Biocompatibility

HOST RESPONSES

Prof. H. H. Lu

Department of Biomedical Engineering
Fu Foundation School of Engineering and Applied Science
Columbia University in the City of New York

BIOMATERIALS

- What is it?
- Why do we need it?
- Where is it used?
- What happens to it in the body?
- Does it work?
- Can it do better?

BIOMATERIALS

What happens to it in the body?

Biocompatibility
Biofunctionality

- **Interaction between biomaterial and living system**
- **Readings: Ratner, Chapters II-2-1 to II-2-5**

- **Biocompatibility: Host Response**
  - **Systemic and local biological response**
  - **Acute and chronic biological response**

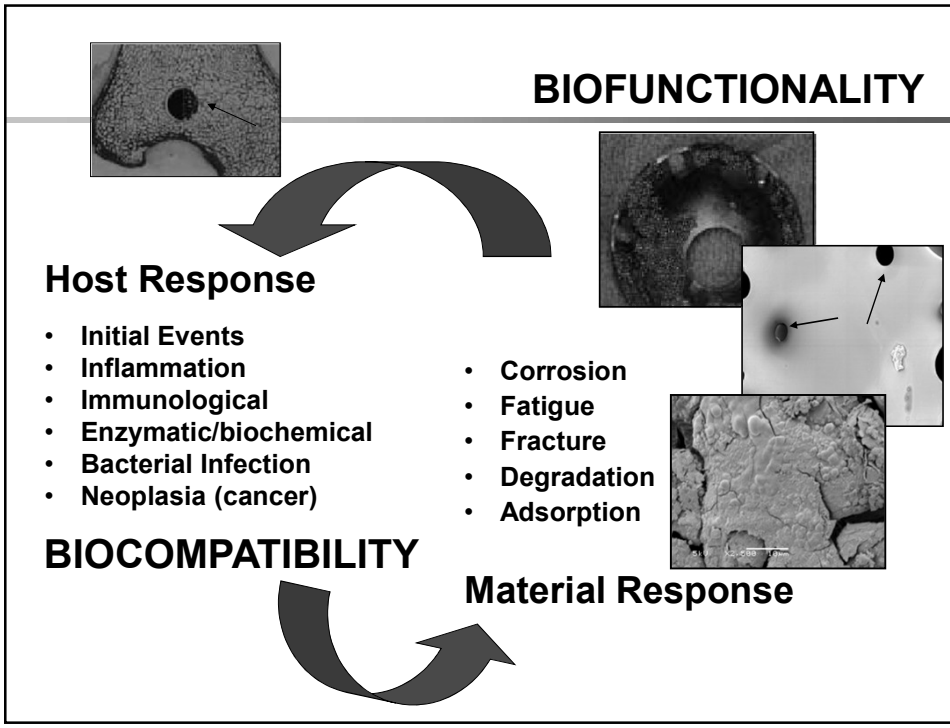
- **Biofunctionality: Material Response**
  - **Response of material to living systems**

BIOCOMPATIBILITY

- **Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application** *(Williams, 1987)*

- ***Biocompatible* material should <u>NOT</u>**
  - **Irritate the surrounding structures**
  - **Provoke a chronic inflammatory response**
  - **Incite allergic reactions**
  - **Induce infection**
  - **Cause Cancer**

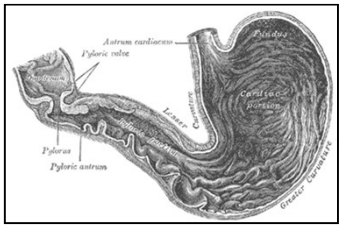
- ***Biocompatibility* vs. *Biofunctionality***



BIOCOMPATIBILITY

- **Biological Environment**
  - **AGGRESSIVE environment when compared to external conditions**
  - **Chemical and thermal parameters**
  - **Instantaneous mechanical loading**
- **Need to accurately predict and design for the *immediate* as well as the *extended* environment that a biomaterial will be exposed to**

BIOLOGICAL ENVIRONMENT



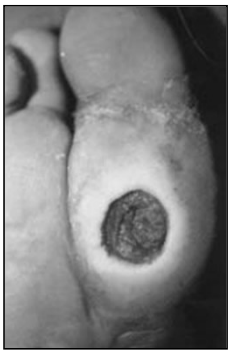
- pH – ranges from 1.0 to 7.35
  - Gastric contents: pH=1.0
  - Intracellular: pH = 6.8
  - Interstitial: pH = 7.0
  - Blood: pH = 7.14-7.35 (diet dependent)
- Temperature
  - T= 37^{o}C core
  - Diseased: 20 - 42.5^{o}C
  - Location dependent
    - Skin: ranges from 0 – 45^{o}C

BIOLOGICAL ENVIRONMENT

- Elemental Composition
  - The body contains many elements in varying concentrations
  - Depending on age, sex, diseases
  - All elements have physiological ranges
- Trace Elements
  - Necessary for physiological function
  - Can be toxic at non-physiological range

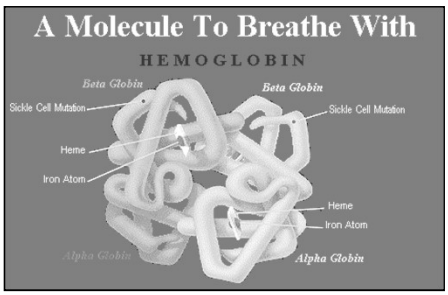
BIOLOGICAL ENVIRONMENT

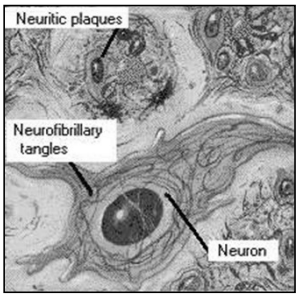
- Oxygen (O), Carbon (C), Hydrogen (H)
  - basic elements
  - present at high concentrations
- Calcium (Ca), Chlorine (Cl)
  - physiological elements
  - low concentrations
  - tightly regulated
  - Disease dependent
    - higher blood [Ca] found in diabetics



BIOLOGICAL ENVIRONMENT

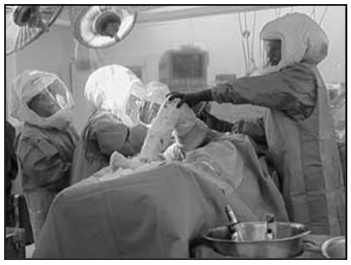
- Magnesium (Mg), Iron (Fe), Zinc (Zn)
  - trace elements
  - minimal but (significant) concentration
  - part of the hemoglobin molecule
- Titanium (Ti), Aluminium (Al)
  - Not found in the body!
  - Al: found in plaques in Alzheimer patients

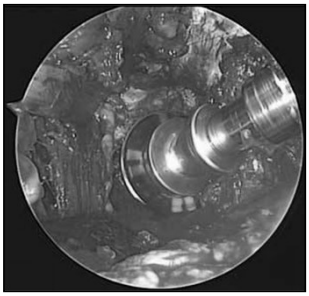




BIOCOMPATIBILITY

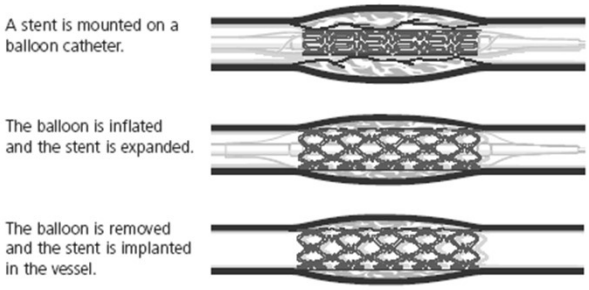
- **Implantation Procedure**
  - **Invasive and Traumatic**
    - **Surgery**
    - **Implantation**
- **Wound healing as a response to surgery**
  - **Results surgeon-dependent**
  - **prep. Acetabulum with acetabular reamers**





BIOCOMPATIBILITY

- **Implantation Procedure**
  - **Insertion of devices**
    - **delivery of cardiovascular stents**
    - **Pressure applied to open the balloon**



J&J Cordis

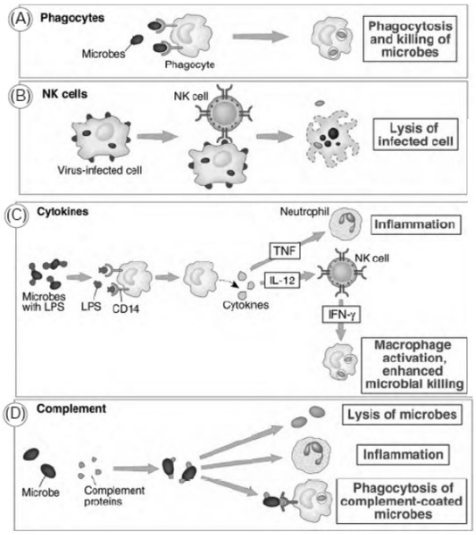
WOUND HEALING PROCESS

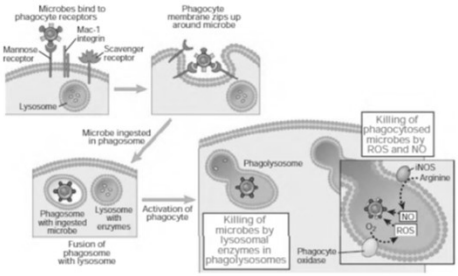
- Two Stages
  - Inflammation
  - Cellular Response to Repair
    - Cellular Invasion
    - Remodeling
    - Fibrous capsule
    - Scar Formation
- Specific vs. Nonspecific Responses

Wound Healing: Inflammation

- *Nonspecific* Response to tissue damage in the biological system
- Arises in response to
  - Trauma, infection, biomaterials, cell death
  - adjunct to S*pecific* immune responses
- Serves to contain, neutralize, or dilute injurious agents or processes
- Initiates the next stage of wound healing
  - Cell-Mediated

Innate Immune System





Ratner, p. 515, Fig. II.2.3.2

Ratner, p. 515, Fig. II.2.3.2

Wound Healing: Inflammation

- ***Initial Events***
  - **Coagulation factor XII is activated when in contact with foreign proteins or material**
  - **Fibrous clot forms from blood (platelets aggregate) and fibrinogen at site of injury**
  - **Localizes the inflammatory response**
  - **Local capillaries dilate and the permeability of the vessel endothelium increases**
    - **kinins – mediators of vasodilation**
  - **Result in increase blood flow to the region**

Inflammation: Initial Events

- **Contact Activation**
  - **Depends on material surface charge**
  - **Factor XII binds to a negatively charged surface**

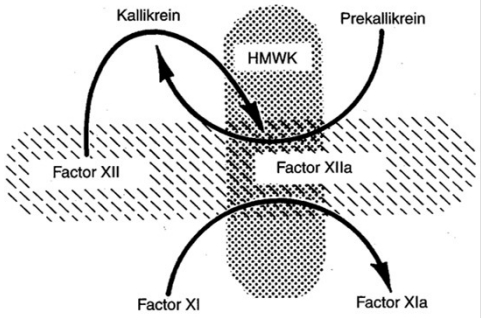


FIG. 4. Contact activation. The initial event *in vitro* is the adsorption of factor XII to a negatively charged surface (hatched, horizontal ovoid) where it is activated to form factor XIIa. Factor XIIa converts prekallikrein to kallikrein. Additional factor XIIa and kallikrein are then generated by reciprocal activation. Factor XIIa also activates factor XIa. Both prekallikrein and factor XI bind to a cofactor, high-molecular-weight kininogen (HMWK; dotted, vertical ovoid), which anchors them to the charged surface.

Inflammation: Initial Events

- **Biological Response to Implant**
  - **Race for the surface !!!**

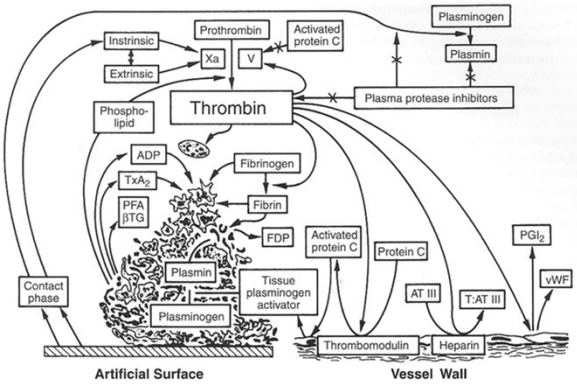


FIG. 5. Integrated hemostatic reactions between a foreign surface and platelets, coagulation factors, the vessel endothelium, and the fibrinolytic system.

Wound Healing: Inflammation

- **Four Classical Symptoms**
  (***Celsus, 25 - 50 A.D., De Medicina***)
  - **Redness (erythma)**
  - **Swelling (endema)**
  - **Pain**
  - **Heat**

- **Magnitude of each is indicative of the degree of the inflammatory response**

Wound Healing: Inflammation

- **Redness (rubor)**
  - **Increased concentration of red blood cells**
- **Swelling (tumor)**
  - **Outflow of plasma to surrounding tissues**
  - **Plasma fraction increases osmotic pressure and induce swelling**
- **Pain (dolor)**
  - **Increased pressure on deep pain receptors**
  - **Kinins may act on nerve endings**
- **Heat (calor)**
  - **Increased metabolism at implant site - pyrogens**

Wound Healing: Cellular Response

- **Cell Invasion**
  - ***Diapedisis***
    - **migration of cells across the vessel walls**
    - **driven by hydrostatic pressure**
    - **lasts up to 24 hours depending on the nature and severity of the initial insult**
  - **White Blood Cells (*neutrophils* and *eosinophils*) move into surrounding tissue within minutes to hours of injury**

Host Response: Cells

- **Inflammation**
- **Remodeling**
- **Fibrous capsule formation**

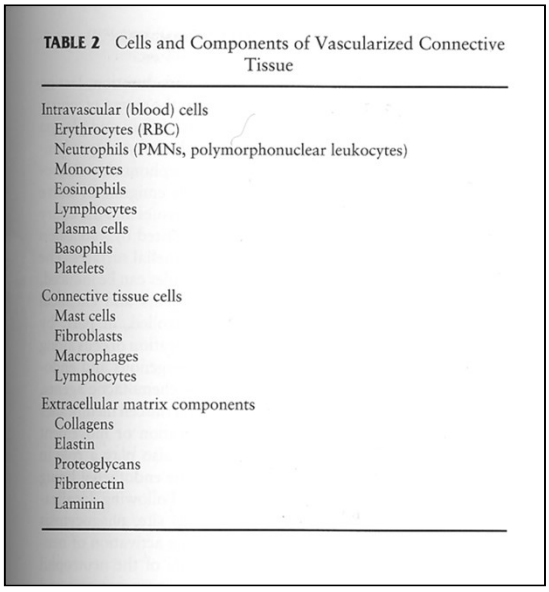


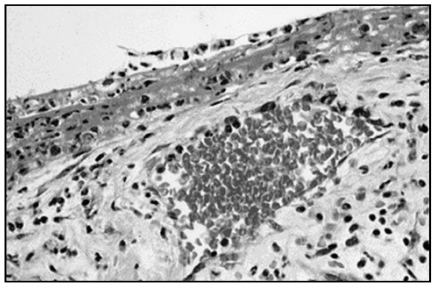
TABLE 2 Cells and Components of Vascularized Connective Tissue

Intravascular (blood) cells
- Erythrocytes (RBC)
- Neutrophils (PMNs, polymorphonuclear leukocytes)
- Monocytes
- Eosinophils
- Lymphocytes
- Plasma cells
- Basophils
- Platelets

Connective tissue cells
- Mast cells
- Fibroblasts
- Macrophages
- Lymphocytes

Extracellular matrix components
- Collagens
- Elastin
- Proteoglycans
- Fibronectin
- Laminin

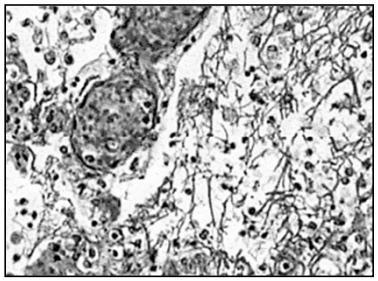
Acute Inflammation



Acutely inflammed appendix

- **pink surface layer of fibrin**
- **cells embedded in the fibrin**

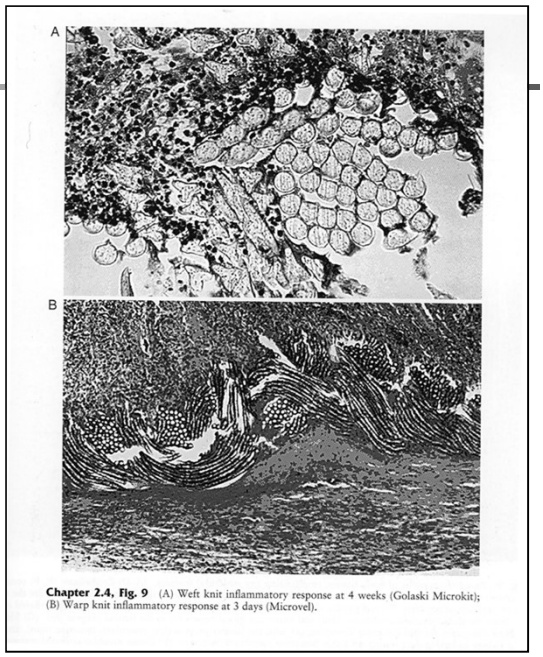
Fibrin strands (pink) with Leukocytes found in alveoli



http://medweb.bham.ac.uk

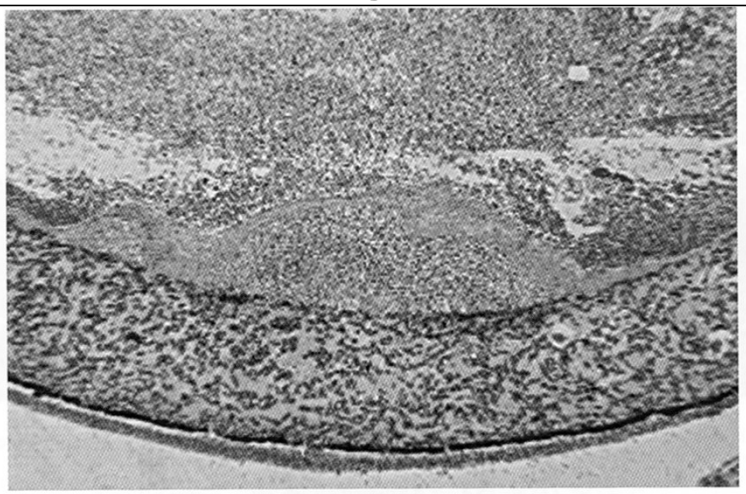
Wound Healing: Inflammation

- **4 weeks**
  - **Polymer mesh**
  - **Massive cell invasion**
  - **Presence of macrophages**



Chapter 2.4, Fig. 9 (A) Weft knit inflammatory response at 4 weeks (Golaski Microkit); (B) Warp knit inflammatory response at 3 days (Microvel).

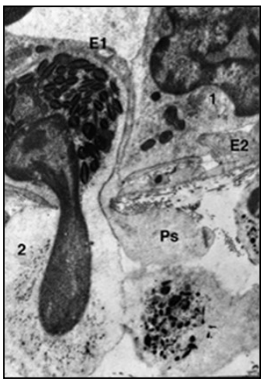
Wound Healing: Inflammation



Chapter 4.2, Fig. 2 Acute inflammation, secondary to infection, of an ePTFE vascular graft. A focal zone of polymorphonuclear leukocytes is present at the lumenal surface of the vascular graft, surrounded by a fibrin cap, on the blood-contacting surface of the ePTFE vascular graft. Hematoxylin and eosin stain. Original magnification 4×.

Wound Healing: Cellular Response

- **Cell Invasion**
  - **Neutrophils**
    - **short lived**
    - **present for 24-48 hours**
    - **non-mitotic**
  - **Replaced by *Monocytes***
    - **differentiate into macrophages**
    - **long lived - up to months**



http://medweb.bham.ac.uk

Wound Healing: Cellular Response

- **Cell Migration – Selectins & Chemotaxis**
  - **Neutrophils and other motile white blood cells emigrate to the injury site recruited by adhesion molecules**
    - **Integrins, Selectins**
    - **Chemotactic factors for neutrophils:**
      - **C5a, bacteria components**
  - **Chemotaxis**
    - **Driven by diffusion and concentration gradients**
    - **Chemotactic factors: Interlukin 1 (IL-1)**
    - **Chemotactic: Tumor Necrosis Factor (TNF)**

Wound Healing: Selectins

- **Comprised of a lectin domain, EGF-like region**
- **Complement regulatory-like molecules**
- **Three selectins have been characterized**
  - ***L-selectin*:**
    - **Found in all leukocytes, binds to activated endothelial cells (ECs)**
  - ***E-selectin*:**
    - **Found on activated ECs, binds to neutrophils, monocytes and some T Cells**
  - ***P-selectin*:**
    - **Found on platelets, ECs and binds to neutrophils, monocytes and some T cells**

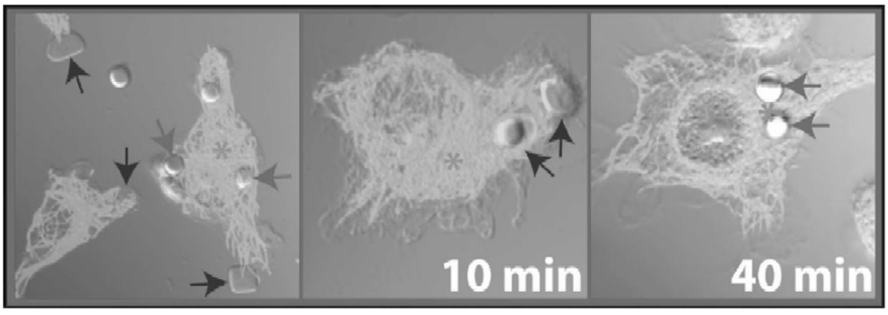
Wound Healing: Cellular Response

- **Phagocytosis - Primary function of neutrophils**
  - **Engulfing and digestion of fragment of tissue or foreign material**
  - **First step: particle adhesion to cell surface**
  - **Opsonisation: antigen presentation**
  - **Pseudopodia extensions surround particle**
  - **Formation of phagocytic vacuole (phagosome)**
  - **Lysosome fuse with phagosome – digestion!**

Phagocytosis

- **Red: cells being ingested by macrophages**
- **Blue: cells ingested (IgG-opsonized)**

Fig. 1. Tight spatial interaction between phagosomes and MTs. RAW264.7 macrophages were allowed to ingest IgG-opsonized red cells and, at the times indicated, the cells were fixed and immunostained for tubulin. Immunofluorescence images are superimposed on the corresponding differential interference contrast images. Red arrows indicate red cells being internalized (phagocytic cups). Blue arrows indicate internalized red cells (formed phagosomes). Purple asterisks denote the MTOC.



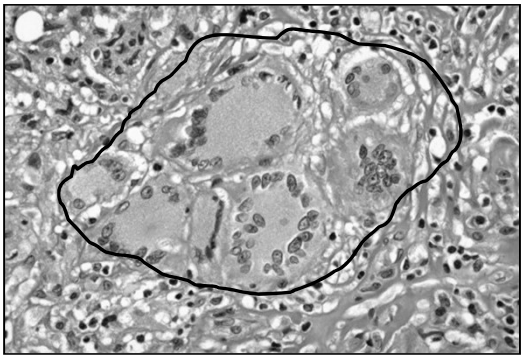
Wound Healing: Cellular Response

- **Phagocytosis**
  - **Function of cells in the immune system**
    - **differ in the number of lysosomes or degradative enzyme pockets (200/neutrophil)**
  - **Initiated by the existence of small particles**
    - **0.1 to 1 mm average size**
  - **Larger particles are ingested by macrophages and foreign body giant cells**

Wound Healing: Cellular Response

Phagocytosis

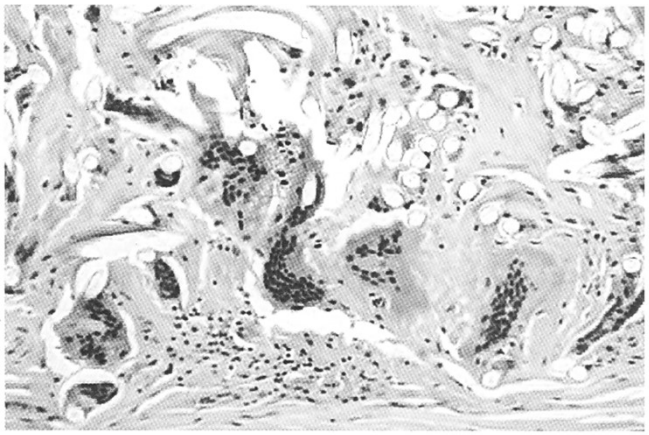
- **Macrophages coalesces to form multinuclear giant cells for encapsulation of large particles**



Wound Healing: Cellular Response

- **Phagocytosis**
  - **Removal of dead tissue and foreign material from the implant site**
  - **If particles are toxic, may results in cell death and accumulation of cell debris (pus)**
  - **Mediated by macrophages**
    - **produces proteases, chemotactic factors, reactive oxygen species, growth factors**
  - **If destructive inflammation persists and no healing occurs within 3-5 days, chronic inflammation commences**

Wound Healing: Immune Response



Chapter 4.2, Fig. 7 Foreign-body reaction with multinucleated foreign body giant cells and macrophages at the periadventitial (outer) surface of a Dacron vascular graft. Fibers from the Dacron vascular graft are identified as clear oval voids. Hematoxylin and eosin stain. Original magnification 20×.

Wound Healing: Cellular Response

- **Biomaterials**
  - **Not phagocytosed due to their size**
  - **Recognized and attached to by leukocytes**
  - **Adsorption of naturally occurring serum factors or opsonins (antigen presenters)**
  - **Degradation and corrosion products are phagocytosed**

Biomaterial-Tissue Interactions

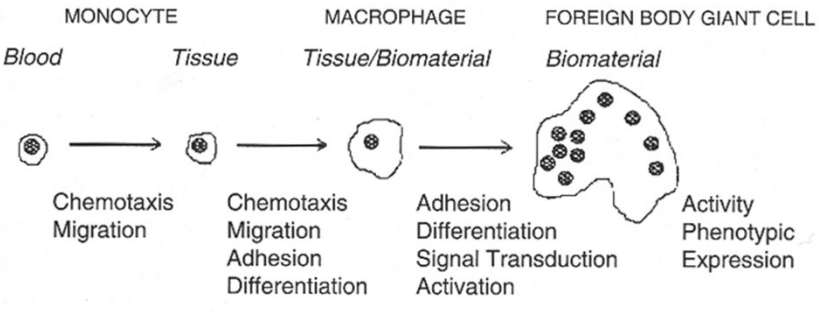
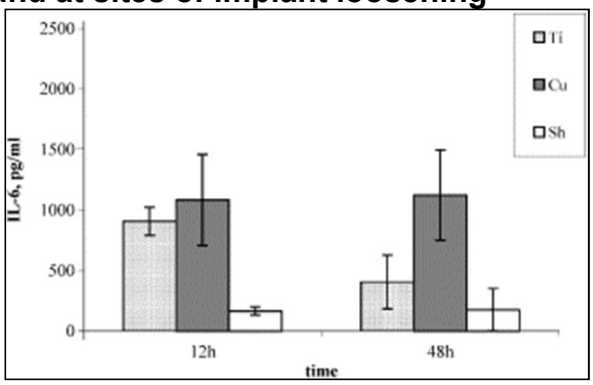


FIG. 5. *In vivo* transition from blood-borne monocyte to biomaterial adherent monocyte/macrophage to foreign-body giant cell at the tissue–biomaterial interface. Little is known regarding the indicated biological responses, which are considered to play important roles in the transition to FBGC development.

Biocompatibility of Ti vs. Cu

- **In vivo (subcu.) secretion of IL-6 (pg/ml) at 12 to 48 hours** ***(Suska et al., Biomaterials, 2005)***
- **Exudate of cells surrounding Cu implants measured higher IL-6 production**
- **IL-6: found at sites of implant loosening**



Inflammatory Cells around Implants

- **Number of inflammatory cells over time increased surrounding the Cu implant vs. the Ti implant**

Time (h)	No. of infl. cells±SEM, % mono, % poly		
	Ti	Cu	Sh
12	150.9±42.5, 20, 80	251.6±83.7, 11, 89	78.1±27.4, 21, 79
48	85.2±43.1, 34, 67	649.5±303.4, 6, 94	22.8±4.2, 50, 50

- **Conclusion**
  - **Toxicity of Cu implant is greater than Ti**

Wound Healing: Remodeling

- A *successful* response to injury or the inflammatory challenge or results in decreased tissue mass as the diseased or injured tissue is removed
- Next step is Remodeling
  - Fibrous capsule formation
  - Encapsulate implant and isolate from surrounding tissue

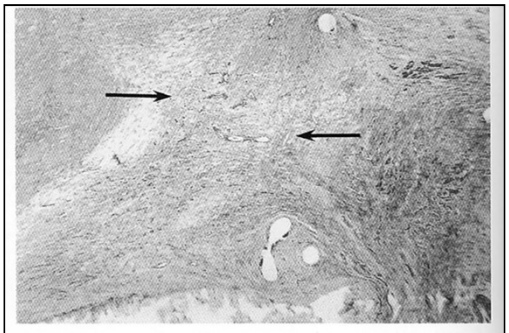
Wound Healing: Remodeling

Tissue Remodeling occurs in stages

- Injury
- Formation of fibrous clot
- Cellular invasion
- Budding of small vasculature and burrowing into implant site
- Migration of fibroblasts to the injury site
- Production of collagen and muccopolysaccharides by fibroblasts
- Forms scar tissue or granulation tissue
- Formation of a fibrous capsule
- Acts as a scaffold for further cellular reconstruction and remodeling

Wound Healing: Remodeling

- **Granulation Tissue**
  - **Soft, pink granular scan tissue**
  - **Formation of new small blood vessels**
  - **Observed at 3-5 days post implantation**



Chapter 4.2, Fig. 4 Granulation tissue in the anastomotic hyperplasia at the anastomosis of an ePTFE vascular graft. Capillary development (red slits) and fibroblast infiltration with collagen deposition (blue) from the artery form the granulation tissue (arrows). Masson's Trichrome stain. Original magnification 4×.

Wound Healing: Fibrous Capsule

Chapter 4.2, Fig. 9 Fibrous capsule composed of dense, compacted collagen. This fibrous capsule had formed around a Mediport catheter reservoir. Loose connective tissue with small arteries, veins, and a nerve is identified below the acellular fibrous capsule.

Wound Healing: Remodeling

- Fibrous Encapsulation
  - Forms in the same manner as scar tissue
  - Acellular
    - if giant cells are present in capsule – corrosion
    - large number of lymphocytes indicates immune response
    - neutrophils or macrophages will interact with each other

Wound Healing: Remodeling

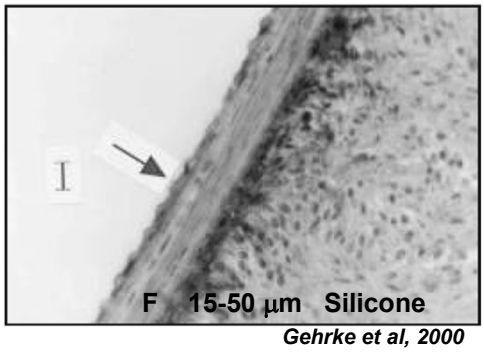
- Fibrous Encapsulation
  - Marks the end of the inflammatory response
  - Maintained by the continuous presence of the implant
  - If the implant is removed or resorbed, the capsule will collapse into a residual scar or become completely remodeled
  - Site of future infection

Factors Controlling Capsule Thickness

- **Chemical activity of the material**
  - **corrosion: metal oxide complexes**
  - **degradation: monomers or ions**
- **Motion between implant and tissue**
  - **thickness increases with relative motion**
  - **extreme motion may result in fluid-filled bursa**
- **Implant shape**
  - **smooth vs. surface features**
  - **capsule thicker at the corners of the implant**

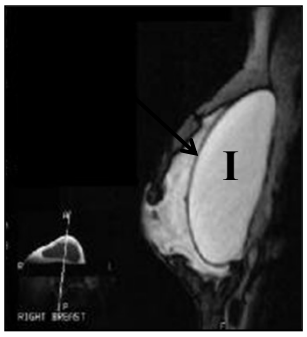
Factors Controlling Capsule Thickness

- **Presence of electrical currents**
  - **Changes in pH or pO_2**
  - **Thickness proportional to current density**
    - **Ex. Ends of electrodes for pacemakers**



F 15-50 μm Silicone

Gehrke et al, 2000



MRI, Silcone

Wound Healing: Remodeling

- A *successful* response to injury or the inflammatory challenge or results in decreased tissue mass as the diseased or injured tissue is removed
- Next step is Remodeling
  - fibrous capsule formation
- Otherwise: Chronic Inflammation
  - Immunological
  - Material specific

Remodeling

- Fibrocapsule formation vs. Chronic Response
- Timeline
  - normal: 1-2 weeks
  - low activity: remodeling by 3 to 4 wks
  - high activity: 6 to 8 weeks

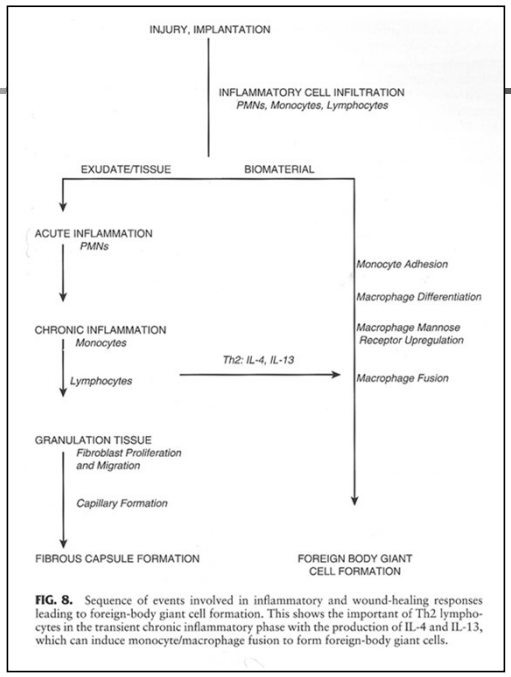
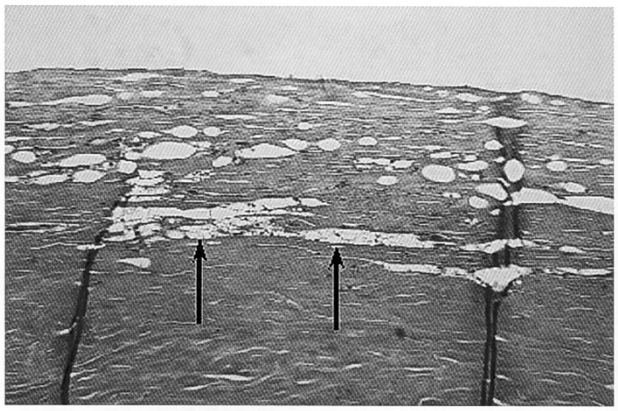


FIG. 8. Sequence of events involved in inflammatory and wound-healing responses leading to foreign-body giant cell formation. This shows the important of Th2 lymphocytes in the transient chronic inflammatory phase with the production of IL-4 and IL-13, which can induce monocyte/macrophage fusion to form foreign-body giant cells.

Wound Healing: Fibrous Capsule



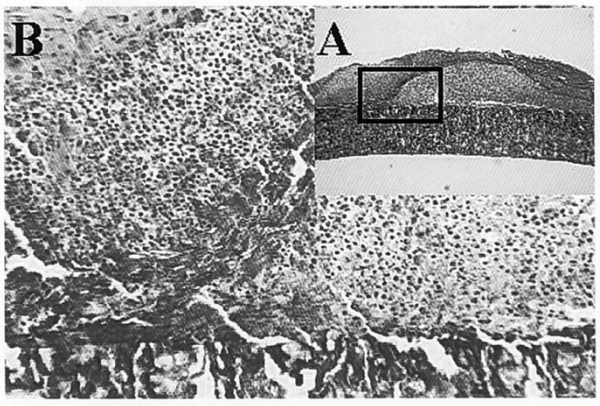
Chapter 4.2, Fig. 10 Fibrous capsule with a focal foreign-body reaction to silicone gel from a silicone gel–filled silicone-rubber breast prosthesis. The breast prosthesis–tissue interface is at the top of the photomicrograph. Oval void spaces lined by macrophages and a few giant cells are identified and a focal area of foamy macrophages (arrows) indicating macrophage phagocytosis of silicone gel is identified. Hematoxylin and eosin stain. Original magnification 10×.

Silicon Implant

Phagocytosis of Silicon

Fibrous capsule

Wound Healing: Inflammation



Chapter 4.2, Fig. 3 Chronic inflammation, secondary to infection, of an ePTFE arteriovenous shunt for renal dialysis. (A) Low-magnification view of a focal zone of chronic inflammation. (B) High-magnification view of the outer surface with the presence of monocytes and lymphocytes at an area where the outer PTFE wrap had peeled away from the vascular graft. Hematoxylin and eosin stain. Original magnification (A) 4×, (B) 20×.

Biomaterial-Tissue Interactions

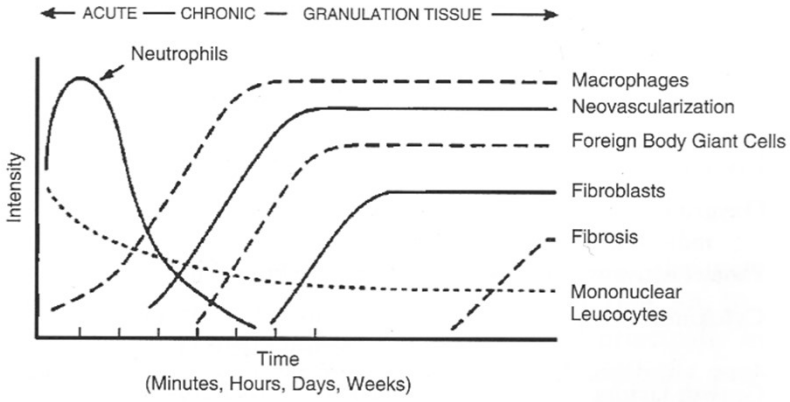
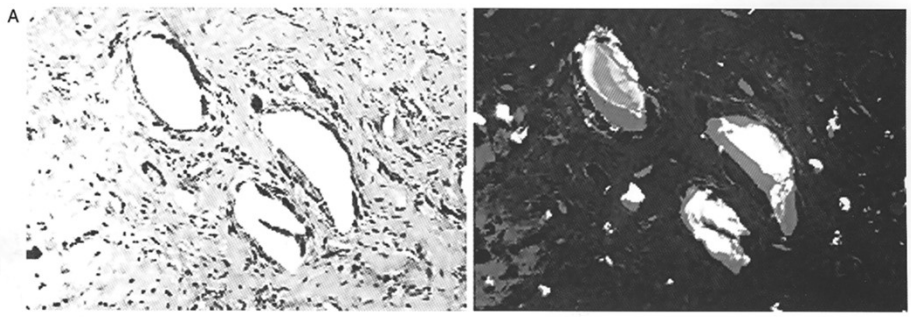


FIG. 1. The temporal variation in the acute inflammatory response, chronic inflammatory response, granulation tissue development, and foreign-body reaction to implanted biomaterials. The intensity and time variables are dependent upon the extent of injury created in the implantation and the size, shape, topography, and chemical and physical properties of the biomaterial.

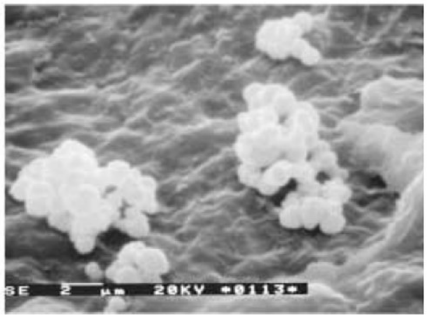
Effects of Wear Products



Chapter 4.2, Fig. 6 (A) Focal foreign-body reaction to polyethylene wear particulate from a total knee prosthesis. Macrophages and foreign-body giant cells are identified within the tissue and lining the apparent void spaces indicative of polyethylene particulate. Hematoxylin and eosin stain. Original magnification 20×. (B) Partial polarized light view. Polyethylene particulate is identified within the void spaces commonly seen under normal light microscopy. Hematoxylin and eosin stain. Original magnification 20×.

BIOCOMPATIBILITY: Infection

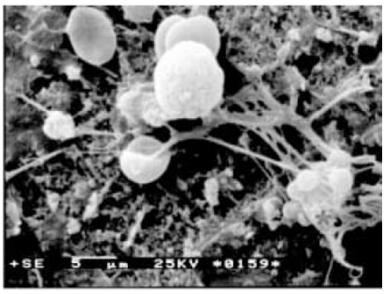
- **Long term infection occurs in months or years post implantation**
  - **Depends on interfacial properties of implants**
  - **Blood-borne pathogens**
  - **Race for the surface!**



Staphylococcus epidermidis **bacteria on polyethylene implant**
Gallo et al., 2003

BIOCOMPATIBILITY: Infection

- **Occurs in surrounding tissue**
- **<1% for most permanent devices**
- **5-10% in the early 1960s**
- **Caused by *staphylcocci***
- **Implant site and patient dependent**
- **Rate of infection correlates positively with length of surgery**



Blood, fibrin, bacteria(*) on Ti-6Al-4V implant
Gallo et al., 2003

Wound Healing: Remodeling

- **Rate of Wound Healing depends on**
  - **Severity of injury**
  - **Size of defect**
  - **Location of defect**
    - ***Ex:*** **Bone, skin: regeneration**
      Cartilage or ligament: fibrous capsule
- **Implant Response**
  - **Capsule Formation**
  - **Tissue Ingrowth: *mechanical* fixation**
  - **Tissue Integration: *biological* fixation**

Biomaterial-Tissue Interactions

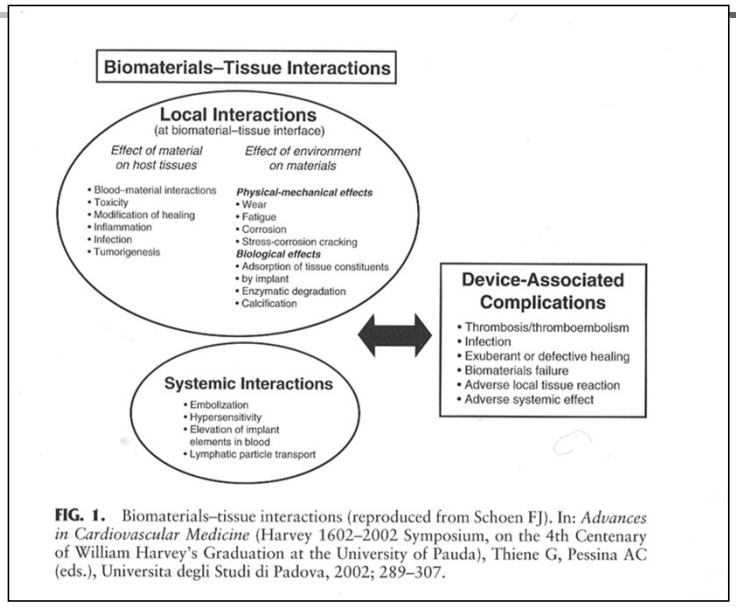


FIG. 1. Biomaterials–tissue interactions (reproduced from Schoen FJ). In: *Advances in Cardiovascular Medicine* (Harvey 1602–2002 Symposium, on the 4th Centenary of William Harvey's Graduation at the University of Pauda), Thiene G, Pessina AC (eds.), Universita degli Studi di Padova, 2002; 289–307.

Summary Host Responses

- Injury
- Formation of fibrous clot
- Cellular invasion
- Vascularization
- Migration of fibroblasts to the injury site
- Formation of a fibrous capsule

- Specific vs. Non-specific Responses
- Blood compatibility
- Readings: Ratner, Sec. 11.2, p.499-583

Infection – Bone Fixation Screws

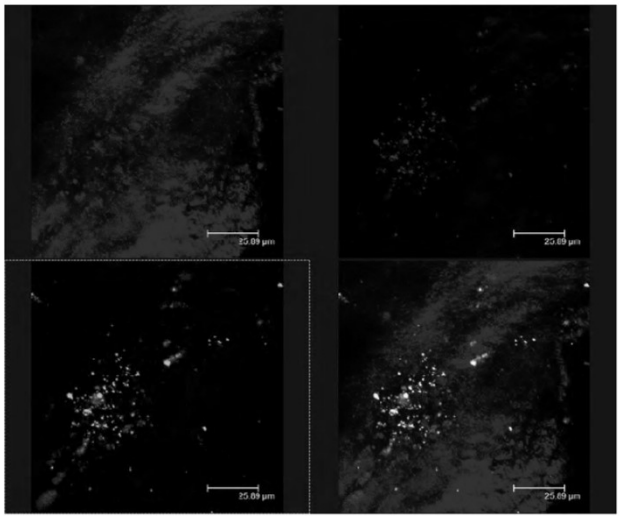


FIGURE II.2.8.2 Biofilm attached to orthopedic screws from a non-union fracture case. The surface of the screw and associated invested tissue are blue and imaged by reflected confocal microscopy. Staphylococci were stained red by fluorescence *in situ* hybridization (FISH) using the Sau probe. General bacteria were stained green with the Eub338 probe. The overlay (bottom left) shows that the biofilm consisted primarily of staphylococci with occasional other types of cocci. Scale bar = 25 μm.