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Biomaterials

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المرحلة الرابعة

BIOMATERIALS

المحاضرة الثامنة

TISSUE RESPONSE TO IMPLANTS

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TISSUE RESPONSE TO IMPLANTS

Nerve guidance channel for nerve regeneration. It is critical to have biocompatible and bioresorbable materials for this type of application.

In order to implant any prostheses, a surgeon has to first injure the tissue. The injured or diseased tissues should then be removed to some extent in the process of implantation. The success of the entire operation depends on the kind and degree of tissue response to the surgical procedure and any interactions between tissues and implants. The local and systemic response of the tissues toward implants comprise an aspect of biocompatibility. Biocompatibility entails mechanical, chemical, pharmacological, and surface compatibility. Some examples of tissue response to various implants are summarized in Table 8-1. The study of tissue response is critical for the success of implants, yet we do not have good "quantitative methods" of measuring tissue responses. The tissue response toward injury may vary widely according to site, species, contamination, etc. However, the inflammation and cellular response to the wound for both intentional and accidental injuries are the same regardless of site.

Table 8-1. Examples of Tissue Response to Various Implants.

Implants	Histology	Infection/complication
Breast	T cells, foreign body giant cells (FBGC), macrophages	Dense fibrovascular connective tissue
Heart valves and pacemakers	Endothelial cell ingrowth, macropharges, FBGC	Tissue valves: calcification, disintegration, mechanical: coagulations, formed elements of blood damages
Hernia	Fibroblasts, macropharges, rare FBGC, collagenous membrane to e-PTFE	Seroma, fistula, infection
Intraocular lens	FBGC	Posterior capsular opacification
Joints	FBGC, macropharges,	Bone cement: granulomatosis, stems and cement: particles, wear debris, osteolysis
Ossicular (ear)	Multinucleated FBGC	Degradation of UHMWPE (ultrahigh-Molecular-weight polyethylene)
Penile	Biofilm formation	Infection, partial extraction, progressive neuropathy

8.1. NORMAL WOUND-HEALING PROCESS

8.1.1. Inflammation

Whenever tissues are injured or destroyed, the adjacent cells respond to repair them. An immediate response to any injury is the inflammatory reaction. Soon after injury, constriction of capillaries occurs (stopping blood leakage); then dilatation. Simultaneously there is greatly increased activity in the endothelial cells lining the capillaries. The capillaries become covered by adjacent leukocytes, erythrocytes, and platelets (formed elements of blood). Concurrently with vasodilatation, leakage of plasma from capillaries occurs. The leaked fluid combined with the migrating leukocytes and dead tissues will constitute exudate. Once enough cells (see Table 8-2 for definitions of types of cells) are accumulated by lysis, the exudate becomes pus. It is important to know that pus can sometimes occur in nonbacterial (aseptic) inflammation.

Table 8-2. Definitions of Cells Appearing in this Chapter.

Types of cell	Description
Chondroblast:	an immature collagen (cartilage) producing cell
Endothelial:	a cell lining the cavities of the heart and the blood and lymph vessels
Erythrocyte:	a formed element of the blood containing hemoglobin (red blood cell)
Fibroblast:	a common fixed cell of connective tissue that elaborates the precursors of the extracellular fibrous and amorphous components
Giant Cell	
Foreign body giant cell:	a large cell derived from a macrophage in the presence of a foreign body.
Multinucleated giant cell:	a large cell having many nuclei
Granulocyte:	any blood cell containing specific granules; included are neutrophils, basophils, and eosinophils
Leukocytes:	a colorless blood corpuscle capable of ameboid movement, protects body from microorganisms and can be of five types: lymphocytes, monocytes, neutrophils, eosinophils, and basophils
Macrophage:	large phagocytic mononuclear cell; free macrophage is an ameboid phagocyte and present at the site of inflammation
Mesenchymal:	undifferentiated cell having similar role as fibroblasts but often smaller and can develop into new cell types by certain stimuli
Mononuclear:	any cell having one nucleus
Osteoblast:	an immature bone-producing cell
Phagocyte:	any cell that destroys microorganisms or harmful cells.
Platelet:	a small circular or oval disk shaped cell(3 μm dia.) precursor of a blood clot

At the time of damage to the capillaries, the local lymphatics are also damaged since they are more fragile than the capillaries. However, the leakage of fluids from capillaries will provide fibrinogen and other formed elements of the blood, which will quickly plug the damaged lymphatics, thus localizing the inflammatory reaction. All of the reactions mentioned above — vasodilatation of capillaries, leakage of fluid into the extravascular space, and plugging of lymphatics — will provide the classic inflammatory signs: redness, swelling, and heat, which

can lead to local pain. When the tissue injury is extensive or the wound contains either irritants (foreign materials including prostheses) or bacteria, the inflammation may lead to a prolonged reaction causing extensive tissue destruction. The tissue destruction is done by collagenase, which is a proteolytic enzyme capable of digesting collagen. The collagenase is released from granulocytes, which in turn are lysed by the lower pH at the wound site. Local pH can drop from the normal values of 7.4–7.6 to below 5.2 at the injured site. If there is no drainage for the necrotic debris, lysed granulocytes, formed blood elements, etc., then the site becomes a severely destructive inflammation, resulting in a necrotic abscess. If the severely destructive inflammation persists and no healing process occurs within three to five days, a chronic inflammatory process commences. This is marked by the presence of mononuclear cells called macrophages, which can coalesce to form multinuclear giant cells (Figure 8-1). The macrophages are phagocytic and remove bacteria or foreign materials if they are small enough. Sometimes the mononuclear cells evolve into histiocytes, which regenerate collagen. This regenerated collagen is used to close the wound or to wall-off unremovable foreign materials such as prostheses by encapsulation with a thin membrane.

In chronic inflammatory reaction, lymphocytes occur as clumps or foci. These cells are a primary source of immunogenic agents, which become active if foreign proteins are not removed by the body's primary defense.

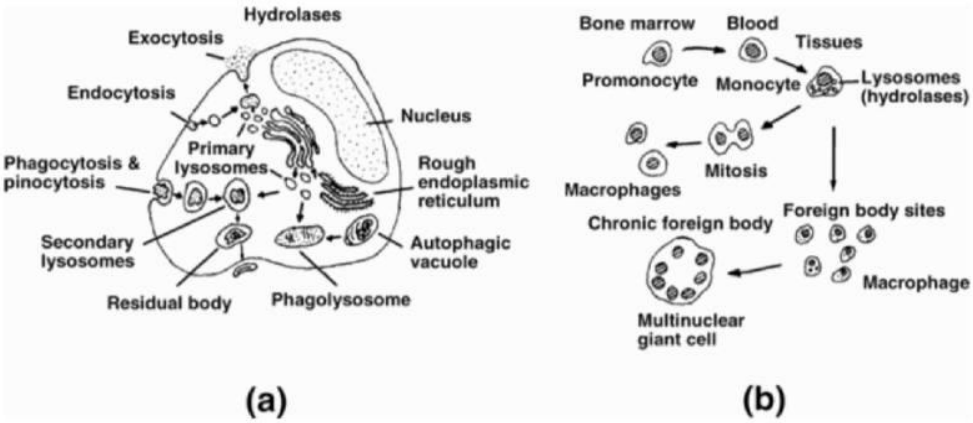


Figure 8-1. (a) Activated macrophage; (b) development of the multinuclear foreign body giant cell.

8.1.2 Cellular Response to Repair

Soon after injury the mesenchymal cells evolve into migratory fibroblasts that move into the injured site while the necrotic debris, blood clots, etc. are removed by the granulocytes and macrophages. The inflammatory exudate contains fibrinogen, which is converted into fibrin by enzymes released through blood and tissue cells (see 8.3). The fibrin scaffolds the injured site. The migrating fibroblasts use the fibrin scaffold as a framework onto which the collagen is deposited. New capillaries are formed following the migration of fibroblasts, and the fibrin scaffold is removed by the fibrinolytic enzymes activated by the endothelial cells. The endothelial cells together with the fibroblasts liberate collagenase, which limits the collagen content of the wound.

After 2 to 4 weeks of fibroblastic activities the wound undergoes remodeling, during which the glycoprotein and polysaccharide content of the scar tissue decreases and the number of synthesizing fibroblasts also decreases. A new balance of collagen synthesis and dissolution is reached, and the maturation phase of the wound begins. The time required for the woundhealing process varies for various tissues, although the basic steps described here can be applied in all connective tissue wound-healing processes.

The healing of soft tissues — especially the healing of skin wounds — has been studied intensively since this is germane to all surgery. The degree of healing can be determined by histochemical or physical parameters. A combined method will give a better understanding of the overall healing process. Figure 8-2 shows a schematic diagram of sequential events of the cellular response of soft tissues after injury. The wound strength is not directly proportional to the amount of collagen deposited in the injured site, as shown in Figure 8-3. This indicates that there is a latent period for the collagen molecules (procollagen is deposited by fibroblasts) to polymerize to their maturity. It may take additional time to align the fibers in the direction of stress and to crosslink procollagen molecules to increase the physical strength closer to that of normal tissue. This collagen restructuring process requires more than 6 months to complete, although the wound strength never reaches its original value. The wound strength can be affected by many variables, i.e., severe malnutrition resulting in protein depletion, temperature, presence of other wounds, and oxygen tension. Other such factors as drugs, hormones, irradiation, and electrical and magnetic field stimulation all affect the normal wound-healing process. It is also noted that scar tissues lack elastin, resulting in noncompliant stiff

collagenous tissue. The scar tissues also contract during healing due to the same reason.

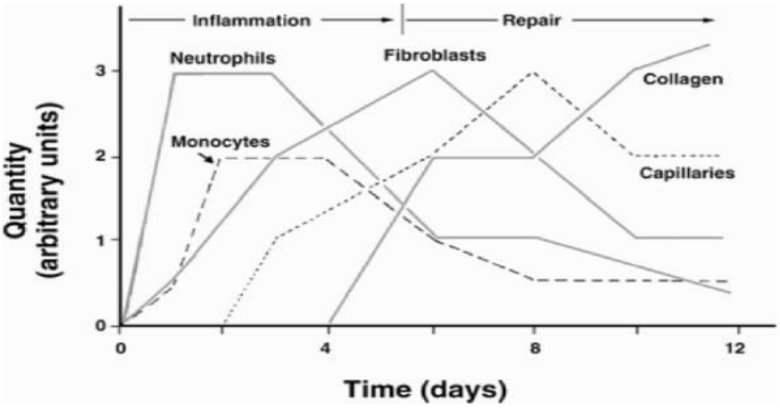


Figure 8-2. Soft tissue wound healing sequence.

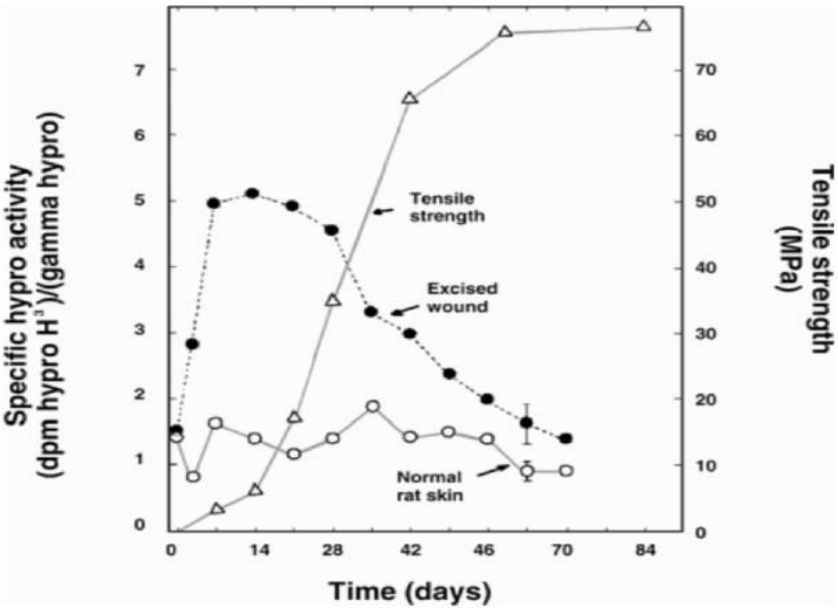


Figure 8-3. Tensile strength and rate of collagen synthesis of rat skin wounds.

The healing of bone fracture is regenerative rather than simple repair. The only other tissue that truly regenerates in humans is liver. However, the extent of regeneration is limited in humans. The cellular events following fracture of bone are illustrated in Figure 8-4. When a bone is fractured, many blood vessels (including those in the adjacent soft tissues) hemorrhage and form a blood clot around the fracture site. Shortly after fracture the fibroblasts in the outer layer of the periosteum and the osteogenic cells in the inner layer of periosteum migrate and proliferate

toward the injured site. These cells lay down a fibrous collagen matrix called a callus. Osteoblasts evolved from the osteogenic cells near the bone surfaces start to calcify the callus into trabeculae, which are the structural elements of spongy bone. The osteogenic cells migrating further away from an established blood supply become chondroblasts, which lay down cartilage. Thus, after about 2 to 4 weeks the periosteal callus is made of three parts, as shown in Figure 8-5.

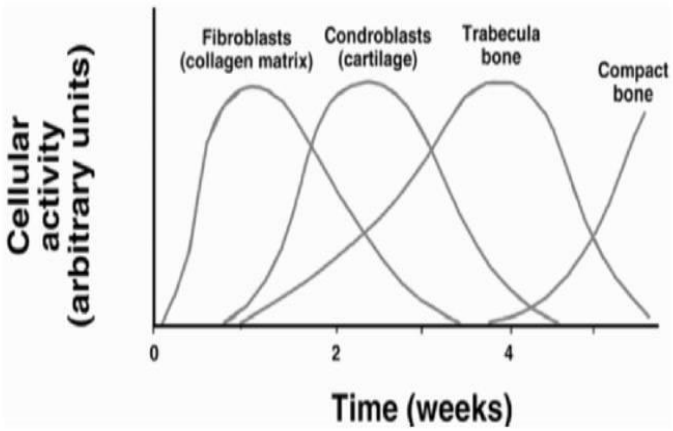


Figure 8-4. Sequence of events followed by bone fracture.

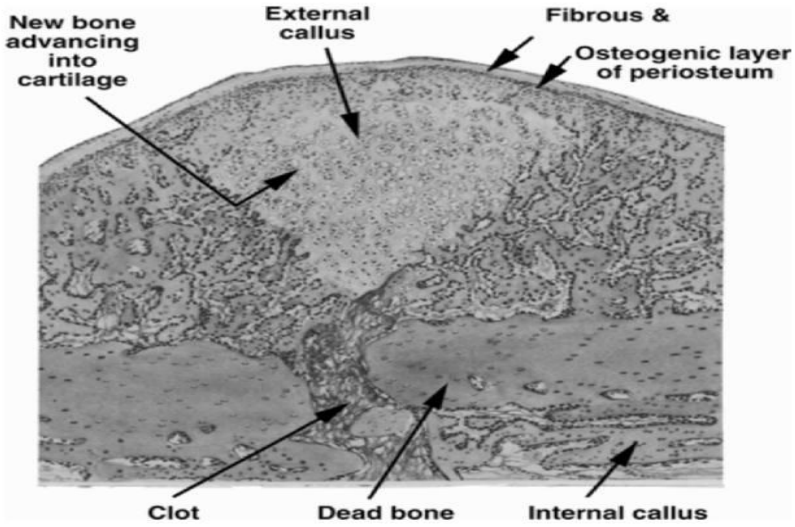


Figure 8-5. Drawing of a longitudinal section of fractured rib of a rabbit after two weeks (H & E stain).

Simultaneous with external callus formation a similar repair process occurs in the marrow cavity. Since there is an abundant supply of blood, the cavity turns into callus rather quickly and becomes fibrous or spongy bone. New trabeculae develop in the fracture site by appositional growth, and the spongy bone turns into compact

bone. This maturation process begins after about 4 weeks. There are many factors contributing to the healing and remodeling of the fractured bone, including energy input (mechanical, thermal, electrical, etc.), pharmacology, and the condition and location of the bone. Some other interesting observations have been made on the healing of bone fractures in relation to the synthesis of polysaccharide on collagen. It is believed that the amount of collagen and polysaccharides is closely related to the cellular events following fracture. When the amount of collagen starts to increase, this marks the onset of the remodeling process. This occurs after about 1 week. Another interesting observation is the electrical potential (or biopotential) measured in the long bone before and after fracture, as shown in Figure 8-6. The large electronegativity in the vicinity of fracture marks the presence of increased cellular activities in the tissues. Thus, there is a maximum negative potential in the epiphysis in the normal bone since this zone is more active (the growth plate is in the epiphysis).

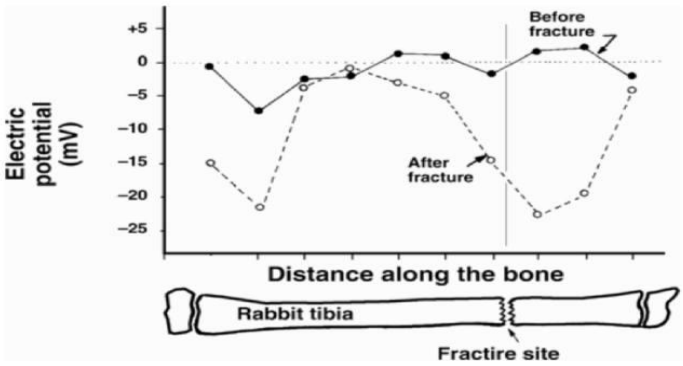
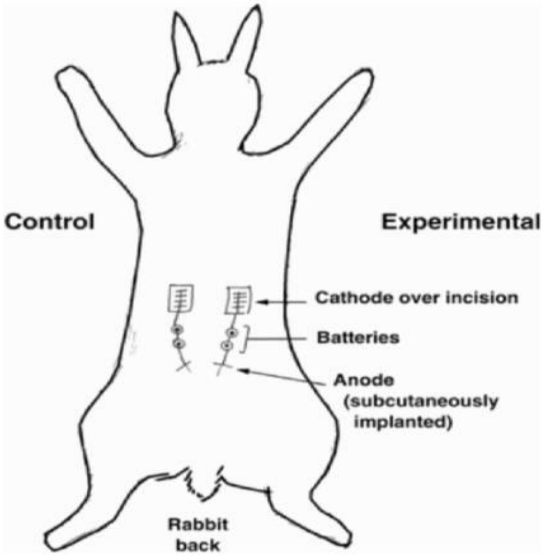


Figure 8-6. Skin surface of rabbit limb before and after fracture. Note that the fracture site has increased electronegative potential.

H.W // Example 1

The healing process of wounds in the skin has often been investigated since such healing is relevant to every surgery. In one study electrical stimulation was used to accelerate the healing of wounds in rabbit skin, as shown in the following figure. The mean current flow was 21 PA and the mean current density was 8.4 PA/cm2. After 7 days the load to fracture of the skin (removed from the dead animals) on the control samples was 797 g, and on the stimulated experimental side it was 1,224 g on average (Konikoff, 1976).



a. Calculate the percent increase of strength by stimulation.

b. The width of the testing sample was 1.6 cm. Assuming a 1.8-mm thickness of skin, calculate the tensile stress for both the control and experimental samples.

c. Compared with the strength of normal skin (about 8 MPa), what percentages of the control and experimental skin wound strengths were recovered?

d. Compare the results of (c) with the result of Figure 8-3.

e. Calculate the corrosion rate in mm/year if a platinum electrode was used.

Answer:

8.2. BODY RESPONSE TO IMPLANTS

The response of the body toward implants varies widely according to host site and species, the degree of trauma imposed during implantation, and all the variables associated with a normal wound-healing process. The chemical composition and micro- and macrostructures of the implants induce different body responses. The response has been studied in two different areas — local (cellular) and systemic — although a single implant should be examined for both aspects. In practice, testing was not done simultaneously except in a few cases (such as bone cement).

Example 2

Describe the major differences between normal wound healing and the tissue responses to “inert” and “irritant” materials. What factors aside from the choice of material can affect the local tissue response to an implant?

Answer

The tissue response to an “inert” material is very much like normal wound healing. No foreign body giant cells appear, and a thin fibrous capsule is formed. The tissue in this capsule differs very little from normal scar tissue. In response to irritant materials, foreign body giant cells appear and an inflammatory response is evoked. There is an abundance of leukocytes, macrophages, and granulocytes. Granular tissue will be formed, serving the functions of phagocytosis and organization, and appears only under circumstances of irritation or infection. Healing is slow and a thick capsule forms. If the material is chemically reactive or mechanically irritating, necrosis of surrounding tissue may result. It has been suggested that the size and shape of an implant should be important factors to consider for what type of tissue reaction it could elicit.

8.3. BLOOD COMPATIBILITY

The single most important requirement for blood-interfacing implants is blood compatibility. Although blood coagulation is the most important factor for blood compatibility, the implants should not damage the proteins, enzymes, and formed elements of blood. The latter includes hemolysis (red blood cell rupture) and initiation of the platelet release reaction. The structure of the platelet is shown in Figure 10-10. The platelets adhere to a surface using pseudopods. Once adhered, platelets release D granule contents, including platelet factor 4 (PF4) and Ethromboglobulin (EGB), and dense granule contents, including adenosine diphosphate (ADP). Thrombin is generated locally through factor XIIa and platelet procoagulant activity. Thromboxine A2 (TxA2) is synthesized. ADP, TxA2, and thrombin recruit additional circulating platelets into enlarging platelet aggregate. The thrombin-generated fibrin stabilizes the platelet mass, as shown in Figure 8-11. Table 8-3 gives the properties of human clotting factors.

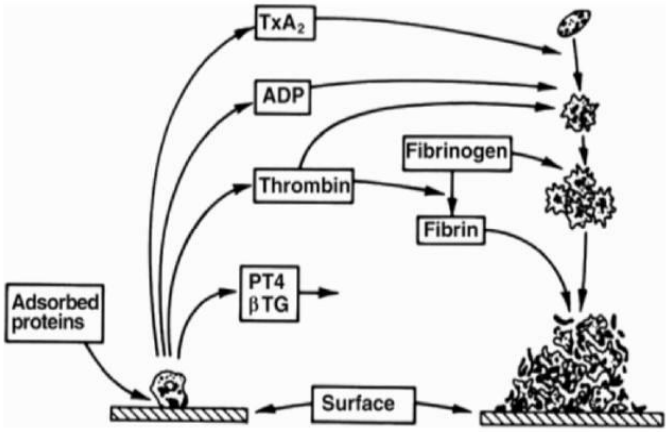


Figure 8-11. Schematic representation of platelet deposition on surfaces.

Table 8.3. Properties of Human Clotting Factors.

Factors	Molecular weight (no. of chains)	Plasma concentration (μg/l)	Active form
Intrinsic system			
Factor XII	80,000 (1)	30	Serine protease
Prekallikreine	80,000 (1)	50	Serine protease
High-m.w. kininogen	105,000 (1)	70	Cofactor
Factor XI	160,000 (2)	4	Serine protease
Factor IX	68,000 (1)	6	Serine protease
Factor VIII	265,000 (1)	0.1	Cofactor
VWF	1–15,000,000[a]	7	Cofactor for platelet adhesion
Extrinsic system			
Factor VII	47,000 (1)	0.5	Serine protease
Tissue factor	46,000 (1)	0	Cofactor
Common pathway			
Factor X	56,000 (2)	10	Serine protease
Factor V	330,000 (1)	7	Cofactor
Prothrombin	72,000 (1)	100	Serine protease
Fibrinogen	340,000 (6)	2500	Clot structure
Factor XIII	320,000 (4)	15	Transglutaminase

The mechanism and route of blood coagulation (thrombi or emboli depending on the mobility) are quite complex. Cascading events can be triggered in blood and tissue, as shown in Figure 8-12. The kinin system is activated by the Hageman factor (Hfa, Factor XII) in contact with collagen, basement membrane, foreign bodies such as bacteria, metal, etc. Blood coagulation pathways can be depicted as shown in Figure 8-13. Again the Hageman factor triggers the cascade in intrinsic pathway while the extrinsic pathways activate Factor VII together with lipoproteins. As discussed earlier, immediately after injury the blood vessels constrict to minimize the flow of blood. Platelets adhere to the vessel walls by contacting the exposed

collagen. The aggregation of platelets is achieved through release of adenosine diphosphate (ADP) from damaged red blood cells, vessel walls, and from adherent platelets.

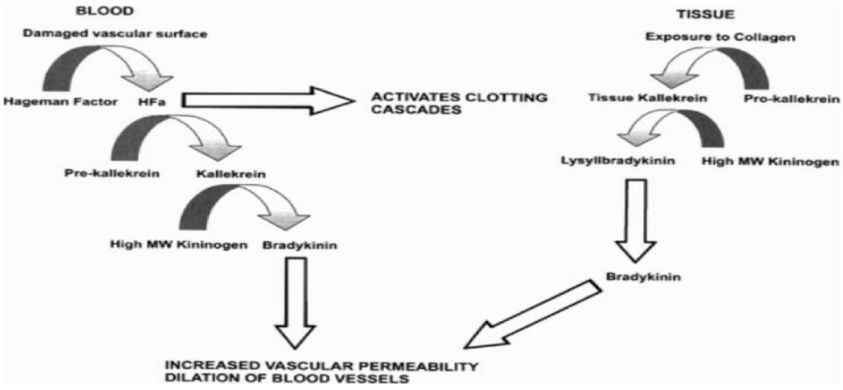


Figure 8-12. The kinin system starts with the damaged blood vessel surface. The highmolecular-weight kininogen and pre-kallikrein circulated in the plasma with Hfa trigger the cascade.

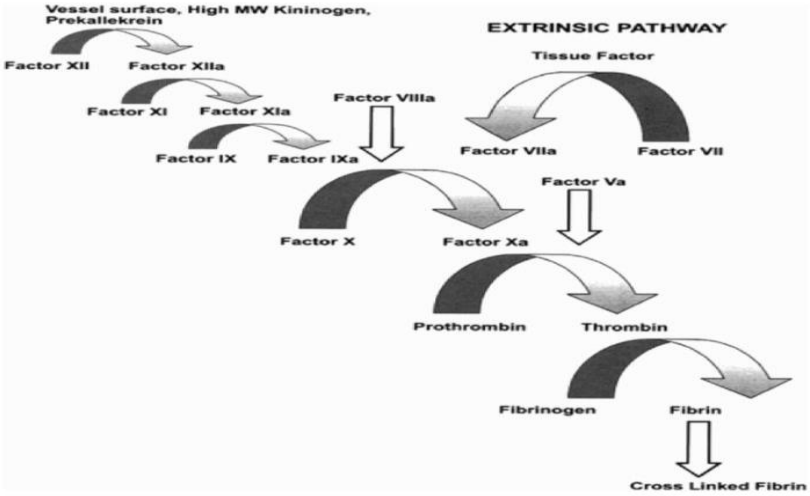


Figure 8-13. Blood coagulation pathways by intrinsic and extrinsic route.

8.4. CARCINOGENICITY

A variety of chemical substances are known to induce the onset of cancerous disease in human beings and are known as carcinogens. Carcinogenic agents may act upon the body by skin contact, by ingestion, by inhalation, or by direct contact with tissues. It is the last possibility that is of primary concern within the context of biomaterials. Early studies showed that sheets or films of many polymers produced

cancer when implanted in animals, especially rats. It was later found that the physical form of the implant was important, and that fibers and fabrics produced fewer tumors than sheets of the same material, and powders produced almost no tumors. Other materials, by contrast, are carcinogenic by virtue of their chemical constitution.

DEFINITIONS

-D granule: Released through the vessel walls after injury, in turn releasing coagulation agents.

-Adenosine diphosphate (ADP): A compound consisting of an adenosine molecule bonded to three phosphate groups, present in all living tissue. The breakage of one phosphate linkage (to form adenosine diphosphate, ADP) provides energy for physiological processes such as muscular contraction

-Aflatoxin: A naturally occurring carcinogenic material. It is formed by mold infestation of food crops such as corn or peanuts.

-Ames test: A screening test for carcinogenic potential of a material. Genetic mutations are observed in bacteria; carcinogenicity is correlated with mutagenicity.

-Biocompatibility: Acceptance of an artificial implant by surrounding tissues and by the body as a whole. The implant should be compatible with tissues in terms of mechanical, chemical, surface, and pharmacological properties.