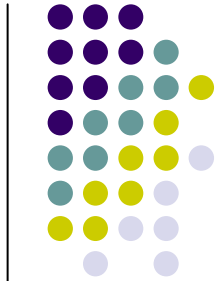


Biological Testing of Biomaterials

Question

What do we mean by the term “*in vitro*?”

Question

Will an *in vitro* test measure parameters that are relevant predictors of what will occur in the body (*in vivo*)?

Question

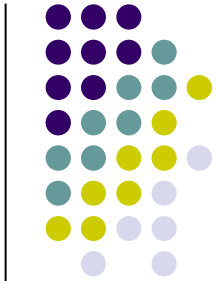
What is meant by the term “animal model”?

What is meant by the term “experimental variability”?

What is meant by the term “cytotoxicity”?

How would you design a test to study the cytotoxic potential of a biomaterial?

Background concepts



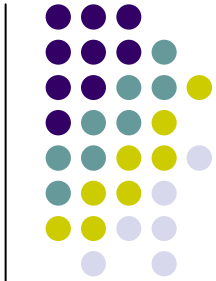
- Toxicity-kills cells
- Potency
- Exposure vs delivered dose
- Target cells

Good tests evaluate target cell toxicity using delivered doses of the test substance in the appropriate media or environment.

Solubility Characteristics

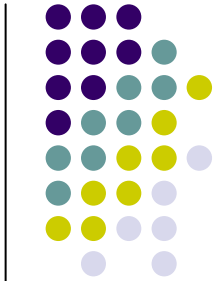
- Biomaterials are water insoluble (1 in 10,000 parts water).
- We are testing other components.

What are they? How do they get there?



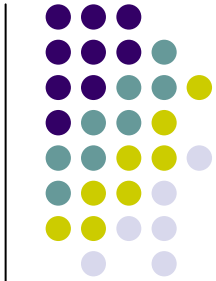
- Plasticizers
- Slip agents
- Anti-oxidants
- Fillers
- Mold release agents
- Trace additives

Historical Perspective



- Cell culture
- Primary cells vs cell lines
- Test standards

Assay Methods

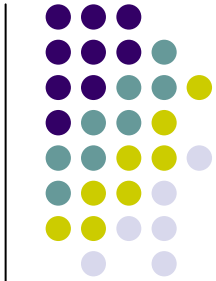


- Direct contact
- Agar diffusion
- Elution

Standard

- Type of cell (L929)
- Number of cells
- Growth phase of the cells
- Passage number
- Dose
- Duration of exposure
- Time of analysis (after exposure or later)
- Test sample size and shape
- Mechanical forces
- Measurement endpoints
- Controls (negative and positive)

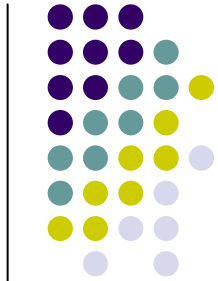
Direct contact assay



- Near confluent monolayer L-929 fibroblasts in culture media 0.8 ml media;
- Material is placed on top of monolayer in center of a 35mm culture;
- Place in incubator 24 hours them fix and stain with H&E
- Examine cells for morphological changes

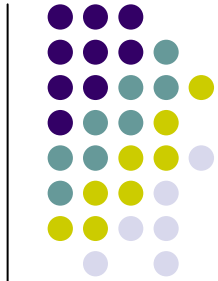
How do you measure toxicity?

Agar Diffusion Test



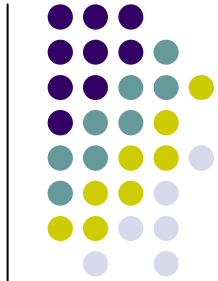
- Over lay monolayer of L-929 fibroblasts with thin layer of agar containing a vital dye (neutral red);
- Place material on top and incubate for 24 hours;
- Fix and analyze.

Elution test



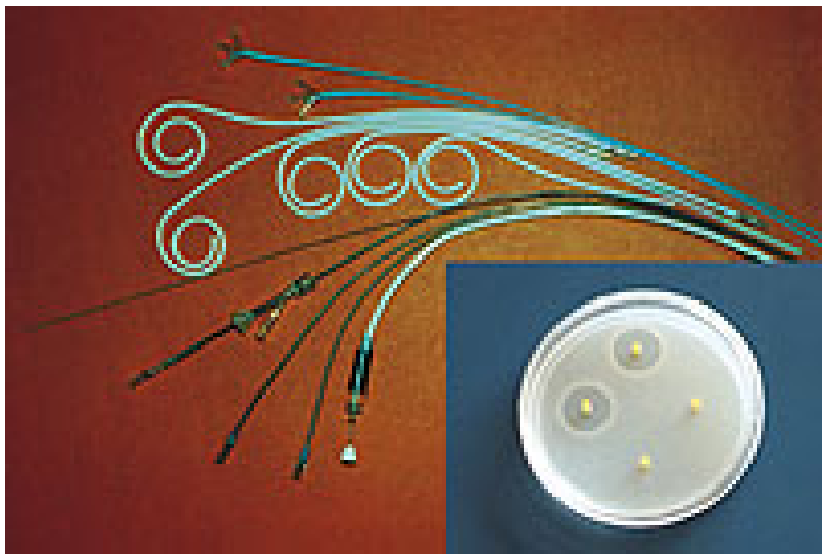
- Incubate material in growth media at body temperature,
- Remove aliquot after some time;
- Add to confluent L929 culture
- Fix and analyze after 24-48 hr;

Biomaterial and Device Testing *(In vivo)*



- Sensitization
- Irritation
- Intracutaneoius reactivity
- Toxicity
- Genotoxicity
- Implantation
- Hemocompatibility
- Chronic toxicity
- Carcinogenicity
- Reproductive and developmental toxicity
- Biodegradation
- Immune response

Surface Coatings



Uses of Surface Coatings

- Anticoagulant
- Increase lubricity
- Antimicrobial
- Enhance imaging
- Adhesion resistance
- Encapsulation-electronic components
- Tissue adhesion-orthopedic fixation

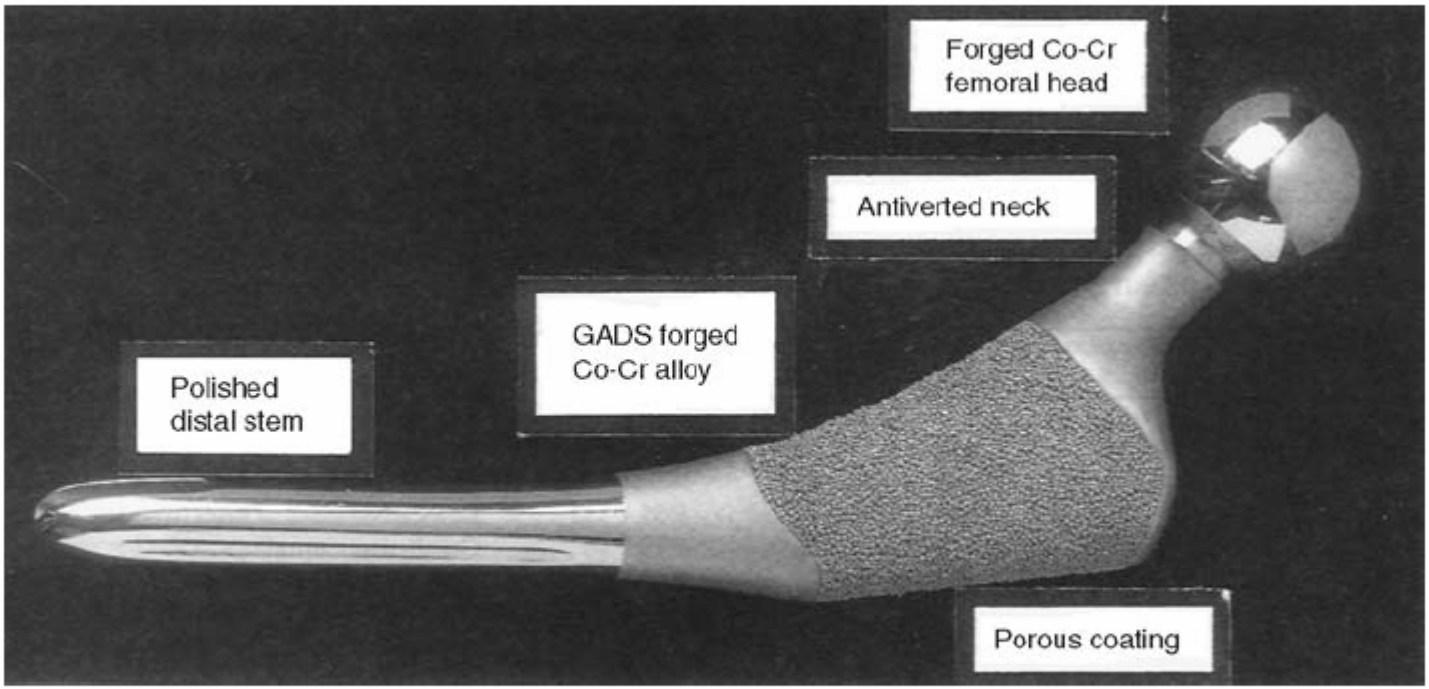


Fig. 2 Porous coated cobalt alloy total hip replacement implant. GADS, gas-atomized dispersion-strengthened alloy

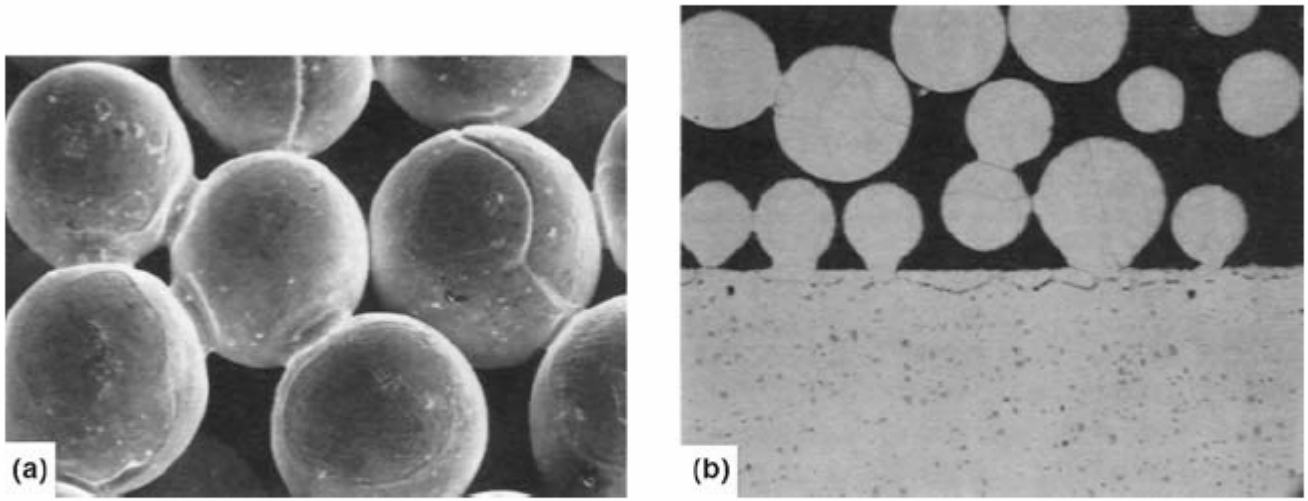
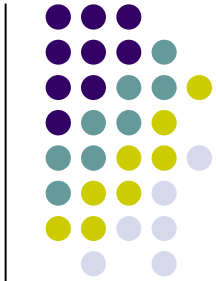


Fig. 1 Porous Co-Cr-Mo coating produced by sintering. (a) Scanning electron micrograph of gas-atomized spheres (beads). (b) Metallographic cross section. Note the necking between the beads. Bead-to-bead bonding is also evident in the cross-sectional view.

Surface coatings

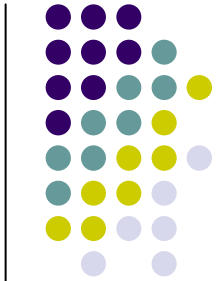
- Angstrom - nm level changes in surface chemistry
- Polymeric coatings
- Change surface chemistry without changing bulk properties (economics);
- Alter binding properties for proteins and other molecules (improves device performance);
- requires less capital investment and less dramatic changes in manufacturing practices (advantage);
- Objective: create a minimally reactive surface that is, one that is invisible to the system or one that is specifically activated to control cell behavior at the interface.

Nonfouling Surfaces



- Polymer coatins
- Protein resistant surfaces
- Stealth surfaces

General strategies to Prevent Device-related Infections



- Minimize contact- Clean Room Conditions
- Kill every thing in contact-Sterilization
- Minimize binding at contact-Nonfouling Surface Coating
- Kill after contact-Anti-infective coatings

Infectious Agents binds to Implant Surfaces

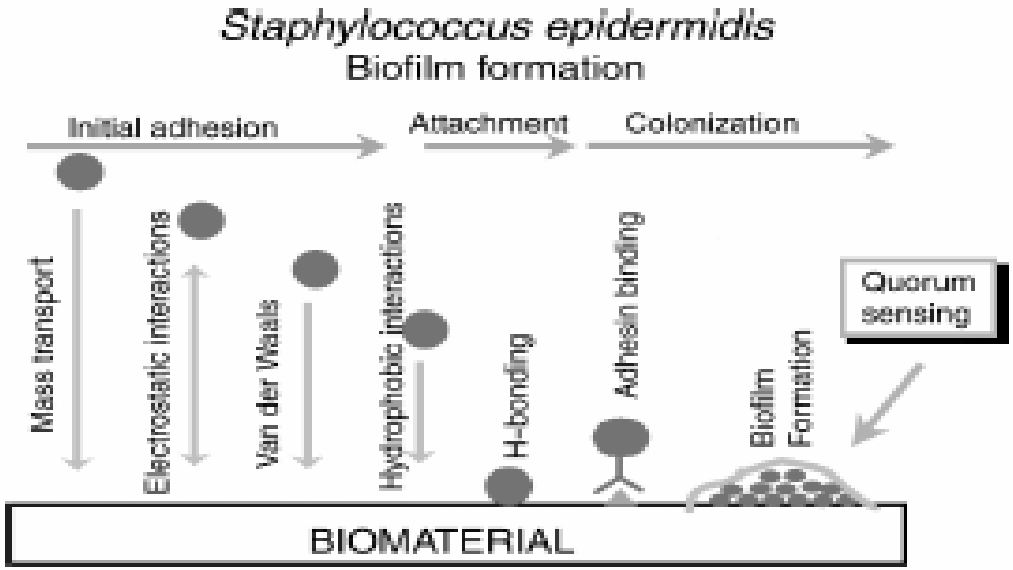


Figure 1 Factors involved in the colonization of a plastic biomaterial by *S. epidermidis*.

Infectious Agents binds to Implant Surfaces

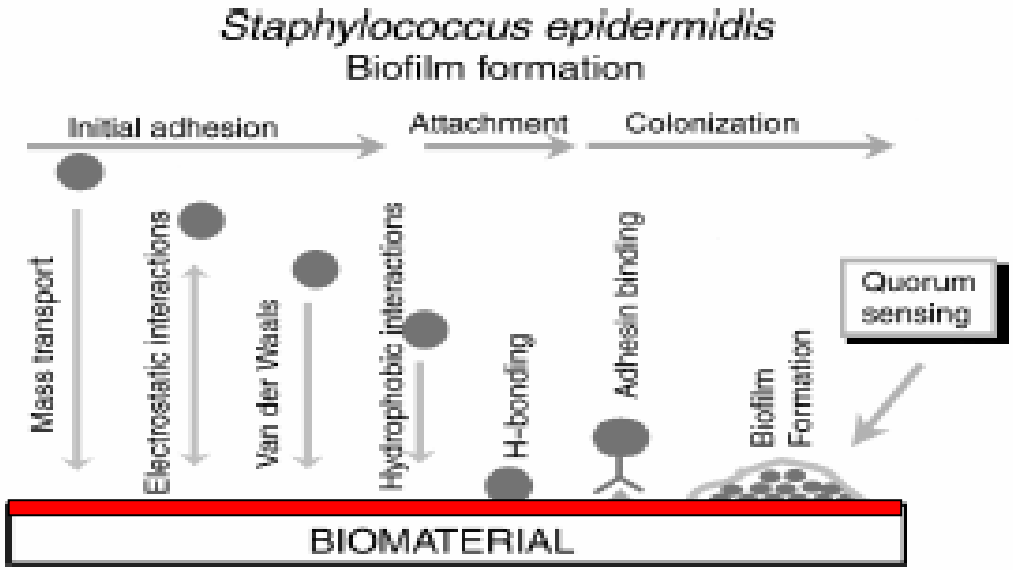
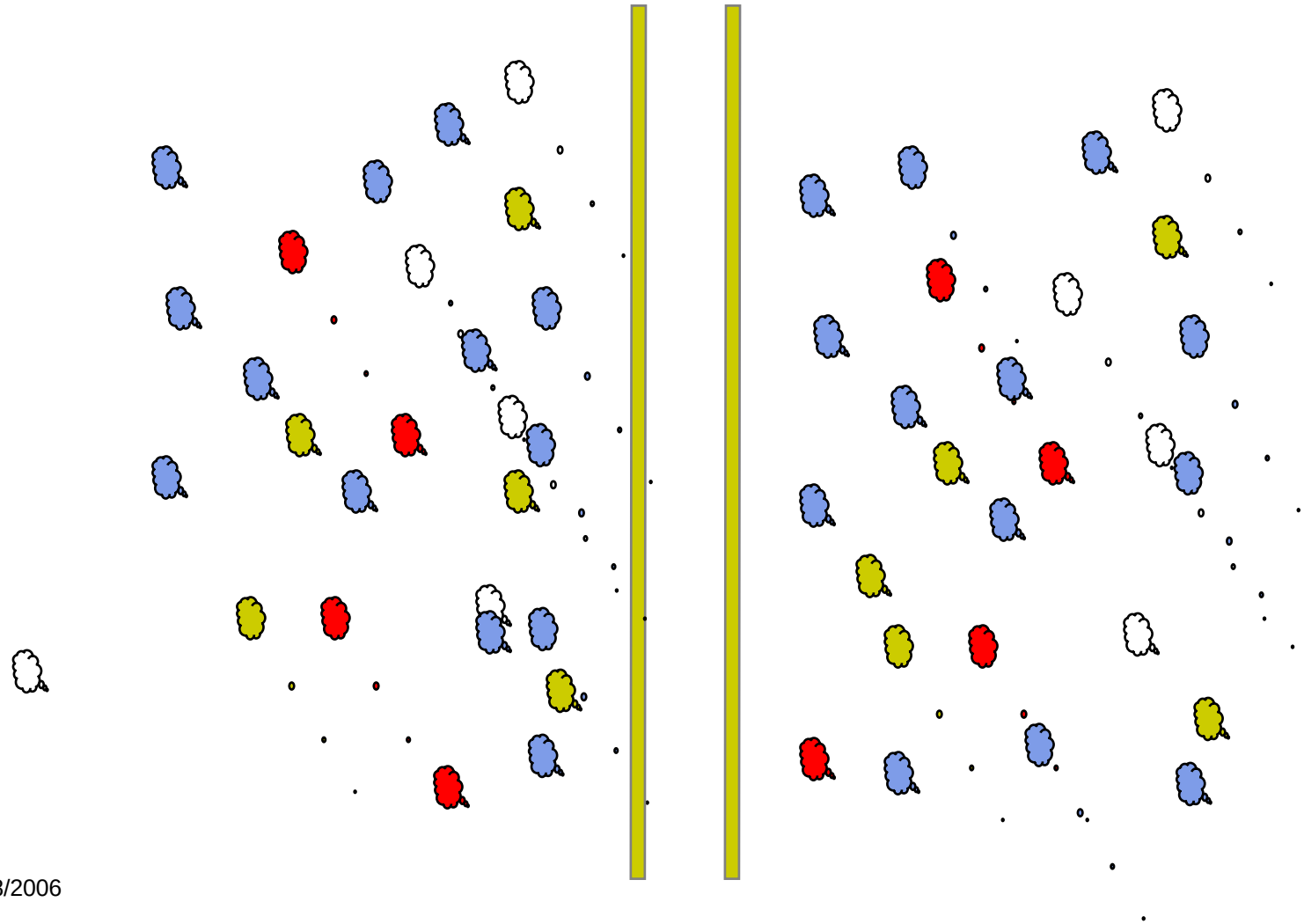
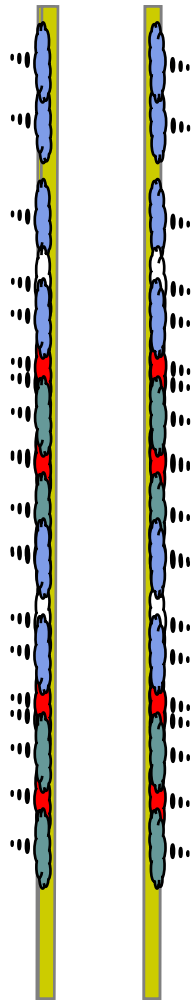


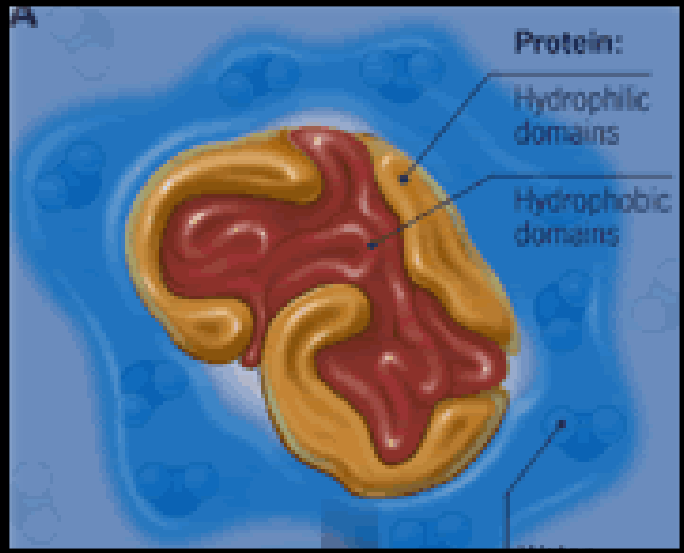
Figure 1 Factors involved in the colonization of a plastic biomaterial by *S. epidermidis*.

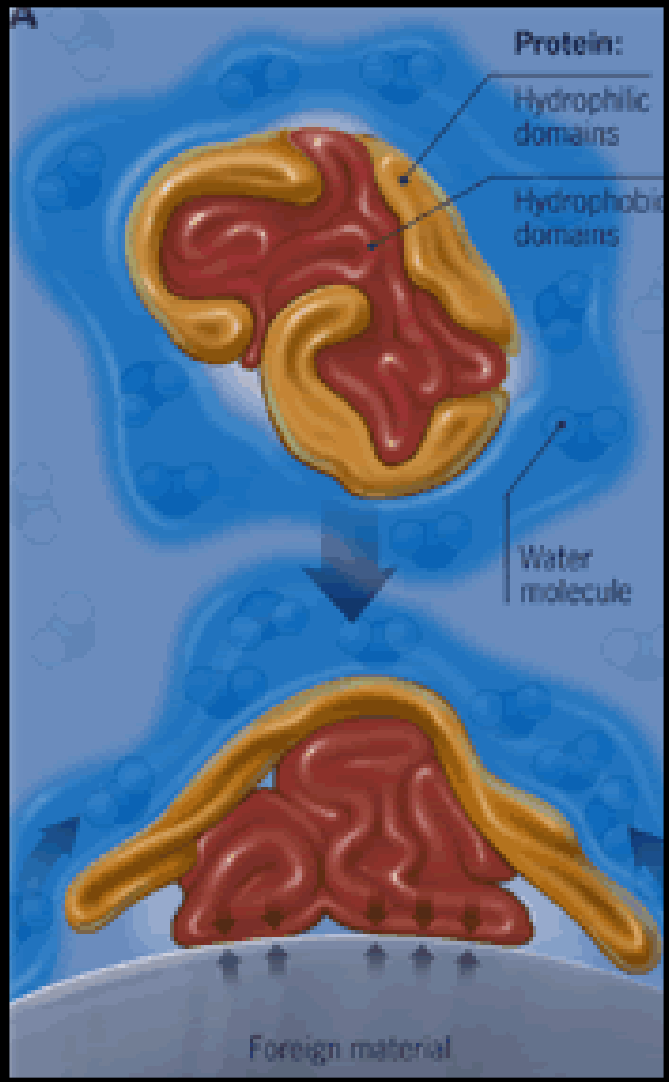
Protein Adsorption *in vivo*

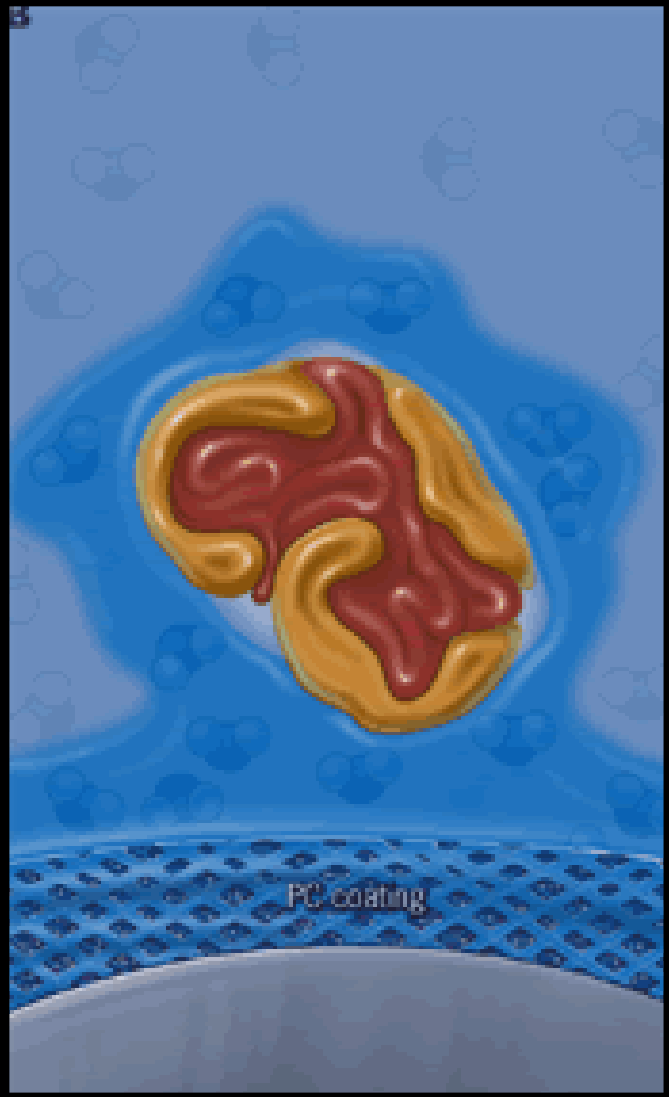


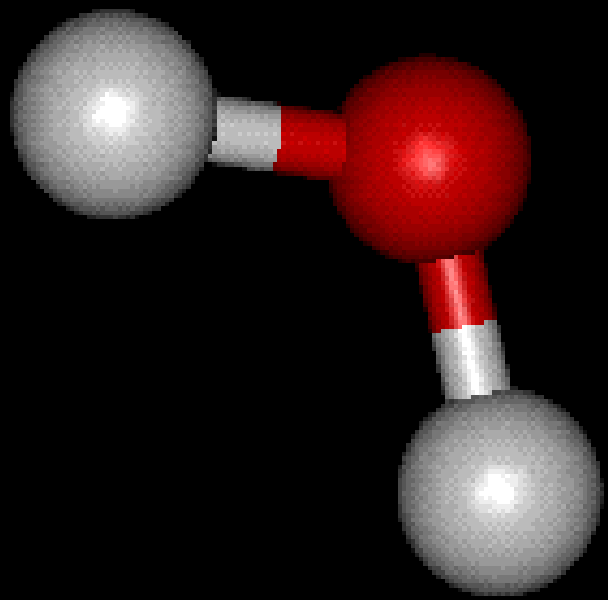
Protein Adsorption *in vivo*

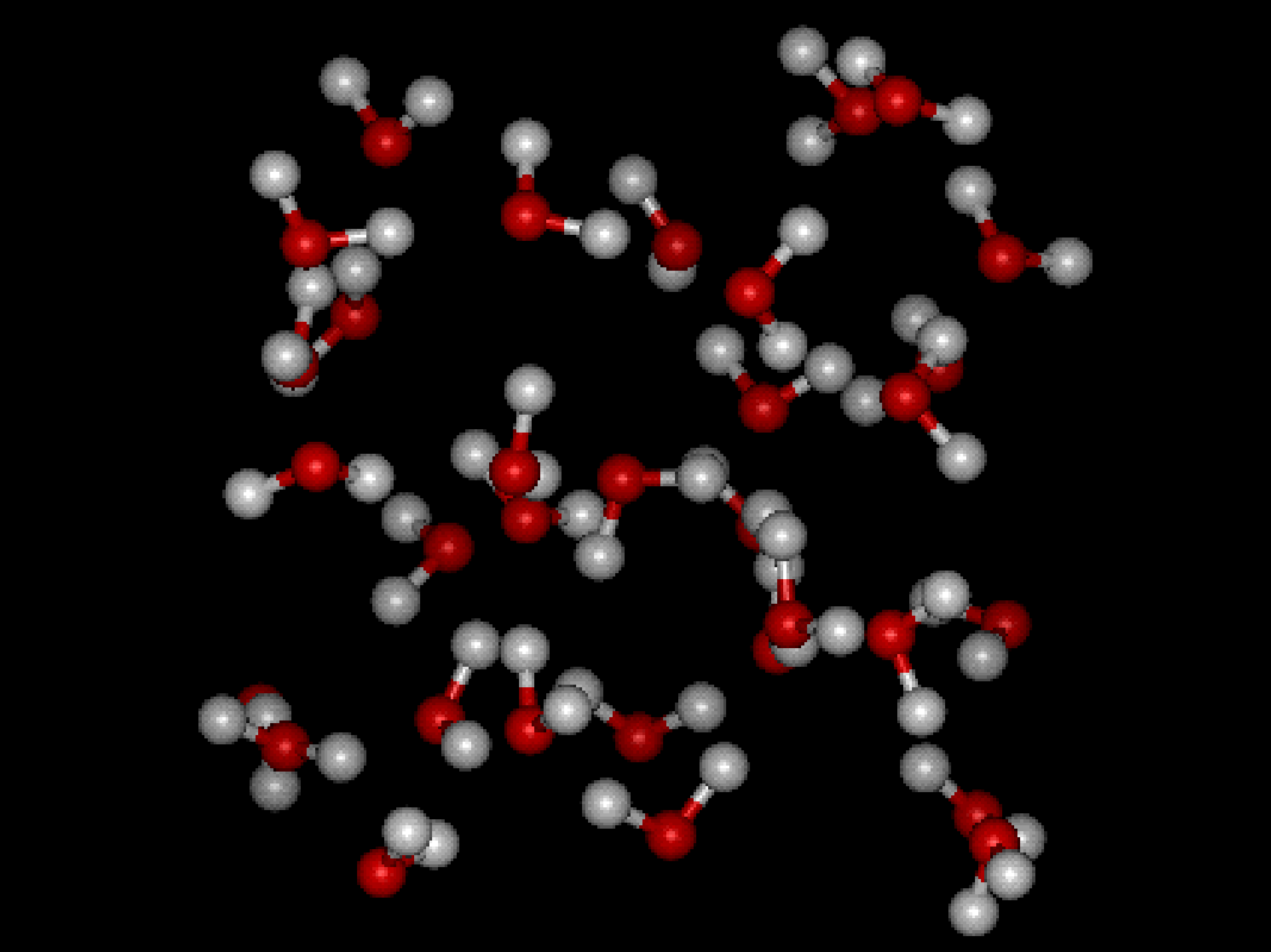



A
Protein:
Hydrophilic domains
Hydrophobic domains

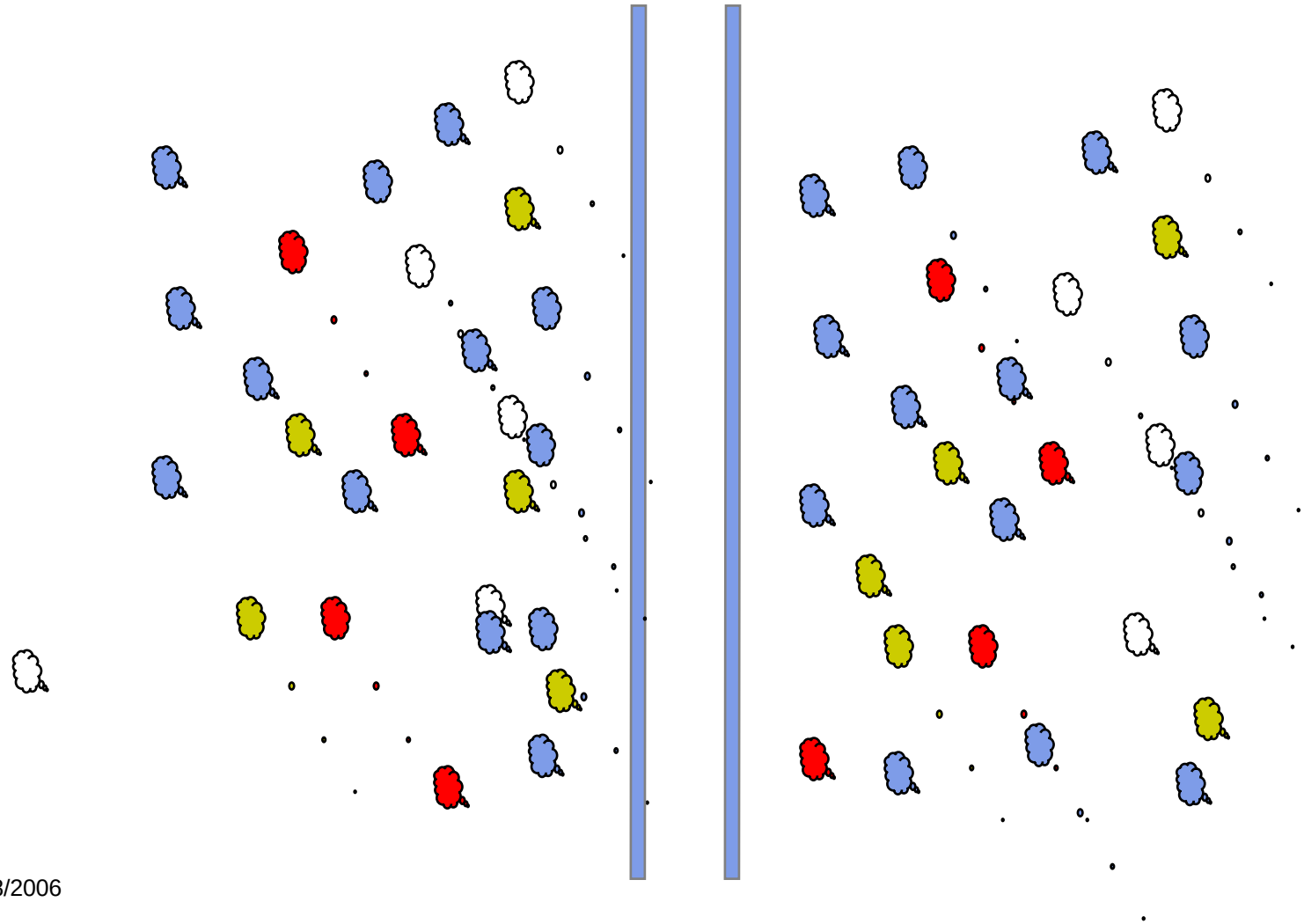

A
Protein:
Hydrophilic domains
Hydrophobic domains
Water molecule
Foreign material


PC coating

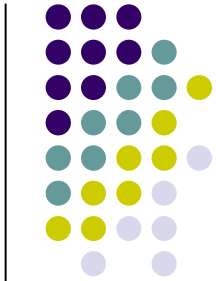




No Protein Adsorption



Uses of Nonfouling Surface Treatments

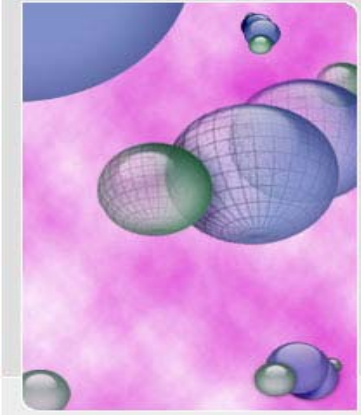


- Inhibit bacterial binding
- Improve hemocompatibility
- Implanted devices urinary catheters
- Diagnostic assays
- IV catheters
- Etc.

Methods

- Plasma or corona treatment in the presence of a reactive atmosphere;
- Adsorption of hydrophilic or neutral chemical species; and,
- Covalent Immobilization of hydrophilic or neutral chemical species.

Surface Modification Using Low-Pressure Plasma Technology



- A plasma is a partially ionized gas containing ions, electrons, atoms, and neutral species.
- commonly selected gases or mixtures of gases for plasma treatment of polymers include oxygen, argon, nitrous oxide, tetrafluoromethane, and air.
- high-frequency generator ionizes the gas into an plasma of reactive particles that react to the surface without damaging the bulk properties
- outermost 10 to 1000 Å of the substrate.

PLASMA APPLICATIONS

- Surface modification using gas plasma is versatile;
- Capable of treating devices with high surface area:

everything from small components like hubs or balloons up to very large and complex substrates, from fibers, nonwovens, wovens, and paper to plastic foils and metal and ceramic parts.

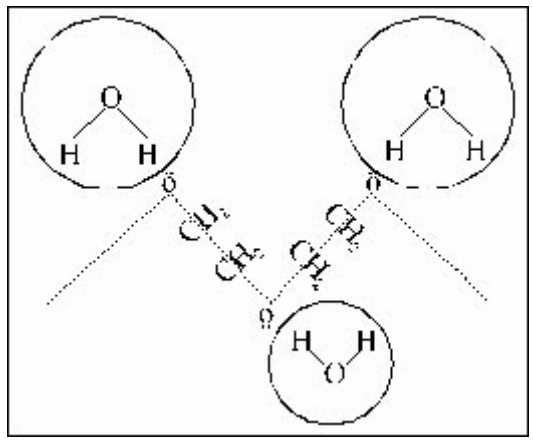
Materials	Surface Energy (dynes/cm)		Water Contact Angle (degrees)	
	before	*after*	*before*	*after*
Hydrocarbons				
Polypropylene	29	>73	87	22
Polyethylene	31	>73	87	42
Polystyrene	38	>73	72.5	15
ABS	35	>73	82	26
Polyamide/ polyethylene copolymer	<36	>73	63	17
Epoxy	<36	>73	59	12.5
Polyester	41	>73	71	18
Rigid PVC	39	>73	90	35
Phenolic	None	>73	59	36.5
Fluorocarbons				
Polytetrafluoroethylene/ polyethylene copolymer	37	>73	92	53
Fluorinated ethylene propylene	22	72	96	68
Polyvinylidene	25	>73	78.5	36

Table I. Typical material surface-tension and contact-angle values

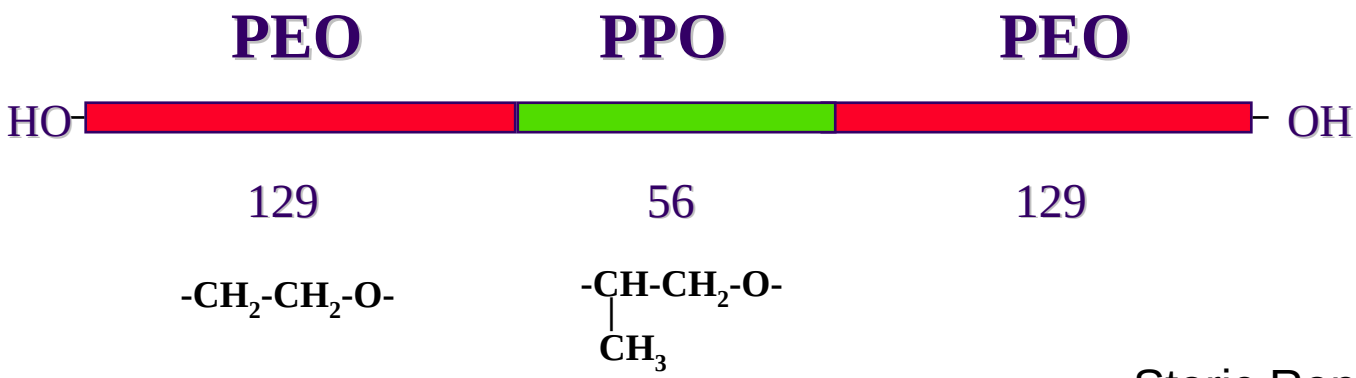
Poly(ethylene glycol) PEG

$[-CH_2CH_2O-]_n$

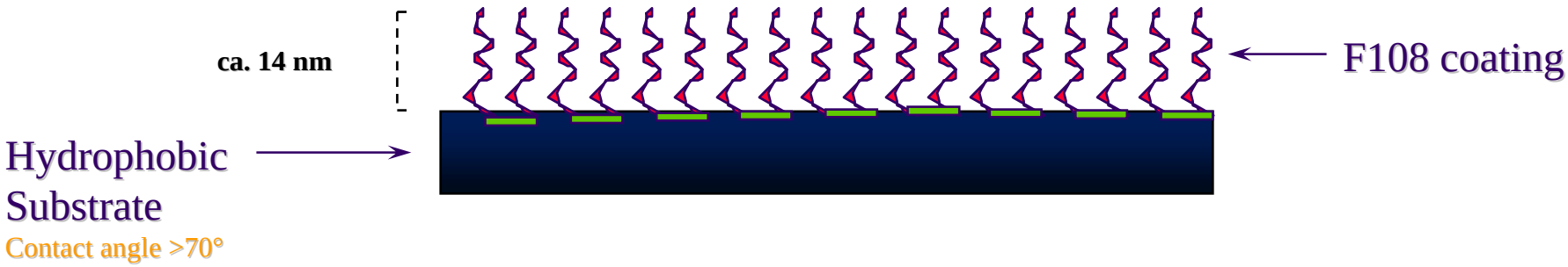
- N = 15-3500; mw 400-100,00
- Binds water
- Steric repulsion



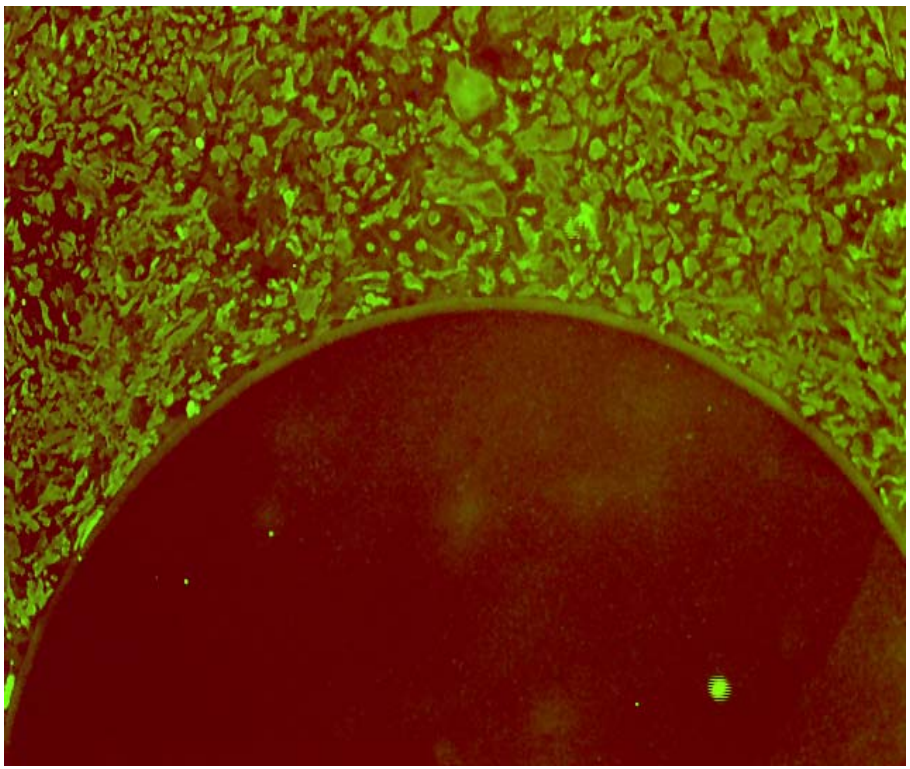
Surfactant-based PEG Coating



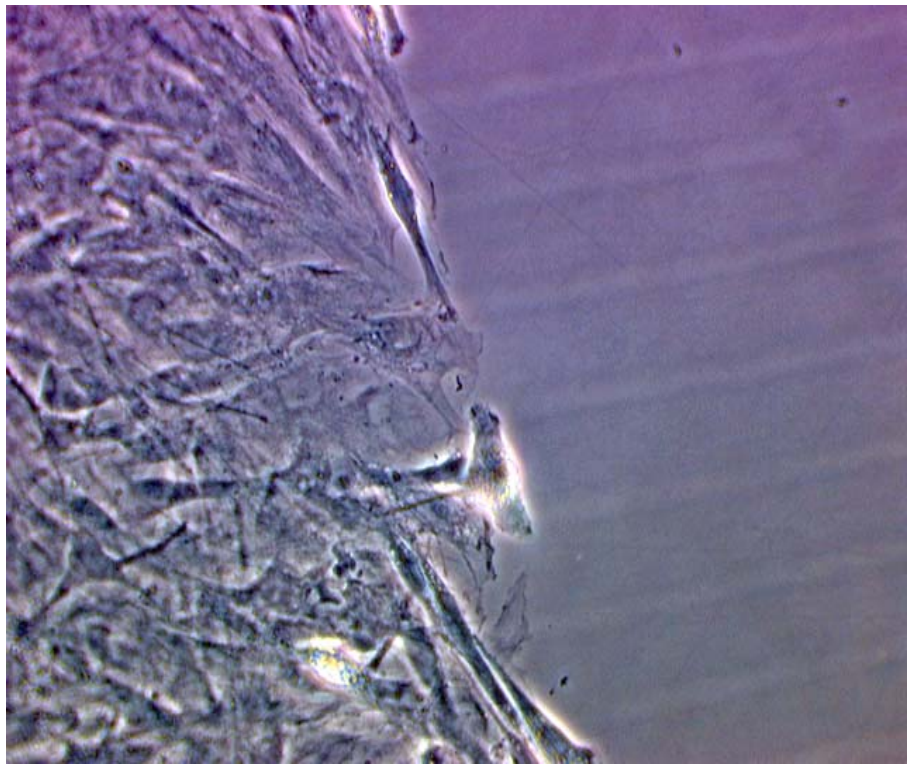
- Steric Repulsion
- Pegylation



Inhibition of Cell Attachment

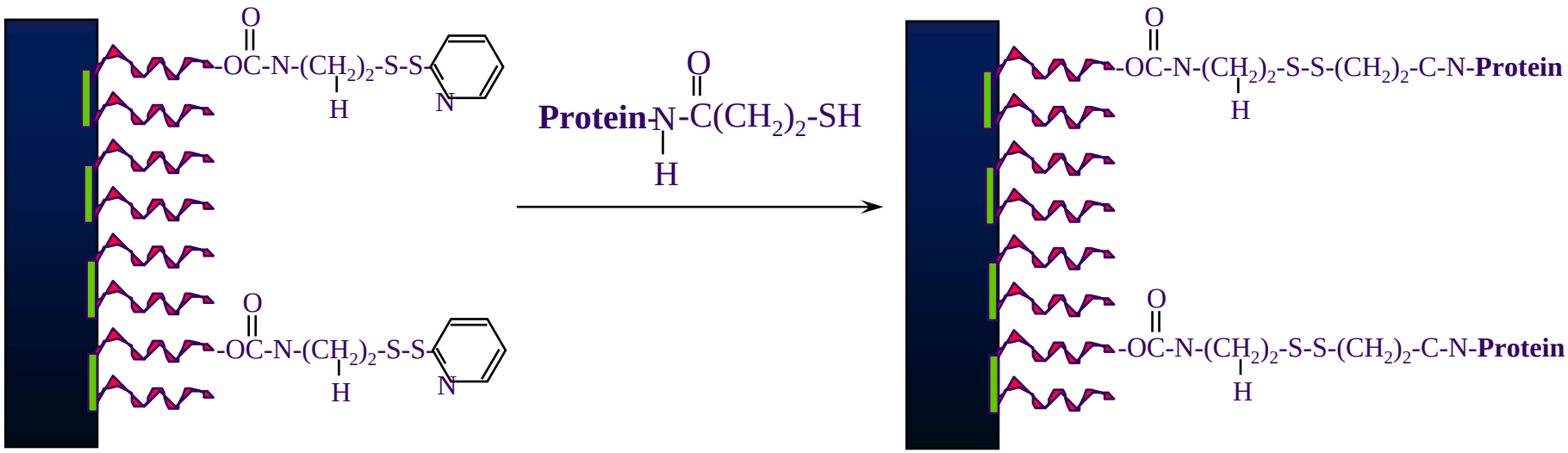


GFAP: Green

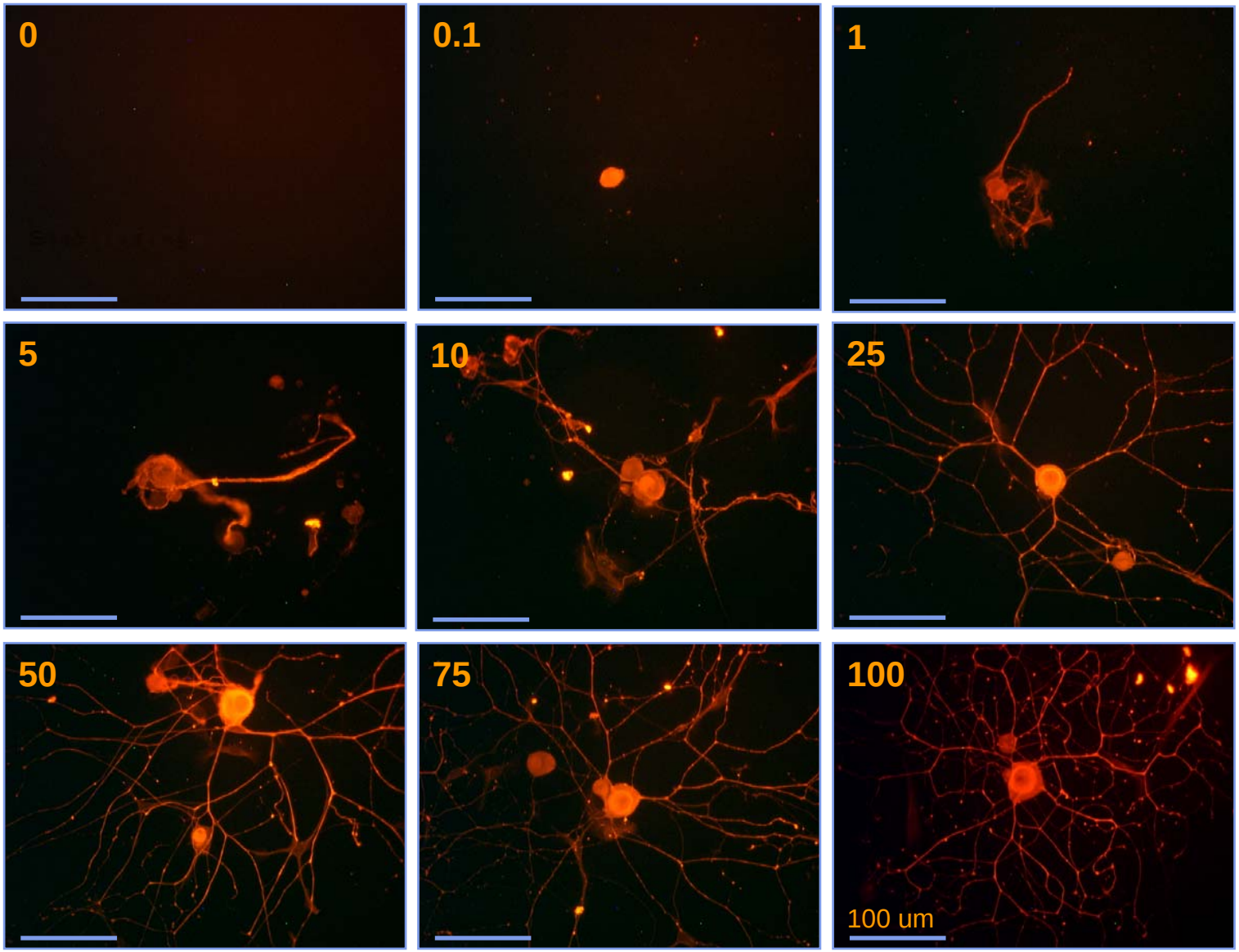


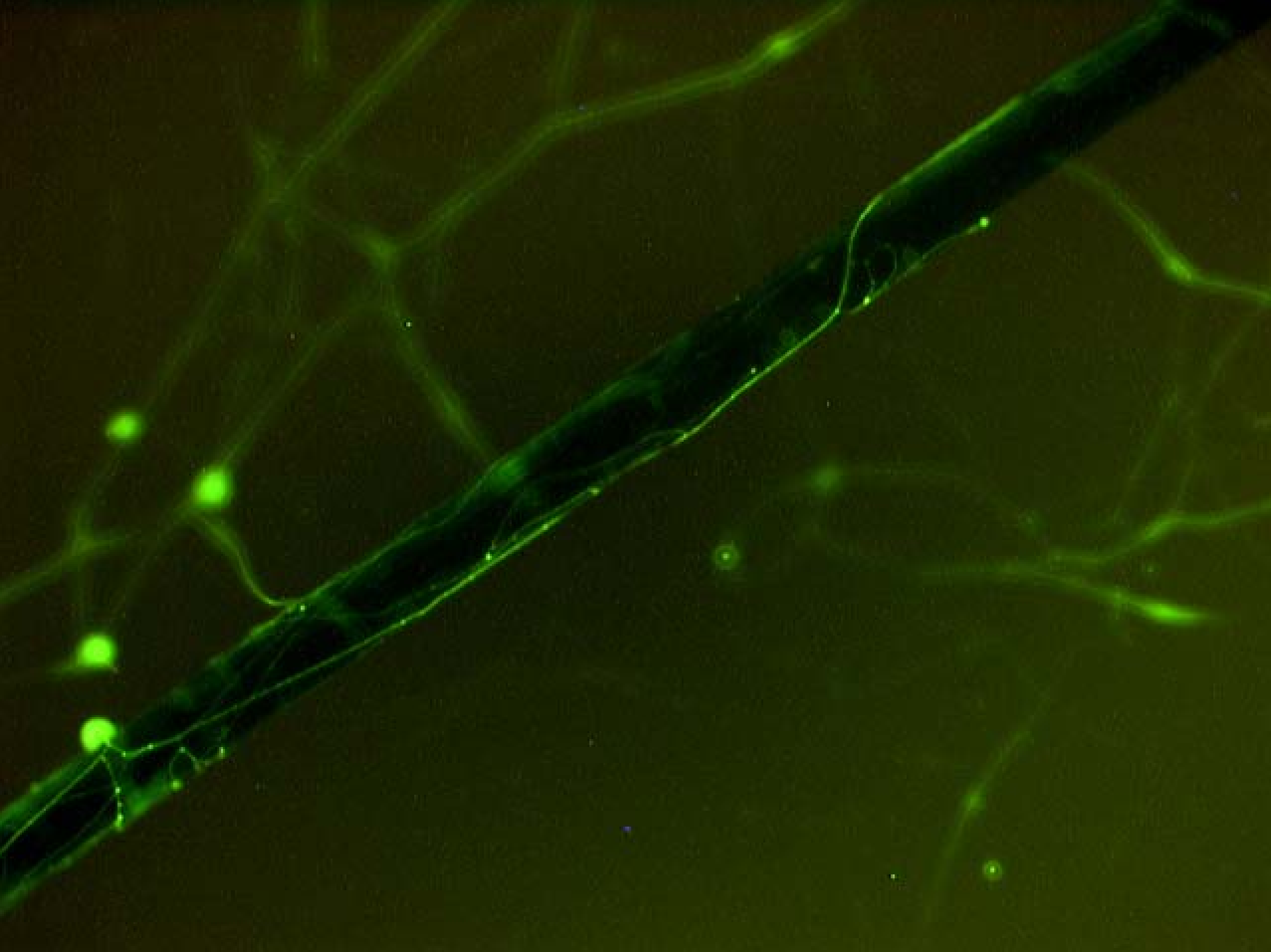
Light Micrograph

Protein Tethering Scheme

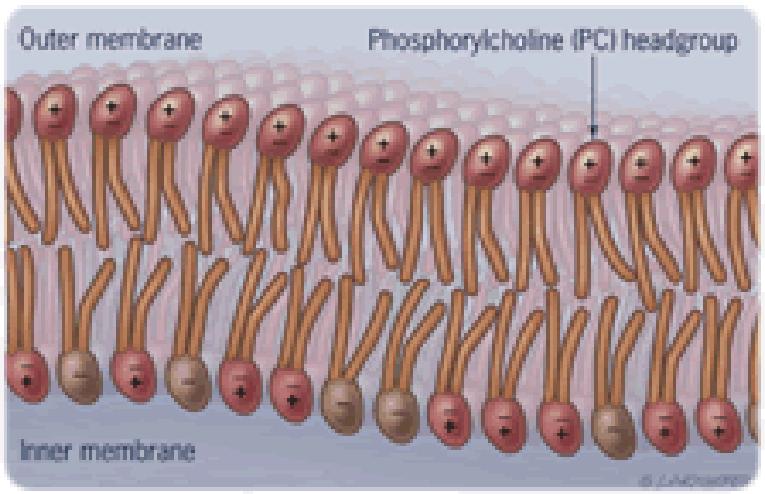


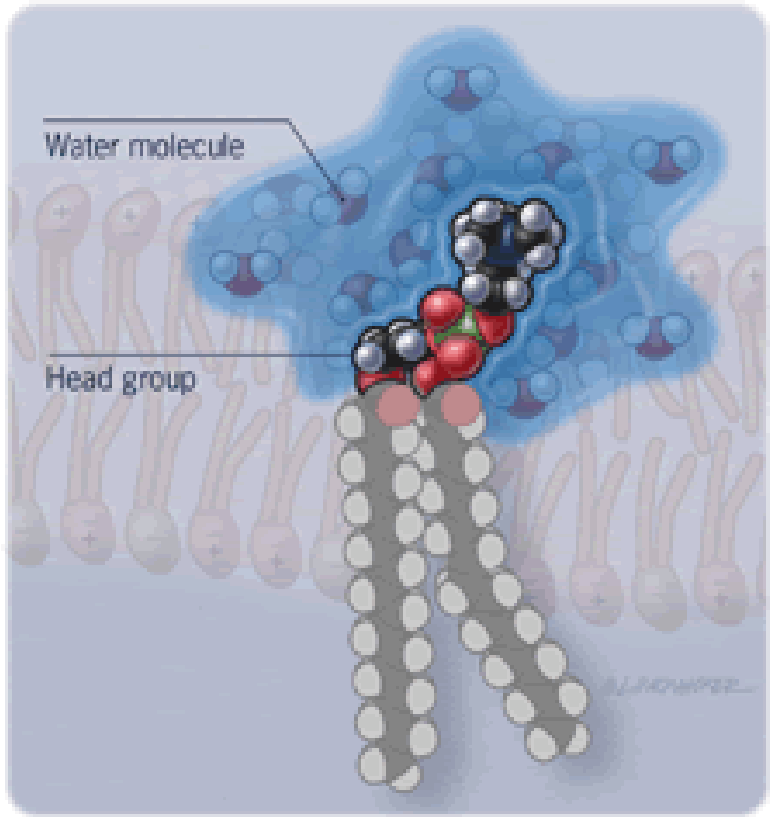
Varying Substrate Ligand Bioactivity

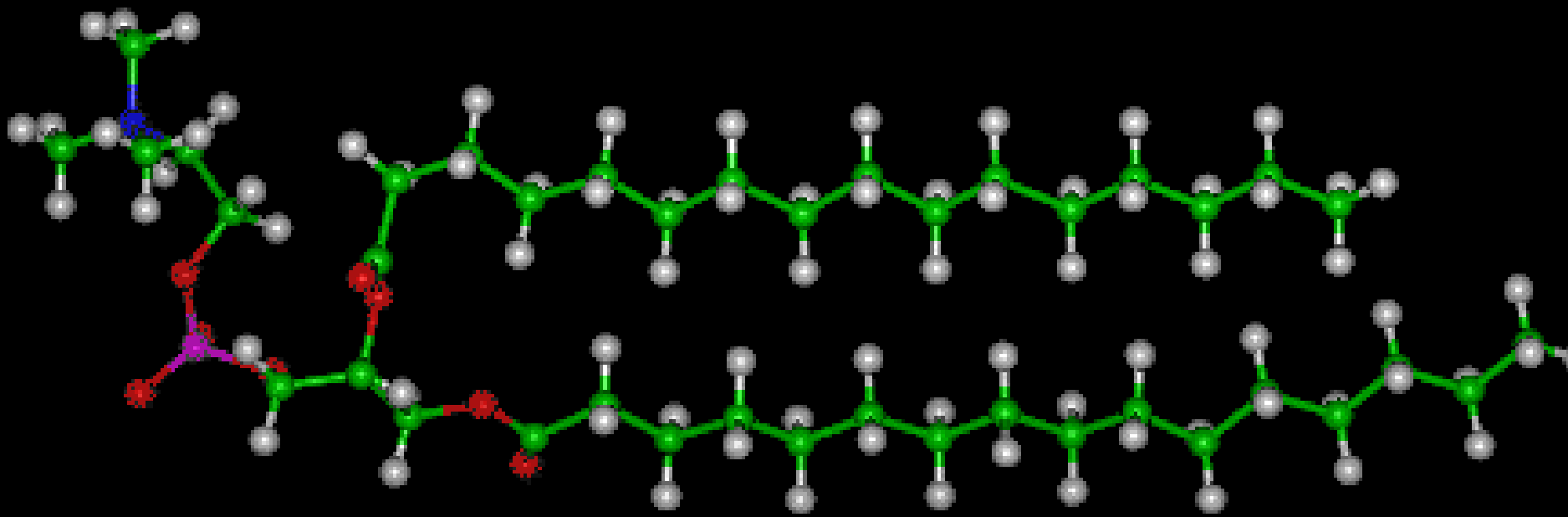




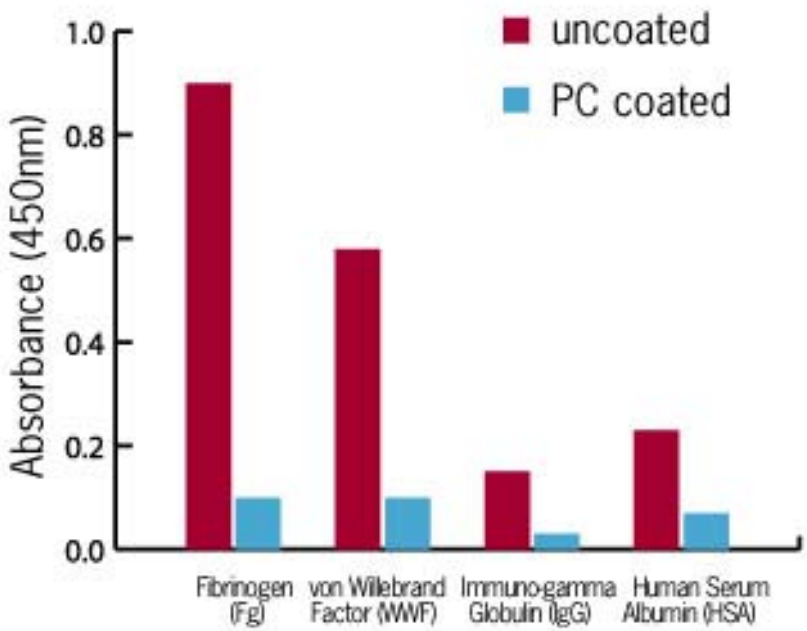
Phospholipids





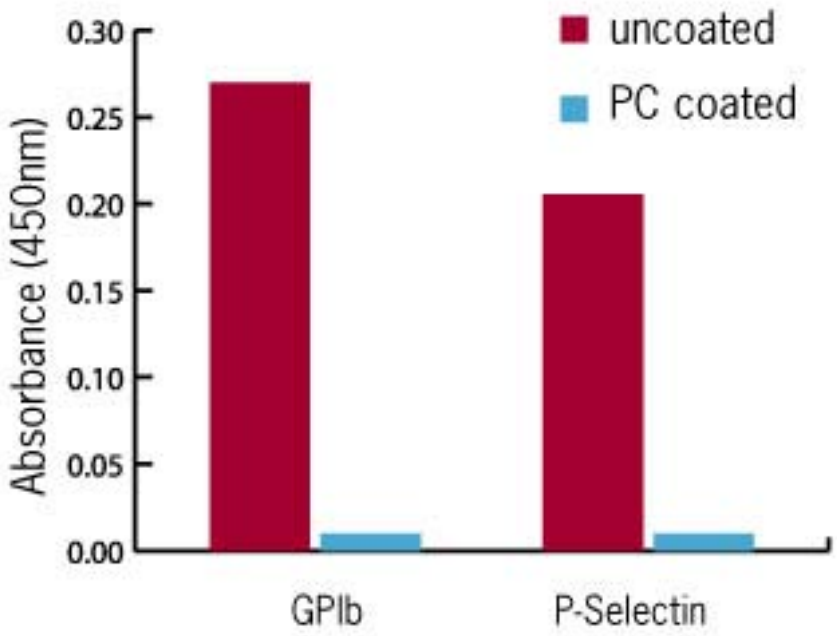


Protein Adhesion *In Vitro*



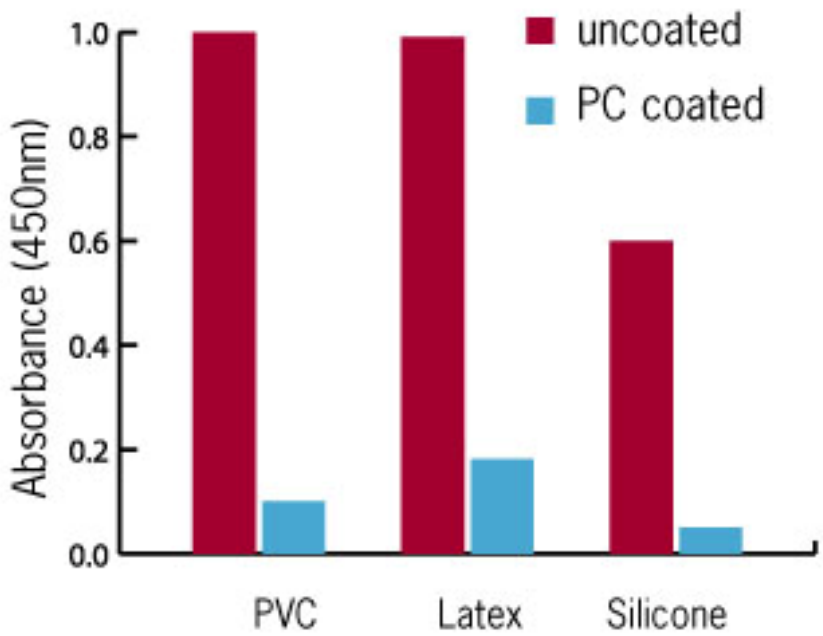
J Chem Edu 79, 321, 2002

Platelet Adhesion and Activation *In Vitro*



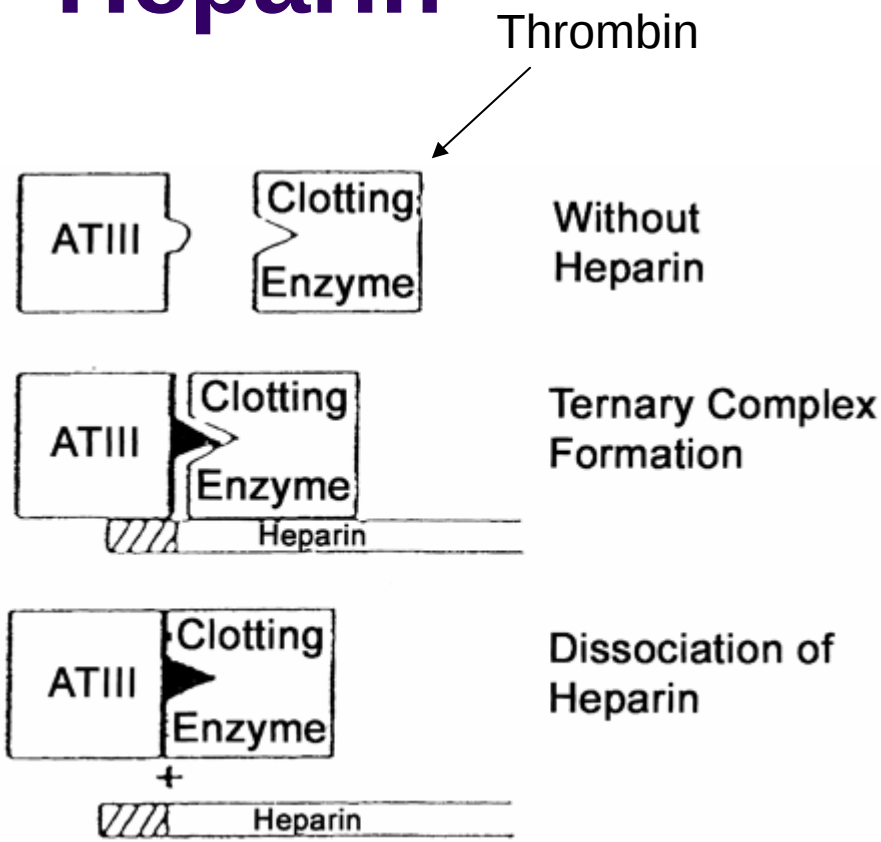
ASAIO 40, M853, 1994

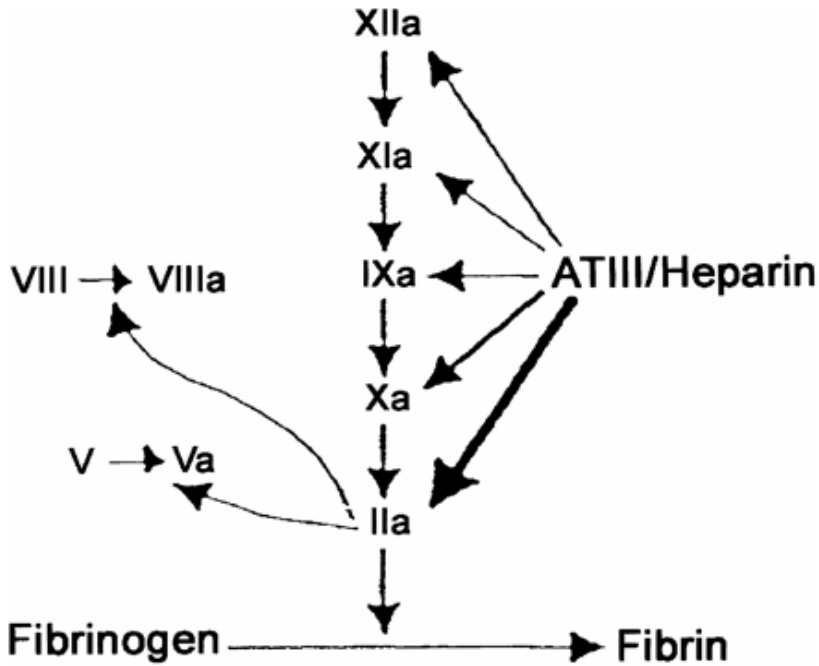
Bacterial Adhesion *In Vitro*



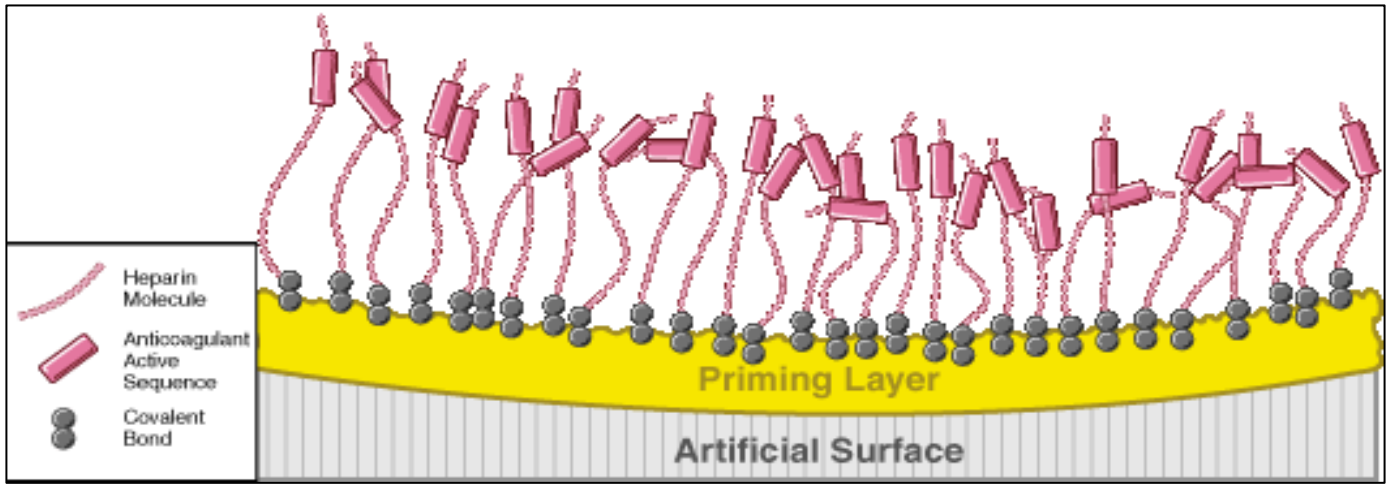
Biomaterials 22, 99, 2001

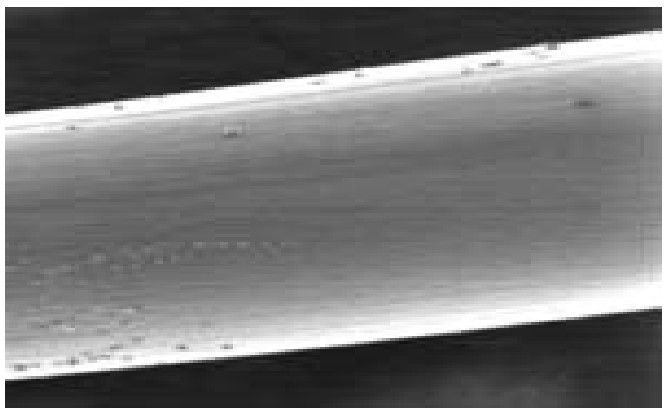
The Anticoagulant Action of Heparin



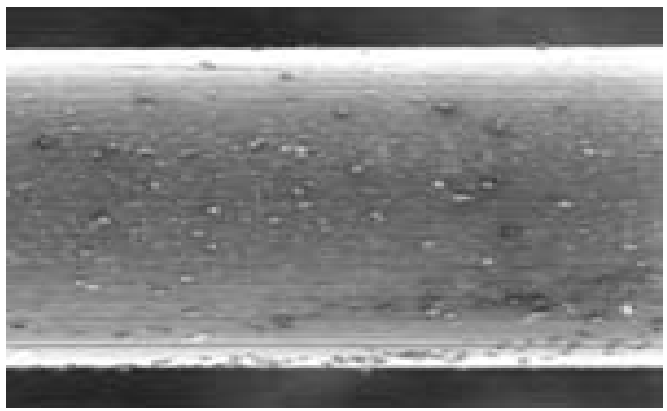


Covalent Immobilization of Heparin



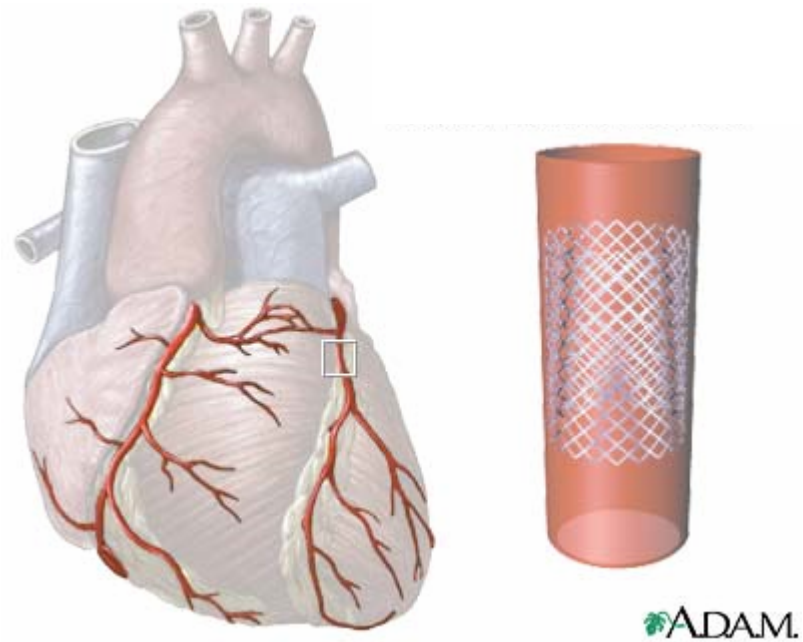


Heparin coated after blood exposure

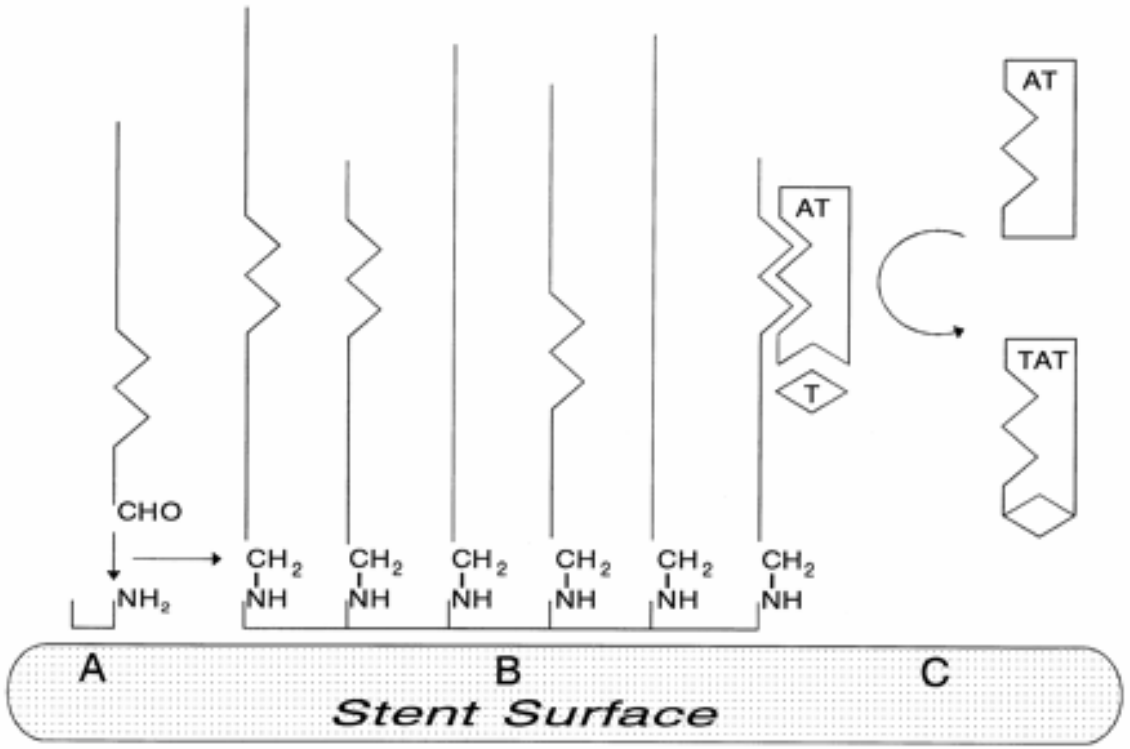


Uncoated after blood exposure with microthrombus on surface

Stents



Stents



General strategies to Prevent Device-related Infections

- Minimize contact- Clean Room Conditions
- Kill every thing in contact-Sterilization
- Minimize binding at contact-Surface coating
- Kill after contact-Anti-infective coatings

Combining Local Drug Delivery and Implantable Medical Devices

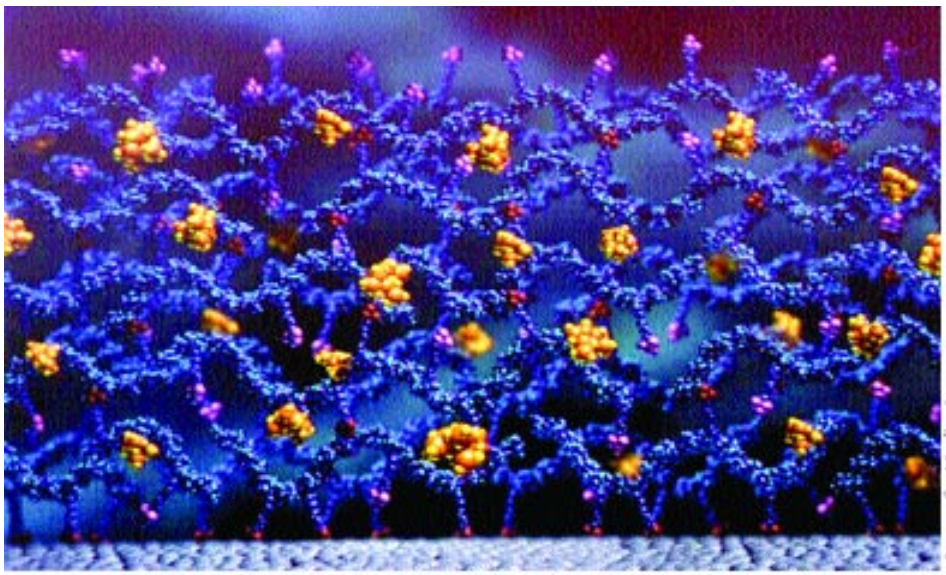


Image: SurModics Inc.

An artist's rendition of a polymeric matrix coating that is eluting drug from the surface of a device.

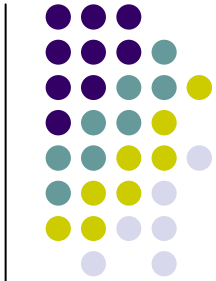
Table I: Examples of devices that could utilise drug-eluting coatings.

Abdominal aortic aneurysm devices
Anastomosis devices
Birth control occlusion devices
Benign prostatic hyperplasia and prostate cancer treatments
Breast implants
Cerebro spinal fluid shunts
Dental implants
Focal epilepsy treatment
Heart valve repair
Implantable biosensors
Implanted drug infusion tubes
Intravitreal drug delivery devices
Nerve regeneration conduits
Neuro aneurysm treatment
Pacing and electro stimulation leads
Pain management
Spinal repair devices
Stents (coronary, peripheral, gastrointestinal)
Vascular grafts
Vena cava filters

Anti-infective Coatings

AST Products (Billerica, Massachusetts) presented data on a surface coating that provides controlled release of antimicrobial agents without an initial burst effect. The coating uses a charged antimicrobial agent, such as a silver ion, that forms an ionic complex with a polymer matrix containing a counter ion, such as a carboxylic group. The silver ions are exchanged when sodium ions from physiological fluids diffuse into the coating matrix.

Anti-infective Coatings



- sequester antimicrobials and antibiotics on the surface of or within devices to reduce the incidence of device-related infections;
- active anti-infective agents in or on the device is secondary to the device's primary therapeutic or diagnostic function;

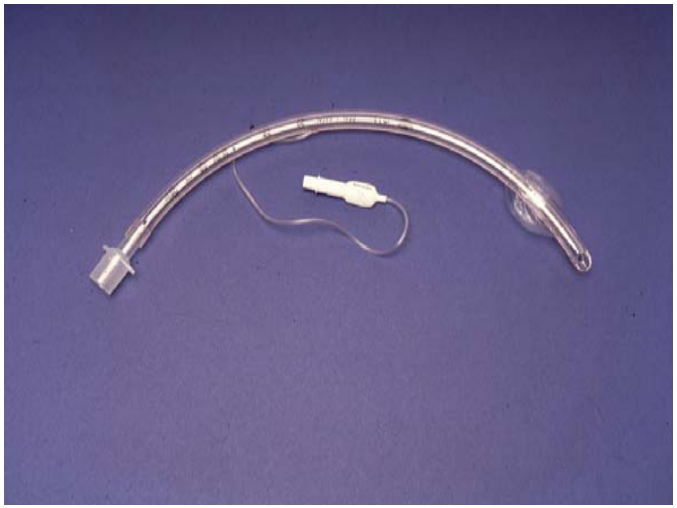
The Central Concept

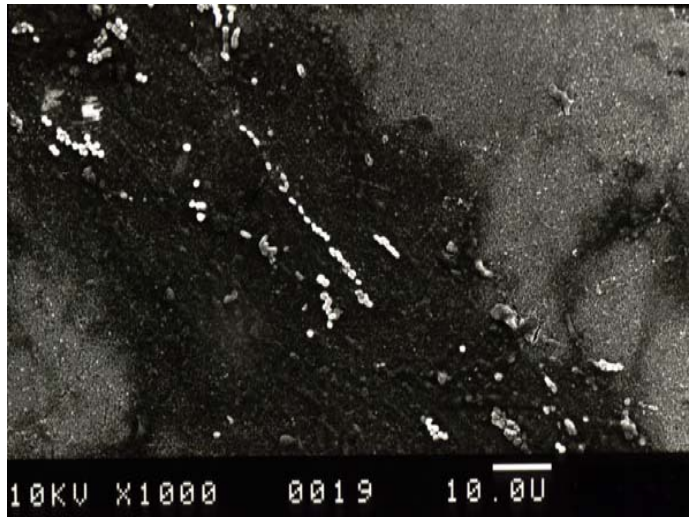
- **Site-specific delivery**-Locating active agents or drugs only at the surface of or in the vicinity of the device to reduce the incidence of device-related infections, which is preferable to administering the same drugs systemically;
- Systemic administration requires maintaining dose levels throughout the body, whereas local administration from the device surface concentrates the drug at the precise site where it is needed;
- Decreases potential for bacterial resistance.

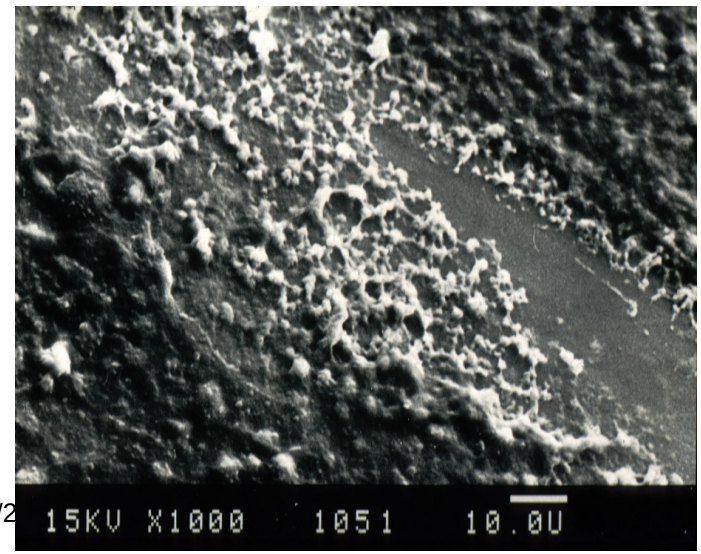
Effective Delivery

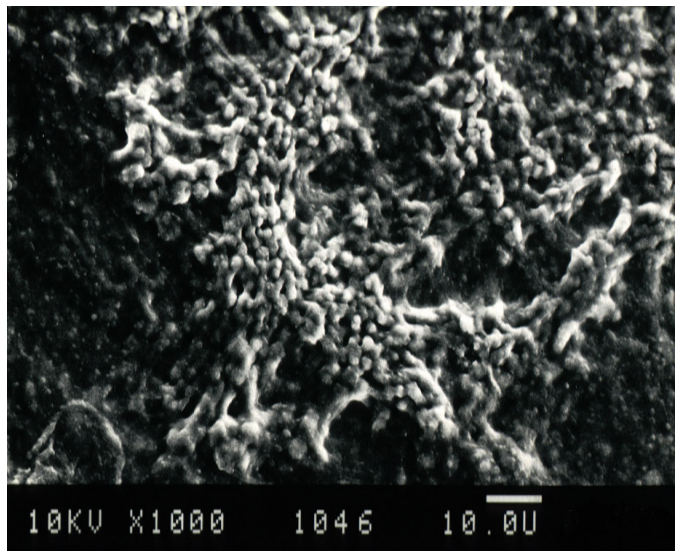
- In order for local administration to be effective there must be sufficient amounts of the agent released from the device, and the duration of release must be appropriate for the condition.
- If there is good elution of drug from the device, drug concentration will be high at and near its surface, but will diminish with distance;

Endotracheal Tubes from ICU at 4, 8 , 12 hrs.



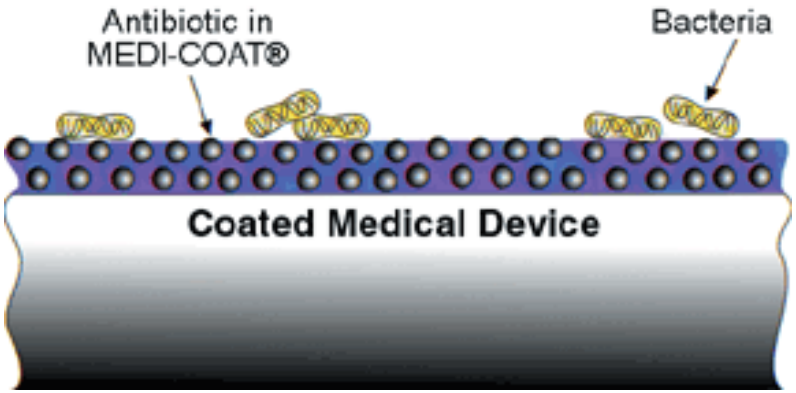


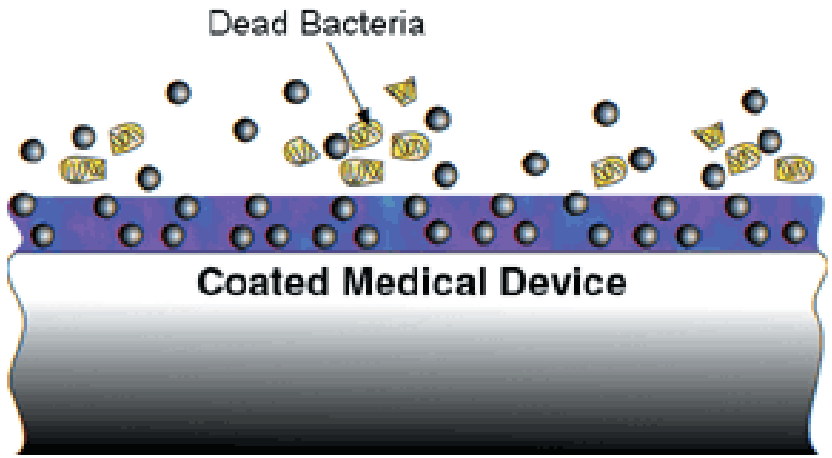




METHODS OF DRUG ATTACHMENT AND ENTRAPMENT

- Adsorption;
- Adding surface charges,
- Covalent immobilization with labile linkage;
- Incorporation into surface coating;





The Ideal Surface Coating

- Biocompatibility
- Drug Availability
- Adhesion
- Durability
- Flexibility
- Coverage
- Sterilizability
- Stability
- Ease of Use
- Cost

Emerging Technologies

- Coatings for enhanced imaging
- Cell coated grafts for tissue engineering
- Coating to enhance regenerative processes
- Coatings for drug delivery

Coating Companies

Polymer Technology Group
SurModics
Carmeda
Hydromer Inc.
AST Products Inc.
STS Biopolymers
Biocoat
Richard James Inc.
Biocompatibles Ltd.
BioChrom
Surface Solutions Laboratories
Spire Corp.
Implant Sciences Corp.
Advanced Polymer Systems Inc.