simulate that real situation.

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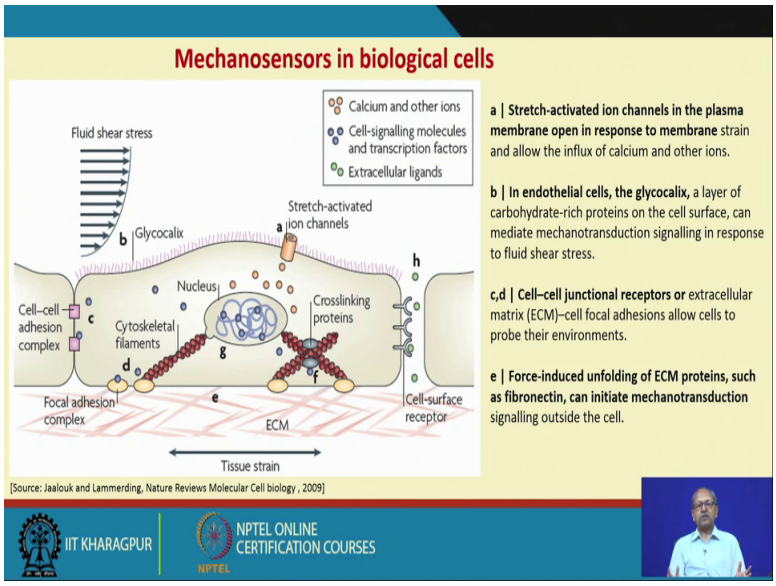

So, what are the mechano sensors in biological cells first one is your stretch activated ion channels means when membrane are stretched these ion channels are activated. In endothelial cells a glicocalix there is a glycoprotein like things which are protruded from that cell surface, these are also acts as an mechano sensor because on flow these moves basically.

Then cell junctional proteins this is atdhe net complex and cell ECM that junctional complex at focal adhesion complex basically. Your force induced unfolding ECM proteins like say fibronectins which are their active sites or some peptide sequence a cryptic not, on force those cryptic sequence are exposed and by which integrands of that cell surface protein they can interact.

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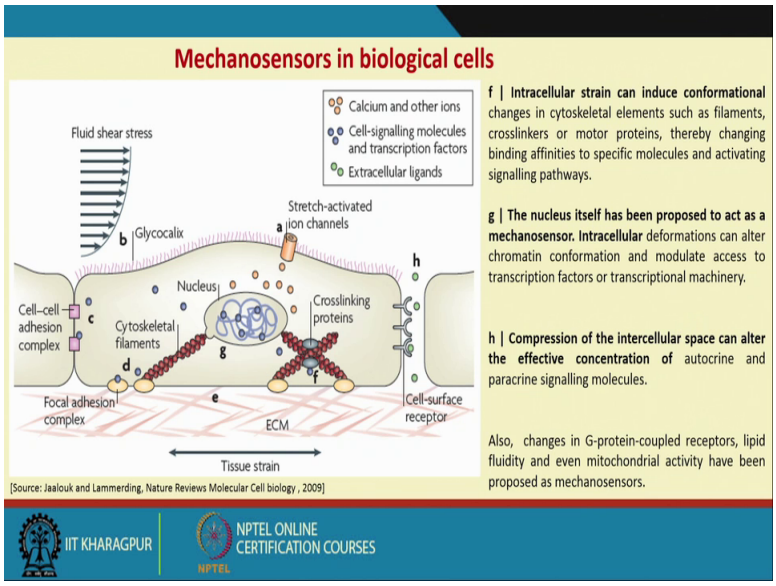

Then intracellular can strain can induced conformational changes in cytoskeleton, by which that motor proteins and other assembling structures are changing and mechanical signals are produced and that nucleus itself actually mechanosensors, because that mechanical force directly may transmit through actin myosin or tubulin fiber to nuclear envelope and then they can activated the chromatids.

That compression of intercellular space can alter the effective concentration of the autocrine or paracrine molecule that is means your effective concentration at that is confines spaces increasing also. Besides this there are others a lot of G protein coupled receptors lipid fluidity also involved during that your fluid shear. So, 2 important mechanosensors or say focal adhesion complex and your lipid rafts are very much important in our study. So, I shall discuss little bit about that focal adhesion complex.

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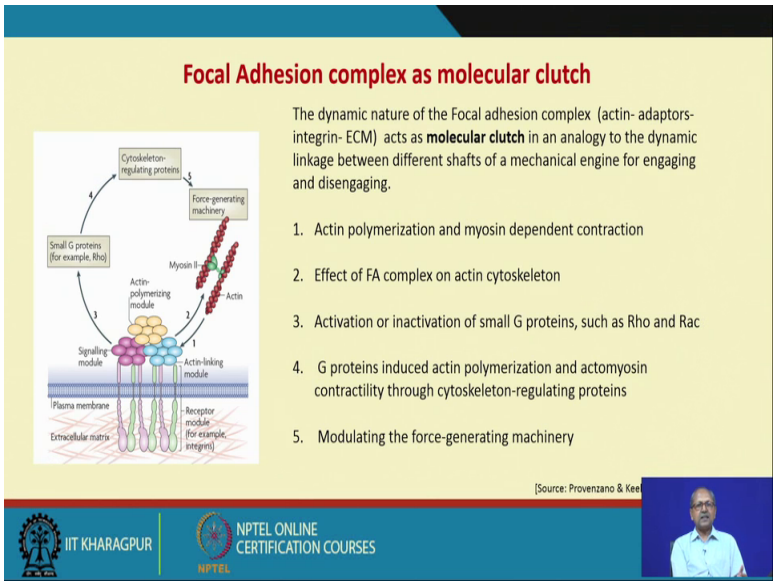

Focal adhesion complex it is a acts like a clutch in a mechanical system which engaged and disengaged between the 2 systems. Here that focal adhesion acts to engaged cell to the ECM and again disengaged cell to ECM and transmit the force accordingly.

The focal adhesion complex basically it is a very dynamic in nature, it is starts initiation then growing then matured then disassociates. The whole the process takes around say maybe 20 seconds or less according to the situation and it starts that when that actin filaments are means polymerized it gives the stress or give the force to that cells and then ECM.

This process triggers that actomyosin complex to form that molecular or compaction force, then it recruits lot of modules this is the adaptor proteins, then signaling molecules then acting polymerizing module. And all these complex again triggered that actomyosin complex may compact and give the force and this your signaling molecule triggers that small G protein or the GTP and etcetera then the triggers that cytoskeleton regulating proteins then force generation.

In that process it is a recirculating and that focal adhesion is growing around say 3 micrometer area and it imparts force or transmit the signals from ECM to cells and topography of ECM and that stiffness of ECM influence that focal adhesion strength.

So, this is you can tell that outside in signal is provided by that focal adhesion complex and when focal adhesion disassociates when that actomyosin complex that contraction force is much much more than that focal adhesion force around say 5 micro Newton 5 pico Newton per micrometer squares. So, at that situation focal adhesion dissociates then again it starts focal adhesion point in other place of the cytoplasm. In that way a focal adhesion transmit their mechanical signal inside the cell and it is converts to chemical signals.

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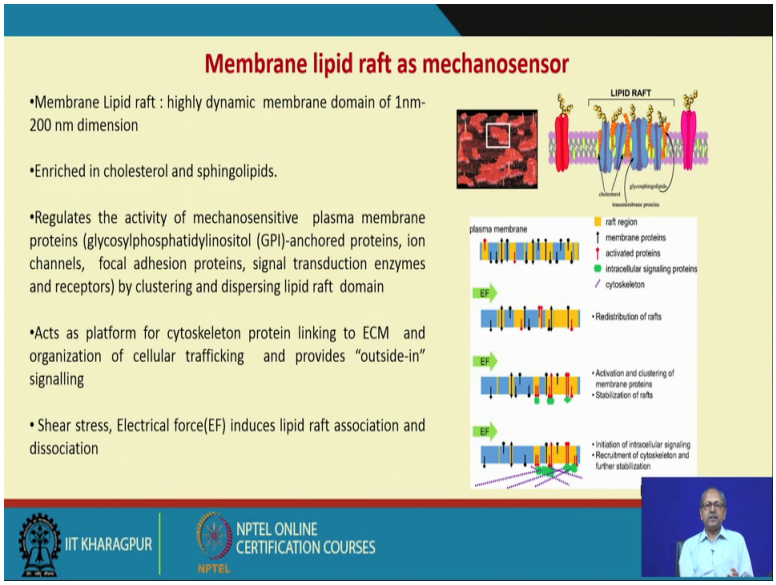

What is a lipid raft? Lipid raft is a micro domain in the cell membrane it is also very dynamic in nature, it is dimension around 1 nano meter to 200 nanometer. It is enriched in cholesterol and sphingolipids it is regulates activated the lot of mecahnosensitive plasma proteins basically plasma proteins or you can say that protein complex in the adhesion to focal adhesion proteins.

And here a acts as a platform for cytoskeleton protein linking ECM and organizing cellular trafficking means that lipid raft can be transmitted from their cell surface to Golgi. Then again it is coming to the cell surface even in going to the focal adhesion also. So, that is why it is very much dynamic and it is size ranges from 1 nanometer to 200 nanometer dimension and it is size and that aggregation depends upon that fluidity of the membranes shears and etcetera.

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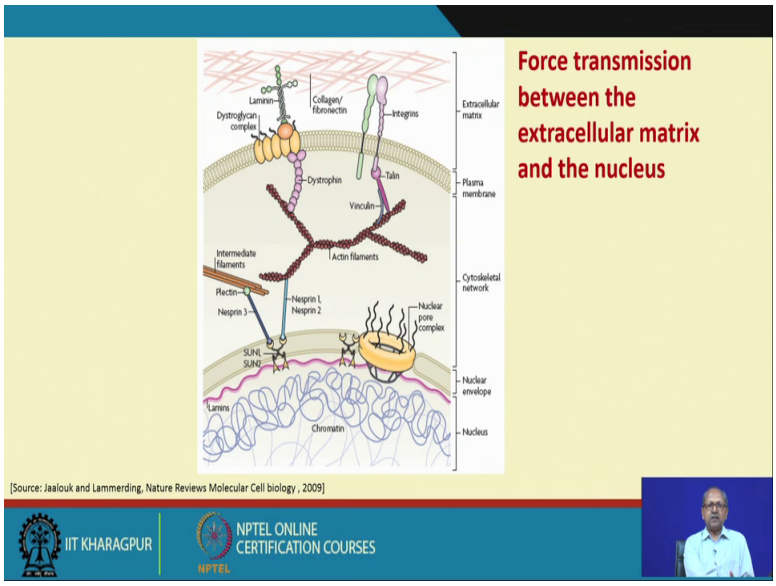

So, as we I told you that that mechanical force directly go to the nucleus by the help of your actin filaments nesprin, then these going to that Lamin, then it is your acting to the chromatin to transcribes it is all the activities or you can tell that messenger RNA TRNA and synthesis.

So, what we can tell that mechanical force from outside going through focal adhesion complex or other mecahnosensors through a actomyosin complex or microtubules then this is directly in contact with that nuclear envelope proteins and it is transmitted to chromatid for their transcription and your other activities.

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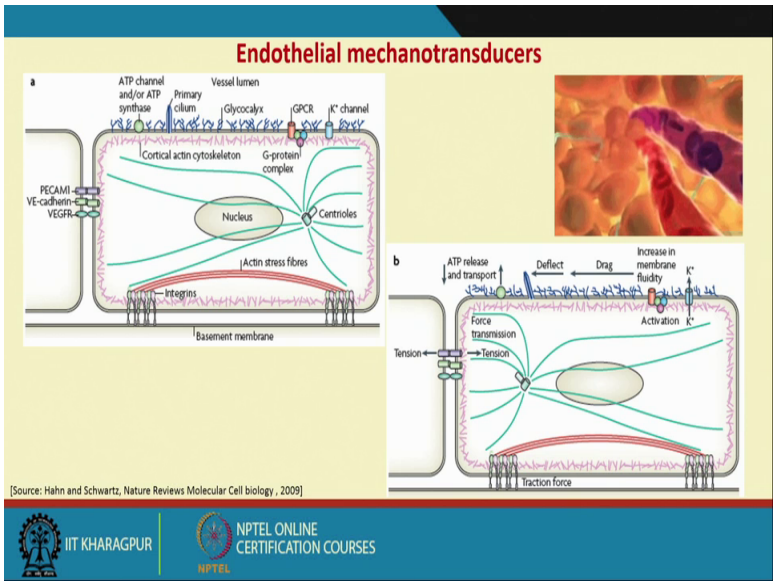

In our body each and every tissues and cells in that tissues faces that mechanical force. So, this is an example how that in endothelial cells which faces that blood flow it faces, that means your mechanical force and it converts to chemical signals. Say in the as I had already mentioned that in endothelial cells they have that a glycocalyx and these glycocalyx are patruded from the cell surface when fluid flow during that stress is developed and under the stress this is bending basically. And this triggers that other ATP released activity then stage activated ion channels and that mechanical force is transmitted to that cell junction and focal adhesion points and it starts that endothelial activity.

And endothelial cells should be properly connected for their proper functioning and that is very much essential for it is integrity. And fluid flow means your blood flow is means types of blood flow whether it is a streamline or turbulent influence at activity.

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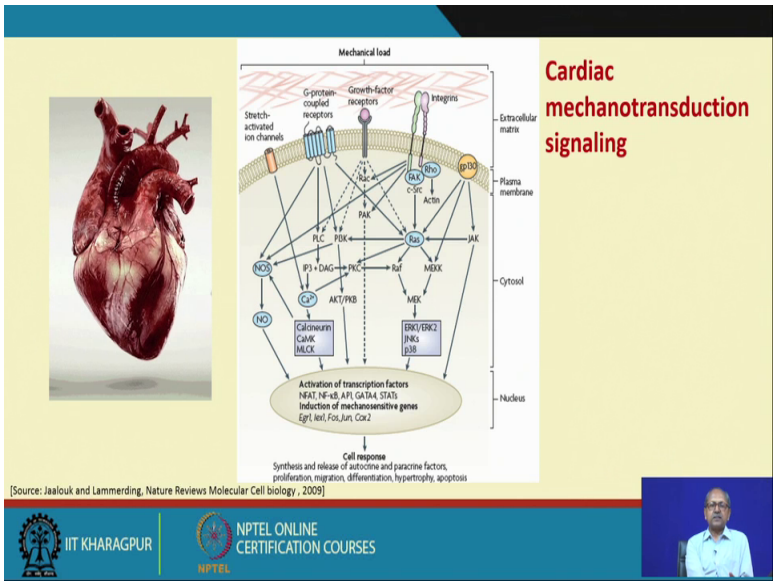

In cardiac muscles that is cells are cardiomyocytes and it is a mechanical force acting organ it generates that force. This is a your seeing that this is a heart beating basically means each and every cell of the myocyte cardiomyocytes beats and that beats starts with their lot of integration of that mecahnosensitive sensors like say stage activated ion generals G protein and integrands. And they converts these mechanical force to chemical signals through RAS NOS nitric oxide then they converts to nucleus like NFAT NF kappa b and etcetera. So, cardiac mechanotransduction signal is mainly that mechanical forces converged to chemical signals inside the cells and do it bricking job.

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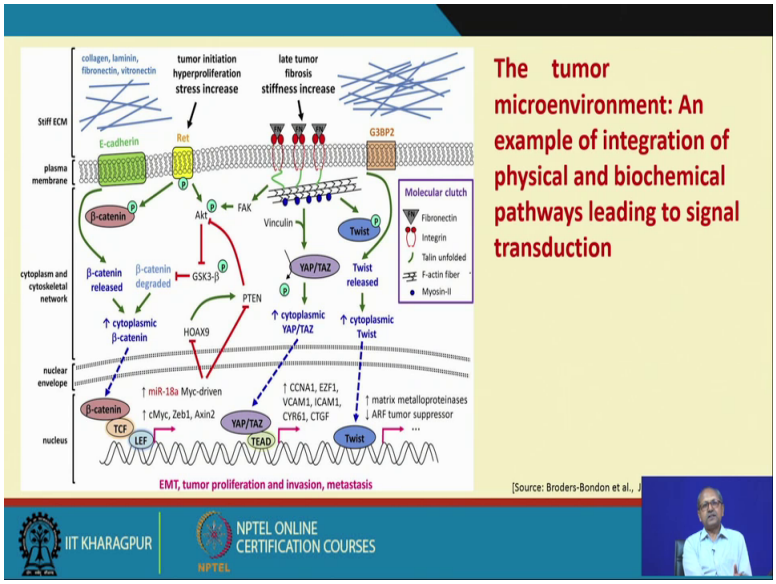

The tumor is a nice example where chemical signals and mechanical signals integrate together and they provide that signal in out outside in signals.

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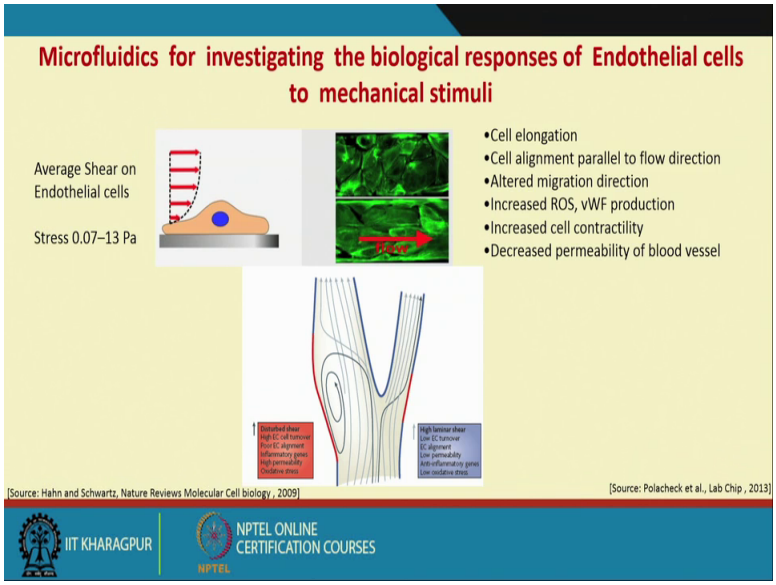

There are few examples I am citings here where that microfluidic platform is essential to understand that physiologically behavior, like say micro confinement and that your mechanical force fluid stress etcetera. This is an example say we know that atherosclerosis pluck in heart disease basically and pluck develops where that bifurcation is there. Have that your blood flows means parallely to that channel means your without any turbulent particularly this portion there is no atherosclerosis pluck developed. Whereas, when the turbulent flow is there that atherosclerosis pluck is developed due to information leakiness lot of things are there is.

Now, question are is a why this is happening to answer this question microfluidic platform is the answer. Because, when your endothelial cells are growing in the microfluidic channel when you are giving the flow around 0.07 to 13 Pascal, then the cells are aligned properly that direction of fluid flow. But if we give a less flow or in turbulent flow then cells are not arranged properly cells are not adhere properly and that means leaky.

So, possibly this is the answer that is from that basic fluid mechanics we know that where the bifurcation is there and that where that channel diameter is higher, then fluids flow becomes turbulent. So, in the turbulent flow cells are leaky, so that is a very

interesting observation we can prove what is happening in the nature in the microfluidic platform.

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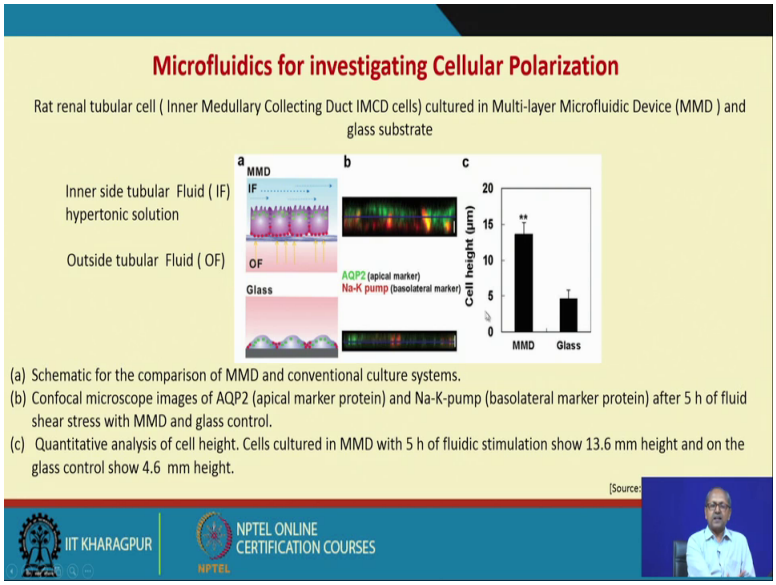

This is an another example without microfluidic platform we cannot understand that physiological phenomenon, in that renal tubular cells epithelial cells had it is means by osmosis or hydrostatic pressure they pass that sodium chloride and activated activates that renal filtration process. If you culture that renal tubular epithelial cell in normal glass plate then polarization we cannot see too much. Polarization means one is your basal layer another is apical surface and both the surface has a different functions they express different proteins.

But if we used some your multi layer microfluidic platform then the cell behaves or polarities nicely visible. And you can see that here that apical surface which faces that hypertonic sodium chloride solutions they express at accompany. Whereas, in the basal layer which is the interstitial flow that is express that sodium potassium pump and at the same time you look for that height; height of the cells is normal in the microfluidic platform here is a normal glass surface it is not flatten.

So, microfluidic platform it gives that cellular means mimicking that physiological system, in that way there are huge amount of examples by which we can understand that your physiology by your using that microfluidic platform.

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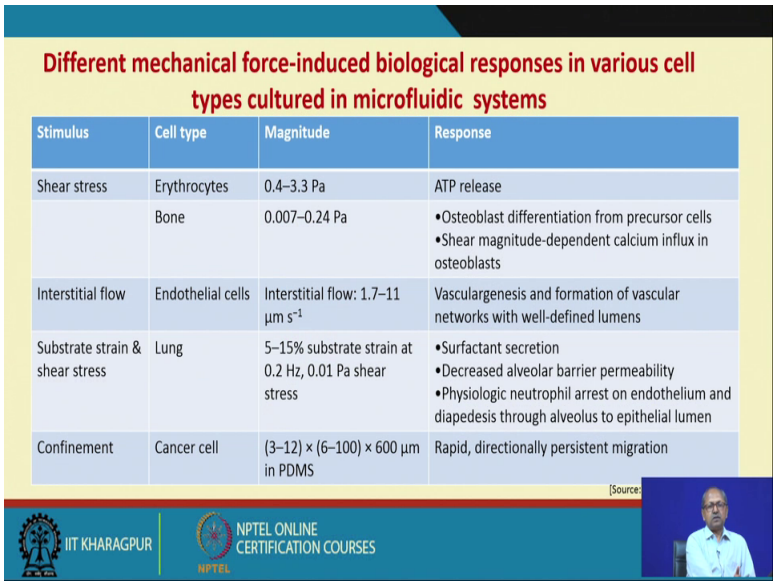

There are examples like say shear stress if you give erythrocyte for a 4 to 3.3 Pascal's ATP release. In the bone if we give with 3.4 Pascal's osteoblasts differentiation Shear magnitude depend on the calcium influx in osteoblasts. In that way there are so and so forth a lot of examples are there. Here I am is showing only few examples like say confinement of the cancer cell rapid directionally persistent migration.

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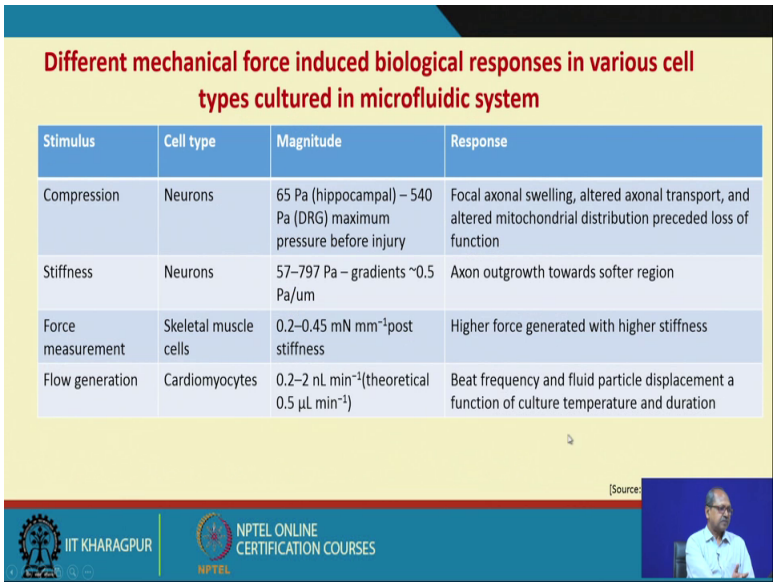

Then compression the neurons at 65 Pascal focal axonal swelling altered axonal transport stiffness in neurons that with this 57 to 797 Pascal's axon outgrowth, in that way a lot of

examples are there. So, so far in the micro fluidics area we are introducing 1 or 2 variables to understand the physiology.

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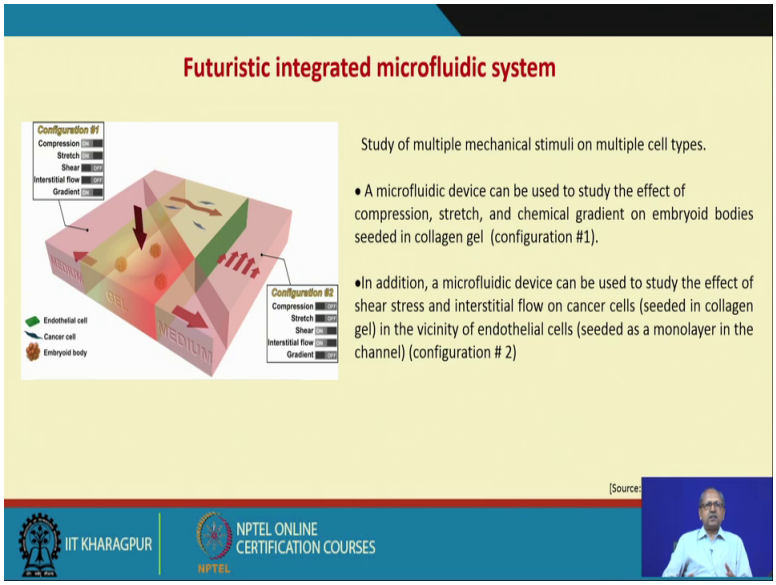

So, next fluidics is coming to that how we can integrate different types of force together to understand that what is happening in real biological system, along with stiffness of the substrate your fine topography or nanoscale topography of the surface. Then shear stress interstitial flow then compression force everything should be integrated in one platform, by which we can understand that physiology of the particular cells in particular biological environment.

I think I can impart some idea about that why bio micro fluidics is necessary to understand that physiological phenomena, and if we can mimic this system in fluidic system. We can develop some disease model and at the same time we can observe some biological phenomenon, so far not possible to observe in the traditional system. So, that is why that micro fluidics is important to understand that biology.

Thank you.