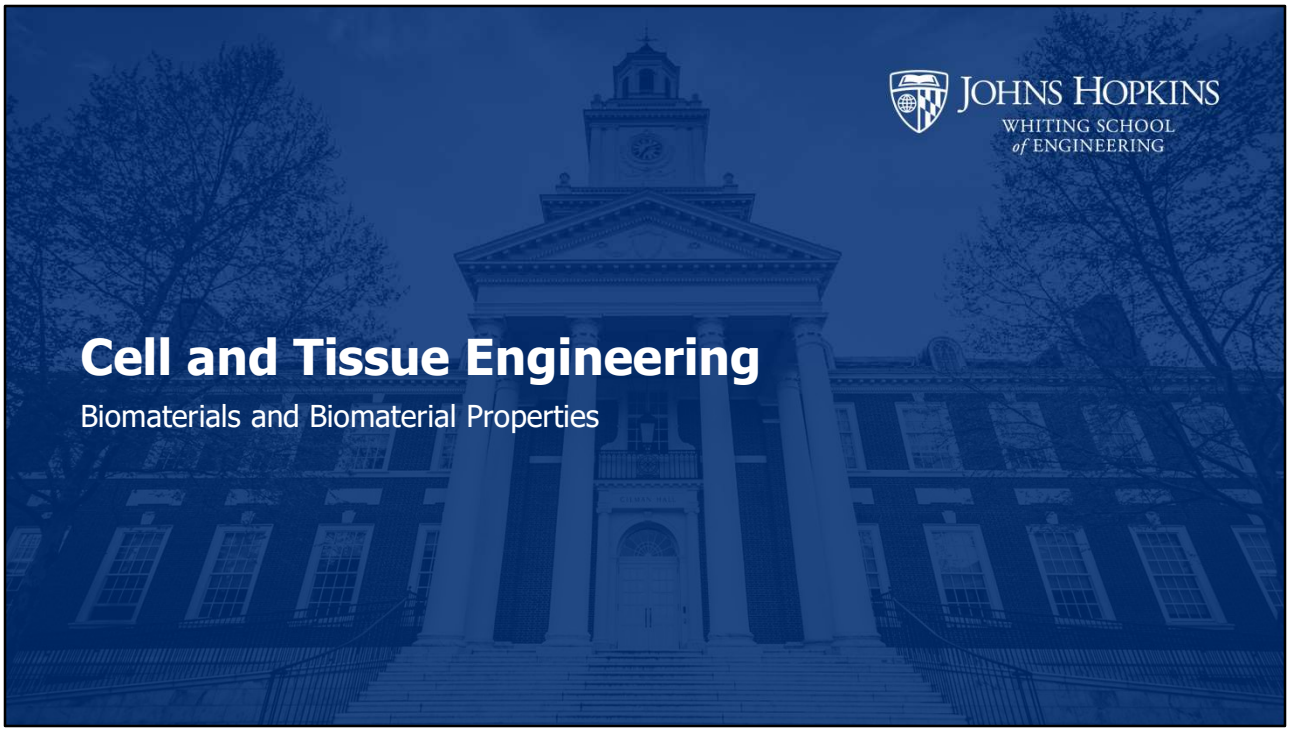
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Cell and Tissue Engineering
Biomaterials and Biomaterial Properties

What is a biomaterial?

- A biomaterial is now defined as a **substance** that has been **engineered** to take a **form** which, alone or as part of a complex system, is **used to direct**, by control of interactions with components of living systems, the **course** of any **therapeutic** or **diagnostic procedure**.

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http://www.journals.elsevier.com/biomaterials/

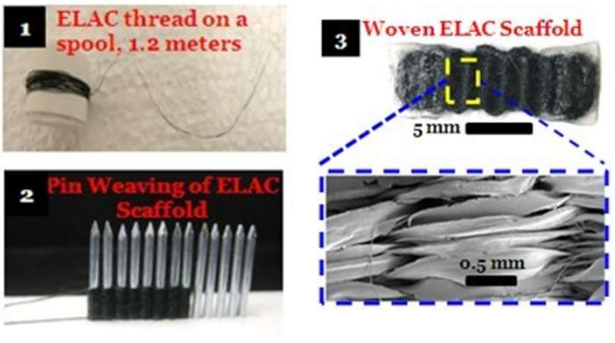
Before we can get into discussing biomaterials we should start with a definition – here is the current definition from the journal Biomaterials.

A biomaterial is now defined as a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure.

There is a lot in this definition – I want you to make note that biomaterials can **stand alone**, they can be used in **conjunction** with other materials, they **always interact** with a biological system, and they are necessarily all for treatments/diagnostics .

What do we need to know about biomaterial scaffolds?

- Properties
  - Surface, bulk, biological
- Types
  - biologic/natural, synthetic
  - Focus on polymers
- Tailoring to the needs of the tissue
  - Subcellular length scale (<10μm)
  - Cellular microenvironment (10-100μm)
  - Supracellular structures (>100μm)



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Engineering.case.edu/groups/akkus_lab/node/9

In today's lecture we are going to discuss three main topics. **Properties** of biomaterials, **types** of biomaterials and **ways to tailor or alter** biomaterials

We will be looking at the biomaterial at several **length** scales
– the **subcellular** scale where biological recognition happens and receptors are engaged.
-- The **cellular** scale where migration, proliferation behaviors emerge and
-- the **supra cellular** scale which includes mechanical and biochemical compliance.

On the right I'm showing you an example of how to view a biomaterial at these different length scales.

This is an image of ELAC – electro-chemically aligned collagen fiber-scaffold that is used for critically sized tendon repairs. You can see how the length scale is different when you are considering different properties of this material and different phases of its fabrication.

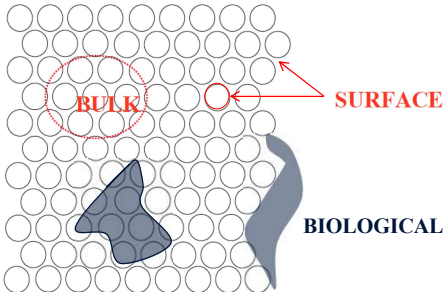
At the top we start with a spool of thread that is over **1meter** in length. This is used to create a scaffold that is over **50mm2** in area. However the fibers themselves are on the order of **10s-100s of microns**.

Biomaterial Properties – surface vs. bulk

Surface Properties
Cell-material interactions
Protein adsorption and resistance
Topology

Biological Properties
Biocompatibility

Bulk Properties
Mechanical properties
Thermal properties
Electrical properties



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Modified from Phys_cmp.tripod.com

To begin we will consider **biomaterial properties** – these can be broken down into **surface**, **bulk** and **biological** categories.

In the cartoon on the right you can see what I mean by these terms.

The surface pertaining not just to the **outer** surface of the material but also the **interior** surface.

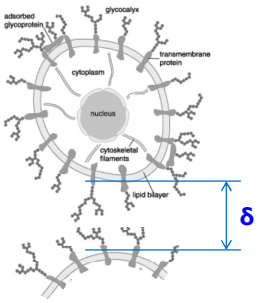
The bulk being properties of the **collective** material and the **biological** pertains to **interaction** with the body in **all locations** of the material.

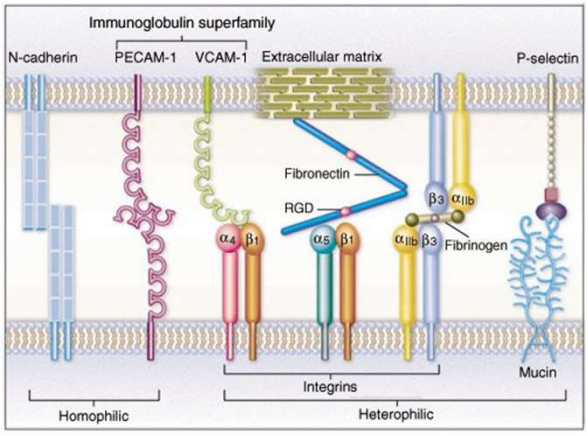
We'll start today by talking about the **surface** properties.

These include things like:
- **cell-material interactions**
- **protein adsorption and resistance**
- and **topology** or topography

Surface Properties – factors determining cell-material interactions

Non-specific and specific interactions





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Modified from Tissue Engineering, Saltzman; Labs.idi.harvard.edu

Earlier, we talked about specific and non-specific interactions involved with cell adhesion –

Non specific – interactions – electrostatic repulsion

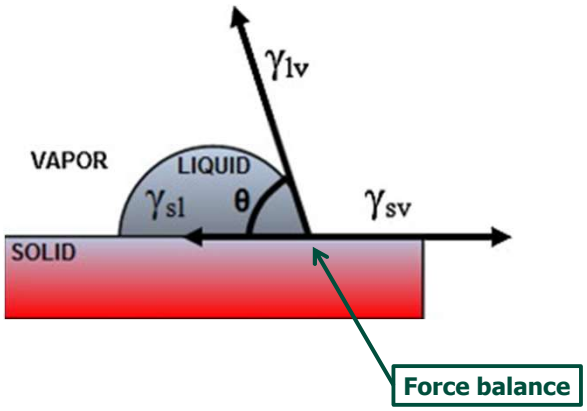
Specific – binding a cell receptor. You may recall integrins, selectins, cadherins fall into this category.

Both types of interactions can be **engineered** into a biomaterial scaffold to **manipulate the adhesive properties**.

Thermodynamic models of cell adhesion (1)

Young's Equation

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



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Paital and Dahotre, 2009

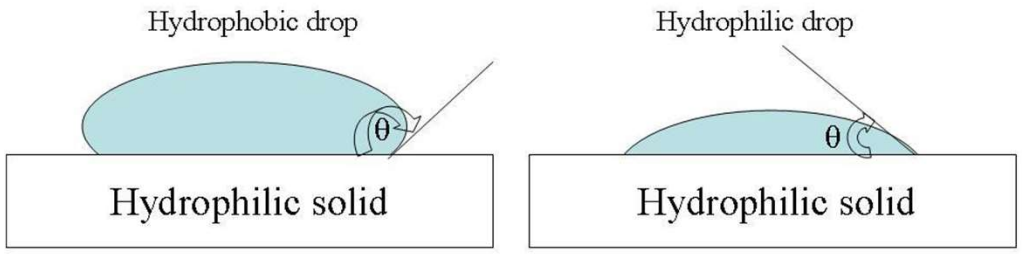
Equilibrium descriptions of adhesion utilize thermodynamic models. In these models, cells are reduced **to liquid droplets** interacting with solid surfaces.

Let's consider **Young's** equation for a moment. This equation describes the interaction between a **liquid,** a **solid,** and a **vapor**

Young's equation relates the **energies** (free energy per unit area) of the different surfaces with the **contact** angle – **theta**. It is essentially a **force balance** where the three phases meet.

γ_{SG} (SV) is solid-gas (Vapor) surface tension, γ_{SL} solid-liquid surface tension, and γ_{LG} (LV) liquid-gas (vapor) surface tension.

Thermodynamic models of cell adhesion (2)



Young's Equation

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

$$F_{adh} = \gamma_{cs} - \gamma_{cl} - \gamma_{sl}$$

Adhesion cell-solid cell-liquid solid-liquid

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Small values of γ_{SL} (Solid-Liquid) correspond to a **hydrophobic** surface – one where fluids bead up.

This is a **positive** and **unfavorable** free energy for cell adhesion.

Large values of γ_{SL} describe **hydrophilic** or **wettable** surfaces. These have a **negative** free energy and are **favorable** for cell adhesion.

If we modify Young's equation for a cell interacting with a **solid** while in a liquid – we get this bottom expression.

Now instead of v for vapor you see c for cell. The **interfacial free energy** is equal to the cell-solid free energy minus the cell liquid and solid liquid free energies.

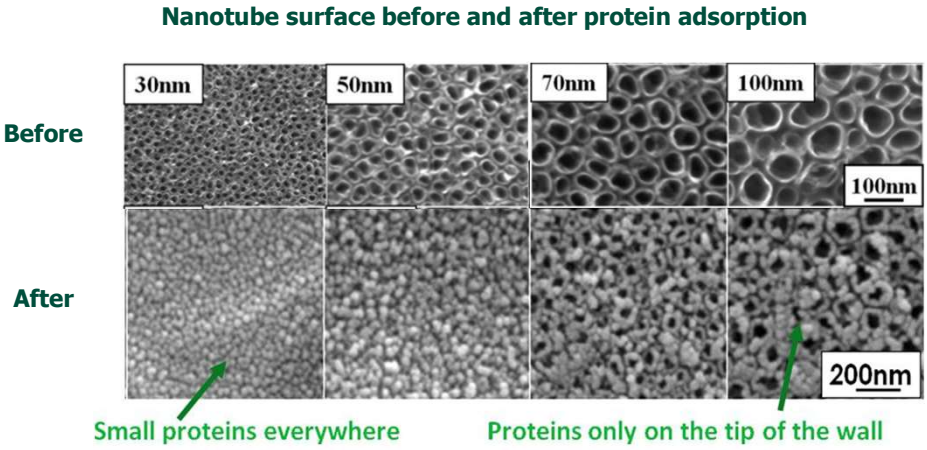
γ_{SL} is often the measured parameter used to calculate γ_{CS} (Cell-Solid)

Receptor-ligand interactions between the cell and solid scaffold typically don't influence the solid-liquid free energy (γ_{SL}) but they do affect the energy between the cell and solid. Therefore we should note that if there are specific interactions we cannot use measured γ_{SL} to solve for the γ_{CS} parameter .

Often we want these specific interactions to lead to features of our cell tissue

engineered product. One way to introduce or engineer specific interactions is to **adsorb protein** to the surface of the scaffold

Protein Adsorption can increase specific interactions



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Modified from Brammer et al 2011

Protein adsorption occurs through **electrostatic**, **hydrophobic** and **non-covalen**t hydrogen bonding

The rule of thumb is that **more hydrophobic** surfaces result in greater protein adsorption.

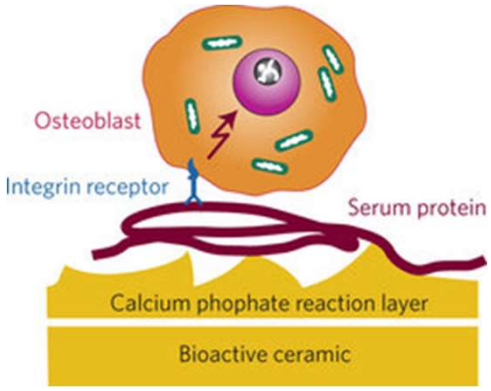
Here I'm' showing you scanning electron micrograph images of **titanium oxide nanotubes** of various **diameters** – 30 to 100nm -- **before and after** protein adsorption.

What I want you to observe in the **after** images is the **location** of protein adsorption. In this case the adsorbed proteins are a mixture from media containing serum.

The small diameter nanotubes **plug with proteins** and appear to have protein **everywhere** while the large diameter tubes only have protein adsorption **on the edges of the wall**.

Its clear that in this case the **structure** of the **material surface** changes the **presentation** of adsorbed proteins and can therefore **influence the response of cells** to these adsorbed proteins.

Protein Adsorption – Differential Affinity



Protein adsorption can influence cell behavior through:

1. Differential protein affinity for the biomaterial surface
2. Conformational changes in the protein due to interaction with biomaterial surface
3. Surface-induced activation of adsorbed protein

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Ducheyne et al 2012.

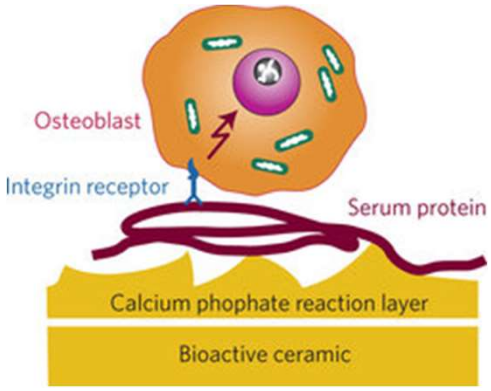
There are several other ways that adsorbed protein can effect cell behavior in a biomaterial scaffold.

The first is through **differential adsorption.**

If you incubate your scaffold in a protein solution – it may be that your scaffold preferentially adsorbs **one type** of protein over an other.

In doing so the surface of your scaffold (because of the protein coating) will initiate **different adhesion receptors**, cue-ing different down stream signaling and ultimately modifying cell behavior.

Protein Adsorption – Conformational Changes



Protein adsorption can influence cell behavior through:

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Ducheyne et al 2012.

Depending on the biomaterial is may also influence **the conformation and functionality of any adsorbed protein** leading again to changes in the way the cells bind and respond.

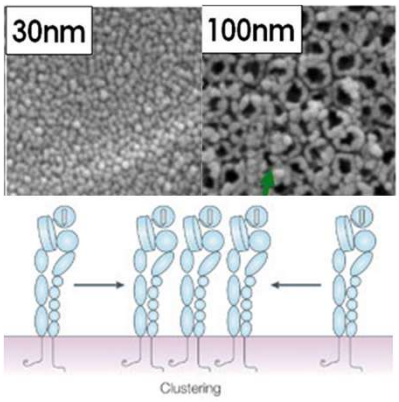
The example schematic I'm showing you on the **left** is one of these cases.

This schematic shows what happens with a **bioactive ceramic material** is used in bone repair.

Dissolution and re-precipitation lead to the formation of a calcium phosphate reaction layer and subsequent adsorption of serum proteins.

The reaction layer changes the conformation of the adsorbed proteins. The end results of this alteration is enhanced bone bonding and bone tissue formation.

Protein Adsorption – Surface-induced Activation



Protein adsorption can influence cell behavior through:

1. Differential protein affinity for the biomaterial surface
2. Conformational changes in the protein due to interaction with biomaterial surface
3. Surface-induced activation of adsorbed protein

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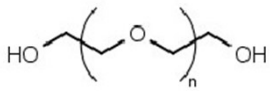
Kinashi, 2005.

Finally, the biomaterial surface an also influence activation – for example – a substrate that adsorbs protein at high concentration can result in clustered and multivalent signaling.

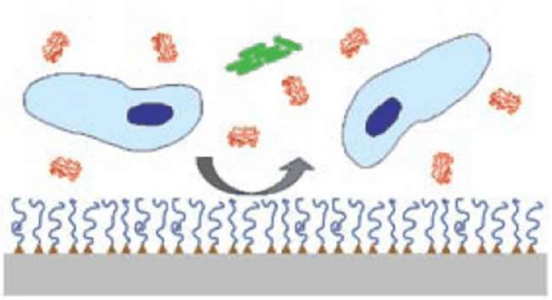
This should remind you of the image we started with – where protein can cluster on the tips of the small nanotubes but is restricted to the edge of the larger ones.

Cells here may experience signals that results from an increase in valance regulated affinty.

Protein Adsorption – Resistance



What about protein-resistance?



PEO or PEG
Polyethylene oxide
or polyethylene glycol

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Glycosan.com; Sigmaaldrich.com

It isn't always the case that we want cells to interact with our biomaterials – in fact there are many cases where we want our scaffolds to resist protein adsorption.

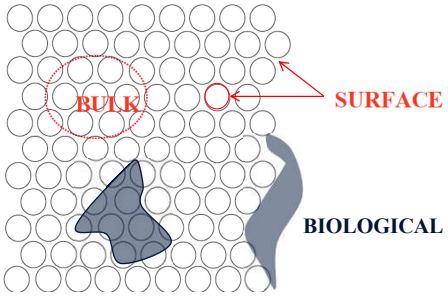
One common material used for these applications **is PEO or PEG**. This is a highly **hydrophilic** material. It will **resist protein adsorption** as well **as cell and bacterial interactions**.

Biomaterial Properties – Bulk

Surface Properties
Cell-material interactions
Protein adsorption and resistance
Topology

Biological Properties
Biocompatibility

Bulk Properties
Mechanical properties
Thermal properties
Electrical properties



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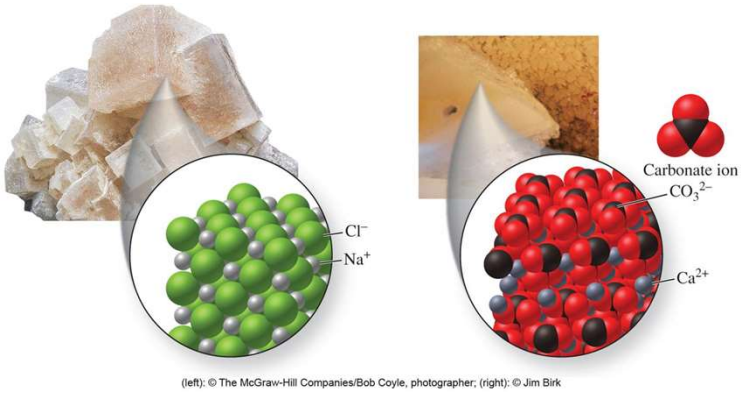
Modified from Phys_cmp.tripod.com

Surface topography is another property of the surface, but we've spent considerable time on this in prior modules so we'll skip it here and move on to **Bulk** material properties

Bulk properties include **mechanical**, **thermal** and **electrical** qualities of the material

Bulk Properties – Material Structure



(left): © The McGraw-Hill Companies/Bob Coyle, photographer; (right): © Jim Birk

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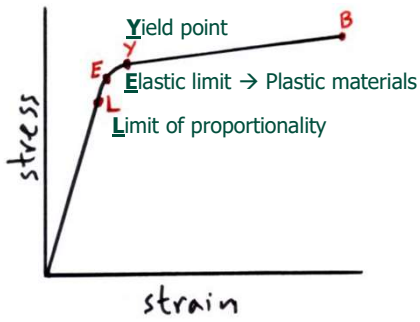
With surface properties we considered **electrostatic** and **van der walls forces**, but with bulk properties well consider **covalent**, **ionic,** and **metallic** bonding.

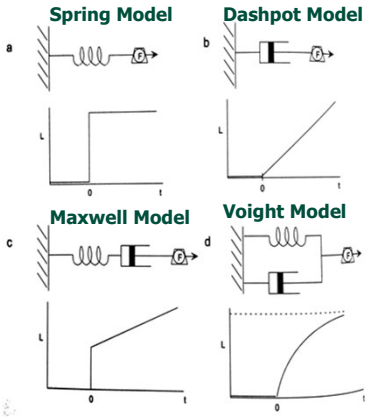
These are the bonds that can change the structure of the material.

For example here I'm showing you the ionic bonds that occur in table salt producing a crystalline lattice structure in the bulk material.

Being in a highly aligned and organized lattice as opposed to an amorphous structure will influence many bulk properties.

Bulk Properties – Mechanical Performance





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Saltzman, Tissue Engineering; Physicsnet.co.uk

In earlier modules, we talked about ways to understand, characterize and analyze bulk mechanical properties.

Recall our dissection of the **stress strain curve**, the characteristics of **fluid-** and **solid-like** behaviors, as well as **viscoelastic** models.

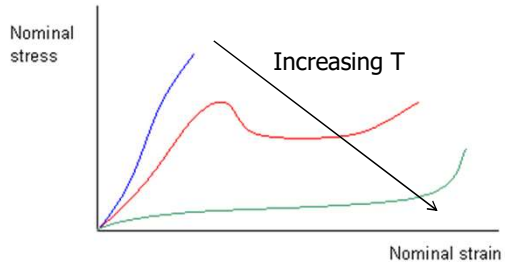
The **internal structure** of the material – that is the organization of the bonds formed that we saw on the last slide -- will profoundly impact the **stress-strain curve** for the material.

As we discussed previously, **matching** the **bulk** mechanical properties of the biomaterial to the **native** tissue is of critical importance to the success of the new material in the body.

Characterization of these mechanical properties can be done using a variety of testing set-ups, including **static tensile** and **compressive testing**, **dynamic loading,** and **rheological** methods.

Bulk Properties – Thermal

Thermal Properties
Transition and melting temperature



(left): © The McGraw-Hill Companies/Bob C

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http://www.doitpoms.ac.uk/tlplib/polymers/stress-strain.php; http://www.pslc.ws/macrog/tg.htm

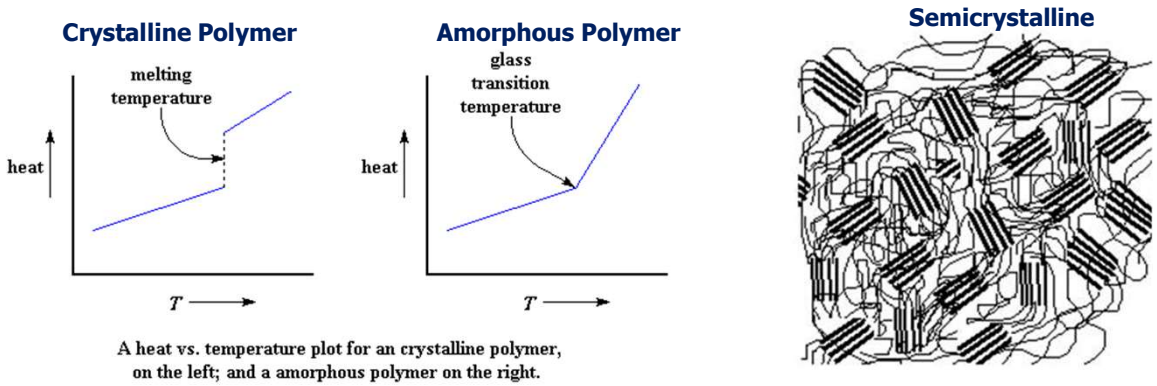
Other bulk properties include both thermal and electrical properties.

Temperature will also influence the stress strain curve – this has to do with thermal properties of the material that change the **bonding and internal** structure.

As the temperature **increase** the material can **melt** or go through a **glass transition**.

Bulk Properties – Melt and Glass Transition

Thermal Properties
Transition and melting temperature



A heat vs. temperature plot for an crystalline polymer, on the left; and a amorphous polymer on the right.

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http://www.pslc.ws/macrog/tg.htm

We think about this mostly during scale-up when the manufacturing process is mapped out. Often, heat is a critical part of manufacturing – like 3D-printing thermoplastics, injection molding, or steam sterilization.

If you start with a **liquid** polymer and let it **cool** to a **solid,** it will go through a **state transition**.

If it is a **crystalline** solid this will happen at the **melting** temperature shown here on this **graph of heat vs. temperature**.

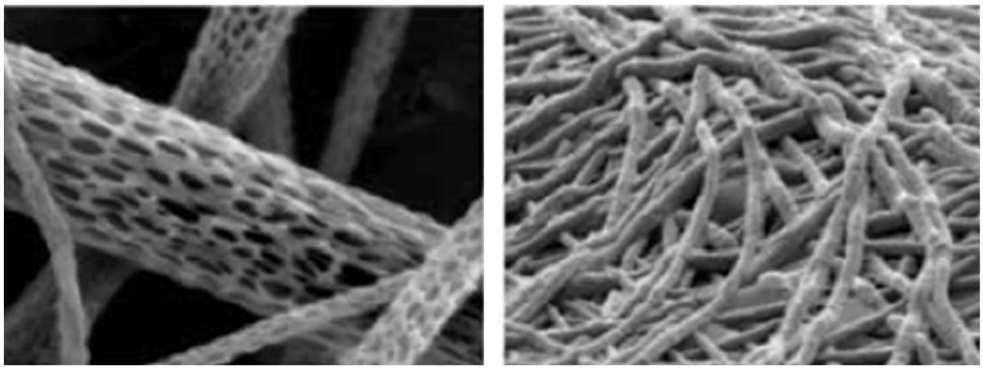
If it isn't crystalline – if it is an **amorphous** solid -- it will go through **a glass transition** temperature. Instead of a break in the curve as we see with crystalline solids, there is a continuous line.

For the **crystalline** solid as heat is removed the temperature stays **constant** during solidification. But with the amorphous solid, as heat is removed the temperature continues to change – **where the crystalline solid will melt all at once, the polymer chains will move and shift around over a long range**.

What this means is that the material behavior around the transition point is variable. Many polymer have both crystalline and amorphous regions, shown on the right, increasing the complexity of their mechanical and thermal properties.

Bulk Properties – Electrical

Electrical Properties
Conductivity
Piezoelectric
Charge storage



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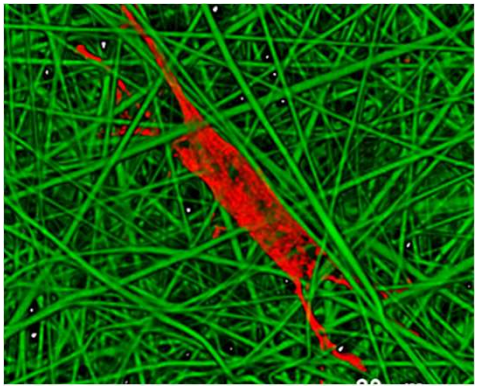
http://www.bioe.psu.edu/labs/Abidian-Lab/research.htm

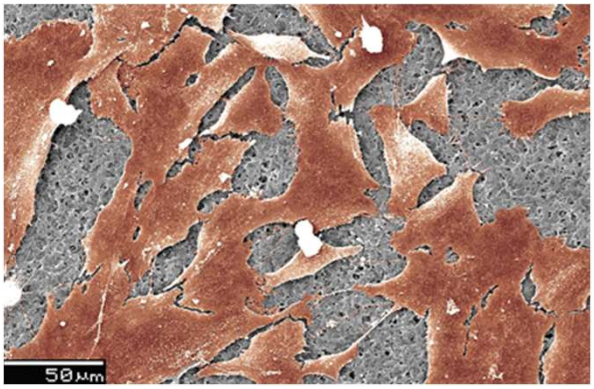
Electrical properties of the bulk have been researched for integration with **sensors** and **electrically active** tissue – including neurons (including the visual system) and the heart.

These images are **conductive** polymer nanotube designed as a **drug release** tool. These tubes can be loaded with drugs and upon **electrical stimulation will shrink** and release their payload.

Similar systems without drug loading have shown that conductive polymer nanotubes improve **adhesion**, **attachment** and **neurite** outgrowth compared to non-conductive fibers. We'll look more at neuron scaffolds in later modules.

Biological Properties – mimicking the native tissue





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Nist.gov; http://rsif.royalsocietypublishing.org/content/6/33.cover-expansion

Finally we will look at the biologic properties of a scaffold, specifically **biocompatibility**.

For a biomaterial scaffold to be biocompatible it must **mesh** with the native environment at **mechanical** and **chemical** levels. The host, specifically the immune system, reacts strongly to non-native non-self intrusions.

This means that **surface** and **bulk** properties will influence biocompatibility.

We'll discuss this in more detail later in this module. For now recognize that all of the factors we've seen – topography, surface free energy and modulus -- will impact the **immunogenicity** of a biomaterial.

Biological Properties – "fitness for purpose"

"Let's face it: you and this organization have never been a good fit."

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The newyorker.com

Before a biomaterial makes it to a human patient it must undergo both *in vitro* and *in vivo* testing for biocompatibility.

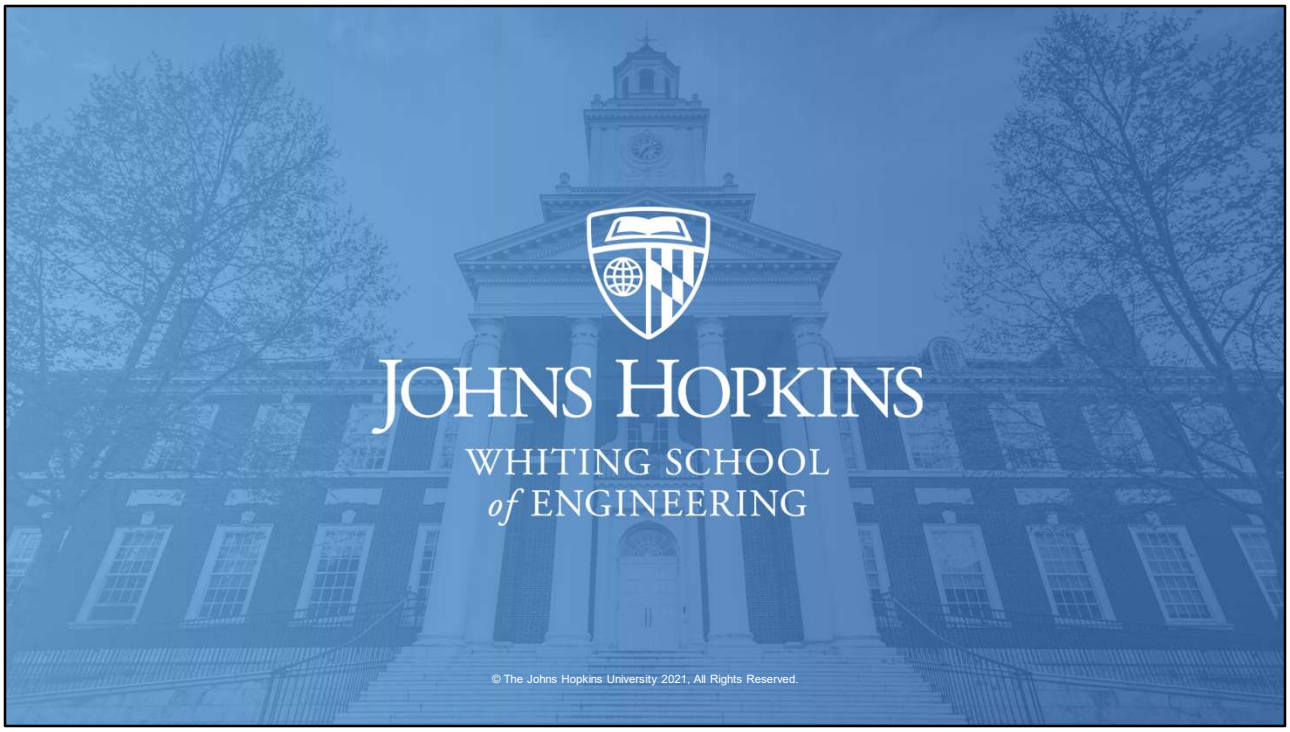
National and international standards follow that in vitro testing must show **fitness for purpose** – that is the a match between the material in **chemical**, **toxicological**, **electrical**, **mechanical** etc. properties.

These tests could either be **exposing cultured cells** directly to the material or **exposing conditioned media** from cells cultured in the presence of the biomaterial to new cells.

The output metrics include **cell viability, proliferation, genotoxic,** and **functional** assays. It is important to consider both **short** and **long** term effects, on the acute region and systemically in the body.

To consider systemic effects rodent models are used – placing the material under the skin, in a muscle, or in the desired anatomic location.

Here you are thinking about **sensitization**, **genotoxicity,** and **carcinogenicity**. These studies often test blood and urine samples, looking at the interface between the material and the body.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