MME 4506 Biomaterials

Biochemical mechanisms of cellular and tissue response to external stimuli

Physiologic functions in mammals are performed by a hierarchical biological structure comprised of organs, tissues, cells, organelles and proteins

Proteins are the building blocks of all organisms

Organelles that synthesize proteins compartmentalize the specific functions of cells

Over 100 specific cell types function in the body

Tissues are formed by cell-to-cell junctions and consist of 4 types: epithelium, connective, muscle and nerve

Organs are assembled from these basic tissues that are glued together by an extracellular matrix which is synthesized by cells

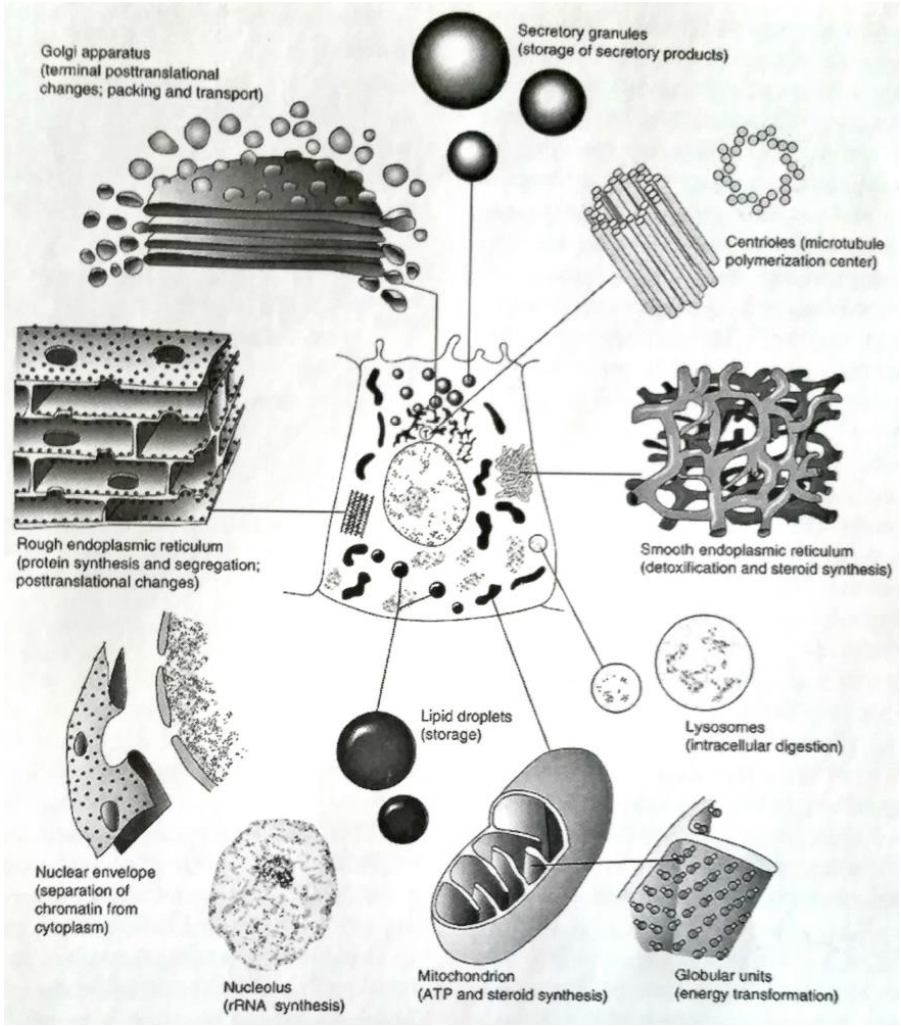
Cells may be viewed as independent collections of self-replicating enzymes and structural proteins that carry out certain general functions including

- Self-replication
- Protection from the environment
- Acquisition of nutrients
- Movement
- Communication
- Catabolism of extrinsic molecules
- Degradation and renewal of intrinsic molecules
- Energy generation

Intracellular organelles exist in a medium containing water, ions, sugars, small-molecular-weight molecules which is called the cytoplasm.

Cell is a structurally highly ordered and functionally integrated assembly of organelles, cytoskeletal elements and enzymes.

By isolating certain cellular functions within distinct compartments, potentially injurious degradative enzymes or toxic metabolites can be kept at useful high concentrations locally without causing damage to other intracellular constituents.



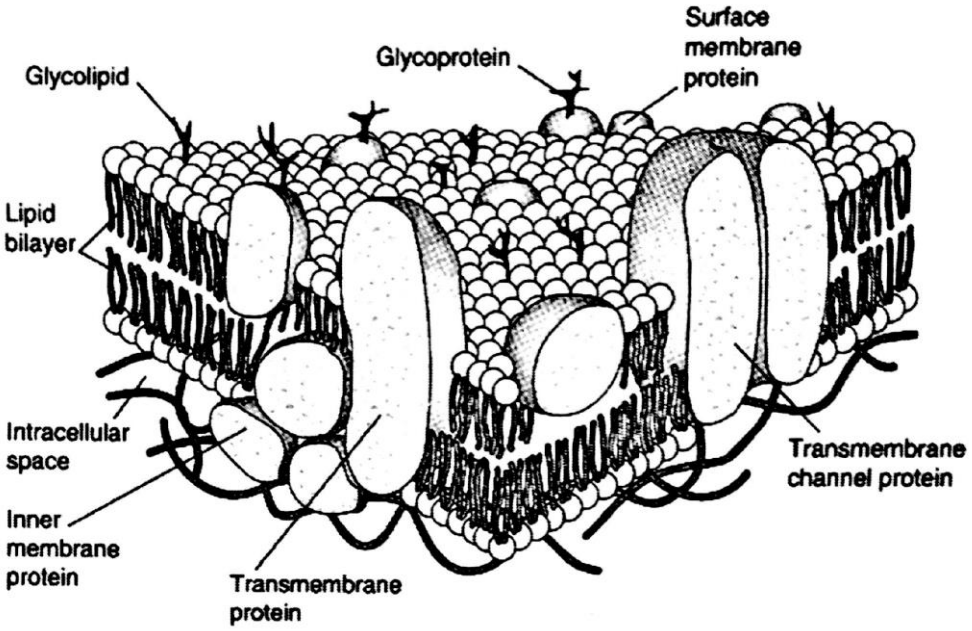
Unique intracellular environments containing low pH, high calcium or high concentrations of an enzyme permit more efficient functioning of chemical processes and enzymes

The plasma membrane protects the cytoplasm from the environment and permits the cell to maintain cytoplasmic constituents at concentrations different from those in the surrounding environment

The plasma membrane is freely permeable to water and impermeant to charged and polar molecules because of its hydrophobic inner core. It is rendered selectively permeable to incoming or outgoing ions, amino acids, etc. by channel proteins inserted through it. Most nutrient acquisition is thereby accomplished by the movement of desired materials through pores or by energy-driven transport.

The plasma membrane is also capable of folding in around external materials and ingesting them (phagocytosis)

It may also express a variety of specific surface molecules that facilitate interactions with other cells, ligands and the extracellular matrix



In addition to basic functional attributes of cells, most cells also exhibit specialization capabilities

Multicellular organisms are composed of individual cells with different specialization of structure and function

These differentiated cells allow a sharing of the performance and coordination of complex functions in architecturally distinct, organized tissues

- For example, striated muscle cells have well organized actin and myosin filaments that slide over each other, enabling cellular contraction.

- Gastric epithelial cells have large numbers of mitochondria to generate the ATP necessary to pump hydrogen ions and acidify the stomach contents

- Skin keratinocytes function as a protective barrier by losing their organelles and becoming scale-like structures filled with durable keratin (a filament)

The structural and functional changes that occur during cellular differentiation are usually irreversible

Increasing specialization results in a loss of cell potentiality and a loss in capacity for cell division

- Nerve and heart muscle cells are highly specialized cells that can neither differentiate nor replicate

Cells constantly adjust their structure and function in response to chemical and mechanical alterations in their environments.

The adaptive responses of cells to achieve a new steady state and preserve viability are
Hypertrophy – increase in size of individual cells
Hyperplasia – increase in cell number
Atrophy – decrease in size
Metaplasia – transformation from one cell type to another
Neoplasia – abnormal tissue growth even after removal of the stimulus

Cell injury develops if the extracellular stresses persist and a cell's adaptive capacity is exceeded. Injury is reversible up to a point and the cell returns to its baseline state (mild heat injury)

The injury is irreversible and leads to cell necrosis if

- The mitochondrial dysfunction can not be reversed (lack of ATP generation)
- Membrane function is strongly disturbed so that the cell cannot keep the concentration differences between the inside and extracellular matrix

Most injury alters the ability of cells to generate energy required to maintain basic cellular processes.

- For example the heart muscle cells adapt to persistent increased loads (due to high blood pressure) by enlargement to compensate for the higher pressures. Heart cells undergo atrophy in long periods of starvation
- The same cells may be reversibly injured if the energy demand is not supplied due to a partially blocked coronary artery. They may undergo irreversible injury and death following complete blocking of artery.

Cell injury occurs under certain conditions resulting from environmental stimuli including:

Hypoxia and ischemia – Decreased oxygen supply due to diminished blood flow

Chemical agents including food components, toxins, hormones, neurotransmitters, synthetic drugs, environmental pollutants, poisons, ethanol, tobacco and toxic biomaterials induce cell injury by

- Combining directly with a critical molecular component or cellular organelle to inhibit its normal activity
- Converting inactive chemicals to toxic metabolites during normal physiologic breakdown by enzymes. The most important mechanism of cell is injury is by formation of free radicals

Infectious biologic agents including viruses, bacteria, fungi, etc. invade a specific cell or tissue type. Also the elimination of infections during healing process can cause cell injury

Physical injury including direct mechanical force, heat, electric shock or ionizing radiation

Genetic defects like mutations in a variety of cellular proteins can lead to cellular dysfunction and injury

There are two basic mechanisms of cell injury due to any cause:

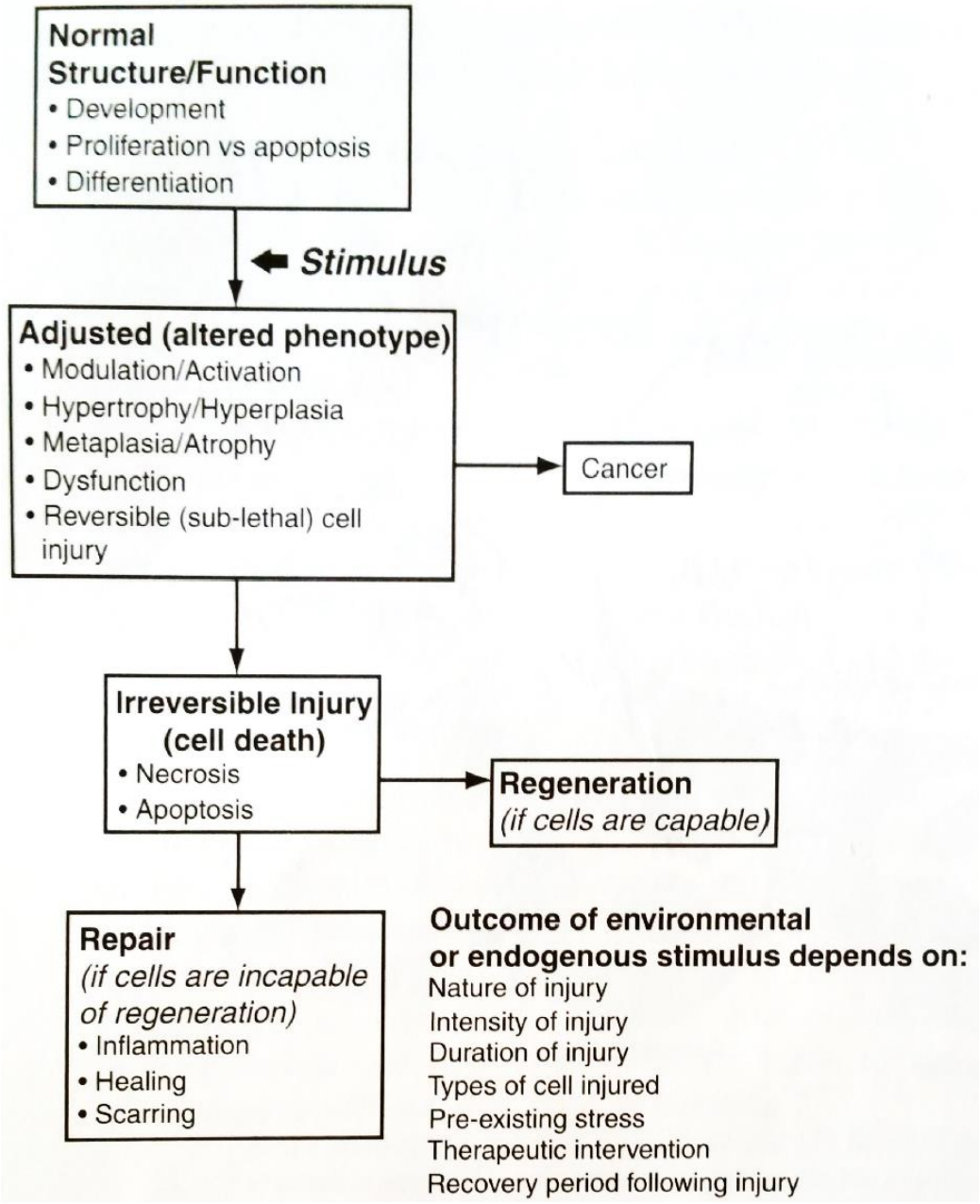
1. Lack of oxygen and oxygen derived free radicals

Free radicals are chemical species with a single unpaired electron in an outer orbital. They are extremely unstable and readily interact with inorganic or organic chemicals. They initiate autocatalytic reactions: molecules that react with them are converted to them, damaging the cell in a chain reaction. The most important ones are OH-, O_2-, NO-. Free radicals formation and cell damage is the injury mechanism of many processes including chemical injury, radiation injury, gaseous toxicity, cellular aging, microbial killing by phagocytic cells, inflammatory damage, tumor destruction by macrophages.

2. Failure of maintenance of intracellular ion concentrations

Cytoplasmic free calcium is an important activator of destructive enzymes. Its concentration is normally kept at extremely low concentrations around 0.1 µM by active calcium transporters. This is enabled by storing calcium in mitochondria and endoplasmic reticulum, and inhibiting diffusion of calcium in the extracellular matrix that is at a concentration around 1300 µM. Ischemic or toxin induced injuries allow a net influx of extracellular calcium across the plasma memebrane followed by release of ions from the inside stores.

Cell injury mechanisms and outcomes



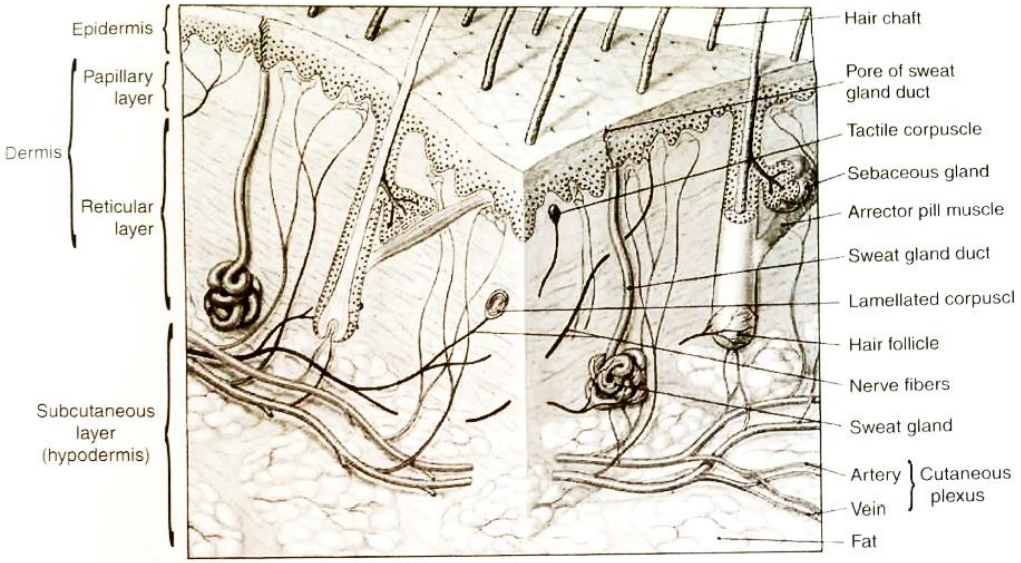
Tissues are composed of three basic components: cells, extracellular matrix, body fluids

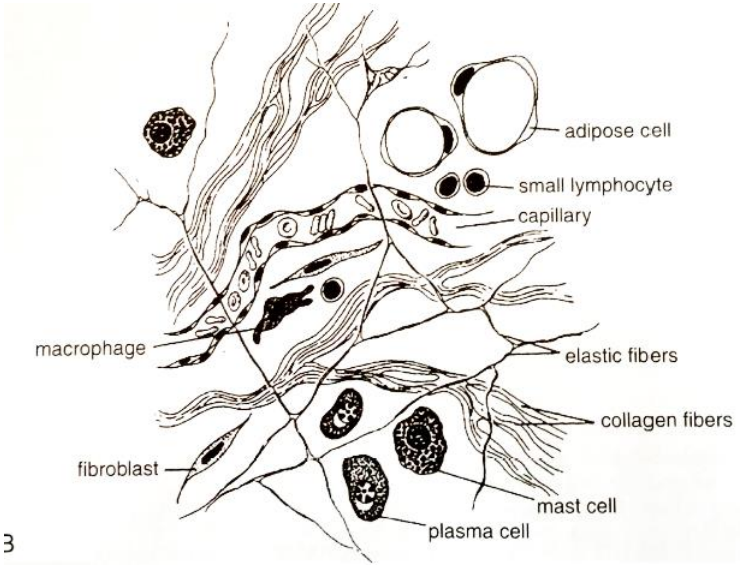
Cells are surrounded by body fluids in which the nutrients and waste molecules diffuse in and out.

Tissue fluid, a clear watery liquid is secreted by the outer surface of capillaries that transport molecules with blood. İntercellular substances between the capillaries and the cells diffuse into the tissue fluid.

As more tissue fluid is produced that can be absorbed back into the capillaries, the excess is carried away as lymph and ultimately mix into the bloodstream.

Most tissues have a rich vascular network that enable perfusion. Other than facilitating exchange of substances between the blood and the tissues, the circulatory system functions to regulate body temperature, to distribute various regulatory substances like hormones, and to distribute immune and inflammatory cells to their sites of action.





Large diameter blood vessels are effective in delivering blood while capillaries are most effective in diffusional transport to and from the surrounding tissues

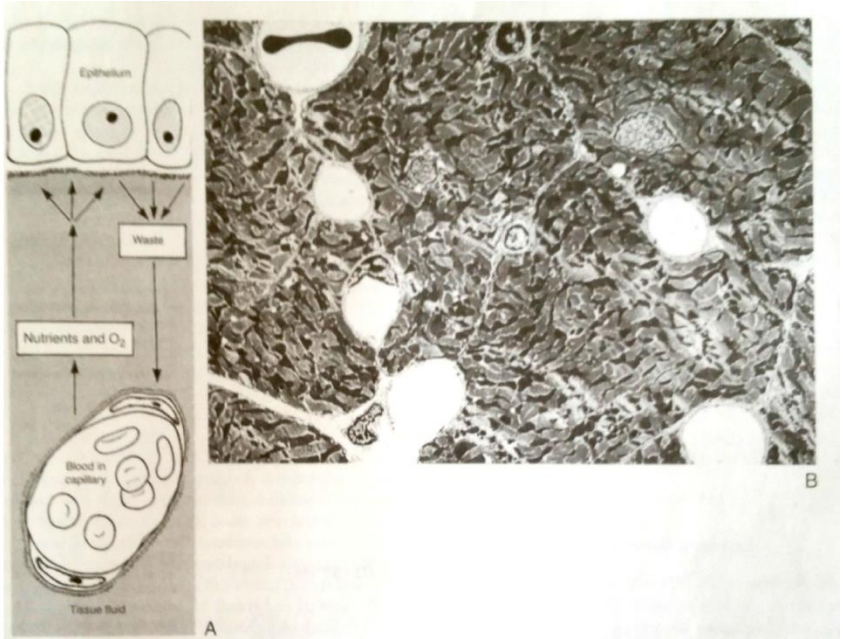
The velocity of blood falls to about 0.1 cm/s in capillaries from 50 cm/s in aorta

In addition very thin walls of capillaries facilitate most exchange of nutrients and waste molecules

Cells are generally located not more than 200 µm from the capillaries, the average diffusion limit of oxygen in highly metabolic tissues.

Thus 3-dimensional tissue formation and growth requires the formation of new blood vessels, a process called angiogenesis

Extracellular matrix, cells and capillaries are physically integrated in functional tissues



The extracellular matrix holds cells together by providing physical support and a matrix to which cells can adhere, signal each other and interact

Adhesive interactions coordinate communication with cell surface receptors, cell cytoskeleton and the nucleus. The resulting intracellular signaling affects a variety of events including gene expression, cell proliferation, mobility and differentiation

ECM consists of large molecules synthesized by cells, exported to the intercellular space and linked together into a structurally supportive composite. The components are collagen or elastic fibers and an amorphous matrix consisting of proteoglycans, solutes and water

Its principal functions are

- Mechanical support for cell anchorage
- Determination of cell orientation
- Control of cell growth and differentiation
- Scaffolding for orderly tissue renewal
- Storage and presentation of soluble regulatory molecules

ECM components and the mechanical forces that cells experience influence the cell functions, shape, polarity through receptors (integrin) for specific ECM molecules (fibronectin). The resultant changes in cytoskeletal organization and intracellular signaling can modify cytodifferentitation. The role of ECM in organogenesis and repair after injury as a scaffold is critical.

Some ECMs are specialized for a particular function such as
Strength as in tendons, bone and teeth
Filtration as in kidney membranes
Adhesion as in membranes supporting epithelia

The ECM is constantly turned over and remodeled in most tissues. Matrix turnover is generally low in normal mature tissues. Rapid and extensive destruction occurs during various adaptive and pathologic states including wound repair, tumor cell invasion and embryonic tissue morphogenesis.

It resembles a fiber-reinforced composite consisting of large interlinked molecules that hold a glyconprotein-water gel in dynamic equilibrium.

The key components of ECM are fibrillary proteins collagen and elastin, amorphous matrix components glycoproteins and adhesive proteins such as fibronectin and laminin

Collagen is a cross-linked protein fibre that forms a tough and flexible matrix in three dimensions

Fibronectin is an adhesive molecule in the ECM that can bind to collagen, heparin, fibrin and cells.

Fibronectin-rich pathways guide and promote the migration of many kinds of cells during embryonic development and wound healing

Elastin is a derivative of collagen that can be stretched. It gives passive recoil to various tissues like heart valves, intervertebral disks and artery walls

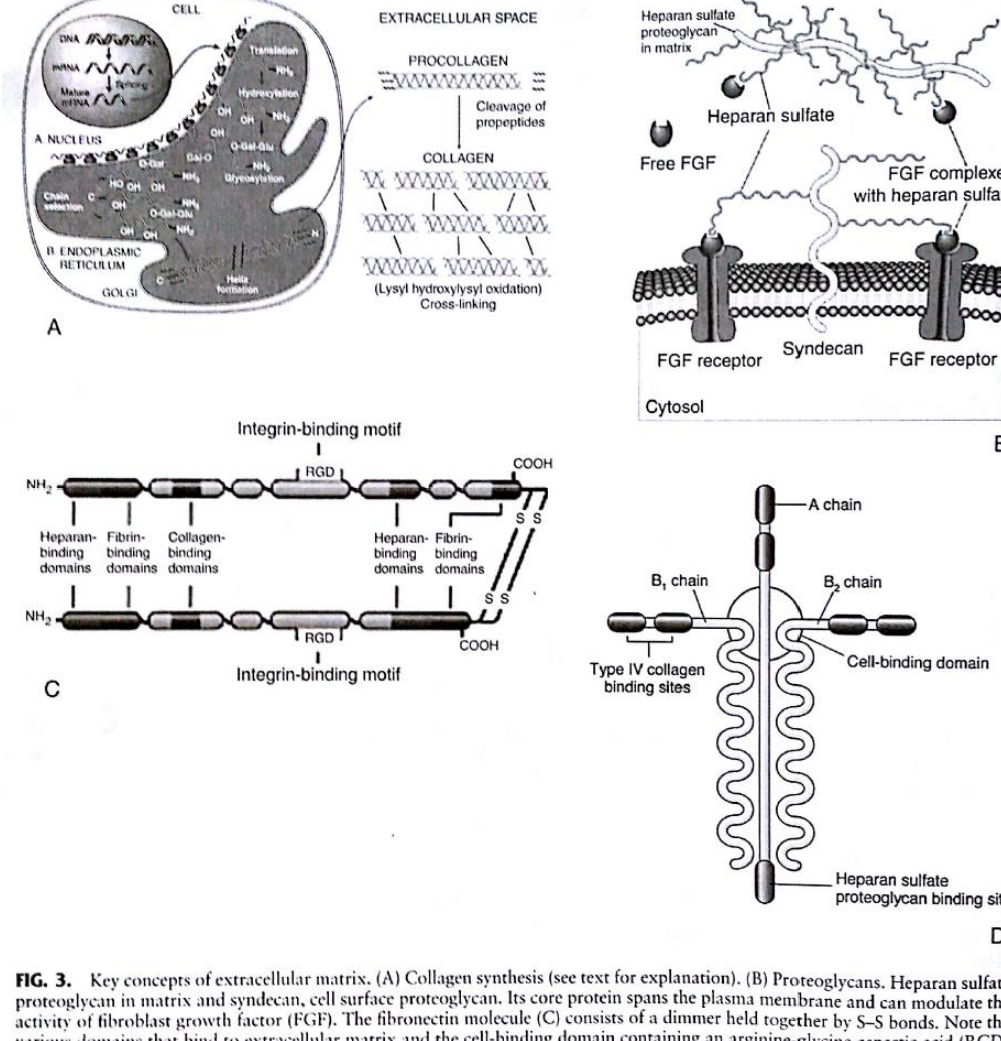


FIG. 3. Key concepts of extracellular matrix. (A) Collagen synthesis (see text for explanation). (B) Proteoglycans. Heparan sulfat proteoglycan in matrix and syndecan, cell surface proteoglycan. Its core protein spans the plasma membrane and can modulate th activity of fibroblast growth factor (FGF). The fibronectin molecule (C) consists of a dimmer held together by S–S bonds. Note th various domains that bind to extracellular matrix and the cell-binding domain containing an arginine-glycine-aspartic acid (RGD sequence. The cross-shaped laminin (D) molecule spans basement membranes and has extracellular matrix (ECM)- and cell-bindin

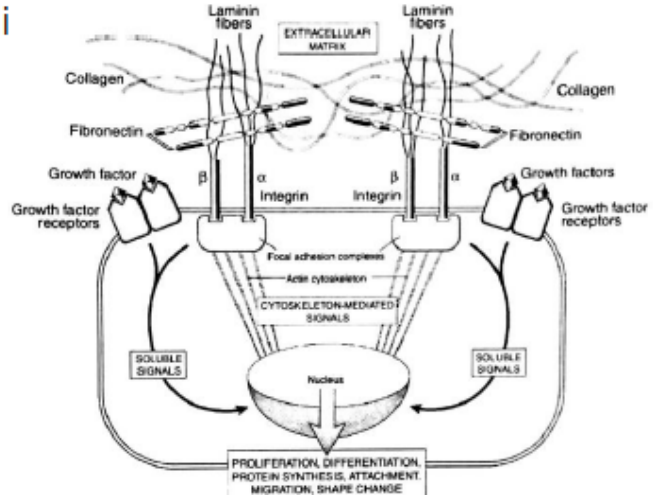
Cells and the ECM interact through specific mechanisms:

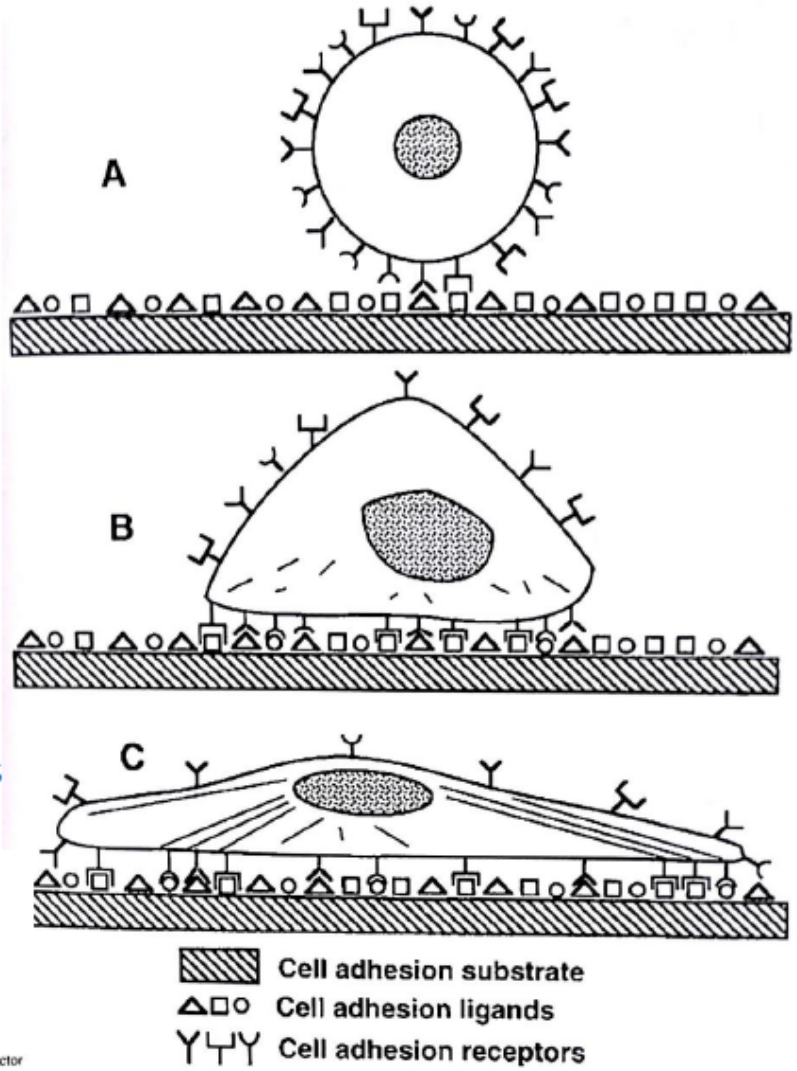
- Initial recognition
- Physical adhesion
- Electrical and chemical communication
- Cytoskeletal reorganization
- Cell migration

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

Integrins can bind ECM proteins, other cell surface proteins plasma proteins and control cell growth, differentiation, gene expression, mobili



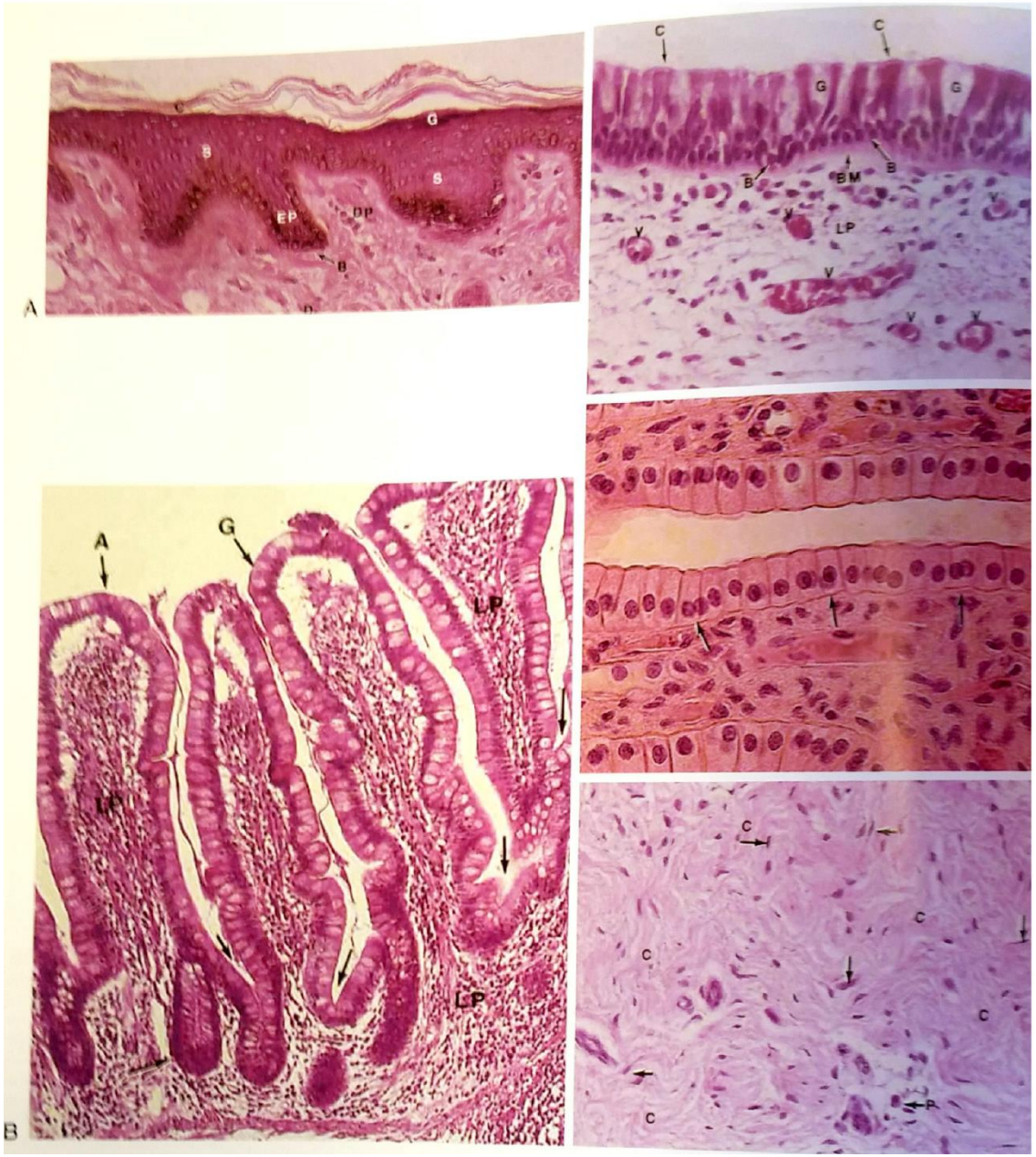


Cells held together by the ECM and fed by capillaries form tissues with distinctly different organization and functions:

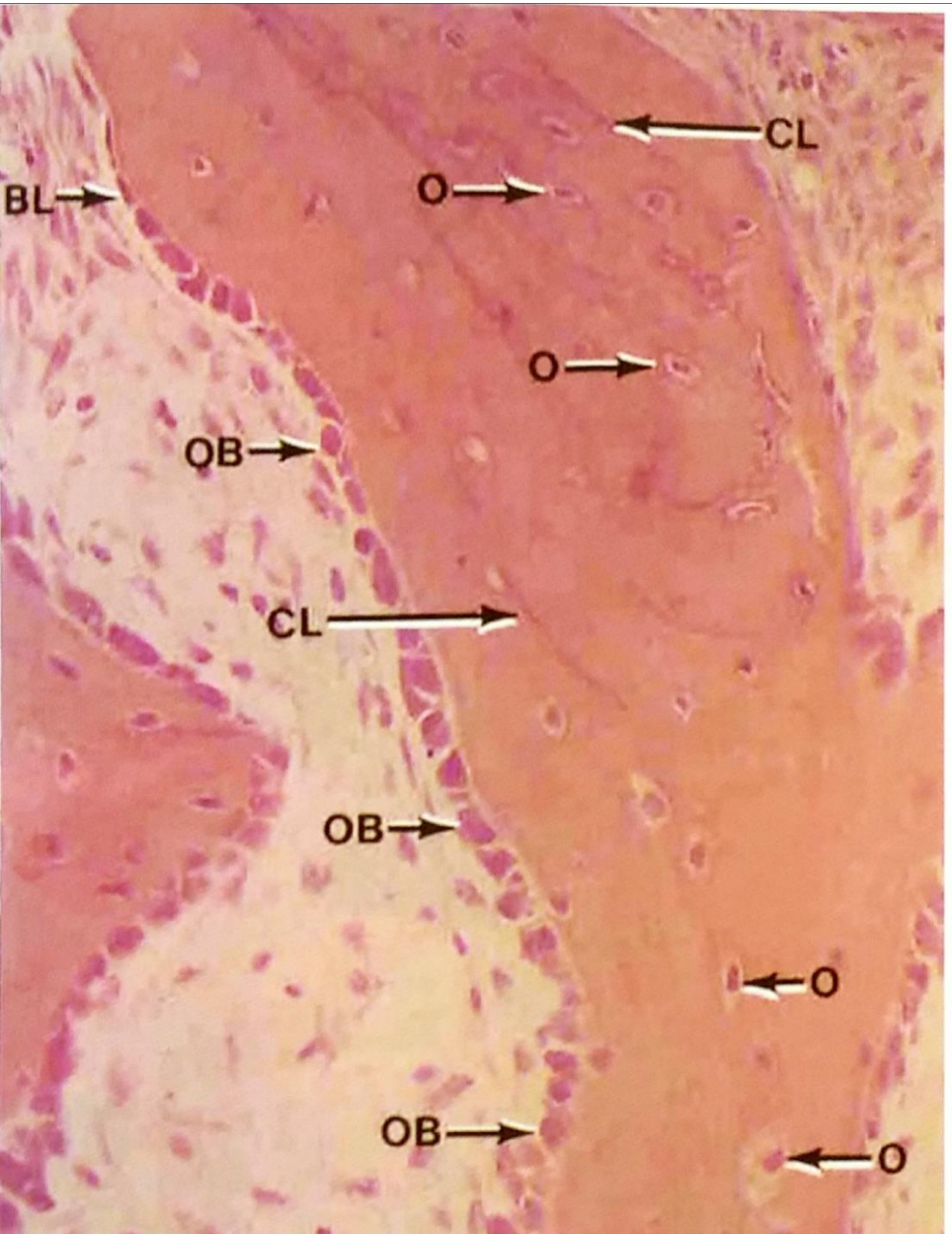
Epithelial tissue		
	Surface	Skin epidermis, intestine mucosa
	Glandular	Thyroid follicles
	Special	Retinal epithelium
Connective tissue		
	Loose connective tissue	Skin dermis
	Dense connective tissue	Tendon
	Special connective tissue	Fat
	Blood tissue	Bone marrow, blood cells
	Supportive tissue	Cartilage, bone
Muscle tissue		
	Smooth	Arterial or intestine smooth muscle
	Skeletal	Arm, leg musculature
	Cardiac muscle	Heart
Nerve tissue		Brain cells, peripheral nerve

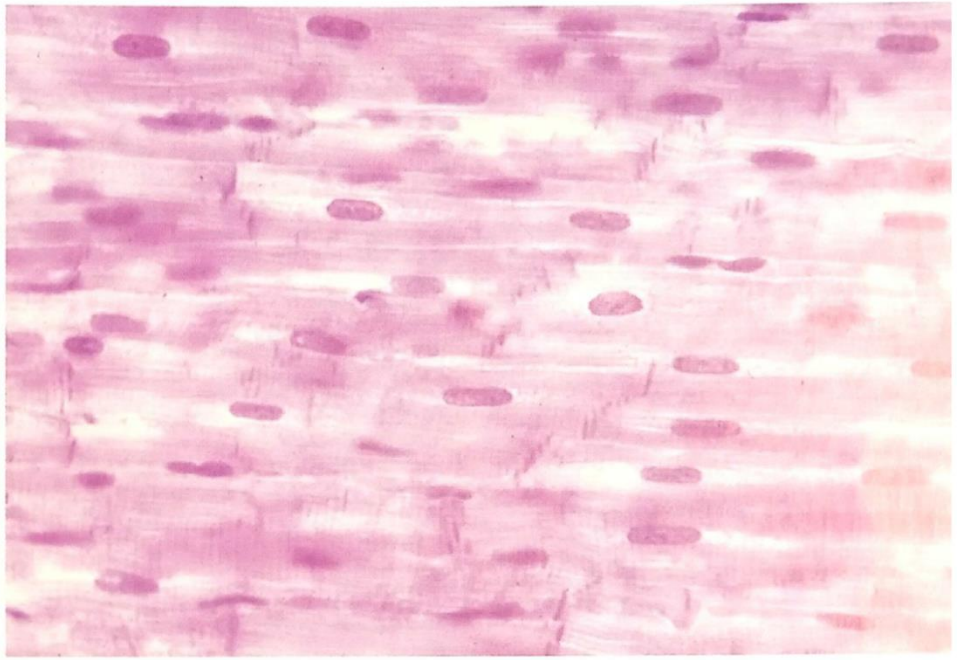
These 4 basic tissues have specific functions and distinctive microscopic appearances

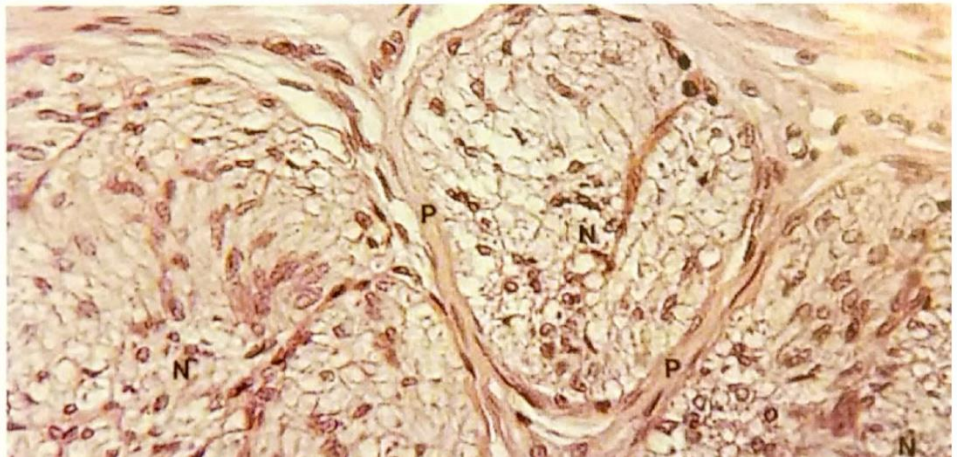
Histology of various tissues



Histology of various tissues







Several different types of tissues combine into a functional unit, organ.

Organs have a composite structure in which epithelial cells typically perform the specialized work of the organ, while connective tissue and blood vessels support and maintain operation of epithelium.

There are two basic organ types: tubular and compact

1. Tubular organs include the blood vessels, the digestive, urinary and respiratory tracts

Each is composed of an inner coat consisting of a lining of epithelium, a middle coat consisting of layers of muscle and connective tissue, and an external coat consisting of connective tissue covered by epithelium.

2. Compact organs have an extensive connective tissue framework, surrounded by a dense connective tissue capsule. The thicker tissue supports the blood vessels and lung channels.

The dominant cells in specialized tissues are called the parenchyma or organ cells.
Parenchymal cells are generally more sensitive to chemical, physical or ischemic injury than the surrounding connective tissue.

Orderly repair and regrowth of parenchymal cells requires an underlying connective tissue as a scaffold.

Cell and tissue injury is followed by inflammation and repair which may take 4-6 weeks

Inflammation is a protective response that eliminates the cause of the injury (diluting, isolating or destroying microbes or toxins), and disposes of both the necrotic cells and tissues that occur as a result of the injury.

It initiates the healing and tissue regeneration process

The immediate and early response to injury, of relatively short duration is characterized by secretion of fluid and plasma protein into the tissue and by accumulation of destructive cells

In the second phase of inflammation that takes a much longer time, lymphocytes and macrophages destroy damaged tissue and lead to repair involving fibrosis and new blood vessel proliferation.

A special type of inflammation involving activated macrophages and multinucleated giant cells is called granulomatous inflammation. It occurs when the injury cause is not removable as in implantation of a biomaterial by a foreign body reaction.

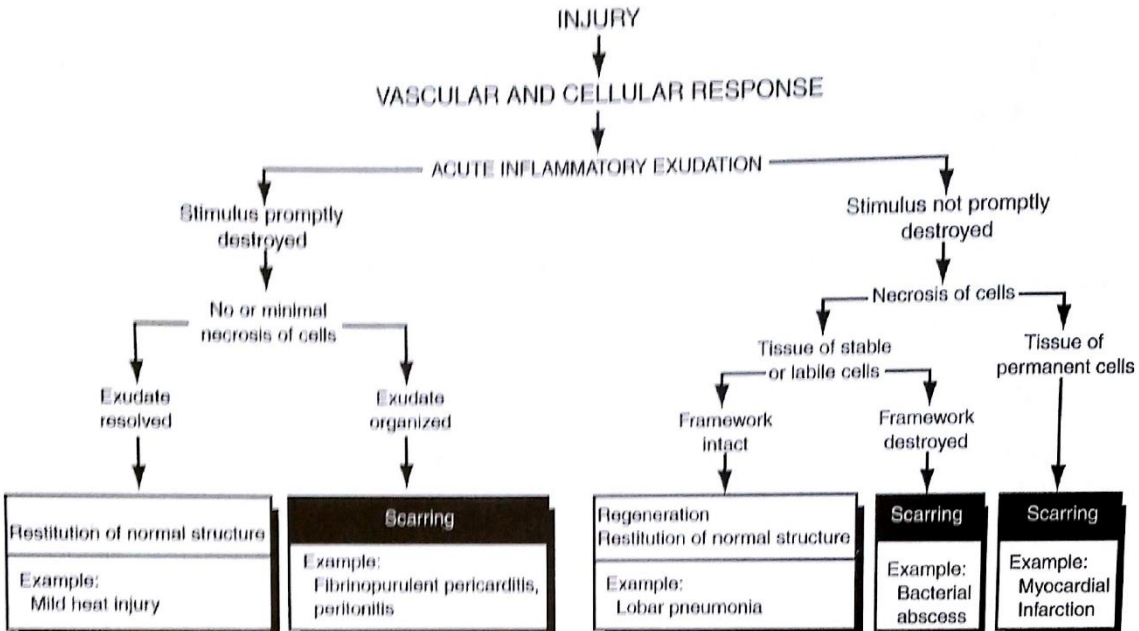
During the repair phase the injured tissue is replaced by native parenchymal cells or by filling up the defect with fibroblastic scar tissue or both

The outcome of repair depends primarily on the tissue type and the extent of the injury

Normal tissue structure and function is restored when
a. Tissue injury is transient
b. Tissue destruction is small
c. Tissue is capable of regeneration

Scarring results when the injury is extensive or occurs in tissue that do not regenerate
It occurs as a series of three processes:
i. Formation of new blood cells (angiogenesis)
ii. Deposition of collagen (fibrosis)
iii. Maturation of the scar (remodeling)

The early scar tissue rich in new capillaries and proliferation of fibroblasts is called granulation tissue.



Inflammation is modulated by chemical mediators from plasma, cells or ECM which regulate the subsequent vascular and cellular events.

The chemical mediators of inflammation include
Vasoactive amines (histamine), plasma proteases, platelet activating factor, cytokines, oxygen-derived free radicals, polypeptide growth factors

Especially important in healing response to implanted biomaterials is growth factors which influence repair and healing by affecting cell growth, motion, differentiation and contractility

The following growth factors are involved in mediating angiogenesis, fibroblast migration, proliferation and collagen deposition in wounds:

- Epidermal growth factor (important in proliferation of epithelial cells and fibroblasts)
- Platelet-derived growth factor (involved in fibroblast and smooth muscle cell migration)
- Fibroblast growth factor
- Transforming growth factor (key role in fibrosis)
- Vascular endothelial growth factor (key role in angiogenesis)

Normal maintenance of ECM and remodeling after injury requires constant collagen remodeling by continuous collagen synthesis and breakdown. It is a highly organized process that involves the selective action of a group of related proteases that collectively can degrade most components of the extracellular matrix.

The enzymes responsible for the destruction of the collageneous matrix are called matrix metalloproteinases (MMP) and are synthesized by macrophages, fibroblasts, and epithelial cells

Myofibroblasts with characteristics of both smooth muscle cells (specialized in contraction) and fibroblasts (specialized in protein synthesis) are especially important in ECM remodeling. They are responsible for the production of and response to tissue forces during remodeling, thereby regulating the evolution of tissue structure according to mechanical requirements.

Other enzymes called tissue inhibitors of metalloproteinases (TIMP) regulate the activity of MMPs and the breakdown of the matrix.

The balance between matrix synthesis and turnover is dependent on the relative amounts of MMPs and TIMPs which in turn depend on the amount of growth factors and hormones that enable their production. Especially transforming growth factor and platelet derived growth factor play an important role in regulation of these enzymes.

Implanted biomaterials are in contact with various cells and tissues (bone connective tissue, cardiovascular muscle tissue, blood tissue) for prolonged periods. Their effective design is required to induce specific chemical and structural information to the surfaces that control tissue formation in a manner similar to cell-cell communication and patterning during embryological development.

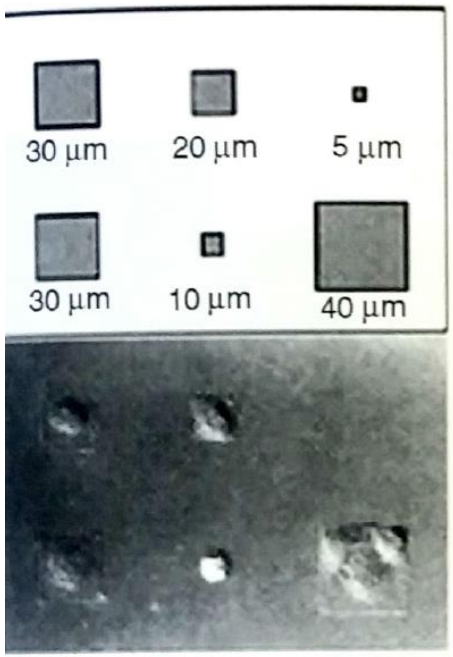
Cell interactions with the external environment are mediated by receptors in the membrane which interact with ligands that adsorb to the material surface.

The chemistry of the biomaterial surface controls the nature of the adsorbed protein layer. Although cells are able to adhere, spread and grow on bare biomaterial surfaces in vitro, proteins adsorbed from the adjacent tissue environment or blood in vivo and secreted by the adherent cells significantly enhance cell attachment, migration and growth.

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. Also integrin receptors transduce biochemical signals to the nucleus upon adhesion.

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.



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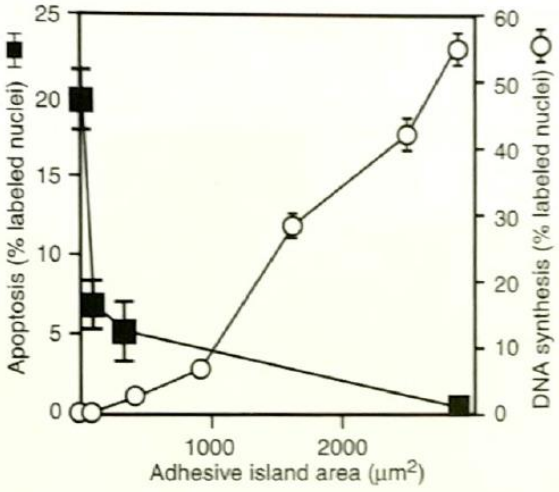

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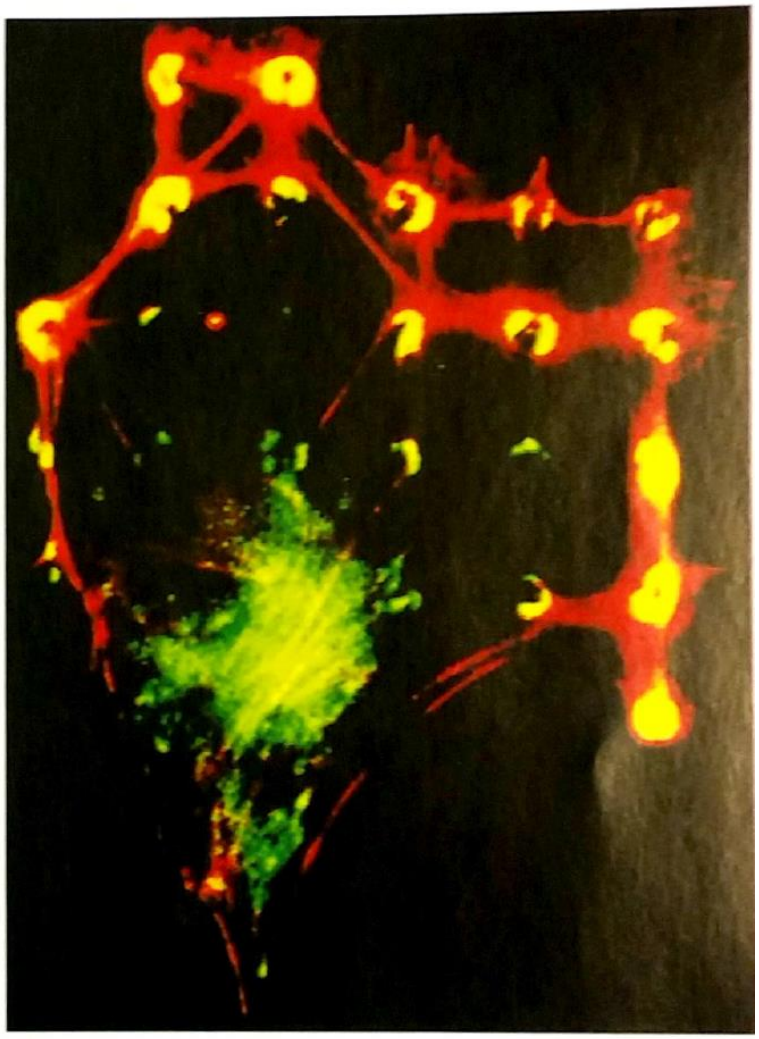
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The ability to proliferate depended directly on the degree to which the cells were allowed to distort physically and not on the actual surface area of substrate bending

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.

Also 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.

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.