Medical Biomaterials
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Lecture - 14
Analytical Tools

Hello everyone. Welcome to the course on Medical Biomaterials. Today we are going to talk about the various analytical tools that are used in area of biomaterial research and lot of tools are being used. So, we are going to spend a quite some time in this particular topic.

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So, if you look at the biomaterials, it is highly interdisciplinary in nature we have materials expertise; that means, metals and ceramics and so on. So, may be a metallurgist or a material scientist we have polymer expertise; that means, a polymer chemist or a polymer technologist, we need experts from chemistry organic chemists then we need experts from biochemistry, who is doing a lot of biochemistry assays then we need expertise from biology who will look at immune responses blood responses and so on. Then we need support from engineering support from engineering who will be designing material. Then all this expertise joined together and which leads to a particular biomaterial. We also will of course, need at a later point of time clinician's medical

practitioner's surgeons and so on. If you are going to take your biomaterial further down into the final market.

So, because of being an interdisciplinary field, the instruments, the tools that are used for analyzing the material are wide and vast. So, it is not possible for one type of expert to comprehend all these, but we need expertise from various disciplines as you can see from here.

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So, what are the tools used in biomaterial research, quite a lot. You want to see the morphological changes more like physical changes, we want to look at whether the material has swollen whether it is shrunk or there is a deformation of the material because it is in contact with the body fluids may be for a short duration or long duration. So, we need to carry out those type of tests, then we need to go into scanning electron microscope to look at the morphological changes in micron layer level, we have to go to atomic force microscope to look at it in nano level.

So, we there is lot of tools that are being used if you are looking at morphological changes. We will spend more time on the scanning electron microscope and atomic force microscope, later as we go along then we are looking at weight loss suppose there is a degradation of the material or resorption we would like to see whether that material has lost it is weight. If it is a drug delivery system you want the material to actually lose weight due to bio resorption.

Then there are going to be lot of surface changes on the material. Material may lose it is hydrophobic or hydrophilic nature, it may the material may lose it is surface energy or gain surface energy, the material may become rough because of being present inside the body fluid if there is flow of blood or if there is flow of urine, they may become rough is that going to have any effect. So, we need to study that next comes changes in mechanical properties if you are using materials especially in the bone or in the knee or where load bearing, I would like to know the stress strain changes is there a change in their flexural strength if we are using it as a diaphragm valve. So, there are mechanical properties you need to understand changes in the mechanical properties you need to understand.

Then comes crystallinity has the material become more crystalline or the material from crystallinity has become more amorphous. Because as the crystallinity changes the degradation pattern may change some of the mechanical properties may change. So, we would like to know whether there is changes in crystallinity sometimes when the material becomes amorphous, it may even solubilize polymer crystalline polymer may be insoluble, whereas when it becomes amorphous polymer it may become soluble. So, we need to understand the crystallinity.

Then comes the thermal changes, has the material change it is phase from one phase to another. So, there are instruments like differential scanning calorimetry thermal gravimetry and so on. So, we can find out the thermal changes of course, in return they are all connected with crystallinity. So, we can study the material especially if they are polymeric in nature. Then molecular weight changes, if it is a polymer I would like to know whether it degraded has it become more oligomeric in nature. So, we can determine viscosity average molecular weight or number average molecular weight or weight average molecular weight.

So, there are many tools like gel permeation chromatography mass spectrometry, which is very useful to determine the mass of the material and suppose if macromolecules are leaching out or if solvents are leaching out we can find out the molecular weight using the mass spectrometer different types of mass spectrometry is there we are going to spend time something like MALDI MS-MS that is mass spec mass spec LC-MS which can separate the various components using the liquid chromatograph then the mass spec. So, on actually.

Then we can look at lot of changes in surface chemistry. This is chemical approach because as the material is inside the body it may get say hydrolyzed it may get reduced it may get oxidized it may get attacked by reactive oxygen species. So, there could be lot of functional groups new functional groups created. Old functional groups might have disappeared. So, I would like to understand that there are many tools there like infrared spectroscopy, Fourier transform infrared spectroscopy, UV visible, Raman spectroscopy, nuclear magnetic resonance spectroscopy, time of flight sims surface, secondary ion this is called a secondary ion mass spec. Time of flight secondary ion mass spec. So, if you are bombarding a surface with the electrons the ions that are produced they are called secondary ions. So, try to characterize them. So, you see lot of these tools. Physical chemical changes that are happening to your biomaterial surface changes bulk changes all these.

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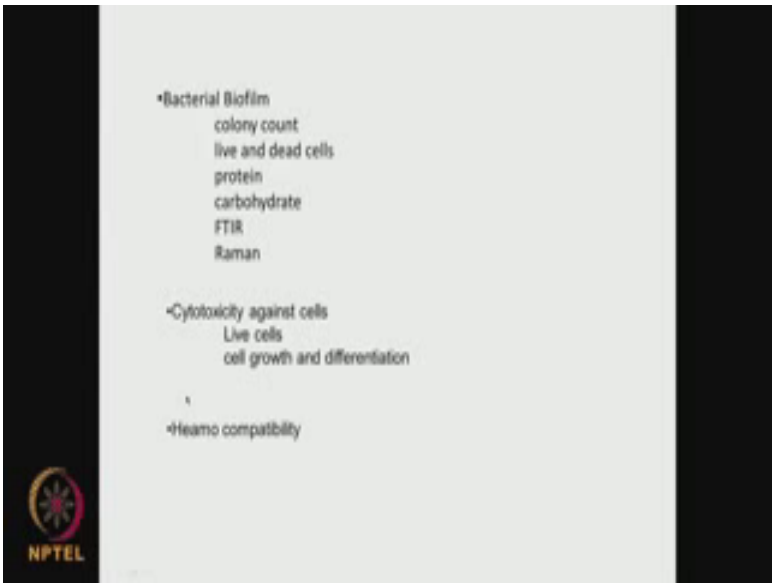

Now, in addition we need to understand the biological changes that has happening. We need to know has the polymer or material solid material like metal or inorganic has acquired attached biofilm. So, I need to find out how many colonies are present in the biofilm. How many live cells vis à vis dead cells? I need to know how much protein is attached in the biofilm. How much carbohydrate is attached all these quantities, I need to know in addition I can perform the Fourier transform infrared to see what are the surface functional groups that has happened there. I can use romans spectroscopy. So, if I want to study the bacterial biofilm; that means, the biofilm that is attached on the surface of

the material be it a polymer be it a metal or a ceramic, I may have to do all these various characterizations.

Then I need to know whether the material is cytotoxic to animal cells. So, if it is going to be cytotoxic, then we do not want to use that material. So, we mean to say we need to see how much life cells are present after say 24 hours after 48 hours. What is the percentage of dead cells I need to do that? So, there are some assays for that then I want to know, how the cells are growing and if I am using this material as a scaffold for growing cells are the cells settling down and then slowly differentiating. So, I need to study that then I need to know the hemocompatibility; that means, if it is a blood contacting device is the material going to be very toxic to the blood will it create responses so that the blood starts clotting on the surface, which is very dangerous.

So, you see lot of biological studies I need to look at I need to look at bacterial biofilm related, I need to look at whether the material is cytotoxic to the animal cells whether they induce any response and then are they problematic to blood. The blood plasma blood protein and other various components of the blood, am I going to have coagulation or I am going to have any other problems related to the blood compatibility.

So, you see we need to have facilities for carrying out physical parameter study, the chemical parameter study and biological parameter study. So, it becomes very difficult for one may be lab to have all these facilities. So, sometimes they may have to do it in collaboration with other groups. I might have covered many of them, but not all of them.

Now we will look many of them slightly more in detail because as I said they have lot of importance.

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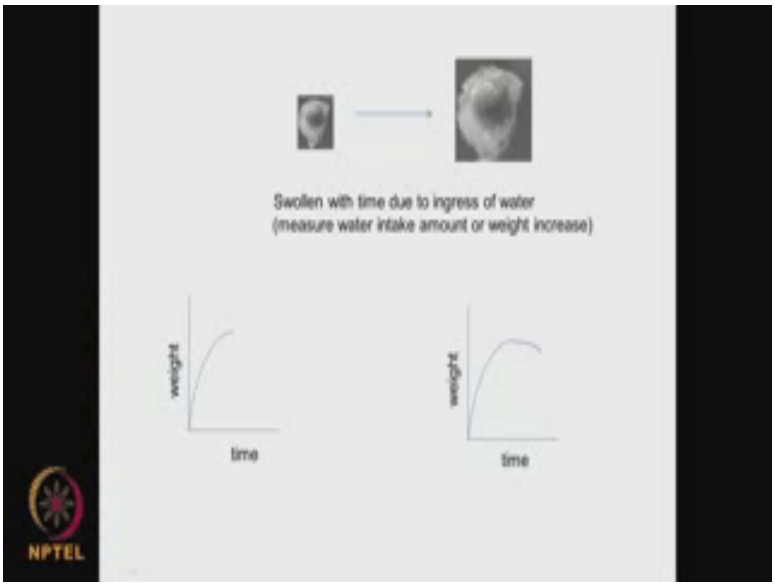

So, we look at it for example, I may have a drug delivery system. I want to know how it gets swollen as a function of time because of ingress of water. So, as the water starts going inside say the polymeric material will start swelling and so, there could be hydrolysis taking place the polymer may be degrading either as a bulk or surface erosion as I said. So, whatever is inside will get released whatever is encapsulated. So, I want to study how the swelling takes place as a function of time. So, it may follow a graph like this. So, as a function of time it may go up and then may flatten out. So, what do you do you measure the weight as a function of time and then you plot it.

Now if the polymeric material also starts degrading. So, after some time the weight may start going down. So, if that happens we can be sure that may be the material itself is degrading. So, if the swelling rate is faster than the degradation rate then of course, you will not see any degradation. So, you will not see decrease in weight, but if the degradation also starts taking place then the weight may go up and start decreasing.

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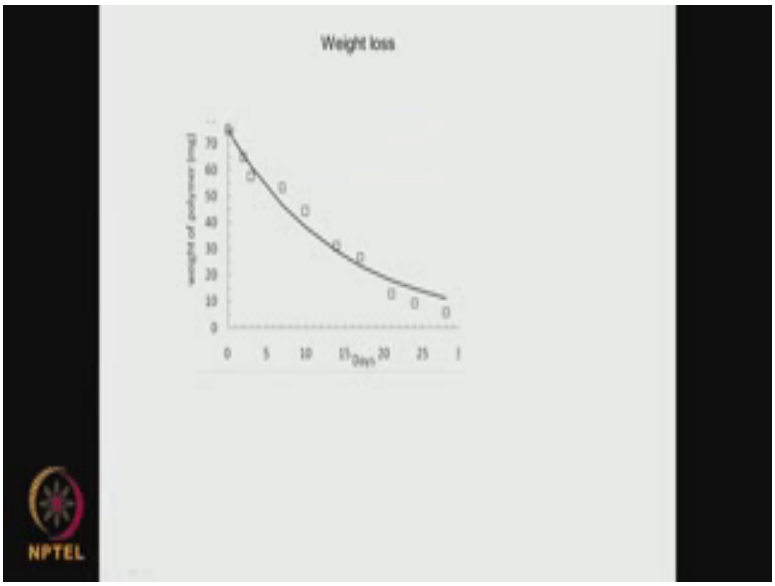

So, with the help of very simple set of physical data collection you will be able to understand the mechanism of swelling of polymeric system. So, if the material is losing weight. So, you may end up having a relationship like this no the weight loss as a function of time it will be going down may be as a first order or may be as a half order and so on. Actually again this data is very useful especially if you are looking at drug delivery systems.

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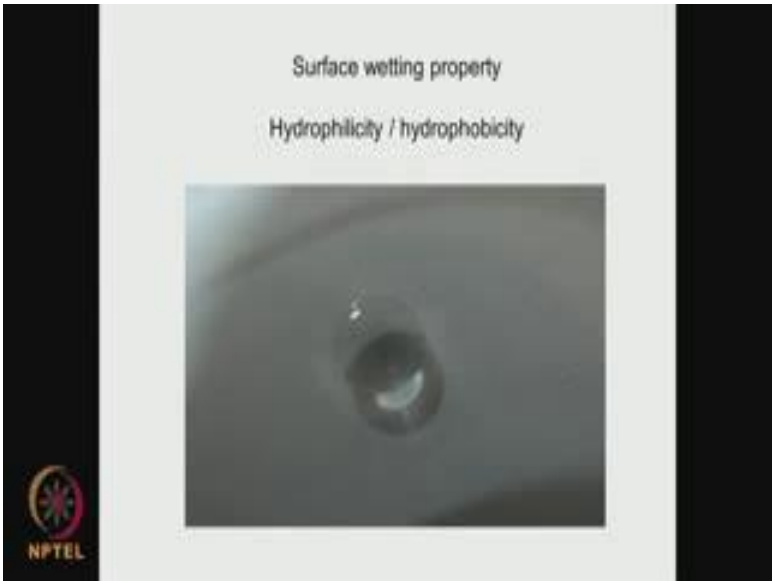

Then comes surface wetting property. This is extremely important because you want hydrophilic material hydrophobic material sometimes cause bacterial attachment sometimes cause cytotoxicity hydrophilic materials are always preferred in such situations. So, the balance of hydrophilicity versus hydrophobicity is very important. So, for example, look at this this is a water droplet on a surface. So, material of course, is highly hydrophobic, that is why the material is not the water is not spreading.

So, the more hydrophilic, it is water will spread out more hydrophobic it is water will not spread out. So, that is very good measure of knowing the hydrophobic hydrophilic nature of the material.

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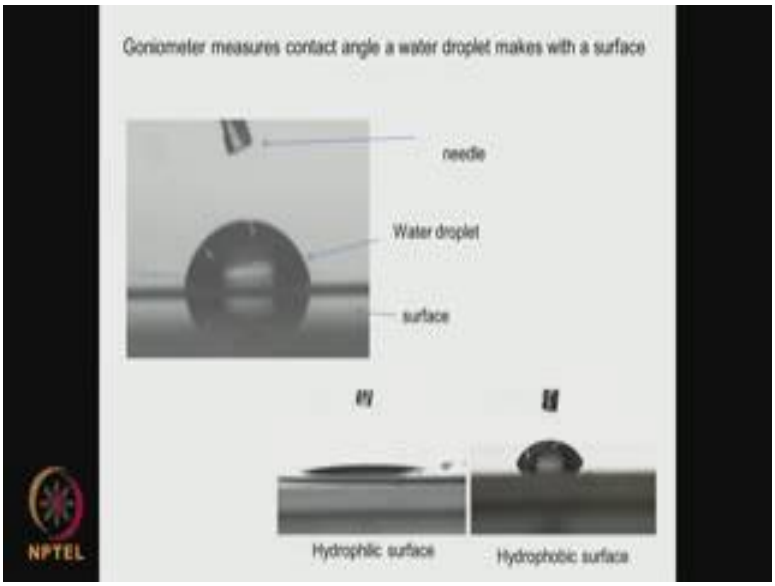

For example, there are instruments there is called a goniometer. It measures the contact angle a water droplet makes with the surface. So, look at this. So, a drop water droplet is slowly placed on a surface. This is the surface this is a needle which is put in a water the water falls in. So, then what do you do angle that is made is measured and from the angle one can tell whether the surface is hydrophilic or whether the surface is hydrophobic, then we can also calculate surface energy so many things.

For example, you can see water droplet spreads very well here. So, of course, the material is very hydrophilic, whereas, here as you can see the water droplet is here like this. So, this is hydrophobic relatively hydrophobic, where as in the previous picture I showed you this must be a extremely, hydrophobic surface because water just sit is on it

is not spreading at all. So, it is relatively hydrophobic this is very hydrophilic. So, the contact angle as we can see this is the angle will be very less. So, lesser the angle we can say the material is hydrophilic 20 degrees 30 degrees 40 degrees whereas 70 80 90 and so on. We can call it hydrophobic surface.

So, it is very important to note this. So, whenever you design a biomaterial and you prepare you need to check the contact angle of the material and tell whether the material is hydrophilic or hydrophobic. As I said hydrophilic material have high surface energy. So, attachment of bacteria is very poor and also the animal cells do not do not have a toxicity towards hydrophilic material whereas hydrophobic material the attachment of bacteria also will be very high, the animal cells could be toxic to that material or the material could be toxic to the animal cells. So, if you have a hydrophobic surface generally try to modify the surface hydrophilic, you may add functional groups like O H or N H and so on.

So, that material is made more hydrophilic. So, goniometer is a very powerful tool to measure the contact angle water droplet makes on a surface of a biomaterial.

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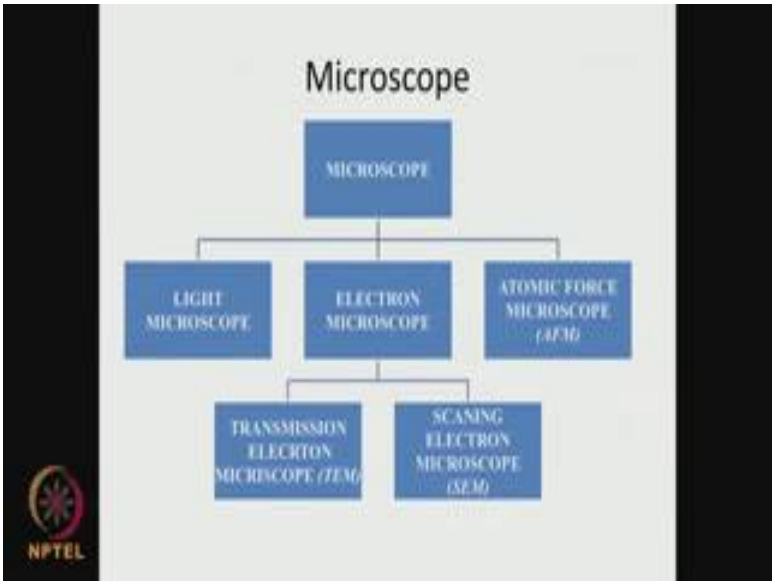

Now, let us look at it microscopes. Microscopes are very important we have light microscope, we have electron microscope, we have atomic force microscope, atomic force. We call it AFM everybody knows light microscope. This is the normally we see under light the of course, the magnification is very limited in light microscope may be

hundred x using oil. So, if we want to see further if I want to see biofilms and if I want to see bacteria, I may go I have to go for scanning electron microscope. If I want to look at nano particles, I may go to transmission electron microscope or even atomic force microscope.

So, depending upon what you want to do you choose different microscope. So, if you are looking at live dead cells using some fluorescent dyes I may start with light microscope. If I want to look at bacteria biofilm, I may have to go to scanning electron and so on actually. So, these are the various categories of microscope the light microscope electron microscope and atomic force. So, I can see in nanoscale 1 nanometer with AFM even with TEM I can see between 1 and 5 nanometers whereas SEM I can see 100 nanometer light may be it could be millimeter going right up to micrometer.

So, this is what is microscope and microscopes are widely used in various areas of biology and especially the area of biomaterials it is widely used.

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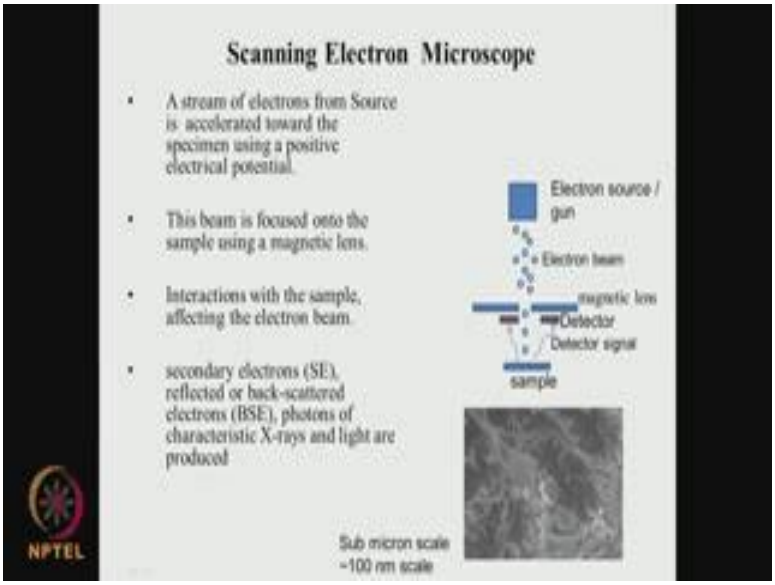

Let us look at each one of them little bit in more detail the scanning electron microscope. What is this scanning electron microscope? So, here the stream of electrons from an electron gun or a source is accelerated. So, it is accelerated by using some voltages and then there is a magnetic lens. So, it is focused this is all done through a positive electric potential because electrons are negative. So, it is focused through a magnetic lens and then hit is the sample. So, secondary electrons are reflected or back scattered it could be

a even photons of characteristic x rays and light. So, here you have a detector and from detector you measure it.

So, we have an electron source a beam is produced focused it hit is the sample. So, you have back scattering electron secondary electrons photons x rays all these are produced as detected here. So, we can look at samples going right up to 100 or even 50 nanometer scale not below that. So, for example, this is say a polymeric surface we are looking at approximately 100 nanometer scale. We can look at see rough surface. So, because of being present in a body fluid for a long time it has acquired roughness it is called a scanning electron microscope.

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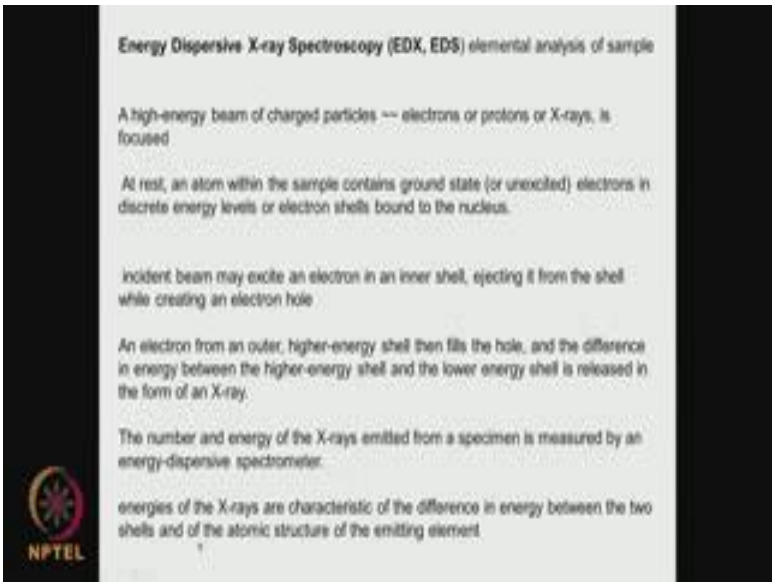

Now, with scanning electron microscope we can also do compositional analysis; that means, I can look the elemental composition on the surface using an attachment called EDX or e d s that is called energy dispersive x ray spectroscopy. So, we can attach that. So, high energy beam of charge particles electrons or protons or x ray is focused on the surface.

So, at rest an atom within the sample contains ground state electrons in discrete energy levels or electron shells bound to the nucleus. So, incident beam excite an electron in the inner shell ejecting it from the shell while creating a electron hole. So, an electron from an outer high energy shell then fill this hole and this difference in energy between the higher energy shell and the low energy shell is released in the form of an x ray, which is

detected that is why it is called energy dispersive x ray spectroscopy. So, what you do you focus a beam of electrons. So, it excites the electrons at the innermost shell ejecting it from the shell. So, it creates an electron hole. So, electron from the outer that is high energy shell fills this hole. Now this difference in energy is liberated in the form of x ray.

That x ray tells you because it is a measure of the difference in energy, which is also a measure of the type of element the number and energy of the x ray is emitted from the specimen is measured by a energy dispersive spectrometer this is how you can measure the composition of various elements present on the surface. So, energies of the x rays are characteristics of the difference in energy between the 2 shells and the atomic structure of the element present. So, the energy difference is characteristics of the shells of the atomic structure of that element. So, from the energy of x rays that are liberated one can tell what type of elements that are present on the surface.

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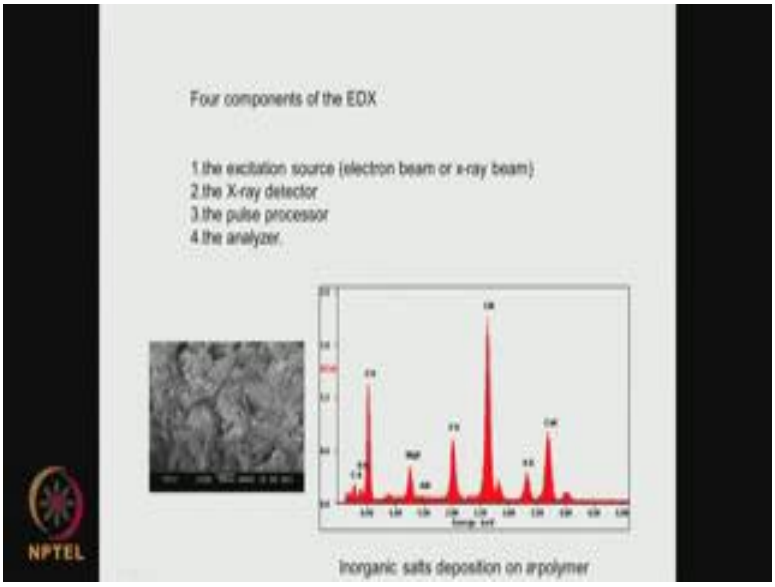

For example look at this picture this is inorganic salt deposition on a polymer. So, we are looking at magnesium phosphor calcium chlorine potassium aluminum carbon and nitrogen of course, so this is how EDX picture looks like this. This is the SEM of that and this is an EDX. So, we are trying to see the salt; what are the elemental composition we can even get the elemental composition of the salts that are present on the surface of this material. So, we can get both the surface morphology as well as the composition of various elements present on the surface.

So, what are the components of the EDX excitation source of course, electron beam or x ray detector pulse processor and the analyzer. So, EDX is very important. So, generally they have an attachment with the SEM. So, we can look at one area of the biomaterial surface either the surface modified or after being implanted explanted. Then you want to know what are the elements that are present we perform the EDX, and we get this type of spectrum then from here we can tell the composition as well. So, that gives nice idea of the composition.

So, with the respect to control we can say what changes have happened. For example, this picture as I said this salt deposition on material surface and what are the elements that are present in the salt like phosphorus calcium potassium magnesium chlorine and then oxygen and so on. So, it is very powerful for you we can get the feeling of surface morphology as well as the elemental composition.

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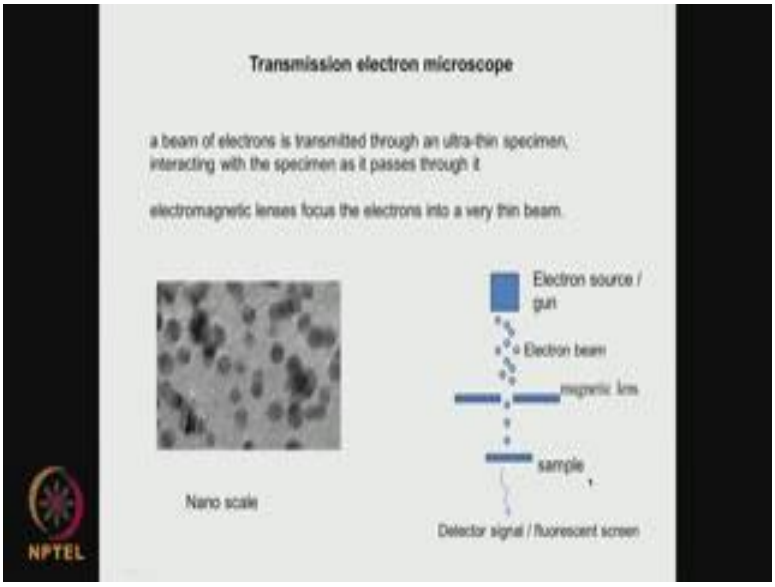

Now, let us go to transmission electron microscope. It is more powerful we can go up to nano scale. We can look at nano particles 10 nanometers 20 nanometers 5 nanometers and so on. So, it is extremely very good in a scanning electron microscope, as I showed you the detector signals come out like this. So, you will have the electron source the detector signal on the same side of the sample, where as in a transmission electron microscope you can see the signal, gets transmitted through the sample. So, we detect it on the other side so; obviously, in the transmission electron microscope if you want

perform some studies the sample thickness should be very minimal, because the signals have to pass through the sample. So, here the beam of electrons is transmitted through an ultra-thin specimen. So, these electrons interact with these specimens and then that gives you some signals which are detected on this side.

Otherwise it is almost the same this portion is almost the same as the scanning electron microscope. So, this is the picture of nano particles produced through a chemical synthetic procedure and detected using a transmission electron microscope TEM as we call it and you can see these all are in nano scale may be about 20 nanometers spherical particle. So, if you want to see. So, nicely we cannot use a scanning electron microscope we need to use a transmission electron microscope then comes atomic force microscope.

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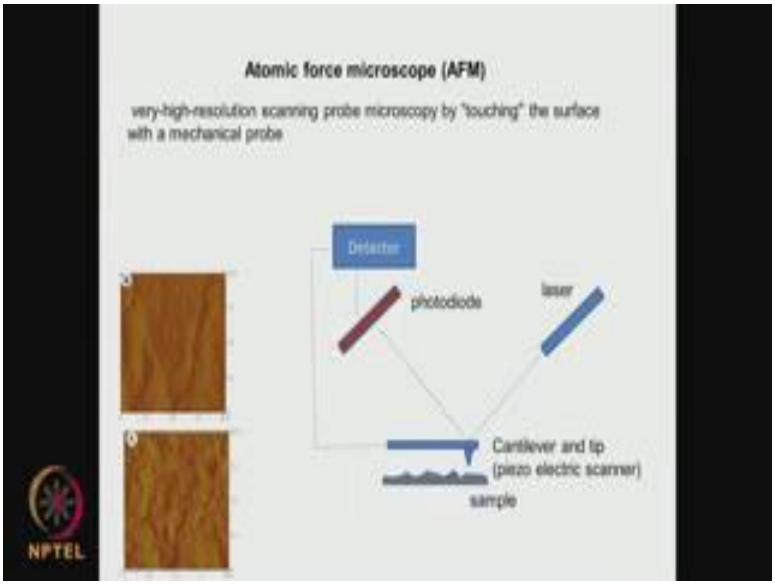

Actually atomic force microscope is not a really a microscope like light or SEM or TEM it is very interesting it measures the changes surface roughness. For example, of a can notilever that moves on the surface of the material. So, it is more like a scanning probe microscopy. It is a probe can notilever probe which travels on the surface and the changes and it is height due to the roughness is measured through a detector and that picture is shown that is what is atomic force microscope. So, it is not like light microscope or scanning electron or a transmission electron. So, what happens is there is a probe which touches the surface it is a mechanical probe. So, there is a laser which is focused on it.

So, as the probe moves because of the roughness of the surface probe may move up and down which is captured here in photo diode and which is detected. So, this particular can notilever moves all across the surface of the sample. And it is upward and downward movement is captured and that is a measure of the surface topography or surface changes. And this is what the picture is and we can get it in nanoscale for example, the surface of a polymer which is incubated for 2 days in body fluid the roughness that has happened.

So, atomic force microscope we can detect in nanoscale that is like TEM, but TEM is more like a microscope whereas atomic force microscope is not a microscope. It is more like touching the surface and the changes in the surface roughness are captured using laser which is shown in the form of pictorial representation as you can see here and that is what it is unlike SEM or TEM, but all these tools are used suppose I want to see if there is a bacterial attachment on the surface, which are in very small level we can see or if there are any nanoparticles embedded on a polymer surface, or I can see the whether the nanoparticles are agglomerated or separated from each other.

So, all these are can be done with atomic force microscope, whereas transmission electron we can look at nano particle nanoscale drug delivery systems scanning electron microscope, we can look at biofilm attachment we can look at bacteria we can even look at the biofilm architecture like last class, I talked about the architecture of the biofilm are there pores in the biofilm or are they smooth. So, there we can use SEM. So, sometimes we need to combine 2 3 different techniques. So, that we get a very good understanding. So, one technique alone might not enough.

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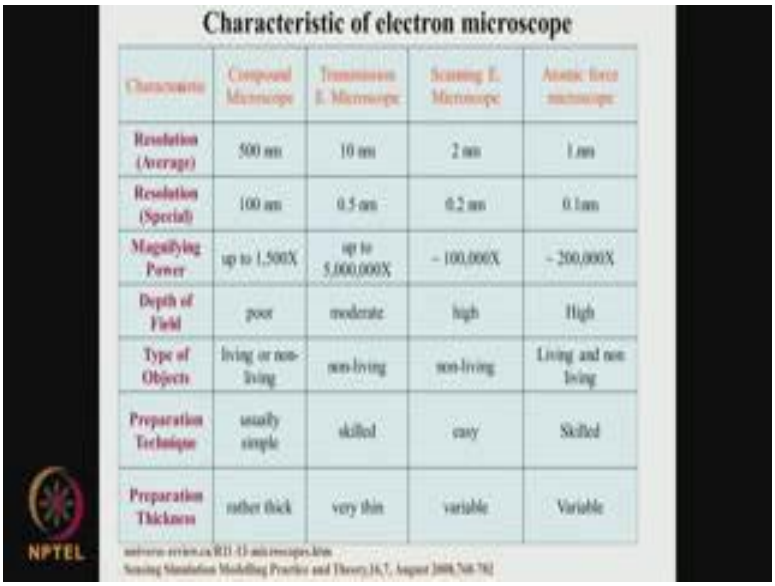

So what are the differences between all these, if you look at resolution normal compound like microscope 500 nanometer TEM will go up to 10 nanometer scanning electron microscope may be 2 nanometers, atomic force 1 nanometer resolution is also like that, magnifying power we can go up quite a lot, depth of field TEM is moderate scanning electron is high atomic force is also high. Depth of field for a compound microscope because you are viewing from the top, we cannot go very low down type of objects. We can see living and nonliving things with compound microscopes.

And whereas with these microscopes what happens with TEM and scanning because we are using electron as a probe it should have very high vacuum. Of course, there are now biological based scanning electron microscope where the vacuum levels are do not very high, but still we need to have very high vacuum in TEM and SEM. So, generally we cannot see live we will always see dead bacterial biofilm where as we can see things live in a compound microscope, and also in atomic force microscope, because you are not applying vacuum in FEM unlike TEM and SEM; preparation technique compound microscope.

It is very simple scanning electron is simple whereas TEM and atomic force, we need to have very skilled operation preparation thickness compound microscope we can have very thick TEM we can go down to very thin surfaces other 2 scanning, and atomic we can have reasonably thin or thick surfaces.

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Characteristic	Compound Microscope	Transmission E. Microscope	Scanning E. Microscope	Atomic force microscope
Specimen Mounting	glass slides	thin films on copper grids	aluminum stubs	-
Field of View	large enough	limited	large	limited
Source of Radiation	visible light	electrons	electrons	Atomic force
Medium	air	vacuum	vacuum	Air or liquid
Nature of Lenses	glass	1 electrostatic + a few em. lenses	1 electrostatic + a few em. lenses	Laser diode+ few emission lense
Focusing	mechanical	current in the objective lens coil	current in the objective lens coil	Magnetic electric and mechanical
Magnification Adjustments	changing objectives	current in the projector lens coil	current in the projector lens coil	x
Specimen Contrast	by light absorption	by electron scattering	by electron scattering	By atom scattering

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Specimen mounting compound microscope has to be on a glass slide, TEM may be on thin films on copper grate scanning can be aluminum or carbon and so on. Field of view compound it is large transmission is limited scanning large atomic force is also very limited. Source of radiation compound microscope we are using light TEM we are using electrons scanning also we are using electrons atomic force, we are using force medium compound microscope is air, TEM is vacuum scanning is vacuum atomic force air or liquid the nature of lens, we have glass here we have one electrostatic plus a few lenses electromagnetic lenses they are called in atomic force, we have laser diode and few emission lenses.

Focusing compound microscope, it is mechanical TEM in you have lens coil current and voltage where as in atomic force it is mechanical forces. Magnification changing objectives current in the projected lens current; so we are using voltage and current in their specimen contrast due to light absorption TEM and SEM, it is because of the electron scattering whereas atomic force it becomes atom scattering.

So, you see lot of difference between these 4 type of microscopy compound scanning TEM and atomic force microscopy. So, each of them have their own advantages disadvantages we use each of them at different levels for different reasons. Sometimes we combine 2 of them together. So, that we get quite a lot of knowledge. So, as I mentioned that you can look at surface morphology, we can look at size we can look at

agglomeration with EDX, we can look at even elemental composition; that means, what are the type of elements that are present on the surface of the material.

So, all these are morphological based studies these are not depth based studies; that means, we do not look at the changes in the bulk, but more from the surface layers it could be few microns' depth only.

Thank you very much and we will continue more in the next class.