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Artificial Heart

Dolly Holt SLCC

Artificial Heart

If a man can grow a heart,
he can build it"
Willem J. Kolff

- William Kolff
- 1957 first implanted in a dog (Western hemisphere, Russia had one in 1930)
- 1982 first implanted Barney Clark
 - Jarvik 7
 - Dr. DeVries
 - Patients living up to 620 days on the Jarvik hearts.

- Two pneumatically powered polyurethane sac-type blood pumps
 - 70 or 100 ml stroke volume
 - Powered by separate units
 - Tilting disc prosthetic inflow and outflow valves provided unidirectional blood flow
 - Air pulses entered the space between the cases to compress and expand the sac, providing the cardiac output
 - Outlet ports had suture cuffs and were attached to the aorta or pulmonary artery by a vascular graft.



www.smithsonianlegacies.si.edu

- Abiocor
 - Completely implantable
 - No sires or ports
 - Weighs 1Kg
 - About size of a grapefruit
 - Motorized hydraulic pump
 - Internal rechargeable battery
 - Lithium battery lasts 30 min
 - External battery pack
 - Transcutaneously transmitting energy to internal battery
 - Polymer is proprietary pothyurethane (AngioFlex)



Abiomed

The AbioCor is the first artificial heart to be used in nearly two decades.

Cardiac Assist Device Complications

- Hemorrhage
- Thrombosis/thromboembolism
- Infection
- Hemolysis
- Device failure
 - Prosthetic valves
 - Pumping bladder
- Transitions of materials and geometries within the pumps were the most vulnerable sites for thrombus formation

Bioinstrumentation

Dolly Holt, SLCC

Clinical Bioinstrumentation

- Ultrasound
- X-Ray
- CT-Scan
- MRI
- PET
- EEG
- ECG
- Pulse Oximeter

Analytical Bioinstrumentation

- Cells, scaffolds, proteins
 - Microscopes
 - Flow Cytometers
 - Spectrophotometers
 - Nanodrop
 - RT and real time PCR
- Micro/Nanoparticles
 - SEM
 - ICPMS
- Organic Compounds
 - Liquid/Gas Chromatography
- Surfaces
 - XPS
 - SIMS
 - AFM
 - Contact Angle
 - FTIR
 - Mass Spectrometry
 - SPR
 - NMR
 - TEM
 - NEXAFS
 - SFG

Surface Analysis

- Property Technique(s)
- Composition ESCA, Auger, SIMS,
- Structure SIMS, ESCA, NEXAFS, FTIR, SFG
- Orientation NEXAFS, FTIR, SFG
- Spatial Distribution Imaging SIMS, AFM, microscopy
- Topography AFM
- Thickness ESCA, AFM, ellipsometry, SPR
- Energetics Contact angle

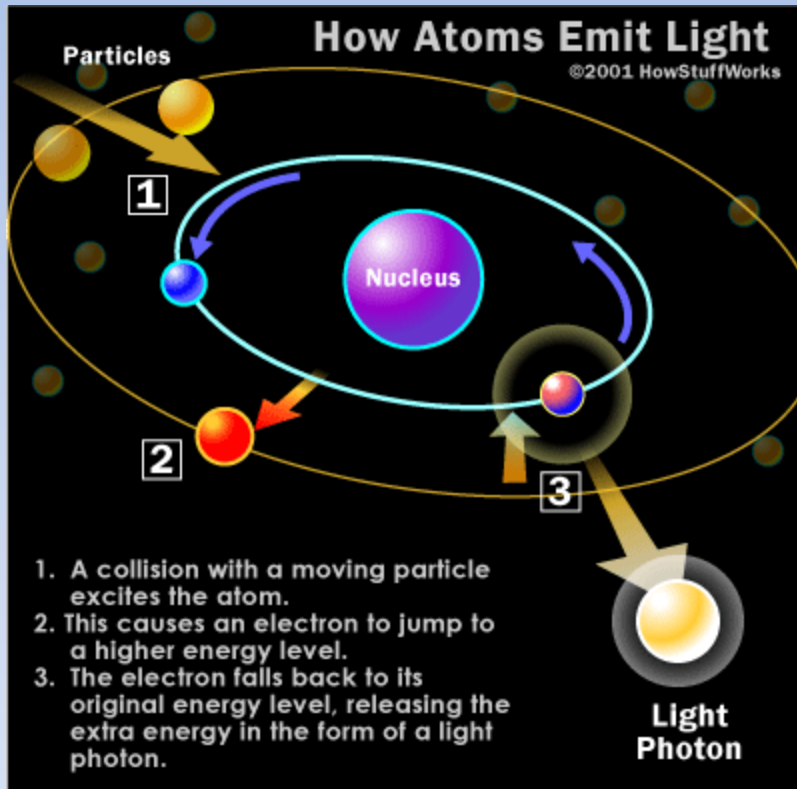
Ultrasound

- Reflects sound and can create an image
- Sonography
 - Type of ultrasound used to see fetuses in the womb
 - Can image muscles, tendons, and internal organs
 - Does not use ionizing radiation like CT
 - Enhances inflammatory response
 - Heat soft tissue
- Produces mechanical pressure wave
- Cannot image through air (lungs, intestinal loops) or bone.



www.examiner.com

X-Ray



[iStockphoto.com/Mostly Mozart](https://www.iStockphoto.com/MostlyMozart)

CT scan

- Computed tomography (CT) or Computed axial tomography (CAT)
- What it can image
 - Soft tissue
 - Bone
 - Blood vessels
 - Contrast agents
- How it works
 - Rotating X-ray (ionizing radiation—creates ions)
 - Compiles cross-sectional images (1 complete rotation) to stitch into a 3D image
 - About 1000 profiles are taken in one rotation and combined to form the 2D slice.



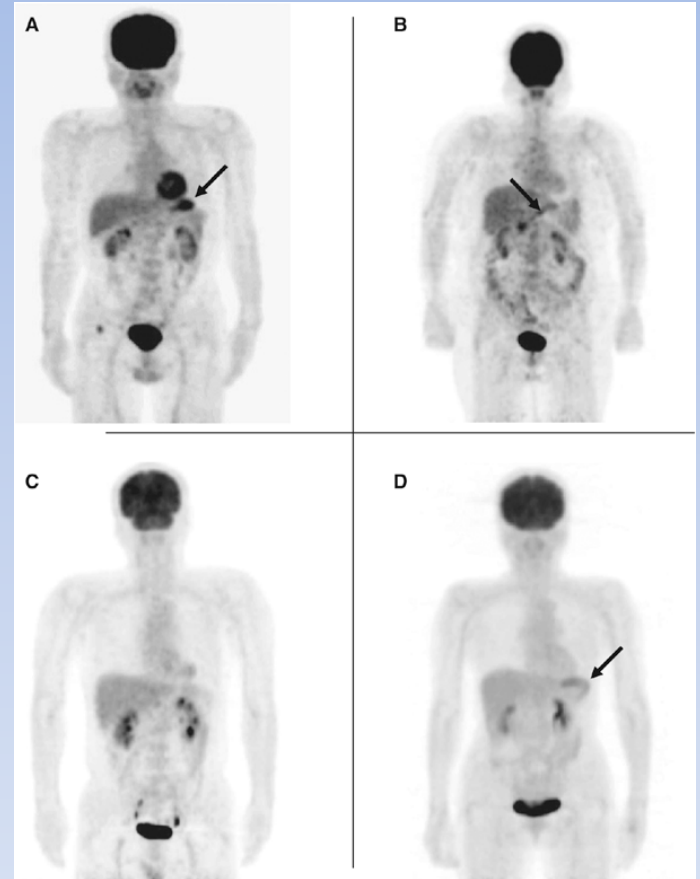
discovermagazine.com

MRI

- Magnetic resonance imaging (MRI)
- Has good contrast between different soft tissues
 - Useful in brain, muscle, heart, cancer (compared to CT or X-ray)
- Uses a powerful magnetic field to align the magnetic moments of the hydrogen atoms in water.
 - Once the field is turned off, the protons (H atoms) decay and release a photon
 - This photon is detected by the scanner
 - Protons in different tissues return to their equilibrium state at different rates producing the difference in imaging.

PET

- Positron emission tomography (PET)
- A radioactive substance is injected (fludeoxyglucose , ^{18}F)
- Coupled with CT or MRI to combine anatomy with PET images.



Microscopy

- Physics of Compound Light Microscopy
- Light Microscopy Modes
 - Bright Field & Dark Field
 - Phase Contrast
 - Differential Interference Contrast (DIC)
 - Fluorescence
 - Confocal Laser Scanning Mode
 - Multiphoton Microscopy
- Applications
 - Cell imaging

Bright and Dark Field Contrast

Fig. 21A

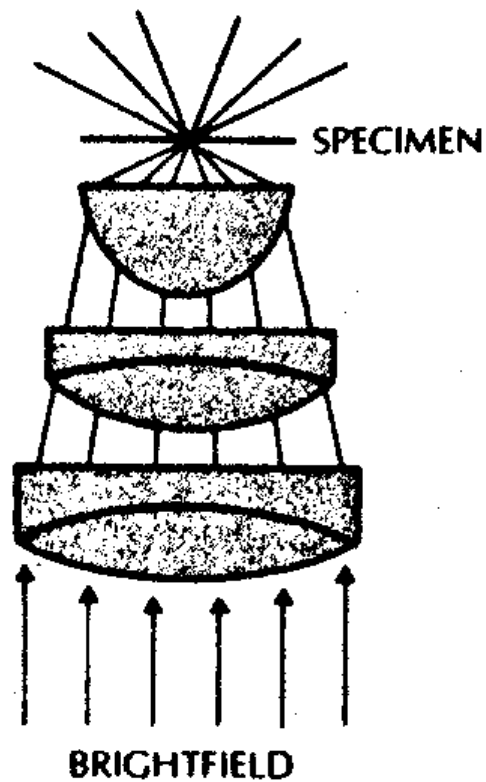
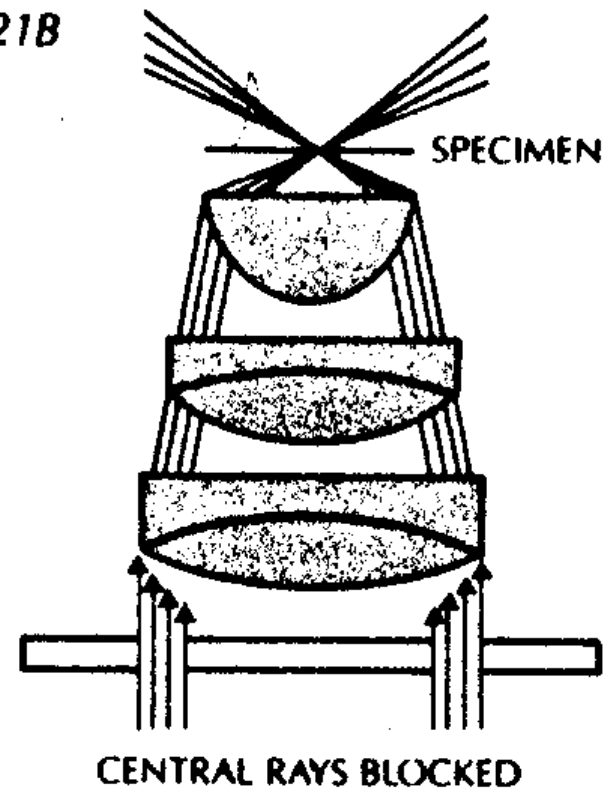


Fig. 21B



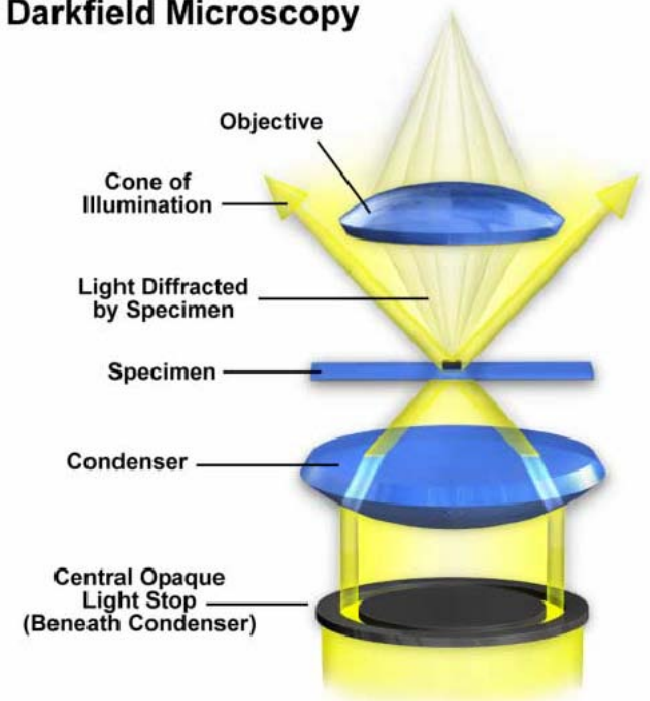
Bright Field Microscopy



Dark Field Microscopy



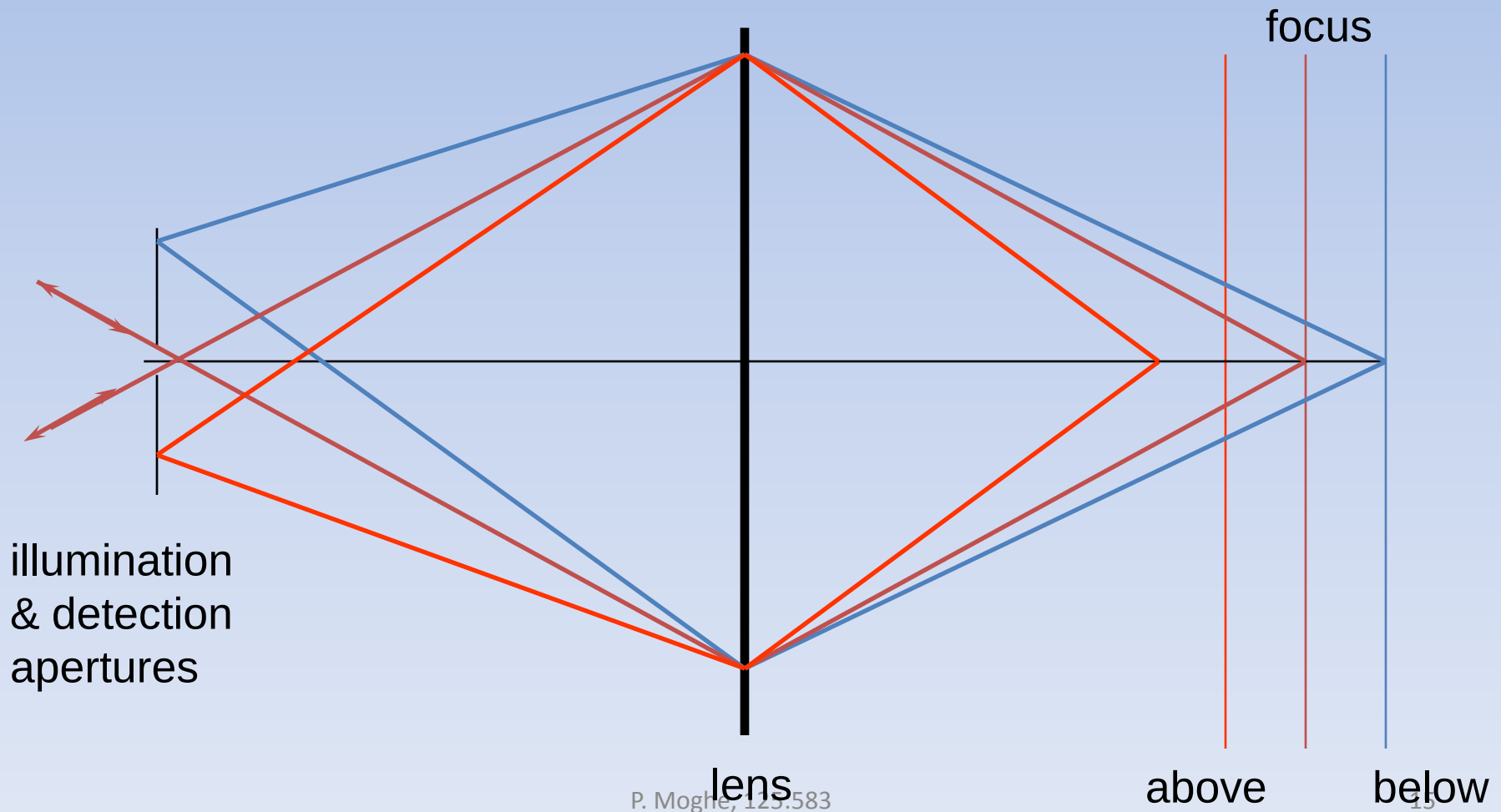
Darkfield Microscopy



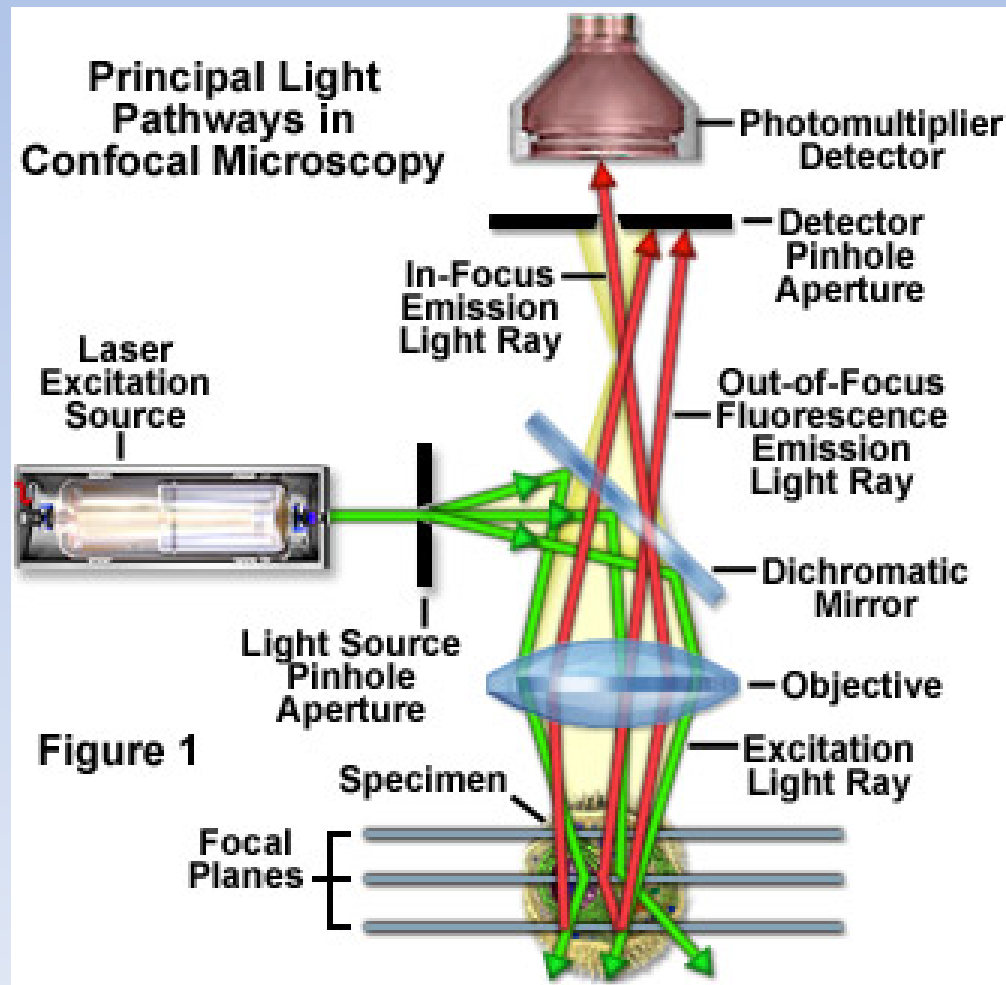
Confocal Fluorescence Microscopy

- Confocal Microscopy
 - Pin hole eliminates out of focus light
- Multiphoton Microscopy
 - Only focus plane is fluorescently excited
 - Increased penetration depth
 - Second Harmonic Generation
- Reflectance
- Allow for simultaneous scaffold/biomaterial and cell imaging

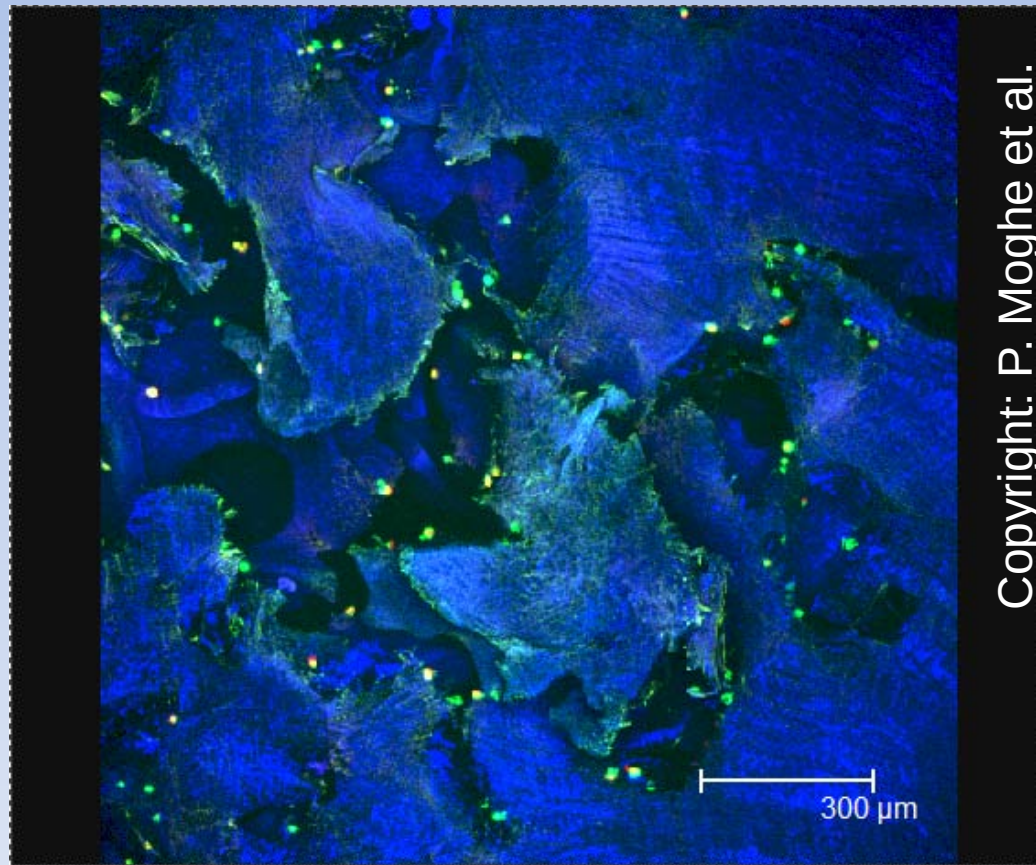
Principle of Confocal Optical Microscopy



Schematic of Confocal Microscopy

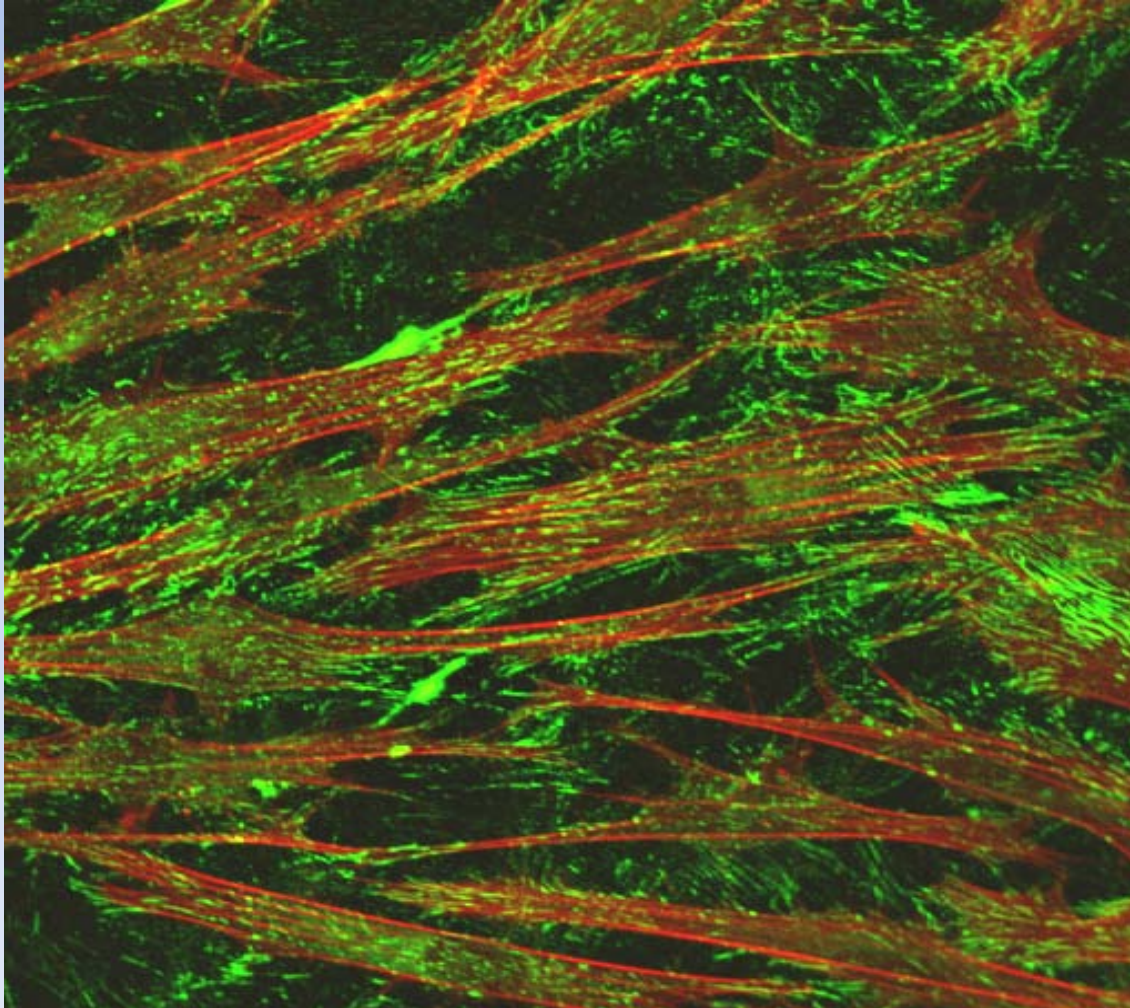


Confocal Imaging for 3-D Projection of Cells in Scaffolds



Moghe, Treiser, Kohn (unpublished)

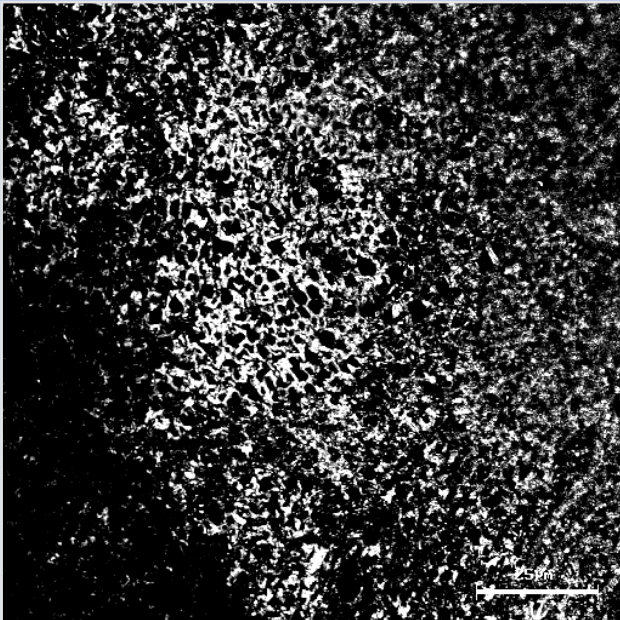
Confocal Image of Cell-Assembled Matrix Proteins



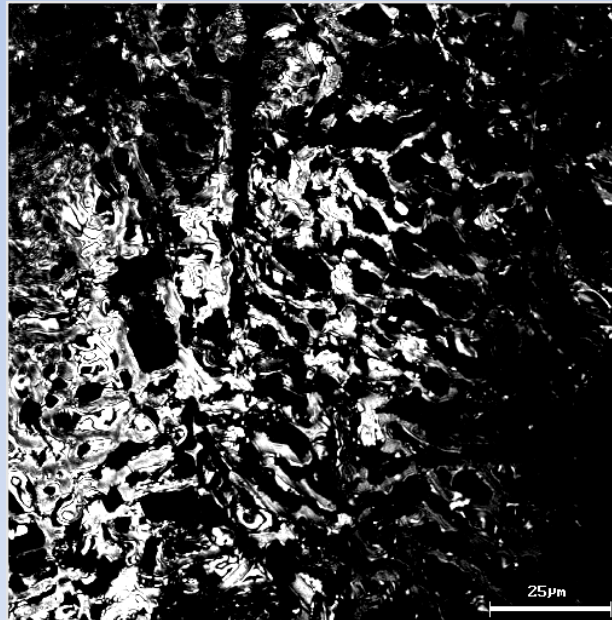
Moghe et al., Unpublished

Confocal Imaging Topographical Imaging of Biomaterials Via Reflection CLSM

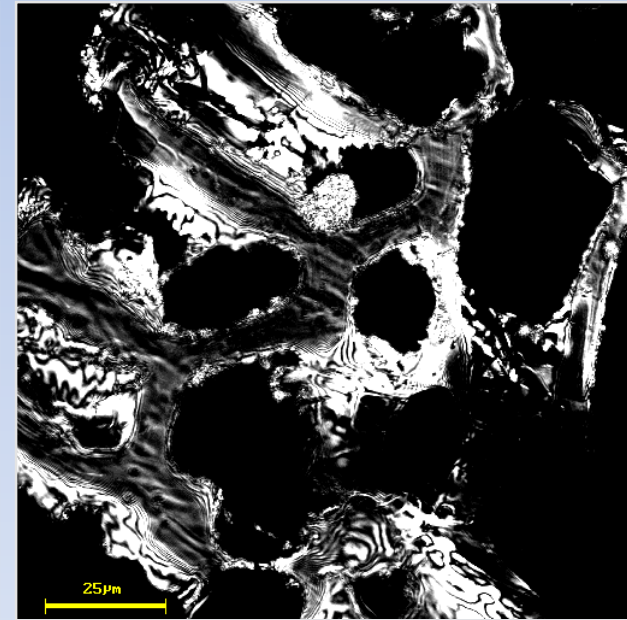
POLYMER SCAFFOLD STRUCTURE



3 μm

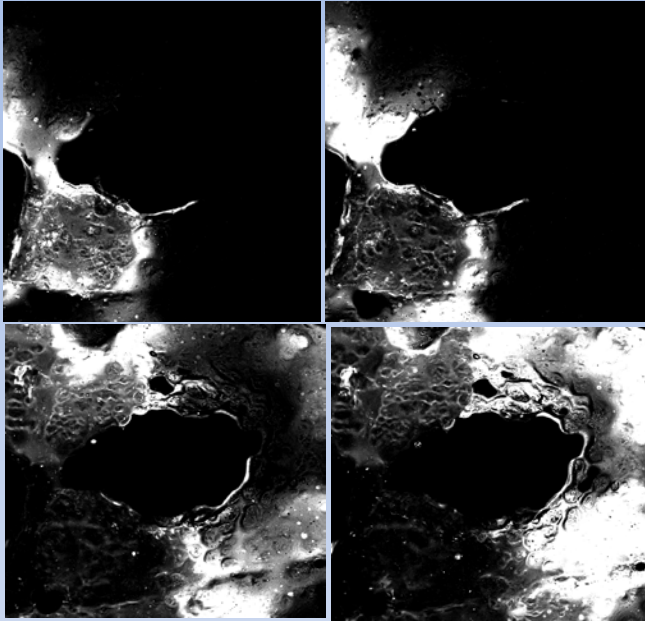


10 μm

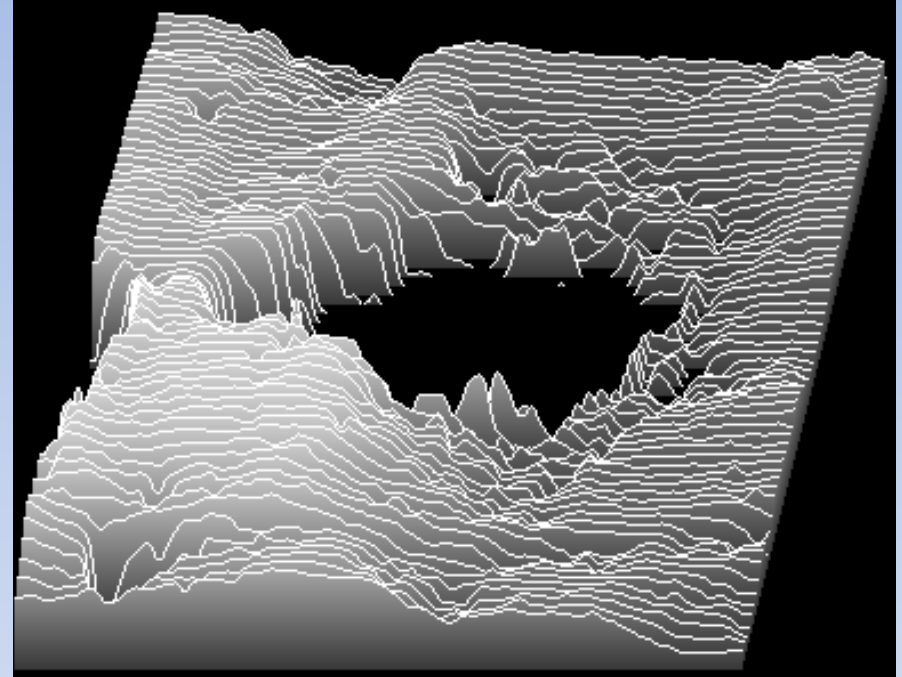


25 μm

3-D Biomaterial Topography Via Reflection CLSM



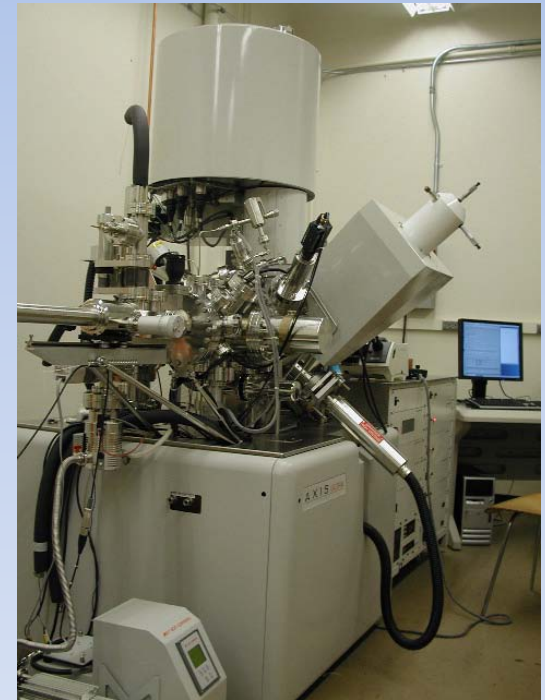
Reflected light images
4 μm increments along
Depth



Topographical reconstruction
of PLGA surface profile

XPS

- X-ray Photoelectron Spectroscopy (XPS)
- Also Known as Electron Spectroscopy for Chemical Analysis (ESCA)



Ratner and Castner, in Surface Analysis--Techniques and Applications, Vickerman (editor), John Wiley (1997) 43.

Advantages of XPS

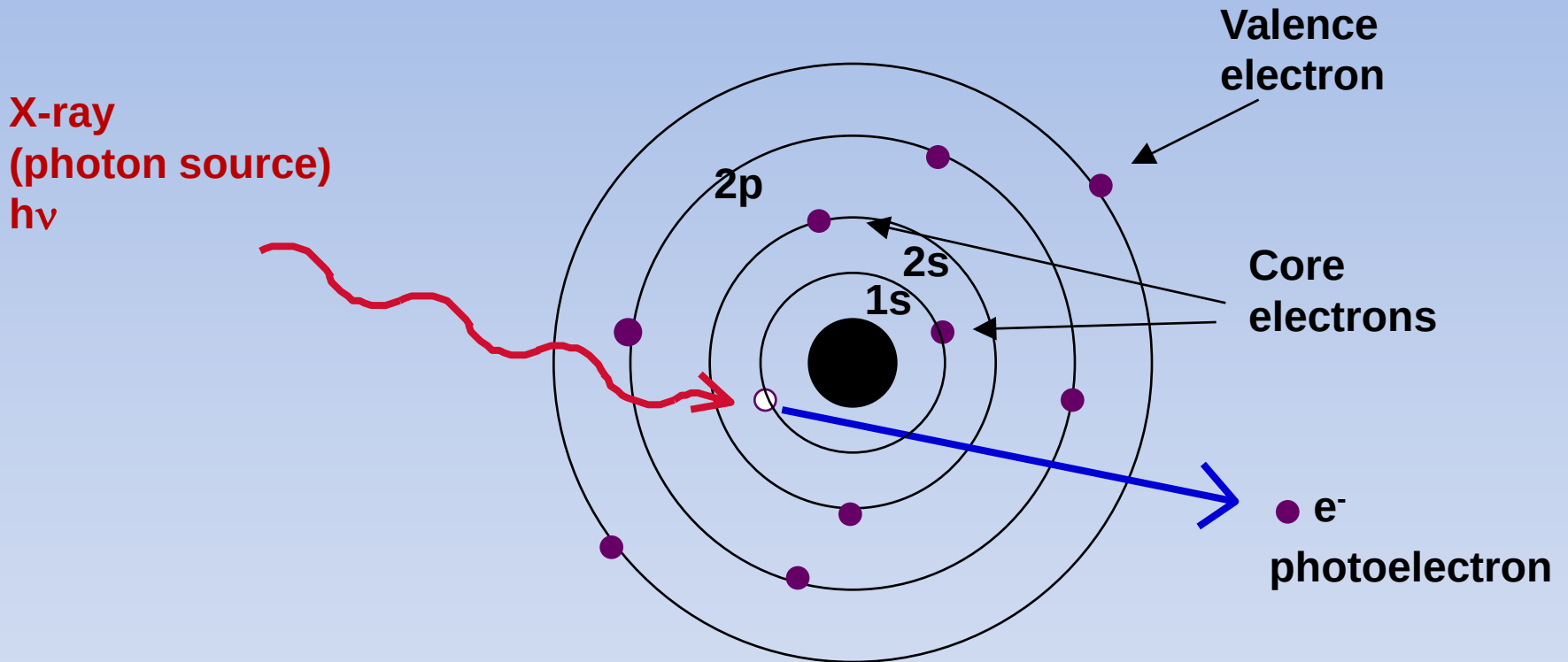
- Quantitative
- Can be used with a wide range of samples with minimal sample preparation
- "Non-destructive"

Information Obtainable from XPS

In the outermost $\sim 100 \text{ \AA}$ of a sample:

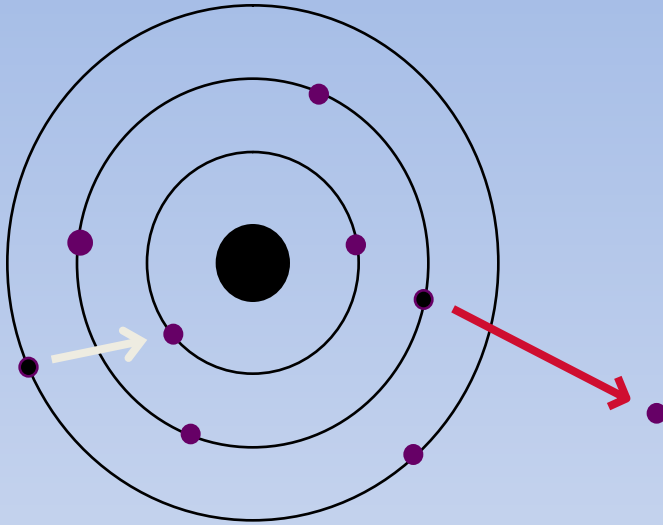
- Identification elements (except H and He) present
(concentrations of ~ 0.1 atomic percent or higher)
- Quantify each element present (error $+10 \%$)
- Determine molecular environment/oxidation state
- Electronic structure of the sample
- Elemental depth profiles
Destructive: $>1000 \text{ \AA}$
Non-destructive: $\sim 100 \text{ \AA}$
- Lateral variations in surface composition (spatial resolution of 8 to 150 mm)

Photoemission

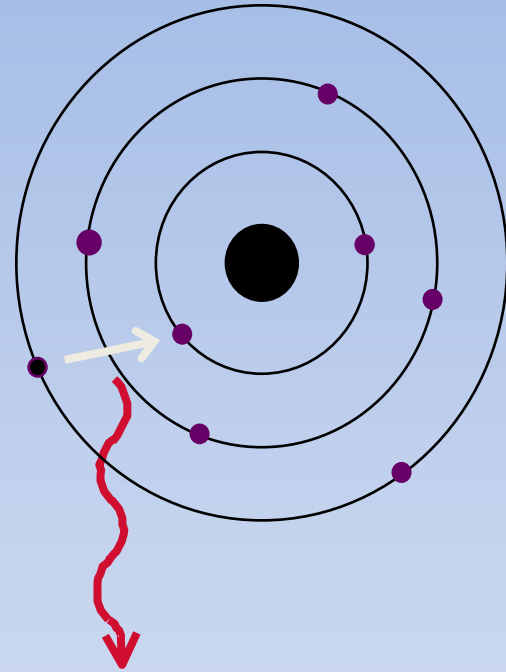


Transfer of energy from the photon ($h\nu$) to the core electron

$$E_b = h\nu - E_{KE}$$

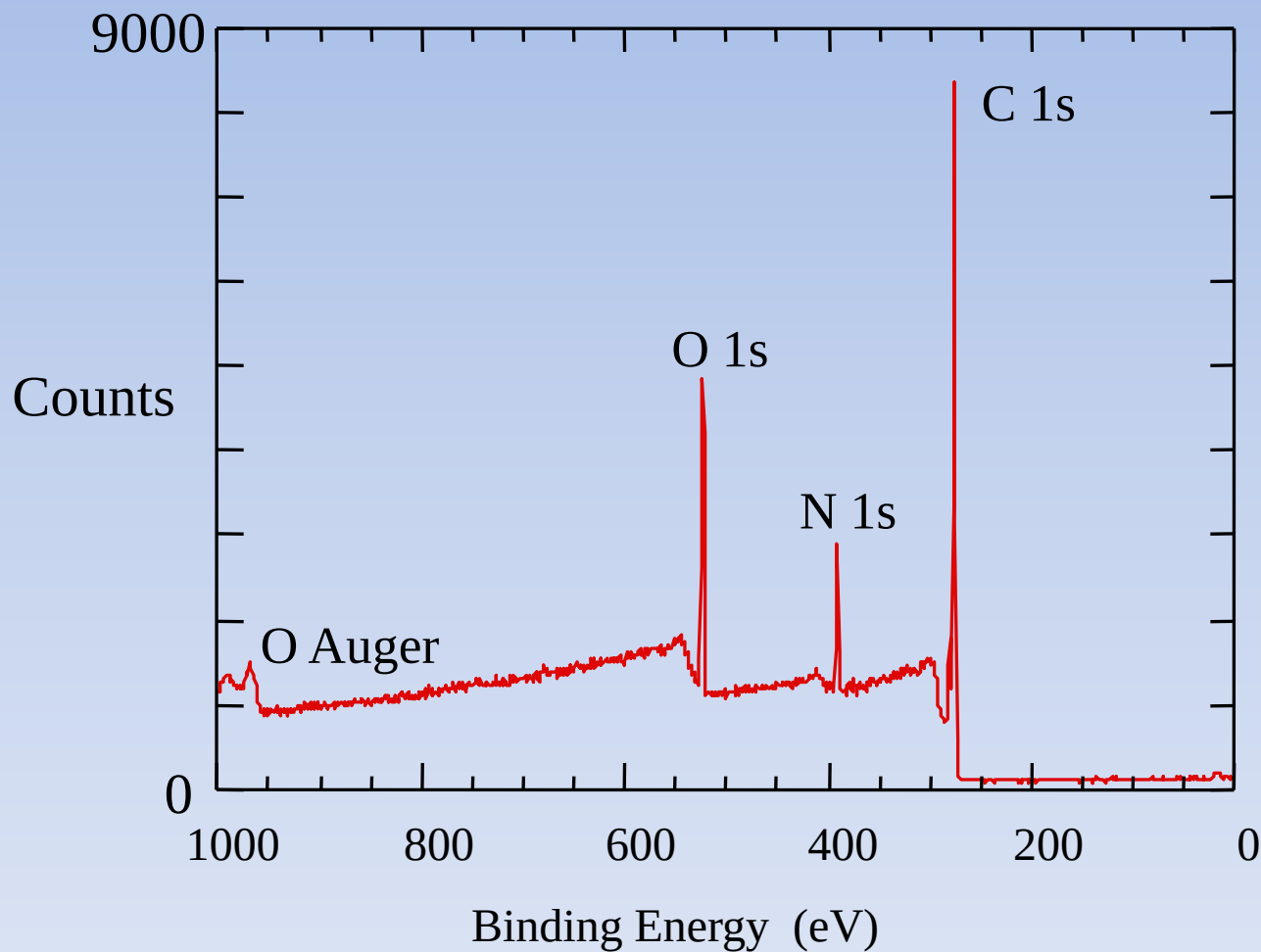


Auger Emission



X-ray Emission

Polyurethane XPS Survey Spectrum



Quantitation Results: Polyurethane Sample

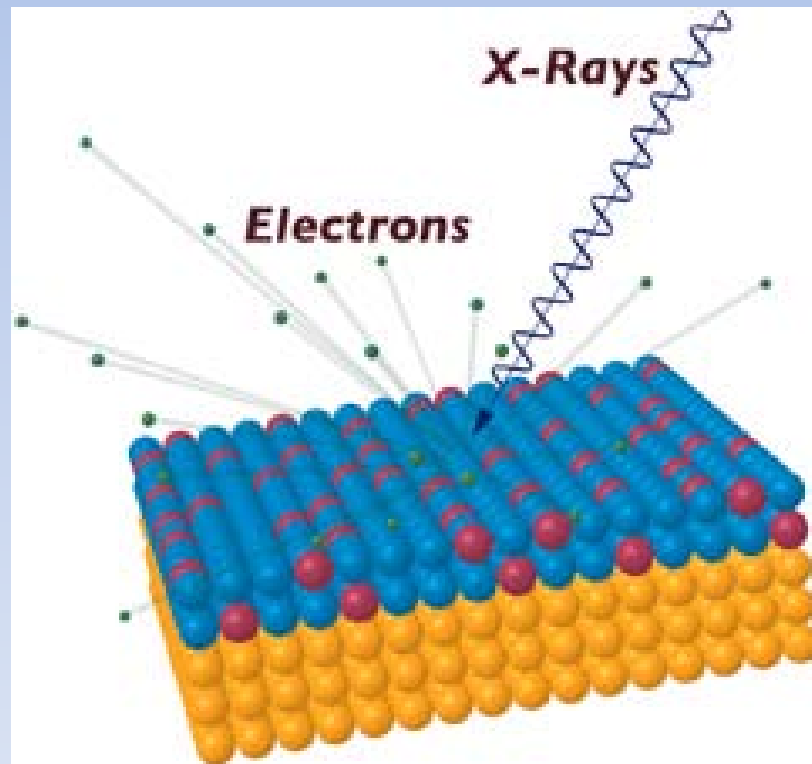
<u>Element</u>	Peak	<u>KE (eV)</u>	Atomic		
	<u>Orbital</u>		<u>s</u>	<u>area</u>	<u>percent</u>
carbon	1s	1200	1.00	26557	76.9
nitrogen	1s	1085	1.80	4478	7.7
oxygen	1s	955	2.93	13222	15.4

Quantitation Results: Polyurethane Sample

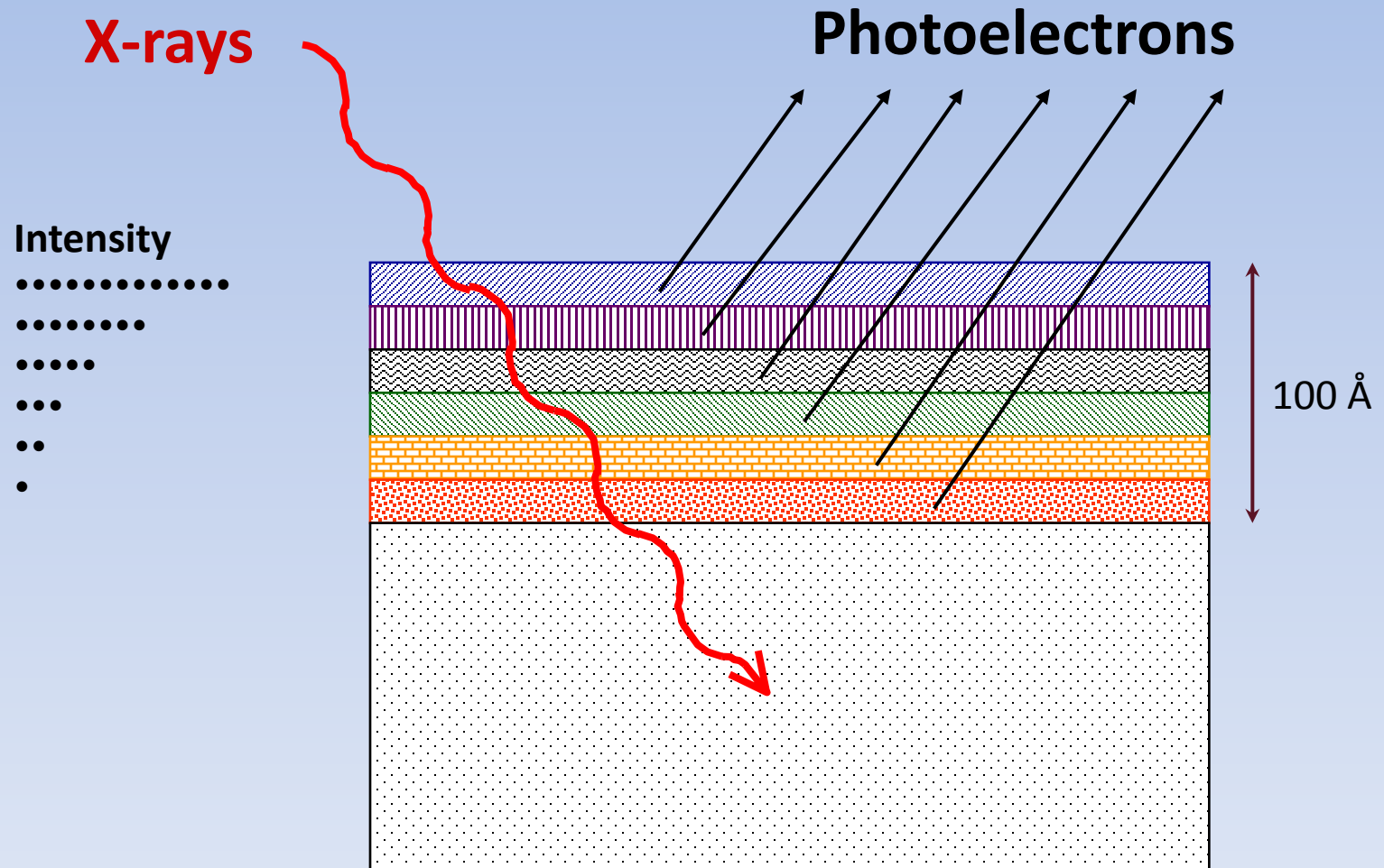
<u>Element</u>	Atomic percent	
	<u>XPS</u>	<u>Stoichiometry</u>
carbon	76.6 ± 1.0	76.0
nitrogen	7.9 ± 0.5	8.0
oxygen	15.5 ± 0.8	16.0

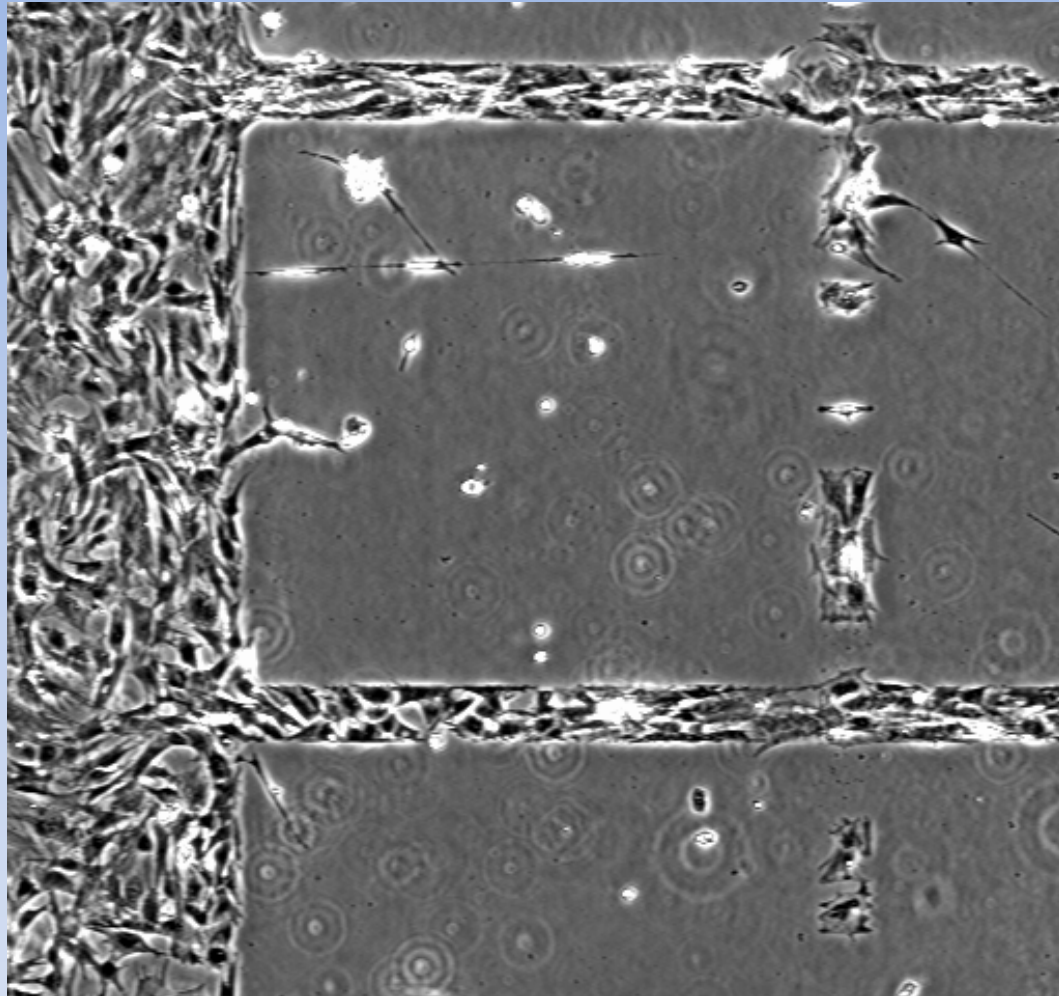
(Number of samples = 8)

Depth Profiling: Non-destructive and Destructive



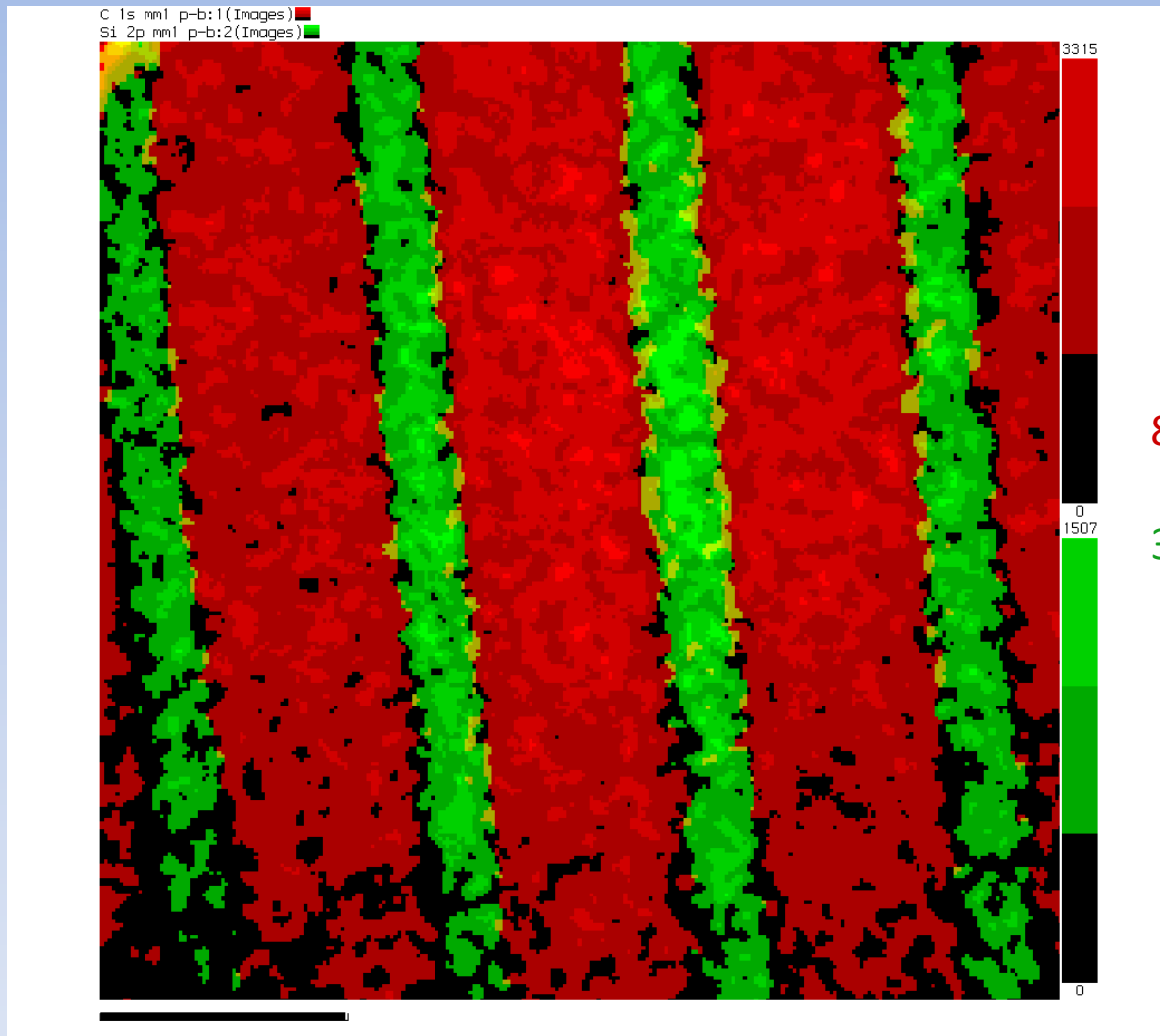
Photoelectron Intensity Decreases Exponentially with Depth from the Surface





Spatial Distribution of Bone Derived Cells on a Micropatterned Surface with Adhesive 100μm Wide Lines Separated by Non-adhesive 900μm Wide P(AAm-co-EG) IPN Squares

Mono XPS Imaging: C1s & Si2p Signals



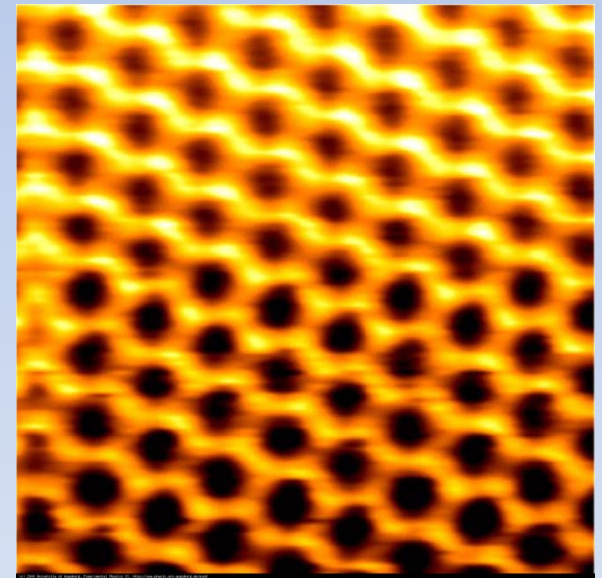
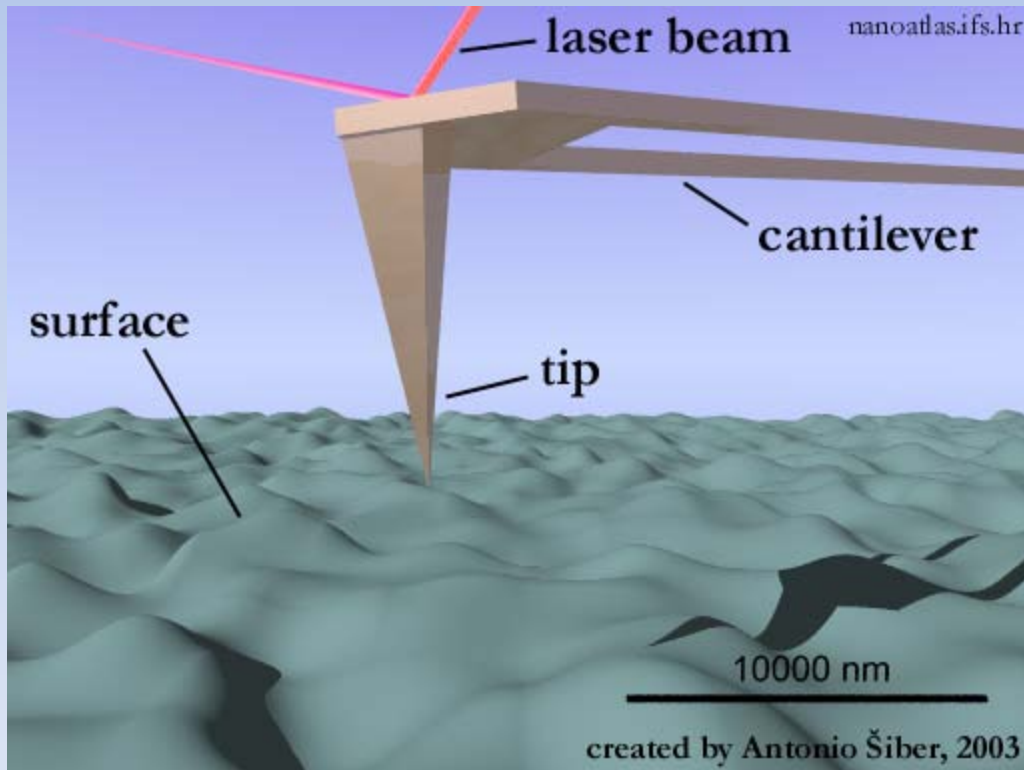
80 nm photoresist lines

30 nm Si lines

100 microns

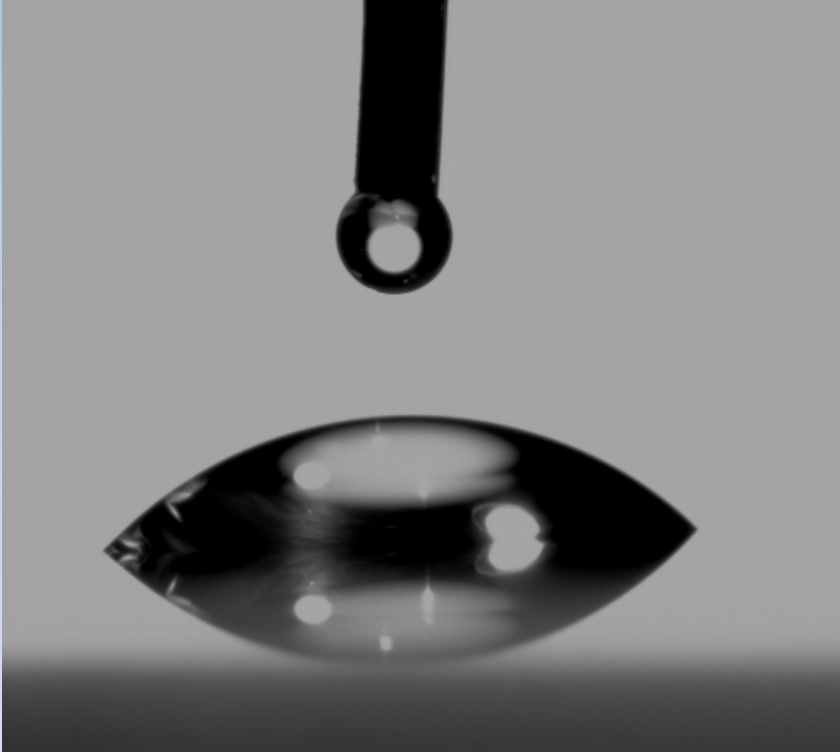
AFM

- Atomic force microscopy (AFM)

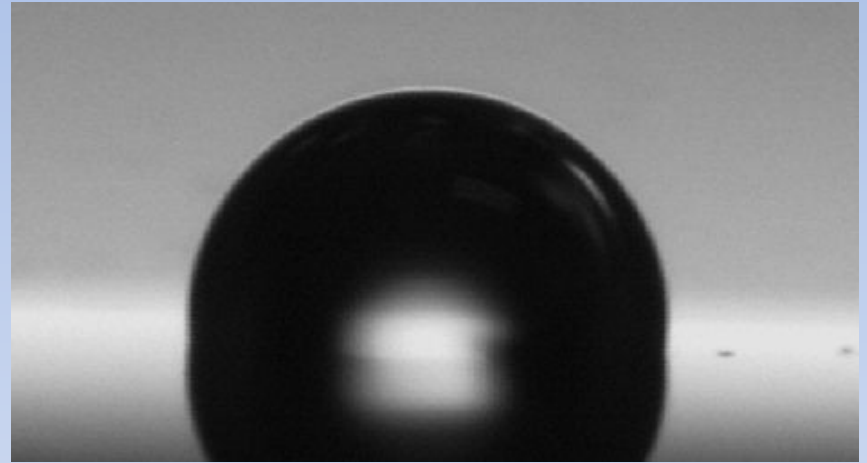


backreaction.blogspot.com

Contact Angle



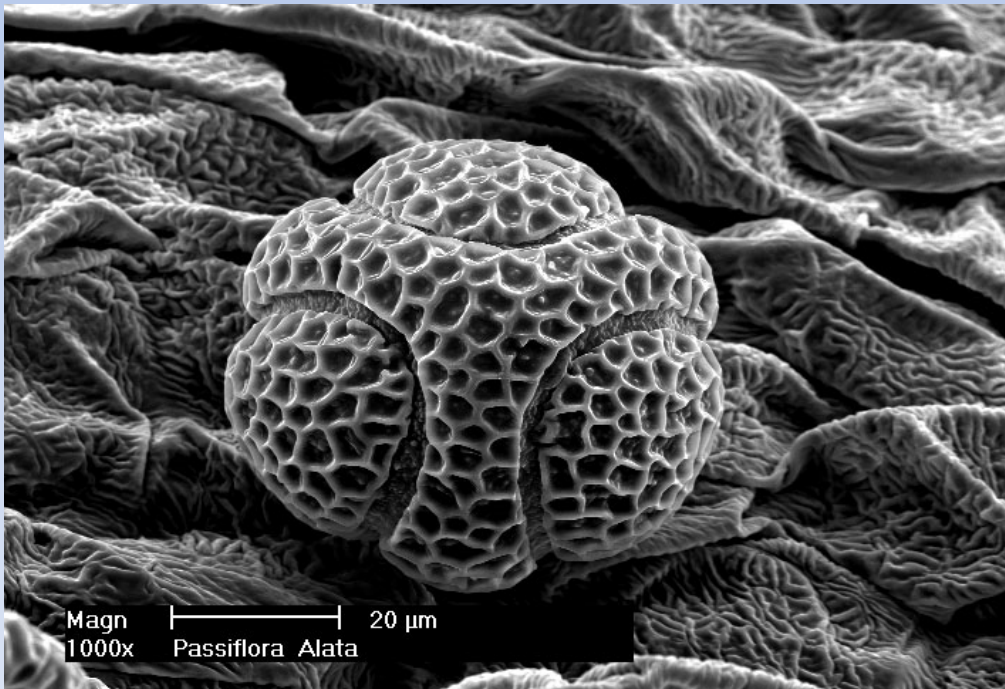
en.wikivisual.com



betterthandiamond.com

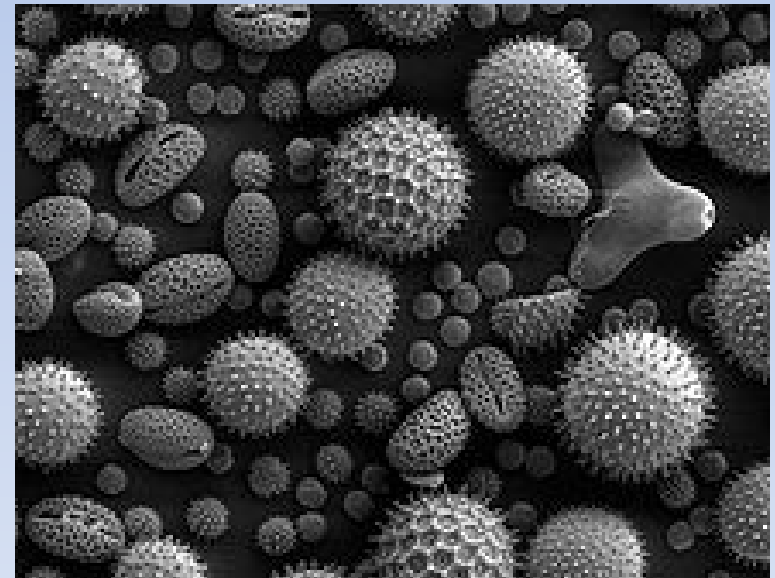
SEM

- Scanning electron microscope (SEM)



[Passiflora flower SEM](#)

www.passionflow.co.uk

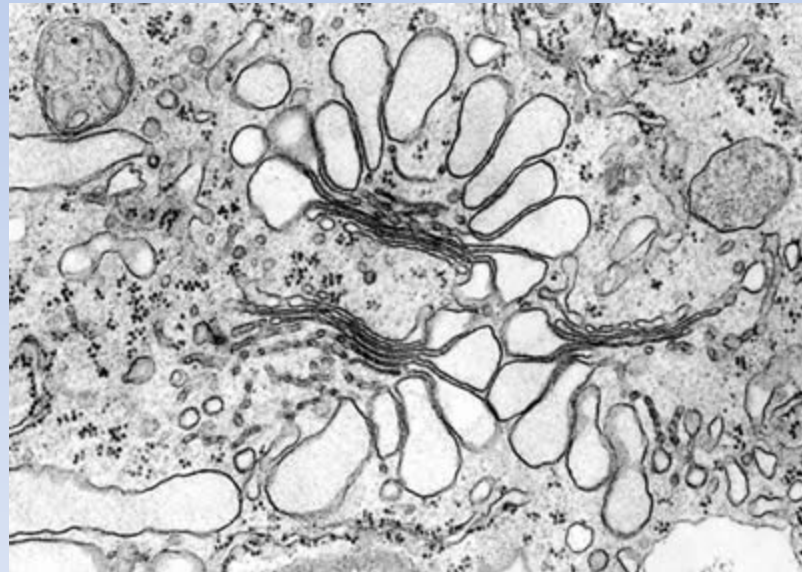


pollen

wikipedia

TEM

- Transmission electron microscopy(TEM)



Golgi

Biomaterials

Dolly Holt, SLCC

Definitions

Biomaterial science- The physical and biological study of materials and their interaction with the biological environment

Biocompatibility- the ability of a material to perform with an appropriate host response in a specific application

bioMATERIALS

BIOmaterials

Biomaterial Considerations

- Formability
- Biological interactions
 - toxicity, decomposition, cellular direction
- Physical Properties
 - surface properties-chemistry, morphology, roughness, mechanical properties-tensile, compressive, fatigue
- Part Design
- Qualification
- Liability

Major Classes of Biomaterials

- **Metals**

- Stainless steel, titanium, and other alloys
- Mostly crystalline solids composed of elemental, positively charged ions. Typical metal properties include- high melting points, high stiffness and strength, high conductivities, isotropic properties

- **Ceramics**

- alumina, other inert ceramics, biodegradable compositions Characterized by ionic or covalent bonding, generally crystalline Properties- Low conductivities, stiff and brittle, bioinert, obvious choice for teeth and bones

- **Polymers**

- Synthetic
- Natural
- Hybrids and composites

Biomaterial Degradation

- Bulk erosion
 - water penetration rate is greater the polymer dissolution rate
- Surface erosion
 - water penetration rate is less then polymer dissolution rate
- Hydrolytic
 - PLGA is hydrolytic and is degraded when water bonds to carbon and breaks the bond
- Oxidative
- Proteolytic
- Enzymatic

Interface

- Interface- the boundary region between two adjacent bulk phases.
- Examples are L/L, G/S, S/S, V/L, L/S. We recognize S/G, S/L, and L/V as surfaces.

Surfaces

- Surfaces are material dependent
 - metals v. polymers v. ceramics
 - can have different chemistries
 - polar, apolar, charge, reactivity, patterned
 - morphology can differ
 - smooth, rough, stepped
 - Molecules in the bulk of a material have a low relative energy state due to nearest neighbor interactions.
 - Molecules at a surface are in a state of higher free energy than those in the bulk
 - in large part due to the lack of nearest neighbor interactions at a surface

Biomaterial Modifications

- **Plasma**- low pressure, high frequency, discharge generates electrons (breaking bonds). Used for chemical transformation, crosslinking and chain breaking
- **Photoactivation**- activation of reactive molecules by light in the gas phase, Br-CN can be broken by light into Br⁺ and CN⁻ then added to a polymer
- **Lithography**- spin coating of a substrate with a photosensitive polymer layer as mask material for deposition or etching process, exposure, and development (Anything exposed through the mask will be removed)
- **Microcontact Printing**- Transfer of SAM precursor with elastomeric stamp onto substrate, master generation by photolithography and similar techniques, stamp is obtained by casting of elastomer over master. Pattern generation by stamping of SAM precursor onto substrate.
- **Chemical Coating**
- **Surface treatment with a natural material**

Common Biomaterial Polymers

- Polyethylene- Hip prostheses, catheters
- Polysulfone- heart valves
- PLA-PGA-PLGA- drug delivery devices, nanoparticles

Biomaterial Topography

- Cells may be able to adhere better to scaffolds with nano-sized pores. Porosity can be changed by altering polymer concentration. Adjusting the thermal bonding time can increase interconnectivities.
- **Roughness**- increases tissue integration, direct cell adhesion.
- **Porosity**- increases cell adhesion, migration, and proliferation

Polymeric scaffold production

- Phase Separation
 - Employs thermodynamic principles to create polymer-rich and polymer-poor phases, resulting in a highly porous network
 - Polymer solution cooled to below T_m of solvent to produce liquid-liquid phase separation and quenched to form a 2-phase solid
 - Remove solvent by sublimation, leaving porous polymer scaffold
 - Advantage
 - Larger pore size and interconnectivity (90%, 100 μm)
 - Bioactive molecules can be included during process
 - Disadvantage
 - Incorporating and releasing large protein structures may result in loss of activity
 - Use of organic solvents: select solvents that are not harsh to bioactive molecules
- Using CAD to create 3D objects. 3-D printing, stereolithography, fused deposition modeling, 3-D plotter, phase-change jet printing. Can control pore size, porosity, and pore distribution, consistency but high cost and time consuming
- Freeze drying- add water to organic PLGA solution to create emulsion. Homogenize mixture, pour into mold and quench (quick freeze) in liquid N_2 . Freeze-dry the scaffold to remove water and solvent, up to 95% porosity and small pore sizes 13-35 μm with interconnectivity. High density porous scaffolds, limitation of pore sizes.

Biomechanics

Dolly Holt, SLCC

Biomechanics

- The study of the structure and functions of biological systems by means of the methods of mechanics

Hatze, 1974

“physics of human movement”

Biomechanics

- The application of mechanical principles to biological systems (i.e. human, animal, plant, organs, cells)
- Developed in the early 1970s
 - Describing the application of engineering mechanics to biological and medical systems.

Hall, Susan J. (1999) Basic Biomechanics.
Boston: McGraw-Hill Companies, Inc.

Applications

- Model development
 - *In silico* modeling of implants, physiology, etc.
 - *In vitro* models of the body, organs, cells, etc.
- Prostheses
- Implants
- Design amusement park rides

Required knowledge

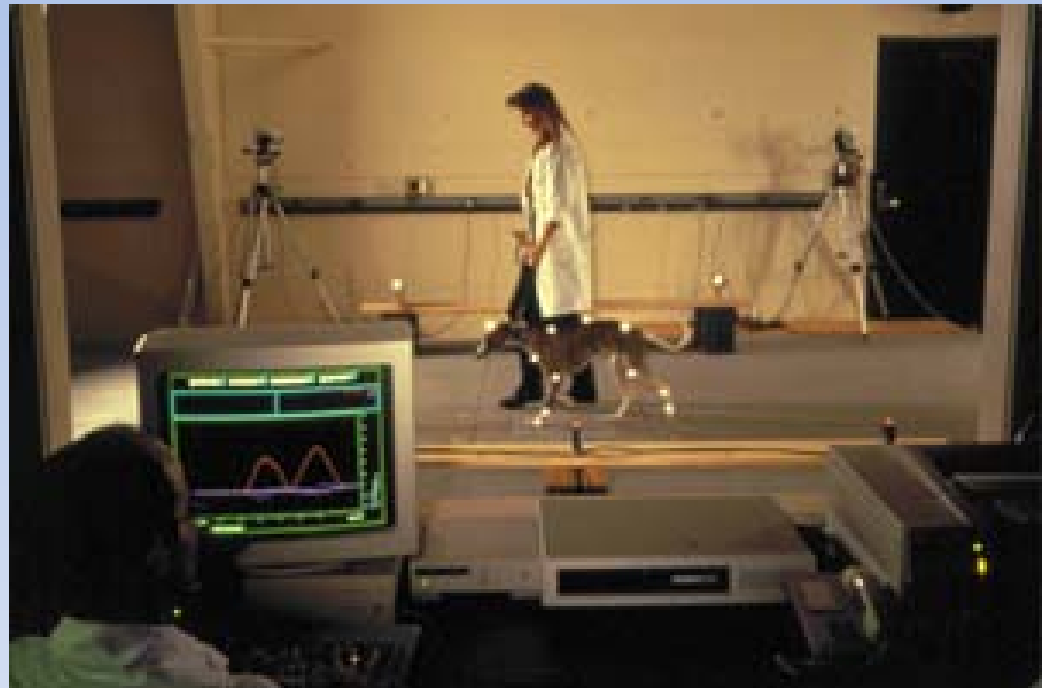
- Biology
- Mechanics
- Math
- Physics

Motion

- Mechanics
 - Study of physical actions and forces
- Kinematics
 - describing movements with respect to time and space
 - how fast, how high, etc.
 - without consideration given to its mass or the forces acting on it.
- Kinetics
 - The study of forces associated with motion
 - Example: Pushing on the table may or may not move the table, depending upon the strength and direction of the push

Types of motion

- Linear (translation)
 - all parts travel the same distance in the same time along the same path
- Angular motion
 - parts rotate around an axis of rotation
- Most movements are usually a combination of linear and angular motion



Measures

- Center of gravity
 - the point about which a body's weight is equally balanced in all directions (Hall, 1995)
- Kinematics: Spatial and temporal components
 - Speed
 - Velocity
 - Acceleration

Kinetics

- Inertia
 - resistance to being moved
- Force
 - a push or pulling

Laws of Motion

- First Law (Inertia)
 - a body continues in a state of rest or uniform motion until acted upon by an external force of sufficient magnitude to disturb its current state
- Second Law (Acceleration or $F=ma$)
 - the acceleration of the body is proportional to the force exerted on it and *inversely proportional to its mass*
- Third Law (action-reaction)
 - every action has an equal and opposite reaction (important for conservation of momentum)

Angular Motion

- When a force is not exerted along a line that passes through a body's center of gravity (eccentric force), the body will experience angular (rotary) motion

Measures

- Angular displacement
 - change in location of rotating body
- Angular distance
 - angle between initial and final positions when measured by following the path of the body
- Angular Velocity
 - angular displacement / time (degree/s)
- Angular Acceleration
 - angular velocity / time (degrees/s²)

Measure

Angular Kinetics

- Torque
 - turning effect on a body measured as the product of force and moment arm length (e.g., tire iron)
- Moment of inertia
 - resistance to rotary motion that results from combination of mass and distribution of the mass of an object

Moment of Inertia: Relative

- Ice skater
- Tuck
- Pike
- Full body rotating around center of mass
- Full body rotating around a bar

Fluid Mechanics

- Drag
 - Fluid force that opposes the forward motion of the body and reduced the body's velocity
- Lift
 - Component of air resistance that is directed at right angles to the drag force

Drag

Fluid force that opposes the forward motion of the body and reduced the body's velocity.

- Surface (hydrodynamic drag)
 - referring to interaction between body surface and the water
- Profile (Form)
 - refers to resistive forces resulting from poor body position

Surface Drag



Water particles attract other water particles and will increase with “roughness of skin”

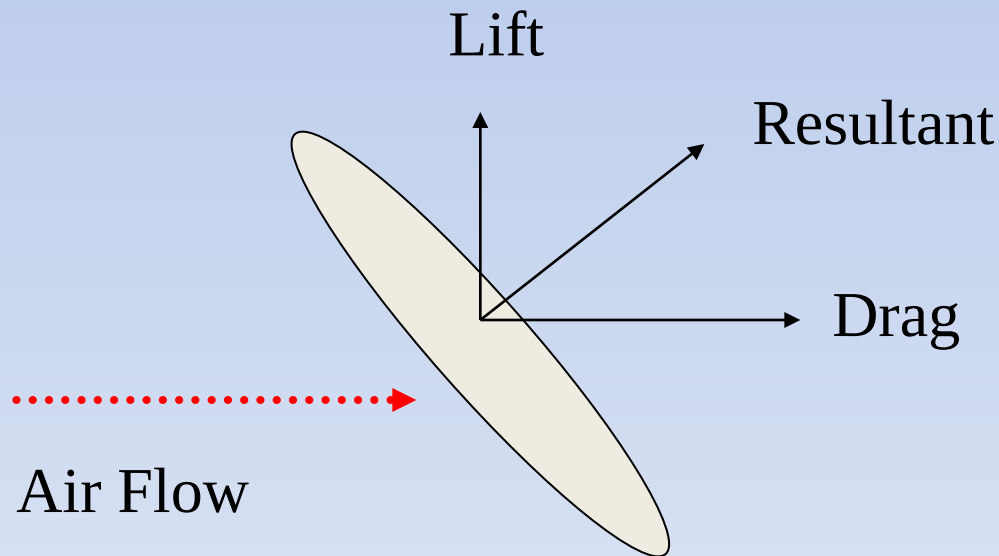
Profile Drag

- Low pressure pocket forms and pulling on the cyclist
 - as velocity doubles, drag quadruples
- Important factors:
 - Shape
 - smoothness
 - orientation (crouch can lower resistance ~30%)
- Design modifications
 - Frame designs on bikes are often “tear-shaped” to reduce drag
- Drafting within 1 m can reduce drag accounting for 6% of energy cost (e.g., ducks flying)



Lift

Component of air resistance that is directed at right angles to the drag force



Lift

High velocity/Low Pressure



Low velocity/High Pressure

- Bernoulli's Law
 - faster air has lower air pressure, and thus the high pressure beneath the wing pushes up to cause lift

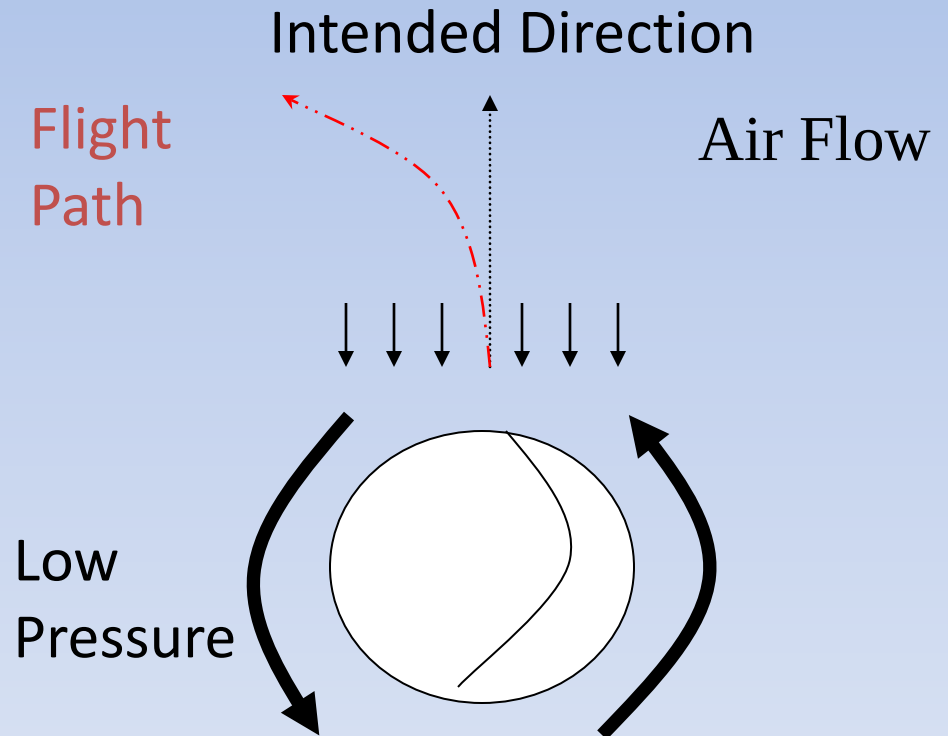
Formula I

- Inverted wing
 - Produce negative lift or downforce to provide greater tire adhesion



Curve Ball

- Magnus Effect
 - As the spinning object pushes the air from one side to the other, it will create a lower pressure zone, making the object move faster on one side.



Summary

- Kinematics
 - linear motion
 - displacement, velocity
 - Angular Motion
 - angular displacement
- Kinetics
 - linear motion
 - mass, inertia
 - Angular Motion
 - torque, moment of inertia
- Fluid dynamics
 - drag
 - lift

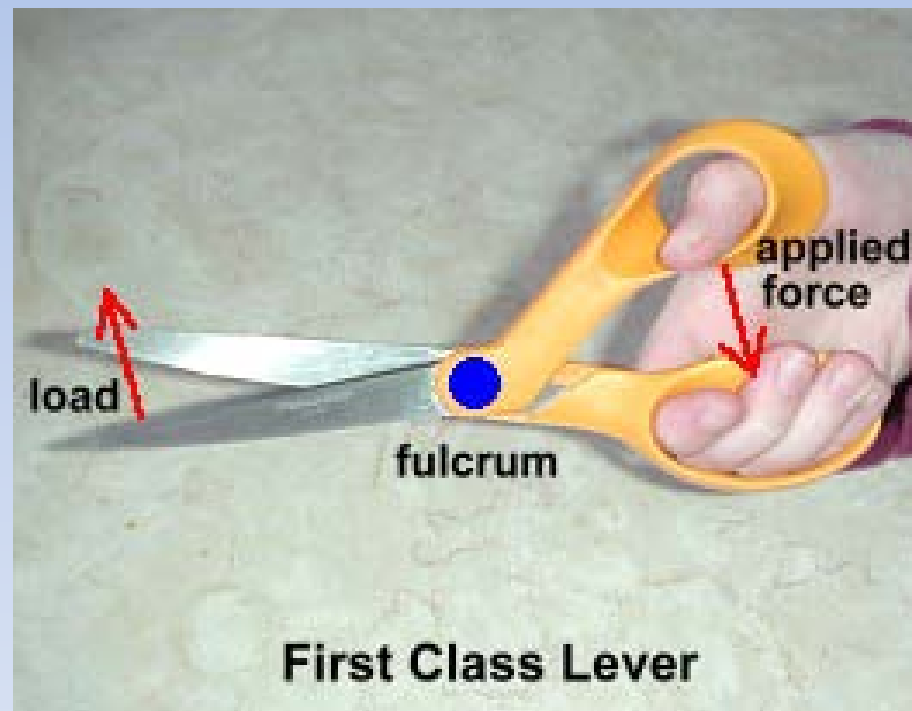
Machines

- The musculoskeletal system
 - series of simple machines
- Machines are used to create a mechanical advantage
 - balance multiple forces
 - Enhance force thus reducing the amount of force needed
 - Enhance range of motion or speed of movement

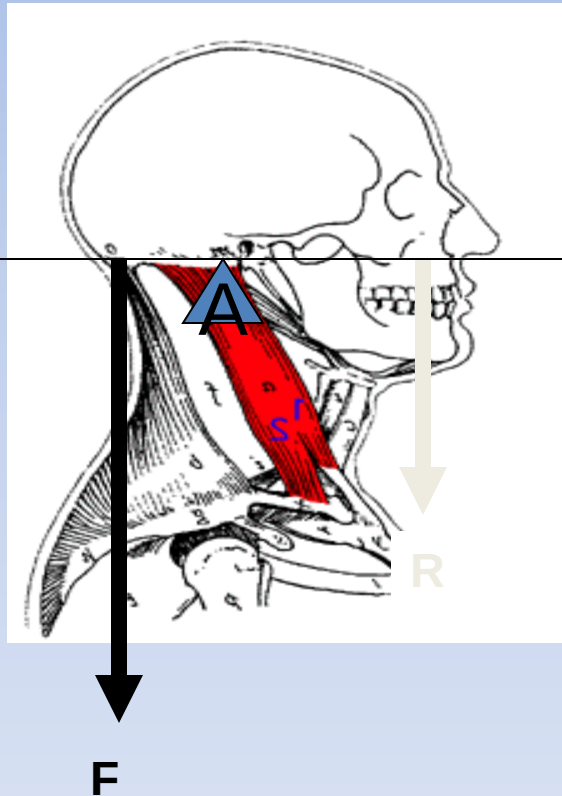
Levers

- Levers
 - used to alter resulting direction of applied force
 - A lever is a rigid bar (bone) that turns about an axis of rotation or fulcrum (joint)
 - The lever rotates about the axis as a result of a force (from muscle contraction)
 - The force acts against a resistance (weight, gravity, opponent, etc.)

First Class



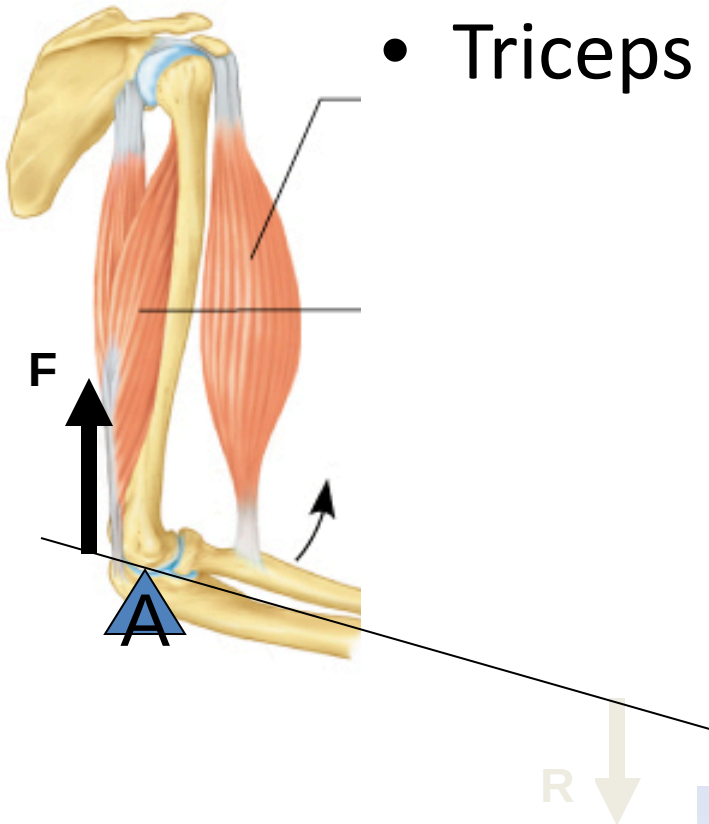
First Class



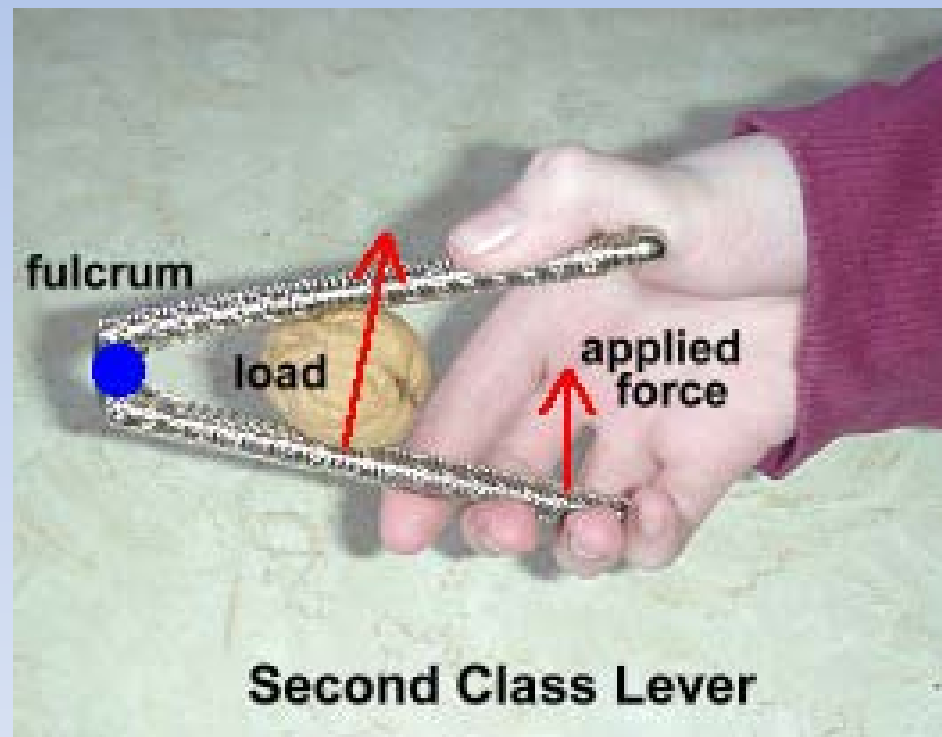
- Neck extension
- Erector spinae and Splenius

First Class

- Elbow extension
- Triceps

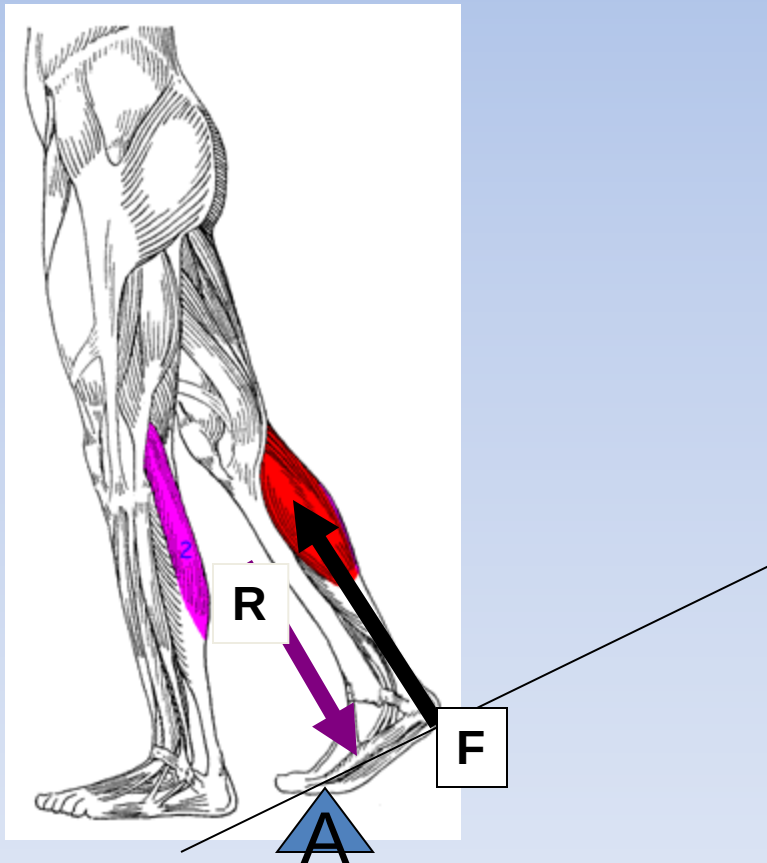


Second Class



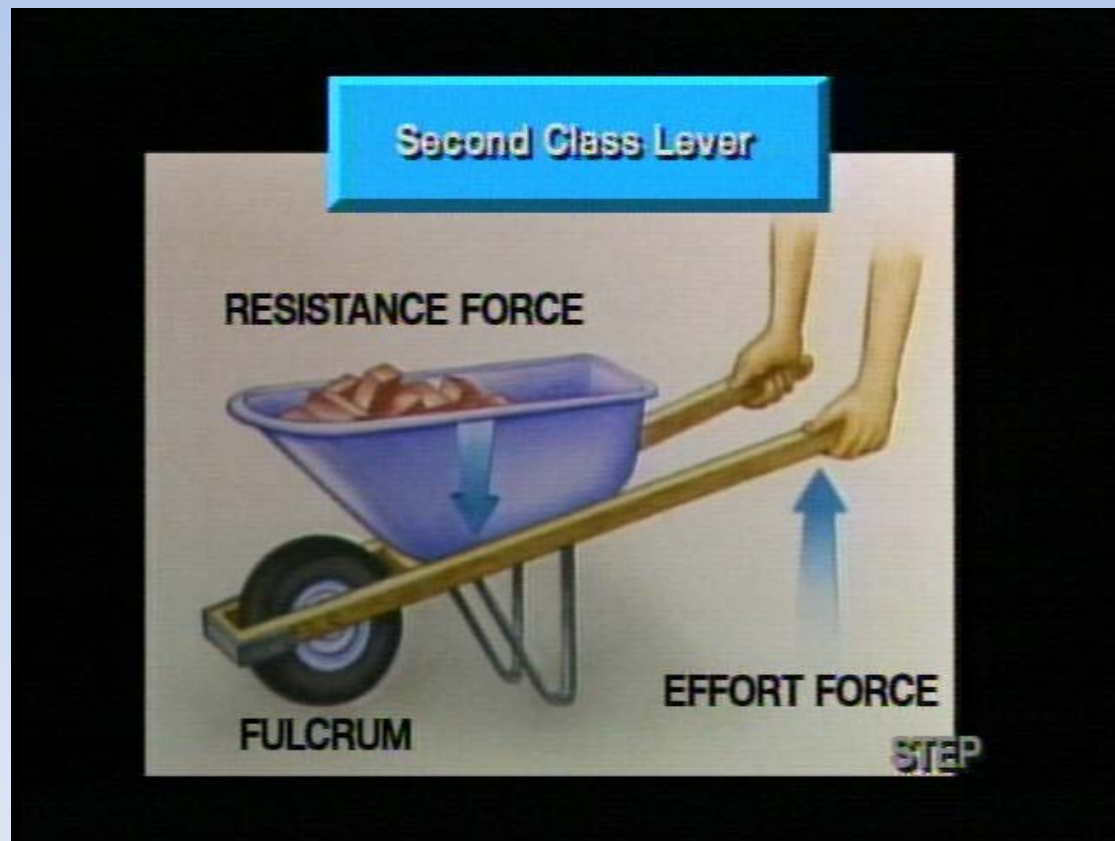
Second Class

- Plantar flexion
- Gastrocnemius and Soleus

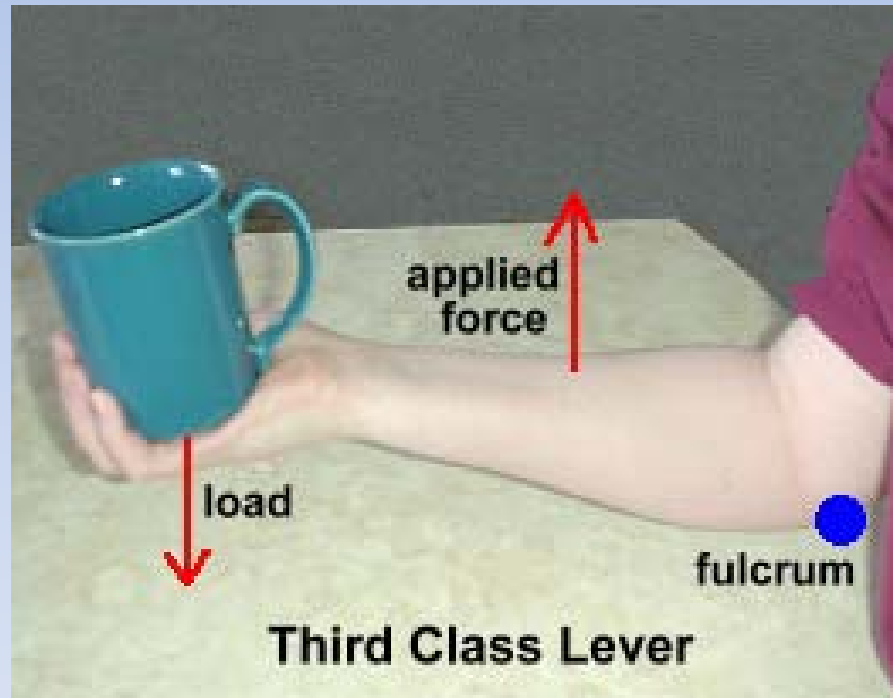


Second Class

- Designed more for force

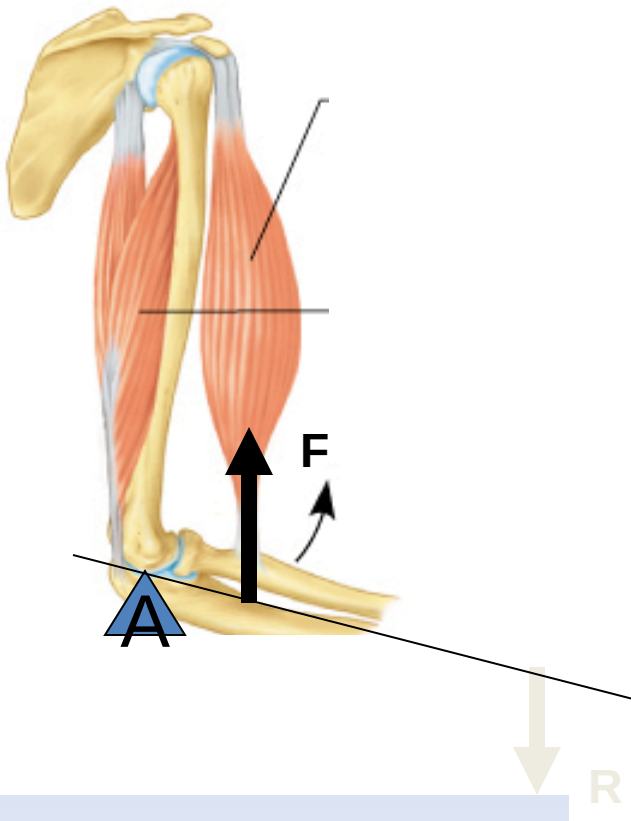


Third Class



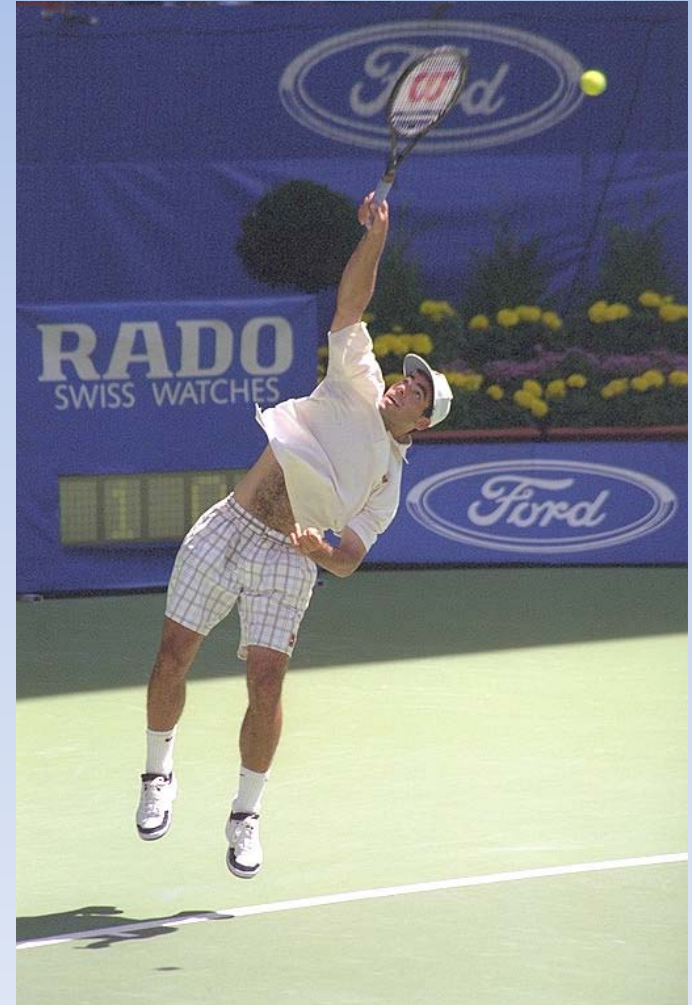
Third Class

- Elbow flexion
- Biceps brachii and Brachialis



Lever Length

- A longer lever would increase speed at the end of the racquet
 - unless the extra weight was too great. Then the speed may actually be slower.

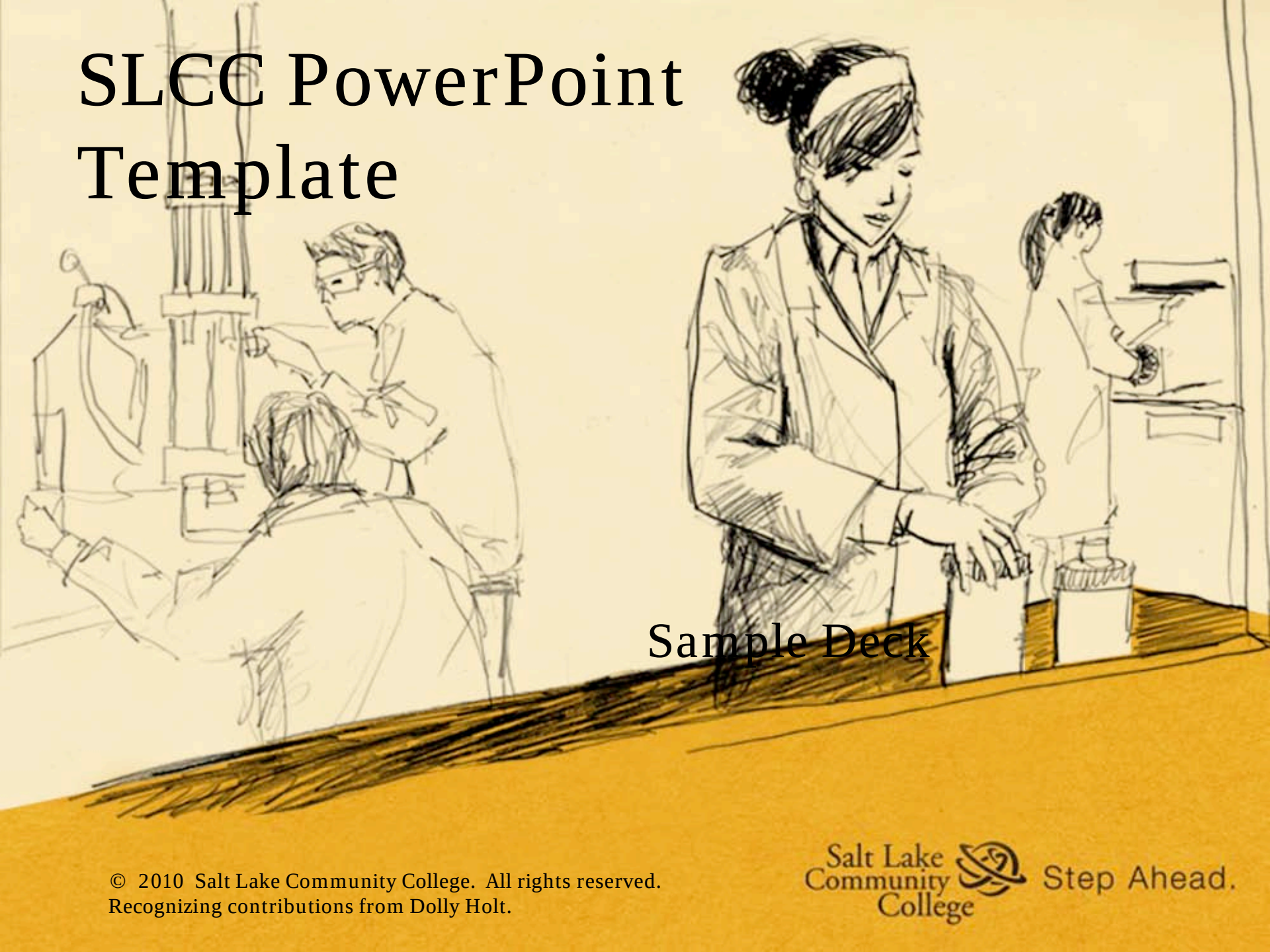




SLCC PowerPoint Template

Sample Deck

SLCC PowerPoint Template



Sample Deck

SLCC PowerPoint Template

Sample Deck



HEADLINE – GEORGIA 44 pt

- This page and the title slide are set up in the Master Templates. Simply hit new slide and select the layout.
- Headline should be all caps.
- Bullets should be lowercase and brief.
- This text is Georgia 28 pt
 - The second level is Georgia 24 pt
 - The third level is Georgia 20 pt

ANOTHER OPTION

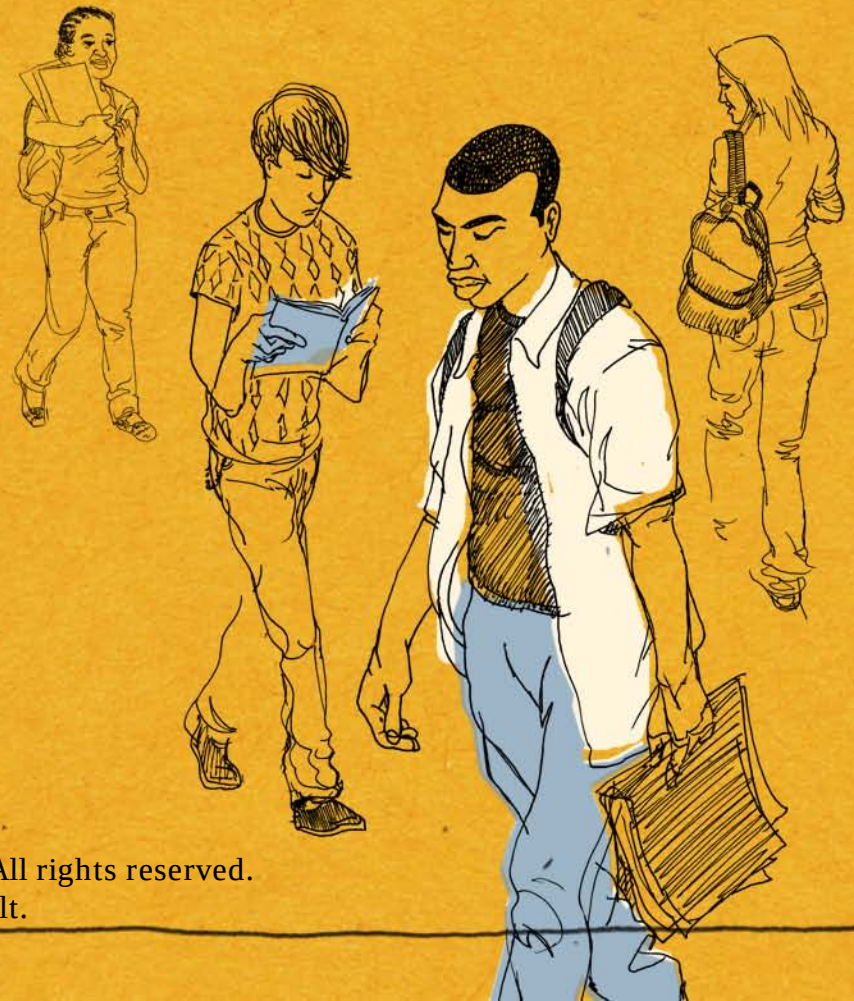
- This slide option will need to be copied/pasted (not a Master template).
- Good option if you want to show a photo.

ANOTHER OPTION

- This slide option will need to be copied/pasted (not a Master template).
- This design would be good for photos, graphs or charts.

OPTION

- This slide option will need to be copied/pasted (not a Master template).
- Illustration slide to break up the presentation.

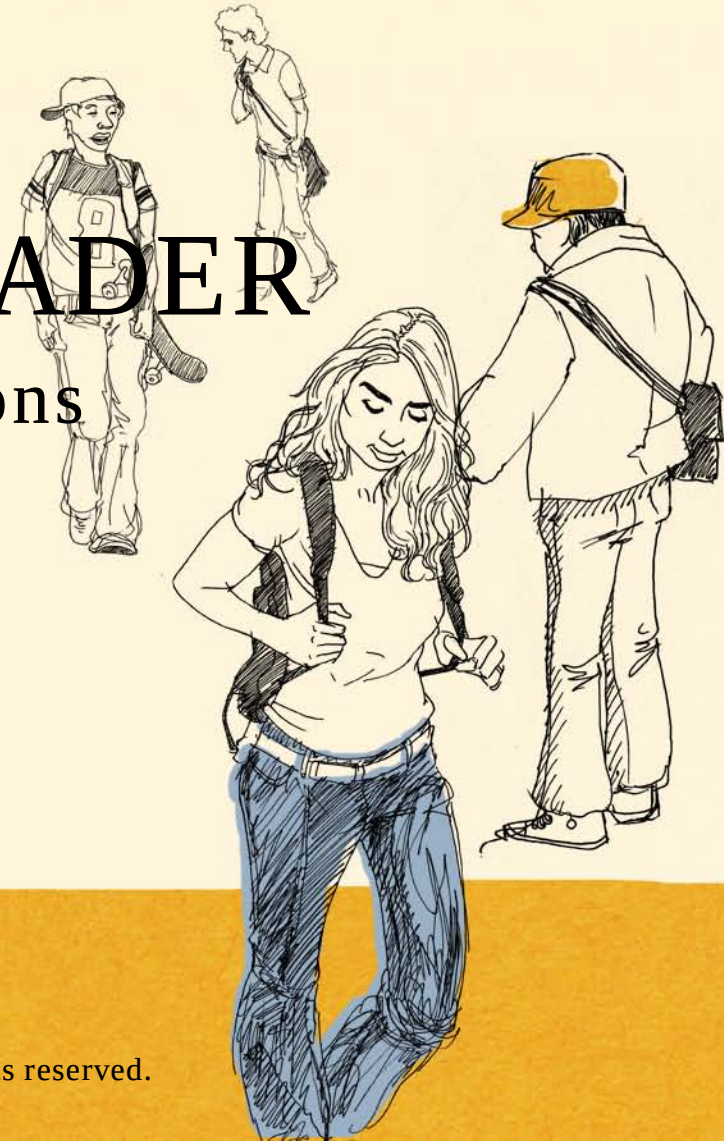


SECTION HEADER

Must copy/paste this slide, when needed.

SECTION HEADER

With Illustrations



COLORS

To select a specific color, click on “more colors” in the font or fill menus. Then click on the “custom” tab. Fill in the RGB formulas shown below.

R: 238

G: 178

B: 17

R: 255

G: 238

B: 198

R: 103

G: 153

B: 200

R: 153

G: 71

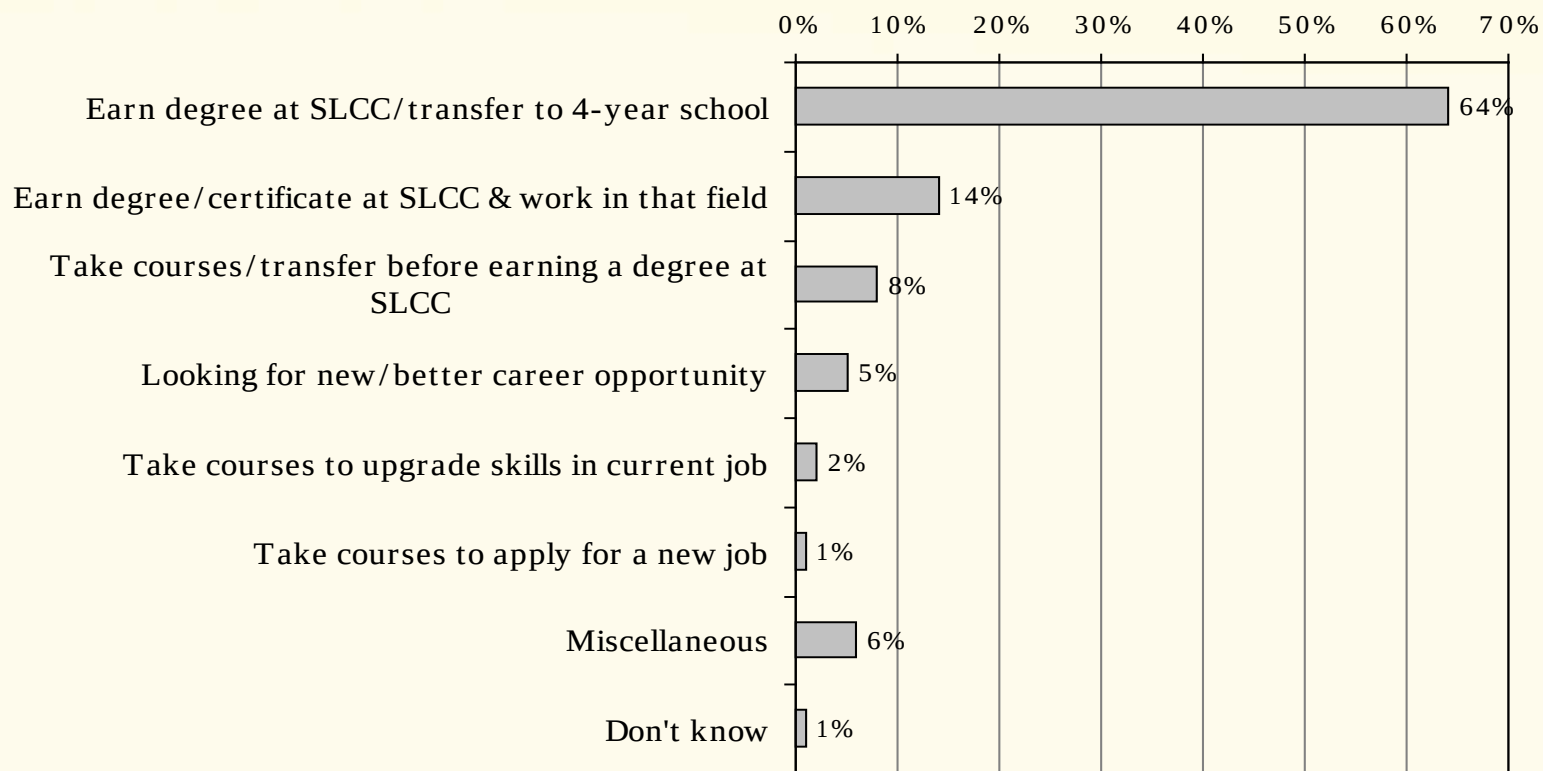
B: 8

SAMPLE CHART

This is what a sample chart could look like:

	Number of Responses	Percentage
Not at all	8	30%
Probably not	3	11%
Maybe	6	22%
I'm leaning towards it	5	19%
Definitely	5	19%

SAMPLE GRAPH



Environmental Health and Safety/ laboratory techniques

Dolly Holt, SLCC

Biosafety Levels (BSL)

- BSL I: BSL-1 is appropriate for working with microorganisms that are not known to cause disease in healthy human humans. This is the type of laboratory found in municipal water-testing laboratories, in high schools, and in some community colleges teaching introductory microbiology classes, where the agents are not considered hazardous.
- BSL II: The facility, the containment devices, the administrative controls, and the practices and procedures that constitute BSL-2 are designed to maximize safe working conditions for laboratorians working with agents of moderate risk to personnel and the environment. Unlike the guidelines for BSL-1, there are a number of immunizations recommended before working with specific agents
- BSL III: BSL-3 is suitable for work with infectious agents which may cause serious or potentially lethal diseases as a result of exposure by the inhalation route. BSL-3 laboratories should be located away from high-traffic areas. Examples of agents that should be manipulated at BSL-3 are *M. tuberculosis* (research activities), St. Louis encephalitis virus, and *Coxiella burnetii*.
- BSL IV: **Biosafety Level 4** is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. Members of the laboratory staff have specific and thorough training in handling extremely hazardous infectious agents and they understand the primary and secondary containment functions of the standard and special practices, the containment equipment, and the laboratory design characteristics. Access to the laboratory is strictly controlled by the laboratory director. The facility is either in a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building.

“aseptic” and “sterile”

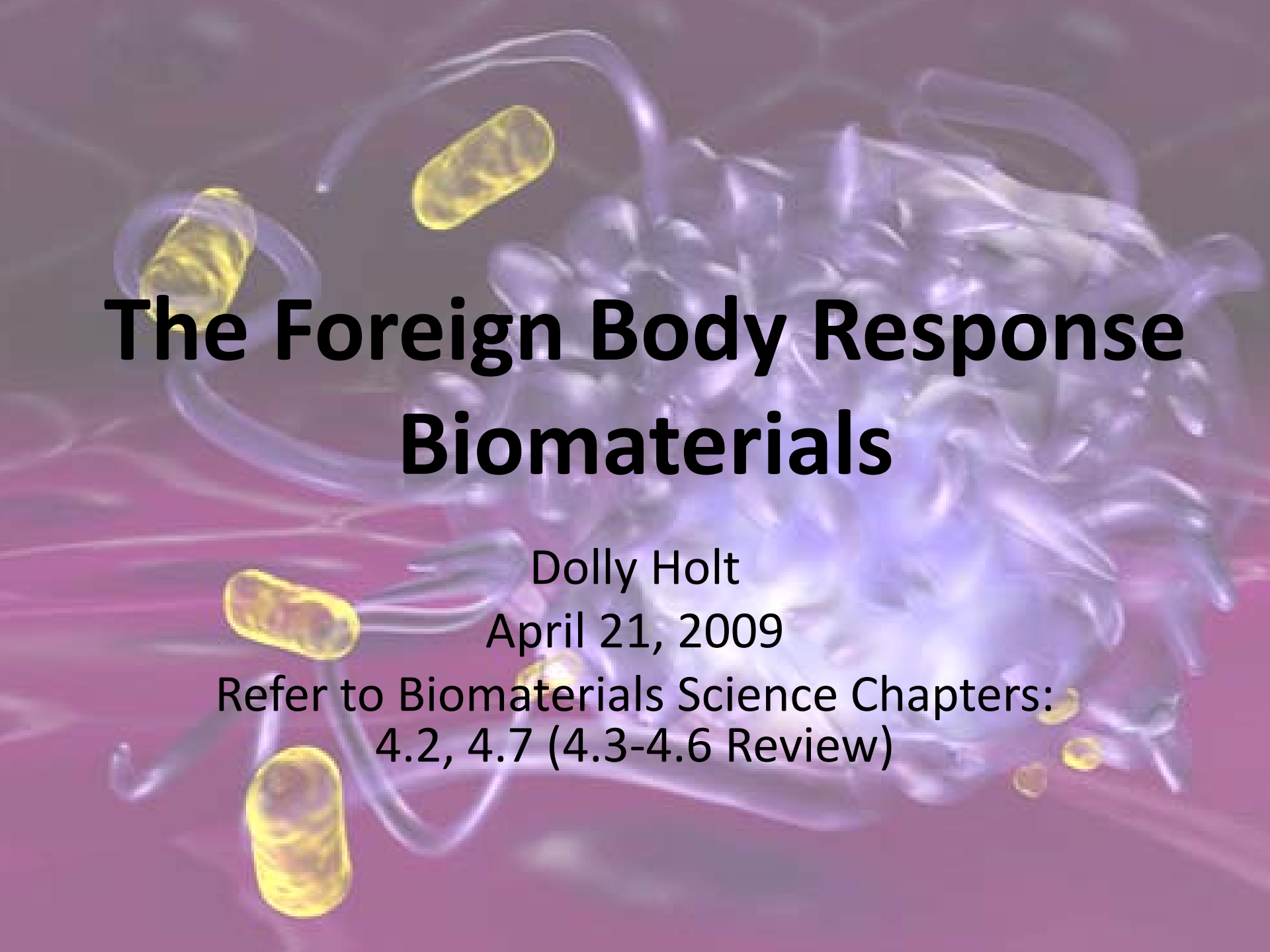
Aseptic-Using methods to protect against infection by pathogenic microorganisms

Sterile- Free from live bacteria or other microorganisms

Sources of contamination for cell cultures

- Fungus
- Yeast
- Bacteria
- mycoplasma
- Viruses
- Other cell lines (eg. HeLa cells, RAW cells)

Guest Lecture by an individual in the office of
Environmental Health and Safety.

A microscopic image showing a dense cluster of cells, likely fibroblasts, with a purple and blue color scheme. Several yellow, rod-shaped bacteria are visible, some of which are being engulfed by the cells, illustrating the foreign body response.

The Foreign Body Response Biomaterials

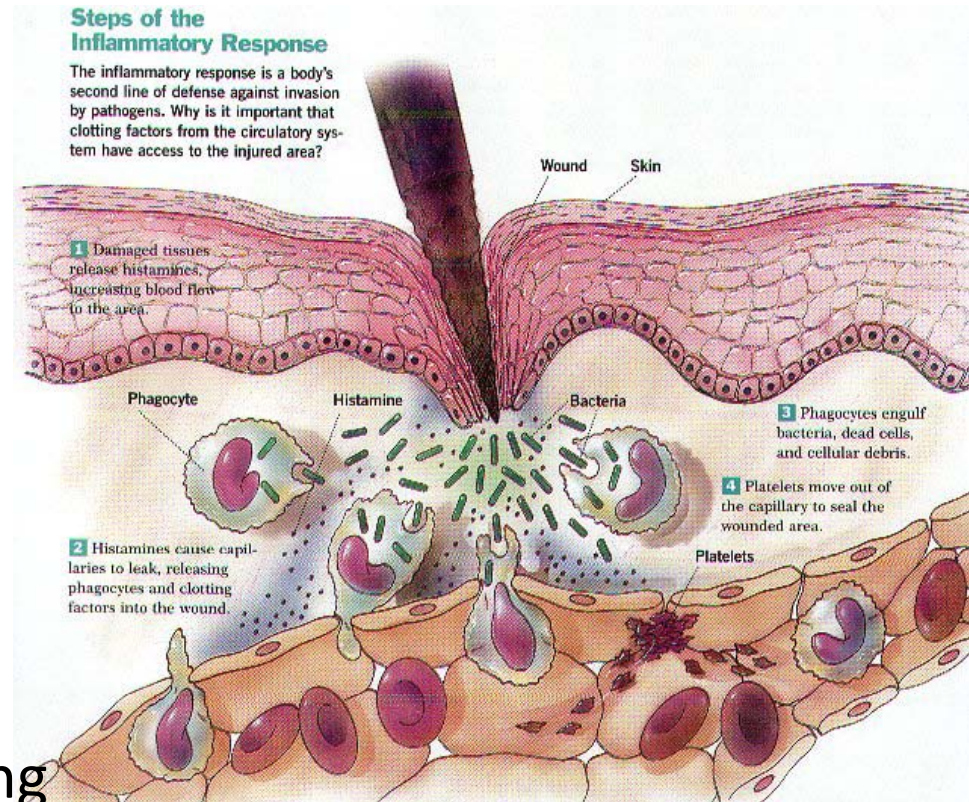
Dolly Holt

April 21, 2009

Refer to Biomaterials Science Chapters:
4.2, 4.7 (4.3-4.6 Review)

Sequence of Events Following Trauma (Review)

- Injury
- Acute inflammation
- Granulation tissue
- Mature extracellular matrix
- Wound contraction, Scar formation, and remodeling



<http://www.biologymad.com/Immunology/inflammation.jpg>

Foreign body reaction (FBR): chronic unresolved wound healing around an implanted material (multiple manifestations)

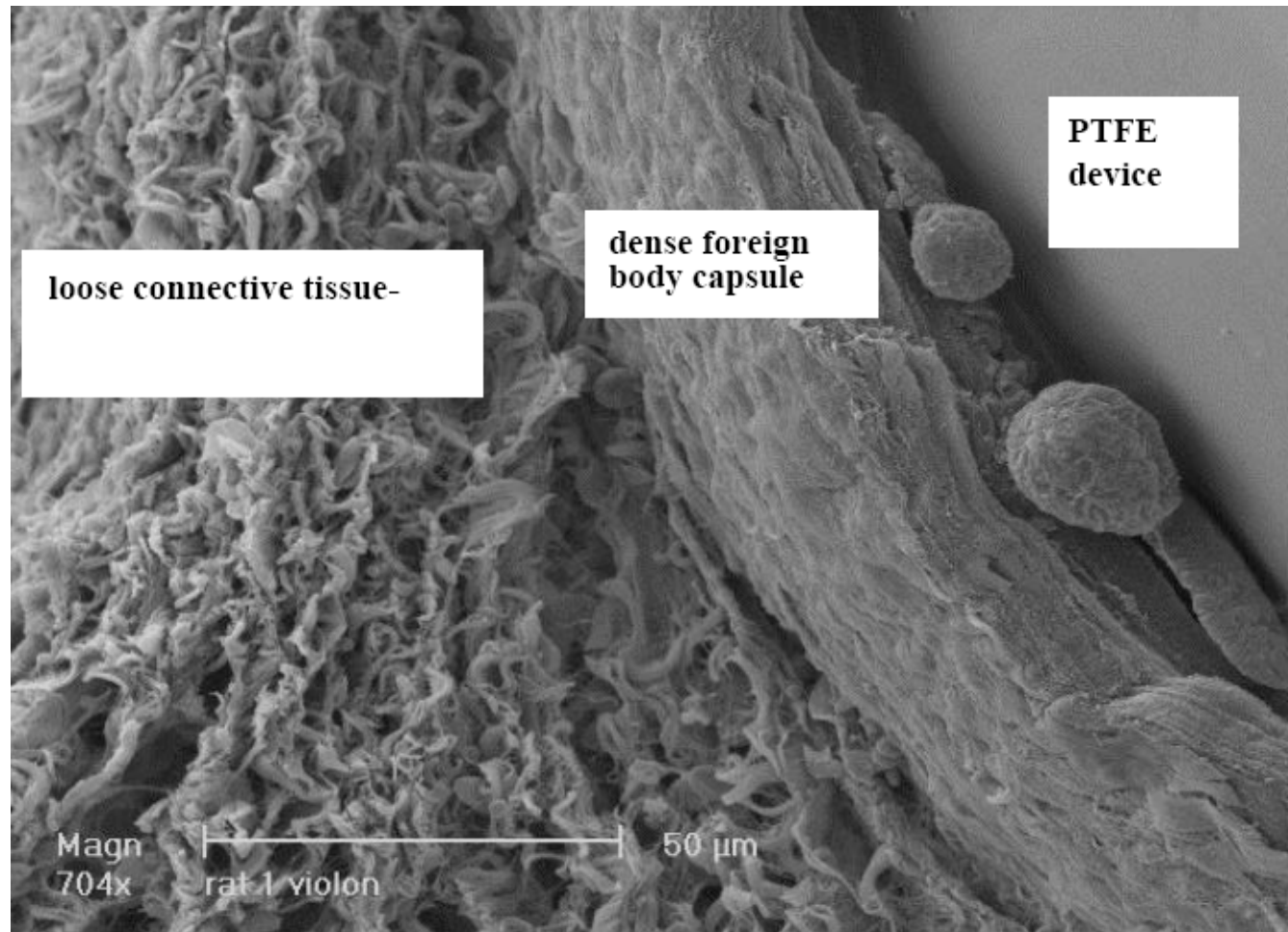


Photo: courtesy K. Ward

Stages of the FBR

1. Injury
2. Blood-Material Interactions
3. Provisional Matrix Formation
4. Acute Inflammation
5. Chronic Inflammation
6. Granulation Tissue
7. Foreign-body Reaction
8. Fibrosis-Fibrous Capsule Development



Stage 1

Injury

Damage to vasculature

- Infiltration of Blood into Extracellular Space
 - Hemolysis and ischemia
 - loss of oxygen and other nutrients -> necrosis
 - Inflammatory cytokines, reactive oxygen intermediate (ROIs) and Nitric oxide (NO) -> Apoptosis and cytotoxicity
- Changes in vascular flow, caliber, and permeability
- Vasoconstriction
- Hemostasis

Stage 2

Blood-material interactions

(seconds, minutes)

Protein Adsorption (Review)

- Cells never “see” surface, only adsorbed protein layer
 - In less than 1 second, lysozyme reached half of its steady-state value (page 241)
- Denaturation
 - Dependent upon protein, time adsorbed, solvent, substrate etc.
 - Not always complete, enzymes retain some activity

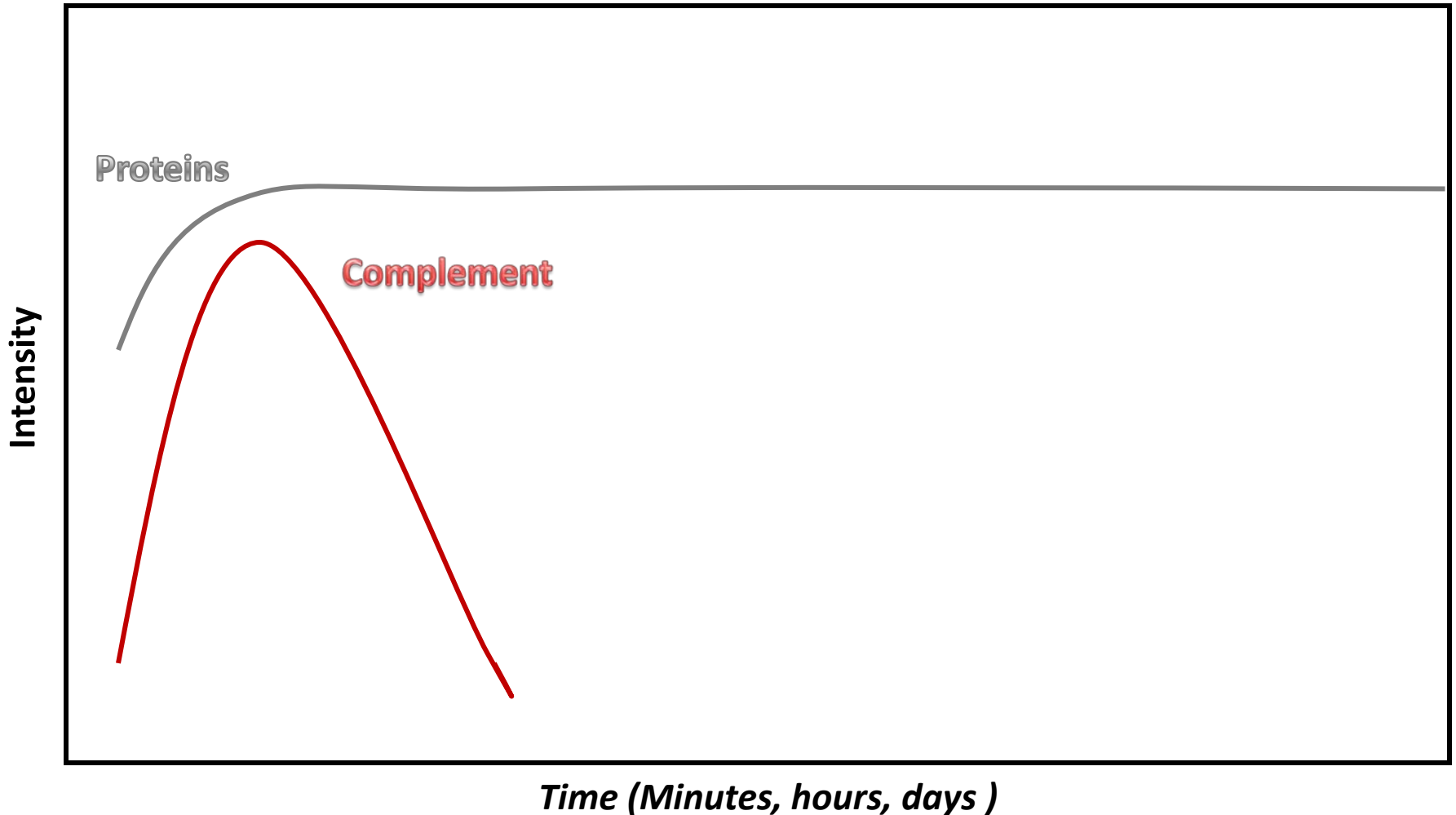
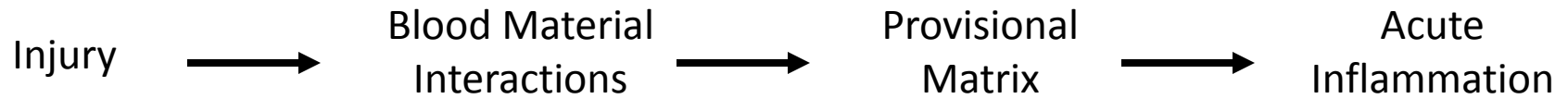
highest
abundance



Protein type	Plasma concentration (mg/ml)	Monomer Molecular weight (daltons)
Prealbumin	10 - 40	54,900
Albumin	35 - 45	66,500 Low M.W.
IgG	6 - 17	150,000
Fibrinogen*	2.0 - 4.0	340,000
Fibronectin*	0.26-0.38	250,000 (but dimeric)

- Est'd $> 10^4$ soluble serum proteins $\rightarrow$ only 20-30% discovered
- **Vroman affect**- albumin arrives first (Fick's Law) and then is replaced by higher affinity proteins which compete for binding
 - Albumin = non-adhesive
 - Fibronectin- very adherent for most cells
 - RGD (Arginine-Glycine-Aspartic Acid)
 - Trace protein, outcompeted by higher abundant proteins

Temporal Sequence of Inflammatory Events



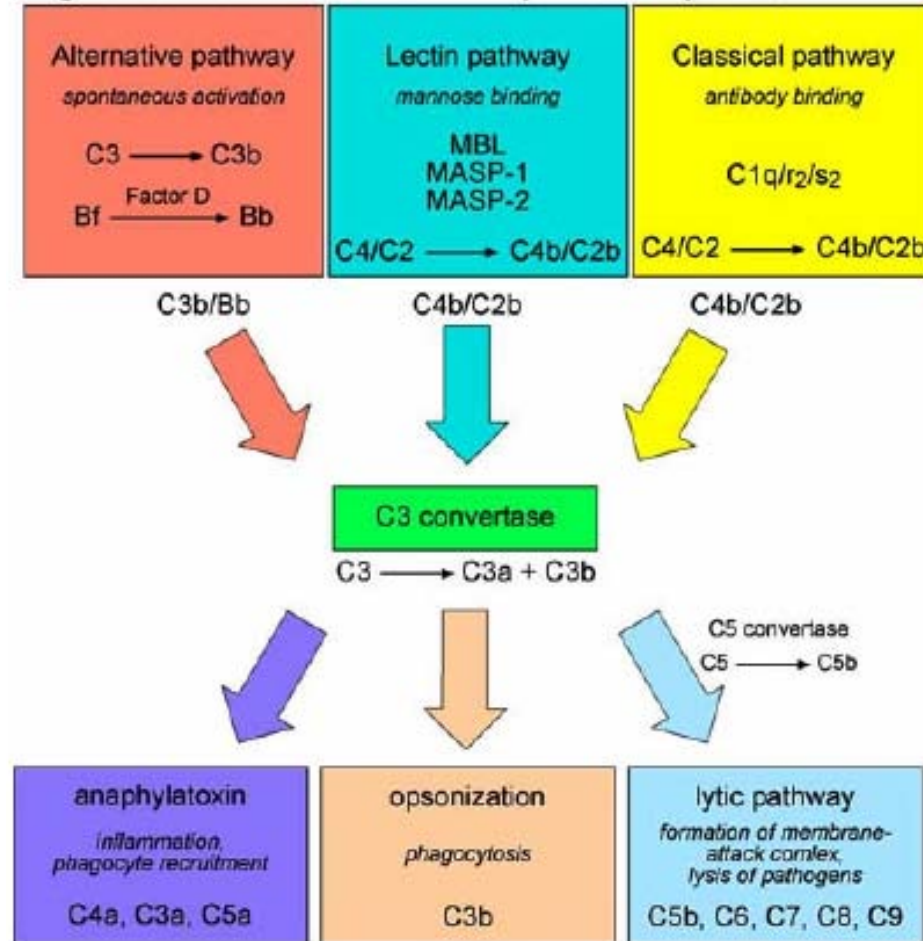
Modified from Anderson, JM. *Cardiovasc. Pathol.* 1993;2 (Suppl 3):33S-41S.

Complement (Review)

General responses

1. Inflammation
 - produces chemotactic substance that amplify the response
 - promote mast cell degranulation which increases vascular permeability
2. Opsonization
 - Tags surfaces for phagocytosis
 - opsonins- plasma derived proteins bind readily to biomaterials and macrophages and neutrophils (activating them)
3. MAC- membrane attack complex
 - Forms on the surface of intruding bacteria
 - Enables free diffusion, and ultimately cell death

Figure 7: Overview of the complement system

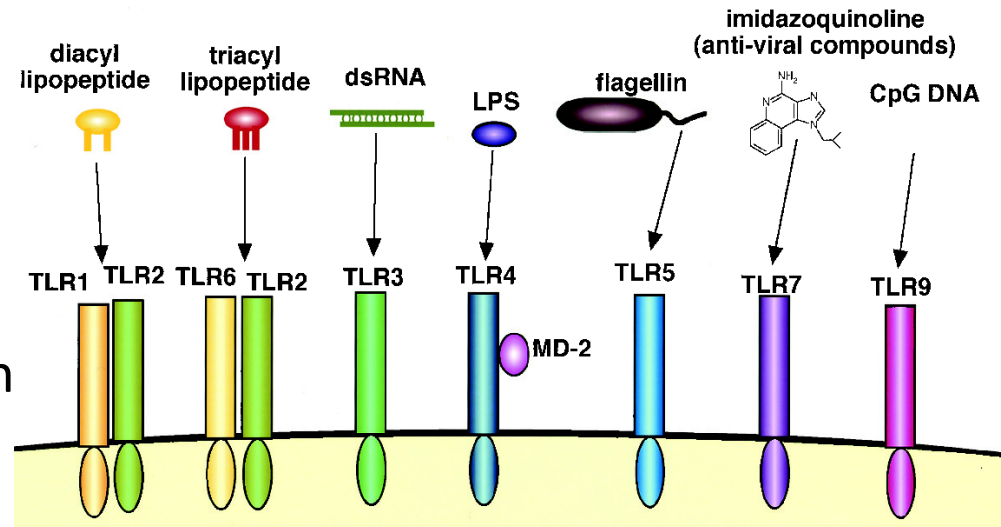
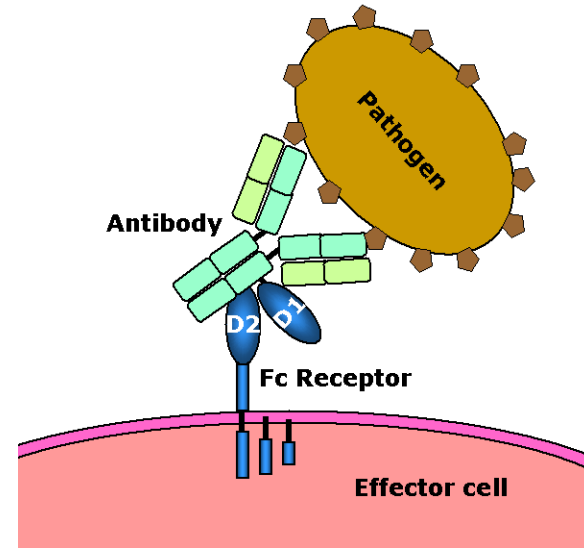


General Features of the Complement Cascade

- Amplify Inflammatory signal
 - Cleaved fragments
- Increase local protein concentration
- Serpins (serine protease inhibitors)
 - keeps the cascade in check
 - Serpin-bound complement is nonfunctional

Macrophage Receptors

- Complement receptor
 - White Blood Cells
 - Activated
- Fc Receptor
- Scavenger Receptor
- Toll-like Receptors
- Macrophage Mannose Receptor (MMR)
 - Upregulated upon activation

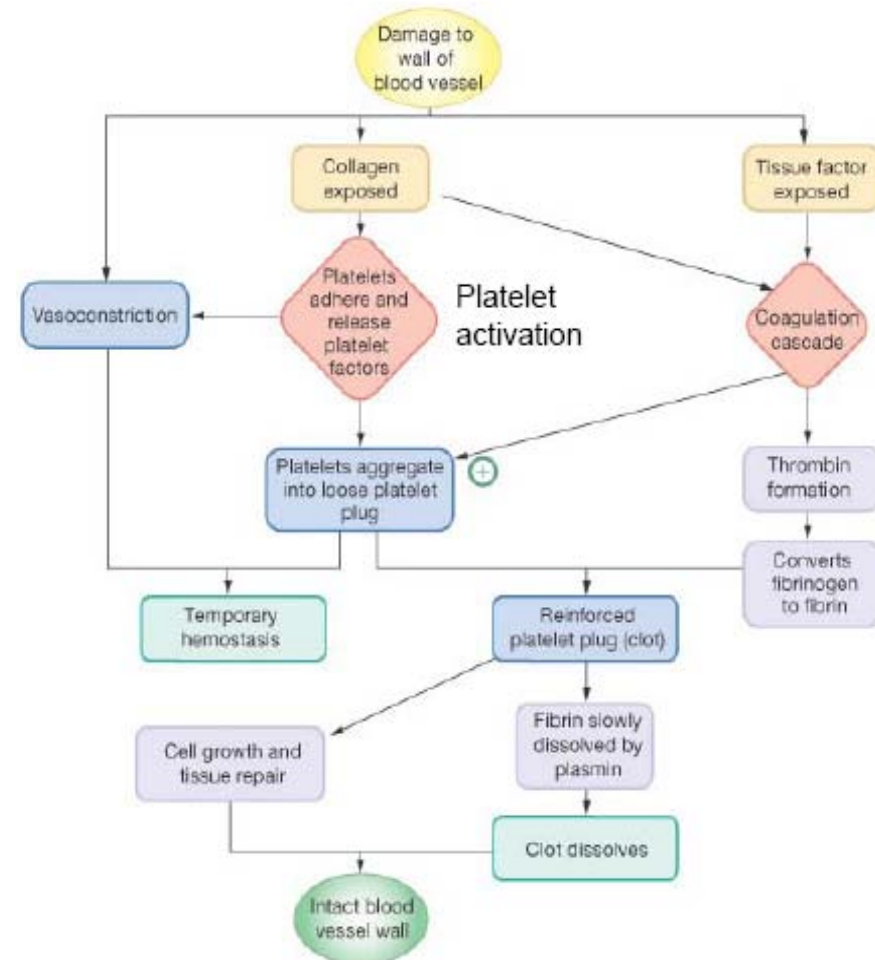


Stage 3

Provisional matrix formation
(minutes to hours)

Provisional Matrix (Fibrin Clot)

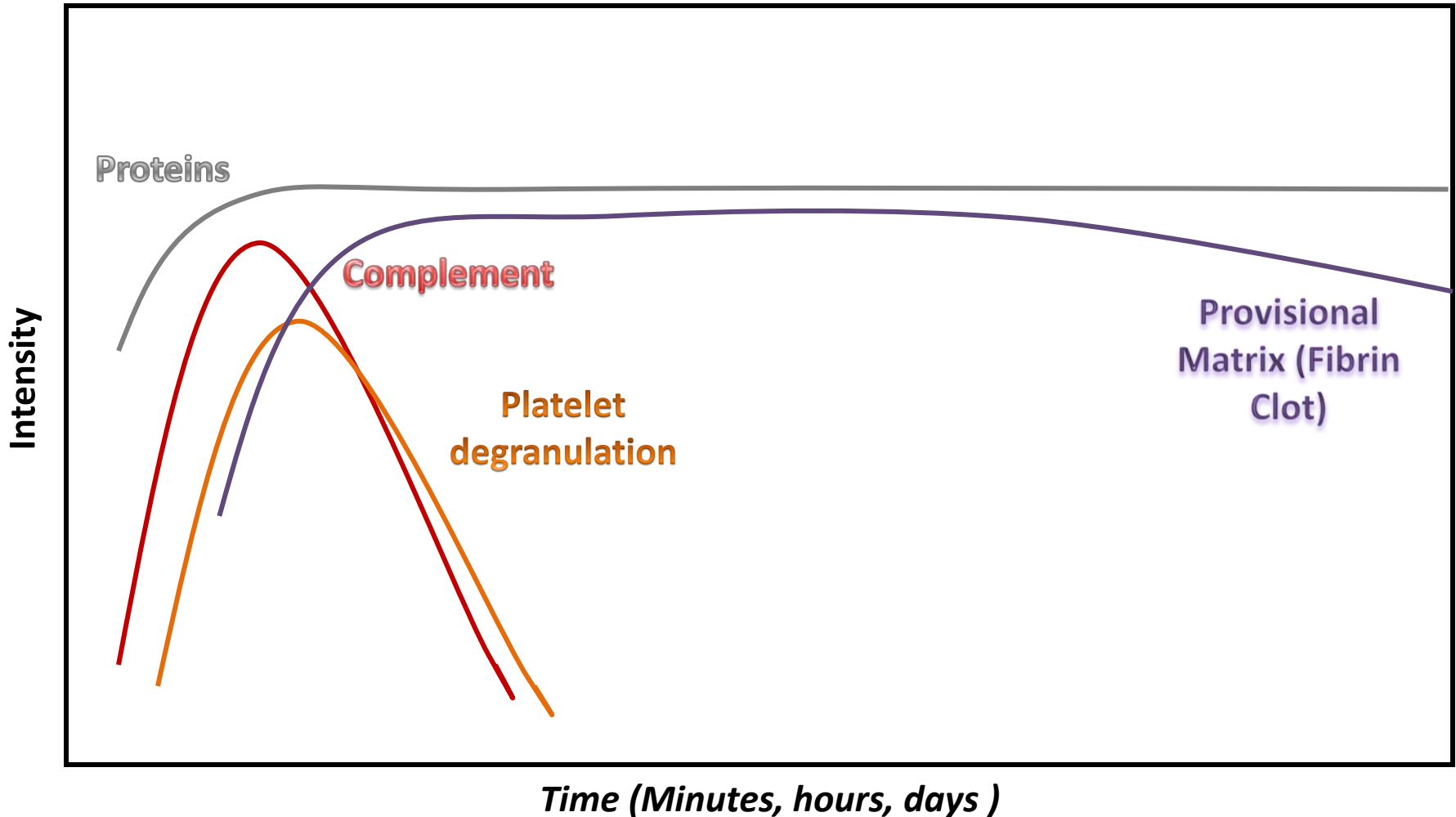
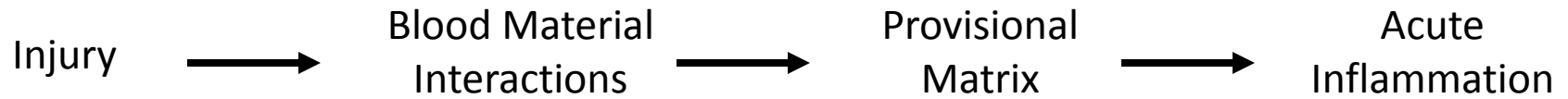
- Collagen or tissue factor Exposure
 - Platelet activation and degranulation
 - Coagulation factors, cytokines, growth factors
- 3D matrix promotes cell adhesion , migration, proliferation, differentiation and their synthesis of new ECM
 - Contains fibrin fibronectin, mitogens, chemoattractants, cytokines, growth factors
- Naturally derived, biodegradable, sustained release system
 - to control subsequent wound healing processes



Coagulation Resolution (Review)

- Serpins-serine protease inhibitors
 - Heparin
 - Enhances antithrombin III binding to thrombin
- Almost 20% of the proteins found in blood plasma are serpins
 - Necessary for suppressing the coagulation and complement cascades that amplify rapidly
- Inhibits the action of their respective serine protease by mimicking the 3D structure of the normal substrate
 - It covalently binds to the protease and marks it for degradation

Temporal Sequence of Inflammatory Events



Stage 4

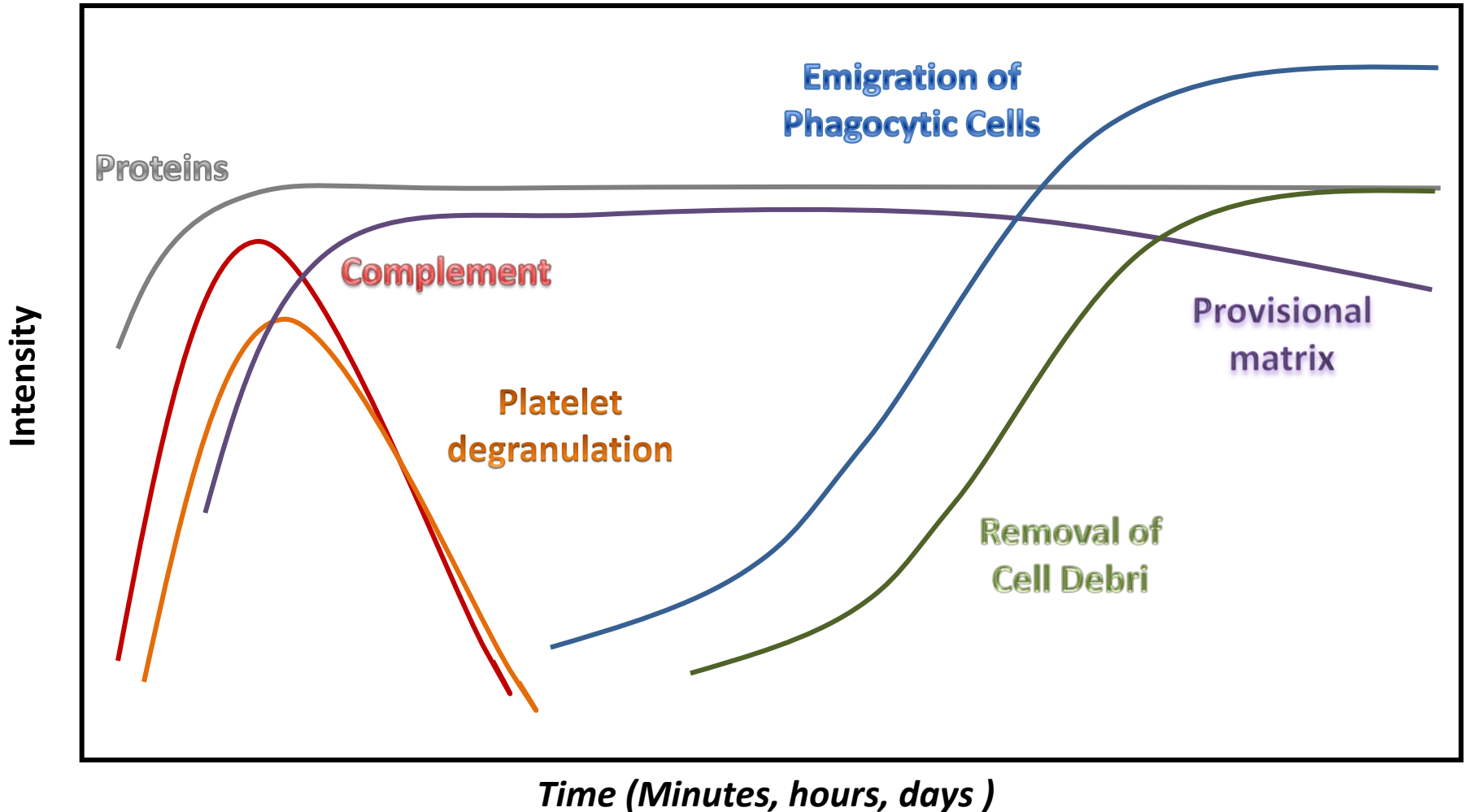
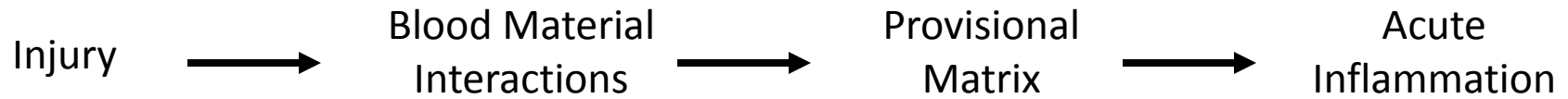
Acute inflammation

(minutes, hours, days usually resolves within 1 week)

Acute Inflammation (Review)

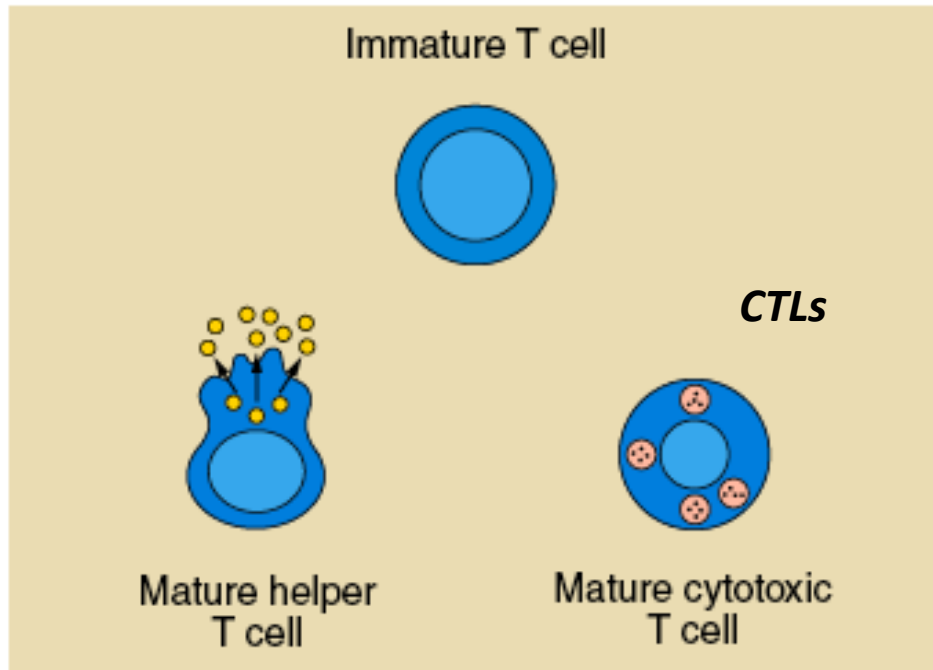
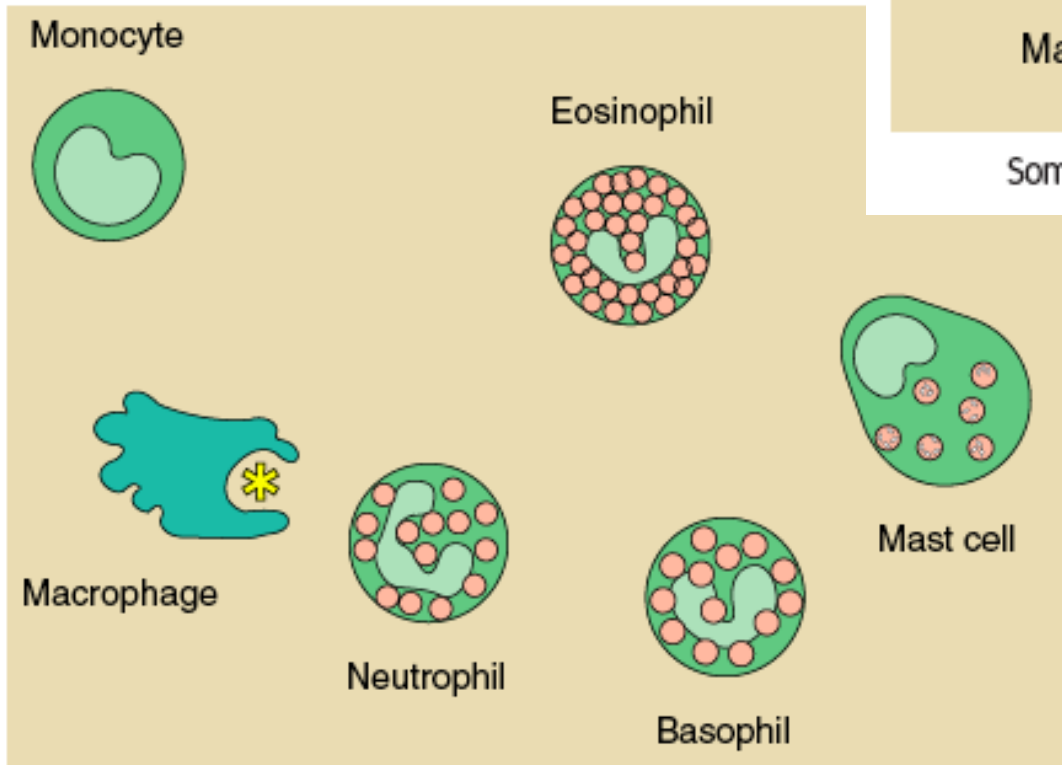
- Edema (exudation of fluid and plasma proteins)
- Emigration of leukocytes (neutrophils)
 - phagocytose and release inflammatory cytokines to increase chemotaxis of these and other cells
 - Removal of damaged matrix and necrotic cell components
- Cell proliferation and recruitment including endothelial, epithelial, stromal and inflammatory cells

Temporal Sequence of Inflammatory Events



Immune cells

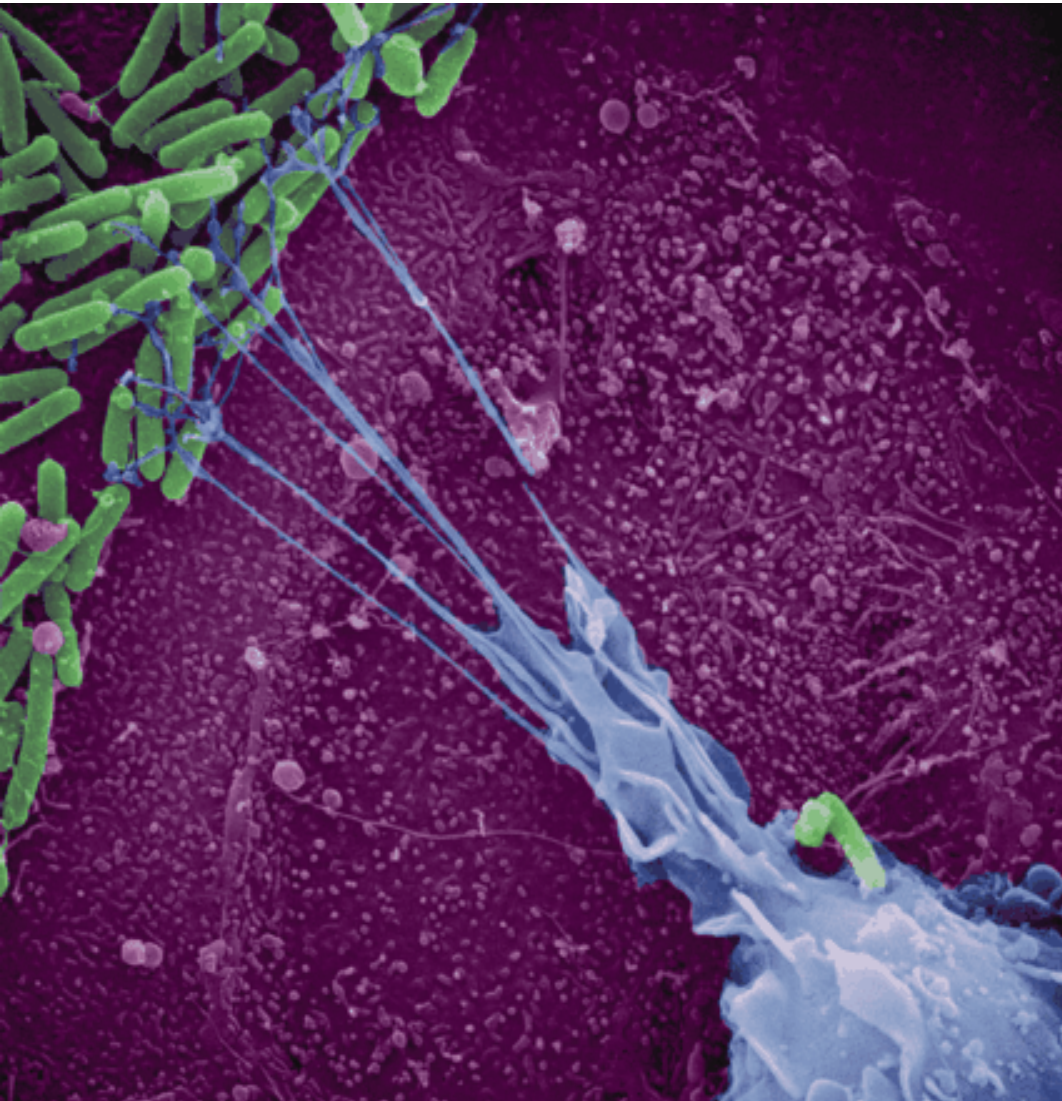
phagocytes



Some T cells are helper cells, others are killer cells.

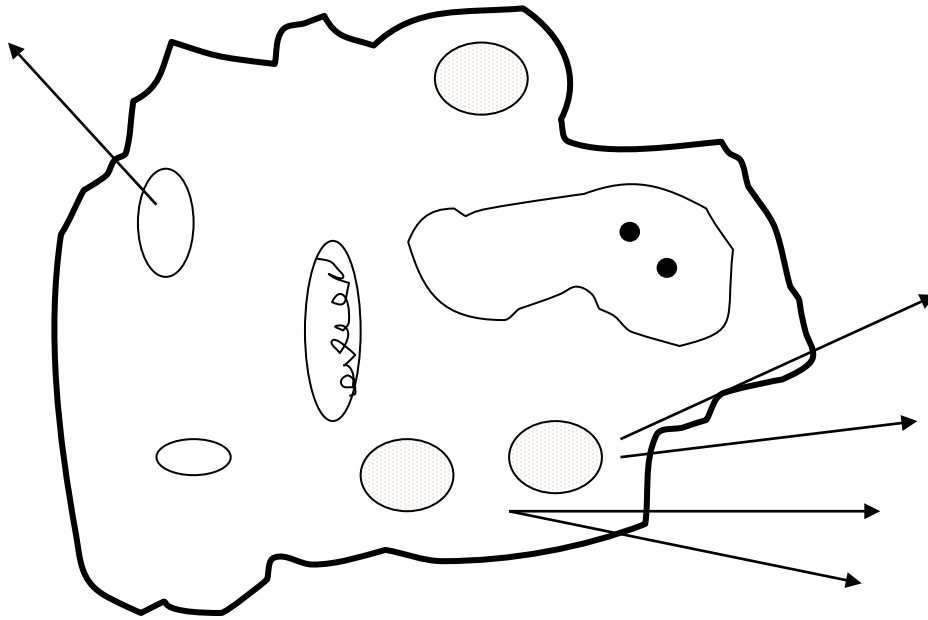
B Cells
Plasma Cells

Phagocytosis: cell engulfment and clearance



- Microbes
- Foreign materials
 - debris
 - implants
 - nanotechnology
 - particles (e.g., wear)
- Size-dependent:
 - >6 microns?
- Frustrated phagocytosis
- accompanied by proteases and powerful oxidative products
- ROS destruction of local tissue sites

Biomaterials degradation from cell-based foreign body responses (Review)



Macrophages and neutrophils release:

- reactive oxygen free radicals
- hypochlorous acid
- superoxide radical
- hydroxyl radical
- peroxynitrite radical
- lysosomal enzymes
 - hydrolases
 - proteases
 - peroxidases
 - phosphatases

- inflammatory mediators responding to all implanted materials
- hostile to biomaterials
- persistent and highly reactive locally
- likely degrading to materials chemistry --> oxidation, hydrolysis, etc.

Stage 5

Divergence from Normal Wound Healing

Chronic Inflammation

(short duration and is confined to implant site)

Chronic inflammation

- Chemical and physical properties of biomaterials, motion at implant site, and infection may all cause chronic inflammation
- Characterized by macrophages, monocytes, lymphocytes, plasma cells, and proliferation of blood vessels and connective tissue
 - Cytokines are responsible for cell migration, differentiation, and tissue remodeling
 - Released factors: PDGF, TGF- β , TNF- α , IL-1
- Chronic inflammation for weeks, months, years may suggest a long-standing infection

Stage 6

Granulation tissue:

(3-5 days)

Granulation Tissue

- Surrounds the foreign body and is considered a normal response to an implant
- Formed by proliferation of fibroblasts and vascular endothelial cells
 - endothelial cells create neovascularization or angiogenesis
 - fibroblasts secrete proteoglycans, collagen (especially type III) which forms a fibrous capsule
 - fibroblasts are responsible for wound contraction
- Macrophages are readily apparent in granulation tissue
- Hallmark of healing inflammation



www.okcreative.com

Absence of foreign body

- Resolution, stabilization and scarring or abscess formation

Stage 7

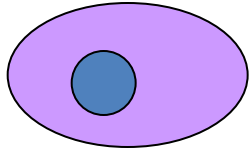
Foreign body Reaction (FBR)

(lasts life of implant)

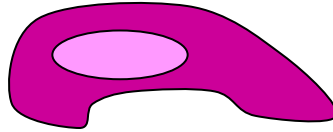
Foreign Body Reaction (FBR)

- Hallmark of FBR are foreign-body giant cells (FBGC) and granulation tissue
- FBGCs form from fusing monocytes and macrophages in an attempt to phagocytose the material
- Different textured surfaces will have a different FBR. Ex. The more textured surface will have more FBGCs
- FBR can block catheters, degrade polymers, blockade biosensors, kill adjacent cells

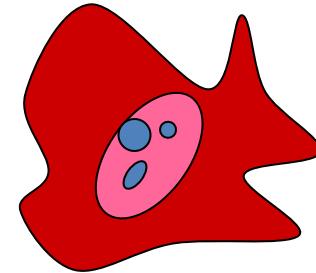
Macrophage Lineages



Hematopoietic
Stem Cell
Bone Marrow



Monocyte
Peripheral Blood



Macrophage
Tissue

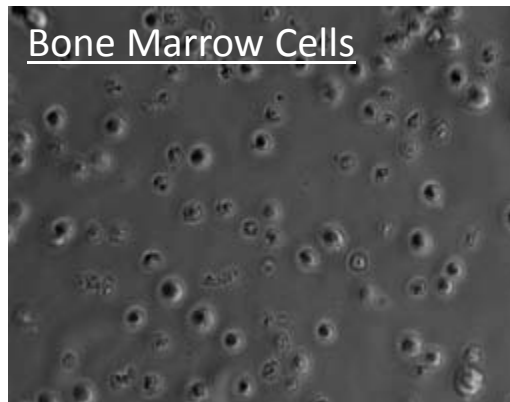
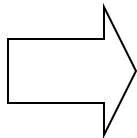
Differentiation

Bone marrow monocytic cells mature into macrophages after 7 days of culture in fibroblast-conditioned media.

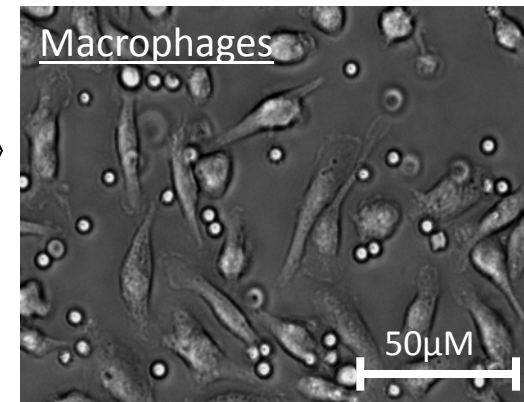
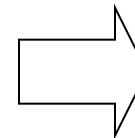


"C57/Black 6"

Harvest cells
from femurs
& tibias



7 days cultured
with L-929
conditioned
media



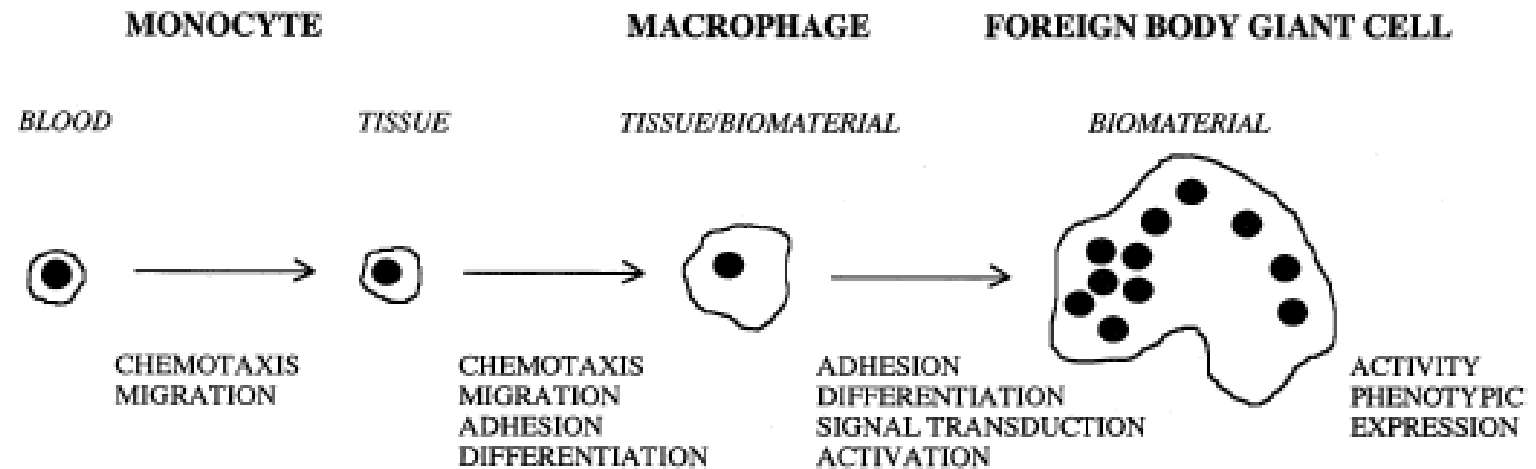
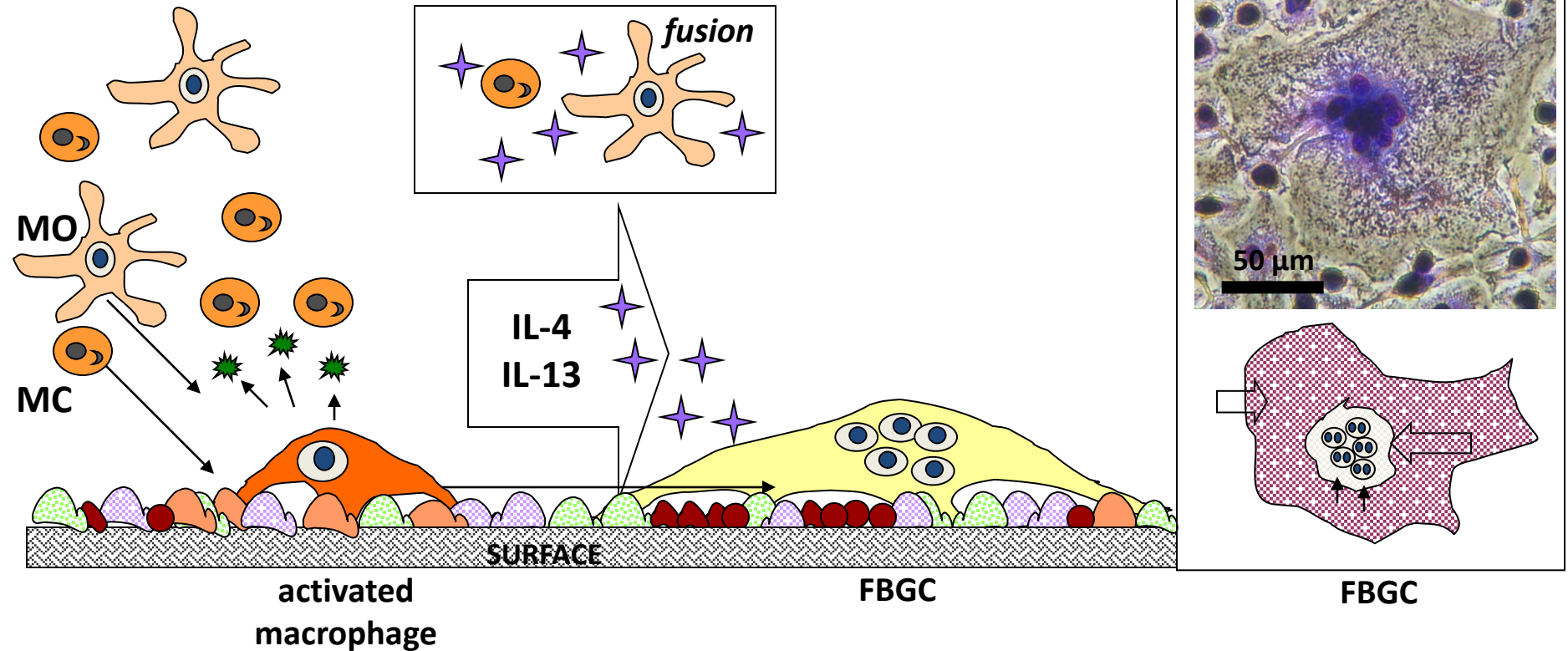


Figure 2 In vivo transition from blood-borne monocyte to biomaterial adherent monocyte/macrophage to foreign body giant cell at the tissue/biomaterial interface. Little is known regarding the indicated biological responses that are considered to play important roles in the transition to foreign body giant cell development.

Monocyte/Macrophage Fusion to Foreign Body Giant Cells (FBGC)

In vitro:



physical cues

+

chemical cues

fusion

Multi-nucleate FBGC

cell-cell contacts

chemokines GM-CSF

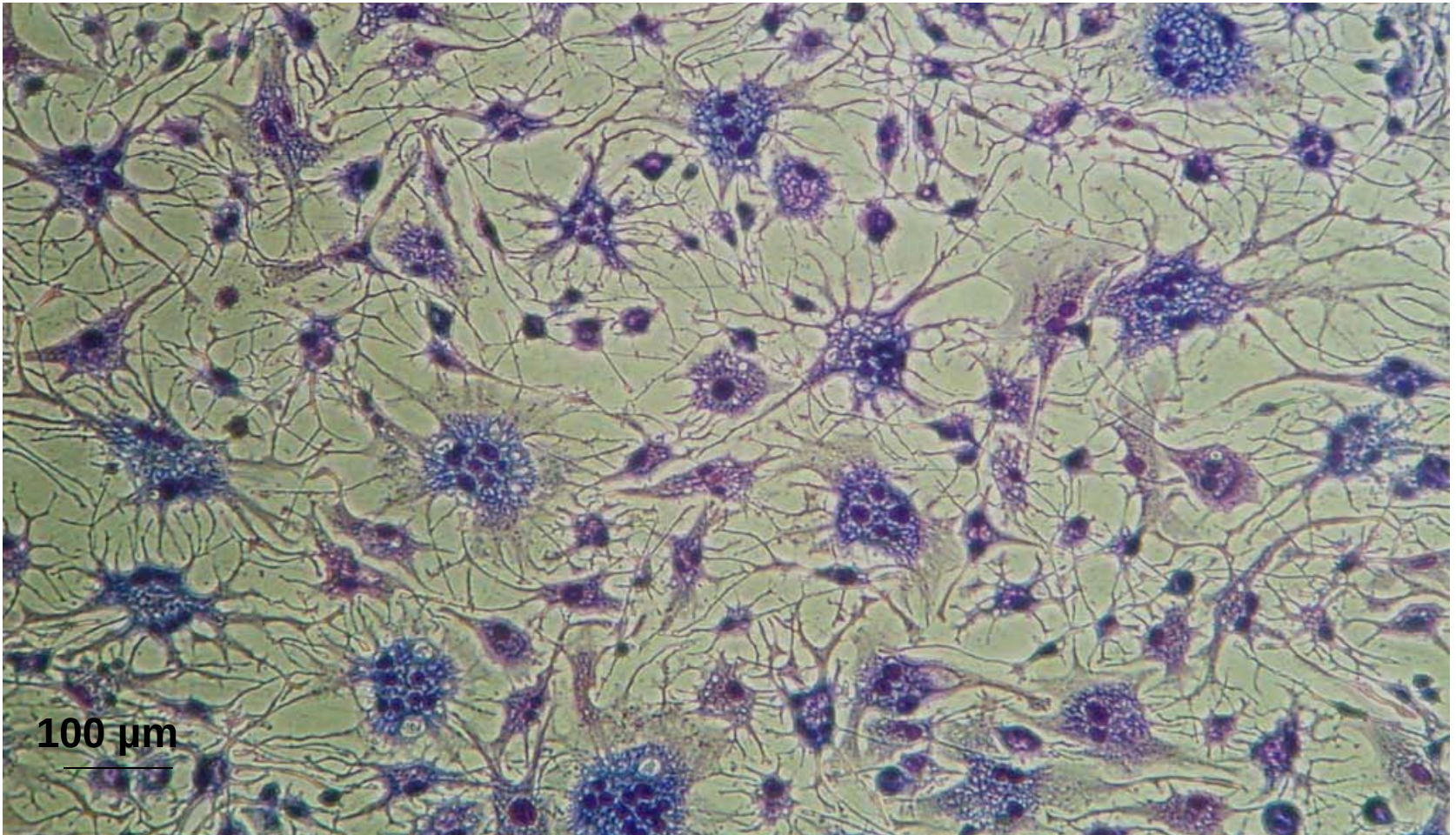
poorly characterized

Cell-adsorbed protein
layer material contacts

cytokines: IL-3, IL-4, IL-13

function *in vivo* poorly understood

➡ *Fused Primary Bone Marrow Derived Macrophages*



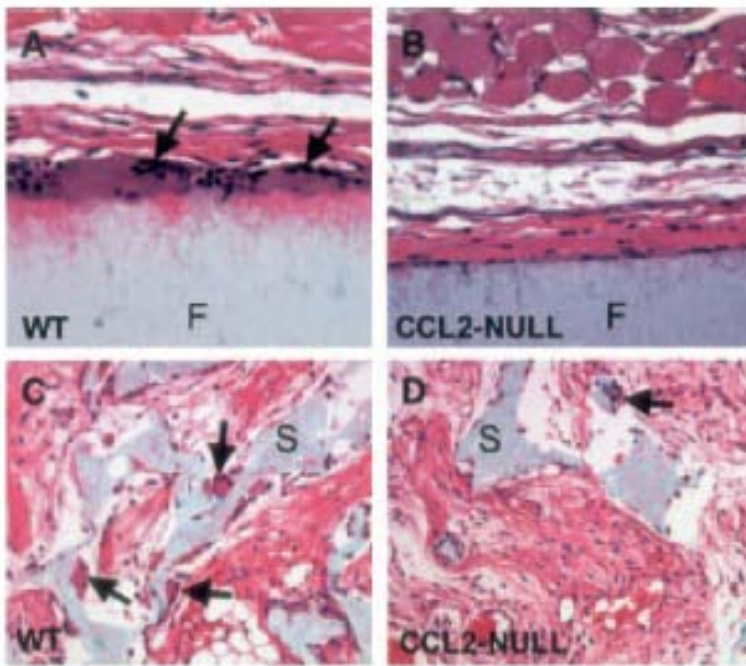
BMMO, Day 10, + 25ng/ml GM-CSF +100 ng/ml IL-4, and 100 ng/ml IL-13

- *~ 50% fusion*

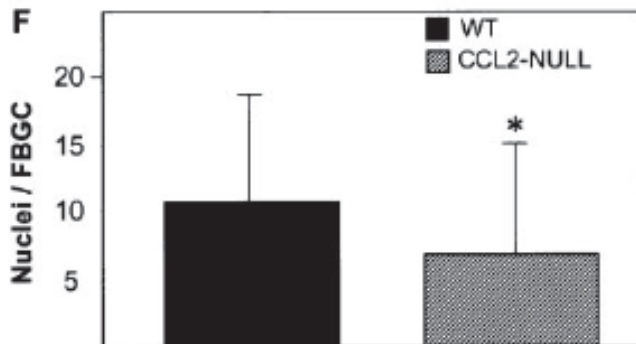
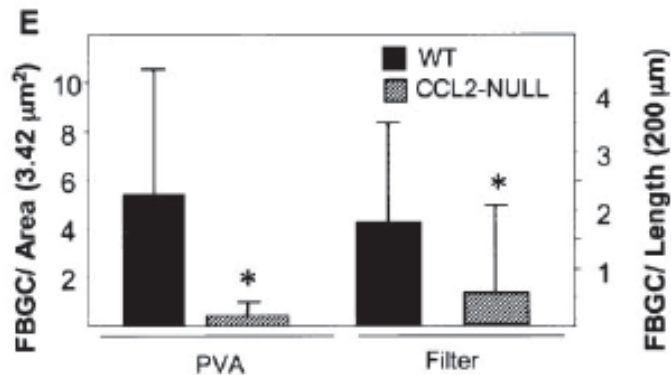
Causal or Correlative?

- Anderson Hypothesis
 - FBGC modulate the foreign body response
 - Secreting the majority of soluble mediators and degradative enzymes
- Kyriakides Hypothesis
 - FBGCs occur concurrently with the FBR
 - The FBR proceeds fully without fused FBGCs

Murine knock out wound healing mutants



- **FBGC** formation was **reduced** by **75%** surrounding filters and **90%** within sponges implanted in CCL2-null mice (B,D, and E)
 - The number of **nuclei per cell** was also found to **decrease** in CCL2-null mice (F)
 - **Abundance of macrophages** in CCL2-null mice but few or no FBGCs (Fig 2 B and D)
- Despite a reduced formation of FBGCs in CCL2-null mice, **deposition of collagen** capsule proceeded **normally** for filter and PVA sponge implants.

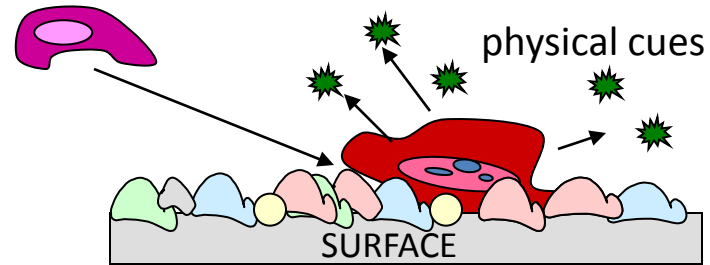
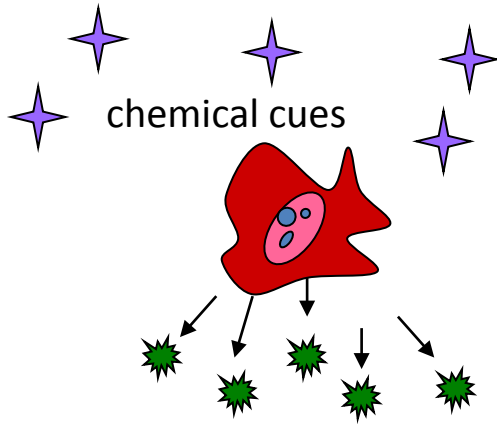


(T. R. Kyriakides et al, *Am. J. Pathol*, 165, 2157 2004)

Kyriakides Conclusions

- Even in a MCP-1 KO mouse that produces **little or no FBGC**, **fibrosis occurs** similar to WT mouse.
- The **lack of MCP-1** does **not inhibit monocyte recruitment** and **development** of the **FBR** when biomaterials are implanted subcutaneously.
 - Though it has been documented that CCL2 null mice cannot recruit monocytes in acute peritonitis.
- **CCL2** is required for **normal macrophage fusion** in regards to frequency of **FBGCs**, **size**, and number of **nuclei**.
 - However studies describing a nearly complete inhibition of FBGC formation has not yet been reported.
- **TGF- β** levels are not altered despite reduction of FBGC formation.
 - Suggesting that macrophages can provide **pro-fibrotic** signals to **fibroblasts**.

Macrophage Activation Occurs Through Multiple Pathways

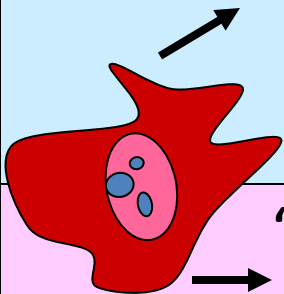


Macrophage activation in response to chemical and/or physical cues directs the immune response

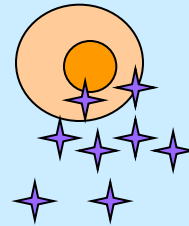
"Classical Activation"

by IFN- γ and LPS

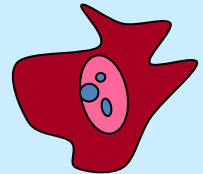
- Microbicidal
- Tissue Damage
- Cellular Immunity



TH1



M1

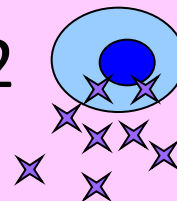


"Alternative Activation"

by IL-4 and IL-13

- Humoral Immunity
- Allergy/Parasite Response
- Tissue Repair

TH2

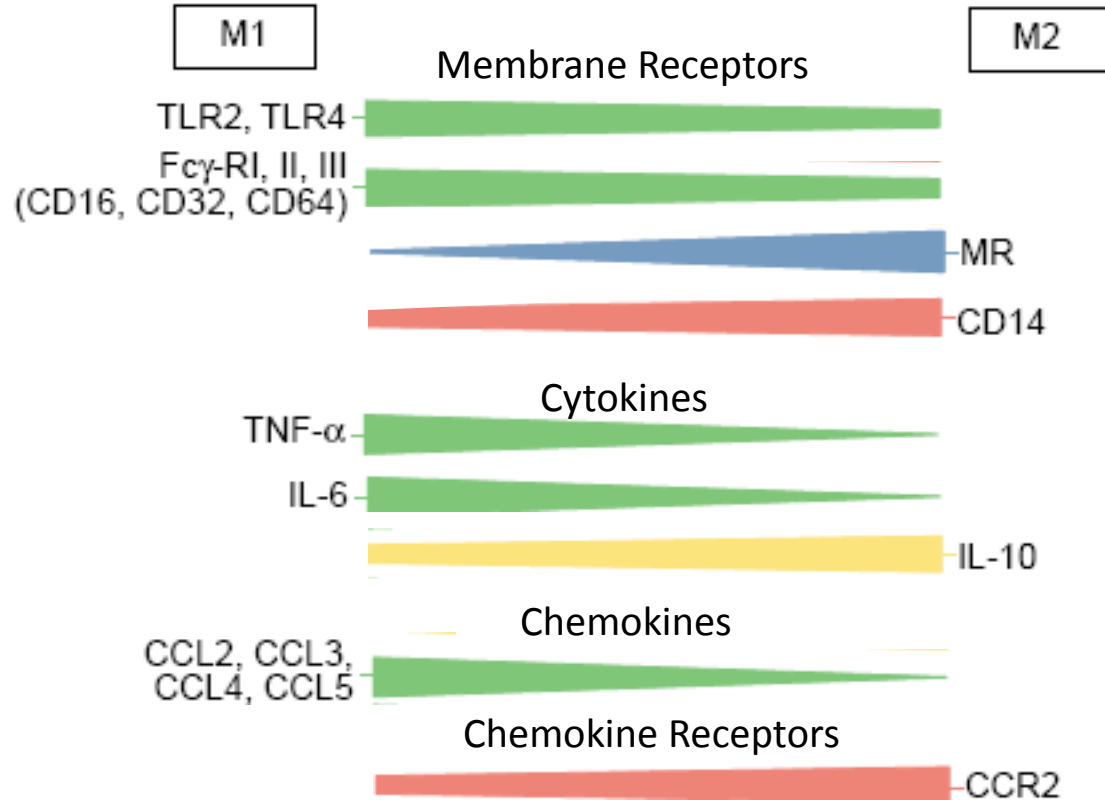
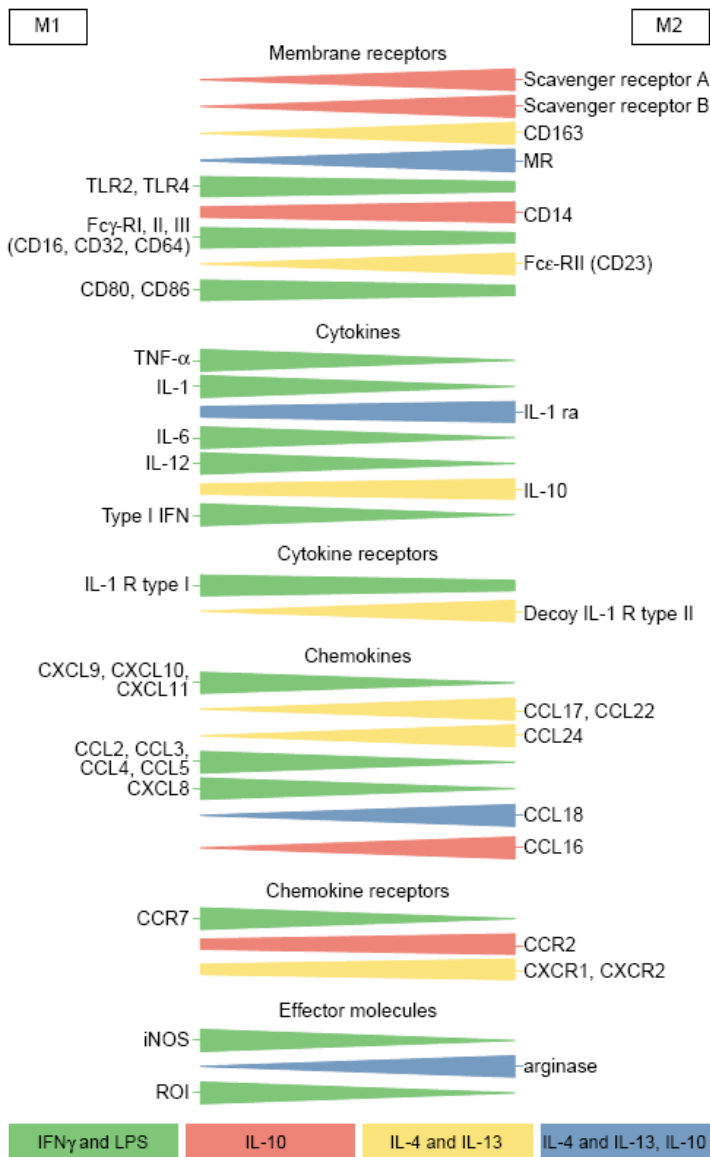


M2



?

M1 vs. M2 Macrophage Markers



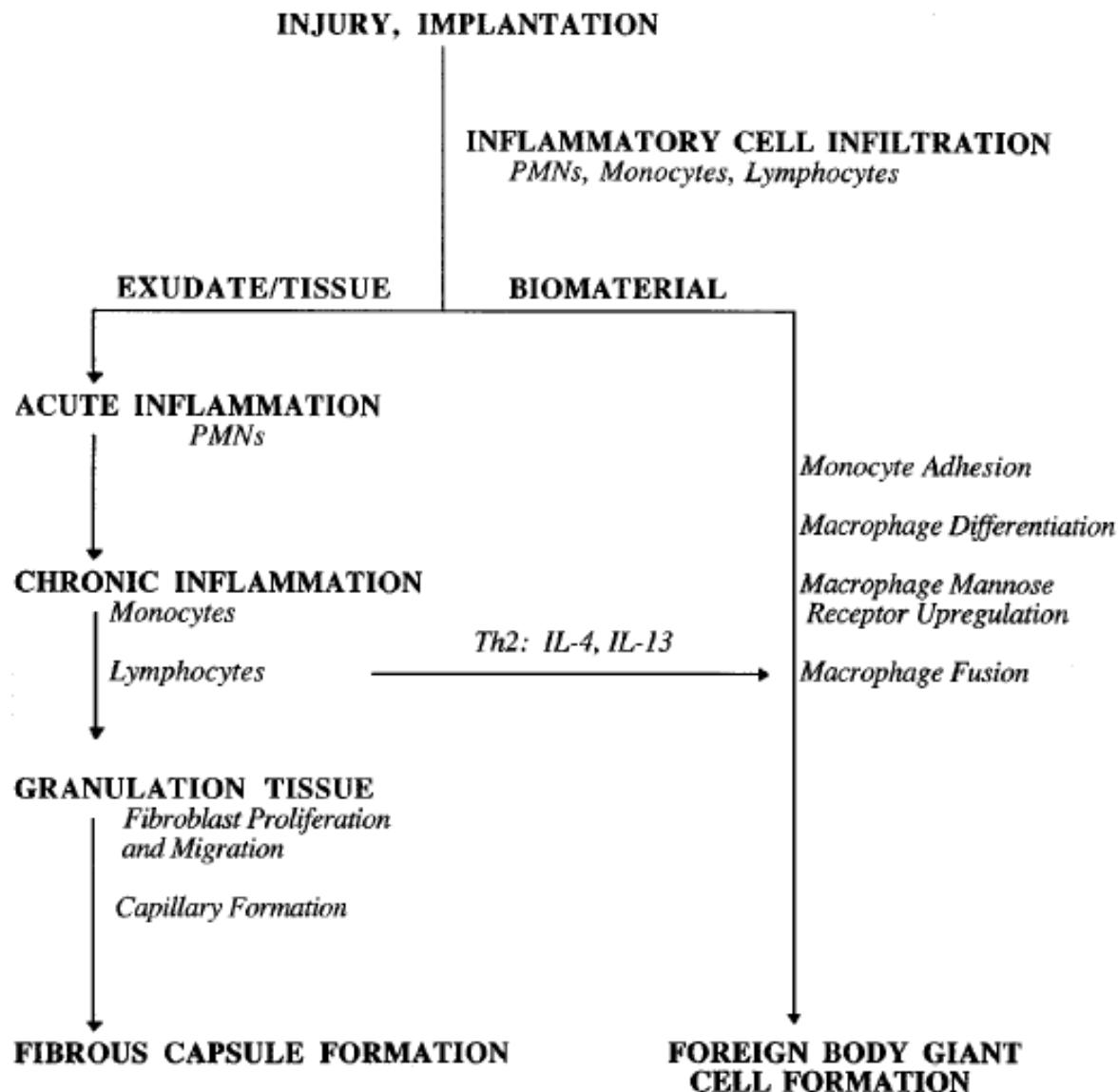


Figure 3 Sequence of events involved in inflammatory and wound healing responses leading to foreign body giant cell formation. This shows the importance of Th2 lymphocytes in the transient chronic inflammatory phase with the production of IL-4 and IL-13, which can induce monocyte/macrophage fusion to form foreign body giant cells.

Stage 8

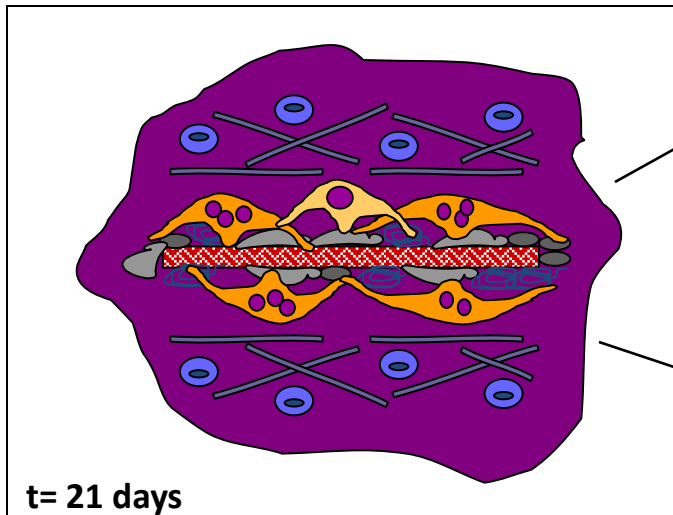
Fibrosis

(lasts life of implant)

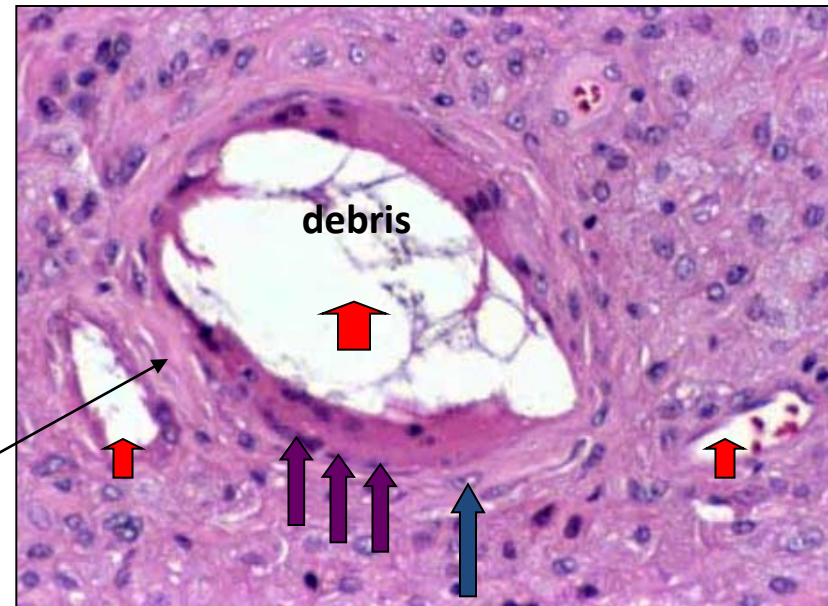
Fibrosis

- Fibrous Capsule Formation
 - end-stage healing response to biomaterials
- Fibroblasts
 - Collagen Type I, III

Foreign Body Response-Encapsulation



*Is it possible to
prevent or minimize
this response?*



www.Gentili.net/thr/osteolys.htm

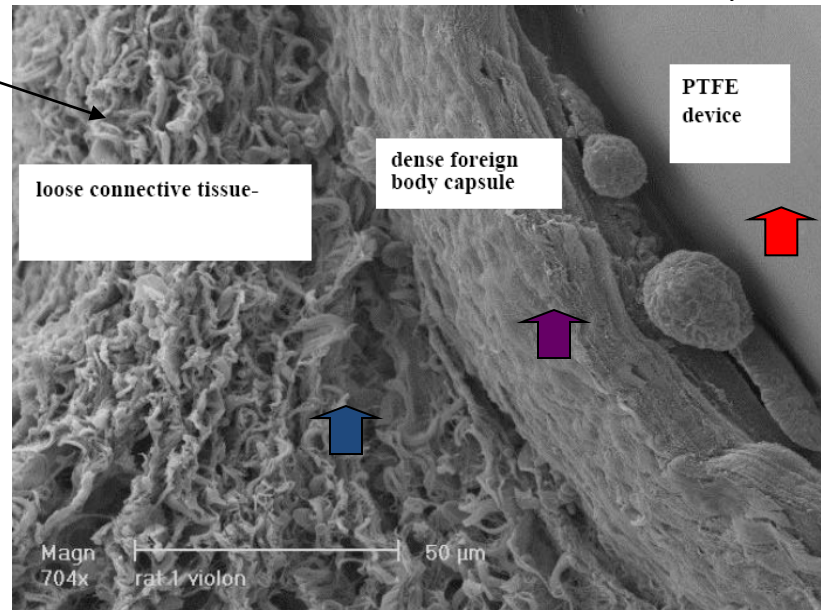
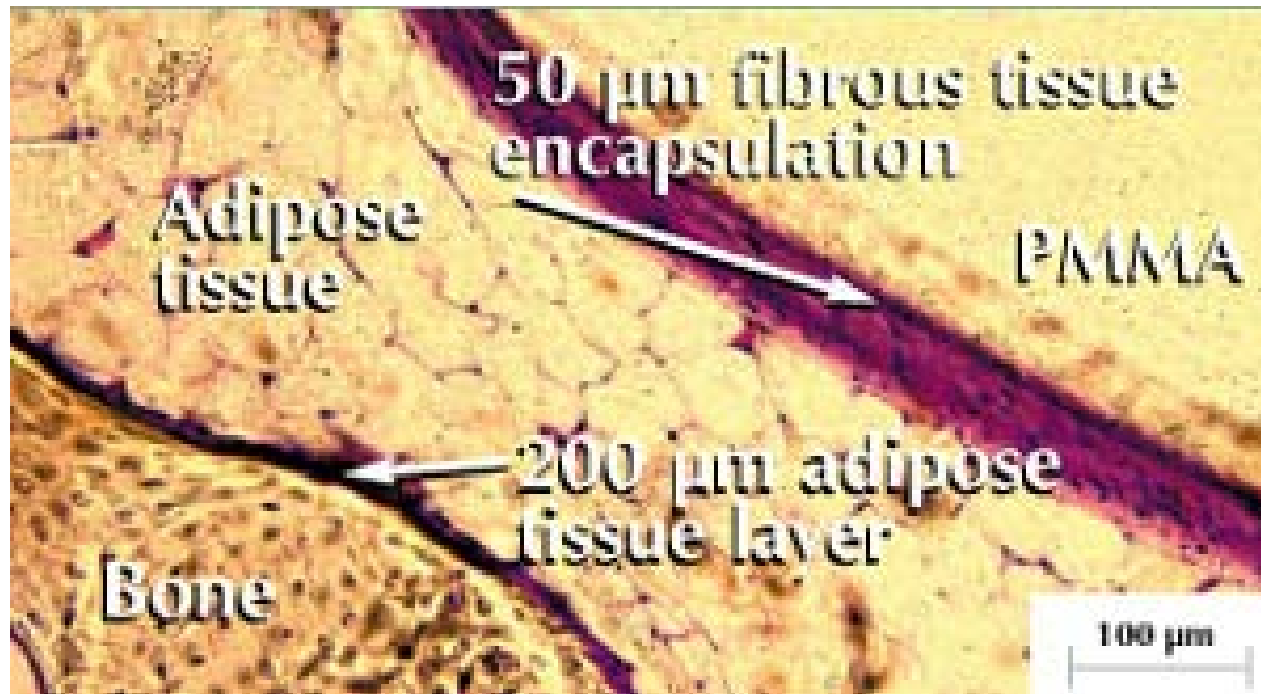


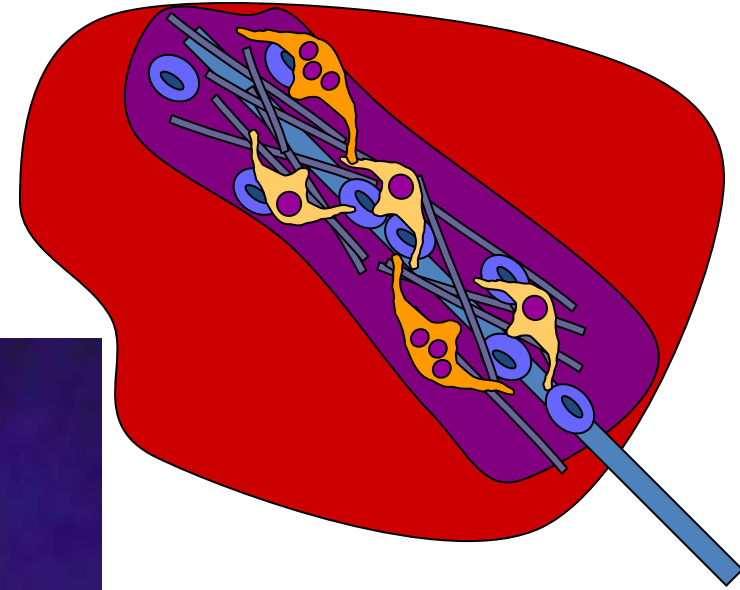
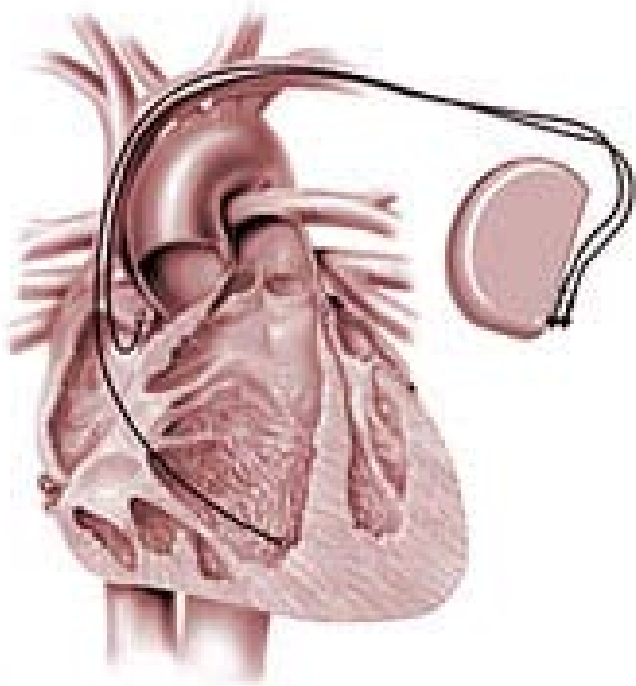
Photo: courtesy K. Ward

Fibrosis and Fibrous Encapsulation



- 78-week PMMA histology (sheep): illustrates soft tissue encapsulation of prepolymerized PMMA

When Fibrosis is acceptable

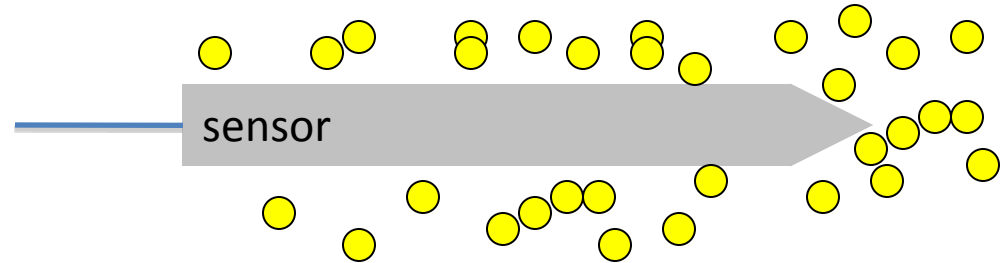


Pacemaker leads, Hernia mesh

Fibrosis from FBR provides adhesion of leads to pericardial tissue or abdominal wall as implant-tissue anchorage

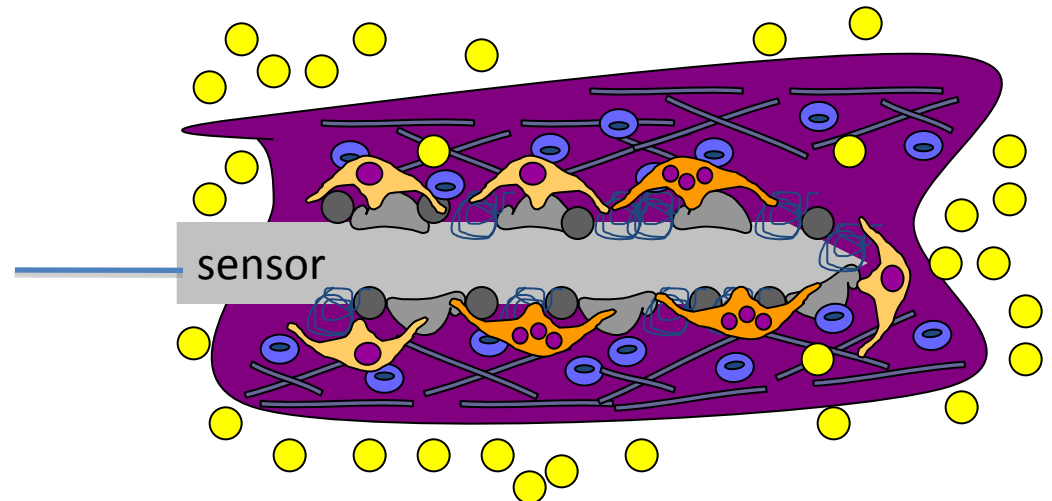
When Fibrosis is clinically unacceptable

Guardian®RT



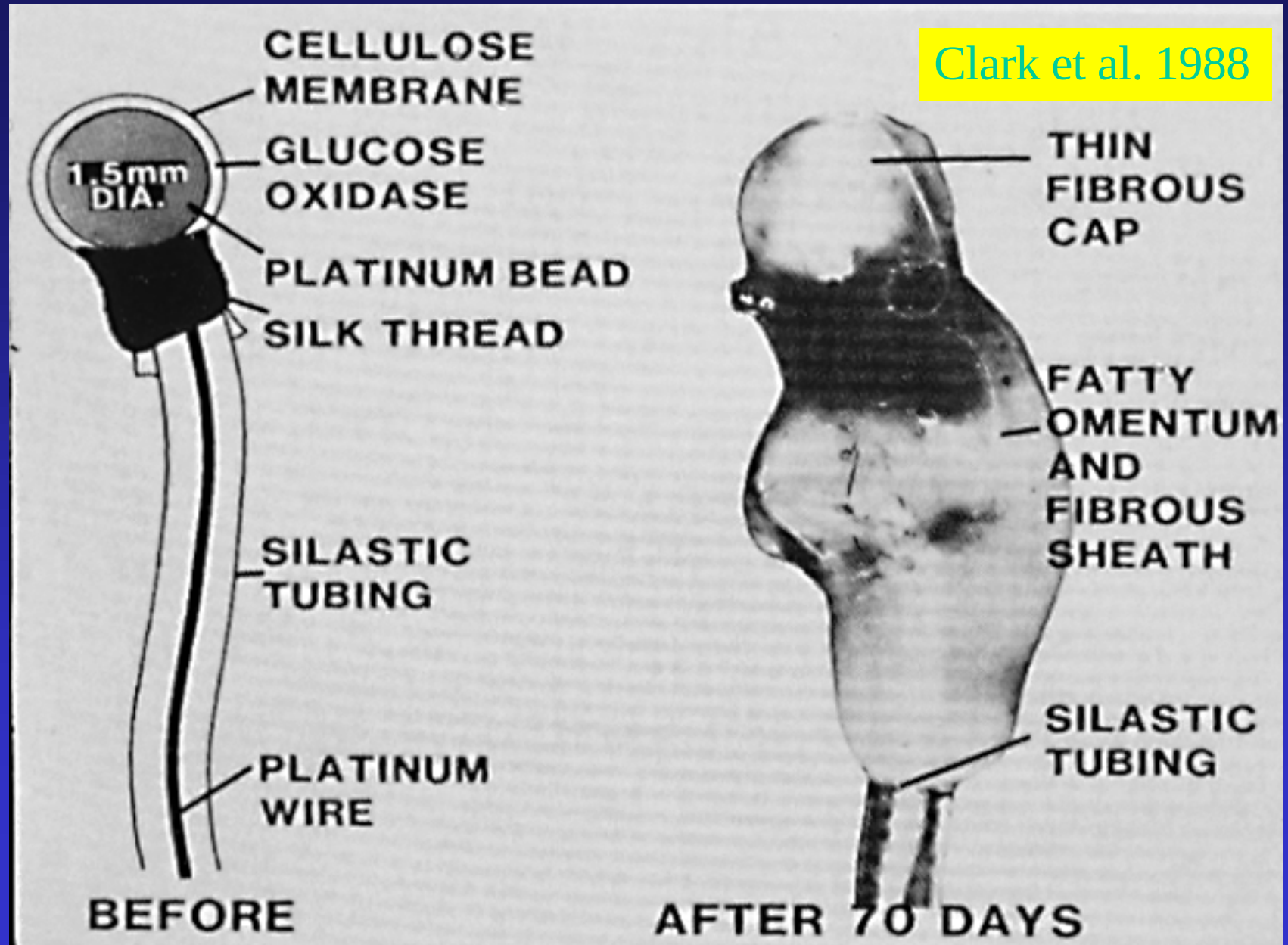
Glucose Sensor

- Over time, FBR walls off sensor
- Requires removal and re-insertion of new sensor every 3 days



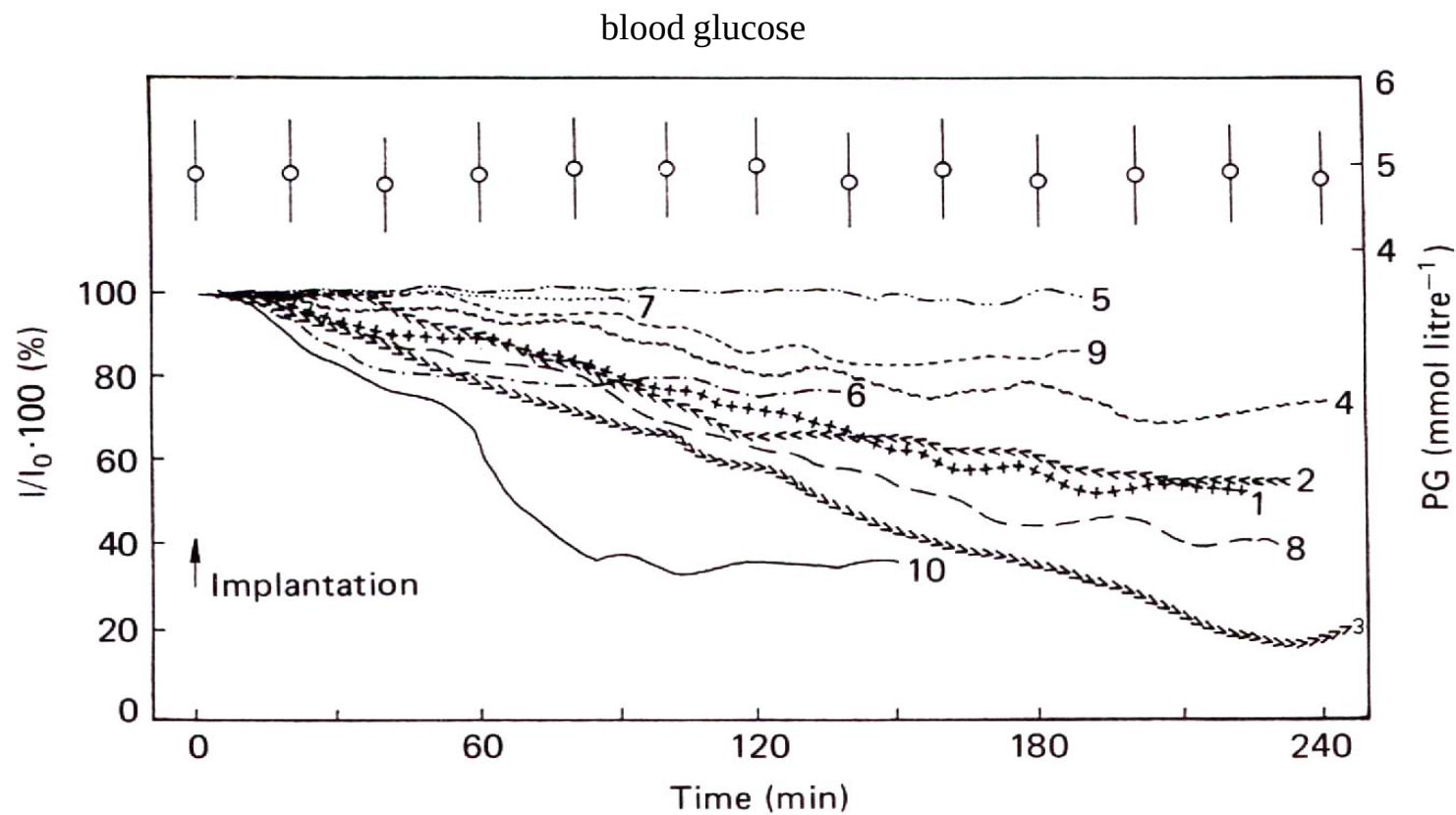
Explanted biosensor: peritoneal implant after 70 days

Clark et al. 1988



Sensor signal decay

B)

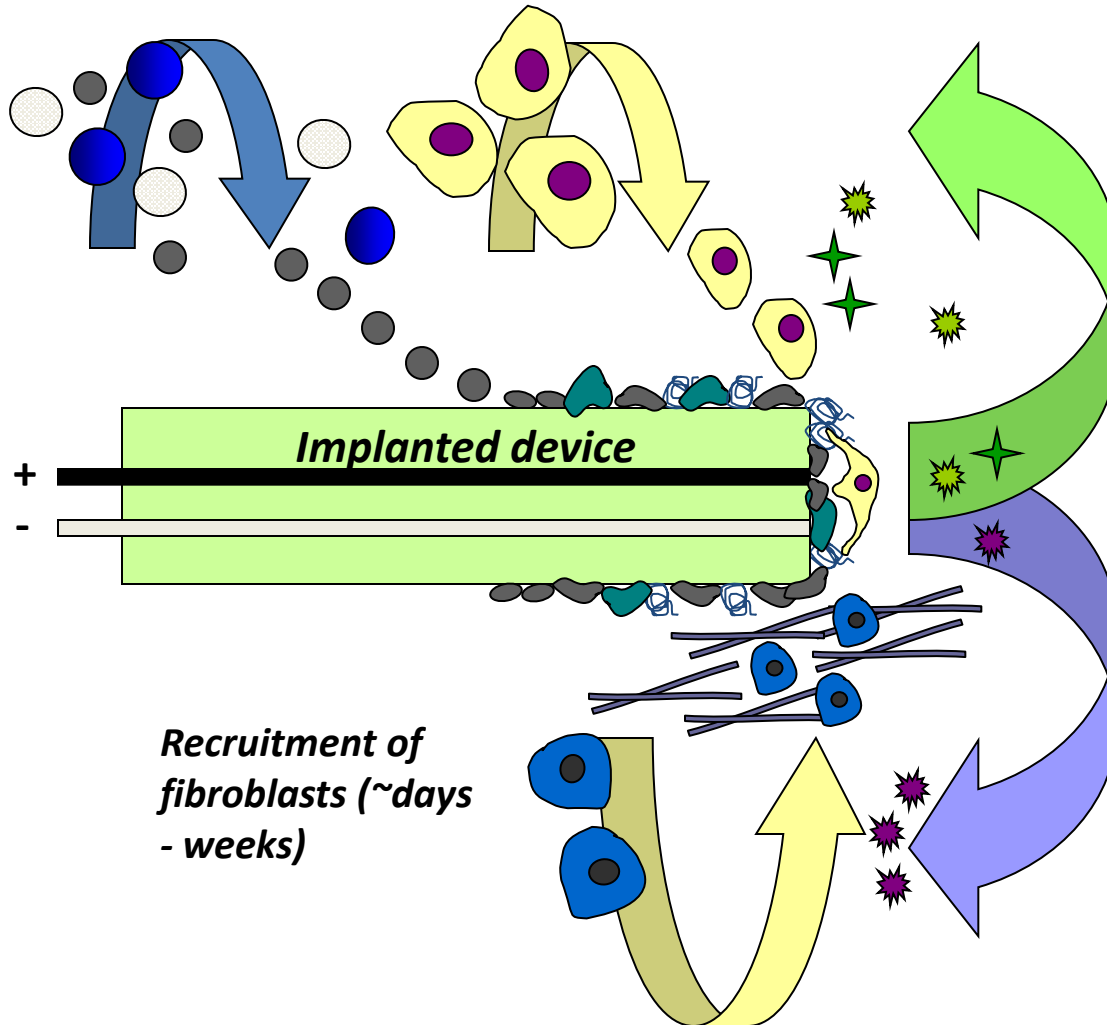


K. Rebrin et al. 1992

**Protein adsorption
(~seconds)**



**Acute cellular interrogation
(~hours - days)**



***The biosensor
implant as a
convenient
reporter of wound
healing***

***Signaling from
activated
macrophages
(~days - weeks)***

***Recruitment of
fibroblasts (~days
- weeks)***

***Avascular fibrous encapsulation (~
weeks - months)***

Time ~ 3 weeks



New Zealand White Rabbit soft tissue histology at 14 weeks from surgery with c.p. titanium implant placed subcutaneously. These next few slides show photos of increasing magnification of a foreign body response to the porous metal.

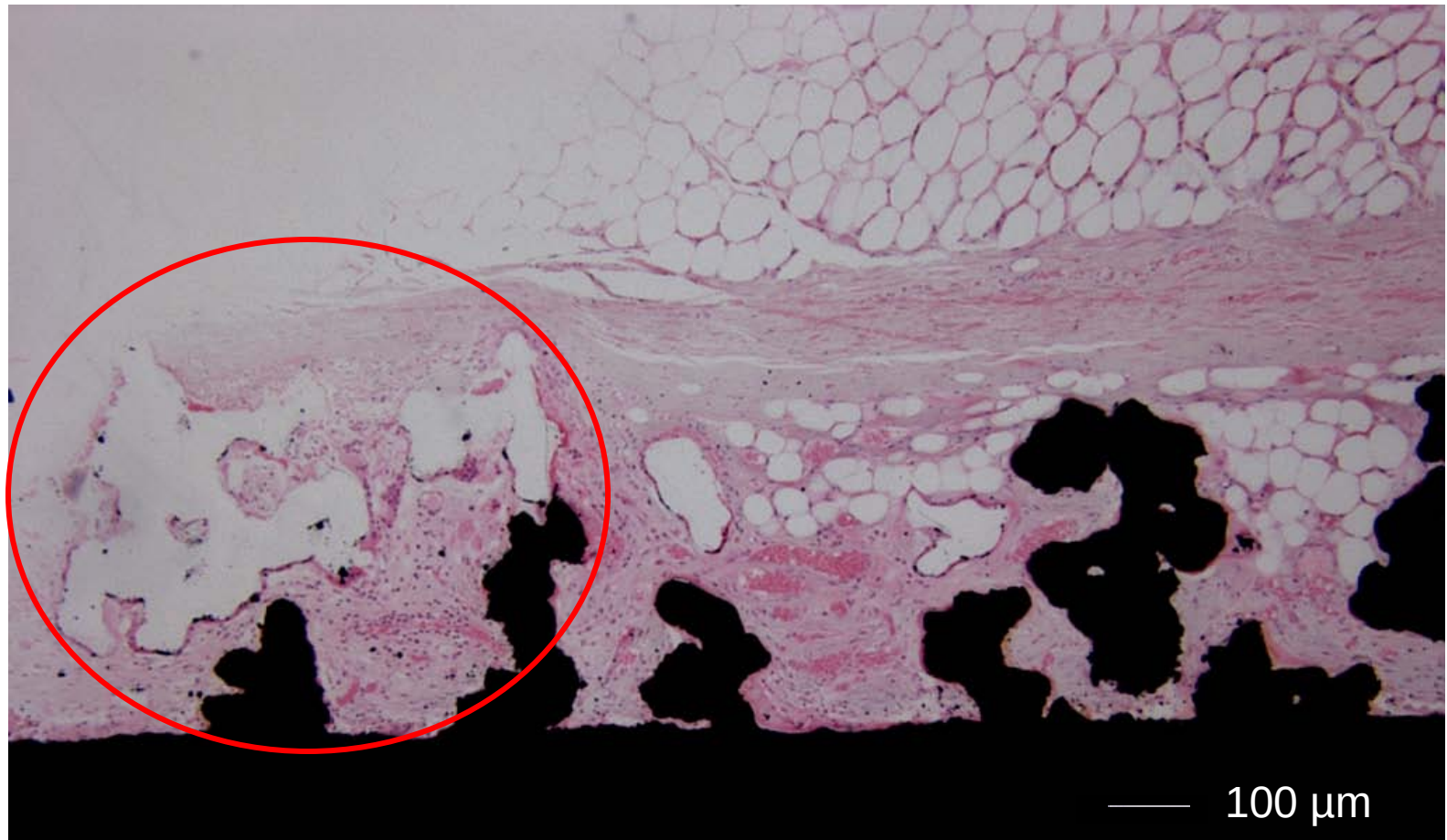
Sections were embedded in PMMA and stained with a modified H&E.

Dark pink = cytoplasm.

Dark Purple = nucleus

Light pink = collagen

Dorthyann Isackson

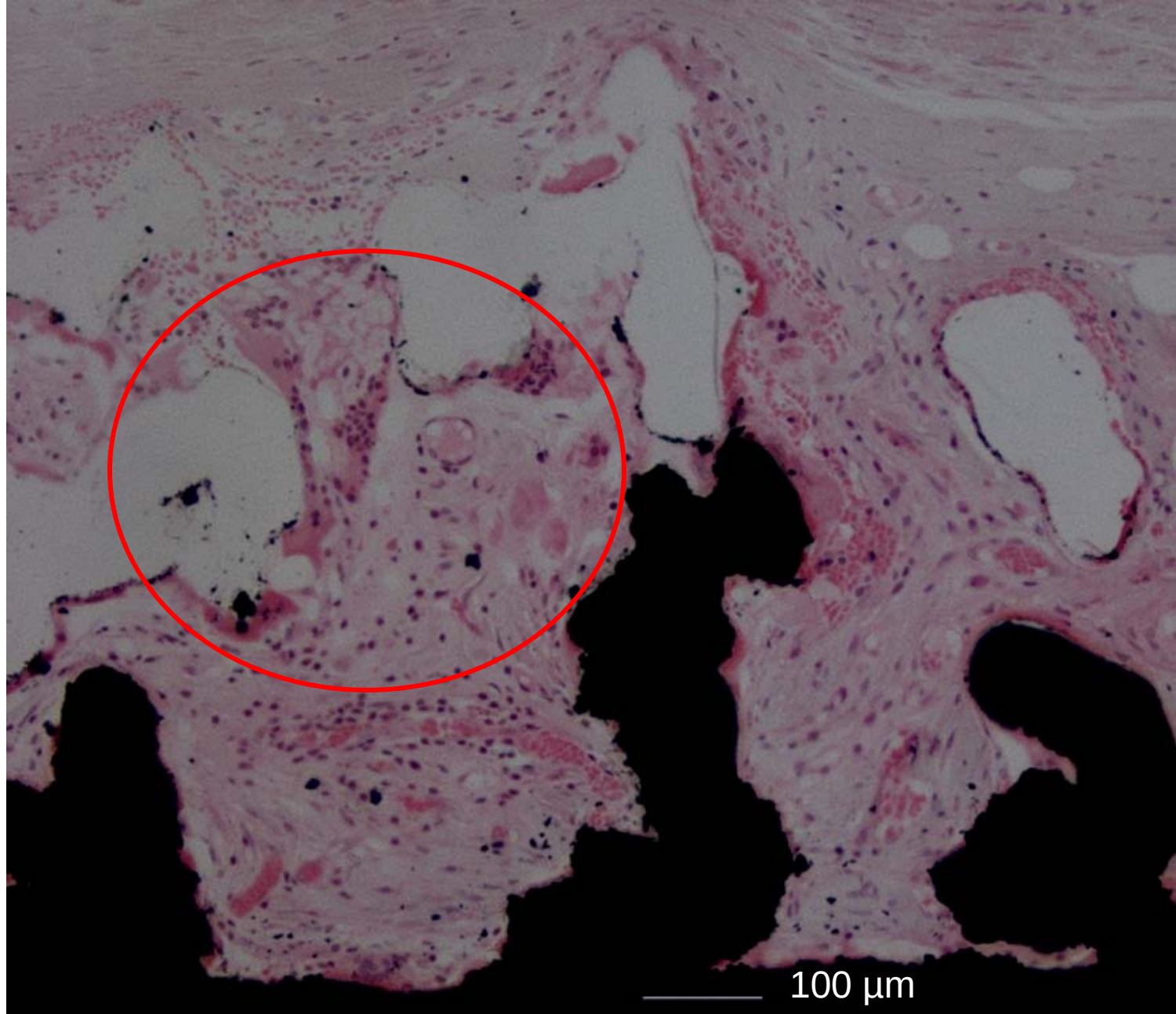


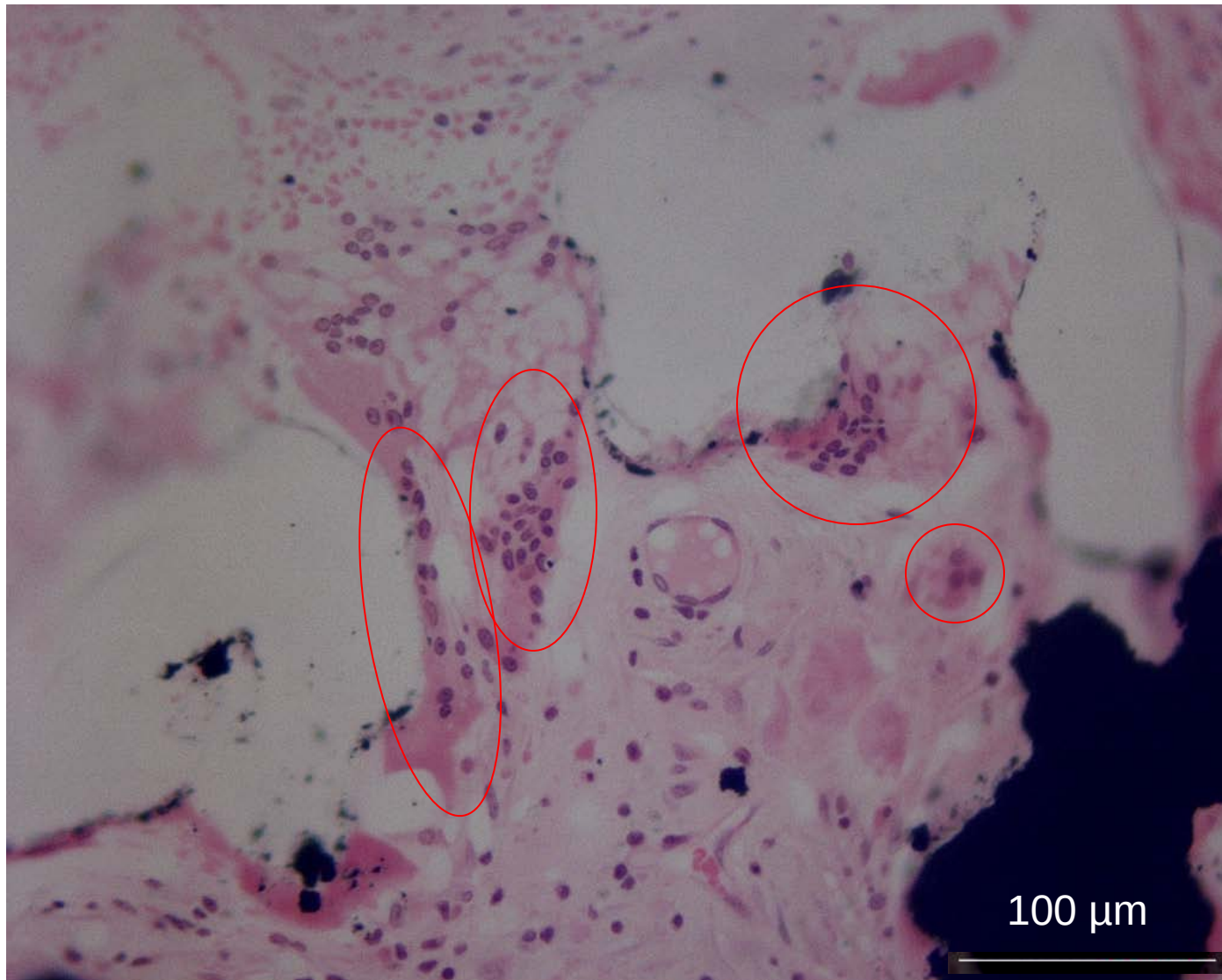
Metal Pore Missing with macrophages and FBGCs lining pore space.
H&E. 4x original magnification.

Metal pore
missing with
macrophages
and FBGCs lining
pore space.

H&E.

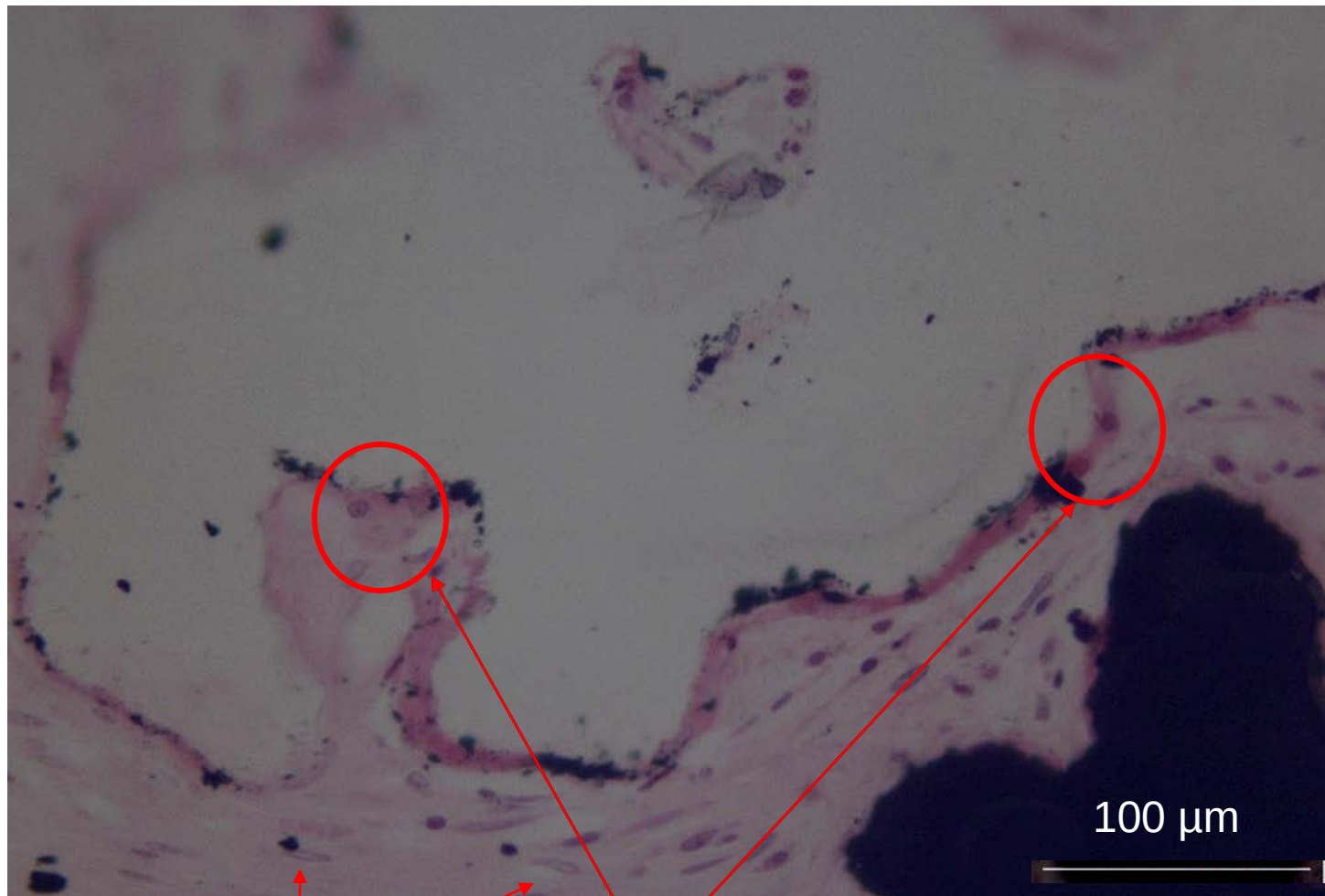
10x original
magnification.





Metal pore missing with macrophages and FBGCs lining pore space. Dark pink is cytoplasm. Dark purple is cell nucleus.

H&E. 20x original magnification.



Fibroblast

Macrophage

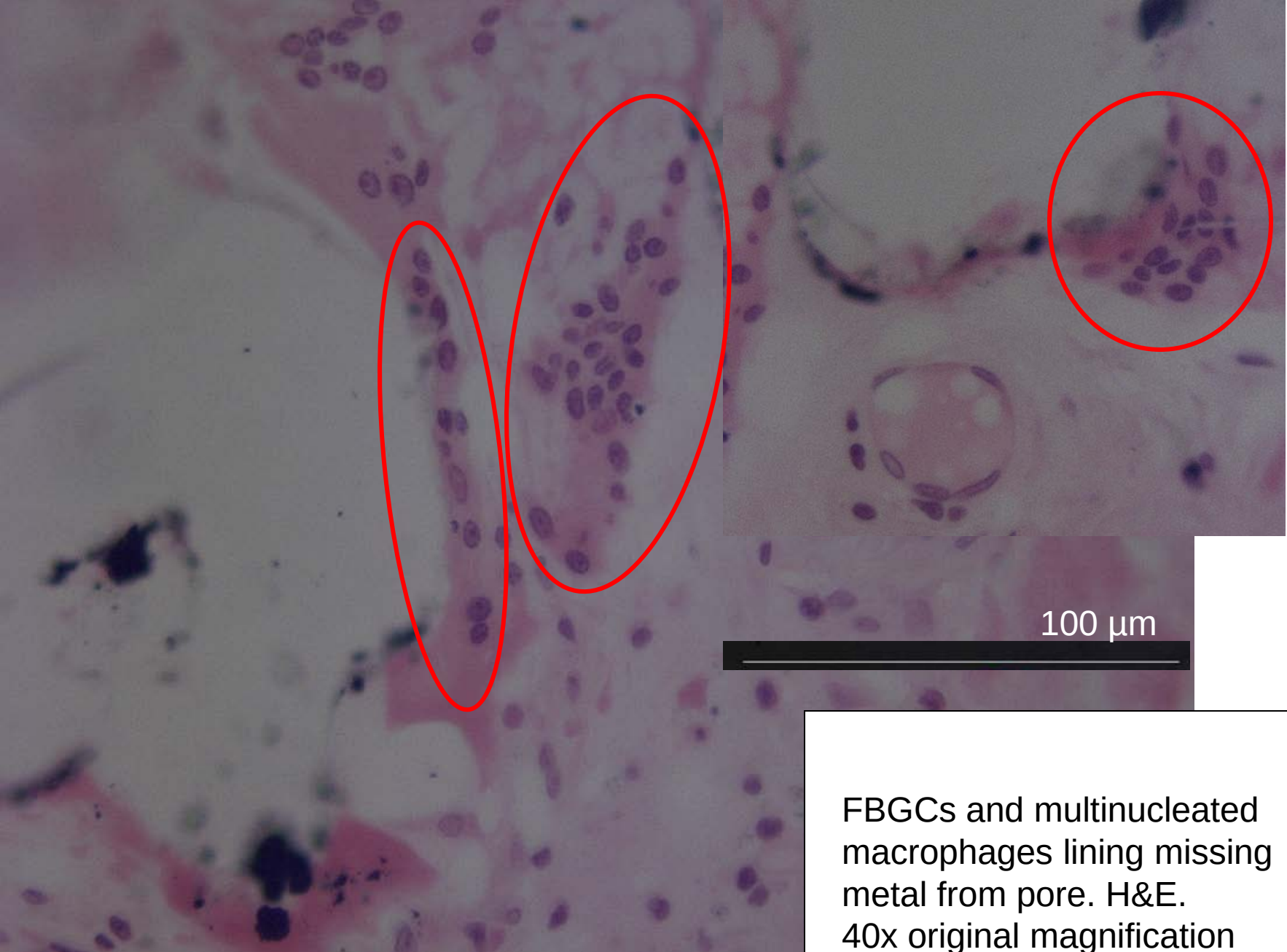
Metal pore missing with macrophages and FBGCs lining pore space.

H&E. 20x original magnification.

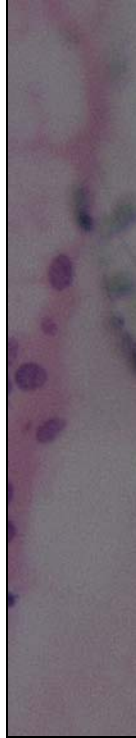
Dark pink = cytoplasm.

Dark Purple = nucleus

Light pink = collagen



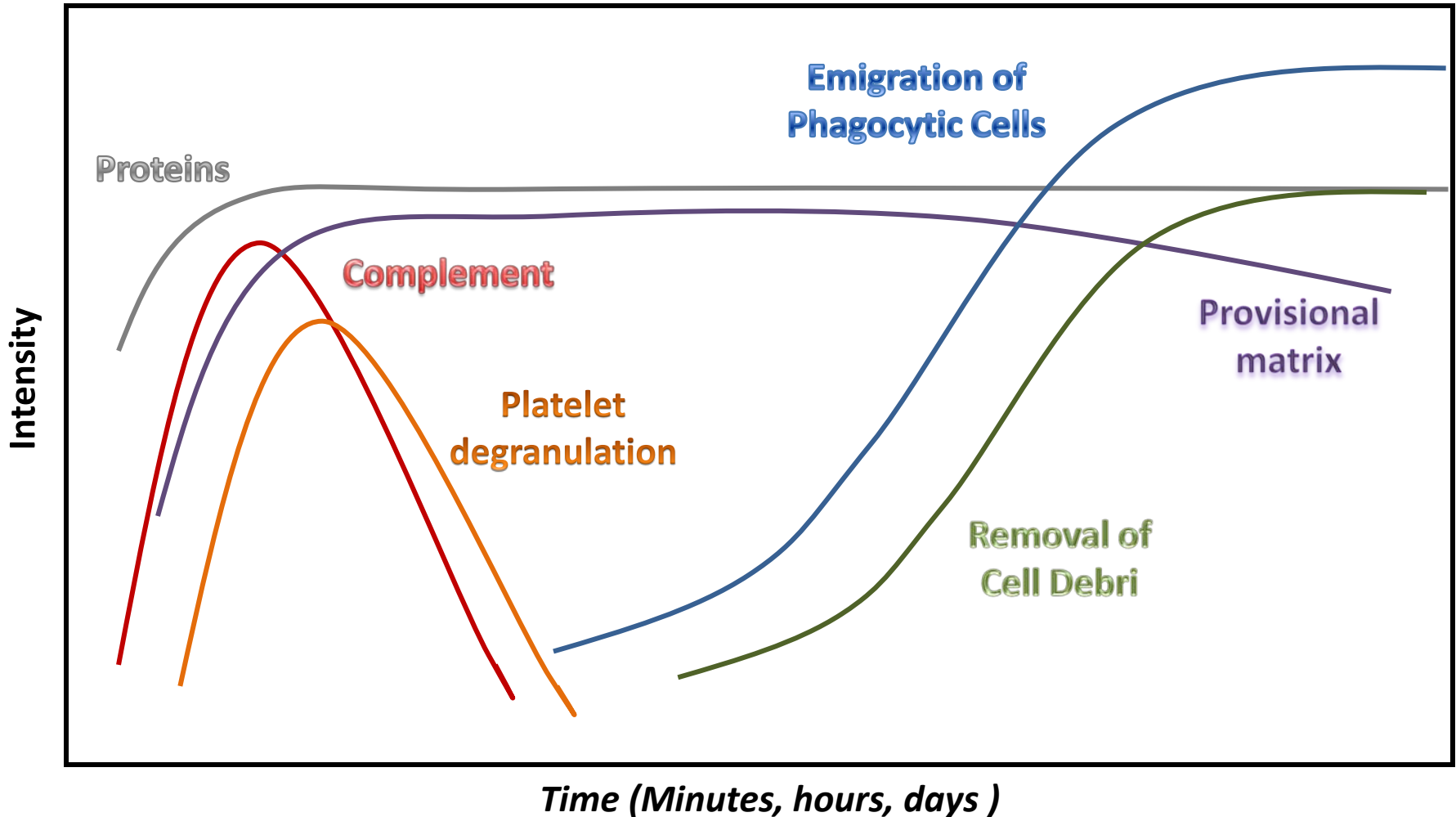
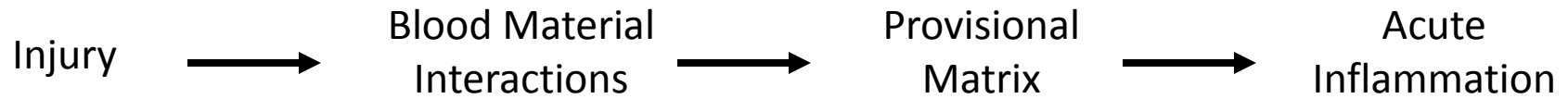
FBGCs and multinucleated macrophages lining missing metal from pore. H&E. 40x original magnification



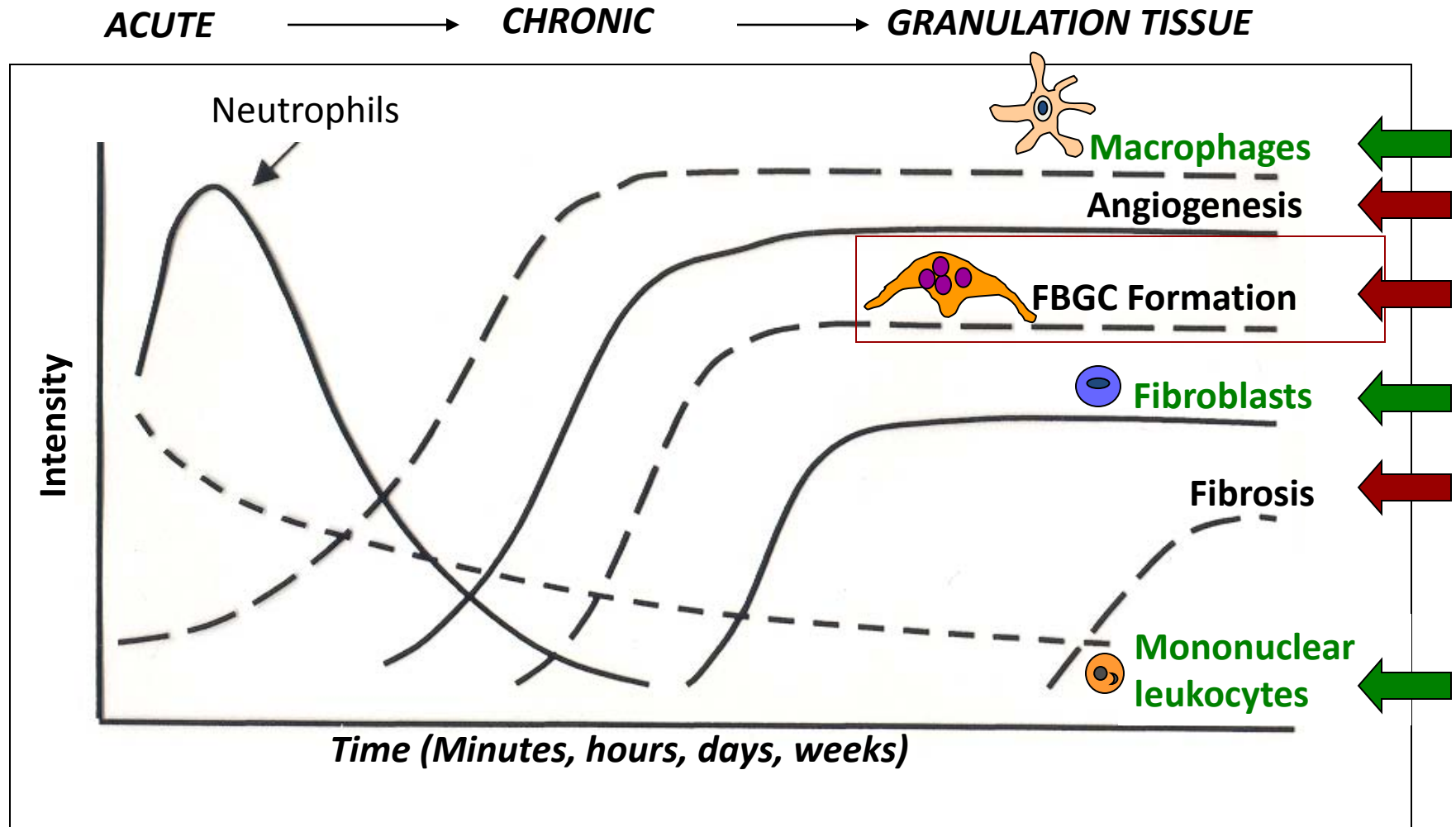
FE
ma
ma
40

Overview of the FBR

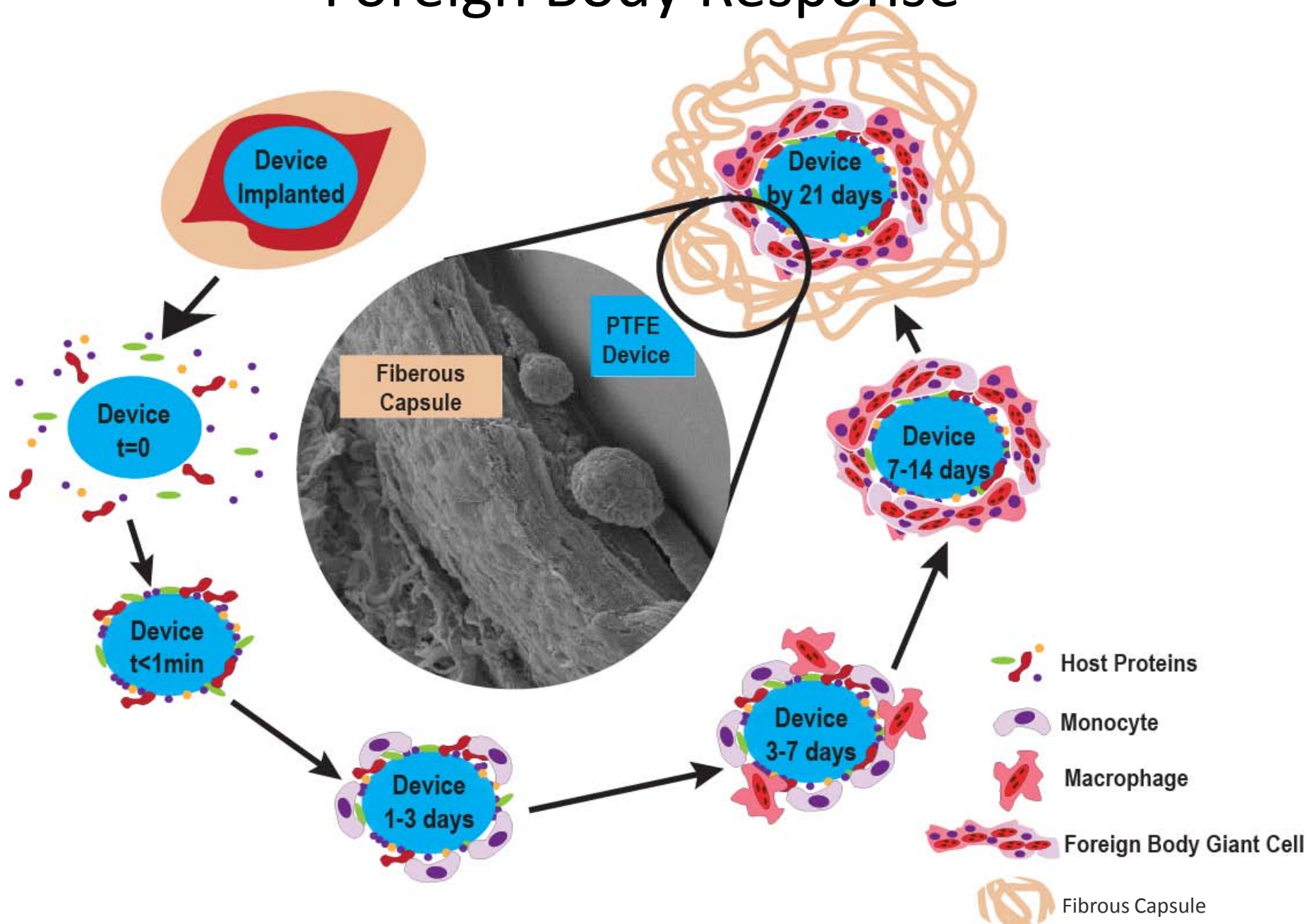
Temporal Sequence of Inflammatory Events



Temporal Sequence of Inflammatory Events



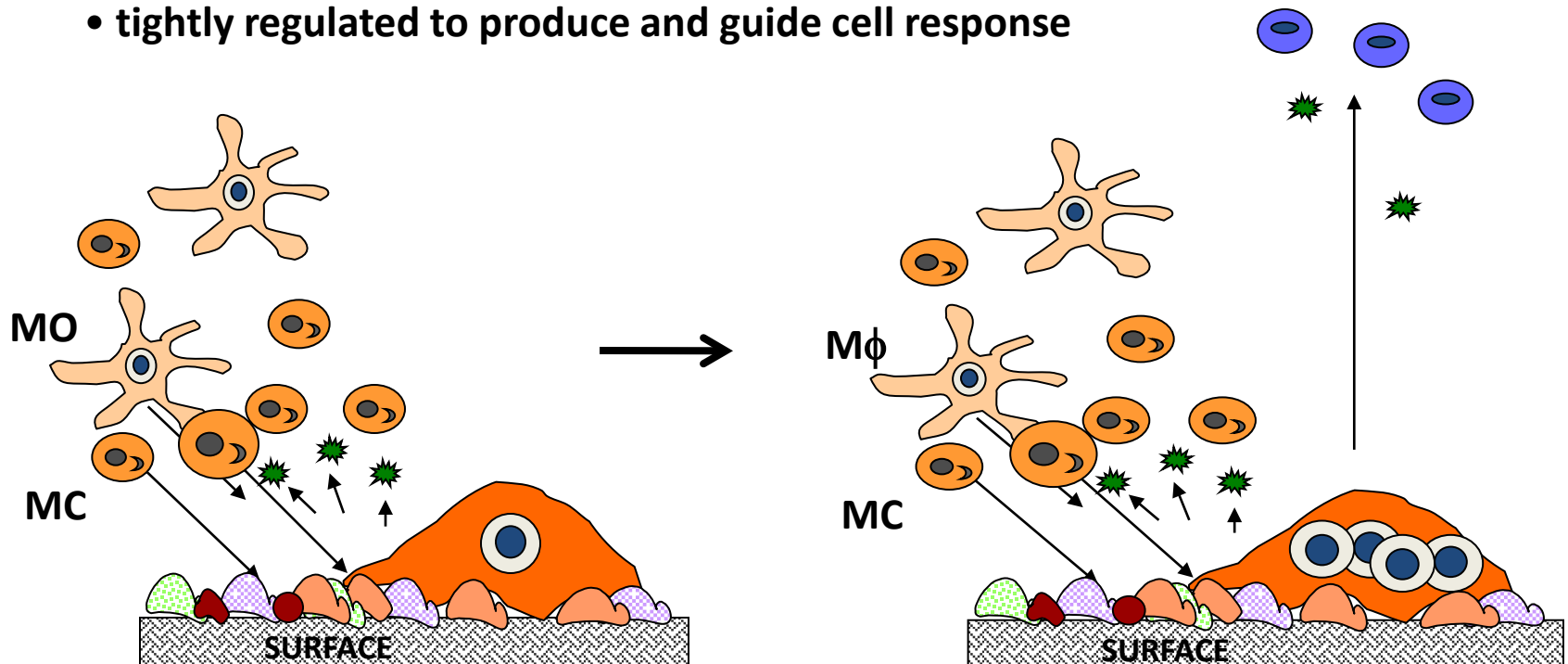
Foreign Body Response



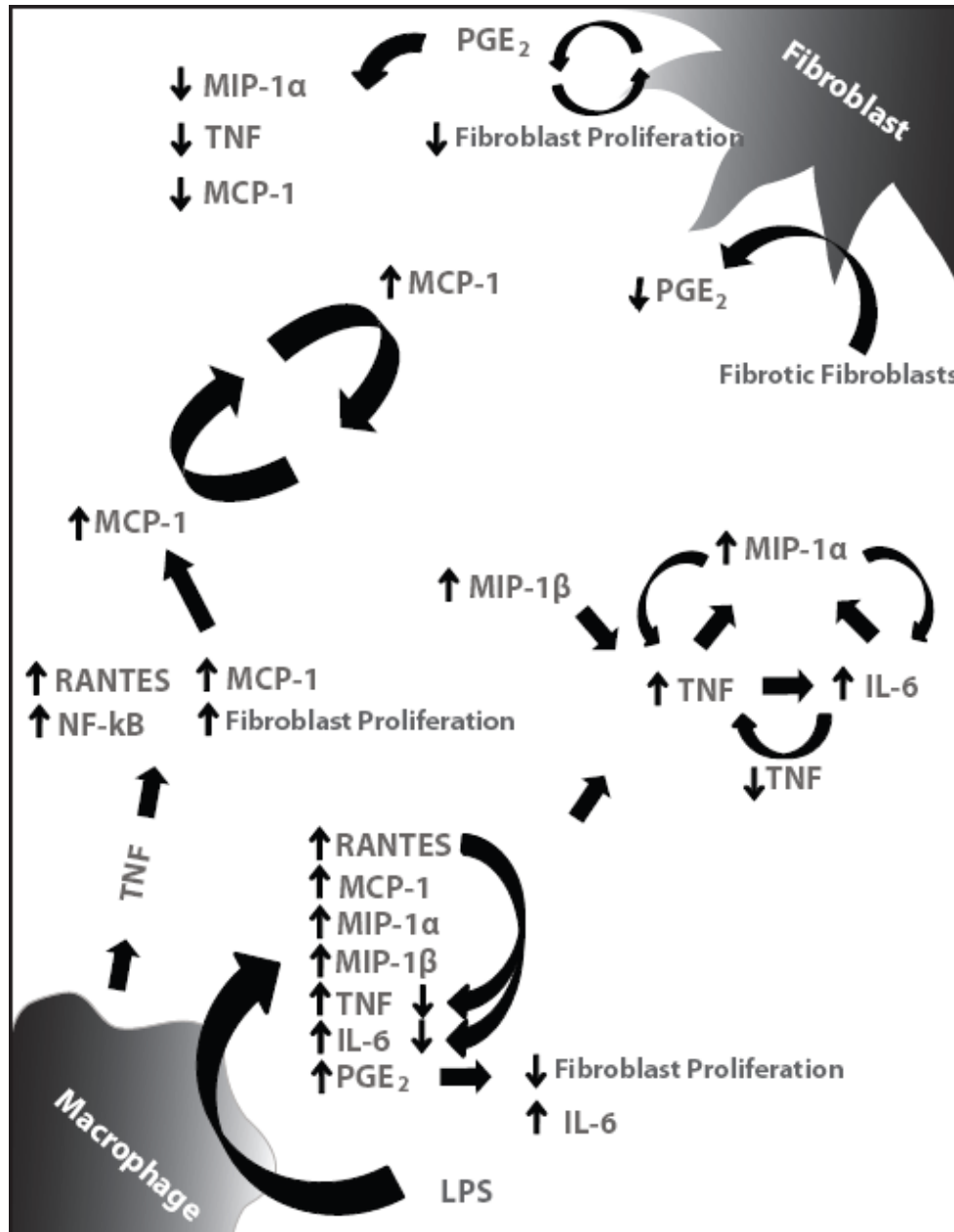
Cytokines

Cytokines: Interleukins and others

- Cytokines: protein signaling molecules between cells
- 33 known for leukocytes (interleukins: ILs)
- also macrophage-specific monokines (MLs) and lymphocyte-specific (lymphokines)
- Vary in size from 8 to 50 kDa proteins
- Production profiles at wound site helps to stimulate cells to action
 - amounts, timing, kinetics of production vs, consumption
 - tightly regulated to produce and guide cell response



Cytokine Signaling is Complex



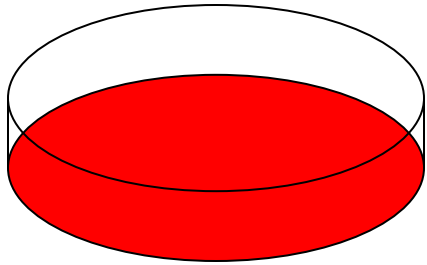
Cytokines in the Foreign Body Response

Wound Healing		Inflammation	
		Anti	Pro
Pro	IL-1ra	IL-1 β <i>VEGF</i>	
Anti	IL-10	IL-6 IL-8 <i>MCP-1</i> <i>MIP-1α</i> <i>TNF-α</i>	

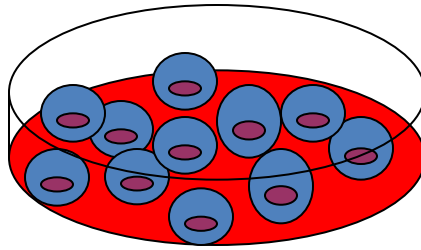
Figure 1. Cytokines have been characterized based on their roles in the foreign body reaction.³ Additions to the original source are italicized.

In Vitro Testing Methods

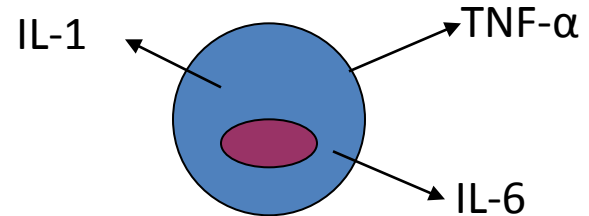
Common *in vitro* technique for testing biomaterials



Prepare Surface



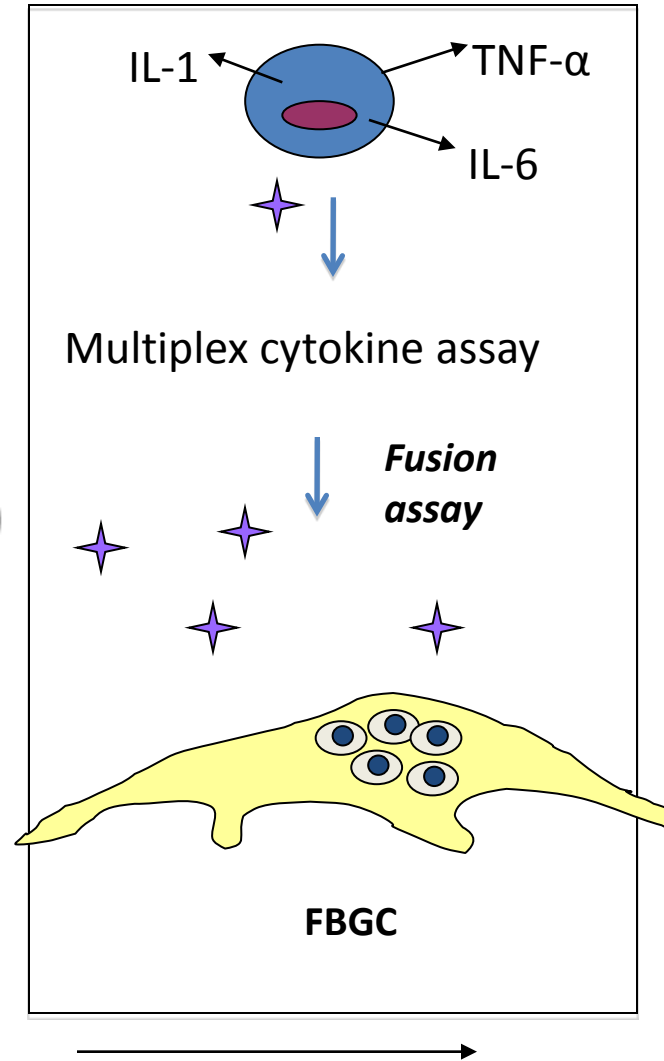
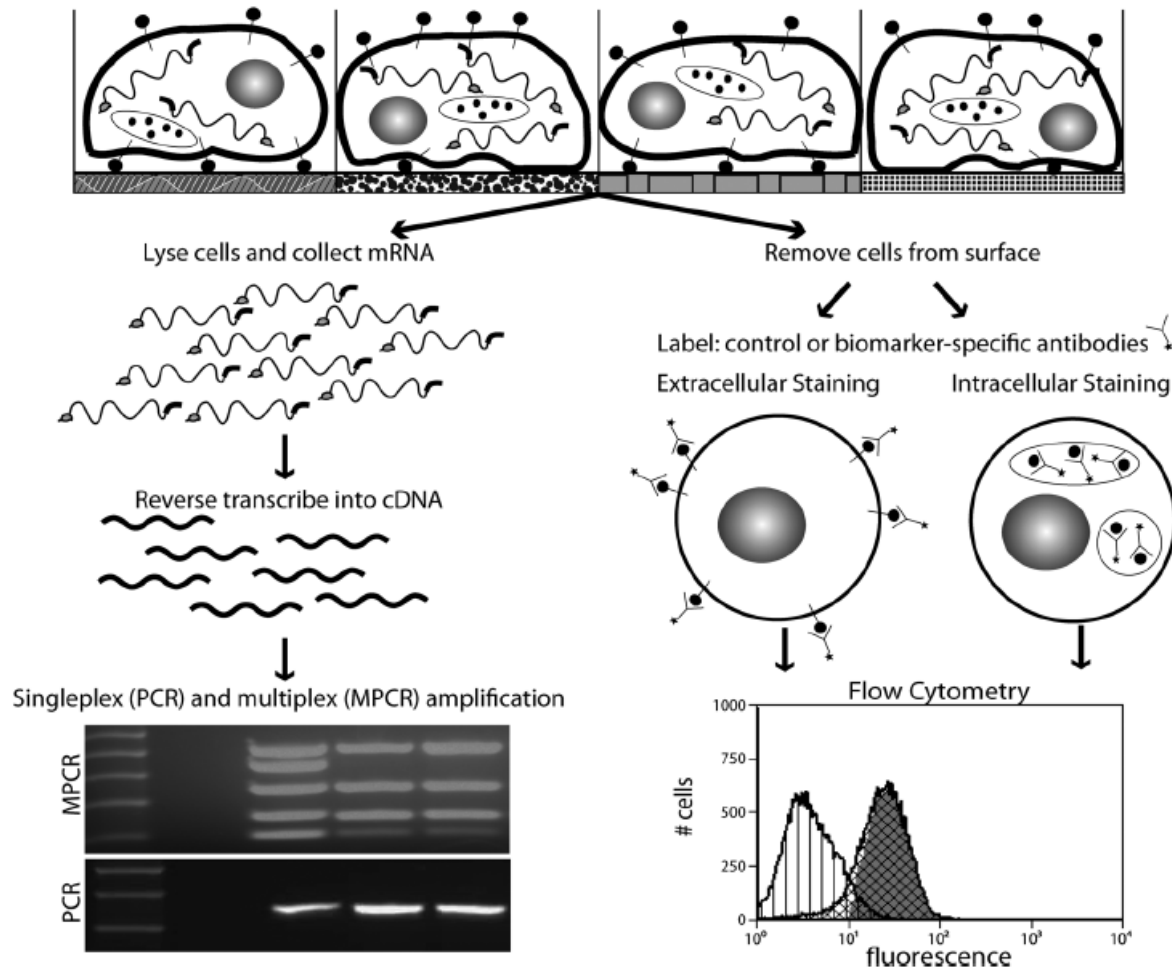
Culture macrophages
on surface



Assay inflammatory
and TH1/TH2
cytokines

- Different cell lines have different characteristics
- Cell Source
- Immortalization techniques
- Are *in vitro* macrophage cell types equivalent?
- Is there any relationship to *in vivo* responses?

Common *in vitro* testing of biomaterials



Tumorigenesis

Tumorigenesis

- Neoplasm “New Growth” = Tumor
 - Excessive and uncontrolled cell proliferation
- Benign
 - More Differentiated, well defined, no proliferative, non metastatic
 - Suffix “oma” i.e. adenoma, chondroma
- Malignant
 - Less differentiated, erratic growth, locally invasive, metastatic
Obstinate ability to adhere to adjacent structures and spread in multiple directions simultaneously.
 - Cancer = Crab-like
 - Suffix “Carcinoma”- epithelium,
 - “Sarcoma”- mesenchym i.e.
 - bone, cartilage, “leukemia”,
 - “lymphoma”



- Animals -> Sacromas are frequent
 - Rodents -> higher rate of foreign body tumorigenesis
- Humans -> Neoplasms are rare adjacent to implants
 - 50 cases reported
 - Developed within 15-25 years
 - Majority are sarcomas
 - Neoplasms can occur spontaneously and may or may not be caused from the implant

Physical rather than chemical determine tumorigenicity

- Large noncontinuous high surface area materials are considered to be most tumorigenic
- Finely shredded or woven forms, where the surface continuity is interrupted “Oppenheimer effect”, lose their tumorigenicity
- Active inflammation prevents tumorigenicity, but mature fibrous capsules enable it
- Wear particles are thought to not be tumorigenic, but particles with high aspect ratios are.

Hypothesis for Tumorigenesis

1. Foreign body reaction
2. Fibrous capsule formation
3. Preneoplastic cells contact implant surface during quiescent tissue reaction
4. Preneoplastic cell maturation and proliferation
5. Tumor Growth

The foreign body probably does not initiate the tumor, however, preneoplastic cells attaching to foreign bodies may lead to tumorigenesis due to the relatively quiescent microenvironment (which is not permitted with sustained inflammation)

Genetic Engineering

Dolly Holt, SLCC

Genetic Engineering

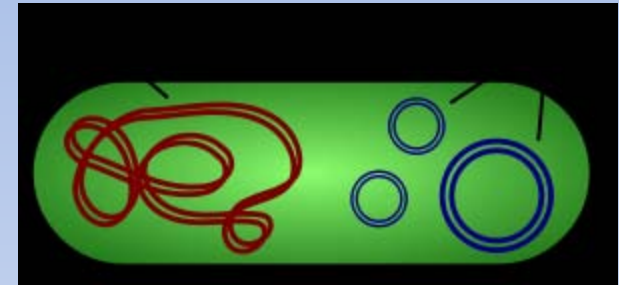
- Alternation of genetic material that does not occur in natural conditions
- Recombinant DNA
- Plant and animal breeding
- Mutagenesis
- Genetically modified organism (GMO)
 - First bacteria
 - 1982 insulin producing bacteria
 - Mice, rats, rabbits, sheep (Dolly)
 - Induced pluripotent stem cells

- Knock-in
 - Insert genetic material
 - FDA approved, antithrombin produced in the milk of genetically engineered goats
 - GFP animals
- Knock-out
 - Remove genetic material
- Knock-down
 - Not genetic, but an antisera or competitive inhibitor
- Transformation
- Transfection

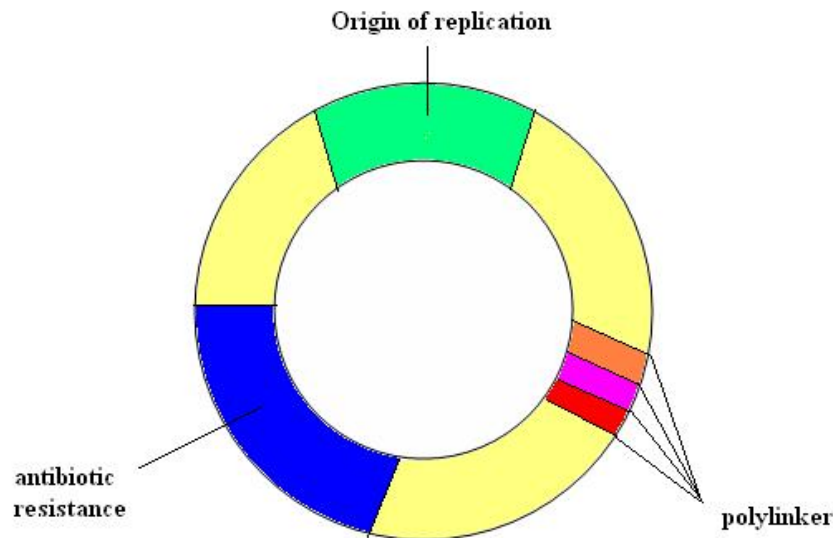
- siRNA
- microRNA
- Plasmids (Vectors)
- Viruses
 - Retroviruses-reverse transcribing viruses
 - Can reverse-transcribe their RNA genomes into DNA to be integrated into the host genome and be replicated along with it
 - Required to replicate itself
- Prions
 - Not genetic, but a protein which can refold other proteins

Plasmids

- Circular bacterial DNA



wikipedia.org



PLASMID

www.genetherapyreview.com

Nanotechnology

Dolly Holt, SLCC

"There is plenty of room at the bottom"

-Feynman

- Seminal speech on December 1959 at CalTech
- " Why can't we compress 24 volumes of Encyclopedia Britannica on a pin head ?"
- " The biological example of writing information on a small scale has inspired me to think of something that should be possible "
- In 1990, IBM scientists wrote the logo IBM using 35 xenon atoms on nickel.

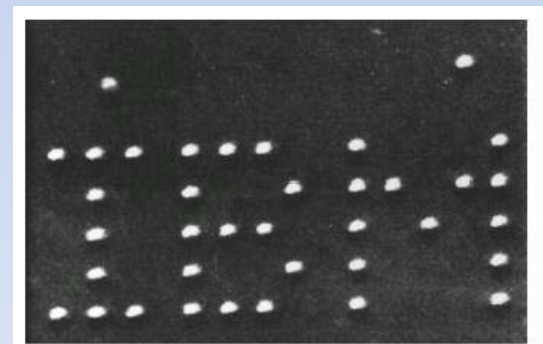
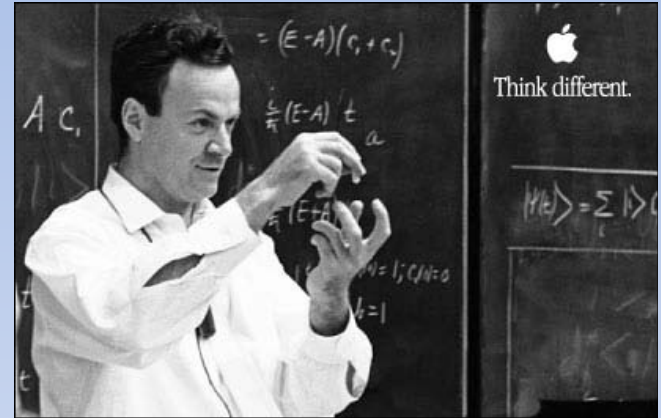
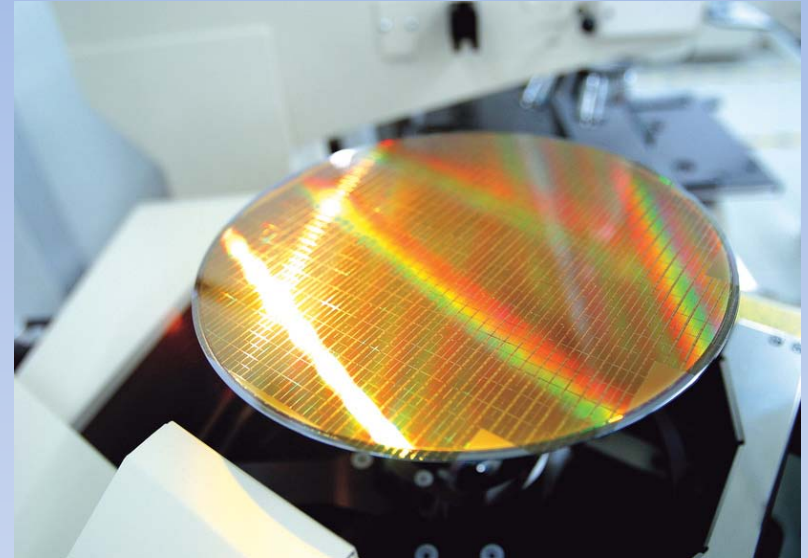


Fig. 2.25. IBM logo spelled out using 35 xenon atoms arranged on a nickel surface by an STM (courtesy of IBM Research Division).

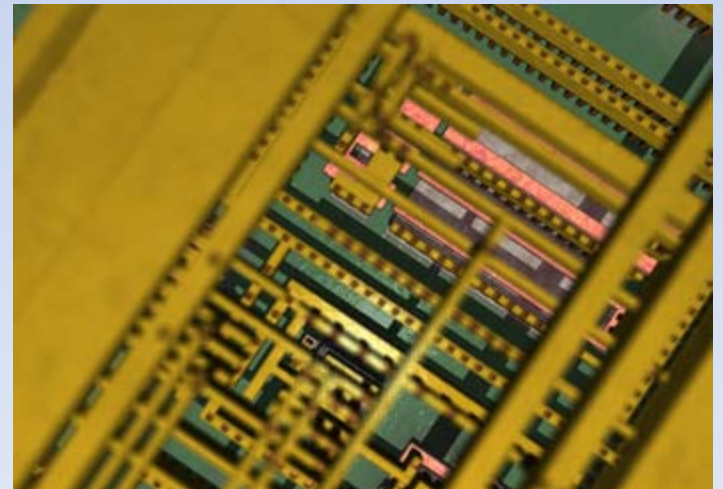
- Microelectromechanical systems (MEMS)
- Nanoparticles
 - Dendrimers
 - Spheres
 - CNT
- Nanostructures
- Nanosurfaces

Semiconductor Industry

- Microfabrication
- Primarily uses silicon
 - Glass, plastics, and other substrates can be used
- Cleanrooms
 - Smoke, dust, bacteria, cells, etc. are micrometers in size
- Active cleaning
 - Wet methods
 - Hydrogen chloride-removes metals
 - Sulfuric acid-peroxide (a.k.a. Piranha)-removes organics
 - Hydrogen fluoride-removes native oxide from silicon surface
 - Dry methods
 - Oxygen, Argon, Hydrogen



www.femtotools.com



wikipedia.org

MEMS/NEMS

- Micro/Nano-electromechanical systems
 - Lithography
- Microfluidic device
 - PDMS Stamp

MEMS Manufacturing

- Microelectromechanical systems
- Chemical Vapor Deposition (CVD)- a controlled chemical reaction produces a thin surface film
- Photoresist- light sensitive coating that is used to transfer the image of a mask onto the surface of a wafer, followed by a reactive ion etch (RIE).
- Lithography
 - Photolithography
 - Electron Beam Lithography
 - Ion Beam Lithography
 - X-Ray Lithography
- Etching
 - Wet etching
 - Chemical degradation
 - Dry etching
 - Ions or vapor phase etchant



We are working on a different kind of micromachine...



Simple Micro Machine

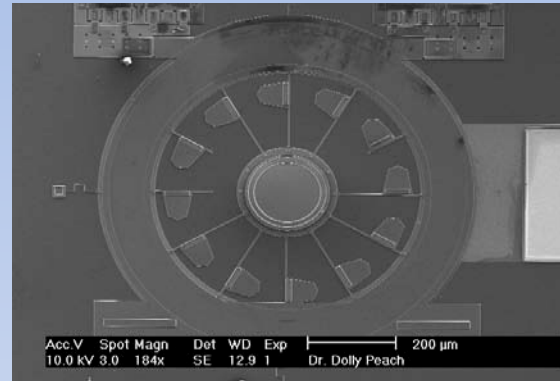
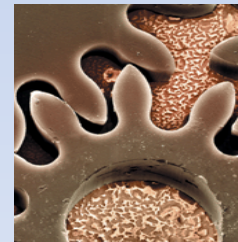
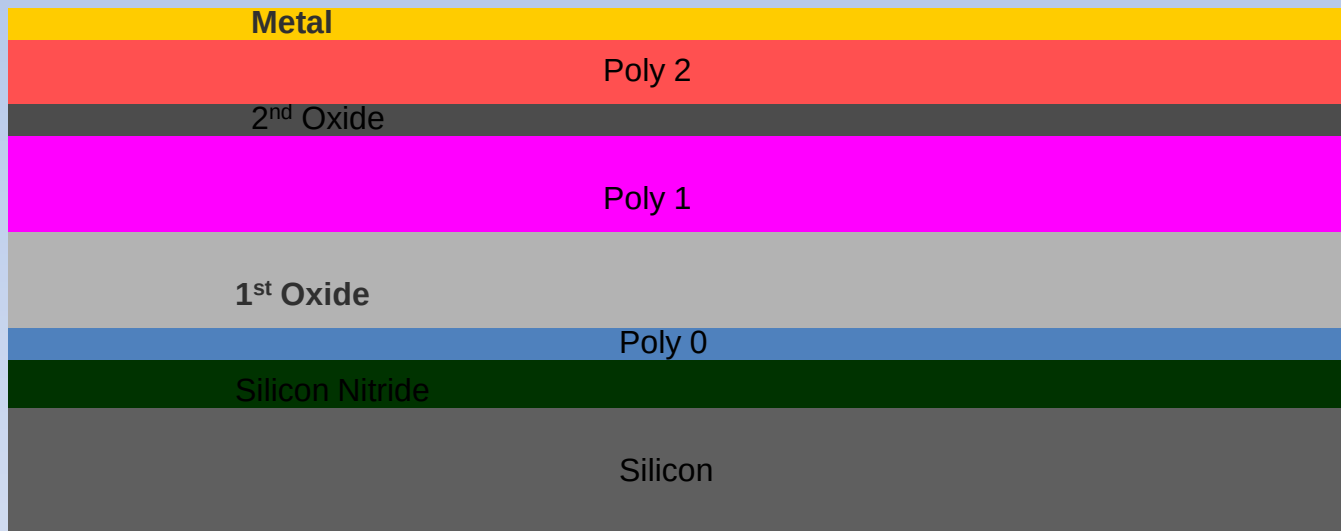


Fig 4. SEM Image: Dolly Creger

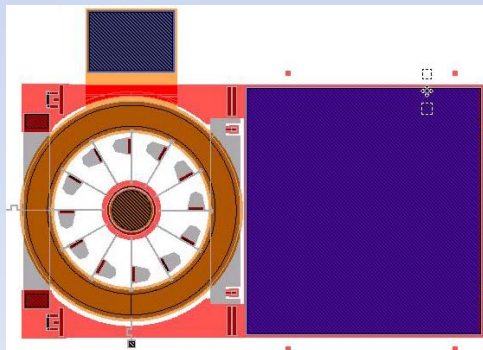
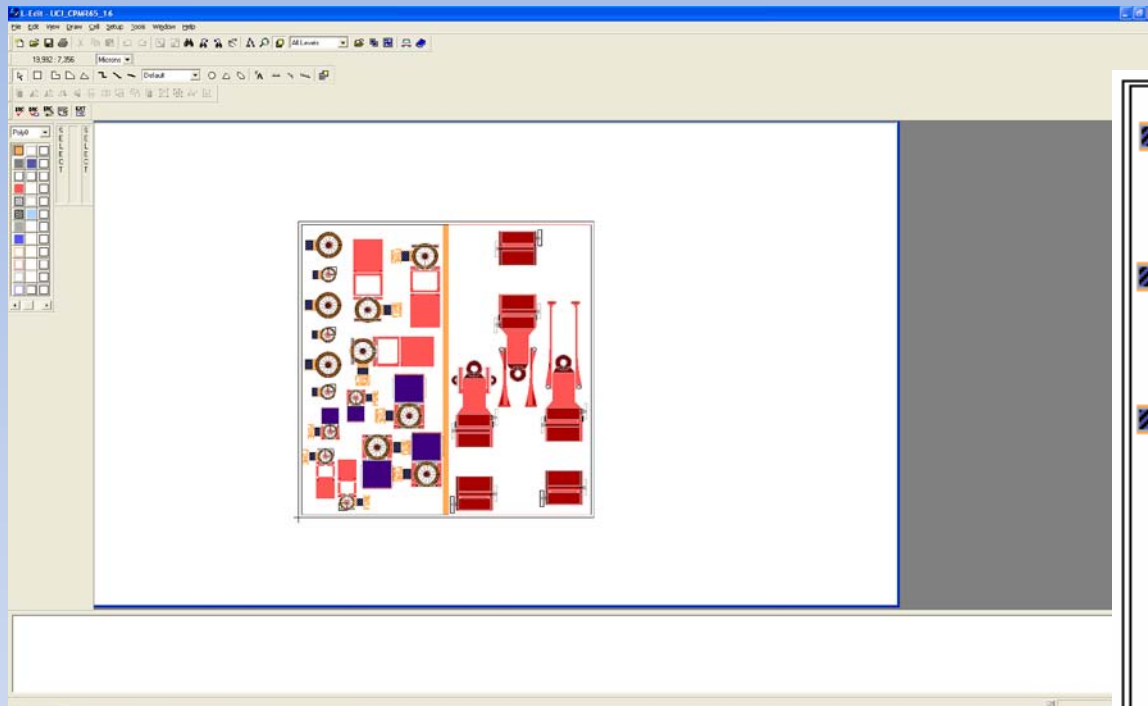


Fabrication

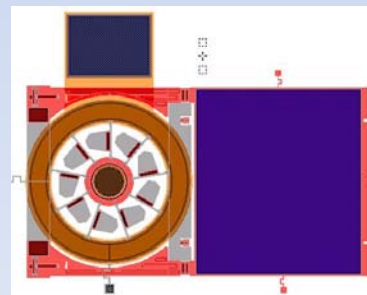
Commercial PolyMUMPs Process



L-Edit



592 μm x 527 μm



990 μm x 941 μm

Mirror

Stiction

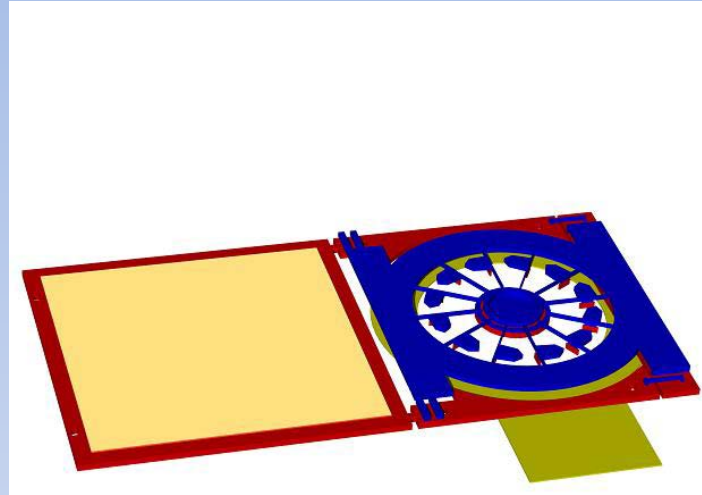
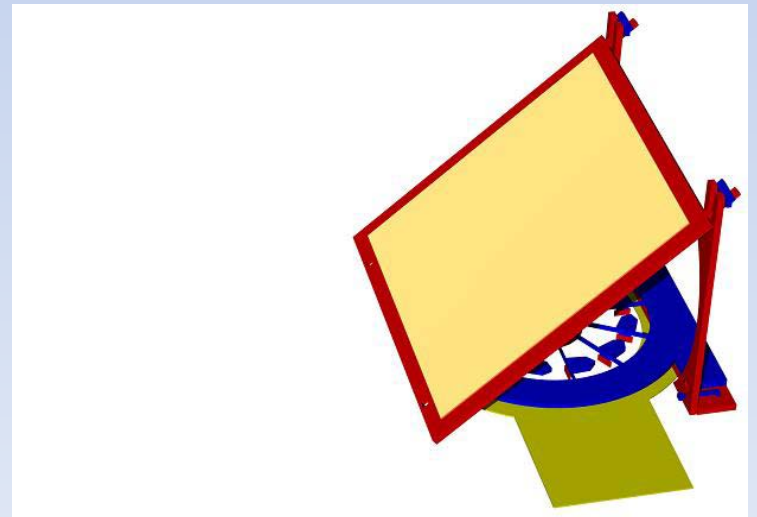
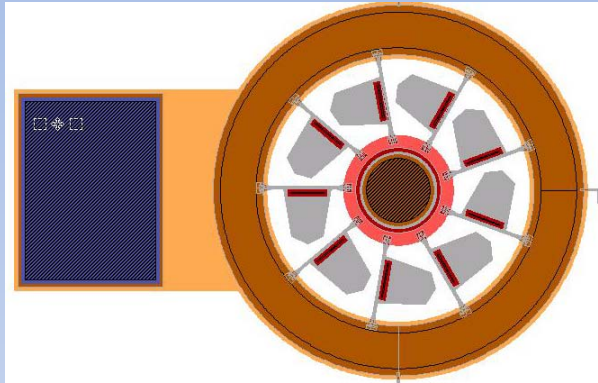


Fig 4 and 5. Mirror Assembly Jessica Ayers UCI

Assembly

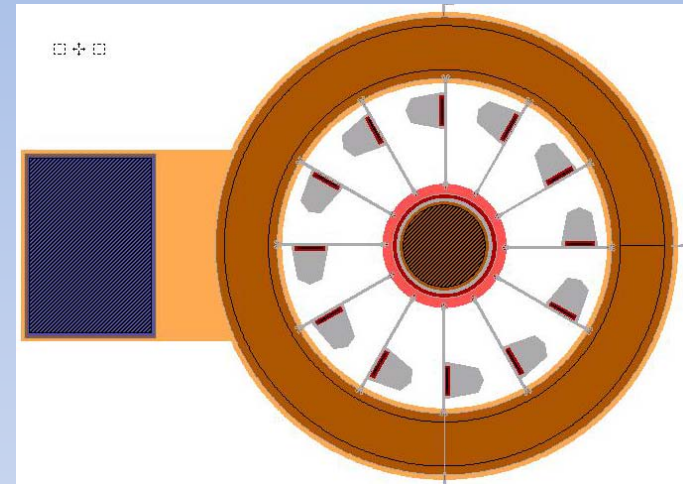


Scratch Drive



520 μm
diameter

Fig 6 and 7. Scratch Drive Array Jessica Ayers UCI



876 μm
diameter

- Electrothermal Actuators
- Microvibromotors, Electrostatic Micro Conveyor, Dipole Surface Drive Microengine
- Electrostatic Linear Inchworm Motors
- Ultrasonic/Magnetic Actuators

Stepping motion of the SDA:

- Electrostatic load deflects the plate.
- Bushing is scratched forward.
- Voltage is reduced and the plate is pulled forward.

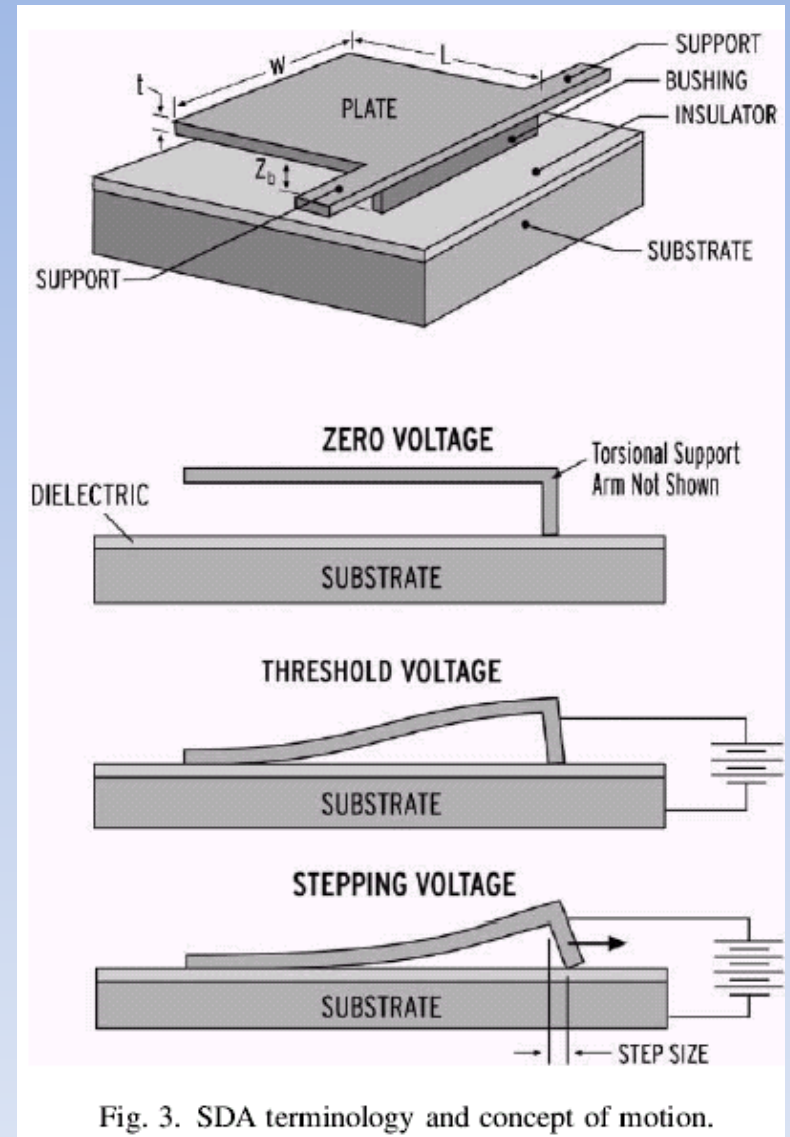
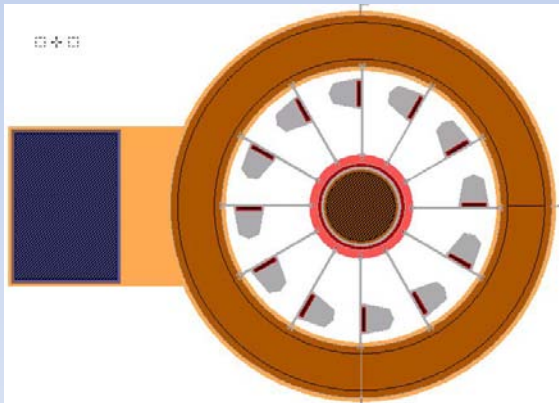
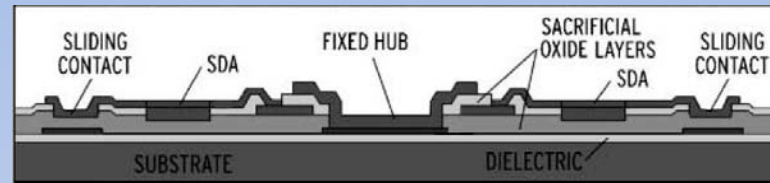
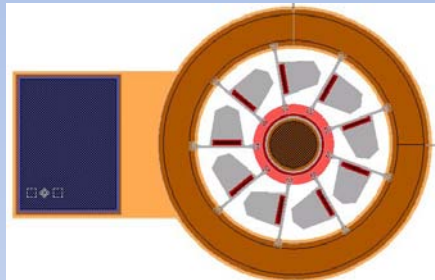


Fig. 3. SDA terminology and concept of motion.

Results

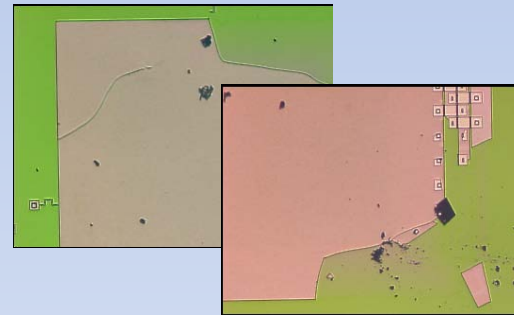
Scratch Drive: $\sim 200\text{V}$.5-1.5 Hz



R.J. Linderman et al./Sensors and Actuators A 95 (2002) 135–142

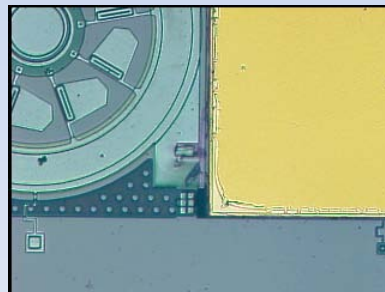
Stiction

- a. Evaporation
- b. Instantaneous vaporization
- c. Leave submerged

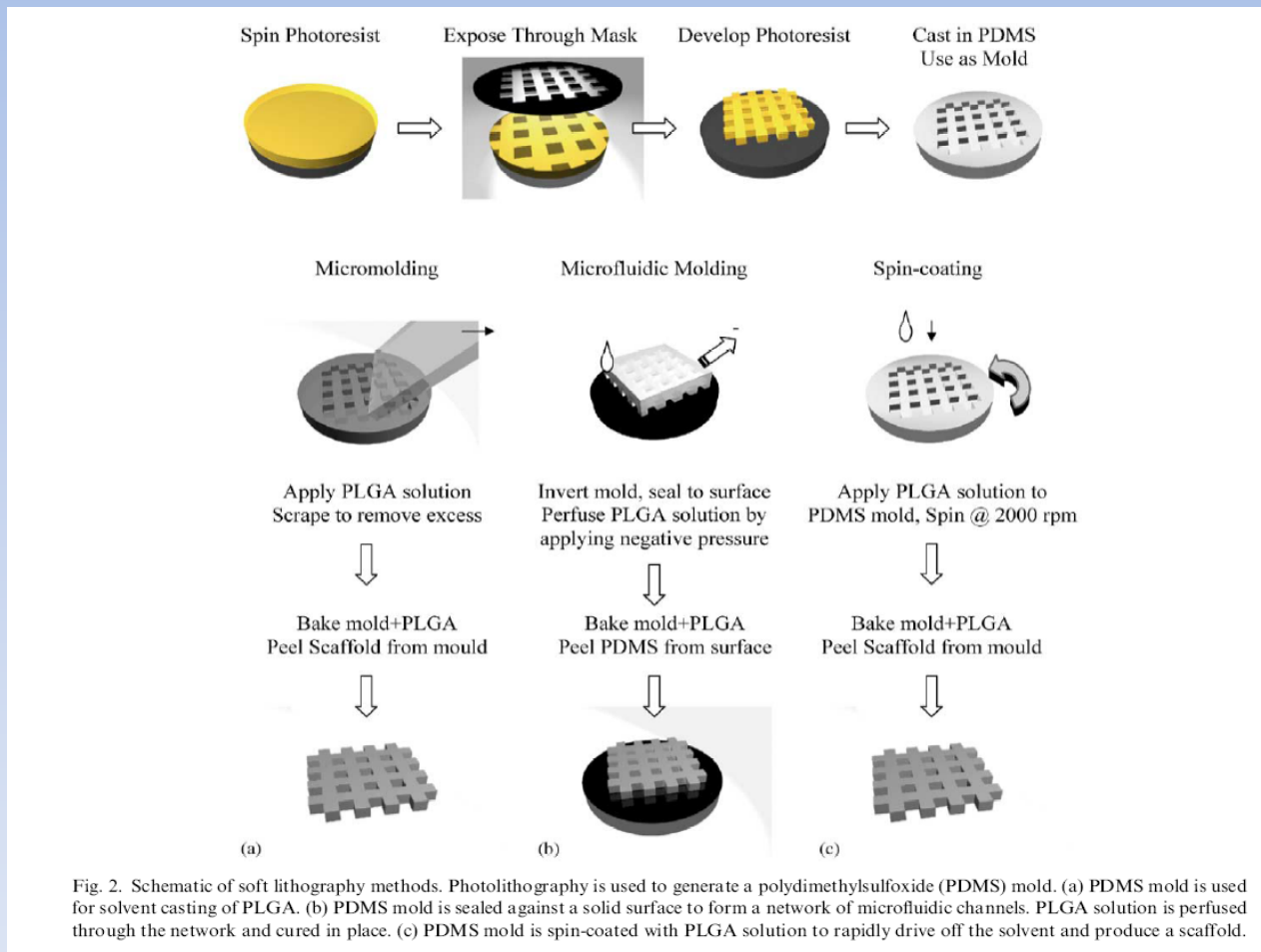


Assembly

- a. Probe Station
- b. Microspatula
- c. Magnetism

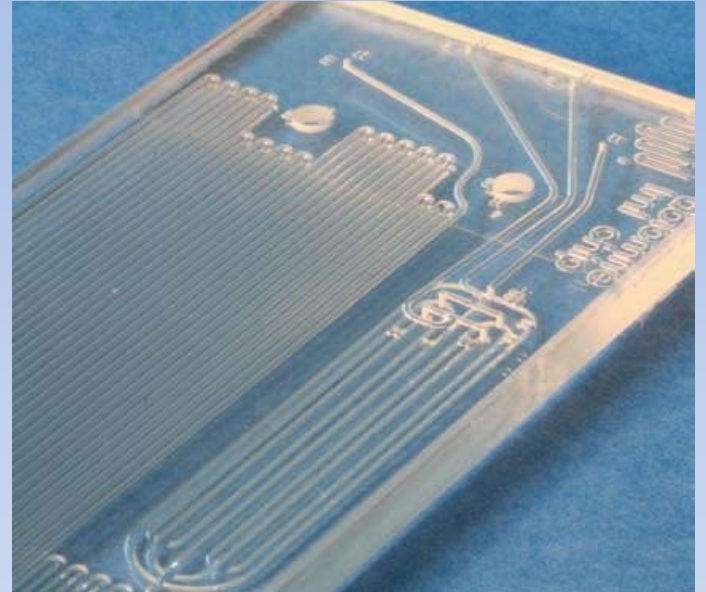


Patterned Surfaces: Soft Lithography

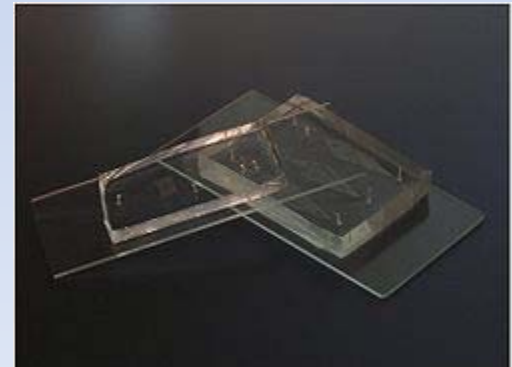


Microfluidic devices

- DNA chips
- Lab-on-a-chip
- Inkjet printheads



www.labmate-online.com

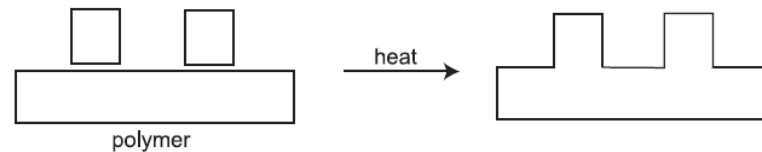


wikipedia.org

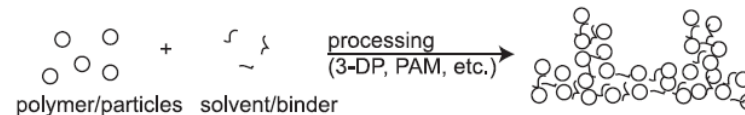
Printing Techniques for Tissue Engineering

Acellular Scaffold Fabrication

Fabrication using Heat



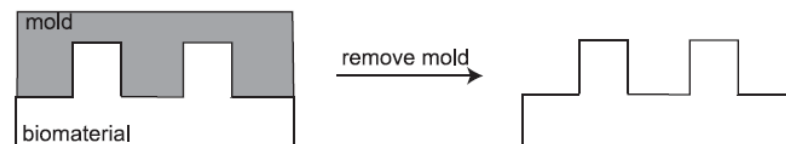
Fabrication using Adhesives



Fabrication using Light



Fabrication by Molding



An Ink-Jet Printer for Tissue Engineering?



Nanoparticles

- Quantum dots
- Carbon nanotubes
- Gold, silver
- Silicon
- Polymer
 - PLGA
 - Polystyrene

Nanoparticle Applications

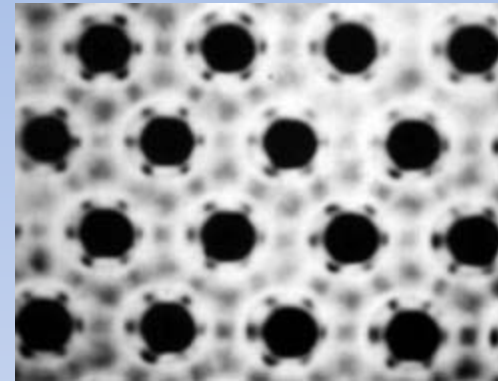
- Diagnostics
 - Contrast Agents
 - Imaging
 - Biosensors
 - Early-warning health monitoring
 - Assays
 - Cytometric Bead Array Assay (CBA)
 - Immunoprecipitation
- Therapeutics
 - Drug Delivery
 - Targeted Drug Delivery
 - Non-viral Gene Therapy
 - Protein, peptide delivery
 - Targetted Chemotherapy
 - Agents for Tissue Engineering

Nanoparticles for drug delivery

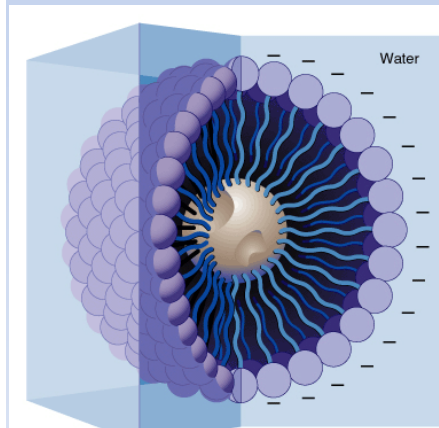
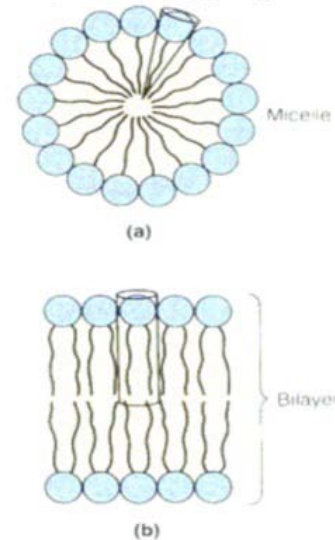
- Polyethelene glycol (PEG)
- Liposomes
 - Doxorubicin
 - Amphotericin B
- Gold
- PLGA
- Targeting
 - Conjugation

Nature's Fabrication Technique: Self-assembly

- Self assembling monolayer
- Lipid mycells and bilayers
 - Self-aggregation of hydrophilic, lipophilic groups
 - First layer creates template for growth of second layer
- Ions can be deposited on charged sites
- This kind of self-aggregation leads to ordered, heirarchical structures
- Techniques to study this atomic-scale morphology



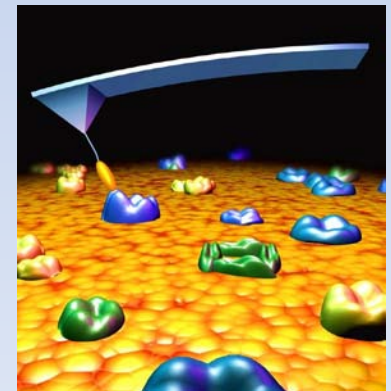
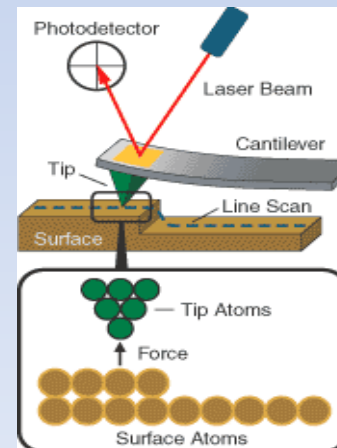
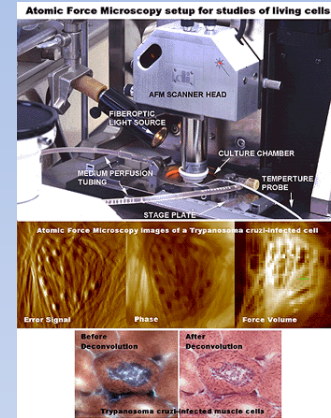
Fatty acid aggregation



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Scanning Probe Microscopy

- Atomic Force Microscopy
 - Surface irregularities
- Scanning Tunneling Microscopy
 - Conducting Surfaces
- Adhesion Force Microscopy
 - Functionalised tips



Enhanced intrinsic biomechanical properties of osteoblastic mineralized tissue on roughened titanium surface

- Nano-indentation
- Acid-etched vs. Machined surfaces
- culturing osteoblasts on rougher titanium surfaces enhances hardness and elastic modulus of the mineralized tissue

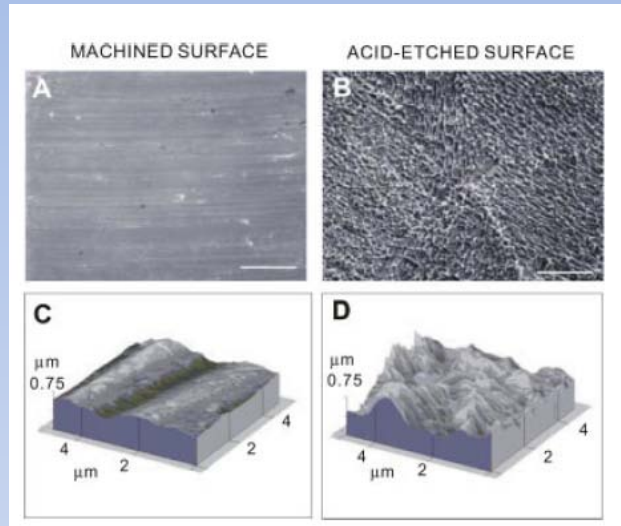
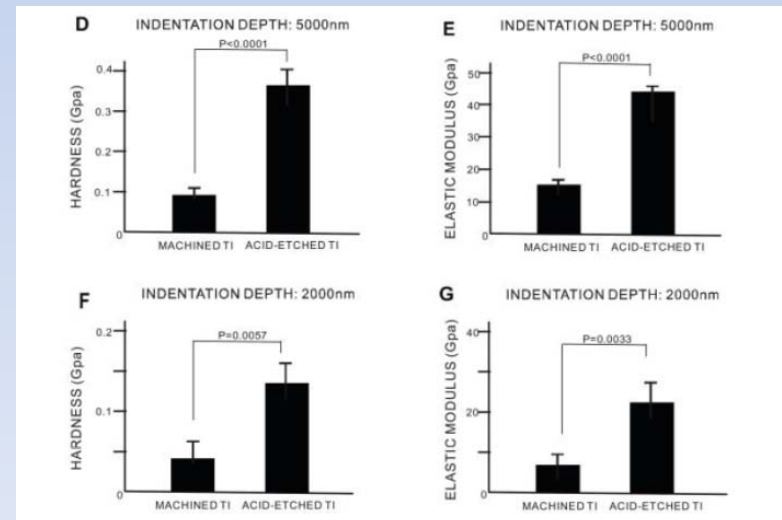


Figure 1. Surface morphology of titanium discs for osteoblastic cell culture. SEM images of machined titanium (A) and acid-etched titanium (B). Bar = 20 μm . AFM images of the machined titanium (C) and the acid-etched titanium (D). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]



Surface modification of SPU

- Segmented Polyurethane – common biocompatible elastomer
- 2-methacryloyloxyethyl phosphorylcholine was added to create nano-domains on surface
- Nano-scale domains reduce platelet adhesion to biomaterial surface

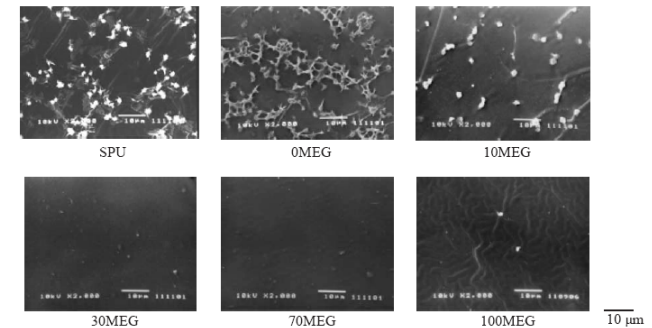


Fig. 5. Platelet adhesion on polymer surfaces.

N. Morimoto et al. / Biomaterials 25 (2004) 5353–5361

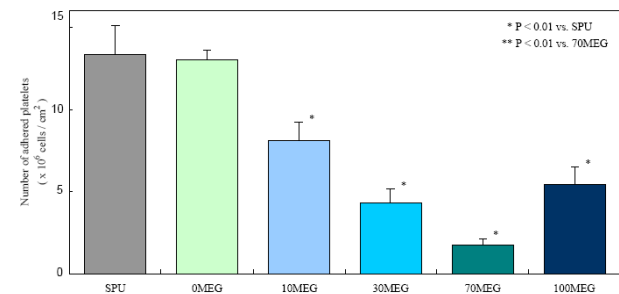
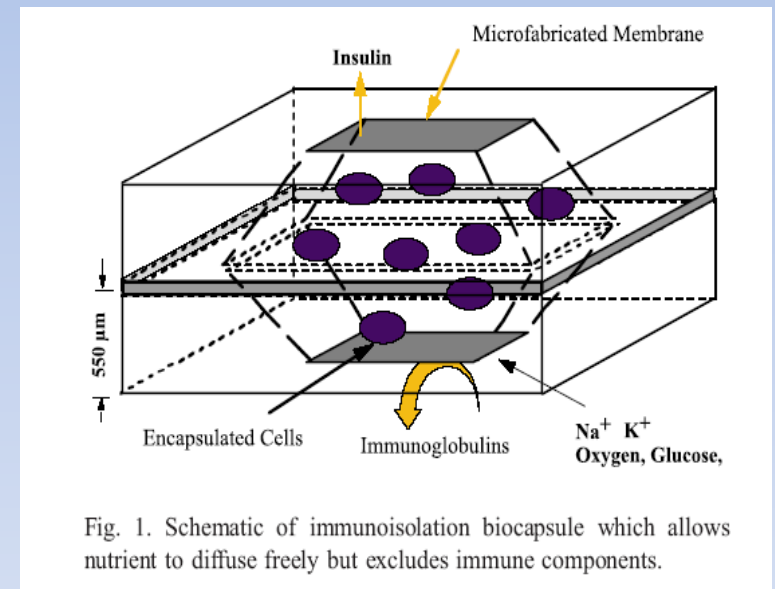


Fig. 4. Number of adherent platelets on polymer surfaces.

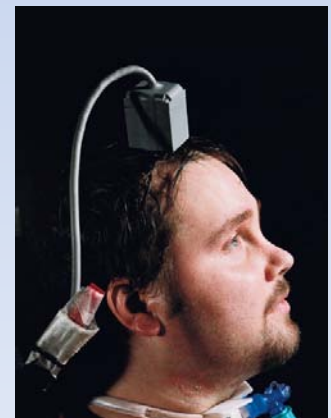
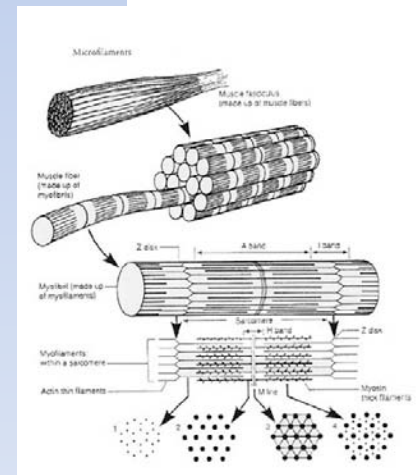
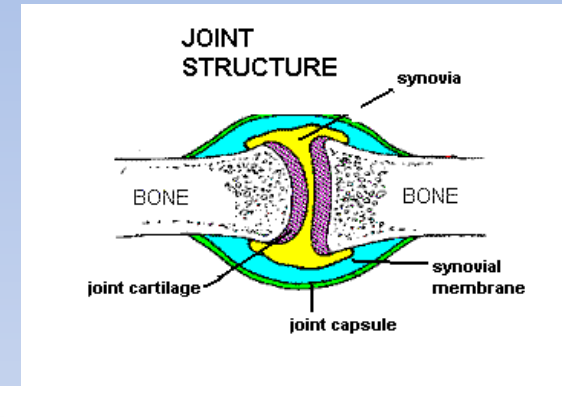
Immunoisolation for Cell-encapsulation therapy

- Liver Dysfunction
 - Encapsulation of Hepatic Cells
- Pancreas Dysfunction
 - Encapsulation of Islets of Langerham
- Disorders of the CNS
 - Parkinson's, Alzheimer's
- Pre-requisites for cell encapsulation
 - continued and optimal tissue/cell supply
 - maintenance of cell viability and function
 - successful prevention of immune rejection
- Nanoporous Silicone-based biocapsules serves as Artificial Pancreas(Desai et al. 2001)



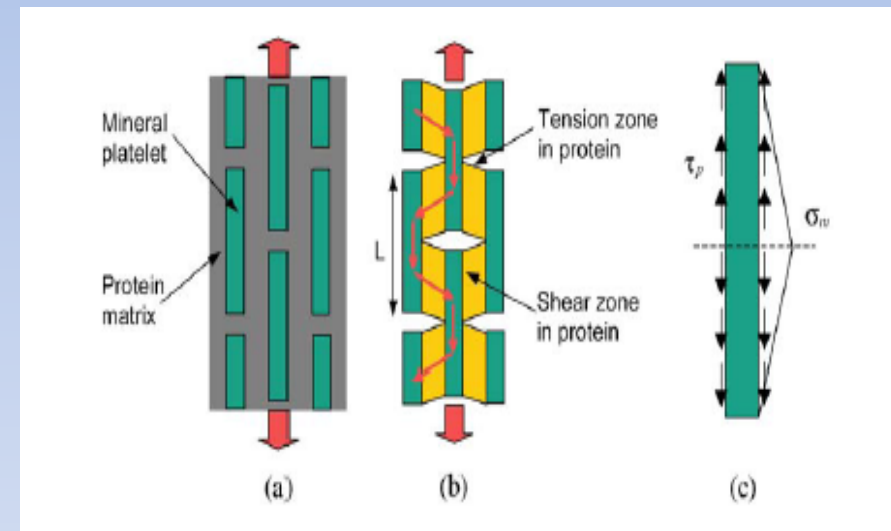
Nanoengineering Bio-analogous Structures

- Bone-cartilage composite ?
- Muscle ?
- Brain-machine Interface ?



Architecture of Hard Tissue

- Staggered mineral platelets (hydroxyapatite) embedded in a collagen matrix
- Arrangement of platelets in preferred orientations makes biocomposites intrinsically anisotropic
- Under an applied tensile stress, the mineral platelets carry most of the tensile load
- Protein matrix transfers the load between mineral crystals via shear
- Biocomposites can be described through tension-shear model described by Ji et. al.



Conclusion

- Nanotechnology allows for better recognition, integration of bionic implants with host tissue
- Allows for precise, targeted delivery of therapeutic agents
- Stealth technology for health monitoring
- Can lead to design of multifunctional biomaterials.

Definitions

Microelectromechanical Systems (MEMS)- micron-scale devices linking mechanical parts to electrical components.

Optical Coherence Tomography (OCT)- Imaging technology which uses infrared light to image cross-sections of biological tissue with micron scale resolution.

Stiction- The surface tension of liquid on MEMS components.

Shear Mode Quartz- A piezoelectric effect in a lateral plane.



Origination of the field of Biomedical Engineering

Dolly Holt, SLCC

Degree

- 1921 in Germany there was one formal program
 - Oswalt Institute for Physics in Medicine
- 1950s the earliest academic programs began to take shape in US
 - Johns Hopkins University, the University of Pennsylvania and the University of Rochester received NIH funding for formal discussions for teaching BME
- Boston University in 1966; Case Western Reserve University in 1968; Northwestern University in 1969; Carnegie Mellon, Duke University, Rensselaer and a joint program between Harvard and MIT in 1970; Ohio State University and University of Texas, Austin, in 1971; Louisiana Tech, Texas A&M and the Milwaukee School of Engineering in 1972; and the University of Illinois, Chicago in 1973.
- 1972 Duke: First undergraduate biomedical engineering program
 - Accredited by Engineering accreditation Commission of ABET
- Combine degrees
 - Pharmaceutics
 - Biology
 - Electrical Engineering
 - Mechanical Engineering
 - Physiology
 - MD

History of Biomedical Engineering

- 50 years ago “Biomaterial” wasn’t used
- Essentially no device manufactures
 - Except those for external prosthetics, such as limbs, fracture fixation, glass eyes, and dental devices
- No regulatory approval process
- No understanding of biocompatibility
- No formal biomaterial courses

History of Biomedical Engineering

- Prehistory
- Era of the surgeon hero
- Designed biomaterials/engineered devices
- Contemporary era

Biomaterials before WWII

- Thebes
 - 3000 year old mummy, wooden prothetic tied to its foot to serve as a big toe.
- Egyptians
 - Used hollowed reeds to look and listen to internal organs of the human anatomy
- Myans
 - Nacre teeth from sea shells 600 A.D. (received bone integration)
- Europe
 - Iron dental implant 200 A.D.
- France
 - 1860 French physician used a rolled up newspaper to listen to a woman's heart beat, leading to today's stethoscope

Biomaterials before WWII

- Cautery
- Sutures
 - Egyptians- linen sutures
 - Europe middle ages- Catgut
 - Greek- metallic sutures (gold wire) others used (lead and silver)
- Contact lens
 - Leonardo Davinci 1508 developed the idea of a contact lens.
 - Adolf Fick (best known for laws of diffusion) developed first glass contact lense 1860
- Most implants prior to 1950 had a low probability of success
 - Poor understanding of biocompatibility and sterilization

Post WWII-the surgeon hero

- After the war, many materials were now available to surgeons
 - Silicones, polyurethanes, Teflon, nylon, and methacrylates, titanium, and stainless steel
 - They were allowed a lot of freedom to test inventions when their patients health and life were at stake
- Intraocular lenses
 - Sir Harold Ridley, M.D.
 - Shreds of poly methyl methacrylate (PMMA) Plexiglass from fighter plane canopies lodged in eyes of fighter pilots without adverse reaction
 - At this point all foreign materials were considered to have an adverse reaction
 - Ridley used PMMA for the first intraocular lens
 - To replace the lens in individuals with cataracts
 - Before this point cataracts lead irreversibly to blindness

- Artificial hip and knee
 - Failed attempts with glass, metal on metal, and Teflon
 - First success in 1961 Stainless steel femoral stem, ball head and plastic acetabular cup
 - high molecular weight polyethylene
 - Fast setting dental acrylics
 - Stainless steel

X-Ray

- Wilhelm Roentgen
 - German Physicist
 - Accidentally discovered X-rays in 1896
 - Cathode-ray tube could make a sheet of paper coated in barium platinocyanide glow even when they were in separate rooms
 - He realized they must be emitting a penetrating ray he termed “X” for unknown ray.
 - At nearly the same time he discovered these rays, he discovered their primary use.
 - By placing his hand in front of the cathode ray, he found that a replica of his internal bone structure was reflected on the paper behind.



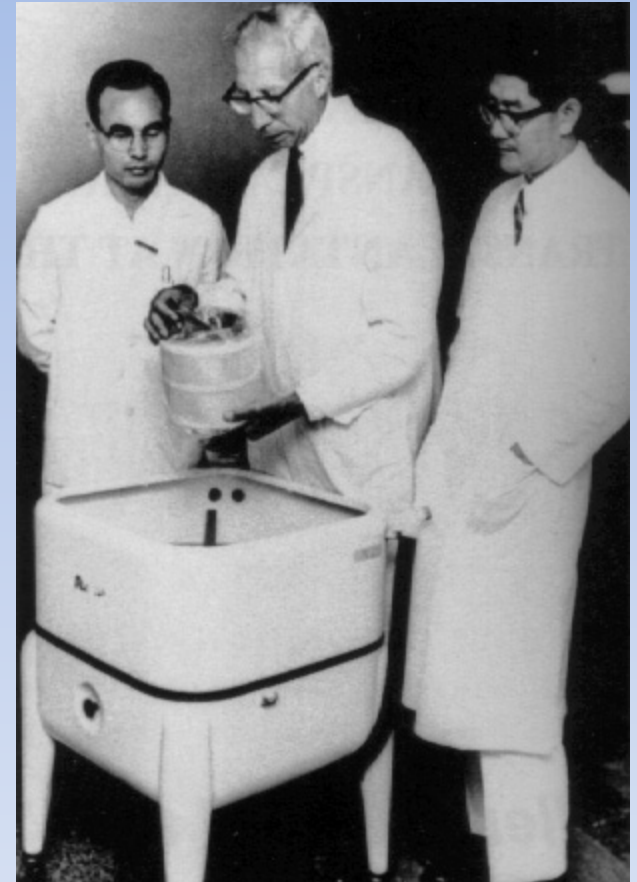
Kevles, Bettyann Holtzmann (1996). *Naked to the Bone Medical Imaging in the Twentieth Century*. Camden, NJ: [Rutgers University Press](#). pp. 19–22. [ISBN 0813523583](#).

History of Tissue Engineering

- 1984 -Earliest cited reference for the term “tissue engineering”
- 1985 -Y.C. Fung proposes center for “engineering of tissues” (denied!)
- The notion of TE as a unifying concept arose in the late 80’s and early 90’s.
- However, the practices of TE are much older:
- Clinical Medicine (16thcentury allografts!)
- Vascular Grafts (early 20thcentury –improved in 1950’s)
- Skin Grafts (cultured *in vitro* in 1970’s)
- Artificial Organs 1940’s to 1970’s (Kidney, Heart –think Kolff)
- Modern Biomaterials (post WWII)
- It is reported that the most significant conceptual breakthrough that leaddirectly to TE was by J. Vacanti—he claims to have conceived of the use of *porous*, biodegradable polymers as 3D scaffolds while observing seaweed on Cape Cod in 1986. A quick call to his friend and collaborator R. Langer lead to the explosion of the field. While a nice story, it is only one part of TE’s genesis. Others were already working on encapsulation, cell sourcing and culture, etc. In 1984 there was one paper written in this area by 2000 there were 214.

Artificial Kidney

- Kidney failure used to lead to an unpleasant death lasting about a month.
- 1910 John Hopkins made first attempts to remove toxins from blood
- 1943 William Kolff used cellulose as a dialysis membrane and began to save lives
- Cleveland Clinic “washing machine artificial kidney”
- Scribner shunt
 - Enabled chronic dialysis possible
 - A U-shape connected between a vein and an artery
 - A Teflon tube to access vessels
 - A Dacron Sewing cuff through the skin
 - Silicone rubber tube for blood flow



Artificial Heart

- 1953 first heart long machine
 - Invented by John Gibbon
- 1966 first left ventricle assist device (LVAD)
 - Michael DeBakery
- University of Utah
- William Kolff
- 1957 first implanted in a dog (Western hemisphere, Russia had one in 1930)
- 1982 first implanted Barney Clark
 - Jarvik 7
 - Dr. DeVries
 - Patients living up to 620 days on the Jarvik hearts.

If a man can grow a heart,
he can build it"
Willem J. Kolff



Contemporary areas of Bioengineering

- Biomaterials and Therapeutics
- Biomedical Polymers
- Biomolecular Engineering
- Tissue Engineering
- Drug Delivery and Nanomedicine

- Biomechanics
- Molecular, Cell, Tissue, Organ and System Levels
- Biosolids, Biofluids, and Biofluid-solid Interactions
- Biological Transport Phenomena
- Biomimetics and Biorobotics

- Bioinstrumentation
- Sensors and Integrated Devices
- Controls and Signal Processing

Areas of Bioengineering

- Cardiac Electrophysiology and Biophysics
- Membrane Ion Transport and Ionic Channels
- Cell Action Potentials and Ion Currents, Ion Regulation, and Contraction
- Cell to Cell Coupling and Spread of Excitation
- Electrocardiography and Volume Conductors
- Experimental and Simulation Techniques
- Neural Interfaces
- Neural-interfaces / Electrophysiology
- Neural Interfaces / Biomaterials
- Computational / Modeling
- Imaging
- Medical Imaging
- Optical Systems, Imaging Methods and Hardware

Protiens

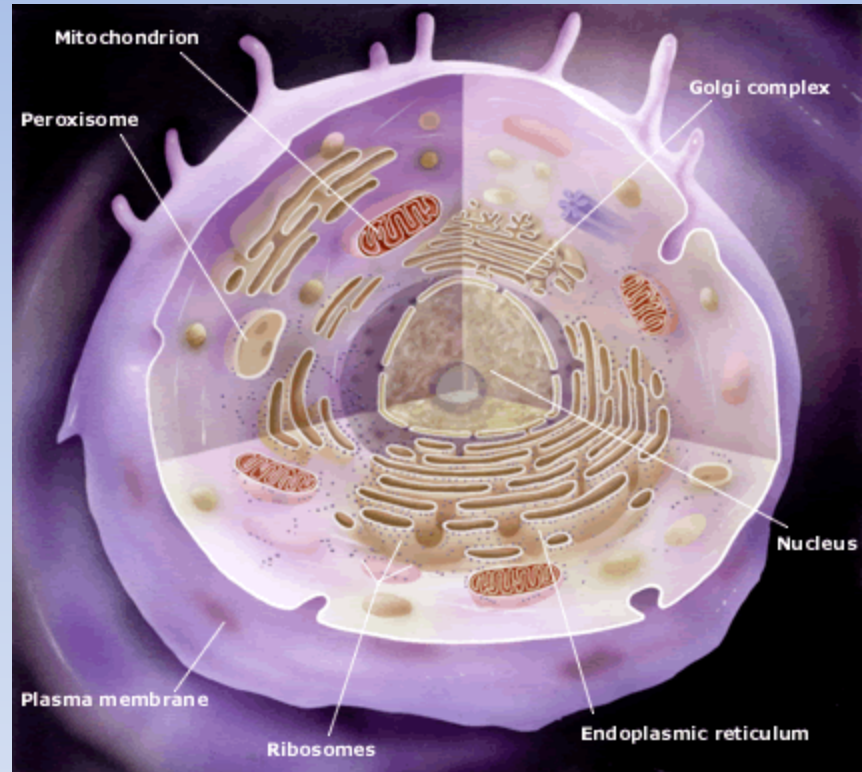
Dolly Holt, SLCC

What are we made of?

- Proteins
 - Collagen
 - Keratin
- Sugars
 - Mucus
- Lipids
 - Cell membranes
- Cholesterol
 - Cell membranes
- Small organic molecules
 - Signaling (eg. NO)
- Minerals
 - bone

Overview of the Cell

- Nucleus
 - mRNA
- Cytoplasm
 - Ribosomes
 - Codon
- Amino Acids
- Protein

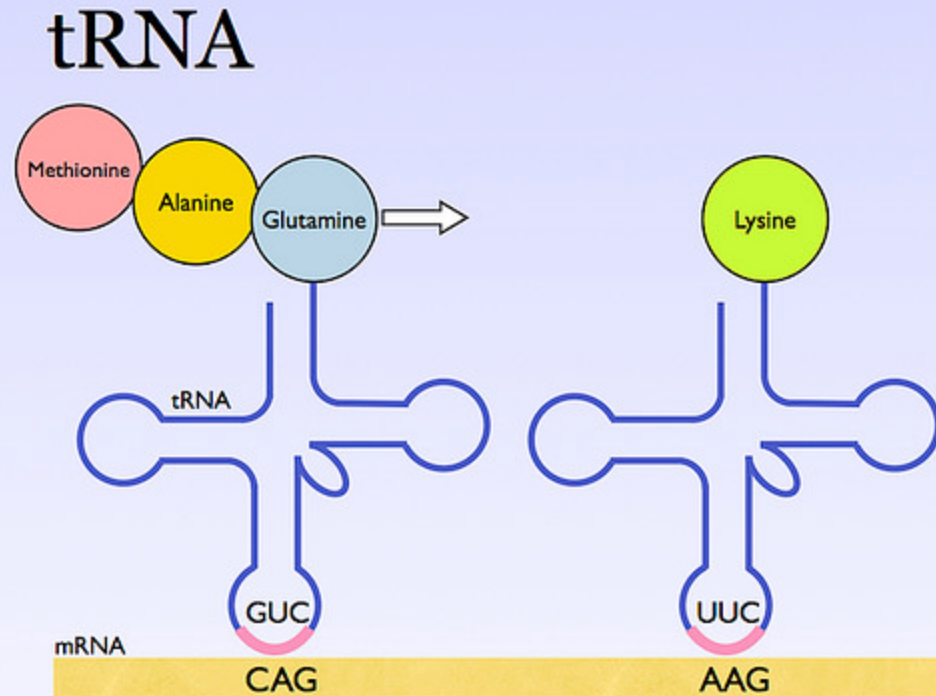


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What are Proteins

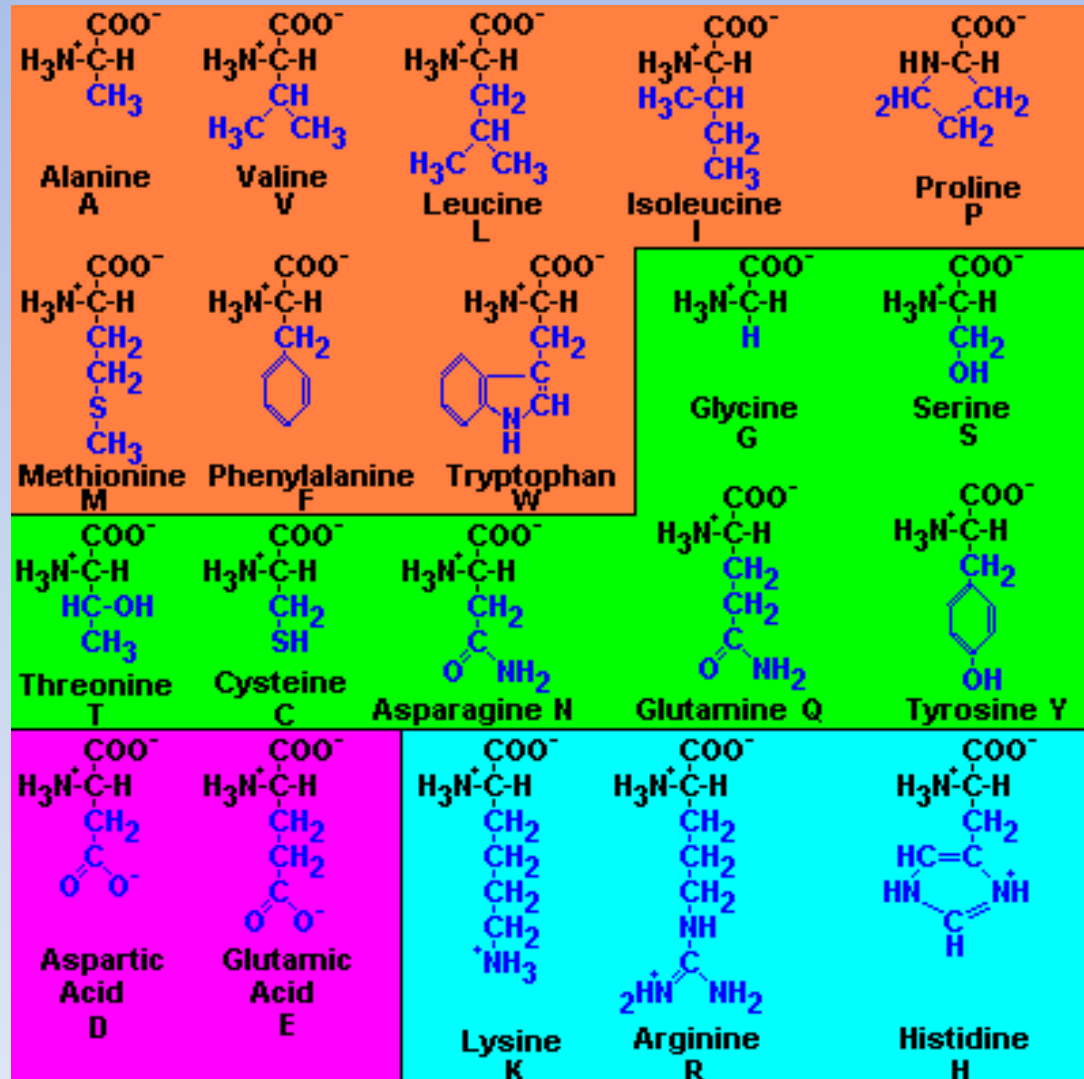
- Amino Acids

© 2009 Magistra Learning Co.

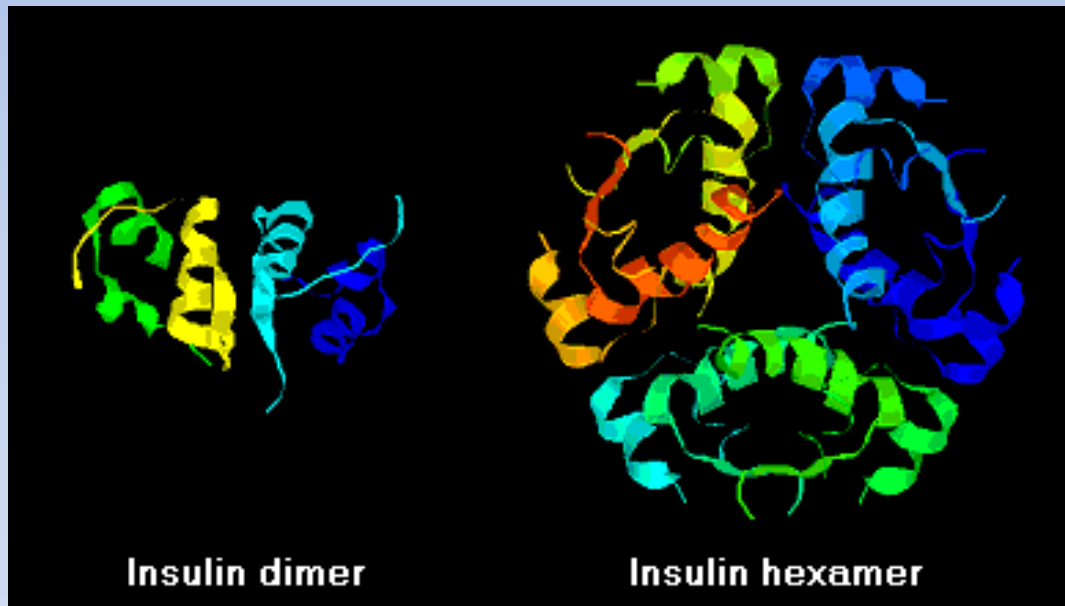


tRNA molecules bind to the mRNA two at a time, linking the next codon's amino acid with the polypeptide chain already in progress.

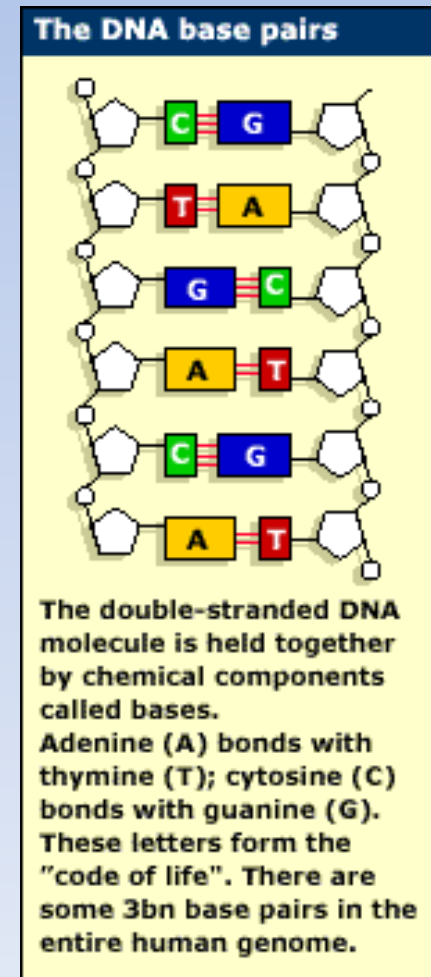
Amino Acids



Protein Tertiary and Quaternary Structure



<http://www.bioss.ac.uk>



Classifications of Proteins

- Extracellular Matrix
 - Collagens, Elastins
- Enzymes
 - Digestive
- Signaling proteins
 - Insulin
- Receptor proteins

Protein Adsorption

Adsorption-process of association of solvates to a material interface.

Physisorption (physical adsorption): long range and weak van-der Waals attraction between adsorbate and substrate (20 kJ/mol)

Chemisorption- short range and strong bonding between adsorbate and substrate (200 kJ/mol)

Protein Orientation

- Specific binding sites
- Activity vs. Inactivity
- Enzyme activity and reactivity
- Hydrophobic vs. hydrophilic
- Charges

Protein Adsorption In Vivo

- Cells never “see” surface, only adsorbed protein layer
 - In less than 1 second, lysozyme reached half of its steady-state value (page 241)
- Denaturation
 - Dependent upon protein, time adsorbed, solvent, substrate etc.
 - Not always complete, enzymes retain some activity

highest
abundance



Protein type	Plasma concentration (mg/ml)	Monomer Molecular weight (daltons)
Prealbumin	10 - 40	54,900
Albumin	35 - 45	66,500 Low M.W.
IgG	6 - 17	150,000
Fibrinogen*	2.0 - 4.0	340,000
Fibronectin*	0.26-0.38	250,000 (but dimeric)

- Est'd $> 10^4$ soluble serum proteins $\rightarrow$ only 20-30% discovered
- **Vroman affect**- albumin arrives first (Fick's Law) and then is replaced by higher affinity proteins which compete for binding
 - Albumin = non-adhesive
 - Fibronectin- very adherent for most cells
 - RGD (Arginine-Glycine-Aspartic Acid)
 - Trace protein, outcompeted by higher abundant proteins

Stem Cells

Dolly Holt SLCC

Could the human body ever be coaxed to completely itself?

“One wouldn't expect a muscle cell to develop into a progenitor cell any more than one would expect a woman to give birth to her own mother.”
Peter G. Schultz



<http://news.siu.edu/news/May05/images/salamander.jpg>

Regenerative Medicine

Regenerative medicine involves the ability cells have to form, organize, maintain, regenerate, and repair tissues in the body.



Stem Cell Classifications

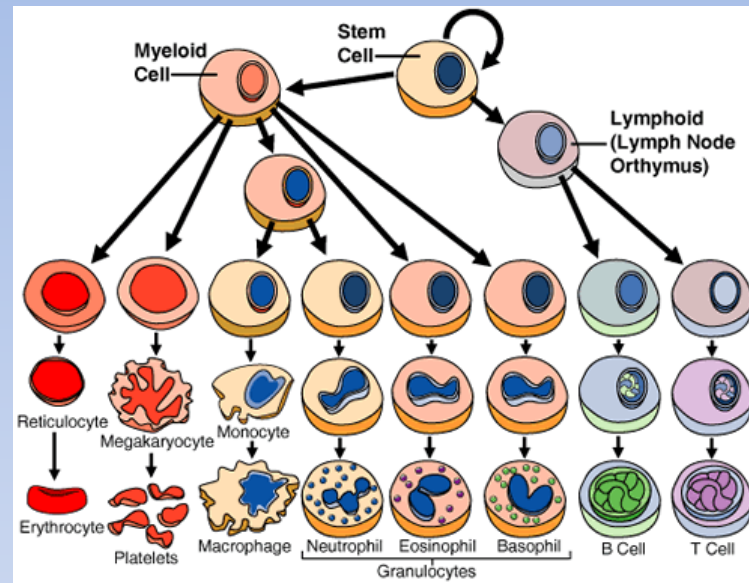
- Stem cell
 - A cell capable of
 - Producing multiple different types of cell types
 - Self renewing (immortalized)
- Multipotent
 - Can give rise to many but not all cell types in an organism
- Pluripotent
 - Can produce nearly all the cell types except those required to support the growth of an embryo (i.e. trophoblast and placenta)
 - Cannot give rise to a complete organism
- Totipotent
 - Can create any cell in body and even a total organism

Overview

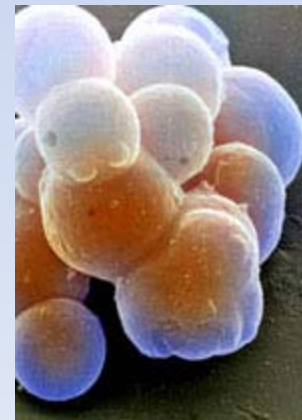
- Embryonic Stem Cells (pluripotent)
- Fetal Stem Cells (multipotent)
- Adult Stem Cells (multipotent)
 - Reversine
- Induced Pluripotent Stem Cells (iPS)
(pluripotent)

Embryonic Stem Cells

- Stem cells are precursor cells that form during the embryonic stage of an individual.
- These cells hold the capacity to differentiate into every type of cell we have in our body.
- "totipotent"--totally potent to form any specific cell type in the body
- The stem cell is an immortal cell.



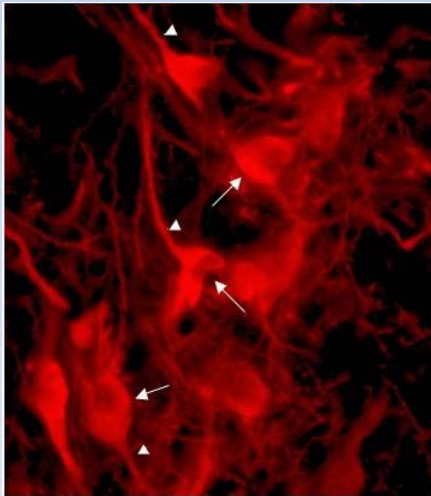
<http://genetics.nbi.gov/images/Polyplurent6.gif>



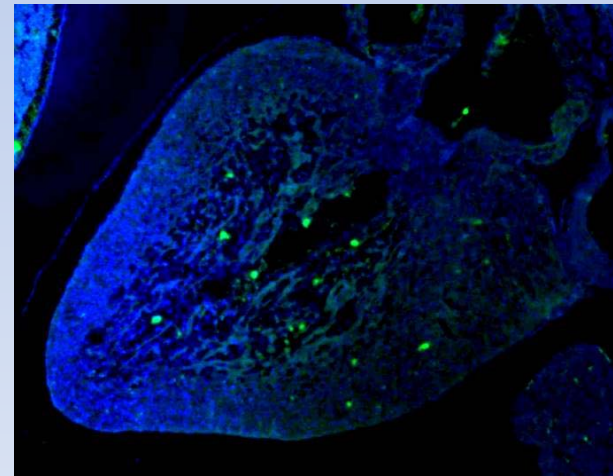
<http://english.pravda.ru/img/2005/03/stemcell.jpg>

Fetal Cells

- Obtained from Maternal Blood
- Umbilical Cord
- Adult leukemia can be combated



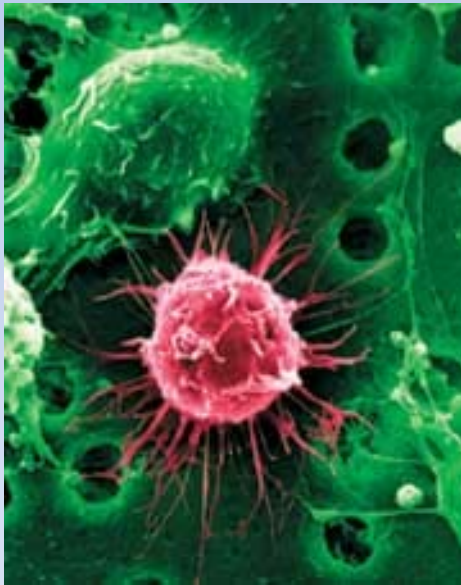
Fetal Stem Cells implanted into the brain
<http://www.liebermanparkinsonclinic.com/>



GFP +ve human fetal mesenchymal stem cells in fetal heart following intrauterine transplantation <http://www1.imperial.ac.uk>

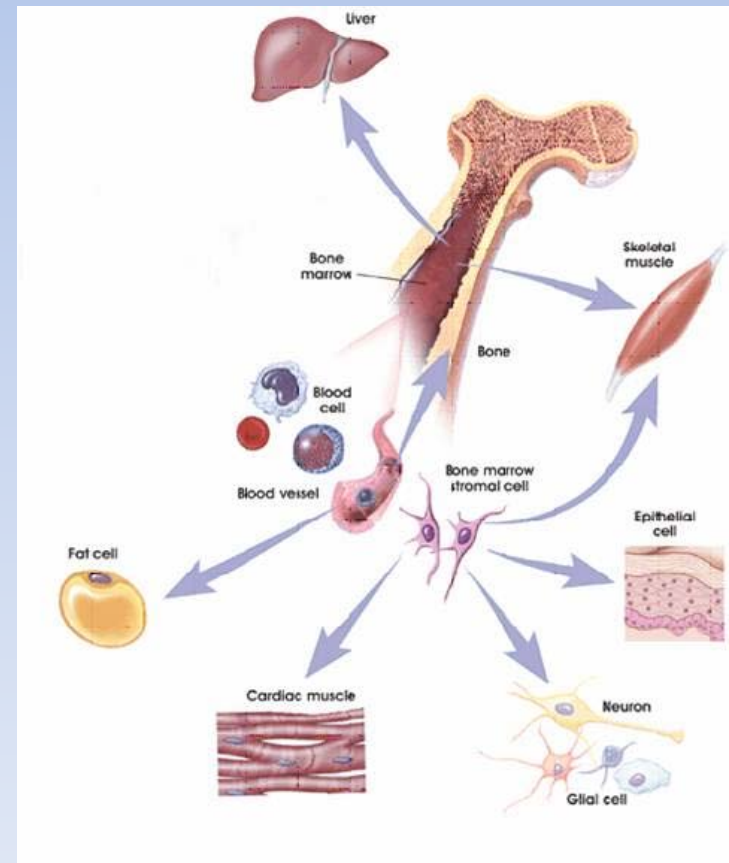
Adult Stem Cells

- pluripotent-able to form many potential specific cell types
- Liver, Spleen, Nasal Cavities, Bone Marrow



Color-enhanced scanning electron micrograph (SEM) of a stem cell collected from human bone marrow.

<http://pub.ucsf.edu>



www1.imperial.ac.uk

Induced Pluripotent Stem Cells

- Pluripotent Stem Cell
 - Equivalent to an embryonic stem cell
- Derived from a non-pluripotent adult cell
 - Eg. Fibroblasts
- Forced expression of specific genes (reprogrammed)
 - Transcription factors (Oct3/4, Sox2, c-Myc, and Klf4 and Oct 4, Sox2, Nanog, and Lin28)

["Making human embryonic stem cells"](http://www.economist.com/science/displaystory.cfm?story_id=10170972). The Economist. 2007-11-22.

http://www.economist.com/science/displaystory.cfm?story_id=10170972.

Induced Pluripotent Stem Cells

Advantages

- Avoids the ethical issue with embryonic stem cells
- Avoids graft-versus-host disease
 - As the cells can be derived from the host instead of a donor
- Risks
 - Viruses are often used to reprogram
 - Initiate oncogenes (cancer genes), resulting in cancer
 - Many of the genes used to create iPS have been linked to cancer
- Potential Solution
 - protein-induced pluripotent stem cells can circumvent both of these issues as genetic alternation is not required
 - Only proteins channeled directly into the cell
 - Low efficiency
 - Developed recently (2009) and risks still need to be determined
 - (Zhou, H. “Generation of Induced Pluripotent Stem Cells”. Cell Stem Cell 2009)

J. Am. Chem. Soc., **126** (2), 410 -411, 2004. 10.1021/ja037390k S0002-7863(03)07390-6

Web Release Date: December 19, 2003

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Dedifferentiation of Lineage-Committed Cells by a Small Molecule

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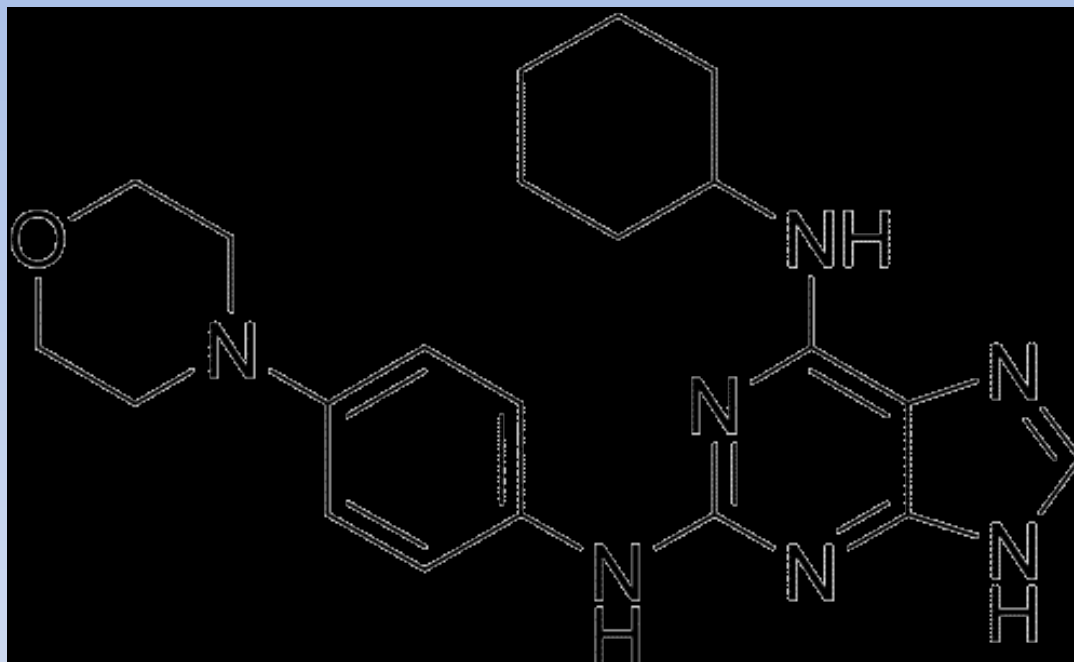
sding@scripps.edu; schultz@scripps.edu

Received July 18, 2003

Abstract:

Combinatorial libraries were screened for molecules that induce mouse myogenic lineage committed cells to dedifferentiate in vitro. A 2,6-disubstituted purine, reversine, was discovered that induces lineage reversal of C2C12 cells to become multipotent progenitor cells which can redifferentiate into osteoblasts and adipocytes. This and other such molecules are likely to provide new insights into the molecular mechanisms that control cellular dedifferentiation and may ultimately be useful to in vivo stem cell biology and therapy.

Reversine



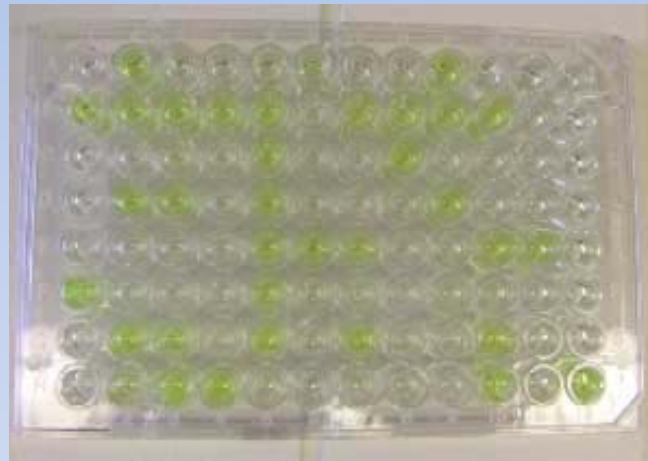
2-(4-morpholinoanilino)-6-cyclohexylaminopurine analogue

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Scripps's Research Institute

Cell-based phenotypic assays

- Myoblasts
- library of 50 000 discrete small molecules
- 4 days to induce dedifferentiation
- assayed for their ability to undergo osteogenesis
- osteogenic-inducing medium



<http://www.plantpath.wisc.edu/soyhealth/virus/techniques/plate.jpg>

**Murine C2C12
Myoblasts**

Adipocytes

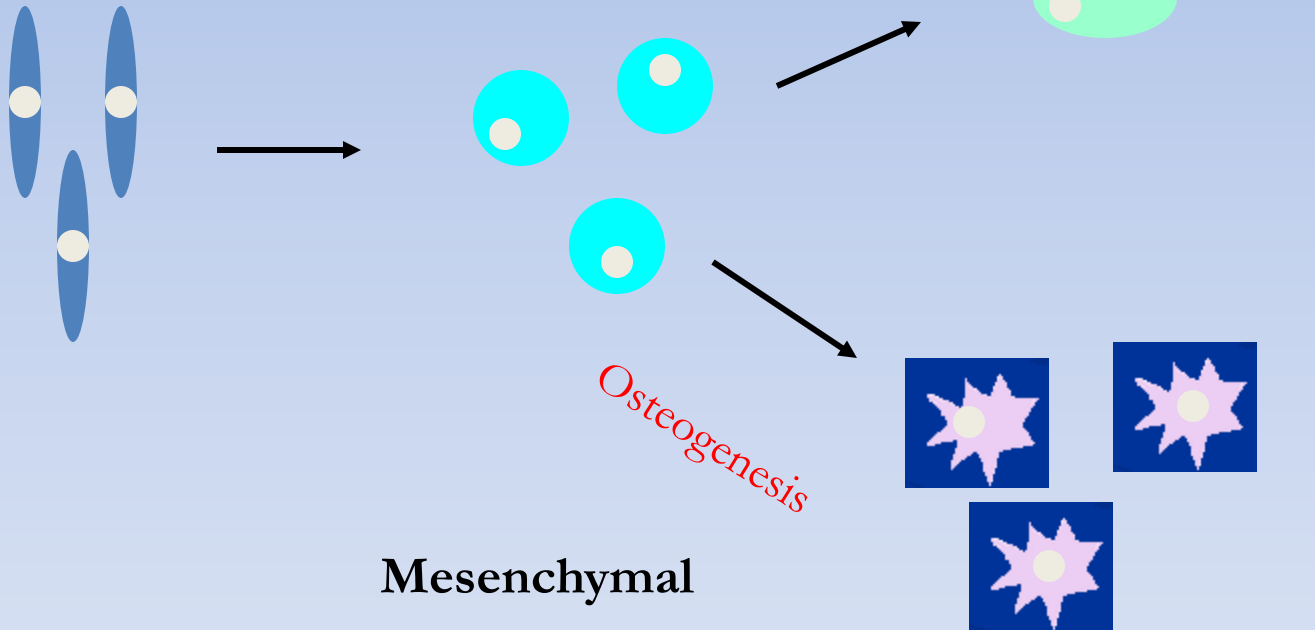
Dedifferentiation

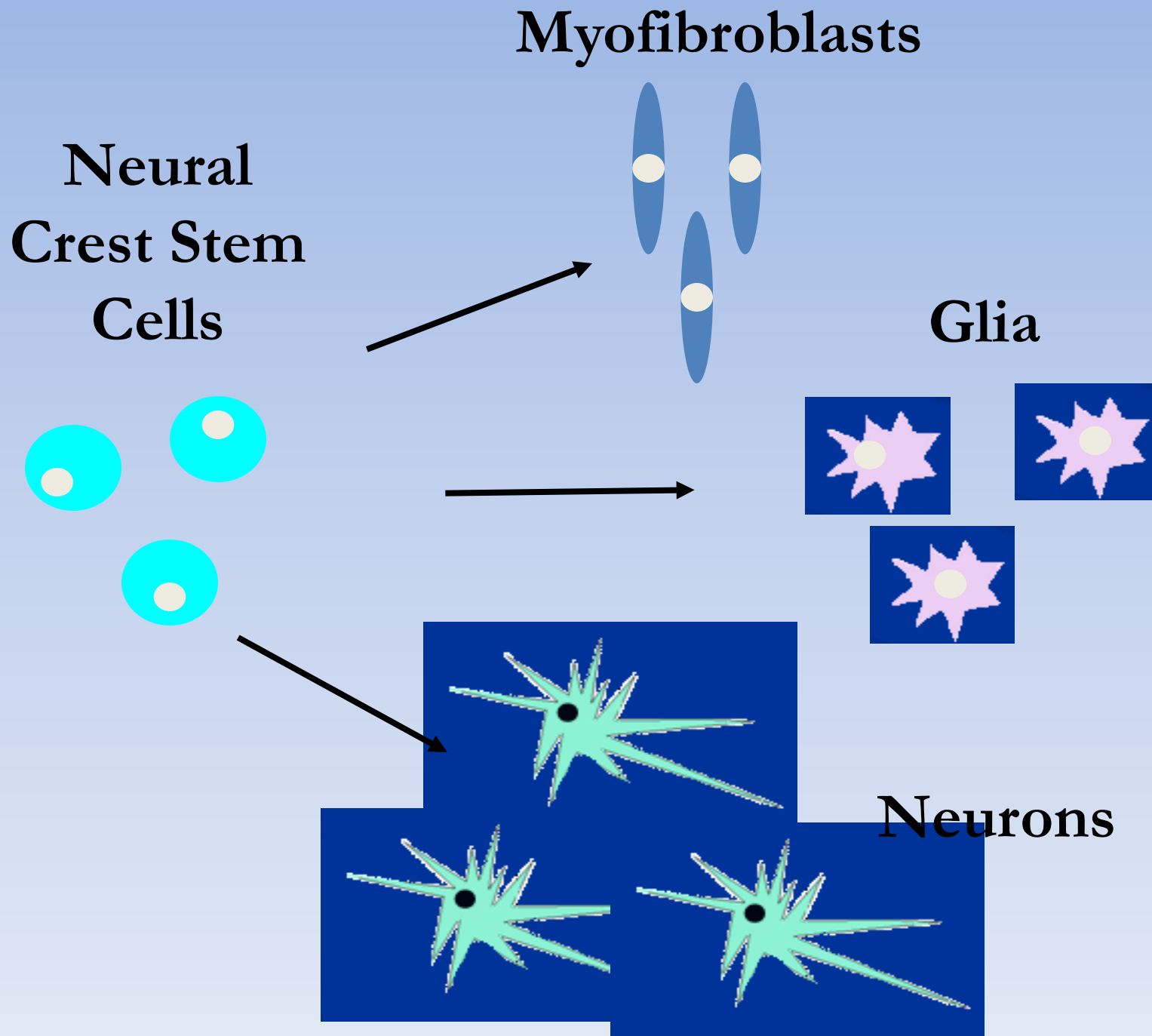
Adipogenesis

Osteogenesis

**Mesenchymal
Progenitor Cells**

Osteoblasts





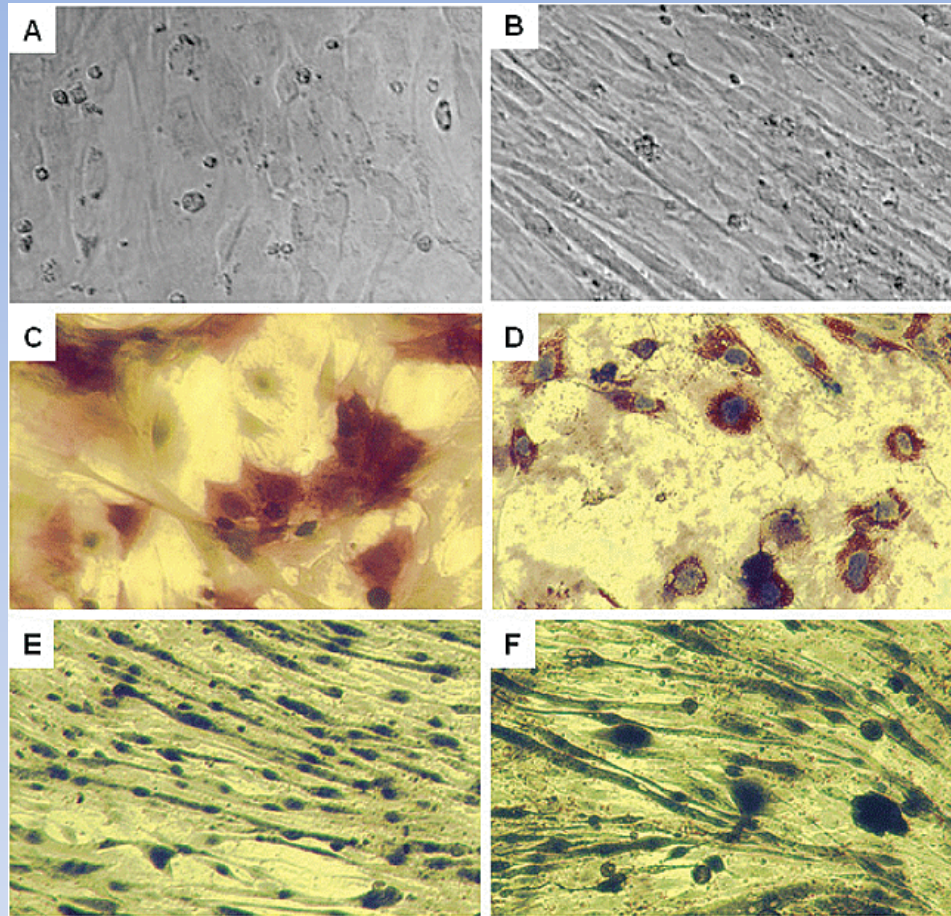


Figure 2 Morphological and histological characterization of cellular dedifferentiation induced by reversine. (A) Phase image of day 4 culture after reversine treatment; (B) phase image of day 4 culture after DMSO treatment; (C) ALP staining (red) of day 7 osteogenic differentiation culture after reversine treatment; (D) Oil red O staining (red) of day 7 adipogenic differentiation culture after reversine treatment; (E) ALP staining (red) of day 7 osteogenic differentiation culture after DMSO treatment; (F) oil red O staining (red) of day 7 adipogenic differentiation culture after DMSO treatment.

Overview

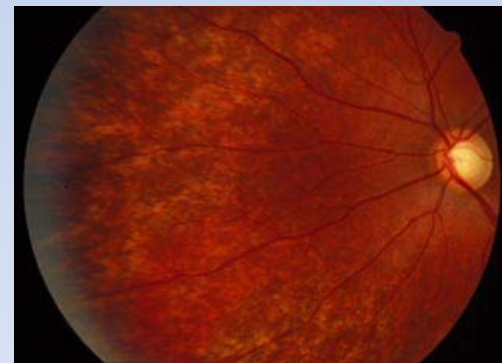
- Applications
- Success stores
- Future directions

Applications

- Spinal Cord Injuries
- Heart Disease
- Parkinson's Disease
- Alzheimer's
- Multiple Sclerosis
- Diabetes
- Ocular Degeneration
- Blindness
- Leukemia



http://cdn.channel.aol.com/aolnews_photos/03/03/20050218094009990001



http://www.visionofchildren.org/images/carrier_detect/Lewis_04.jpg

Success Stories

Heart Tissue Regeneration

- Beaumont procedure
 - Sixteen-year-old Dimitri Bonnville
 - The entire front wall of his heart was dead
- University of Frankfurt 2003
 - Transplantation of patient's own blood and hematopoietic (blood-forming) stem cells into their heart arteries
 - higher levels of heart-pumping capability



<http://www.image.cbsnews.com>

Success Stories

Spinal Cord Injuries

- Laura Dominguez 2001
 - olfactory mucosa transplantation
- Susan Fajt 2001
 - adult stem cell procedure
 - she was able to walk with the aid of braces



<http://www.pbs.org/wnet/innovation/images>

Success Stories

Corneal Reconstruction

- Michael May
 - was exposed to a chemical explosion as a child, losing his left eye and becoming blind in his right
 - limbal stem cell transplantation
 - corneal degeneration, blindness, and other ocular diseases
 - within two years had recovered his sight
- Jon Newton
 - ocular disorder known as Stevens-Johnson syndrome
 - Lost his eye sight
 - Less than 18 months later he had 20/30 vision

Future Direction: Controlled Differentiation

- TWS119 is molecule that will differentiate stem cells into neurons.
- Cartilage
- Bone
- Fat
- Spleen is a source of stem cells associated with limb regeneration.

Massachusetts Hospital

- Hox11 is a protein associated with embryonic limb development

Conclusion

- Embryonic Stem Cells
- Fetal Stem Cells
- Adult Stem Cells
 - Reversine
- Induced Pluripotent Stem Cells
- Applications
- Success stories
- Future directions

References

- Shuibing Chen, Qisheng Zhang, Xu Wu, Peter G. Schultz,* and Sheng Ding* Dedifferentiation of Lineage-Committed Cells by a Small Molecule. *J. Am. Chem. Soc.*, 126 (2), 410 -411, 2004. 10.1021/ja037390k S0002-7863(03)07390-6. 2003.
 - http://www.tissue-engineering.net/index.php?seite=News_detail&action=show&nr=131
 - THE TECHNOLOGY PROGRAMME **AUTUMN 2005 COMPETITION FOR FUNDING** Regenerative Medicine Technologies
 - <http://www.wired.com/news/medtech/0,1286,57944,00.html>
 - http://www.theinfoshop.com/study/bc14719_bone_joint.html
- Regenerative Medicines: Bone and Joint Applications
Pub Time: 2003/07 Published by : Business Communications Co., Inc.
Distributed by : Global Information, Inc.
- www.genomecanada.ca/GCglossaire/glossaire/index.asp
 - <http://news-service.stanford.edu/news/2004/august4/med-steinberg-84.html>
 - <http://www.biomedcentral.com/1534-5874/3/47>
 - Paraplegic improving after stem-cell implant
<http://www.indystar.com/articles/5/209449-5235-047...>
- Cord Blood Transplantation Now a Viable Option for Adult Leukemia Patients
<http://releases.usnewswire.com/GetRelease.asp?id=4...>
- Spleen Surprise Source of Stem Cells
<http://www.forbes.com/lifestyle/health/feeds/hscou...>

Supplementary Slides

Different types of stem cells

- Tissue-derived stem cells or progenitor cells
 - ability to differentiate to several (multipotent) or one cell type
 - blood stem cells, multipotent
 - bone marrow, foetal liver, umbilical cord or peripheral blood
 - mesenchymal stem cells
 - found in bone marrow, foetal liver, adipose tissue, maybe elsewhere
 - multipotent, can differentiate to several cell types (bone, cartilage, muscle, other cell types such as neurons or liver cells to limited extent)
 - MAPC, multipotent adult progenitor cells, Verfaillie et al., rare cells
 - skin, intestine, muscle, other rapidly renewing organs
 - nervous tissue, even in adult, but in limited numbers
- Embryonic stem cells
 - pluripotent, can differentiate to all cell types and divide indefinitely
 - isolated from the inner cell mass of embryos 5-6 days after in vitro fertilisation
 - specific demands for culture as non-differentiated cells

Stem cell transplantation standard treatment within the next few years?

- Severe neurological disorders (stroke, injuries, Alzheimer's and Parkinson's diseases, spinal cord injury, MS, ALS, JNCL etc)
- Cardiac failure
- Liver failure
- Diabetes
- Cancers
- Cartilage and bone diseases and injuries

Stem cells in cardiac repair

- Satellite cells from skeletal muscle
 - arrhythmias, do not function well in cardiac muscle
- Cardiomyocytes from mesenchymal stem cells
 - do not integrate to the cardiac muscle
 - may help in revascularisation
- Cardiomyocytes from embryonic stem cells
 - several methods to differentiated (Mummery et al. 2005)
 - functional in animal experiments

Neural repair

- Clinically extremely important indication
- Adult neural stem cells are not capable of correcting major injuries in the body
- Adult neural stem cells very difficult to sample for culture
- Cells from olfactory epithelium used with some success
- Mesenchymal stem cells with limited potential
- Embryonic stem cells most potential in this respect
 - differentiation in vitro feasible

Human ES cells

- First cultures in 1984
 - Fishel, Edwards, Evans (Science 1984) (see also Edwards, Nature 2001, RBMonline 2002)
- The first permanent hES cell lines
 - Thomson et al. Science 1998
 - Reubinoff et al. Nature Biotechnol 2000
 - Derived using foetal mouse fibroblastas as feeder cells
- In 2005, about 250 hES cell lines in the world



A blastocyst five days after in vitro fertilization

hES cell lines can be derived from the inner cell masses
of donated blastocysts

Safety of embryonic stem cells

- The risk of infection has to be eliminated
 - EU directive from March 2003
 - GMP quality for handling of the cells
- No animal-derived materials in the process
- Non-differentiated ES cells may form teratomas
- Differentiated cells safe in animal experiments

What is an optimal surface for ES cell growth ?

- A material onto which the cells attach well, grow as colonies, which can be taken out mechanically
- Transparent, allows follow-up and imaging
- Secretes substances which keep the cells non-differentiated
 - it is also possible to add the substances to culture medium
 - conditioned medium from mouse fibroblasts has been used, but that one from human fibroblasts does not work well (Susanne Ström, submitted 2006)

Mouse ES cells to neural cells

- Ying, Q. L., and Smith, A. G. (2003). Defined conditions for neural commitment and differentiation. *Methods Enzymol* 365, 327-341.
- Ying, Q. L., Stavridis, M., Griffiths, D., Li, M., and Smith, A. (2003). Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. *Nat Biotechnol* 21, 183-186.

Human ES cells to neural cells

- Reubinoff, B. E., Itsykson, P., Turetsky, T., Pera, M. F., Reinhartz, E., Itzik, A., and Ben-Hur, T. (2001). Neural progenitors from human embryonic stem cells. *Nat Biotechnol* *19*, 1134-1140.
- Reubinoff, B. E., Pera, M. F., Fong, C. Y., Trounson, A., and Bongso, A. (2000). Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. *Nat Biotechnol* *18*, 399-404.

Successfully used in experimental animals

Stroke, Parkinson's and Alzheimer's diseases, spinal cord injury, peripheral nerves, MS, ALS

- Brustle, O., Jones, K. N., Learish, R. D., Karram, K., Choudhary, K., Wiestler, O. D., Duncan, I. D., and McKay, R. D. (1999). Embryonic stem cell-derived glial precursors: a source of myelinating transplants. *Science* 285, 754-756
- Chiba, S., Ikeda, R., Kurokawa, M. S., Yoshikawa, H., Takeno, M., Nagafuchi, H., Tadokoro, M., Sekino, H., Hashimoto, T., and Suzuki, N. (2004). Anatomical and functional recovery by embryonic stem cell-derived neural tissue of a mouse model of brain damage. *J Neurol Sci* 219, 107-117.
- Horner, P. J., and Gage, F. H. (2000). Regenerating the damaged central nervous system. *Nature* 407, 963-970.
- Kim, J. H., Auerbach, J. M., Rodriguez-Gomez, J. A., Velasco, I., Gavin, D., Lumelsky, N., Lee, S. H., Nguyen, J., Sanchez-Pernaute, R., Bankiewicz, K., and McKay, R. (2002). Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature* 418, 50-56

Spinal cord injury related research

- **The promotion of oriented axonal regrowth in the injured spinal cord by alginate-based anisotropic capillary hydrogels.** Prang P, Muller R, Eljaouhari A, Heckmann K, Kunz W, Weber T, Faber C, Vroemen M, Bogdahn U, Weidner N. Biomaterials (2006). Alginate-based highly anisotropic capillary hydrogel scaffolds seeded with neural progenitor cells were implanted into acute cervical spinal cord lesions in adult rats. The research displayed induced directed axon regeneration across the artificial scaffold.
- **Physical and biological performance of a novel block copolymer nerve guide.** Lietz M, Ullrich A, Schulte-Eversum C, Oberhoffner S, Fricke C, Müller HW, Schlosshauer B. Wiley InterScience (2005). Block copolymers made from trimethylene carbonate and ϵ -caprolactone were used as nerve guides filled with Schwann cells implanted into lesioned spinal cords of adult rats. Promising axonal regrowth was observed.

Tissue derived vs embryonic stem cells

- tissue derived
 - No rejection, if own cells
 - grow well from many tissues (mesenchymal stem cells, skin, cartilage)
 - grow slowly from many tissues (neural, heart)
 - difficult to isolate (nerve)
 - not functional after isolation in many tissues
- embryonic stem cells
 - unlimited growth
 - can be differentiated to almost any cells
 - are immunogenic

Tissue Engineering

Dolly Holt, SLCC

Tissue Engineering

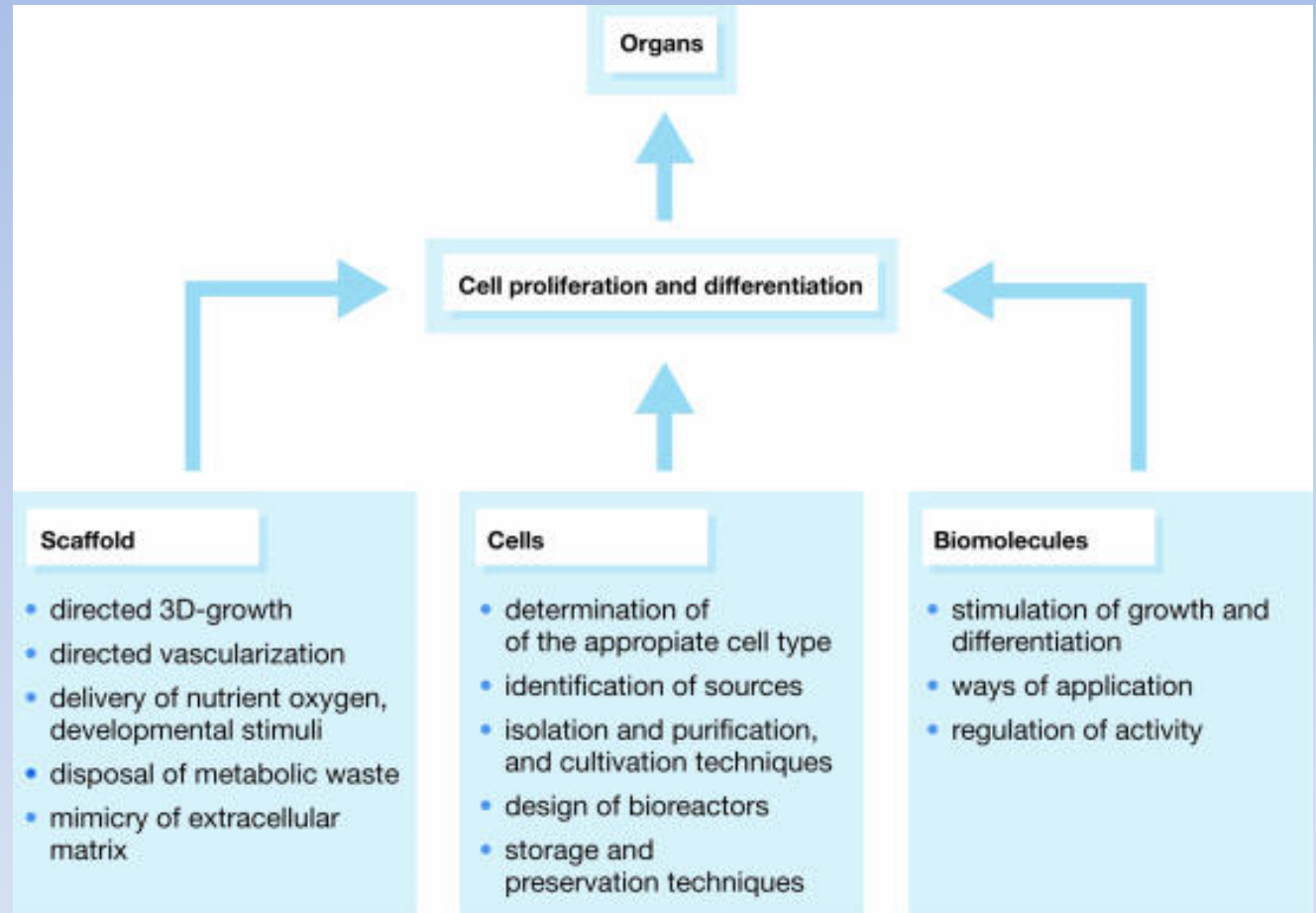
- Technology combining genetic engineering of cells with chemical engineering to create artificial organs and tissues such as skin, bone, heart valves, and cartilage for joints.



- **Engineering:** The application of scientific and mathematical principles to practical ends such as the design, manufacture, and operation of efficient and economical structures, machines, processes, and systems.
- **Tissue engineering:** The use of a combination of cells, engineering materials, and suitable biochemical factors to improve or replace biological functions in an effort to effect the advancement of medicine.
 - Probably the first definition of tissue engineering was by Langer and Vacanti who stated it to be "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function".

Three Pillars of Tissue Engineering

- Scaffold
 - Natural/synthetic 3D construct to support cellular growth
- Cells
 - Stem cells, fibroblasts, cardiomyocytes, etc.
- Signals
 - Biomolecules, Drugs, protein, small molecules, etc.



Types of Grafts

- Autograft
- Isograft
 - Twins
 - Inbred mice
- Allograft
 - Same species
- Xenograft
 - Different species
 - Baby Fae
 - Heart valves

History of Tissue Engineering

- 1984 -Earliest cited reference for the term “tissue engineering”
- 1985 -Y.C. Fung proposes center for “engineering of tissues” (denied!)
- The notion of TE as a unifying concept arose in the late 80’s and early 90’s.
- However, the practices of TE are much older:
- Clinical Medicine (16thcentury allografts!)
- Vascular Grafts (early 20thcentury –improved in 1950’s)
- Skin Grafts (cultured *in vitro* in 1970’s)
- Artificial Organs 1940’s to 1970’s (Kidney, Heart –think Kolff)
- Modern Biomaterials (post WWII)
- It is reported that the most significant conceptual breakthrough that lead directly to TE was by J. Vacanti
 - He claims to have conceived of the use of *porous*, biodegradable polymers as 3D scaffolds while observing seaweed on Cape Cod in 1986. A quick call to his friend and collaborator R. Langer lead to the explosion of the field. While a nice story, it is only one part of TE’s genesis. Others were already working on encapsulation, cell sourcing and culture, etc. In 1984 there was one paper written in this area by 2000 there were 214.

History of Tissue Engineering

- Dozens of tissue engineering companies emerged in the past ten years, and only a few have survived.
- Tissue engineered skin is currently the only FDA approved tissue engineered product.
- Gail Naughton was the first to invent this which she called Dermagraft, which has only been approved for diabetic foot ulcers. She started up a company known as Marrow-Tech to commercialize her invention.
 - This company then transformed into Advanced Tissue Sciences (ATS), which then went bankrupt.
 - Now Smith and Nephew hold the patent for Dermagraft. Organogenesis Inc. went bankrupt along with ATS in 2002.
 - However, due to a simple distribution agreement Organogenesis held with Novartis, they were able to retain their intellectual property during bankruptcy and have since been able to return.

Classes of Materials

- **Metals**- stainless steel, titanium, and other alloys
 - Mostly crystalline solids composed of elemental, positively charged ions. Typical metal properties include- high melting points, high stiffness and strength, high conductivities, isotropic properties
- **Ceramics**- alumina, other inert ceramics, biodegradable compositions Characterized by ionic or covalent bonding, generally crystalline Properties- Low conductivities, stiff and brittle, bioinert, obvious choice for teeth and bones
- **Polymers**-modified biological/natural polymers (collagen), biocompatible synthetic polymers, hybrids and composites

Natural Materials

Type 1 collagen-cell adhesion and activation, cosmetic injections, bone scaffold additives, nerve tube

Hyaluronic acid- Water retention, cell migration and adhesion, surgical adhesion, device coating, controlled release carriers

Chitosan-deacylated derivative of chitin, good mechanical properties, bone scaffold additives

Natural Materials

- Collagen- advantage: natural fibril orientation and mechanical strength maintained. Naturally used in the ECM.
 - Disad: It is difficult to control the mechanical properties, biodegradability, and batch-to-batch consistency. There are also potential problems with pathogen transmission and immuno-rejection.
- PLGA- It has been redily studied and is FDA approved
 - Disad- It is not the most biocompatible substance, there are better out there.

Degradation

- Can happen through bulk erosion
 - water penetration rate is greater the polymer dissolution rate
- surface erosion
 - water penetration rate is less then polymer dissolution rate.
- Hydrolytic
 - PLGA is hydrolytic and is degraded when water bonds to carbon and breaks the bond.
- Oxidative
- Proteolytic
- Enzymatic

Extracellular Matrix (ECM)

- collagen proteins
- Carbohydrates—hyaluronic acid
- Proteoglycans
- Fibrinogen
- Fibronectin
- Laminin
- vitronectin

types of cell and tissue “grafting?”

- Autograph- same individual
- Isograph- identical twin
- Alograph-same species
- Xenograph- other species

Cell Culture

- Primary cell sourcing comes from a living animal or human, from an embryo, or from a germ cell.
 - Dissection enzyme digestion leads to cell culture,
- finely chopping leads to primary explants,
- or further dissection if necessary leads to organ culture.
 - Organ culture (histology structure maintained), explant culture (cells migrate to form outgrowth), dissociated cell culture (cells form monolayer), organotypic culture (different cells co-cultured; organotypic structure recreated). A serial subculture is multiple flasks of cells obtained from one passage.

Immortalized Cells

Hayflick limit

After a number of population doublings most primary cells undergo this phenotype shift that leads to cell death, termed the “hayflick limit” or replicative senescence. It causes the cell lines to die out.

Immortalized cells

Culturing of primary cells is generally limited to a number of passages which is cell-type specific. To development of cell lines, initially a primary culture is established from the primary cell source. If one can successfully pass the primary culture, then a primary cell line is established. Such cell lines can only be passed a finite number of times. If a cell line is successfully transformed, then a continuous line is established.

Transformation is the process by which a primary cell line becomes an immortalized cell line. No, an immortalized cell will not live for ever, but immortalized cell lines are able to undergo mitotic replication indefinitely, or at least along time. Immortalized cell lines are created from primary cell lines by induction in order to create models of primary cells.

Stem Cells

Stem Cells

can renew itself indefinitely and differentiate into other cell types. A stem cell is not committed to a specific function until it received a signal. Stem cells can be obtained from embryos, umbilical cord, bone marrow, liver, spleen, olfactory stem cells found between cornea and sclera.

Totipotent- can give rise to complete organism

Pluripotent- can give rise to all tissues but not complete organism

Multipotent- able to differentiate to certain human tissues

Encapsulation Devices

- **Encapsulation**- when living cells are isolated from their host by a selective barrier
- **Immunoisolation**-shielding of implanted cells from the host's immune system
- **Microcapsules**- dimensions less than 600 μg , usually in the form of spherical beads, implanted in high numbers to achieve an adequate dose.
 - Hydrogel-based- cell-hydrogel emulsion, droplets formed by extrusion.
 - Common materials- alginate, collagen, HEMA-MMA
- **Macrocapsules**- tubes or sheets with inner dimension $>500 \mu\text{m}$, outer dimensions 1-10 cm, mechanically and chemically stable.
 - Phase-inversion process- thermoplastic polymer in two-solvent system, insoluble phase forms voids, removal of solvents, cells introduced after fabrication. Polysulfone, PAN-PVC, Polyvinyl alcohol
- **Components of encapsulation devices**
 - **Membrane**-contacts both the host and the cells, selective barrier, determines geometry.
 - **Matrix**- mechanical support of cells, facilitates cell behavior.
 - **Cells**-from what source did they come from: allogenic, xenogenic, large amount
- Encapsulation removes the possibility of vascularization so you need to design in the mode of nutrient delivery (or waste removal).
- Nutrients have a limit diffusion distance of about 500 μm

Types of Tissue Engineering

- **Barriers**-(membranes and tubes) cell activity on one surface while precluding transverse movement of surrounding cells into that surface. Eg. Periphrical nerve regeneration, must allow axonal growth, must prevent growth of fibroblasts to get in the way.
- **Gels**- Provide a hydrogel scaffold to encapsulate or provide a specialized environment for isolated cells. Semi-permeable to limit cell-cell interaction yet allow nutrients through implant. Non-degradable for cell encapsulation to maximize long-term stability. Eg. Encapsulation of xenografted cells.
- **Matrices**- three-dimensional porous architecture. Allows cell ingrowth and outgrowth as well as diffusion of small molecule nutrients and macromolecule proteins. Porosity, pore size, pore orientation, fiber structure and important design parameters effecting cellular and tissue responses. Resorbable templates for tissue regeneration.