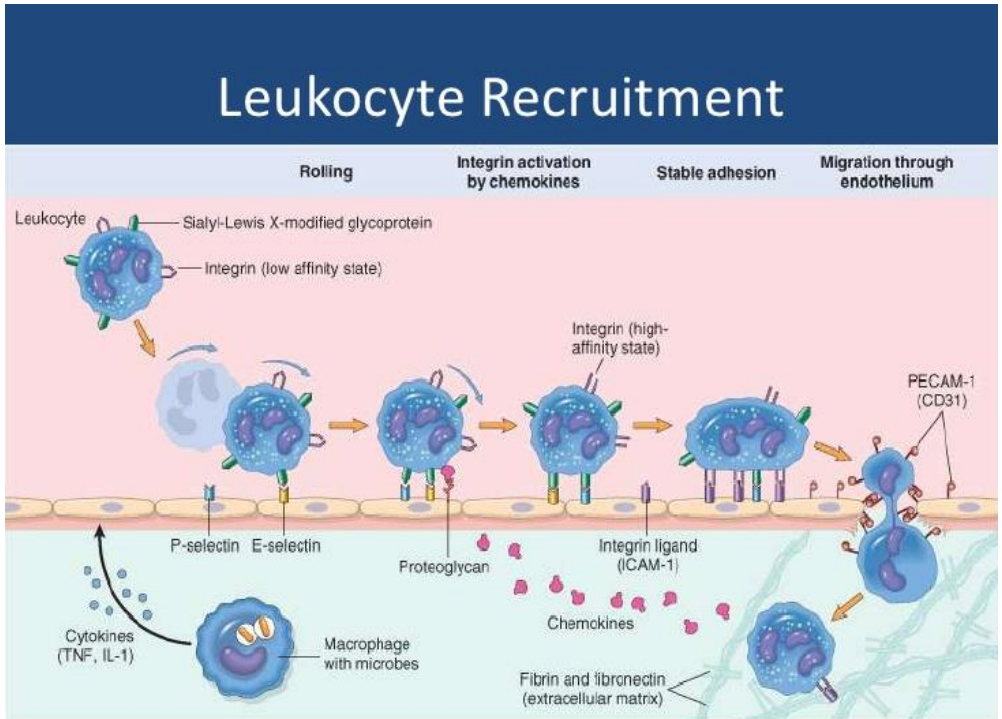
MME 4506 Biomaterials

Cellular response to protein adsorbed biomaterial surfaces

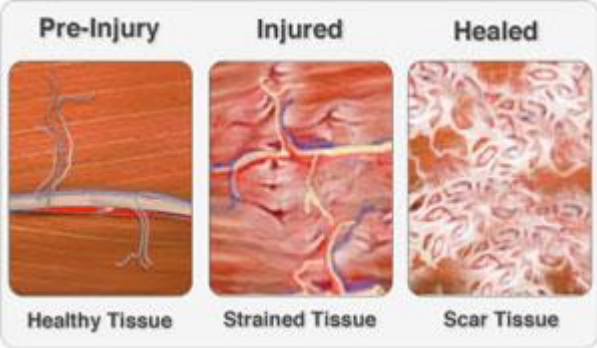
The replacement of injured or diseased tissues with donor tissues or organs is not feasible as these materials are attacked by the immune system.
Synthetic biomaterials are extensively used in medical applications because of the absence of immunologically recognizable biologic molecules on their surface.

Normal wound healing consists of two stages:

1. Inflammation starts once damage to vascularized connective tissue occurs
    - Damage to the circulation causes release of danger signals that recruit inflammatory cells (leukocytes: neutrophils and monocytes)
    - Fibrous clot forms from blood cells and fibrinogen. It is a provisional matrix that provides both structure and biochemical to direct healing (cytokines, growth factors, etc.)
    - Local capillaries dilate and the permeability of the vessel endothelium increases



2. Repair
    - Cellular invasion
    - Remodelling
    - Scar formation



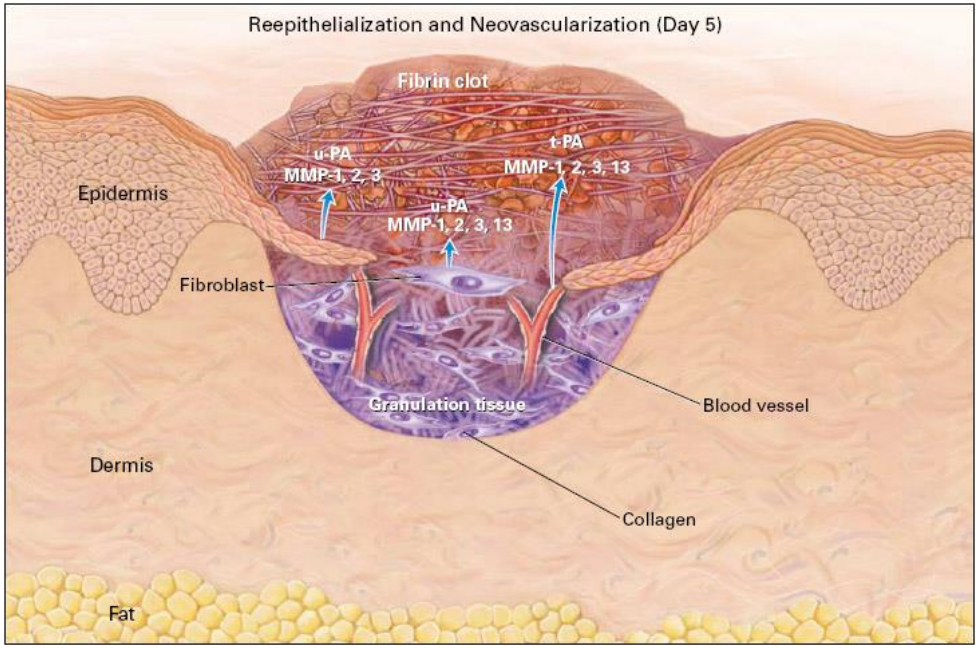
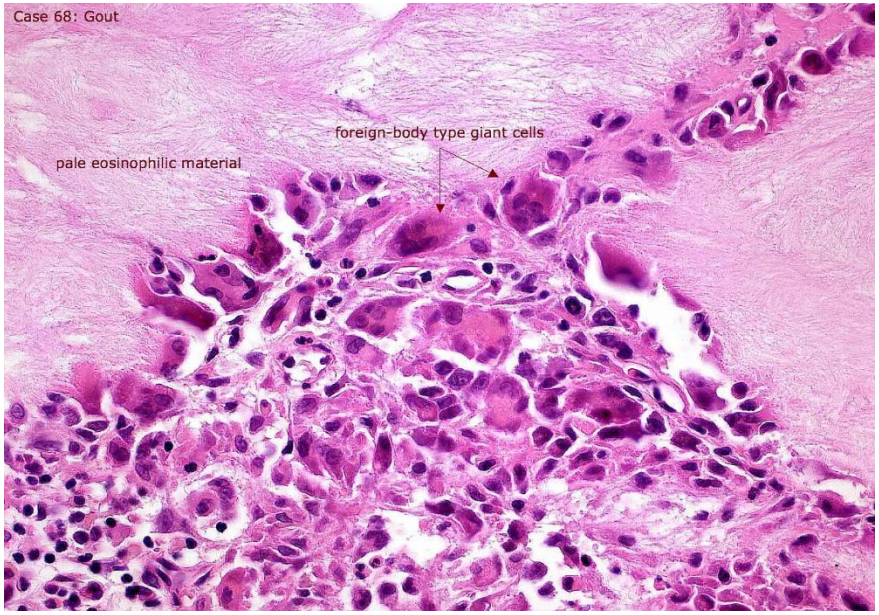


Figure 2. A Cutaneous Wound Five Days after Injury.

Blood vessels are seen sprouting into the fibrin clot as epidermal cells resurface the wound. Proteinases thought to be necessary for cell movement are shown. The abbreviation u-PA denotes urokinase-type plasminogen activator; MMP-1, 2, 3, and 13 matrix metalloproteinases 1, 2, 3, and 13 (collagenase 1, gelatinase A, stromelysin 1, and collagenase 3, respectively); and t-PA tissue plasminogen activator.



Millions of medical devices are implanted into humans each year with reasonable levels of success

FDA and other regulatory agencies ‘‘stamp’’ these medical devices as biocompatible

However there is still no clear understanding of the origins of biocompatibility

Materials that do not leach biologically reactive substances will heal in the body in a manner now considered biocompatible.

Not all non-leaching materials are equally biocompatible irrespective of surface properties

The body reacts similarly to nearly all materials that we call biocompatible and walls them off in an avascular, tough, collagenous bag, up to 200 micrometers thick

There are typical biological responses to implanted biomaterials that change their functionality:
In clotting of blood and foreign body reactions, the body recognizes and responds to biomaterials on the basis of the protein layer on their surface.
Adhesion proteins are recognized by the integrin receptors that are present on most cells.

The adsorption of adhesion proteins to the biomaterial surface converts it into a biologically recognizable material which results in different degrees of cellular response

Cells can adhere to an implant surface, release active compounds, recruit other cells, grow, replicate and die. These processes often occur in response to the protein on the surface.

Cell processes lead to desirable and undesirable responses that physicians and patients observe with implants

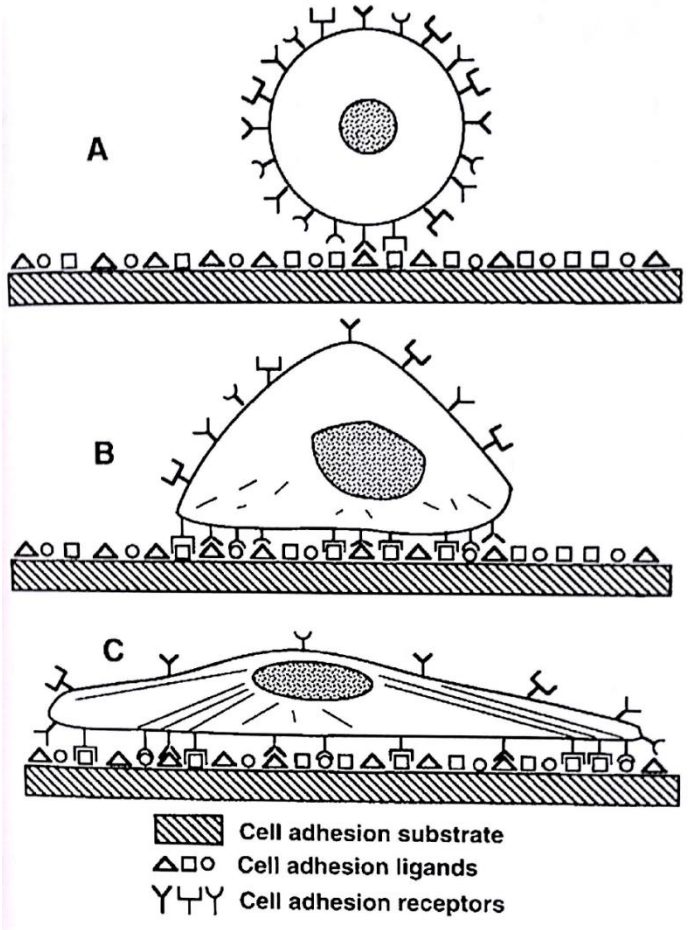
Cell processes at artificial surfaces are also integral to the unwanted buildup of marine organisms on ships, bacterial biofilms on implants and the useful growth of cells in bioreactors used to manufacture biochemicals.

Cells and the ECM interact through specific mechanisms:

- Initial recognition
- Physical adhesion
- Electrical and chemical communication
- Cytoskeletal reorganization
- Cell migration

Adhesion receptors on the cell also act as transmembrane signaling molecules that transmit information about the environment to the inside of cells and mediate the effects of signals initiated by growth factors

The ligands with which cells interact are immobilized in the ECM and not in solution



While biomaterials developed in vitro are highly bioactive, (induce cell adhesion and differentiation), they do not heal normally in the body

Uncontrolled biological encapsulation directly confounds the performance of many implanted devices. The presence of this capsule seriously degrades the performance of implant electrodes, drug delivery systems, and breast implants by preventing intimate contact between device and tissue.

The reaction associated with this foreign body response (long term, low level inflammation and macrophage activation) may also

- inhibit the healing of vascular grafts,
- trigger capsular opacification found with intraocular lenses,
- lead to the extrusion of percutaneous devices,
- increase device calcification,
- induce contact lens discomfort

So it generally leads to complications and less than desirable outcomes associated with today's medical devices.

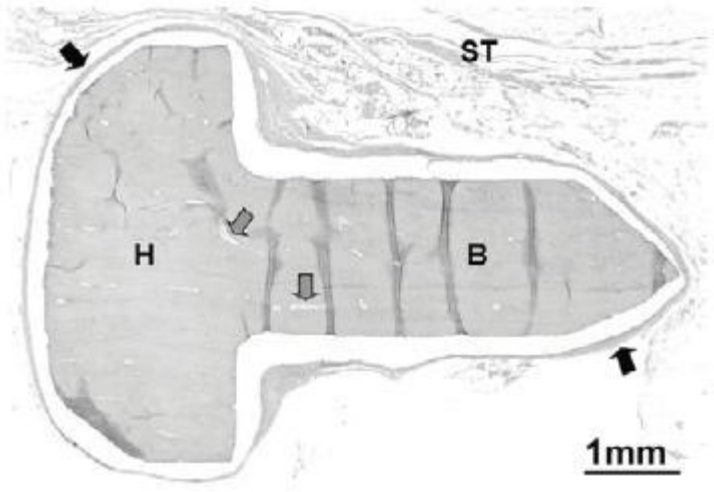


Figure 3. Photomicrograph of DCBB-pin 14 days after implantation in subcutaneous tissue (ST) involved by a fibrous capsule (black arrows), showing the head (H) and body (B) composed by compact structure with some small pores (gray arrows). HE.

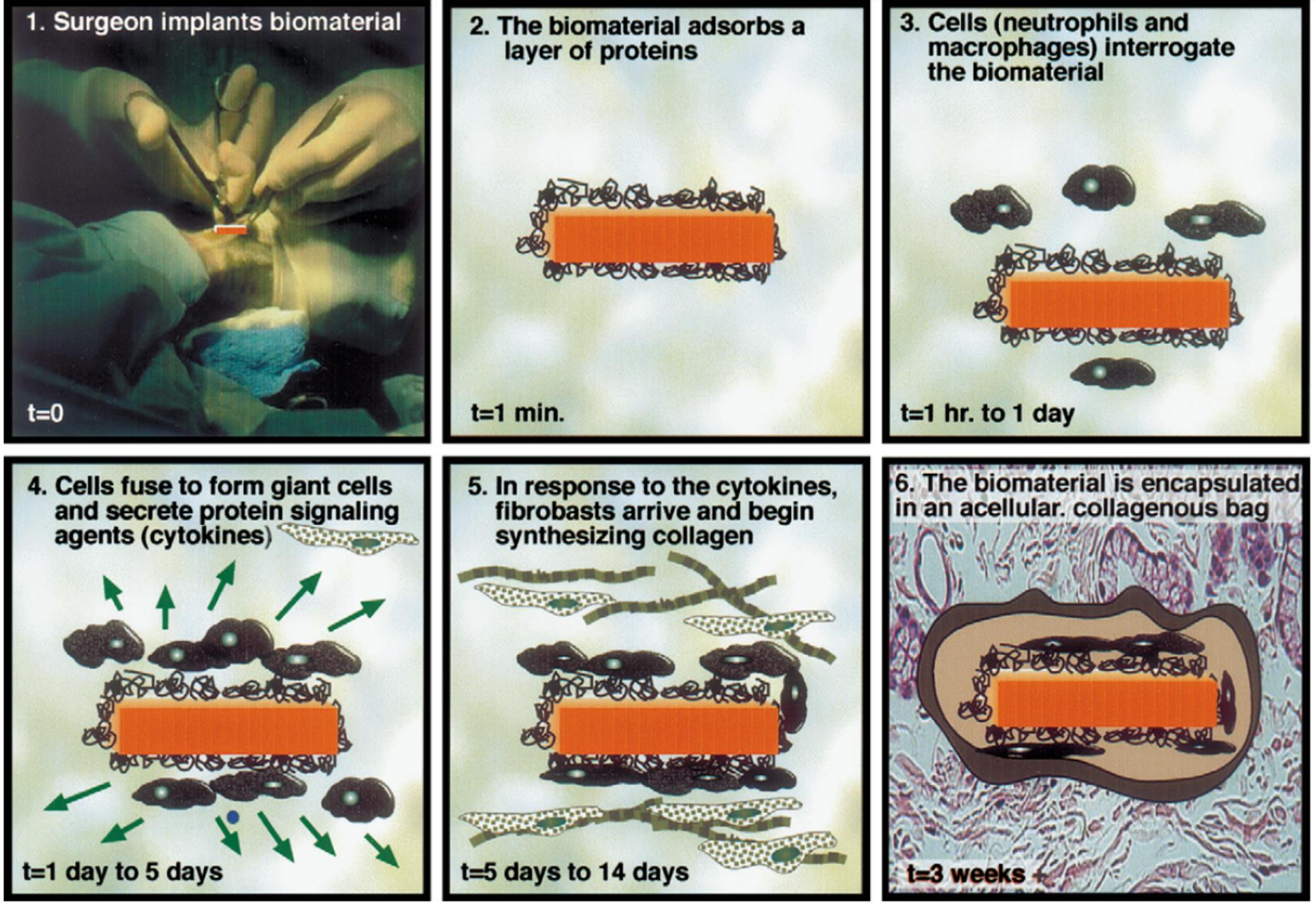


Fig. 9. The foreign body reaction is the normal reaction of a higher organism to an implanted synthetic material and is schematically illustrated here. (1) A surgeon implants a biomaterial in a surgical site (an injury). (2) Quickly, the implant adsorbs a layer of proteins, the normal process for a solid surface in biological fluids. (3) Cells (neutrophils and then macrophages) interrogate and attack the "invader," i.e., the biomaterial. (4) When the macrophages find they cannot digest the implant, they fuse into giant cells to engulf the object. However, it is too large to completely ingest. The giant cells send out chemical messengers (cytokines) to call in other cells. (5) Fibroblast cells arrive and begin synthesizing collagen. (6) The end stage of the reaction has the implant completely encased in an acellular, avascular collagen bag. There are macrophages between the collagen sac and the implant.

The response of the body to implantation of a biomaterial

1. • Incision and Implantation
2. • Acute inflammation
   • Recruitment of neutrophils and monocytes
3. • Monocyte differentiation into macrophages
   • Macrophage interaction with the biomaterial
4. • Chronic inflammation
   • Macrophage fusion into FBGC and formation of fibrous capsule

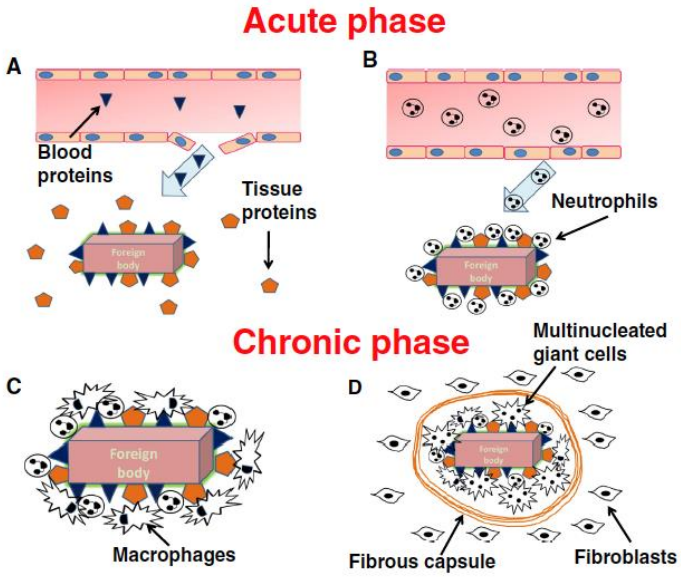


Figure 1. Schematic representation of the acute and chronic phases of the tissue foreign-body reaction (adapte

After protein adsorption the macrophages adhere to the biomaterial

The proteins adsorbed on the material surface are non-specific and of high variety

Macrophages do not recognize the surface of a biomaterial because of the non-specific proteins

As a result macrophages spread on the surface as they try to phagocytose it. They cannot digest or engulf this large mass, so, to increase their effectiveness, they fuse together to form multinucleated giant cells. These cells still cannot engulf a macroscopic medical device.

The giant cells signal to the body that there is a large foreign body to be walled off.
The fibroblasts arrive and generate the collagen capsule

Immune response to biomaterials

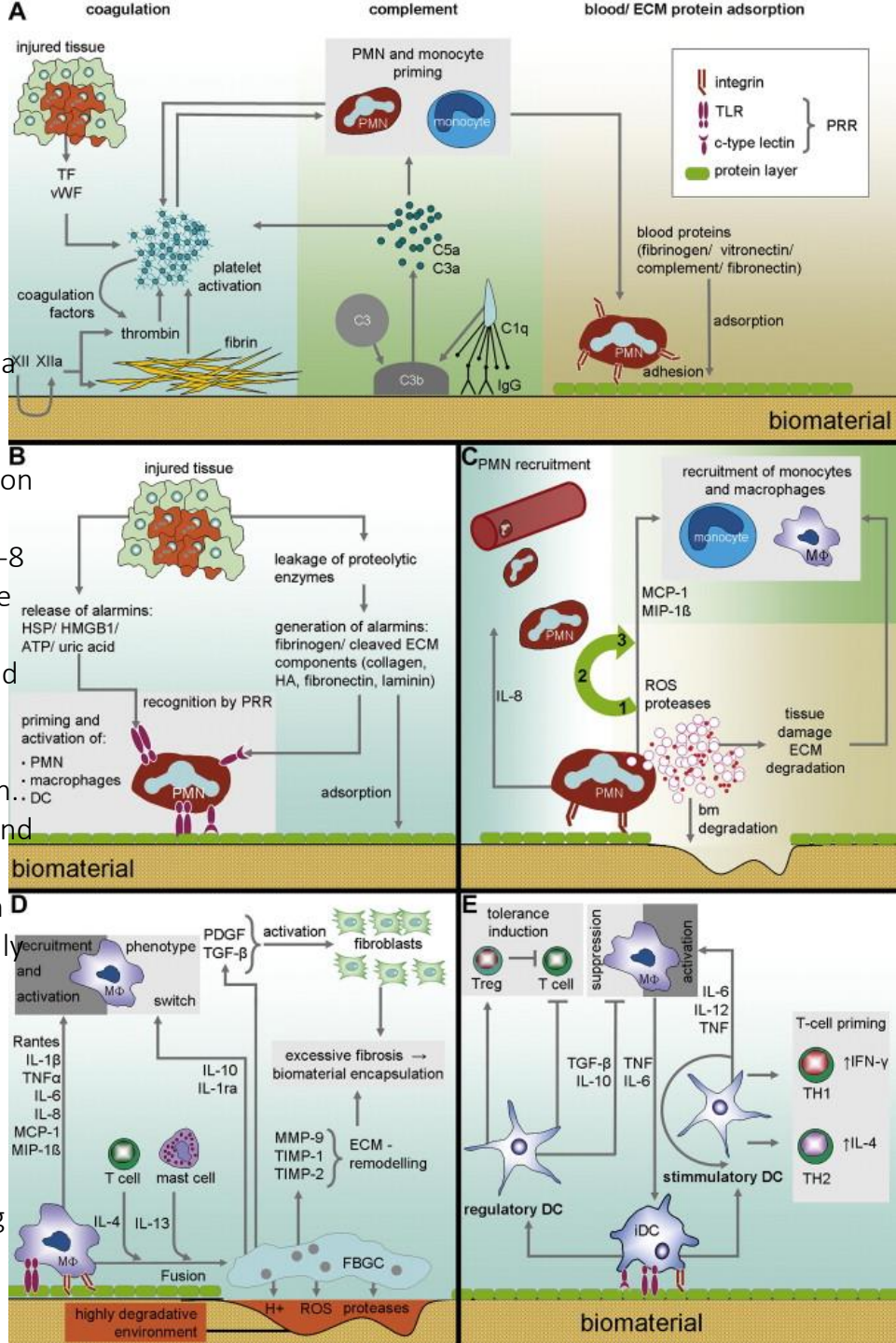
A) Adsorption of blood proteins and activation of the coagulation cascade, complement and platelets result in the priming and activation of PolyMorphoNuclear leukocytes, monocytes and resident macrophages.
There are two major proteins that cause the macrophages to recognize foreign materials: Immunoglobulin G and C3b

B) Danger signals (alarmins) released from damaged tissue additionally prime the immune cells for enhanced function via PatternRecognitionReceptor engagement.

C) The acute inflammatory response is dominated by the action of PMNs. PMNs secrete proteolytic enzymes and ReactiveOxygenSpecies, corroding the biomaterial surface. IL-8 released from PMNs enhances PMN influx and priming. In the transition from acute to chronic inflammation, PMNs stop secreting IL-8 in favor of cytokines promoting immigration and activation of monocytes and macrophages.

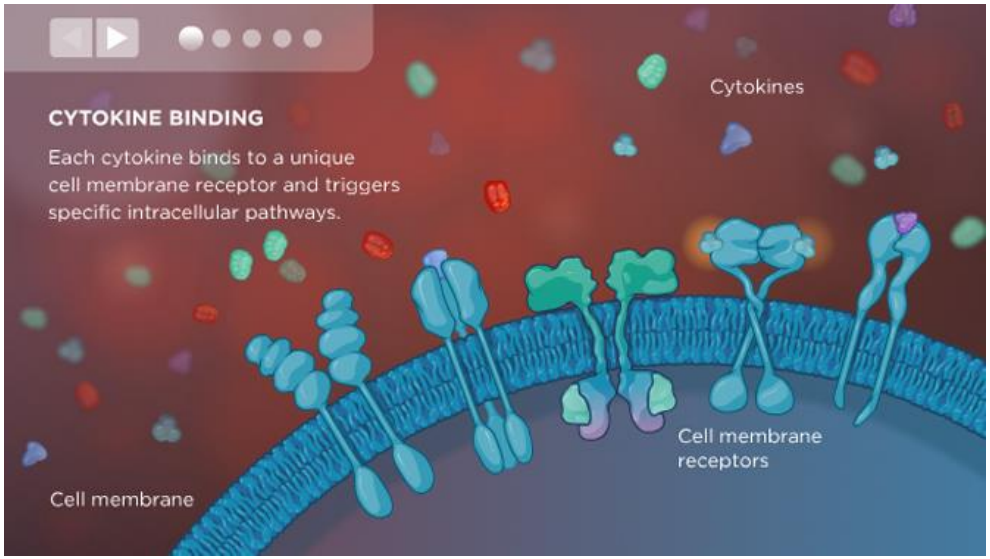
D) Macrophages are the driving force of chronic inflammation. Constant release of inflammatory mediators like TNFa, IL-6, and MCP-1 results in permanent activation of macrophages. Fusion-inducing stimuli like IL-4 and IL-13 promote the fusion of macrophages to ForeignBodyGiantCells, which form a highly degradative environment on the biomaterial surface. Furthermore, FBGC promote ECM remodeling and fibroblast activation resulting in excessive fibrosis and biomaterial encapsulation.

E) Macrophage-derived cytokines and PRR engagement activate DendriticCells on the biomaterial surface. Depending on the nature of the stimulus, DCs mature to either immunogenic or tolerogenic subtypes, amplifying or suppressing the inflammatory response.

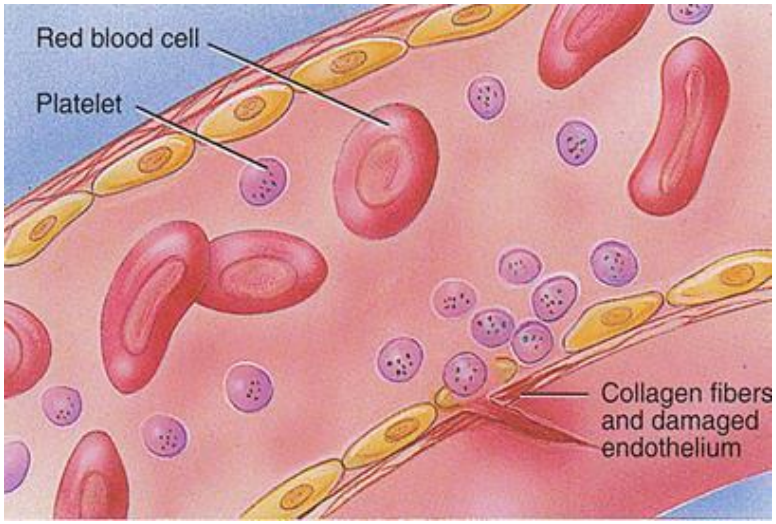


All cells communicate by the release and detection of signaling agents (cytokines) through a network made up of multiple and interactive signaling pathways.

The state of a cell (shape, structure, biological activity, etc.) depends on the signals it receives from its biological environment.



For example, platelets normally circulate in the bloodstream in a passive state.



Upon vascular injury a signaling cascade is initiated that activates the platelets for their role in healing the vascular injury.

For this reason, whole blood must be treated (e.g., heparinized) when removed from the body to keep it from coagulating

Cells adhered at the implant surface finally secrete extracellular matrix molecules that fill the spaces between cells once fibrous capsule is formed and chronic inflammation is over.

ECM provides attachment structures for more proteins and cells.

Formation of small and large blood vessels provides this new tissue with nutrition and removal of wastes.

Foreign body reaction is associated with the following events that are not observed in normal healing:

- Unresolved acute inflammatory events
- Chronic inflammation and persistence of inflammatory cells
- Limited angiogenesis
- Excessive fibrosis

These effects are strengthened if

a. There is infection in the wound site
b. Biomaterial surface is not biocompatible
c. Motion of the implant
d. Implant site has access to blood supply
e. Implantation is done by large incisions

The thickness of the fibrous capsule and the period of healing increases as a result of these adverse effects

In a normal wound, the macrophage cell ‘‘orchestrates’’ wound healing

In the presence of an uncomplicated wound, the macrophage turns on the pathways leading to normal healing by first cleaning up the wound site and then secreting the appropriate cytokine messenger molecules.

These soluble messengers activate processes in the cell types needed for healing (fibroblast, keratinocyte, osteoblast, etc.).

The surfaces of today’s biomaterials present in the wound site, turn this normal healing process off.

The response of cell and tissues to biomaterial surface depends on many biomaterial related factors including, chemical, physical and mechanical stimuli from the surface.

- Stress concentrations at corners of an implant

Two samples made of the same material, one a triangle shape and the other a disk, implanted in soft tissue will show different healing reactions with considerably more fibrous reaction around the triangle

- Rigidity

It is known that rigid biomaterials promote cell spreading and growth in the presence of soluble proteins.
In contrast flexible scaffolds that cannot resist cytoskeletal forces promote cell retraction and differentiation and inhibit growth.

- Implant topography and roughness texture

Smooth surfaces provide less surface area for protein adsorption and cell support

- Corrosion and degradation

Release of metal oxide complexes or monomers from the implant cause severe inflammation

- Adhesive proteins on the biomaterial surface

Preadsorption of certain proteins on to a solid surface greatly increases its adhesiveness to many kinds of cells. Such proteins are called adhesive proteins.

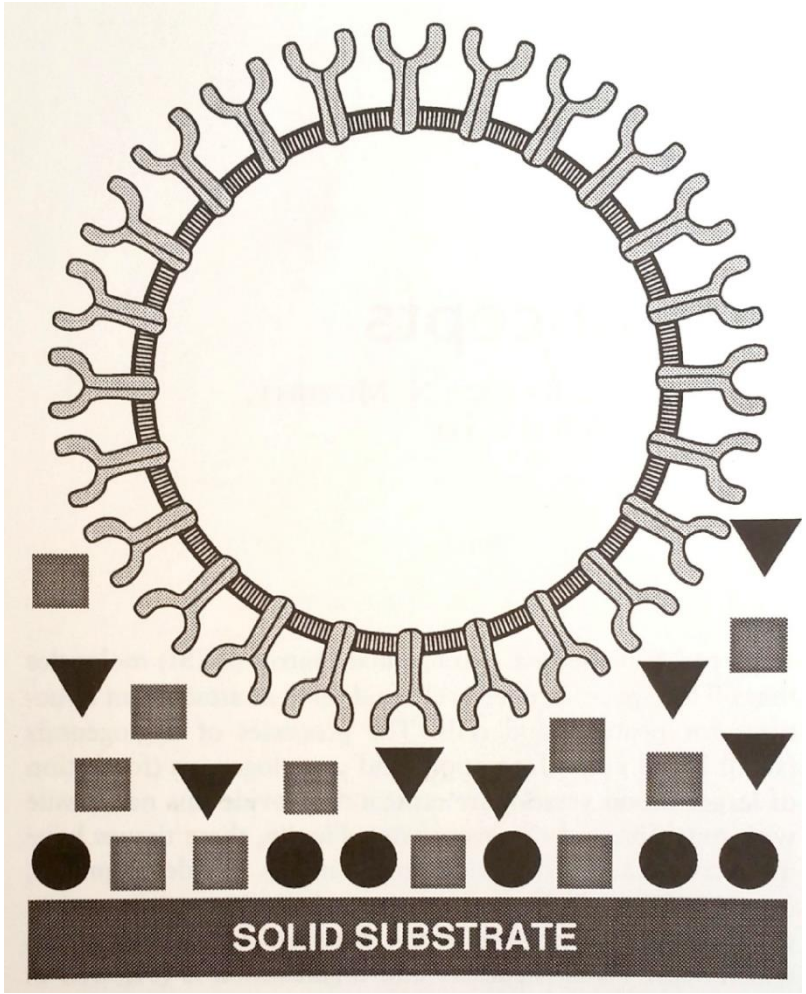
The increased adhesiveness is caused by receptors on membranes of many cells that bind specifically to these specialized proteins.

These receptors called integrins mediate cell interactions with foreign surfaces when the special protein is adsorbed.

Integrins recognize and cause the cells adhere only to surface-bound form of that protein which has different biological activity when it is in solid phase than the solution phase.

The dissolved, plasma-phase adhesion proteins do not bind to integrins unless the cells are appropriately stimulated. So the adsorption of proteins to surfaces changes the integrin-adhesion protein interaction.

The type of surface to which the adhesion protein is adsorbed affects the ability of the protein to support cell adhesion.



Effect of protein layer on cellular response

The principles that determine protein adsorption to biomaterials are

- Monolayer adsorption and consequent competition for available adsorption sites
- Driving forces for adsorption are the intrinsic surface activity and bulk concentration of the protein
- Surfaces vary in selectivity of adsorption
- Biological activity of the adsorbed protein varies on different surfaces

Generally all proteins are known to have an inherent tendency to deposit very rapidly on surfaces as a tightly bound adsorbate that strongly influences cell response to surfaces.

The particular properties of surfaces and specific properties of individual proteins, together determine the organization of adsorbed protein layer.

The nature of that layer determines the subsequent interactions of many different types of cells with the surfaces.

Experiments on biomaterial surfaces that are preadsorbed with adhesion proteins show the increased adhesiveness clearly.

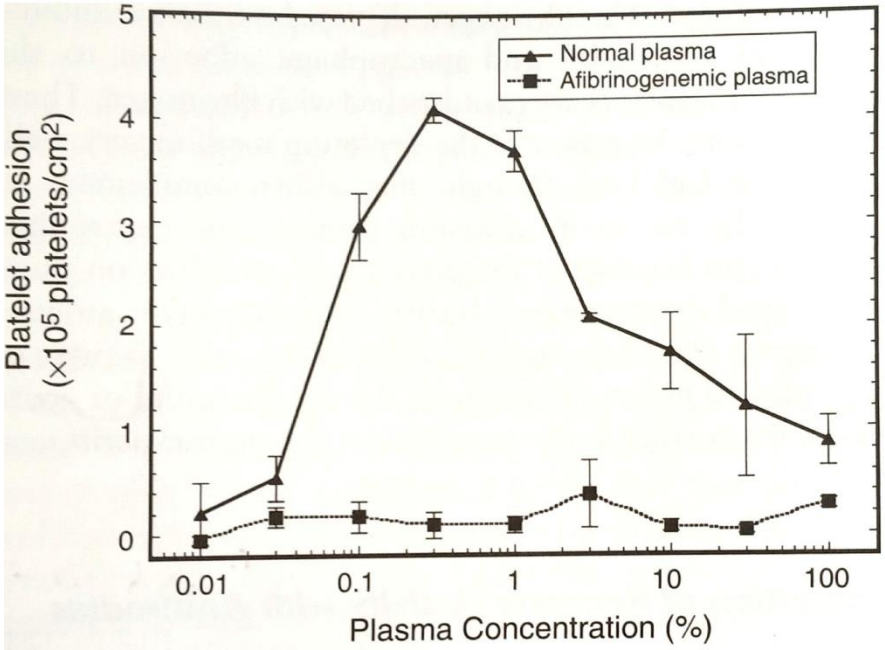
It is seen that platelet depositon onto polymeric vascular tubes in dogs is greatly increased when fibrinogen or fibronectin are preadsorbed to the surfaces.

Implants are exposed to complex mixtures of proteins such as plasma or serum, so the adhesion protein must compete with many others to adsorb to the surface. A given adhesion protein that is present in the plasma may adsorb very little compared to other proteins because of competition for limited surface sites.

Thus, selective depletion from the complex mixture is a more biologically correct way to understand the role of adhesion proteins on adhesion than preadsorption with pure adhesion proteins.

Selective depletion means that only one of the proteins is removed from the mixture at a time and adsorbed surfaces are exposed to the mixtures, then tested for cell attachment.

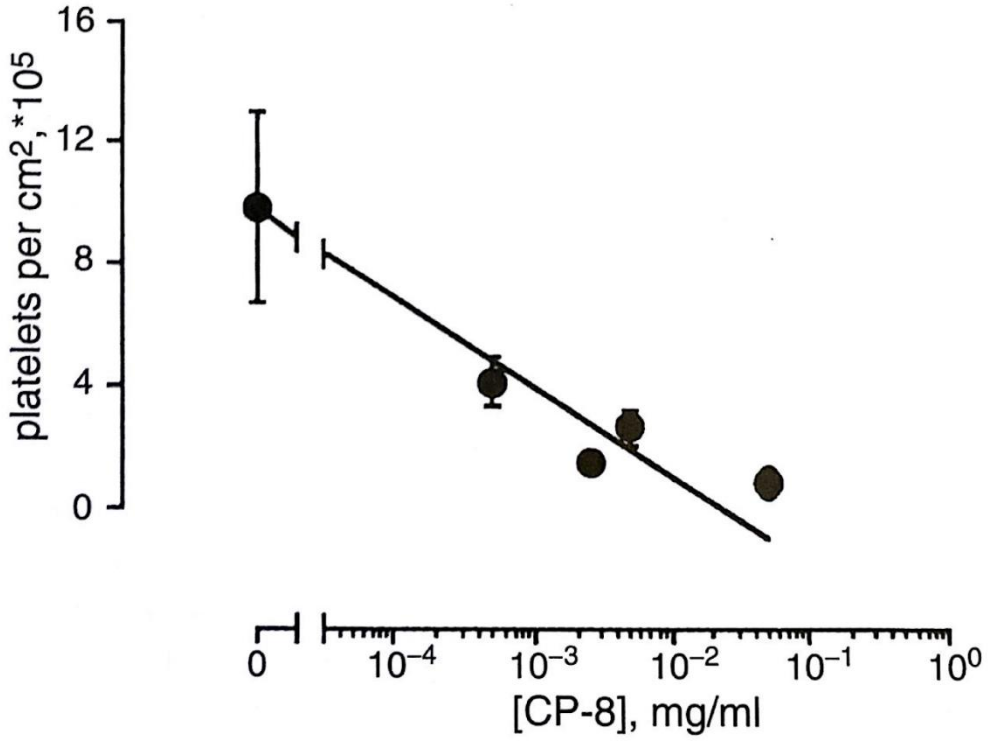
Platelet adhesion to surfaces preadsorbed with plasma that is deficient in fibrinogen is much less than to the same surface preadsorbed with normal plasma. In contrast removal of fibronectin or vitronectin from plasma has little effect on platelet adhesion. It appears that too little of these proteins adsorb from plasma due to competition from other proteins.



The effect of adhesion proteins on cellular adhesion is also demonstrated by inhibiting the integrin sensitivity to adhesive proteins

The recognition of adhesive proteins in blood plasma by platelets is inhibited by addition of an antibody that binds to the receptor, blocking access to the adhesion protein.

Platelet adhesion to surfaces preadsorbed with blood plasma is inhibited by an antibody called anti-glycoprotein CP8 that is fed to platelets in albumin containing buffered saline suspension

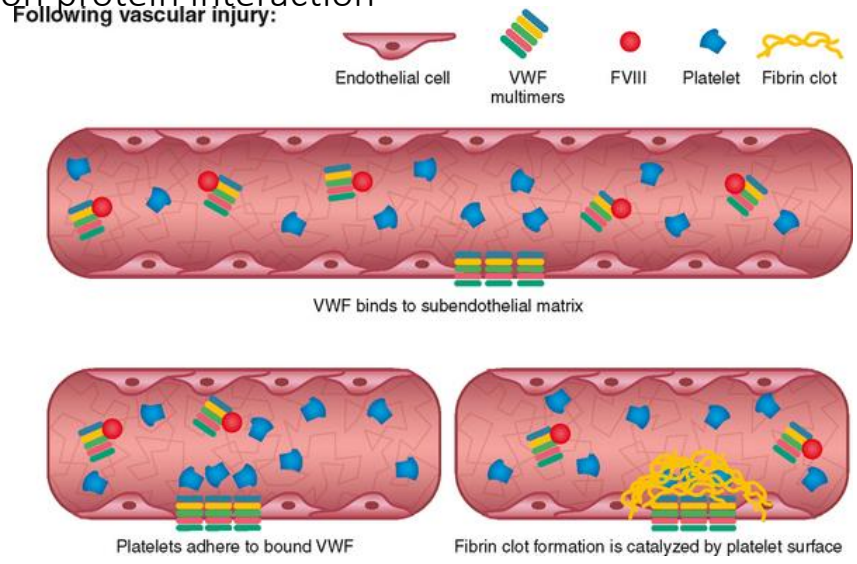


Overall it is concluded that synthetic foreign materials acquire bioreactivity only after first interacting with dissolved proteins.
The principal means by which the transformation from an inert, nonthrombogenic polymer to a biologically active surface takes place is the interaction of the proteins with the surface that mediates cell adhesion

Studies on platelets provide information on why and how adsorbed proteins are influential in cell-biomaterials interactions.

Sensitivity of platelets to adsorbed proteins is because:

- Some proteins in plasma are strongly adhesive for platelets: fibrinogen, fibronectin, vitronectin, von Willebrand factor
- Platelets have two kinds of integrins that bind specifically to a few of the plasma proteins, mediating adhesion
- Concentrating, localizing, immobilizing effects of the adsorbed proteins at the interface modulates the integrin-adhesion protein interaction



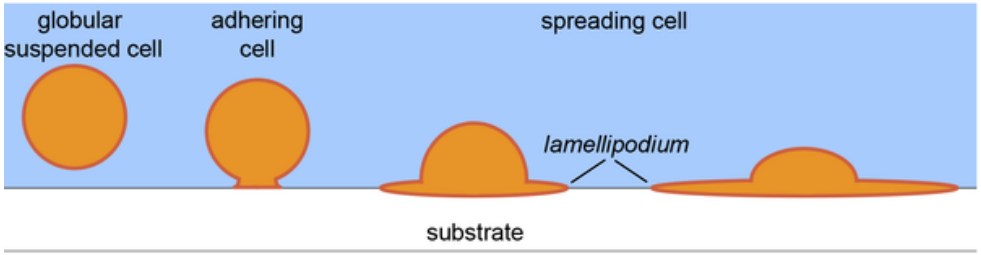
Platelet adhesion is strongly dependent on surface chemistry.

Cells are able to adhere, spread and grow on bare biomaterials surfaces in vitro.
However proteins adsorbed from the adjacent tissue environment or blood, and the proteins secreted by the adherent cells enhance cell attachment, migration and growth.

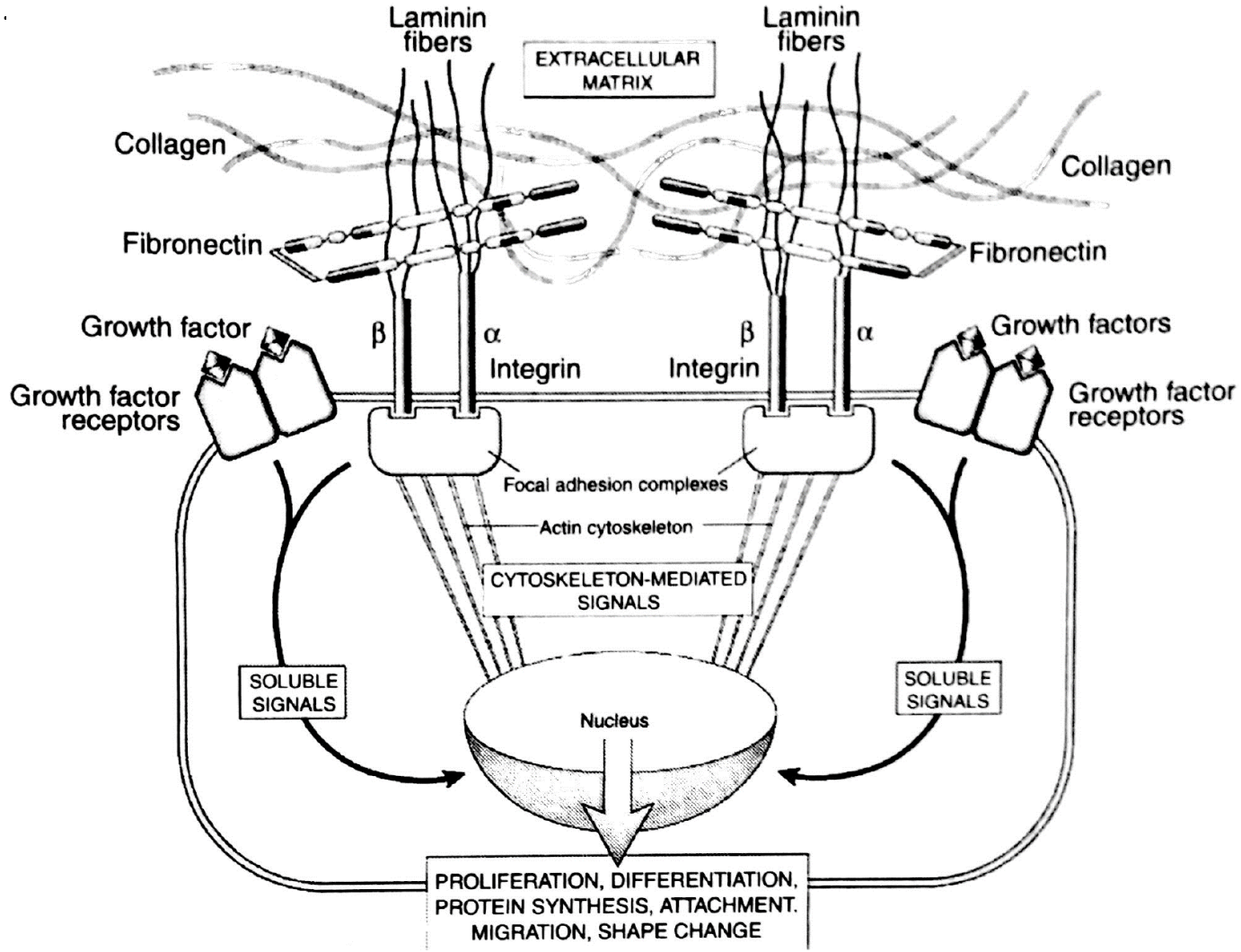
Cell-surface integrin receptors transduce biochemical signals to the nucleus by activating the same intracellular signaling pathways that are used by growth factor receptors.

The more cells spread, the higher their rate of proliferation (growth)

Cell binding to both the biomaterial surface and the extracellular matrices is also critical to cell-growth control through mechanical forces mediated by the changes in cell shape and cytoskeletal tension.



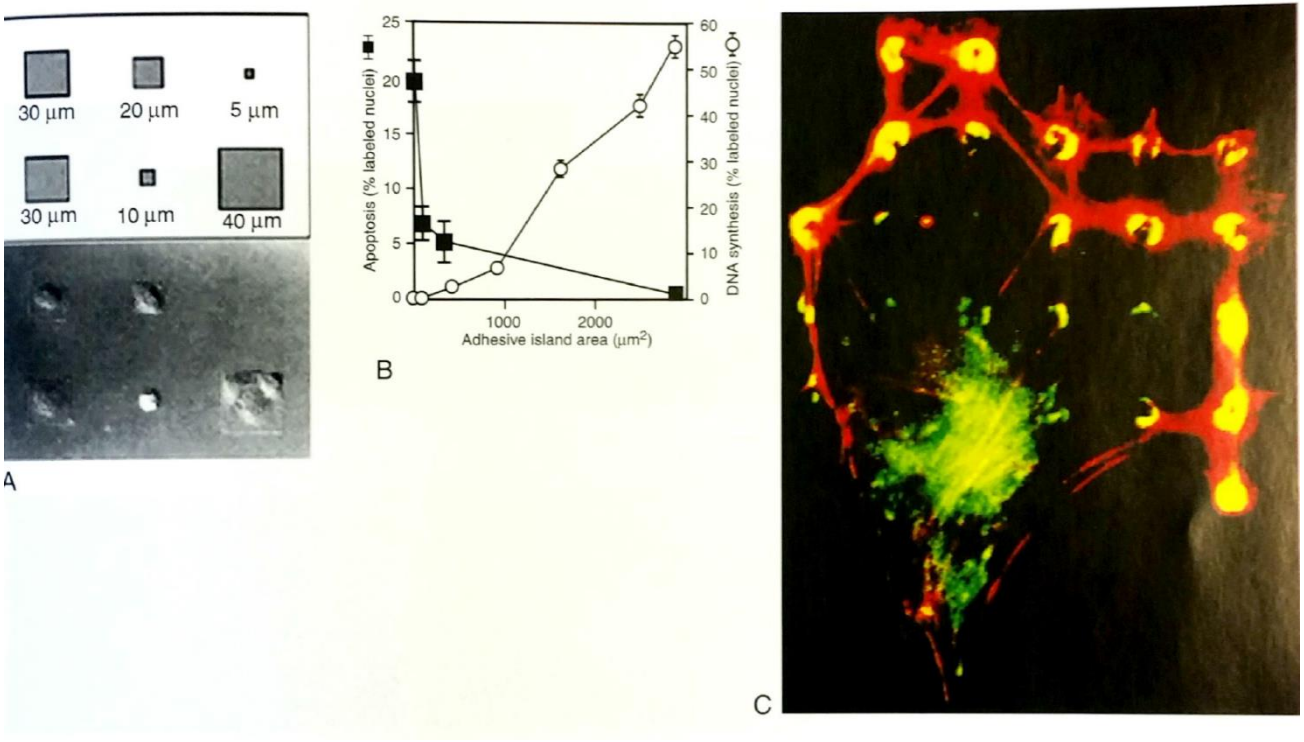
Integrins can bind ECM proteins, other cell surface proteins, plasma proteins and control cell growth, differentiation, gene expression, mobility by transducing biochemical signals to the nucleus upon adhesion.



Cell adhesion to biomaterials is mediated by cytoskeletally associated receptors in the cell membrane. Cell growth is controlled through mechanical forces produced by the cytoskeletal tension and the changes in cell shape.

Most cells require attachment to a solid surface for viability, growth, migration and differentiation. The nature of that attachment is an important regulator of those functions. Rigid substrates promote cell spreading and growth, in contrast flexible scaffolds that cannot resist cytoskeletal forces inhibit cell growth and promote differentiation. The reason is the surface-bound ECM on the substrate, its nature and properties that are affected by adherent cells producing the ECM.

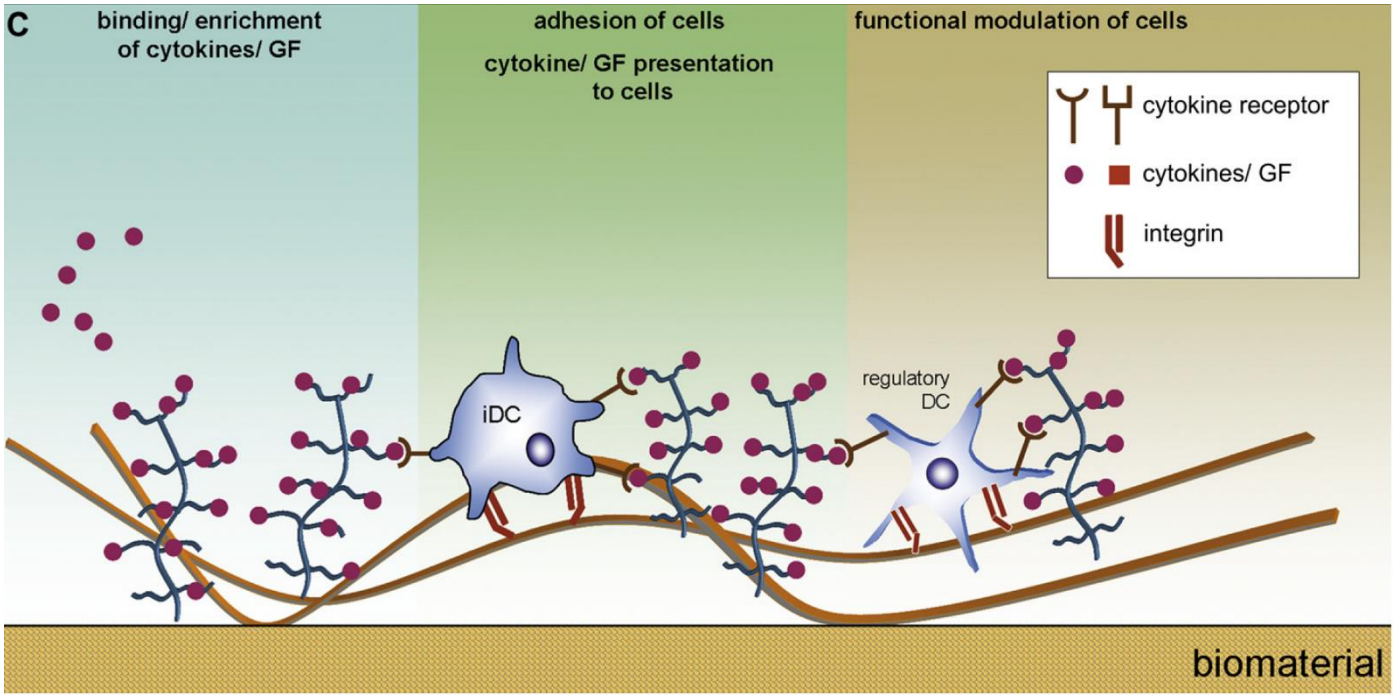
The more cells spread, the higher their rate of proliferation.
The importance of cell spreading on their proliferation is seen in experiments using endothelial cells cultured on microfabricated surfaces containing fibronectin-coated islands of various shapes and sizes.



The ability to proliferate depended directly on the degree to which the cells were allowed to distort physically and not on the actual surface area of substrate bending

Immunomodulation of biomaterials

The binding domains of the cell-cell and cell-ECM interactions can be mimicked by a multifunctional cell-adhesive surface created by specific proteins, peptides, and other biomolecules immobilized on a biomaterial. Proteins containing the amino acid sequence of adhesion proteins can also bind to integrin receptors and are seen to promote adhesion and spreading of endothelial cells.

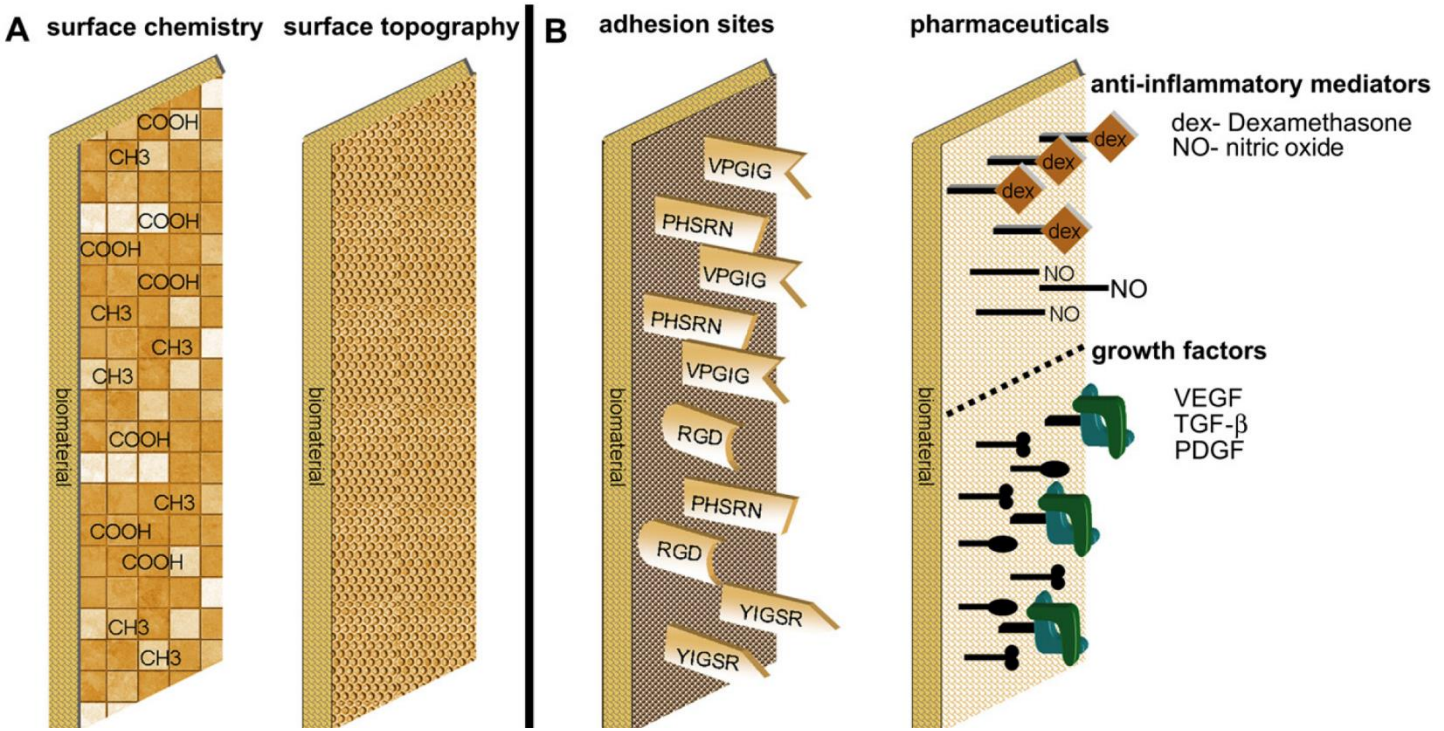


A biomaterial surface can contain specific chemical and structural information that controls tissue formation, in a manner analogous to cell-cell communication and patterning during embryological development.

Chemical and physical modification of surfaces through biomolecule immobilization, patterning and texturing are useful approaches in promoting cell adhesion. Especially covalently immobilized growth factors are thought to modulate wound-healing by directly altering cell response positively.

For example molecular modifications of resorbable polymers drive specific interactions with cell integrins and direct cell proliferation differentiation and ECM production.

Similar tissue engineering approaches to stimulate highly precise reactions with proteins and cells at the molecular level include molecular design of scaffolds that are seeded with cells in vitro for subsequent implantation



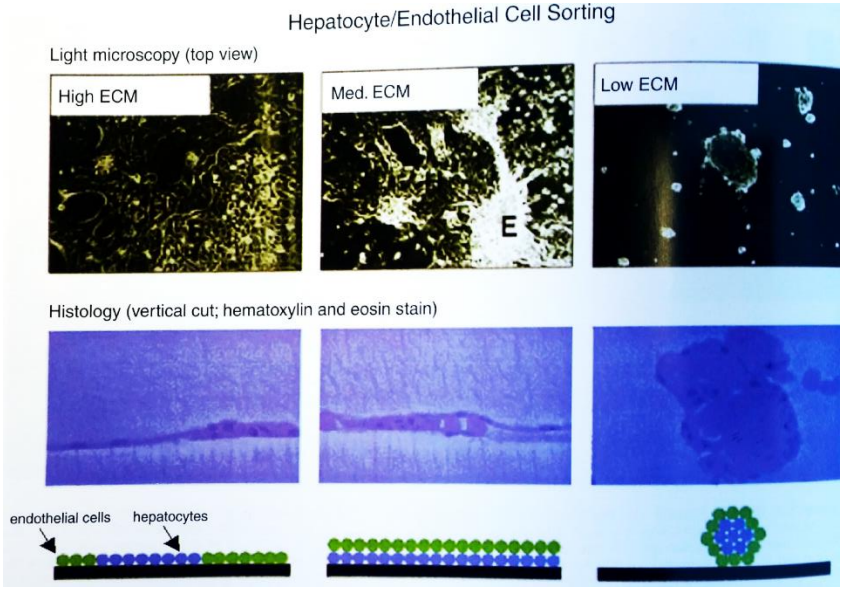
Proteins that induce desirable cell behaviors like adhesion and spreading have been incorporated into biomaterials to control tissue reactions. These protein sequences support the adhesion and spreading of human endothelial cells but not smooth muscle cells, fibroblasts or blood platelets.

Another tissue engineering study area is the use of chemically patterned surfaces to control cell behavior by creating adhesive and non-adhesive regions and chemical gradients.

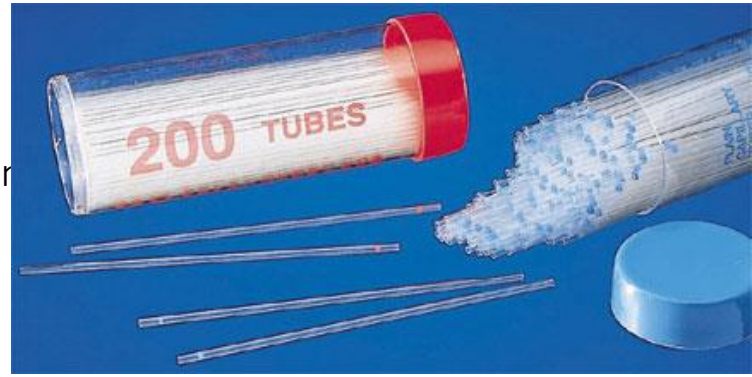
A two dimensional organ can be grown on a tissue engineering scaffold by varying the size and chemistry of the various regions (thereby the type, architecture, directional migration and function of cells)

Thus engineering biological responses is enabled by new technologies for ligand immobilization and micropatterning such as photolithography and self-assembly.

An example is the engineering of hepatic tissue (liver) constructs in which hepatocytes and endothelial cells self-sort to form liver cell plates



In the case of nonclotting vascular grafts, the manipulation of cell-integrin interactions with engineered ligands that prevent platelet cell adhesion, collagen production and promote endothelial cell adhesion on synthetic biomaterials can improve biomaterial function.



Also biomolecules such as enzymes, antibodies, affinity protein cell receptor ligands, and drugs have been chemically or physically immobilized on and within biomaterials.
Living cells may also be combined with biomaterials.

These hybrid combinations of natural and synthetic materials give biological functionality to the synthetic biomaterial.

There is a wide and diverse range of materials and methods available for immobilization of biomolecules and cells on biomaterial surfaces.

The important molecular criteria for successful immobilization of a biomolecule are

- a large fraction of the available biomolecules should be immobilized
- a large fraction of those should retain an acceptable level of bioactivity over an economically and clinically appropriate time period

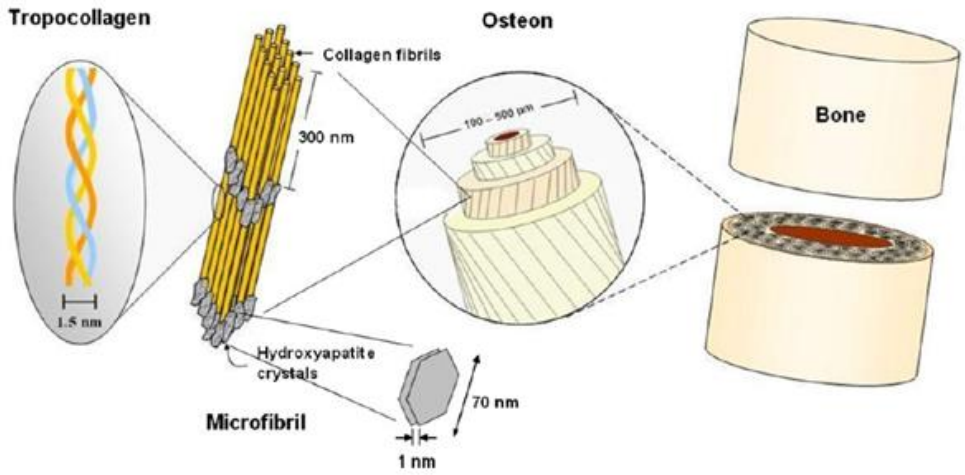
Case study: The behavior of bone cells in a healthy person

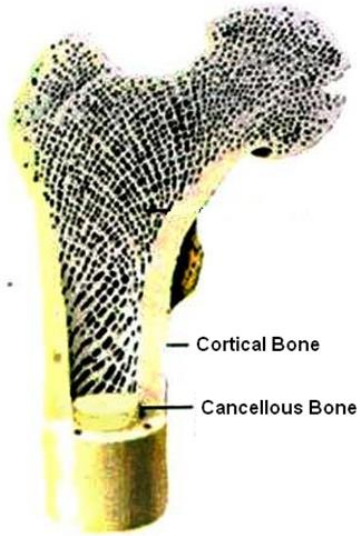
Bone is a composite material having a matrix of a 3D collagen network in which nanosized hydroxyapatite crystals are dispersed.

Bone is a living organ populated by living cells. There is not much porosity in bone structure. The collagen network is covered by layer of tightly bound water and the crystals are tightly packed within the network.

Osteocytes are the main bone cells which help maintenance of the active bone tissue. They dissipate energy by aerobic glycolysis. They need oxygen and glucose and produce carbondioxide and water.

Bone also contains some glycoproteins which contain loosely bound water through which the oxygen and the nutrients can diffuse to the osteocytes and carbondioxide resulting from cell metabolism can be disposed.



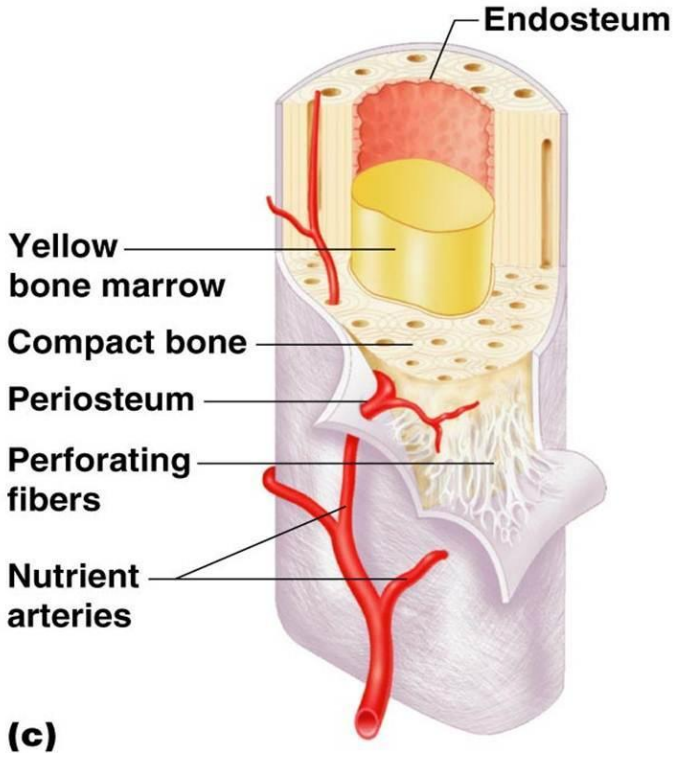


Normally metabolism of bone tissue is different at day and night time.

The metabolism is not so fast in the situation of night rest, and the transport by diffusion and through arteries is sufficient.
The situation of the bone extracellular fluid is at a high pH and apatitic crystals remain stable

During the day the metabolism is elevated and the bone s loaded. Part of the loosely bound water is squeezed out of bone by compression and it is sucked in again from the envelopes of the periosteum and endosteum upon unloading

This transport by flow is strongest where the highest stresses occur in the bone and the enhanced metabolism of osteocytes under stress is maintained by the flow. (carbondioixde removal and oxygen supply)

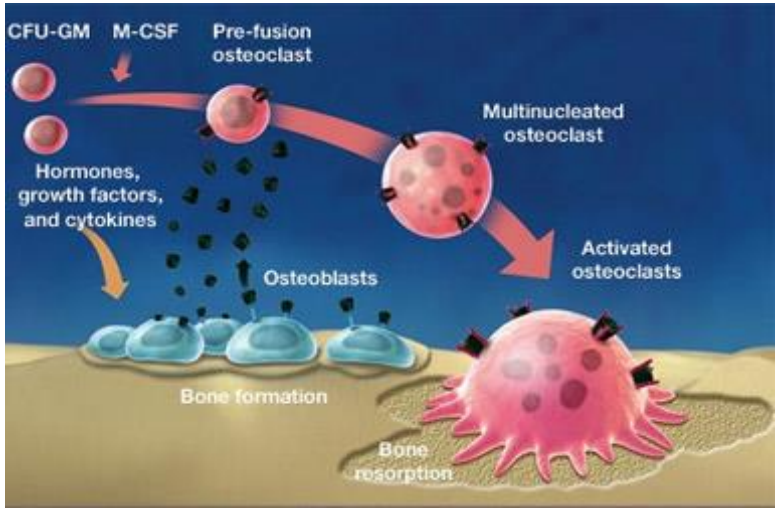


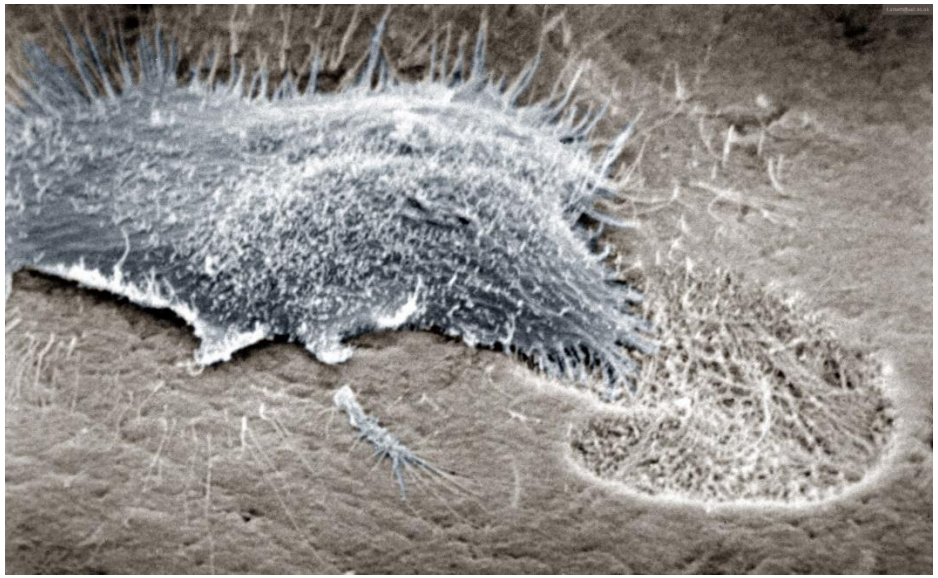
As people age, their bones age by hydrolysis of the glycoproteins. As these constituents degrade with time, the bone loses its loosely bound water so that diffusion as well as transport by flow is retarded.

Carbondioxide removal is lagged and the pH in the local bone extracellular fluid decreases. As a consequence some apatites that are highly soluble under acidic conditions dissolve and the bone loses compactness and strength.

The retardation of metabolism can go so far that necrosis of the osteocytes happens. At that situation the pH of the bone extracellular fluid restores due to lower metabolism and the toxins of the dead cells destroy the local collagen network where newly formed nanoapatitic crystals deposit.

The toxins of the dead osteocytes trigger the activity of osteoclasts. These cells are responsible for cleaning up of the bone tissue and dissolution of damaged crystals. They dissipate energy by anaerobic glycolysis which produces lactic acid instead of carbondioxide.





Osteoclast activity around the dead osteocytes increases the excretion of lactic acid and the local pH goes down to dissolve the bone mineral.

As a consequence an osteoclast can dig a tunnel through the bone structure and remove the newly formed nanoapatite crystals around dead osteocytes.

An osteoclast works in tandem with an osteoblast which is responsible for the build up of the collagen network. During the night it restores the collagen network in the tunnel behind the osteoclast.

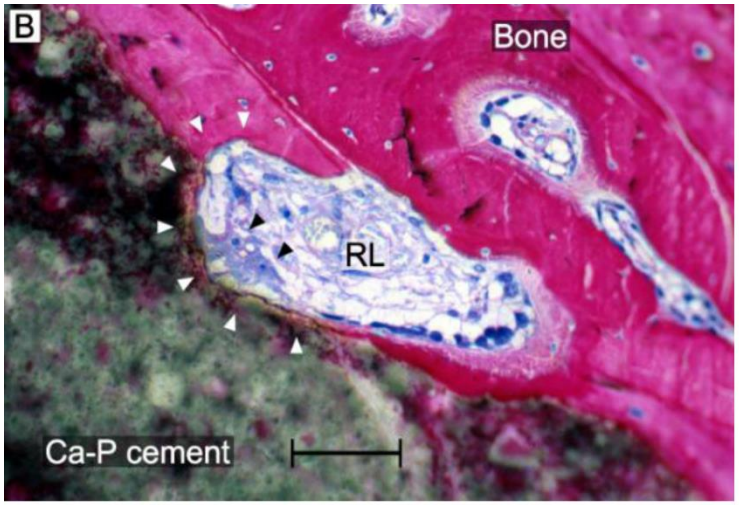


Figure 4: Remodeling lacuna (RL) with the cutting cone headed towards the cement. Osteoclast-like cells (black arrowheads) resorb bone and Ca-P cement (white arrowheads). Original magnification 40x

At night time osteoblast also collects potassium, calcium and phosphate ions from the local loosely bound water. It swells with water and ions and forms dendrites which fill the whole tunnel space and the newly formed collagen network.

With the new active day loading starts and the dendrites of the osteoblast are squeezed to let the cytosol containing ions out. The local supersaturation of the bone extracellular fluid in apatites causes instantaneous precipitation of apatite nanocrystals.

During the daytime mechanical activity, the tandem of osteoclast and osteoblast move further through bone in a new tunnel and the metabolism around newly formed bone mineral slows down.
Local pH gradually increases as a result and hydroxyapatite which is the most stable calcium phosphate at high pH forms in the new collagen network by the end of the day.

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Table 2. Calcium phosphates and the pH range in which they precipitate homogeneously.

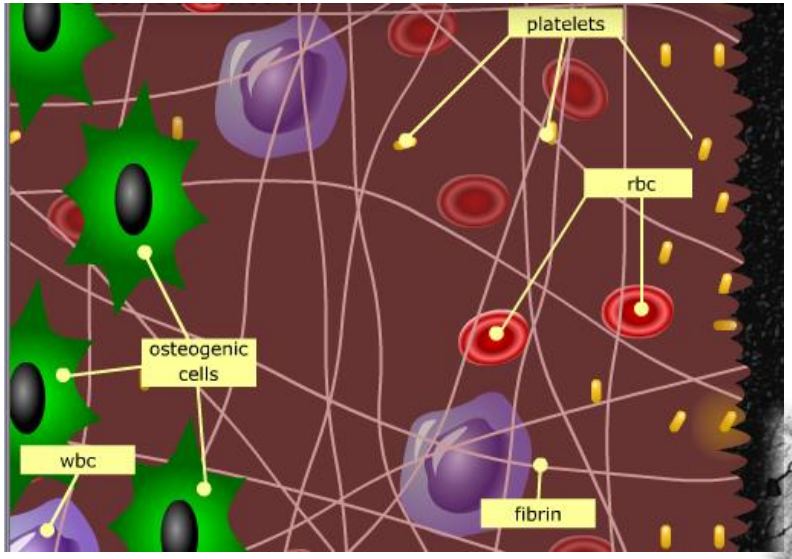
Abbreviation	Precipitate	pH range
MCPM	$Ca(H_2PO_4)_2.H_2O$	0.5 < pH <2
DCPD	$CaHPO_4.2H_2O$	3 < pH <5
KCA	$Ca_5K_{4-x}Na_x(HPO_4)_4(PO_4)_2(H_2O)$	5.5 < pH <7
CDHA	$Ca_9(HPO_4)(PO_4)_5OH$	7.5 < pH <9
HA	$Ca_{10}(PO_4)_6(OH)_2$	10 < pH <12

Slow growth of apatite crystals with gradual increase in pH with time packs the collagen network so that the bone can carry the full load.

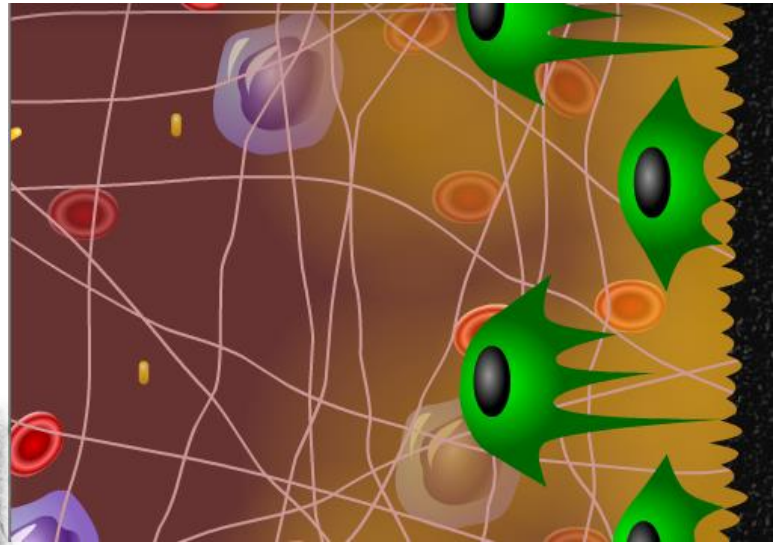
At the end of bone remodeling the glycoproteins are restored and the bone contains enough loosely bound water again for the transport of nutrients and removal of wastes.

Bone Regeneration around an implant

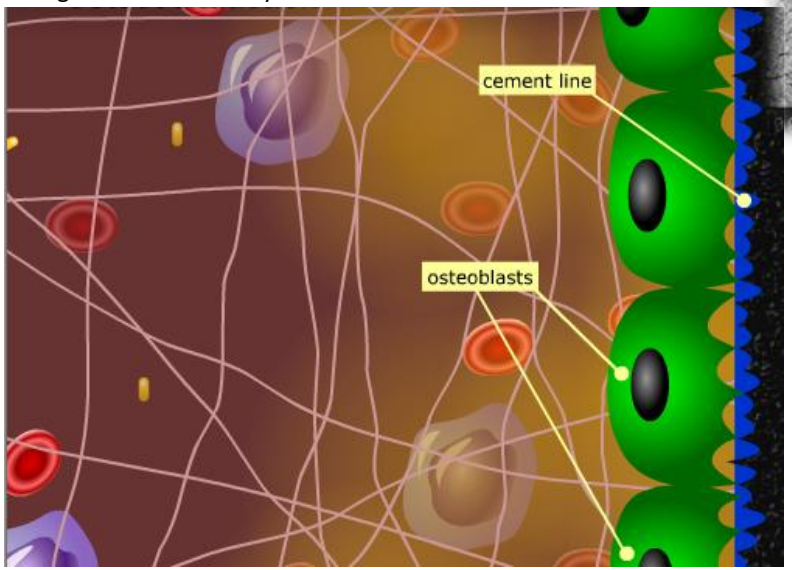
Stage 1. Non-collagenous bone protein adsorption on the surface

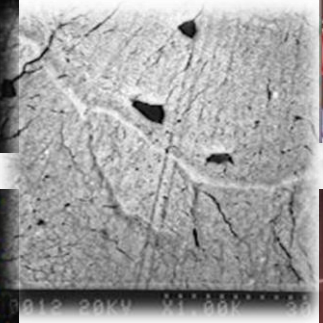


Stage 2. Migration of osteogenic cells to the surface



Stage 3. Cement line synthesis on the surface





Cement line- A hypermineralized matrix interlocked to the submicron topography presented by the surface (Davies et al. 2003)

Stage 4. Collagenous extracellular matrix formation