Tissue Engineering
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Lecture - 13
Material Characterization - Part 1

Good morning everyone, today I will be talking about Characterization of Materials. Until now, you might have already studied what are biomaterials and its types and what is it is applications.

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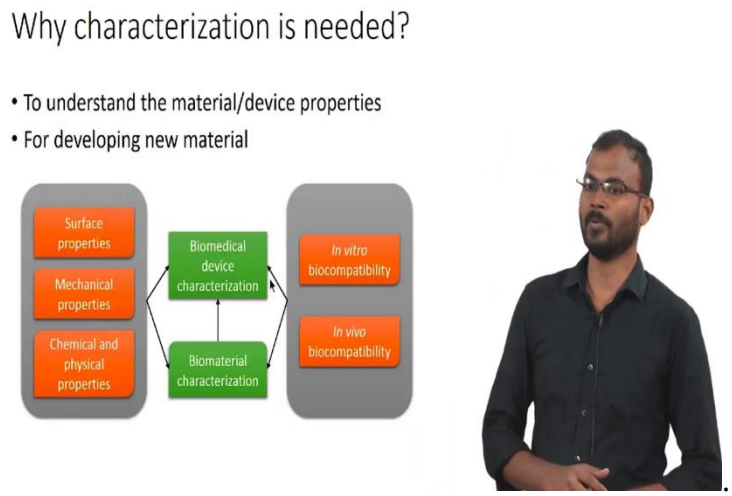

Now, you have to learn why we need to characterize the materials. To understand the material properties, we need to characterize the biomaterials. Understanding the material properties will give us an idea about whether the material is accepted as an implant or a medical device. Thus, the biocompatibility of the material is primarily dependent on its property. So, to find out it is properties, we have to characterize the materials.

For developing new materials also you have to understand the new property. We can identify that the material mimics the existing natural organs and other applications. Also, to identify these properties, you need to characterize the materials. The material characterization is primarily divided into two categories; one is based on engineering properties such as surface properties, mechanical properties, chemical, and physical

properties. The other is dependent on the biological relationship with the material, which is *in vitro* biocompatibility and *in vivo* biocompatibility.

The surface property is a property primarily dependent on the material surface, which interacts with the host system. When a material is implanted into the body, the first point of contact is at the interface of the material surface and the host cells. Hence, any surface of the biomaterial should be biocompatible so that it would not be rejected by the host mechanism.

Mechanical properties are important while you are considering an implant such as hip prosthesis and other heavy load-bearing applications. You need to have a good mechanical property, which will be understood by characterizing the bulk properties of the material.

Physical and chemical properties involve understanding the material composition and what are its thermal properties. If you are using a scaffold or hydrogel for tissue engineering applications, you have to understand the porosity and permeability and how much the water intake it can have. So, those properties and all come under chemical and physical properties.

In vitro biocompatibility is a must biological characterization before going for the clinical application; we have to primarily test the material with *in vitro* and *in vivo* testing. *In vitro* biocompatibility studies identify whether a material is suitable for the cell adhesion, whether there is an immunogenic response from the cells, whether there is a protein absorption, whether there is blood coagulation occurs on the material surfaces and so on.

In vivo biocompatibility involves testing the material in animal models like rats, pigs, rabbits and dog models, etc., to check whether the material has the same function when you are introducing into a human body. All these properties have to be characterized both at the biomaterial level and the biomedical devices level. If I am developing new material, and if it is biocompatible, we cannot say that it will be exactly replicated in the devices also because the device will have different structures and different functions compared to the material itself.

Hence, we have to check at the biomaterial level first; then, you have to go for biomedical applications also. One important point is that all these properties cannot be applied for all the material. It depends on what type of material, some are a nanoparticle, some are films,

and some are scaffolds. So, each of these materials will have to be characterized based on the material properties, what type of characterization you need to understand, and what type of application you are going to look into.

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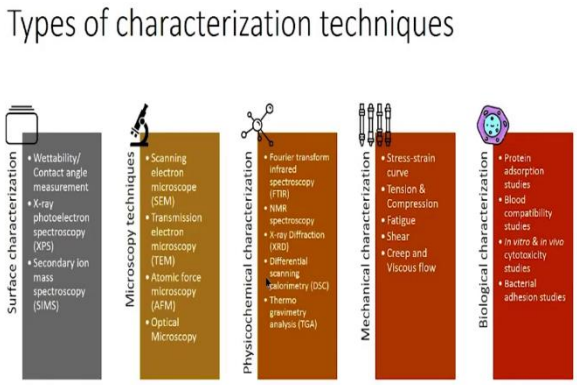

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Coming into characterization techniques, these are the predominantly five different types. There are a lot of techniques available for a lot of properties, but these are the widely used techniques used in the biomaterial field. Surface characterization techniques such as wettability or contact angle measurement is a technique where you will find a material surface is hydrophobic or hydrophilic; hydrophobic is water-repelling, and hydrophilic is water-attracting.

X-ray photoelectron spectroscopy will calculate the elemental composition of the material, what are the elements present on your material surface, and what are the chemical bonds present on the material surface.

Secondary ion mass spectroscopy is also a surface technique, where you can find out the particular atomic composition of the material. Whether how many electrons available on the material surfaces can be calculated by secondary ion mass spectroscopy.

Microscopy techniques also come under surface characterization, but there are a lot of microscopic techniques available. One of the mainly used technique is SEM, scanning electron microscopy, that is used to observe the morphology of the material surfaces. Then,

transmission electron microscopy, which is used to see the inside the material. So, I will explain both of those techniques later. Atomic force microscopy or AFM will identify the surface roughness of the materials. By AFM, you can check how much of the material surface is smooth or whether it is rough. Those parameters will greatly affect cell attachment and other biological properties. Then an optical microscope will have a variety of microscopic techniques such as phase contrast, fluorescence microscopy, confocal microscopy, and so on.

Physicochemical characterization involves identifying the physical and chemical properties of the materials. FTIR, which will characterize the functional groups present on the materials. By NMR spectroscopy, we can find out what are the bondings and elements available on the materials.

X-ray diffraction will tell whether the polymer is amorphous, crystalline, or semi-crystalline. DSC and TGA analysis are thermal property characterization techniques where you will find out the melting point, degradation temperature, glass transition temperature. Those kinds of thermal properties can be found out using a DSC and TGA analysis.

Mechanical characterization is usually important for bulk or load-bearing applications. These experiments involve tension and compression; how much of a load it can withstand and how much of compression it can withstand. The bulk properties identification shows how long the material is stable without any breaking or defect due to different loads. Creep and viscous flow are used to identify the mechanical properties at varying the time and frequency of the loads used for the application.

Then biological characterization involves a lot of studies based on the application intended for the use. Protein absorption study is very important because when you implant material into a host system, the first interaction between the host system and the material is the protein absorption at the material interface. Hence, if there is a protein absorption occurring on your material surface, then that will lead to cell attachment.

Based on the application, the property can be modified. If you want your material not to have any attachment to cells, it should not have any protein absorption on the material surface. For example, when you are going for blood-contacting devices, there you do not need blood cells to attach onto the material surface because it will lead to the formation of a blood clot inside the devices, which will lead to complications and failure. So, for those

applications, we have to check for protein absorption studies. And if you are going for a tissue engineering application like wound healing, you need to have cells attachment onto the surfaces. So, for that application, you need protein absorption on the material surfaces.

So, these are the basic techniques, other than this there are still a lot of techniques are available which is specific for each of the application, we have to look into.

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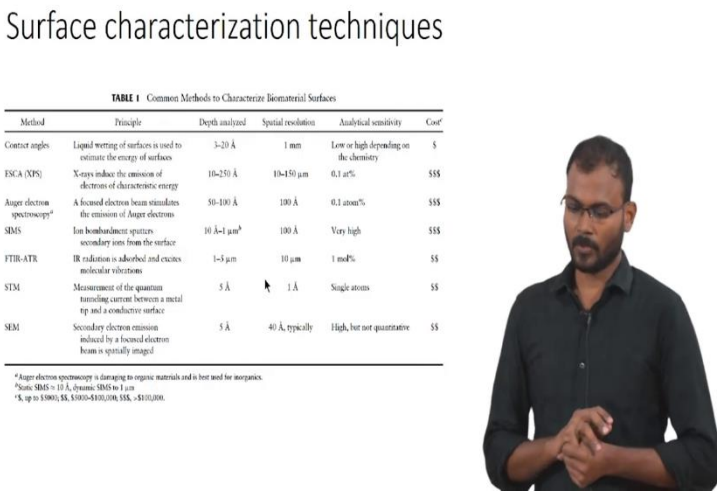

So, coming into surface characterization techniques, as I told you earlier, contact angle analysis, XPS, Auger electron spectroscopy, which involves the auger electrons, then secondary ion mass spectroscopy, which is known as SIMS, FTIR, STM is scanning tunneling microscopy, then scanning electron microscopy. Each of the techniques has a different resolution of how many properties we can identify and the cost they have given.

All these atomic related composition finding techniques will be expensive. The basic techniques, like contact angle analysis, FTIR, and others, are slightly lesser. So, while developing new material, you can first optimize those materials using the basic techniques, then you can go for a higher-end technique to confirm whether that material has been properly characterized based on your application.

So, each of the applications I have told you already. Let us go into contact angle measurement.

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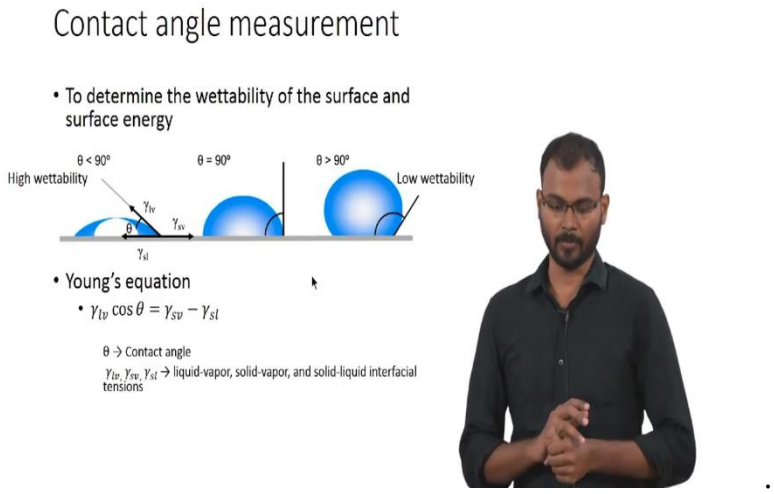

Contact angle measurement is done to determine the wettability of the surface and to estimate the surface energy. When a material is introduced, first, liquid medium from the host system will spread on the material surface. This primarily affects how the proteins are absorbing? How are the cells absorbing on the material surface and all?

To find out that wettability property, that whether the material is hydrophobic or hydrophilic, we have to use the contact angle measurement. The angle between the solid-liquid interface and the liquid-vapor interface is the contact angle. So, this relation has been given by Young's equation, which is

$$\gamma_{lv} \cos\theta = \gamma_{sv} - \gamma_{sl}$$

Where θ is the contact angle and γ_{lv}, γ_{sv}, γ_{sl} are liquid-vapor, solid-vapor, and solid-liquid interfacial tensions, respectively.

Based on the contact angle, we can modify the surfaces. For example, existing material is available, and you want to improve the surface properties of that material. So, you modify the material surface using different coating techniques such as polymer grafting, plasma treatment, or some other chemical treatment. Based on the surface treatment, the contact angle will vary. Thus, varying the surface property, you can have better cell attachment, better protein adsorption, and so on.

If the contact angle is above 90°, then the material surface is hydrophobic. If the angle is below 90°, then the surface is hydrophilic. In some cases, the angle will be above 150°, then that material exhibits a superhydrophobic surface, which is also known as lotus effect, based on how the water droplet on a lotus leaf will retain its bead-like structure on the leaf surface. That is called the lotus effect, due to the microstructure available on the lotus leaf.

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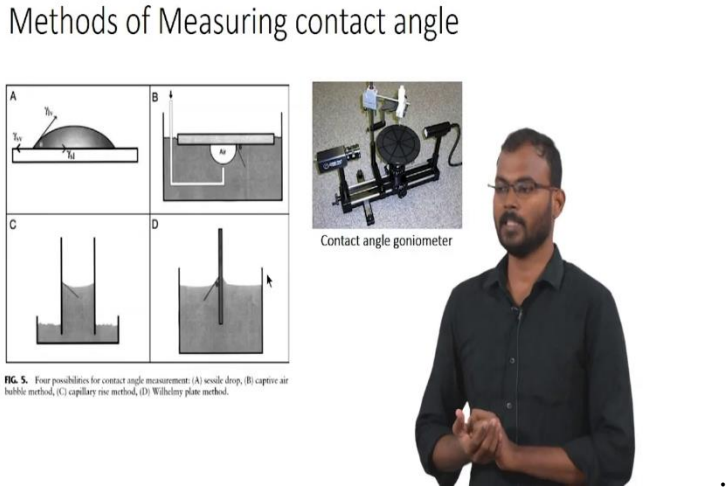

For measuring the contact angle, we use the instrument called contact angle goniometer. There are different methods of measuring the contact angle. One is a sessile drop method, which is the widely used technique. There is a material surface, and you put a drop on the material surface, and you measure the angle between the solid-liquid interface and the liquid-vapor interfaces. You draw a tangent at the point of contact and measure the angle between them. So, that will give you the contact angle, and you can identify whether the material is hydrophobic or hydrophilic.

Then the captive air bubble method, which is similar to the sessile drop method; however, instead of using a liquid, we use an air bubble to measure the contact angle. So, you have a material surface, and it is immersed in a liquid solution, then you introduce a drop on the bottom of the surface. So it will form a bead-like structure that will give you the angle between the liquid interface and the solid surface, which is the contact angle. So, where we can use the captive air bubble measurement? When your material is absorbing a lot of water. Because in the normal sessile drop method, the water-absorbing material will

hydrate quickly, and absorb all the water into the material itself. However, in the captive air bubble method, the contact angle will be proper because the material is already hydrated with the liquid present.

Then the capillary rise method is used to measure the contact angle in small diameter grafts and tubes. It is mainly used to characterize whether the coatings are properly done and the surface morphology is uniform on the inner surface. Here, we will use the meniscus to find the angle between the liquid surface and the solid surface.

Then the Wilhelmy plate method, where we will have thin metal plates, and we can measure the contact angle and the surface tension, introduced on the plate. Usually, both sides will have the same angle, and by that, we can find out the contact angle.

The advantages of using the contact angle are cost-effective, and you can use it anywhere immediately after preparing material surfaces. The major disadvantage is that if there is a very rough surface or nonuniform surface, it will have different angles all over the material surfaces, causing its difficulty in measuring.

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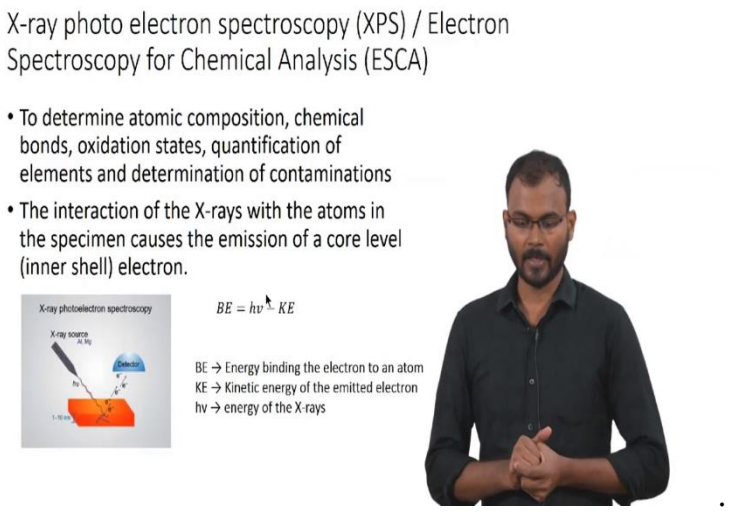

The next technique is X-ray photoelectron spectroscopy, also known as Electron Spectroscopy for Chemical Analysis. It is a technique where you can find out what are the elements present on the material surface. This characterization technique is very useful if you have a material, and you do not know what are the elements present on the surfaces.

If you can do this analysis, then it will show all the elements present in the material surfaces.

XPS is only for the surface characterization technique, where it will go up to a depth of ten angstroms. So, up to that depth level, it can measure the elemental composition. This is mainly used when you are checking for contamination of a material surface or if you are modifying a material surface and checking whether the introduced modification has an effect. You can check all these using this XPS method, and it is a confirmation technique for most of the surface modification applications.

It works on the principle that when an X-ray is introduced onto the surface, it will excite the electrons. The excited electrons will be detected using the detector, and it will identify the elements based on the binding energy details obtained from the electron intensity. It will show the binding energy and what category it belongs to, whether it belongs to carbon, oxygen, nitrogen or sulfur, etc. So, it works on the principle that binding energy (BE) equal to the energy of the X-rays (hγ) minus kinetic energy (KE).

$$BE = h\gamma - KE$$

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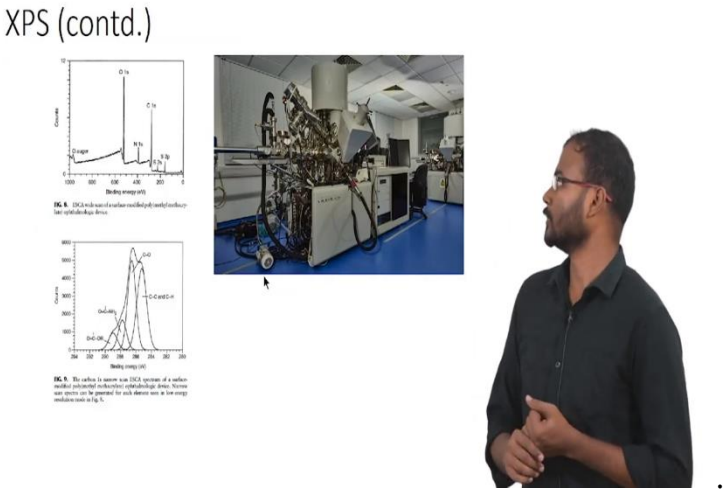

So, this is an XPS instrument, and this is how the spectrum data will look like. XPS has two methods of analysis, one is wide spectrum analysis, and another is a core spectrum analysis.

In wide spectrum analysis, you can identify the elements present throughout the surfaces. You can see at the top graph there is a wide spectrum analysis, where you can see the oxygen, carbon, nitrogen, sulfur peaks, and so on. It will show all the elements detected on the material surfaces. From this spectrum, you can identify the elemental percentage on the surfaces.

Whereas, in the core level spectrum, you can identify what type of chemical bonds formed between the elements. For example, the carbon-based spectrum would be around 285 eV of binding energy. If you look at that carbon-based spectrum present at the binding energy, you can see different peaks for the different states of carbon binding. The different bonds, such as C-C bond, C-O bond, acid bonds, and ester bonds, will have slightly different binding energies based on their properties. So, all these bonds can be identified properly using the core level spectrum analysis. So, by this core level spectrum analysis, you can find out the oxidation state, the valence electrons, valency state of the elements. If you are covalently attaching a molecule, you can find out whether the bonding has been properly done on the material surfaces. So, all those things can be confirmed using the core level spectrum.

One of the disadvantages of using XPS is that the sample would be analyzed under a high vacuum condition. So, if your sample is not stable in a high vacuum condition, it is difficult to do XPS. Also, XPS analysis is a bit expensive and a time-consuming process.

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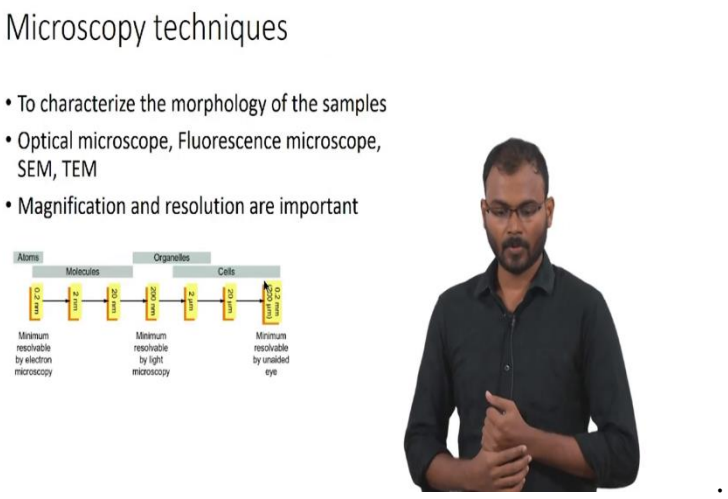

Microscopic techniques also come under surface characterization techniques only, but it has a wide range of applications. Like a fluorescence microscope can see how the organelles and how the nucleus looks inside the cells. The transmission electron microscope will look inside the material and not only on the surface.

To visualize the sample qualitatively, we primarily use microscopic techniques. Each of the microscopic techniques has a different depth and different resolution it can go up to. So, it can go from atoms to the cells and much higher materials also. The size will range from millimeter to nanometer, and some recent microscopic techniques can go up to angstrom levels also. For light microscopy, you can see up to 200 nanometers, and if it is electron microscopy, you can go up to nanometer size.

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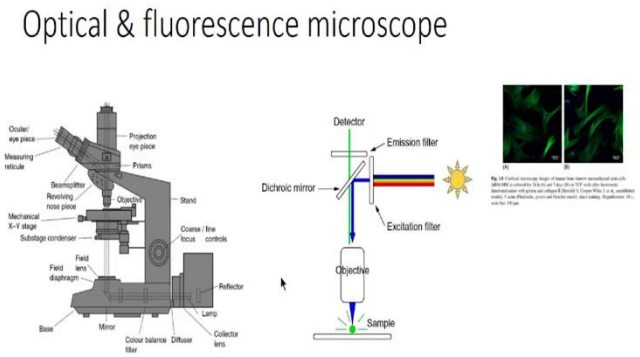

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This is a schematic representation of an optical microscope and a fluorescence microscope. In the optical microscope, we will have an objective lens and light source, and you will keep a sample inside, and based on the light source, the sample would be observed through the lens and eyepiece to see whatever sample you inserted inside.

The optical microscope has a lot of types. The phase-contrast microscope uses small phase shifts in the light, passing through the samples or cells based on the background. The cells and their surfaces scatter the light from the illuminated light. So, that cells would be dark, and lights that are not scattered would be brighter. Other types include bright field microscopy, confocal microscopy, fluorescence microscopy, and so on.

In fluorescence microscopy, you will have a fluorescence sample, which gets excited due to the light of a specific wavelength. If you have a fluorophore inside the sample that will get excited and that excited wavelength can be observed as an image. The image here is a fluorescence microscopy image, where the green elements are the actin filaments, which are stained using phalloidin, and the blue structures are the nucleus that is stained using Hoechst dye.

If you are designing an experiment that involves nanoparticle encapsulation or introducing a drug into cells. To observe whether the encapsulation or the mechanism of the desired drug molecule, a fluorophore can be tagged, and all these are observed under the fluorescence microscope to check whether that what happens.

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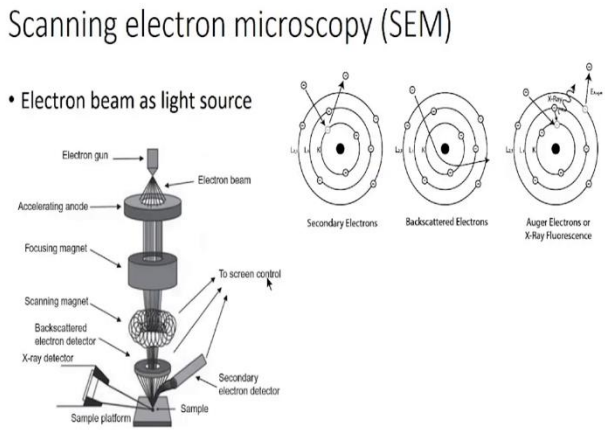

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In general, the optical microscopy has a lower resolution; you can go up to 200 nanometers only you cannot go below that. For that purpose, we move into scanning electron microscopy. This is a schematic representation of the Scanning electron microscopy; the major difference is that here you have an electron beam as the light source.

The electron beams are passed through the electromagnets, which will control the electron beam, and it will focus on your sample, and based on the sample property, you can visualize the images. There are two types of detection available in scanning electron microscopy, one is the secondary electron, and another is a backscattered electron.

Secondary electron mode is used whenever your electron source excites the other electrons on the material surface, this excitation causes the electron to be scattered and then absorbed under secondary electron detector. The major advantage of using secondary electron is that it gives details of the surface topography.

For backscattered electrons, the electron which is used in the source gets reflected by the sample surface. The reflected electron is deducted, and this mode is the backscattered electron detection method. The backscattered electron imaging is useful when we want to know the distribution of the elements on the material surfaces. Hence whenever surface topography of the sample is needed, we can use secondary electron detection mode, and whenever surface composition is needed, backscattered electron detection mode is used. Then Auger electrons spectroscopy, which is another different type of spectroscopy, where the deduction is based on Auger electron. So, Auger electrons are the electrons that are excited from the core level, will be replaced from the outer electrons, that electrons will produce the energy. So, that will be observed under the detector. This method is a surface-sensitive spectroscopic technique that will show the chemical and elemental composition of the surface. In scanning electron microscopy also you can find out the elemental composition, where it will show the percentage of elements present. So, that is called an X-ray energy dispersive method. However, auger electron spectroscopy is much more sensitive and detailed.

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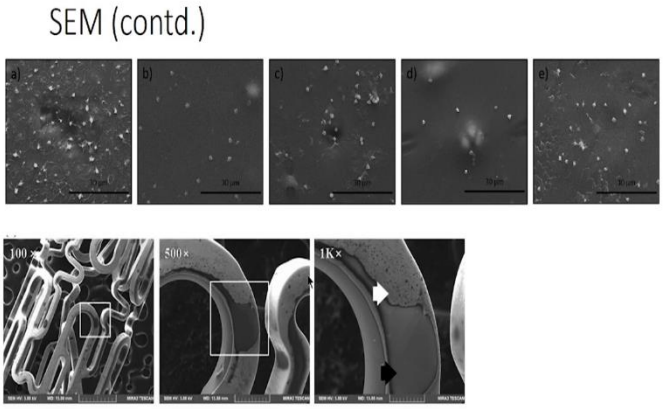

So, this is how the SEM images look like. As you can see, the first five images are the blood platelets adhering to the material surface. A is a control sample, which is an unmodified surface, and all the other four are the surface-modified samples. Different modification techniques are used to study the effects of platelet adhesion on the material surface. As you can see, after the surface treatment, the number of platelets adhered were reduced. So, that can be observed under scanning electron microscopy.

Then the bottom picture is a vascular stent, where they have coated another material onto the stent. So, to observe the coating has been properly done on the vascular stent, they have observed using scanning electron microscopy.

There are two parameters which are important in microscopy, one is magnification, and another is the resolution. Magnification determines how much of an enlargement you can do to the image of a sample; resolution determines how detailed your images are and how much you can distinguish between two objects. Magnification depends on the instrument lens property, and resolution depends on the ability to distinguish at that magnification. This is important because the resolution is as much as important as magnification. If you have a highly magnified image with a poor resolution is not that useful. For example, in SEM, if I go above 10 nm size, the image would not be detailed. So, you have to go to higher-end equipment. Hence, the resolution is very important when you are looking into microscopy.

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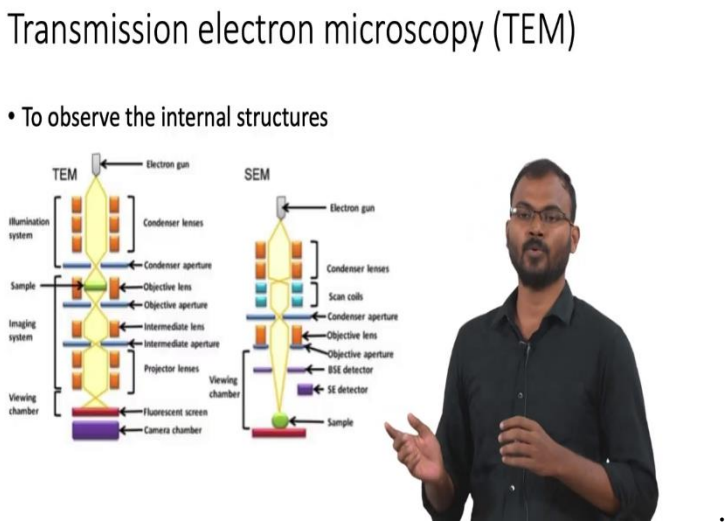

The transmission electron microscope, TEM, also works on a similar principle as SEM, but the idea is that the electron beam will be passed through the sample. So, transmitted electrons through the sample and gives the sample image.

As we can see in the SEM the sample is at the bottom, and in SEM, if it is reflected based on backscattered or secondary electrons the images would be observed, but in transmission electron microscopy the sample would be at the center, and the electron beam would be passed through the sample, and that will give the what is present inside the material.

If your material is porous structure and all, then the electron would not have any inhibition passing through that porous structure, you would easily see the inside structures. If you have materials with a compact structure or tightly packed property, based on the density of the material, the electron will have a different transmission, thus producing images with altered contrast. Based on that, the image would be observed under the transmission electron microscopy.

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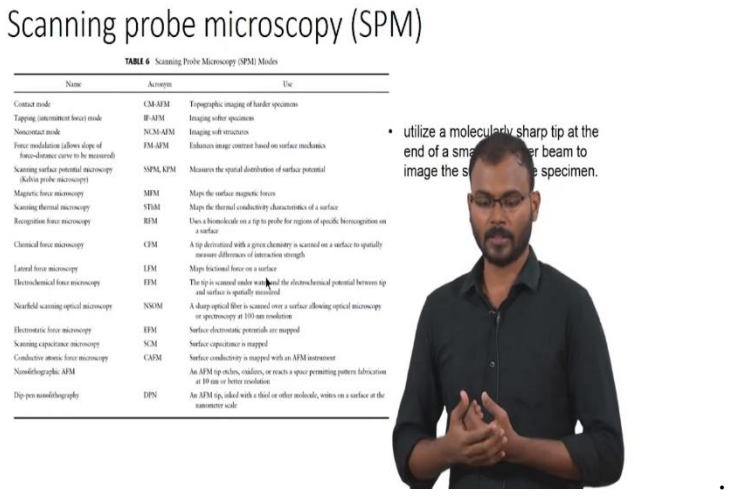

Then coming into scanning probe microscopy, until now, we have discussed different microscopes based on the different light source and electron source. However, you also can have a physical probe that will give feedback from the surface, which will be fed into the detector, and that will give us a visualization of the sample surface. So, that is the concept for a scanning probe microscope, and it has a lot of different techniques based on

the interaction between the probe and the sample and also the application. One of the widely used scanning probe microscopies is AFM, atomic force microscopy.

Atomic force microscopy is widely used to measure the surface roughness of the sample on the scale of nanometers. In atomic force microscopy itself; it is having different types such as noncontact, contact, and tapping methods. Based on the sample properties and the desired observation, you can choose any of these methods.

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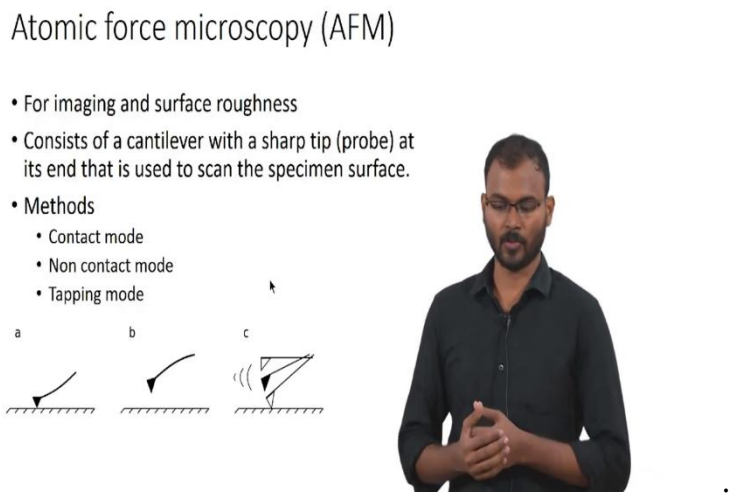

So, we will see about the atomic force microscopy. The physical probe on the AFM is called cantilever with a sharp tip balanced by a piezoelectric object which will expand and contract based on the voltage applied. In contact mode, the cantilever would be placed on the top of the material. The cantilever will touch the material surface and move along different directions. If your material has a flat surface, the movement will be straight without any disturbances. Hence, the voltage difference would be constant all over the surface, thus leading to produce a flat image. If you have a rough surface, it will move up and down. So, that will have the voltage difference that would be detected using the detector, and it will have that rough surface image observed.

In Tapping mode, what happens is that the cantilever will oscillate at a frequency within the resonant frequency of that cantilever and taps the surfaces in a much gentler way then contact mode. Based on the atomic surface of the material, the movement would be

interfered by different interactions on the surface of the material. So, that change would be observed under the voltage difference, which will be represented as images.

In non-contact mode, it is again vibrating at a high frequency above the resonance frequency where it would not touch the surface at all. However, van der Waals force and other long-range forces available on the surfaces will have an effect on the noncontact mode; that effect will be observed on the into an image. So, why these three modes of AFM are available? If some samples are very sensitive, and if you are observing under the contact mode, the sample will get damaged. To avoid that, people will usually go for tapping mode. Tapping mode is one that is widely used modes in AFM. The non-contact mode is used when you have a very sensitive sample; For example, hydrogels or other thin films, you do not want to touch the sample at all. So, these are the three different techniques.

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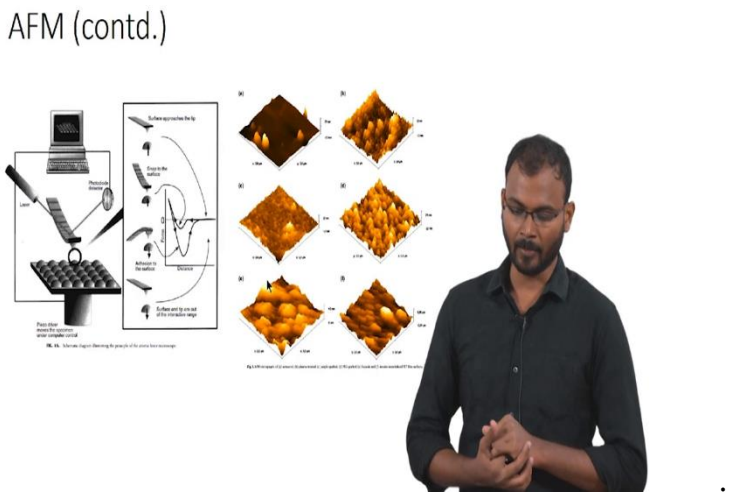

So, this is a schematic representation of AFM, and this is how the image looks like. you have a plain surface, which is a, and it has some ridges observed. If you modify the surfaces, it leads to having a rougher surface that will be observed as a 3D contour; we can see how the images with different surface roughness are.

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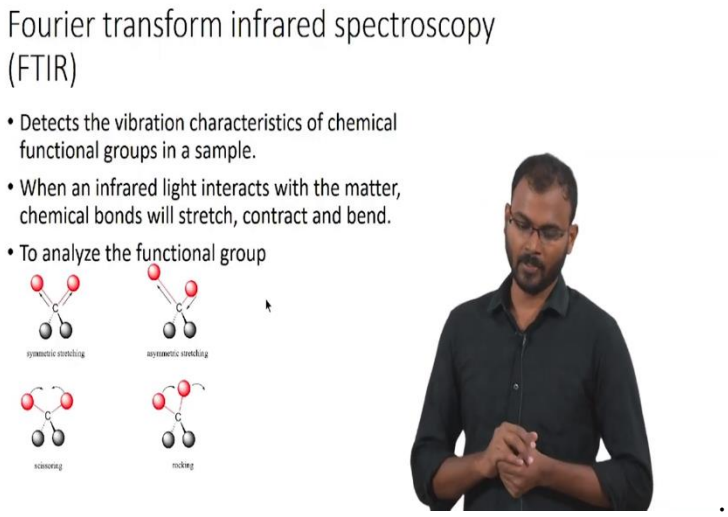

Fourier transform infrared spectroscopy is a characterization technique to identify the functional groups present on a molecule. When a molecule is exposed to IR radiation, it will exert vibrational, stretching, contraction, and bending motions. So, those molecular exertion properties can be converted into Fourier transform infrared spectroscopy spectrum.

If you are having a molecule such as methane CH_4, if you are exposing it into the IR spectrum, that molecule will have a different bending based on the bond between the atoms. So, that can be observed under an FTIR spectroscopy. There are different types of stretching; symmetric stretching, asymmetric stretching, scissoring and rocking. If you are having an extreme nucleophile, it will have a repulsion between the neighboring nucleophile. That stretching will be observed under FTIR spectroscopy.

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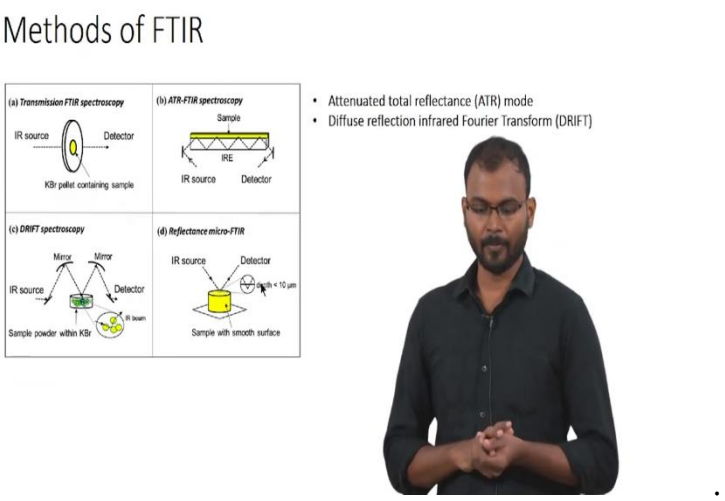

There are different methods of FTIR available. FTIR can be used for surface characterization and also for bulk composition. Each of the different functional groups of a molecule will have different stretching and bending. Based on that, we can identify the functional groups. It can be done for surfaces, powder samples, scaffolds, and liquid samples. So, I will first explain the methods. Transmission FTIR spectroscopy is the widely used method, where you keep the sample in the path of the IR, and it will detect the spectrum, and it will show how much of the light has been transmitted through the sample and based on that it will plot the spectrum.

Then ATR-FTIR spectroscopy is the attenuated total reflectance FTIR, where you have a reflective surface at the bottom, where the light source would be reflected to and fro to the sample, and the reflective surface and the final amplified light would be detected using detector.

Then drift spectroscopy, it is diffuse reflection infrared Fourier transform where it is similar to the transmission FTIR spectroscopy, but the IR would diffuse up to a particular depth, and then it would be reflected back into the detector.

The reflectance micro FTIR is a new technique where you can characterize the micro-sample. The IR source will be reflected, and it can be detected for a very small amount of sample with high sensitivity. For a liquid sample, you can form a thin film using a casing

where you can drop the liquid onto that. So, that thin liquid film can be measured using transmittance FTIR spectroscopy.

If you have an opaque material, transmittance FTIR is not possible because IR cannot pass through. If the sample completely blocks the IR, you cannot observe any spectrum. So, to avoid that obstacle, we will use ATR FTIR spectroscopy, which will have a reflective surface.

And for transmission spectroscopy, we use KBr as a standard molecule for preparing the sample. This is because KBr does not have any absorption spectrum in the IR region. Hence, if you are mixing the KBr with your sample and checking for the spectrum that will have only the spectrum of your sample.

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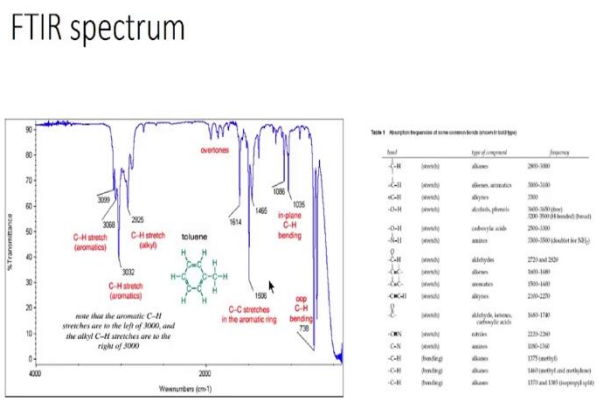

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So, this is how a normal FTIR spectrum looks. This is an example of toluene where you have the C-H stretching, C-C stretching in the aromatic ring. This is because C-C at a normal bonds it would differ from the aromatic ring C-C stretching due to the constraint exhibited by them. So, each will have a different wavenumber.

Based on different wavenumbers, we will identify what the functional groups present on the sample are. These are the commonly available functional group. Alcohols and carboxylic acids will have a range from 3000 to 3300 cm^{-1}. And if you are looking into

ester bonds and amine bonds, you can look around 1700 cm^{-1}. Like that, different functional groups will have different characteristic spectrums.

So, the FTIR technique will show, what are the functional groups available on a sample. But if your material which is having the same functional groups, then it would be difficult to identify, because the same functional groups will overlap with each other. Hence, you cannot have the exact mechanism to identify in FTIR. However, it is a technique where you can optimize the reactions, but you cannot fully depend on FTIR.

Ok.