Tissue Engineering
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Lecture - 14
Material Characterization - Part 2

Good morning everyone. In the previous class, we learned about what are surface properties and what are the different techniques we can use for characterizing those surface properties, today we will be learning about the bulk properties

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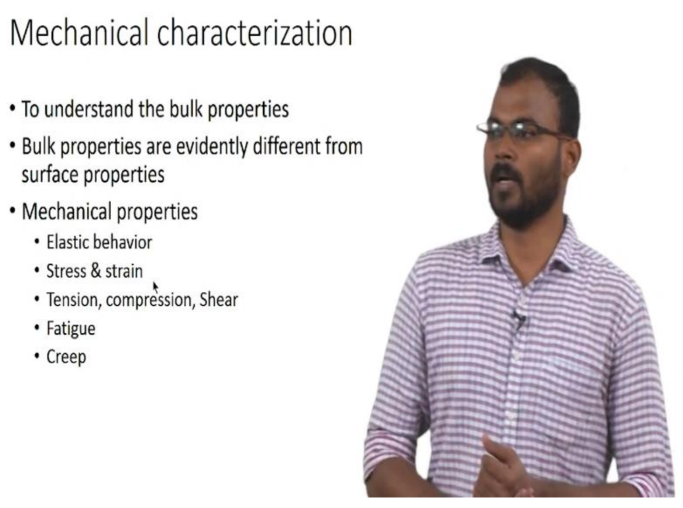

What are the bulk properties? So, other than the outer surface of the material, all other section is bulk section only; For any material to be used for the implant first that bulk property has to be satisfied. It has to be mimic the already existing natural material. Then only we can go for surface analysis and other things for making it more biocompatible

So, what are the bulk properties? They are the mechanical properties of a biomaterial and vary based on the application. These properties include stress and strain, tension, compression and shear stress, fatigue, creep, and so on. Bulk property is totally different from the surface, where it would not have much interaction with the system. It is significant for the orthopedic implants, where the load-bearing applications are involved.

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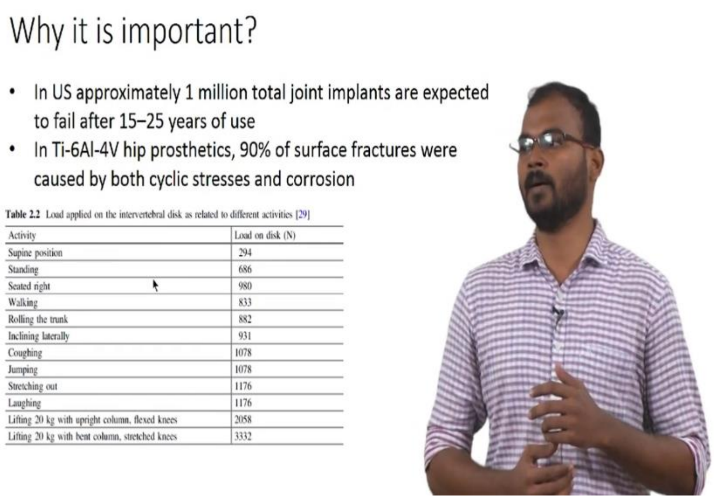

So, why it is important? Because, if you consider the orthopedic implants, once you surgically insert it into the body, it has to be there for lifelong, and it has to maintain its function all over that period. To have that capability, we have to characterize those mechanical properties of the implants.

For example, consider an intervertebral disc that is inserted into the vertebra of a human. For each position, the load applied to that intervertebral disc is different. As you can see in a normal supine position, which is lying down, the load on the disc would be 294 Newton only, but if you are standing and walking and all, it increases gradually due to the force applied on the vertebra and if you are lifting any objects, that load would be increased further.

Hence, your material has to have that wide range of load-bearing capacity. So that it can be in the host system for a prolonged period of time. Because if there is a failure and if you are inserting an implant at the age of 30, then around 50, if it fails, you have to go for again go for surgery, which has a higher risk and complications. So, the bulk property thus plays a major role in the longer patency of the implant.

What are the major obstacles in these bulk properties? One such is corrosion; due to the excessive usage of the implant inside the body, the material can break down into smaller pieces, which will cause further complications in the biological system.

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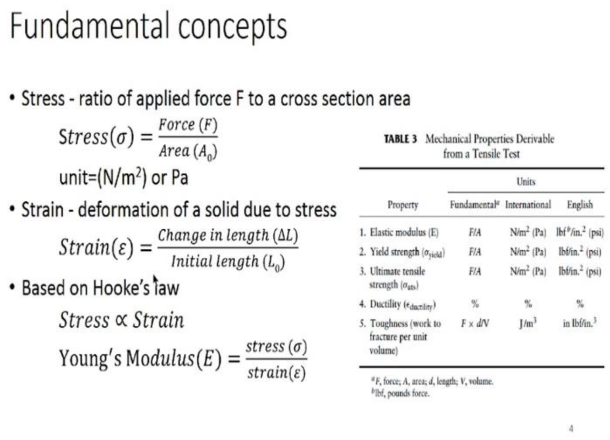

Property	Units		
	Fundamental[a]	International	English
1. Elastic modulus (E)	F/A	N/m^2 (Pa)	lbf[b]/in.^{2} (psi)
2. Yield strength (σ_{yield})	F/A	N/m^2 (Pa)	lbf/in.^{2} (psi)
3. Ultimate tensile strength (σ_{uts})	F/A	N/m^2 (Pa)	lbf/in.^{2} (psi)
4. Ductility ($\epsilon_{ductility}$)	%	%	%
5. Toughness (work to fracture per unit volume)	$F \times d/V$	J/m^3	in lbf/in.^{3}

[a] F, force; A, area; d, length; V, volume.
[b] lbf, pounds force.

The fundamental concepts in the mechanical properties are as follows;

Stress, when a force applied onto the surface that is called stress. The mathematical equation of stress is equal to force by area.

$$Stress(\sigma) = \frac{Force\ (F)}{Area\ (A_0)}$$

So, you are applying a force on a specific cross-sectional area of material, that is the stress. Based on this stress, the material will go deformation. If there is a dental implant so, while you are eating, that stress would be applied to the dental implant that it has to maintain integrity. That deformation is called strain.

The Strain is change in the length by original length or initial length.

$$Strain(\varepsilon) = \frac{Change\ in\ length\ (\Delta L)}{Initial\ length\ (L_0)}$$

Based on these two factors, there is a relation called Hook's law, which introduces a new parameter called Young's modulus, which is a ratio of stress by strain.

$$Young'sModulus(E) = \frac{Stress\ (\sigma)}{Strain\ (\varepsilon)}$$

This is an important and commonly used parameter in all of the biomaterials for defining a mechanical property of that material. Other properties related to these tensile strengths are elastic modulus, yield strength, ultimate tensile strength, ductility, and toughness. So, these properties I will explain while explaining the stress-strain curve.

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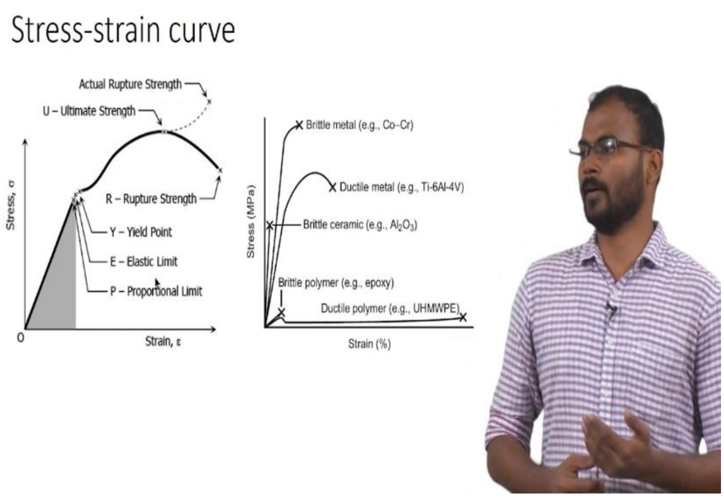

Now, you know what stress is and what is a strain. You have a linear relationship based on this stress and strain; if there is an increase in stress there would be an increase in strain. The initial proportionate linear increase in the region is called the elastic region. So, in that elastic region, what it represents is, due to the strain, if it deforms into a different structure, then it can retain back into its original structure; it is like a rubber band whether you extend it. So, if you leave, it can come back into the original shape and length. So, in that region is called the elastic region.

There is a particular region were if you extend it furthermore it will cause breakage of the rubber band. That region is called the plasticity region, where it cannot come back to its original length. So, the initial region is called an elastic region. Up to this, the stress and strain would be very linearly proportional. Then after it, the yield point is there. So, after this yield point, the material cannot go back to its original state if you are having an implant, polymer materials used for vascular grafts, catheters, and so on. So, if the force applied to the material is going beyond that yield point, its shape can change into a different one. So, that will cause problems while introducing into the biological system.

Hence, your material has to be below that yield point of the replacing material or the application. The ultimate strength is the maximum load that material can withstand. Considering a hip prosthesis, if you are introducing into the system, the maximum load where that material can withstand even if it deforms, that is the ultimate strength.

After that, if you continue to give the load, then that material will break. So, that is called rupture strength. This is a typical stress-strain curve, but for different types of biomaterials, the stress-strain curve varies because polymers will have a higher elasticity whereas, ceramics has no elasticity at all. As you can see, the brittle ceramic aluminum oxides it has an initial Young's modulus. Then after that, if you give a load, it will break. There is no point of elongation or anything for the ceramic. Then for brittle metals like cobalt-chromium alloys and titanium also somewhat similar with increased Young's modulus. For ductile metals like titanium alloys, there would be a little bit elongation, then after which it breaks down.

In a polymer, there is a thermosetting polymer such as epoxy polymers those kinds of polymers it is very brittle in nature. So, it does not have that elastic point. These epoxy polymers are used for the adhesive surgical glues and adhesives purposes. So, that they will immediately seal up the wounds without any leakage, those materials would be very brittle and would not have any elasticity.

Then ductile polymers such as low-density polymers, polyethylene, carbohydrate polymers, and so on will have a higher range of elasticity. They also include polymers such as PVA, chitosan, polyethylene glycol, normal plastic polyethylene. If you extend these materials, it can go for the very long length so that these polymers will have a different Young's modulus and different plasticity region.

Based on this, you have to decide the application you are going to use, you have to decide the material and characterize whether these materials have the same property as that of naturally occurring materials. For example, if you are introducing new vascular grafts, they should have the mechanical property such that it can withstand the force of the bloodstream passing through that graft. If you are going for a hip prosthesis, you have to look for how the loads applying to the region around the hip prosthesis and check whether it does not break or does not fall above the yield strength.

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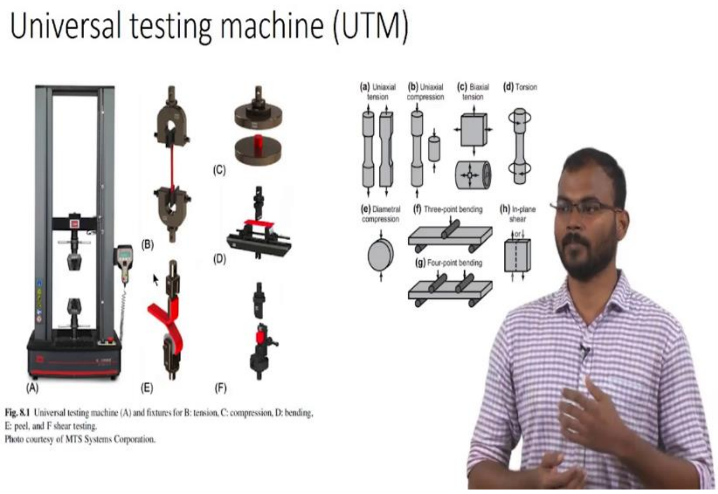

How we can find out all these parameters Young’s modulus, ultimate strength, fracture, and rupture point and all. All of these properties can be found using the universal testing machine. In this machine, you can do a variety of tests where each of the tests will give different parameters. This is how the instrument will look like. The bottom one is called a mounted head, and it is fixed and unmovable. So, you can see the two clamps. In between those two clamps, we will fix the sample.

The top one is a movable head. In that one, the load cell would be there at the top. The load cell will apply the force on the sample. And the tension will be applied to the sample. So, that will introduce stress, and then the change in length will be calculated by the sensors. From that, we can calculate the ultimate strength, yield strength, and rupture point and Young’s modulus and so on. For different applications, different clamps would be available, as you can see. One is for tension; Tension stress is where you elongate a sample. So, that is the tension stress. Compression is where you compress the sample. So, that how much the load it can compress into that material surface.

Then bending, where the material would be kept, and there would be bending at a different points. So, there is based on the application. For example, if you have screw plates in the legs and disc prosthesis, there the bending would be different based on the how many screws and nuts you are introducing into the plates. Based on that, you can go for threepoint bending, four-point bending to exactly characterize how that will function inside the biological system.

Then torsion, there would be a twisting occurs on the sample. This kind of testing will be done for the ligaments, where the ligaments would have to be turned so that it does not break or rupture during that torsion stress. Then in-plane shear is mainly for hip prosthesis where there would be a metal contact. There would be shear stress applied whenever that ball and cub would be moving over the plane. So, that will be found out using the shear stress in-planar testing.

So, all this can be done for different types of samples such as metals, polymers, ceramics with different sample dimensions. Using this instrument, you can find out all the mechanical properties. As you can see in that sample dimension, it looks like a dog bone shape. So, it is an important factor that if you are keeping a single lengthy rectangular sample result in breakage when you are clamping the sample. So, that is not the actual stress applied to that sample. So, they make a dog bone shape so that the sample at the ends would be thicker. Hence, it would clamp properly, and the breakage would occur at the middle position of the sample only. So, that will give the exact value of how that stress-strain observation will be occurring.

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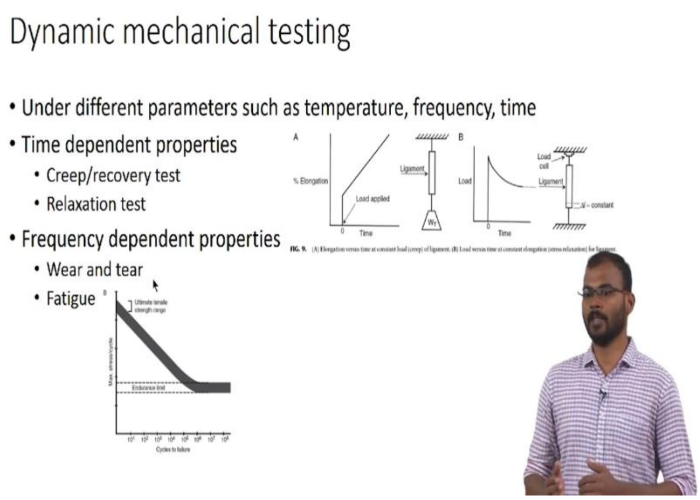

Usually, we consider that when a stress is applied, the strain will instantaneously produced. So, that is how our idea was, but it does not follow that all over the period. Consider different parameters, such as temperature, frequency, and time. Based on these parameters with constant stress also, it can have a different strain. In the stress-strain curve, if you can see, there is applied constant stress, which varies the strain. So, that is dynamic mechanical testing.

So, why this is important? Temperature, as I said earlier, dental implants where you can have a change in temperature like while eating ice cream or the hot soup. So, the temperature changes. Hence, the implant should maintain its integrity all over the range. So, we do dynamic testing to identify whether that affects that mechanical property. At the same normal room temperature, if you are applying a load on to your material, that will have a different effect when the temperature is very cold. Those things have to be calculated. Then frequency varies how much time a material is being used for that specific application. Consider the hip prosthesis, where it has to move while walking; how much distance you are walking determines the frequency.

For example, consider the heart valves; it has to function a lot number of times. For a minute, it has to beat for around 72 times. For a minute, it is like that, then for the day, how much beats? If it has to be there for a year and for a prolonged period, how much time the valves have to open and close? So, over those periods, what happens is the material is in contact with the nearby surfaces, there would be some corrosions. There would be some surface etching and erosion that will reduce the efficiency of that material. So, those factors have to be tested before clinically using that heart valves.

What are these properties that change based on this are frequency and time. So, these are the widely used properties; Creep or recovery test and relaxation test. The creep or recovery test is where the stress should be maintained constant, whereas the strain would vary. So if you consider a ligament. As you can see in the figure, the weight wt has been loaded. So, when it is given at that time point, it will not elongate if it is within that linear range of the stress-strain curve.

After a particular period of time, it tends to elongate. So, that is that percentage elongation changing. That is what it is changing how much time can affect that strain based on constant stress. So, that is a creep or recovery test. Then the relaxation test is the exact opposite of the creep test where the strain would be kept constant the elongation would be kept constant, but the load will vary. So, how much load it can vary to maintain that elongation. These things would be very useful when you are designing new material; so that the material should not go to a point where it cannot recover its original state back.

For those things, these time-dependent properties will involve, and to identify that materials prolonged usage, we will study these time-dependent properties. Frequency-dependent properties, as I have said earlier, the number of frequency your material is being used like in the heart valves and hip prosthesis.

Wear and tear it is a major problem in orthopedic implants where the material would be in contact with the nearby materials. Due to the high number of frequency we are using that material inside the biological system, the surface will get eroded, and that will lose its functionality, lose its efficiency.

Fatigue is again the same thing wherein fatigue; you are below the yield point only. The number of the load we are giving is below the yield point only, but due to the over usage of the implants for that application, the material will lose its efficiency, which is called fatigue. It will lose its functionality. This usually happens when the material you are designing it should be almost similar to that natural material like the metal implants, it should be similar to the bone already present. If it is above, also what happens is that there is a stress shielding effect, it can happen.

Stress shielding effect is an effect where if a material can have a load of around 500 Newton, if we make that material into a 1000 Newton it can have that much capacity of a 1000 Newton material what happens is, it can have that all the load by itself and it will not pass on to the nearby bones. So, what happens due to that is the bones surrounding that material will lose its integrity, and it would be weaker compared to the normal bones. So, that is, by this material, it is shielding the stress of the nearby natural materials and thereby reducing its integrity. That is one of the major problems in orthopedic implants. So, those things will come when fatigue is also involved. It can seal the stress of the load and thereby reducing the integrity of the bone implants.

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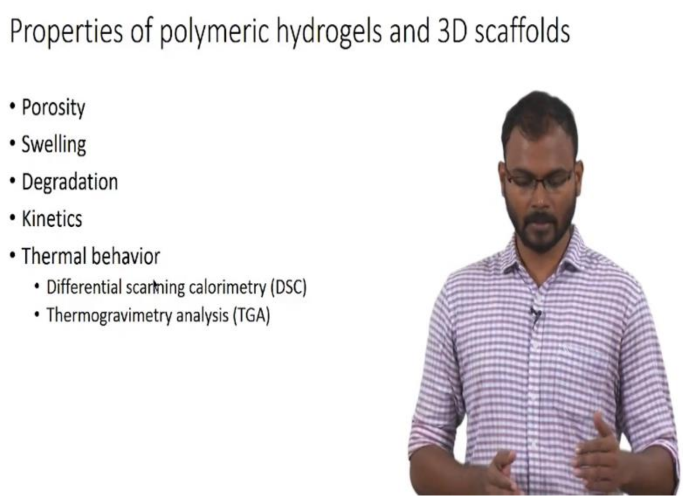

These are the basic properties and characterization we can do for the bulk properties of the material. Consider hydrogels and scaffolds. Those have a different property rather than these polymers films and metal implants. So, you might have already studied what are hydrogels and scaffolds. Hydrogels are gels that can have a higher amount of water intake, and scaffolds are the polymeric scaffolds that can be used for cell or tissue regeneration process. What are the properties important for these hydrogels and scaffolds? Some basic properties which define them?

You can do all the surface characterization and bulk characterization for these hydrogels and scaffolds. Other than those properties, these properties also involved porosity, swelling, degradation, kinetics, and thermal behavior.

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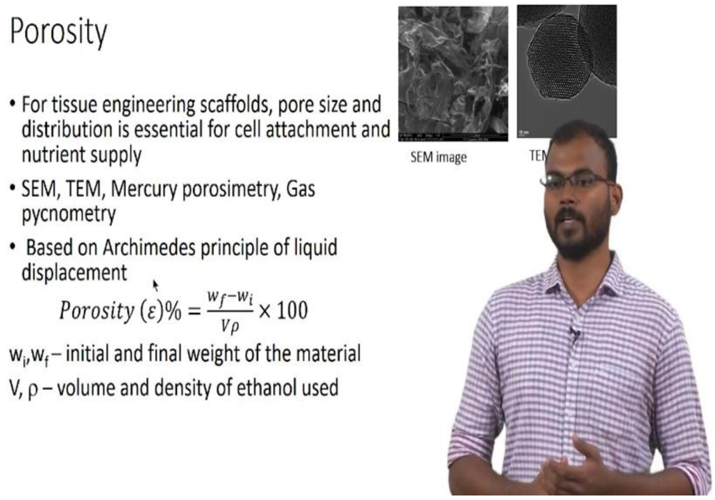

Porosity for scaffold porosity is an important factor because porosity will help in cell attachment in-depth, and the number of pores will help in the transfer of nutrients inside and outside the scaffolds. To identify the porosity of the material, tissue engineering scaffold normal techniques like SEM, TEM mercury porosimeter, and gas pycnometry can be used. In TEM and SEM, you can visually see the porous structure.

As you can see in the SEM image, the porous scaffold is seen, but this in SEM image, you can see at the surface only. So, you cannot say it confirms that inside also the porous structure would be there. However, inside, it would be a compact bulk structure, and the surface would be a porous structure. In TEM, you can say that the material is completely porous because, due to the transmitted electrons, you can see what is inside the material.

If material is completely porous inside, you can find out using that thing other than these sophisticated techniques; you can use mercury porosimeter and gas pycnometry, which uses an Archimedes principle of liquid displacement. How the experiment for finding the porosity goes is that you will have a liquid in mercury porosimeter. You will insert the porous structures inside, and you apply a vacuum also. So, the air would be sucked out of the system, and all the pores inside will be filled by mercury.

Then you take out the scaffold and weigh the amount of scaffold before keeping into that mercury, and after that, and based on the volume, you can actually calculate the porosity. So, a gas pycnometer it involves gas and commonly used another liquid is that ethanol.

Why ethanol is because it will not involve in swelling of the materials, and most of the materials would be hydrophilic.

If you are using water, it can absorb water, and it can swell, it is not actually the porous volume. The volume would be based on the polymer scaffold. So, using ethanol will be better than the water experiment

$$Porosity\ (\varepsilon)\% = \frac{w_f - w_i}{V\rho}$$

Wf, Wi are the initial and final weight of the material, and V, ρ are the volume and density of the ethanol used for the experiments.

So, how much volume and ethanol density of the ethanol you are using, that will give you the porosity of the scaffold.

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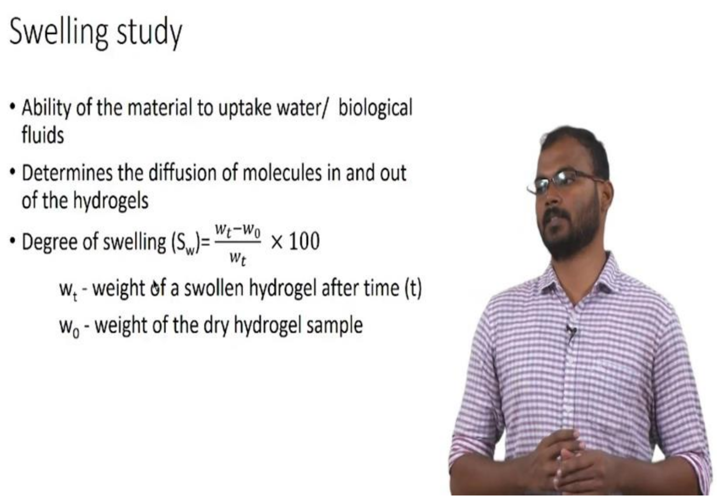

The swelling study is predominantly used for hydrogels because the uptake of water majorly influences the kinetics of the molecules loading and releasing from the gels. So, a material that can swell faster can mean that the water intake is higher so that the nutrients or any other drugs or molecules which you are incorporating into the hydrogel can be released faster. If swelling is lower, then the release would be slower. So, that defines how the kinetics mostly in drug delivery application, how these kinetics would be affected by this swelling study.

For swelling analysis similar to that, what you do is you weigh the samples before keeping it into the water (W_0), then you keep it in water, then you again weigh the sample (W_t). The percentage degree of swelling is that,

$$Degree\ of\ swelling\ (S_w) = \frac{W_t - W_0}{W_0} \times 100$$

[***In the presentation this equation is incorrect]

So, usually for hydrogels for the application wise, you can actually use this degree of swelling to understand that the rate of drug release and all.

If it is an uncrosslinked polymer hydrogel, the swelling would be faster, and if it is a crosslinked hydrogel, the swelling would be slower. So, to understand the kinetics of the release profile, you can study this swelling.

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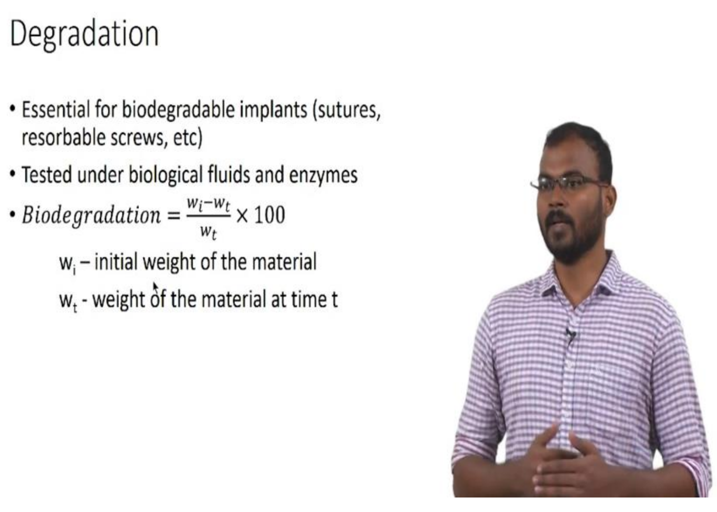

Degradation is another important parameter for both the hydrogels and scaffolds because nowadays, most of the implants are coming as a biodegradable implant. When it is implanted into the body instead of having another surgery to remove those implants, these things can degrade by itself, such as the sutures, resorbable screws, where you implant it for the metal plates and the sutures for internal sutures.

Over the period, initially, it should function like a normal suture where it should have all the mechanical property and the physical property like the normal suture, then after over the period of time when the wound heals the biological system should degrade that, and it should be washed away from the system.

So, this would be tested with the biological fluids and enzymes based on the application you are using. So, the biodegradation is calculated by

$$Biodegradation = \frac{W_i - W_t}{W_i} \times 100$$

W_i and W_t are the weight of the sample initially and at time t, respectively.

[***In the presentation this equation is incorrect]

This will help you understand how long material can degrade. Based on that you can design them; so, if you want your material to be degraded within a week or within a month, then your biodegradation should be there within the month; that is the final value will be around a 0 or so, that it should be completely replaced by the natural tissue.

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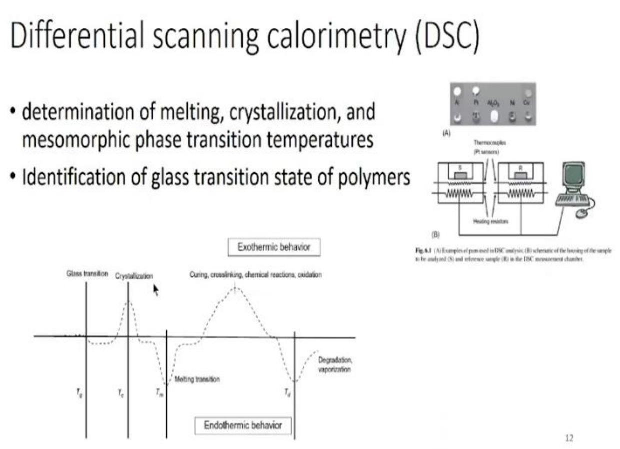

So, those are the normal physicochemical properties for the scaffolds and hydrogels, now we are coming into thermal behavior. Thermal behaviors like I said used for characterizing the implants for dental implants and normal polymeric materials also; you have to understand the effect of temperature on the material. So, that can be found out using a DSC and TGA analysis. These two are commonly used techniques other than this; there are other thermal characterization techniques also.

DSC, Differential scanning calorimetry is used to identify the material melting point, crystallization point, then the glass transition temperature, then the phase change of the polymers. All those things can be found out using a differential scanning calorimeter. The instrumentation involves, as you can see in the picture, there are two components; one is a reference, and another is the sample. In the sample pan, we will keep our material, and in the reference pan, it would be an empty pan, or you can have comparable reference materials.

You will apply a constant temperature to both the sample and reference at the same time at the same temperature. So, what happens is that if your material exerts heat, like releases heat by exothermic reaction or absorbs heat by the endothermic reaction, so there would be a change in the heat flow that would be detected. Based on that, you can plot this DSC thermogram where you can find out the melting point, crystallization point, and degradation point of the materials. As you can see, polymeric materials, if it is amorphous polymers, it will go a glass transition change. So, it can go from brittle nature into a rubbery nature.

Above glass transition temperatures, the molecule will be arranged randomly. So, that will have a rubbery nature, and that is the temperature called glass transition temperature. After the glass transition temperature, it will have molecules arranged in an orderly fashion where you can find out the crystallization temperature after that molecule tends to melt. So, that is the melting temperature then if there is a chemical reaction due to the temperature So, that is the exothermic behavior you see then finally, the degradation occurs where the material is completely degraded due to that temperature.

All these temperatures are very essential when you are using a thermoresponsive gel and other temperature involving implants and devices. It can also be used for the characterization of new biomaterial, whether you are having a crosslinked or the mixtures of two materials; there would be a difference in temperature shifts in the glass transition temperature, and change in melting temperature also will occur.

Because higher the crosslinking, the melting temperature will vary. Based on that, we can identify that your crosslinking has occurred, or your material properties have changed due to the incorporation of a new molecule into your samples and so on.

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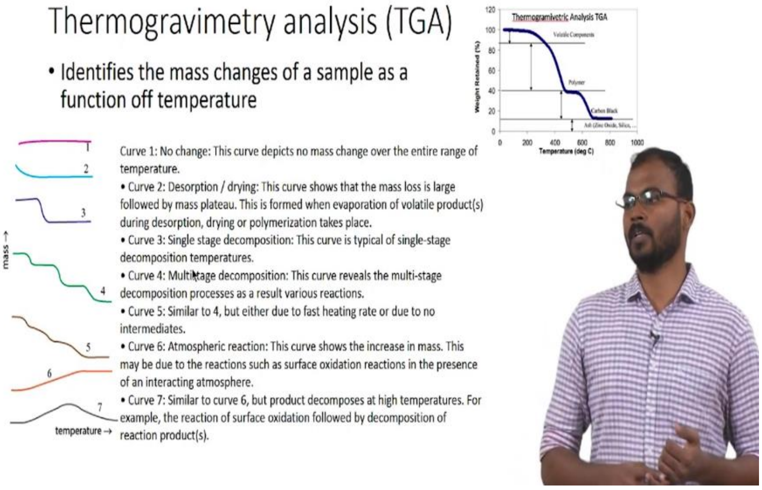

Thermogravimetry analysis is similar to DSC only; the mechanism is similar, but here it involves as the name suggests the gravimetry, which is a weighing of the material. So, the instrumentation is almost similar nowadays. The advanced instruments have both DSC and the TGA available combined. In the DSC, you can observe the change in temperature due to the material when the heat is supplied; Here, due to the heat supplied, you can measure the minute change in the weight. So, you will use a sample of around 20 mg or 5 mg only for each of them. A minute change in temperature can be observed using this thermogravimetric analysis.

This mainly helps us to identify how degradation occurs for the material. A typical thermogram will look like that image in the right corner; So, in DSC and TGA, there would be a constant increase in temperature they will be supplying. The initial increase in temperature there would be volatile compounds like moisture, water adsorbed, or any solvents present it would get evaporated from that system. You can see there is a decrease in the weight percentage, which is the initial stage.

Then the second stage would be the decrease in the weight due to polymer degradation. So, that is in the second stage; then, the final stage where it is completely turned out into ash, which is a degraded polymer, everything has been completely turned into carbon.

The interpretation for this TGA analysis would be, if your material is not affected by any temperature, the line would be very straight. Then in curve 2, we can see there is a sudden decrease, and it became stabilized that usually denotes that your material has some moisture or some solvents are there, which is getting evaporated, but not a degradation of a compound or molecule present on the scaffolds, that is the curve 2.

Then in curve 3, it's a single-stage decomposition. If your material is made up of a single polymer or single material or something ceramics, there would be a single degradation of that particular molecule only. So, that can be observed due to the single loss of the weight. If your material is having multiple polymers like interpenetrating networks where you have a couple of polymers or alloys where you use silver titanium alloys. So, those will have a; each material will have a different degradation temperature. The lower degradation temperature first step would be those materials will get degraded, then simultaneously based on the degradation temperature, it varies.

In step 5, if it is not stepwise, if it is just gradually decreasing, it means that you are actually heating it very fast so that your instrument cannot measure the exact weight of that material as scaffolds present. There can be some increase in weight also, that happens when your material is reactive to the atmosphere present inside the system. Usually, it would be inert atmosphere such as we can use such as nitrogen or argon, but if you are using a normal atmospheric condition, there would be oxidation occurs, which can lead to an increase in the weight of the material.

The final one is again after decomposition; those decomposition products can interact with the atmospheric compounds and reactive species so, that it can lead to an increase in that weight. This can also happen in magnetic samples; there would be an increase in weight. By characterizing these thermal properties, you will know that your material is pure or if whatever you are incorporating into your material can have any thermal effect on the final material. So, those things can be analyzed using these techniques.

Understanding both the bulk properties and the surface properties will have a clear idea of how exactly you want to use that material for your applications. After finishing these two characterizations, you have to go for your biological characterization, where you can check for protein absorption, cell attachment, and if you are working on an anti-bacterial adhesion or biofilm formation, to avoid biofilm formation. So, those things will come under the biological characterization of how effectively it can improve or decrease the attachment of the biological system.

So, before doing that you have to understand these parameters and these characterization techniques has to be done for all the materials you are trying to do.