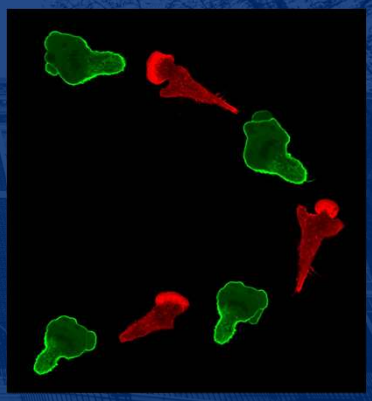
Cell and Tissue Engineering

Cell Adhesion and Migration
Part 1: Cell Adhesion

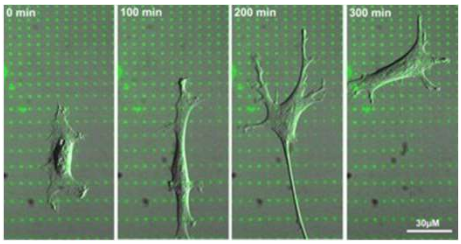


Martin Bergert,
Max Planck Institute for Molecular Cell Biology and Genetics

Welcome to Module 7. In this this lecture we are going to focus on cell adhesion.
There are diverse types of cell adhesion ranging from cell-cell interactions to cell –matrix interactions.
These adhesions are integral to the creation and the maintenance of tissues in the human body.
As cell and tissue engineers we must understand how these adhesions function if we wish to use or disrupt them for our designs.

Why is cell adhesion so important?

Haptotaxis



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www.zi1.uni-karlsruhe.de; www.mayo.edu

The growth and function of most cell types are adhesion-dependent.

The strength of adhesion determines things including migration speeds and cell-cell aggregation.

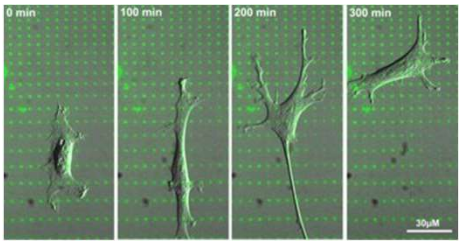
You may recall this image from module 5 where we discussed haptotaxis. In the body there are often gradients of ECM molecules.

Changes in ECM composition influence the adhesion and migration of cells leading to a host of pathological conditions including tumor metastasis.

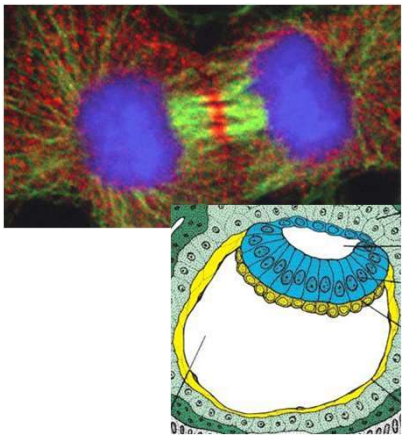
After we tackle cell adhesion in this lecture we'll transition to cell migration.

Why is cell adhesion so important?

Haptotaxis



Cell-cell adhesion



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Today we are going to cover cell-cell adhesion like you can see in this fluorescent image.

In this image you can see that this adhesion involves proteins beyond just the plasma membrane.

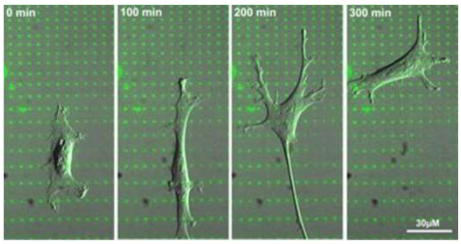
Here you can see how the cell-cell adhesion physically links the cytoskeleton of one cell to the cytoskeleton of the other cell.

These connections transmit mechanical signals between cells.

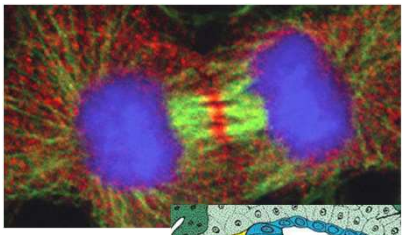
(cartoon image)
Cell-cell adhesion can define cell polarity as we saw in our morphogenesis module, and set up barriers between tissues.

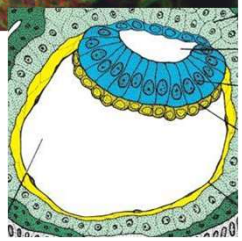
Why is cell adhesion so important?

Haptotaxis

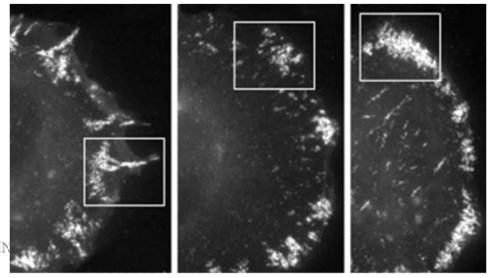


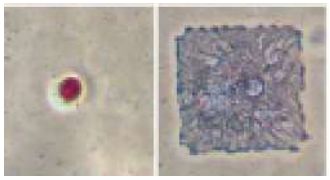
Cell-cell adhesion





Cell-matrix adhesion





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McBeath, et. al., 2004 www.zi1.uni-karlsruhe.de; www.mayo.edu; studyblue.com

The other type of cell-adhesions we'll discuss today are cell-matrix adhesions.

In this B&W fluorescent image you can see focal adhesion at the edge of the cell. These can also connect directly from the extracellular matrix to the cytoskeleton and are used in cell migration.

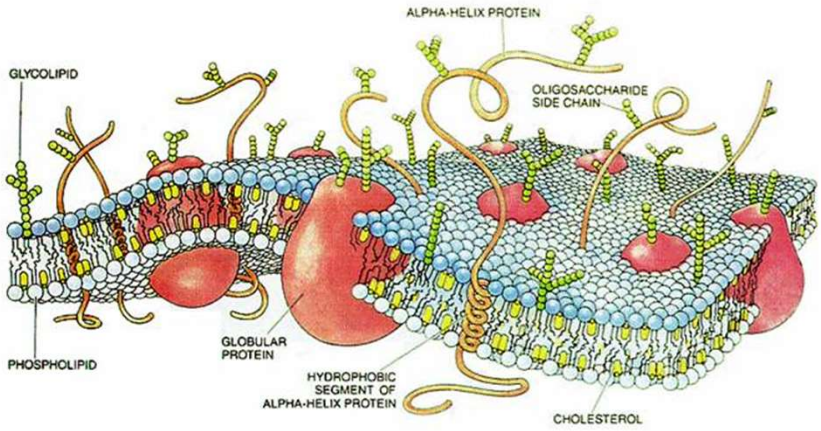
We saw previous that cell shape can regulate cell function and cell phenotype. Here a small shape giving rise to an adipose phenotype and a large shape giving rise to an osteogenic phenotype. This was done through arrangement and availability of cell-matrix adhesions.

Cell adhesion happens near the cell membrane

5% carbohydrate

50% protein

45% lipid (abundant anionic phospholipid phosphatidylserine give the membrane a net negative charge)



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Wikipedia.org

Before we get into cell-cell and cell-matrix adhesion let's take a minute to talk about where adhesion happens and the mechanics behind it.

Adhesion proteins are found in the cell membrane – that is the phospholipid bilayer that surrounds the cell. This bilayer membrane is coated in carbohydrate molecules and also contains membrane associated and membrane spanning proteins.

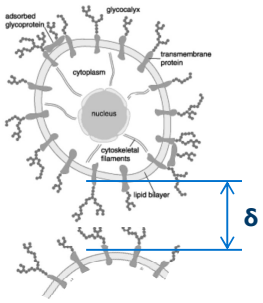
You can see a **spanning protein** running from one side of the membrane to the other (highlight alpha-helix protein).
You can also see **a membrane associated protein** sitting on just one side (highlight oligosaccharide side chain).

These phospholipids give the cell membrane a net negative charge.

Percentages By weight of the membrane composition are shown here.
You may have expected that the membrane was mostly lipid, but in fact it is so packed with proteins that these make up the largest fraction.
Some of these proteins are for binding other cell, the matrix or soluble molecules. This cartoon shows you examples of proteins that span the membrane connecting the extracellular space to the intracellular space and the proteins that sit in the membrane communicating with just one site.
Many of these proteins will work together creating signaling complexes at the cell surface. Each component of the plasma membrane will influence cell interactions with the environment.

In this lecture we are most interested in the membrane bound proteins that form junctions with other cells and the underlying ECM.

Mechanics of cell adhesion



Poly(L-lysine)

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Tissue Engineering, Saltzman; ammrf.org

As a cell's membrane approaches a surface – whether that be a matrix surface or the membrane of another cell -- there are both attractive and repulsive forces which depend on the separation distance delta shown here.

The balance of these forces will determine if the cell moves away or adheres and perhaps spreads, migrates and grows. If you were developing a scaffold for at tissue engineered product you would certainly want to know if cells would run from your construct or happily set up shop.

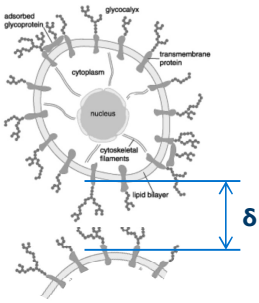
Recall that electrostatic, steric, and Van Der walls interactions do not require specific binding of surface molecules like the cell-adhesion that we're discussing today.

IN fact as two cells approach each other the electrostatic forces are always **repulsive** since the outer surface of the cell has that net negative charge.

A surface can have either a negative or positive charge depending on its composition.

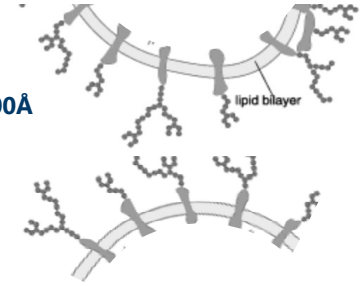
in this bottom image you can see cell is spreading on poly-L-lysine. Poly-l-lysine for example is a common substrate coating used in cell culture because of its positive charge which is conducive for cell attachment.

Mechanics of cell adhesion



δ → 100-300Å

Repulsive forces
Charge
Osmotic imbalance
Compression of surface molecules



Poly(L-lysine)

Attractive force
van der Waals (>200Å)
~1000dynes/cm^2

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Tissue Engineering, Saltzman; ammrf.org

As the two surfaces get close together, water gets pushed out of the this gap space causing an **osmotic imbalance**. There is a higher concentration of proteins in the gap between the cells compared to just adjacent. This pulls water into the gap generating a repulsive force which becomes greater as the gap between the cells decreases.

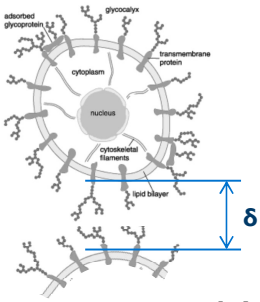
Note that Cell adhesion occurs at distances between 100-300 Angstroms.

Compression of cell surface molecules also acts as a repulsive force.

Van der Walls - interactions between polarizable but uncharged molecules are attractive forces and dominate the forces as distances greater than 200 angstrom. These are on the order of 1000dyne/cm2. This is above the shear force in a venule but below those felt in an arteriole

Mechanics of cell adhesion

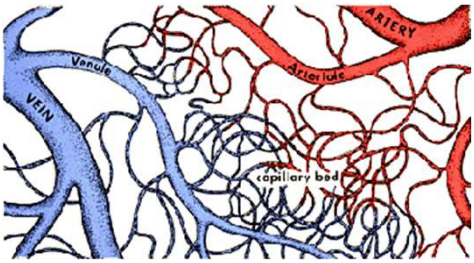
Attractive force
van der Waals (>200Å)
~1000dynes/cm^2

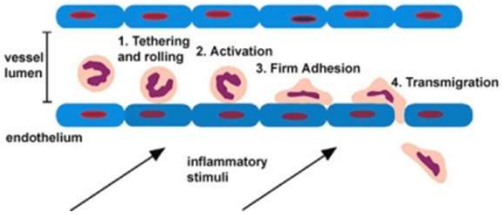


Poly(L-lysine)

Shear force in venule
~1-10dynes/cm^2

Shear force in arterioles
~10,000dynes/cm^2





Leukocyte adhesion cascade

Tissue Engineering, Saltzman; ammrf.org; start10g.ovh.net

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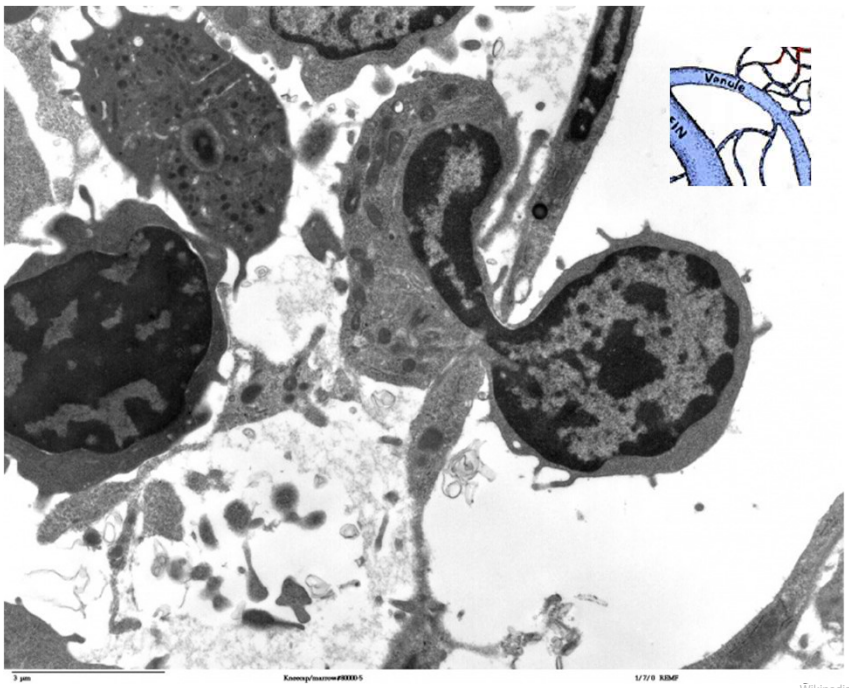
When you think about this force balance between attractive van der walls forces and shear force exerted from the blood flow you can see how this regulates the extravasation sites for immune cells.

For an immune cell to leave the blood stream and get to an injury site in the tissue it must first adhere to the wall of the blood vessel, then crawl through a gap between adjacent endothelial cells.

In order to adhere attraction forces must dominate and that happens in the post capillary venules.

Cell Extravasation



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Here you can see a white blood cell that has adhered to the wall of this venule and is moving out into the tissue.

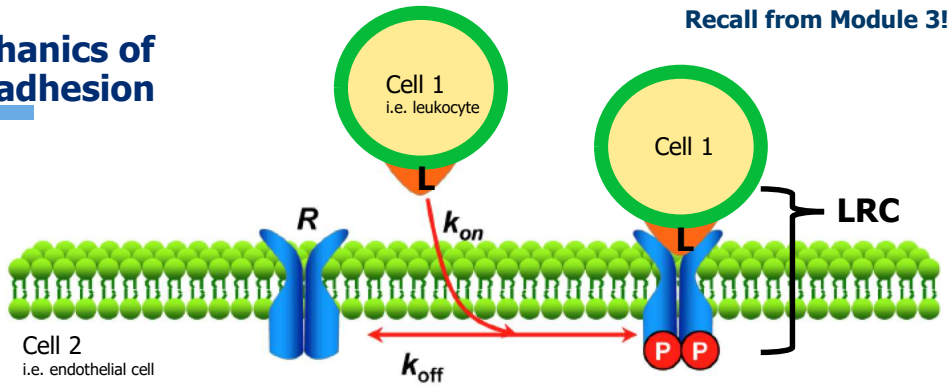
You can see the hour-glass shape of the cell as it is halfway across the endothelial barrier.

It is adhered and is now transmigrating from the venule, between two endothelial cells into the tissue space. You can see there are other inflammatory cells beyond the vessel wall that have already extravasated.

Perhaps this cells was called to clean up an infection or injury.

Mechanics of cell adhesion

Recall from Module 3!



$$L + R \underset{k_{off}}{\overset{k_{on}}{\Leftrightarrow}} LRC$$

$$\frac{[L][R]}{[LRC]} = \frac{k_{off}}{k_{on}} = K_d = \frac{1}{K_a}$$

k_{on} – forward rate constant
k_{off} – reverse rate constant
K_d – dissociation equilibrium constant
K_a – association equilibrium constant

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After the attraction of van der walls forces, cells (including white blood cells) use specific receptor ligand bonds to continue to promote adhesion.

IN module 3 we covered receptor ligand binding kinetics using the laws of mass action. Here you see that the reverse rate constant divided by the forward rate constant is equal to the dissociation equilibrium constant (Kd)

The constants tell us about the affinity of the receptor ligand pair.

Bond strength estimations

Tensile strength for affinity bonds

δ (Å)	K_d (M)	F (µdyn/molecule)
10	10^{-6}	6
1	10^{-12}	120

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adapted from Tissue Engineering, Saltzman; livescience.com

Low affinities are on the order of Kds of 10-6 molar and high affinities are on the order of 10-12 Molar.

Recent research has demonstrated that receptor ligand affinity is directly related to bond strength or tensile strengths.

Low affinity bonds having lower adhesion or tensile strength and higher bonds having higher strength.

Bond strength estimations

Tensile strength for affinity bonds

δ (Å)	K_d (M)	F (μdyn/molecule)
10	10^{-6}	6
1	10^{-12}	120

Assume
1) average bond of 40μdyne/molecule
2) Bonds spaced 1μm apart on the cell surface

4,000dyn/cm^2 produced from specific binding

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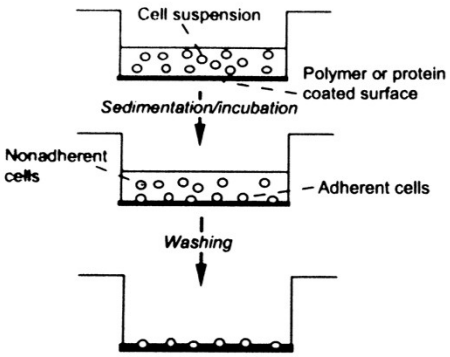
adapted from Tissue Engineering, Saltzman; livescience.com

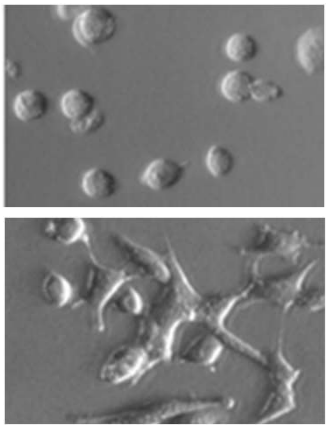
Mathematical models have been developed to take in values for bond length and binding constants – and estimate bond strength. These models predict that the highest affinity bonds have strength even higher, that is on the order of 250udyne per molecule with the average hovering around 40udyne per molecule

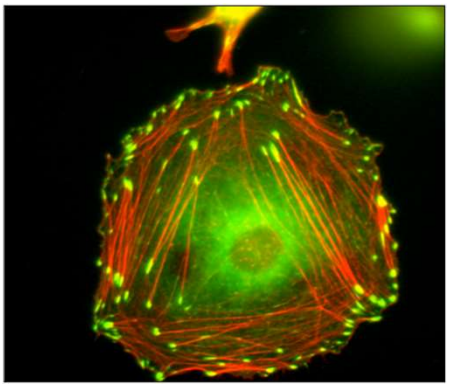
If we assume …. The average bond strength of 40udynes and an average spacing of adhesion molecules to be 1um apart this gives us about 4000dynes/cm2 which is in the ballpark of adhesion strength for non-specific binding interactions.

Methods for measuring cell adhesion

Sedimentation-detachment







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Tissue Engineering, Saltzman; Sithanandam, et. al., Nature Pediatrics.Wisconsin.edu

Because adhesion strength is important to many applications – tissue engineering included. Methods of have been developed to specifically measure the these strengths.

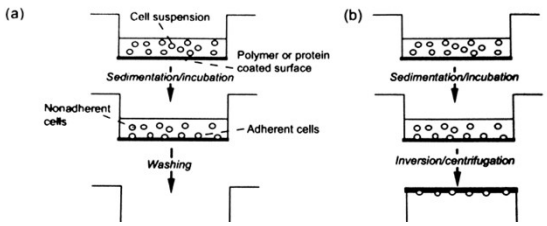
In the sedimentation method cells are left to settle in a culture setting. After a period of attachment cells are washed removing nonadherend and loosely adherent cells. In this microscopy image, you can see balled up, unattached cells on top and adhered cells spreading out on the substrate on the bottom.

The extent of adhesion is determined by the number of cells that remained adherent or the number of cells that were removed with washing. This may be coupled with microscopy measurements of cell spreading or counts of adhesion complex staining.

What's nice about this method is that its easy to preform in most labs – requires limited equipment and expertise. However it is difficult to control the fluid force used to wash the cells and therefore its hard to compare results from one lab to the next.

Methods for measuring cell adhesion

Sedimentation-detachment **Centrifugation**



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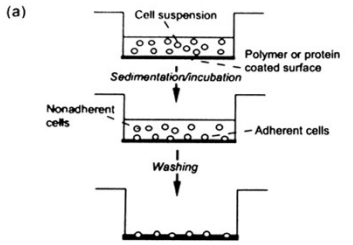
Tissue Engineering, Saltzman; Sithanandam, et. al., Nature; Cole Parmer, Eppendorf

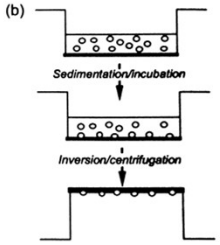
One way to use constant detachment force is through the use of a centrifuge. Instead of washing the cells, the tissue culture plates are put in a centrifuge that can be set to desired speeds. After the centrifugation force is applied the dish is simply inverted to remove cells that are not adhered. Analysis is the same as in the sendimentation method. Or alternatively done using radio-labeled cells for a quick an accurate cell count.

Methods for measuring cell adhesion

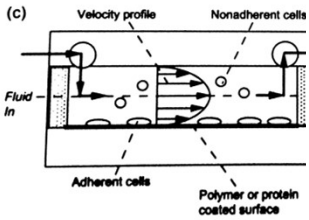
Sedimentation-detachment

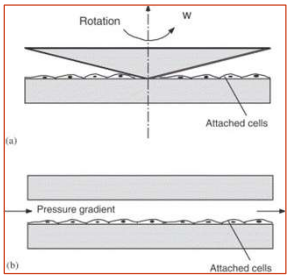


Centrifugation



Fluid flow chambers





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Tissue Engineering, Saltzman; Ohashi and Sato, 2005

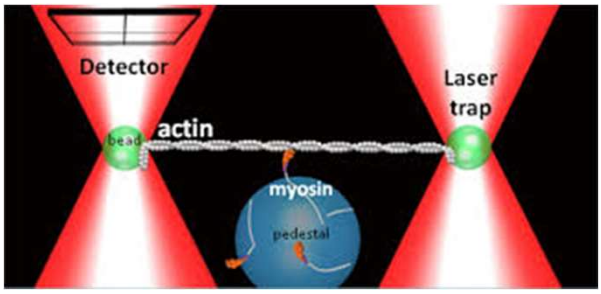
Fluid mechanical force can also be used in a controlled fashion via a flow chamber. The simplest are flow between two parallel plates however this results in a non-constant shear stress down the length of the chamber.

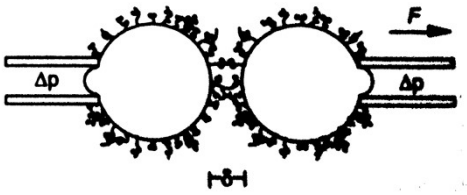
On the right I'm showing you a plate and cone device where the cone rotates, produce constant shear stress across the entire monolayer of cells.

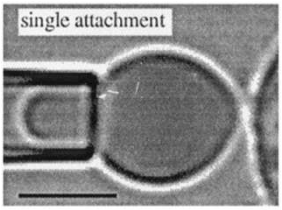
These devices can be used not only to measure adhesion strength of attached cells but also the kinetics of cell attachment and cell rolling.

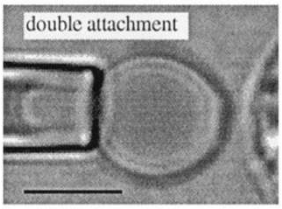
Methods for measuring cell adhesion

Micropipette or Laser Trap









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Tissue Engineering, Saltzman; Chesla, et. al., 1998; Debold 2012

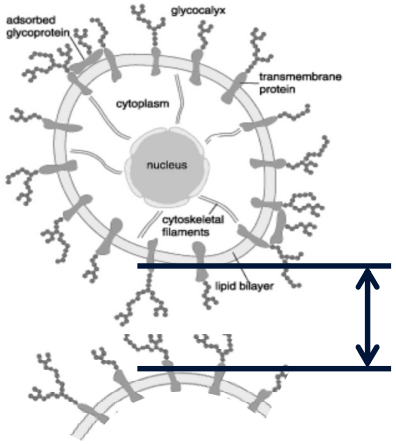
Another tool to measure bond strength involves the use of micropipettes or laser traps to hold cells (or beads coated with adhesion proteins).

Once an adhesion is made, movement of the calibrated pipets -- as you can see in the single and double attachment images -- will give a quantitative read out on bond strength.

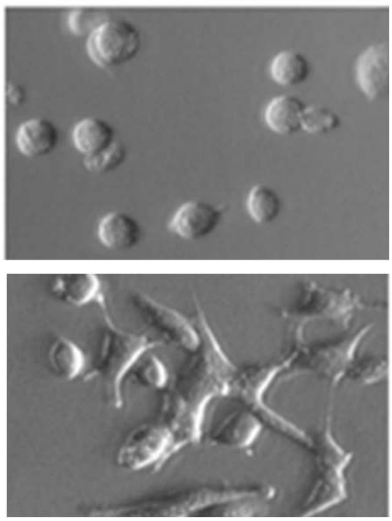
For example in this cartoon of the laser trap we are measuring the force generation of a myosin motor pulling on this actin filament between these two beads in opposing laser traps.

Rewind and Review

Forming a bond



Measuring adhesion and bond strength



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Lets take a second to review what we've covered in this lecture.

We began by discussing how non-specific forces bring cells close to other cells and surfaces – and how the balance between attractive and repulsive forces regulates this.

Next we looked at methods for measuring adhesions and bond strength.


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