Lecture #3, 1/18/02

Implant

Surface Properties

Protein Properties

Organization of Adsorbed Protein Layer (< 1 second)

Cellular response to the adsorbed surface

Cells adhere, release active compounds, Recruit other cells and grow

Organize into tissue

Cell Adhesion: Good or Bad

Example: Teflon vascular prosthesis
Inside: blood compatible
Outside: firmly attach to surrounding fibrous tissue

Protein Adsorption – Not new!!!

History dates back to early 1800's
"interfacial coagulation"

1920 – 40's: Langmuir studied behavior of proteins at air-water interfaces

Recent interests:

Proteins at solid-liquid interfaces

"Protein Engineering"

Proteins:

Soluble: Present in biological fluids

Insoluble: e.g. collagen, fibronectin
Deposited in fibrous form by cells during foreign body capsule formation

Structure of Proteins: Important in Biomaterials

Types and proportions of amino acids determine properties (charge, hydrophobicity, surface tension)

Effect solubility and adsorption properties

Sequence of amino acids: lead to distinct 3-D structures.

Spatial arrangement: hydrophobic residues inside and ionized and polar residues outside.

Tightly folded structure: Charged wax droplets in water.

Protein adsorption is TRUE adsorption!!!

Compare surface phase conc. to bulk phase conc.

Mixture of Proteins:

Competition occurs

Surface Activity

Two main driving forces:

1) **Relative bulk conc of each protein**
2) **Surface activity**

Example: Adsorption of Proteins in Blood Plasma to Polyethlene

	Enrichment
Fibrinogen	**1.3**
Globulin	**.33**
Albumin	**.88**
Hemoglobin	**79**

What happens when a protein adsorbs on a surface?

Adsorption of protein is usually irreversible

Because of strong immobilization:

Processes involving biomaterials may be altered greatly at surface (e.g. transport)

Cell are attracted to surface
(Cells will attach to protein on surface but may not attach to dissolved protein).

Ex: Platelets and fibrinogen

Orientation of proteins in important:

Because of amino acid sequence, areas that promote cell binding may be hidden by surface or may be sticking out.

Typical Protein Adsorption Isotherm:

So, protein adsorption can effect cellular

responses to surfaces:

How does this happen? Ex: Platelets in blood

Platelets carry certain receptors that bind to certain proteins in the blood.

Only a few types of proteins will bind to these receptors.

Soluble proteins will not bind to these receptors.

But once proteins are bound to surface, platelets will bind to these proteins.

Platelets will not bind to same proteins that are on other surfaces.

So……..

Properties of Surfaces and Properties of Proteins

Determine organization of adsorbed protein layer

Determines cellular response to surfaces.

REVIEW OR INTRO TO CELLS

Cell Theory???

All living things are made up of cells and their products.

Multicellular organisms: made up of numerous cells

Unicellular organisms: one-celled

A Little History:

1930's & 40's:

- **Observed cells with microscope**
- **All cells were alike**
- **Cells were different sizes**
- **Used stains to reveal DNA - All cells had DNA**

1950's:

- **Found two distinct types of cells**
  Prokaryotic - No defined nucleus
  Eukaryotic - Defined nucleus

Later:

- **Discovered that these cells differed in many ways.**

Eukaryotic Cells Include:

All plant and animal cells (multicellular)
Unicellular:
Algae
Ameba
Fungi (molds and yeasts)
Protozoa

Prokaryotic Cells Include:
All bacteria
Blue-green Algae

Properties of Prokaryotic Cells:

- **Simple and small: very few organelles (0.5 - 3 µm dia)**
- **Shape can be spherical, rodlike, spiral**
- **No defined nucleus - Naked DNA**
- **Contain a cell wall**
- **Contain a plasma membrane**

  Gram - bact.: have additional outer membr.

- **Exist alone as single cells: Do not work together to control environment. Versatile: Can adapt to environment.**
- **Grow rapidly: double in size, mass & # in 20 min.**

  Rapid growth and versatility make them desirable for research.

- **Organelles include Ribosomes: Organelles on which proteins are structured.**

<u>Properties of Eukaryotes</u>

- **Complicated and larger**
  Many organelles (well organized)
  Usually 10 times larger than Pro.

- **Nucleus surround by membrane: DNA**

- **No cell wall (Except plant cells)**

- **Most coexist and interact in a cooperative manner to control environment**

- **Contain plasma membrane + membranes around organelles**

- **Organelles:**
  Endoplasmic reticulum, Nucleus, Ribosomes, Mitrochondria/Chloroplasts, Golgi Complex Lysosomes, Vacuoles

- **Contain organized cytoskeleton which consists of fibrous proteins in cytoplasm**
  Microfilaments
  Microtubules
  Intermediate Filaments

  Gives strength and rigidity and controls movement.
- **Doubling time can be hours**

- **Exception: RBC's**

  No organelles
  No nucleus
  No structured cytoskeleton - membr. contains structural proteins.

Approximate Chemical Composition of Typical Cell

	Bacterial (% Tot. Wt.)	Mammalian (% Tot. Wt.)
H2O	70	70
Protein	15	18
DNA, RNA	7	1
Phospholipids	2.5	3
Other lipids	---	2
Polysacc.	1.5	2
Others (inorg, hybrid)	4	4

Bacteria

Two classes:

Gram +

Ability to retain a crystal violet iodine stain when treated with organic solvents

Gram -

Overhead of Cells:

Both have a cytoplasmic membr. (phospholipids and proteins)

Surrounds the interior of the cells and serves as a barrier between cell interior and exterior.

Everything outside of cytoplasmic membr. is the cell wall - Provides structure and shape: Differs greatly between Gram + and Gram -

Cell Wall

Gram +

Thick layer of murein - Peptidoglycan

Keeps cell from bursting in hypotonic environment.

Gram -

Very thin murein

Outer membrane

Contains LPS in place of phospholipids

LPS contains hydrophilic chains on the surface and excludes hydrophobic compds.

Lipid nature of outer membr. excludes hydrophilic compds

Channels in membr are used for transport

Space between inner and outer membr. -

Periplasm

20 - 40% of cell vol. Contains murein layer and a gel like solution of proteins that facilitates nutrition and inactivates toxic chemicals

Outside the Membrane or Murein Level: Polysaccharide containing structures- Glycocalyx

- **Capsule**

  High MW polysacc.

  Provides a protective barrier

  Viscous and permits cells to attach to surfaces.

  Synthesis of capsules is not necessary

    Seen on cells in natural envir. but not in laboratories.

      Why?

Vary in thickness and can be firmly or loosely attached.

S-Layer:

Glycoprotein subunits arranged in a crystalline array.

Role is not completely known

Protection???

Adhesion????

Cell Surface Appendages

Flagella - organs of locomotion

helical filaments of protein that rotate and propel cell thru fluid

 # can differ: some have 1, some have hundreds.

Play a role in adhesion

Pili - Protein structures

Straight rods: Do not rotate

Play a role in attachment

Sex pili: play a role in attachment of mating pairs.

Specific adhesion: Receptors on tips of pili (Adhesins) bind to specific receptors of other cell surfaces.

E. coli has 100 to 300 pili

Fibrils : Glycoproteins

Shorter than pili

Play a role in adhesion

Inside Bacterial Cell

Very simple: Consists of ribosomes and free DNA

3 parts:

Cytosol region: packed with ribosomes
Nuclear region: DNA
Cytoplasm: Fluid occupying remainder of cell

Other Important Things About Bacteria:

- Most do not utilize light energy
- Reproduce by binary fission (division into 2 daughter cells)
- Some bacteria are aerobic - need oxygen
  Some are anaerobic- don't need oxygen
  Some are both
- Some can form endospores
  - Dormant forms of cells: can resist heat, radiation, chemicals
  - When killing bacteria usually require T>120°C

EUKARYOTIC CELLS - MAMMALIAN CELLS

More complicated!!! (Especially inside)

Organelles

- **Nucleus:**

  Large and spherical. Carries DNA and RNA

  Contains a double membrane - Nuclear envelope

  Contains nucleolus - organelle that is cite of ribosome construction.

- **Vacuole:**

  A space in the cytoplasm of plant cells filled with H20 and solutes surrounded by a single membrane - plant cells have one vacuole

  Called vesicles in animal cells - smaller and thousands. Play a role in transport of nutrients.

- **Ribosomes:**

  Most numerous

  Site of protein synthesis – (RNA is translated into protein). - the more protein the cell is making the more ribosomes it will have (hundreds or thousands)..

  Two types:

  Free in cytoplasm: make proteins used by cell

  Attached to endoplasmic reticulum: make proteins for export (digestive enzymes)

- **Endoplasmic Reticulum;**

  Rough: has ribosomes attached
  Smooth: does not have ribosomes attached - site of synthesis of fatty acids and phospholipids

- **Golgi Vesicles (Golgi Body)**

sac like structures; membrane bound, stacked loosely on top of each other.

Secretory proteins synthesized in the rough ER move to golgi vesicles

Sort these proteins and direct them to their proper destinations "Traffic Police" - Do this thru enzymes (Add signals or tags).

How proteins move from ER to Golgi vesicles is controversial but they think vesicles play a role.

Evidence also supports the transport of proteins from Golgi vesicles to outside by "secretory vesicles" which bud off Golgi vesicles and fuse with membr. of cell releasing contents.

Sometimes they fuse right away. Other times they store protein until cell receives signal to release

- **Lysosomes:**

Contain enzymes that breakdown proteins, polysacc. and lipids

Examples
protease: digests protein and converts to amino acids.

ribonucleases and doxyribonucleases degrade RNA & DNA into mononucleotide building blocks

phosphatases: remove phosphate groups from nucleotide and phospholipids

Also enzymes that degr. polysacc. and lipids into smaller units.

All enzymes work at low pH (~4.8) and are inactive at neutral pH found in cells and extracellular fluid.

Lysosomes are maintained at low pH by a hydrogen-ion pump in the membr.

If enzymes are released, no degradation will occur

In addition, cells contain proteins that bind and deactivate some degradative enzymes

Lysosomes degrade many membranes and organelles that have outlived usefulness

How aged or defective organelles are marked for degradation and transported to lysosomes is unknown

<u>Macrophages</u>: ingest harmful bacteria

Lysosomes contain lysozyme: degrades peptidoglycan in cell walls

Endocytosis occurs (plasma membr. invaginated) to form a closed vesicle with harmful bacteria. This vesicle fuses with lysosomes and bacteria are killed

Diagram of Endocytosis:

- **Mitrochondria:**

  Largest organelle. Breaks down energy yielding organic molecules (i.e. ATP)

  Has own DNA and ribosomes: can make itself

THE CYTOSKELETON

Mammalian cells do not have a cell wall to maintain structure.

Cytoskeleton: adds structure to the cell

Invisible when looking through light microscope

Usually not shown in pictures of the cell

However, its important!!!

- **Adds structure (keeps the cell's shape)**
- **Anchors organells in place**
- **Moves parts of the cell when necessary**

Protein filaments that make up the cytoskeleton:

Microfilaments:

Thin: Approx 6 nm in diameter

Made up of protein called actin

Actin can assemble into a filament network and disassemble by polymerization and depolymerization: Cell can change shape and deform.

Microtubules:

Larger: Diameter approx 24 nm

Made up of protein, tubulin

Also major component of cilia and flagella

Used by cells to hold shape

Intermediate Filaments:

Diameter: 7 – 11 nm
Less is known about these

Red Blood Cells

Have a phospholipid membrane

No structured cytoskeleton

Peripheral and integral proteins: Play a role in transport of nutrients and structural integrity of the cell.

CELL MEMBRANES

Why does a cell need a membrane?

It must keeps its molecules of life (e.g. DNA, RNA, proteins) from dissipating away

It must keep out foreign molecules that damage or destroy the cells components.

What else is a membrane good for?

Cell must communicate with environment.

Ex: Bacteria detects high conc of lactose: must synthesize proteins to take in and metabolize lactose.

How does it know to do this???

Membrane proteins gather information about the environment (receptors)

Has to pump in nutrients and release toxic products of metabolism.

How does the cell do this???

Membrane proteins (Transporters)

Two types of membrane proteins:

Intrinsic: embedded in lipid bilayer and extend through it.

Extrinsic: on the surface of the lipid bilayer.

Already talked about the lipid bilayer structure:

Hydrophobic interior

5 nm thick

Semipermeable

Impermeable to large molecules

Impermeable to charged ions

Permeable to lipid soluble low MW Molecules

Permeable to water (not well understood)

MEMBRANE PROTEINS

Transmembrane Proteins

Cells pump ions in and out through their membranes. How do they do this?

With Transmembrane Proteins

Example:

Sodium-Potassium Pump

Inside cell: high conc K+, low Na+
Outside cell: high Na+, low K+

Maintained by active transport:
Na+ is pumped outside
K+ is pumped inside

Driven by ATP

Transmembrane proteins also transport polar molecules.

Example: Glucose in RBC's (Facilitated Diffusion)

Receptors:

Acquire information from the outside and relay this information into the cell through the plasma membrane (involves extrinsic and intrinsic proteins)

Example:

Growth factor receptors:

Should the cell grow?
Triggered by growth factors in the medium

GFs bind to the cell surface GF receptors.

Signal transduction occurs: information is sent through the cell via various proteins and enzymes to tell the cell to grow.

Receptors also play a role in adhesion.