Nanotechnology in Agriculture
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Lecture-32
Fabrication of Nano-Micro Devices to Study Force Generation in Muscles

Welcome back to the lecture series in application of nanomaterials in agriculture. So in the previous class we were discussing that how cerium oxide affects the muscles physiology, cerium oxide nanoparticle. So, in that context I told you that there are interrogate physiology of the muscle once you understand that it will be very easy for you to appreciate that how this whole contraction profile, force generation profile is being modulated by the presence of antioxidant nanomaterial.

And apart from it I told you that will be talking about some of the tools micro fabricated, nano fabricated tools which are being use to understand muscle physiology and as a diagnostic tools for muscle growth and muscle development and their respective force generation. So let us catch up where we left in the last class, in the last class if you remember, so this is where we were as we talked about the development of muscle.

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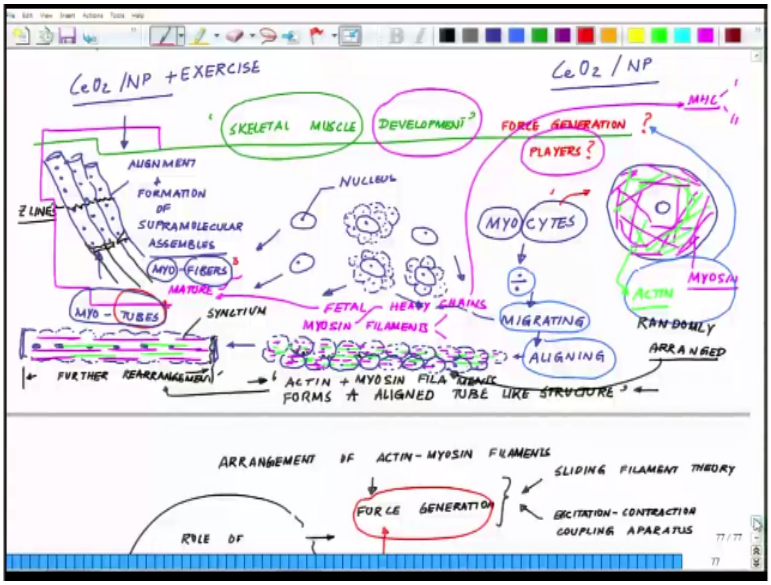

And there we talked about the alignment of the actin and the myosin filament as we could see.

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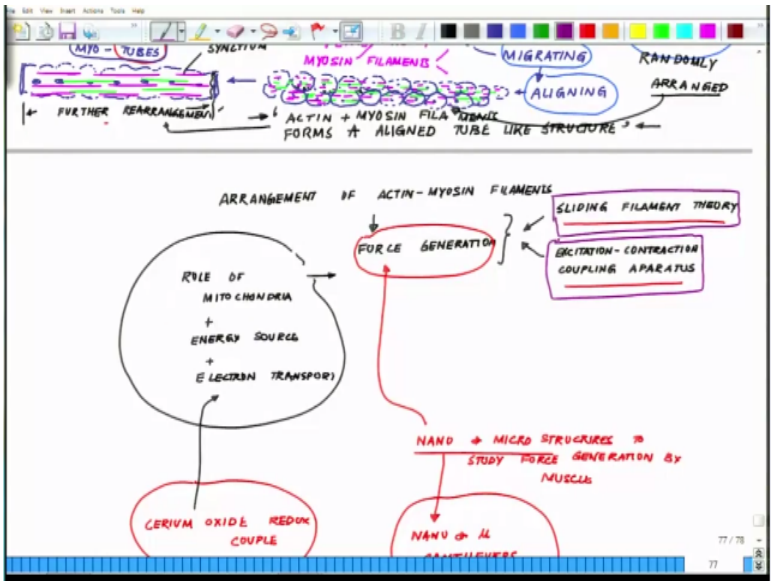

So, now today we will talk about these 2 aspects, sliding filament theory and excitation contraction coupling apparatus and how cerium oxide is influencing it.

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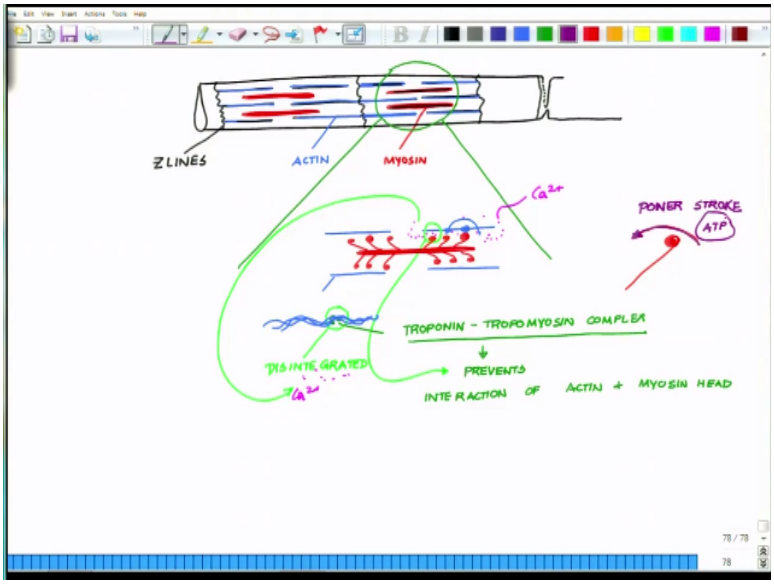

So, when you look at a myo-tube cross-section of a myo-tube is something like this will becomes myo-fibers. If I told you that there is a very interesting arrangement of the actin and the myosin filaments so something like this. And if you go to any textbook referring to actin-myosin filament arrangement is skeletal muscle, you will come across this kind of wonderfully drawn picture is I am not interest in the colors yet now soon I am going to do this ok.

So, now you see there is a pattern here, so if I had to put the pattern say if it look something like this. So, I am translating these lines which I am making the red now, so these filaments what you see which I am now coloring them in red. These are the myosin filament and the myosin filament as I told you changes their protein subtypes as we mature. So some of them of thick, some of them are thin and depending on that they have a different force generation potential.

And the one now I am putting them in green sorry blue these are the actin filaments ok which are much more thinner ok. So now this is how this arrangement of myosin and actin is being orchestrated by nature. And the lines what I am drawing here the jagged lines these are the z lines and in between these myo-tubes you will see some kind of gaps like this and then again another myo tubes starts and this will continue ok.

And we will talk later about those kind of jagged portions or the grooves which are formed, here you are having the actin filaments, here you are having the myosin filament ok. If you look at this picture pretty closely will observe something very neat, picture is more like this. Here is a myosin, myosin has these kind of heads top of them, these are called the myosin heads, something like this ok and on top of that you are having this actin filaments rolling like this.

Now if you look at the actin filaments these are super molecular assemblies of multiple proteins something like this. And in the normal condition this actin filaments are in proximity with the myosin heads but they are not in touch, they cannot interact with the actin filaments because of the blockages which are present out here, they something called these are different proteins present on actin filament troponin-tropomyosin complex.

There are multiple proteins which are involved in it that is not important. Now this troponin-tropomyosin complex it prevents the interaction of actin and myosin especially the myosin head and the actin filament, actin filament and myosin head ok. Now when there is a increase all of us sudden there is a increase of one of the cataions which is calcium Ca^{2+}. This blockage between the myosin head and the actin because of the troponin and tropomyosin complex is this complex gets disintegrated transiently.

As soon as this complex troponin-tropomyosin complex gets disintegrated in the presence of calcium ions, the myosin head binds to the actin filament here and it the something called a power stroke. So this head moves like this and when it moves like this there is a contraction which is observed in the actin and the myosin filament. And this is where the force generation is, so the force with which this myosin head is moving is what we call the muscle force all of them move like this, like this and this is what generates the muscle.

Now for this movement which is technically call as power stroke, you need ATP ok, now does this strike in the (()) (08:21). This is now to let us correlate what is happening when you talk about the presence of ATP there.

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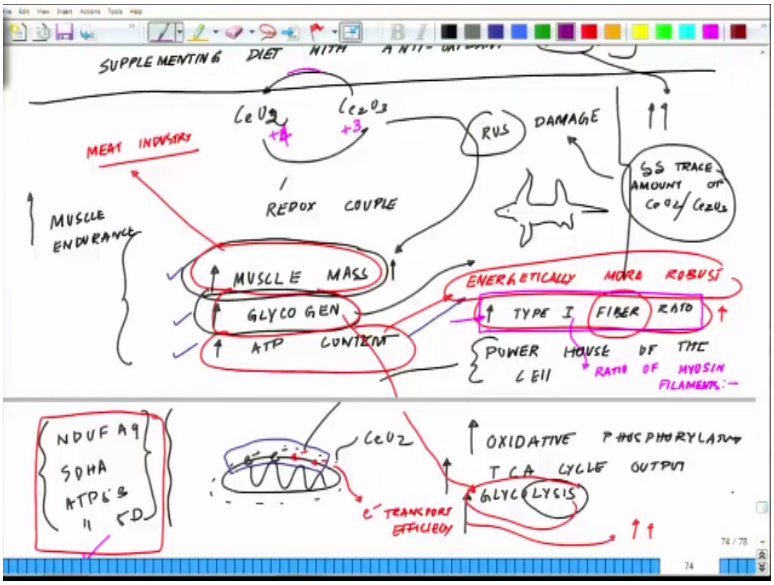

And we are talking about the ATP content here, in the presence of cerium oxide redox couple. So, you realize in order for a process like this to occur you need to really appreciate how 1 simple autocatalytic redox couple can change the muscle performance, so this is where this whole ATP thing comes.

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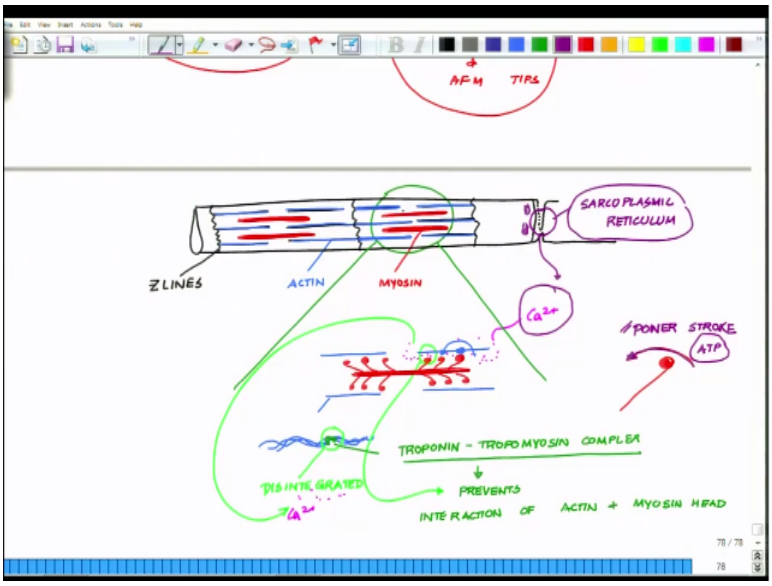

So, if you are more ATP then the power stroke is going to get will be able to enacts it is action better ok. And of course I will request you to go through the sliding filament theory which is just now essentially I taught you and there is another which is called excitation contraction coupling apparatus which is present somewhere out here in this zone which essentially regulates this calcium.

Because this calcium cannot remain there forever, the calcium has to be dumb there and it has to be pull back in a fraction of a movement and that is done by an organelle called sarcoplasmic reticulum. And one more thing what I want you to highlight here as you must have seen that these muscles have higher power generation it means the mitochondria which is involve which is lined up this part all over those mitochondria are far more efficient in cerium oxide treated animals.

So, this is the part what I wanted to highlight that a simple molecule can make a whole range of difference in the performance of muscle, with this brief idea about muscle biology and the role of cerium oxide nanoparticle I will move on to a diagnostics aspects, a approach by which you do not have to kill any animal or sacrifice any animal and yet you can understand the muscle physiology better what are nanotechnology has to offer in terms of diagnostic tools.

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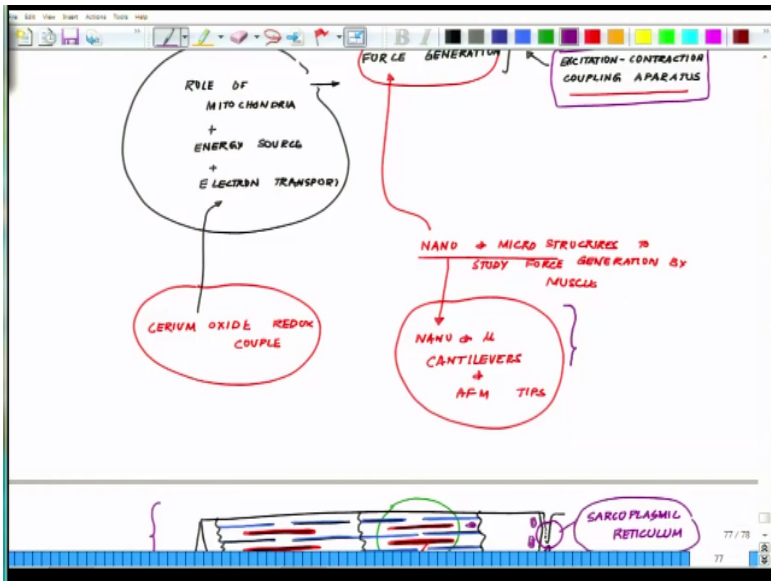

So, there will be talking about as we promised about nano and micro cantilevers it is ok. So, since now we have talked about the force generation out here or a stroke and the force generation.

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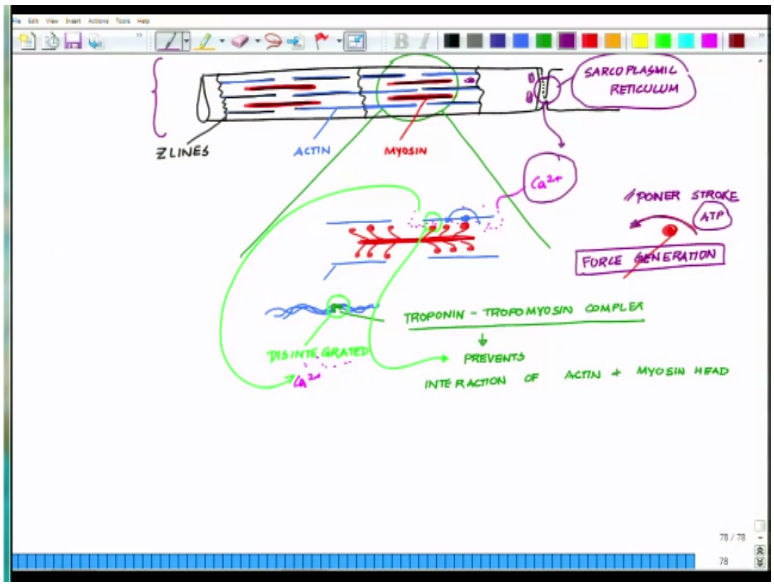

So, we needed a tool to study force generation of the muscle of course you can do it invivo in animals as well as you can do it invitro. Invivo much of the tools unknown to the exercise physiologist, invitro there are upcoming tools which are using different kind of nano devices, micro devices to study in these kind of force generation. So here I will talk about one such micro nano device which follows one of the techniques what we discuss very early in the course how we can develop nanomaterial.

You can develop nanomaterial from bottom of approach from top down approach ok. So, today we will talk about one of the micro nano device which is develop by top down approach ok.

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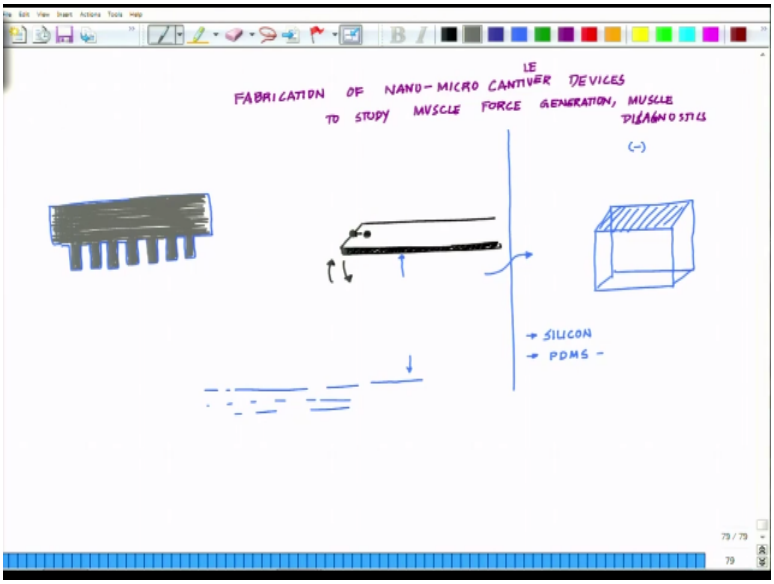

So, today we will talk about fabrication of micro and nano cantilevers for studying or for diagnostics of muscle or it could be any kind of muscle ok. Fabrication of nano-micro depending on what size you are wait for the aspect ratio nano-micro cantilever devices to study muscle force generation, muscle diagnostics ok. So, now what is really a cantilever, so all of you have must been at some point or other into swimming pool.

We must have seen a swimming pool in television or internet or somewhere, say in a swimming pool you must have seen that diving board ok. So, you have these diving boards where people kind of jump ok, so if you look at a diving board there is something like this, there is a plank dropping out like this ok. So, this is how the plank is been attached and this plank can move like this as well as this, so the person stands here or maybe slightly closer here.

The plank will be vibrating at a particular frequency right and of course you are having a person which jumping in water over here and the plank is at a height. So essentially this is a classic situation of a cantilever, now this you have seen, now think of it if I could make such cantilevers

in a micro dimension in order to measure forces of nano newton, femtonewton and those kind of stuff.

Now what does now this is from where you can understand the whole perspective, now I am reducing the size ok aspect ratio. Now what I will do I will take a block like this I am going on a top down approach ok, say for example I take a silicon wafer like this. It could be made on silicon, it could be made on PDMS poly dimethyl sulfoxide you guys can please look at to these kind of compounds PDMS is I think it is a dow corning patented compound poly dimethyl sulfoxide silicon wafers if you take a wafer like this.

Now what you do you have to have a mask, mask which will say what kind of shape you are asking. Say for example talking about the mask suppose I have a mask like this array of suppose I want a array of cantilevers like this ok. Now I am shading the part which are solid and the one which are not solid ok. So, now let me shade it fully that will kind of give you an idea how these kind of fabrication happen ok.

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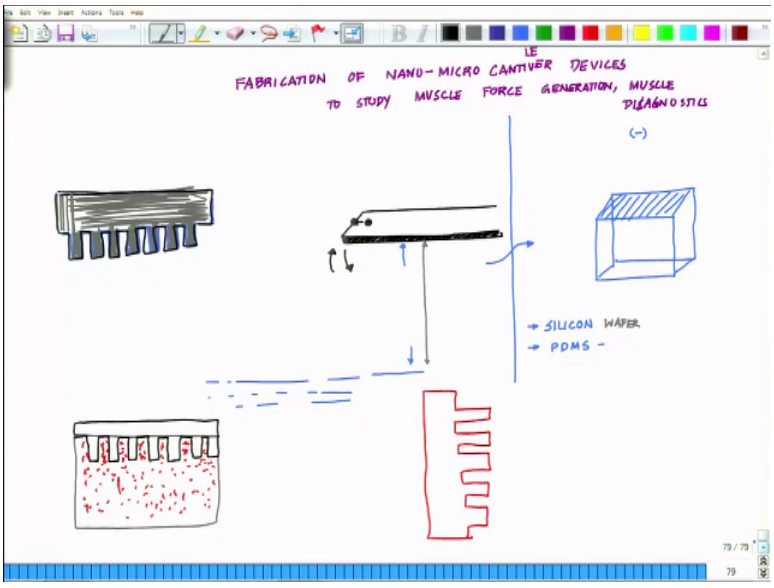

Now here I have a mask oh shit ok, now I want this pattern to be made on this block, what I will do I will keep this mask. So, say for example I have this thin wafer of a certain thickness, now this also has to be determined what will be the height from the base I want. So, the wafer what I

am using a silicon wafer whatever I am using or a block whatever I am using I have to decide what should be the thickness of it ok.

Now what I will do I will keep the mask on top of it something like this I am putting the mask in black now and the wafer is underneath. Now using either a chemical technique or a laser base technique, lithography technique I am going to etch all those part where I am putting a red. I am going to heat away this part I am going to ensure by using certain chemicals or something I will leave the part which is in black line and all these par will be etched out.

So, what have you obtaining is something like this exactly like this where I will have a array of cantilevers sitting like this is the side view I am drawing ok. And of course the depth you have decided depending on the thickness of the block. This is precisely one of the top down approach by which micro cantilevers are being made. So, this kind of give you an idea when we talked pretty early the top down approach you are breaking this stuff.

You are kind of you know bringing down the size and all those things, now depending on the size of the mask whatever is provided you can actually decide what dimension what aspect ratio of cantilever you are asking for you can go to the micron range, you can even go down to nano range, you can go to even 100 meter, 100 nano meter range all up to you what kind of things you want to diagnose. In case of muscle general we go for a micron range ok, now how we do the diagnostics.

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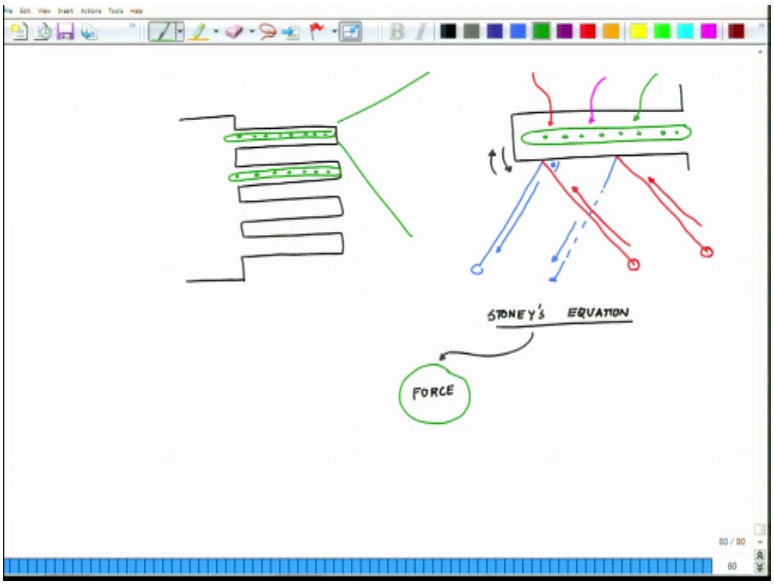

Now after reaching to this point what you do, you allow the muscle cells to grow on top of these cantilevers. In order for them to form myo-tubes and as I told you the myo-tubes are the smallest unit of muscle. So, once the myo-tube grows on top of it like this say for example you have the myo-tubes going like this. So, these myo-tubes will start generating force, so they will be contracting either in the presence of some drag or something or all by themselves at time ok.

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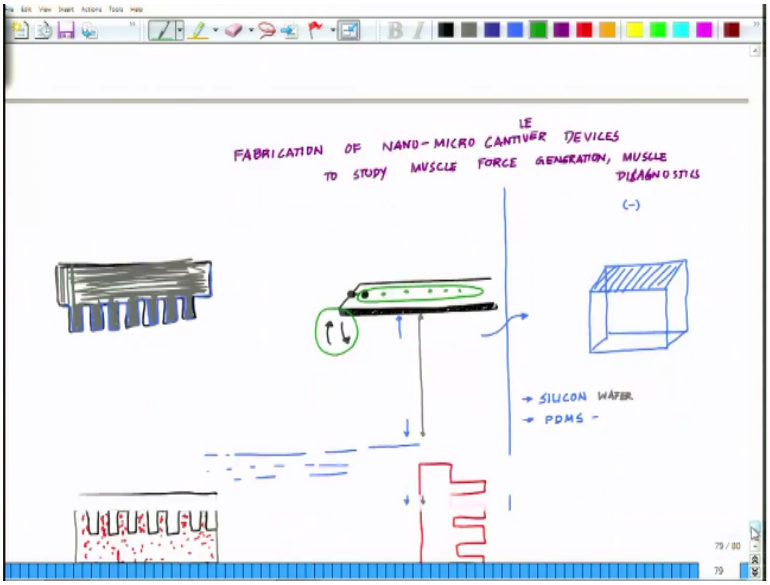

Once these myo-tubes will contract what will happen exactly the same situation as I told you if this is a myo-tube sitting on top of it. This cantilever is going to vibrate at a certain frequency ok, now if you could make an arrangement something like this. Say for example I am just taking 1

cantilever into the picture for the diagnostic part purpose, say for example this is a myo-tube which is on top of the cantilever and this is the cantilever.

Now this cantilever is vibrating at a particular frequency right, now from here underneath make an arrangement to bounce a laser beam like this somewhere maybe here or here depending on. You are bouncing a laser in this direction on the cantilever when there are no muscle, no myo-tube, that laser beam will bounce out from here ok. Now this angle what the laser beam is making with the cantilever is going to change.

If a cantilever beam is going down based on that angle at the something called please look into the literature on this Stoney's equation. Stoney's equation is almost 100 years old now or more than 100 years old, Stoney's equation will help you to calculate the force what is being generated out there. So now if you know the force then depending on what kind of drug molecule or what kind of pathological situation the muscle is or what kind of material is working on muscle. We have a very simple tool by virtue of which you can quantify the force generated by the muscle.

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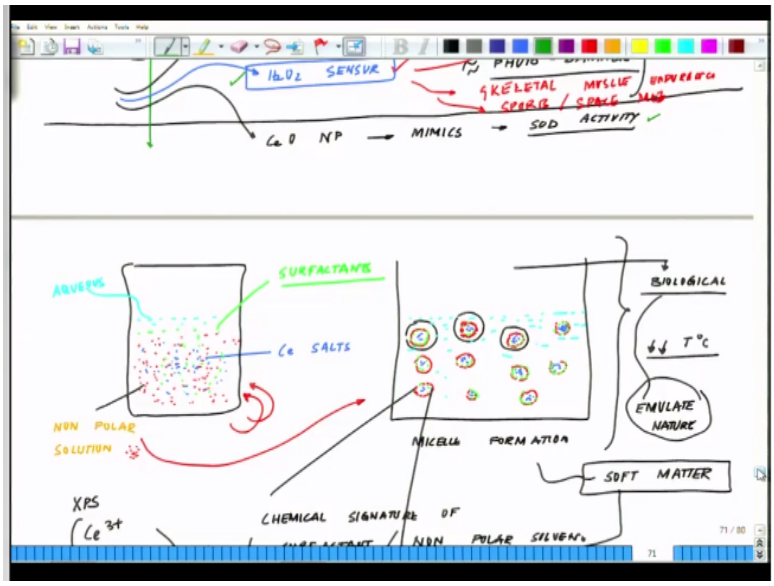

Now you see when you talk about diagnostics of using all kinds of nano tools.

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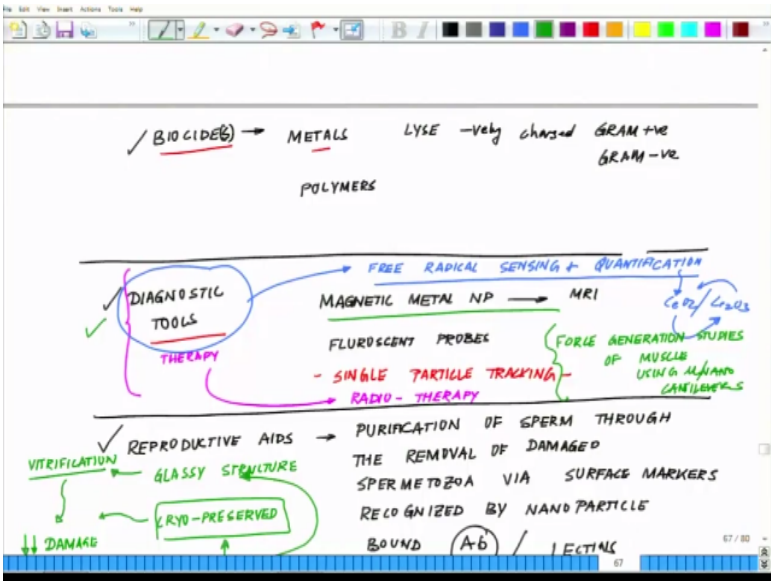

If you remember when you talk about all the applications out here diagnostic tools produced about force generation studies of muscle using micro/nano cantilever. So, these are some of the very interesting emerging tools which are coming very handy in the animal industry, if they are being used in a particular fashion ok. So, I will provide you the relevant literature which will kind of help you to appreciate that these are already proven thing.

And much of the invitro diagnostics are relying on these kind of tools, so this is I am going to close and will move further in this line with different kind of other tools where nanotechnology is coming pretty handy in the animal production, thank you.