CHAPTER 2

Host reactions to Biomaterials and Their Evaluation
2.1 Introduction

- Biomaterials and medical devices are now commonly used as prostheses in cardiovascular, orthopedic, dental, ophthalmological, and reconstructive surgery, in interventions such as angioplasty (stents) and hemodialysis (membranes), in surgical sutures or bioadhesives, and as controlled drug release devices.
- Most implants serve their recipients well for extended periods by alleviating the conditions for which they were implanted.
- However, some implants and extracorporeal devices ultimately develop complications-adverse interactions of the patient with the device, or vice versa-which constitute device failure and thereby may cause harm to or death of the patient.
- Complications result largely as a consequence of biomaterial-tissue interactions, which all implants have with the environment into which they are placed. Effects of both the implant on the host tissues and the host on the implant are important in mediating complications and device failure (Ch.4.1: Fig.1).
This chapter contains overview discussions of the most important host reactions to biomaterials and their evaluation, including nonspecific inflammation and specific immunological reactions, systemic effects, blood-materials interactions, tumor formation, and infection.

To a great extent, these interactions arise from alterations of physiological (normal) processes (e.g., immunity, inflammation, blood coagulation) comprising host defense mechanisms that function to protect an organism from the deleterious external threats (such as bacteria and other microbiologic organisms, injury and foreign materials).

Chapter 6 addresses degradation mechanisms in biomaterials (i.e., the effect of the host on biomaterials).

Several key concepts of biomaterials-tissue interactions are emphasized here in an effort to guide the reader and facilitate the use of this chapter.
• **In contrast to** living organ transplants, **biomaterials** are not generally “rejected.”

• **The process of organ rejection** denotes an **inflammatory process** that results **from a specific immune response** and which causes tissue death, which **synthetic biomaterials** typically do not generate.

• The **usual response to biomaterials** comprises **nonspecific inflammation**. However, **tissue-derived biomaterials** (such as bioprosthetic heart valves) may express foreign histocompatibility antigens and be **antigenic** and capable of eliciting an **immune response**, including antibodies and antigen-specific T cells.

• Nevertheless, it is important to **understand the following**:
1. Tissue immunogenicity does not necessarily induce immunologically mediated device dysfunction.

2. Specific immunological responses can be not only a cause of but can result from device failure.

3. Although mononuclear inflammatory cell infiltrates (containing macrophages and lymphocytes) are characteristically associated with organ/tissue rejection on histological examination, mononuclear inflammatory infiltrates are themselves nonspecific and comprise a largely stereotyped and generic response to tissue injury. Therefore, the presence of mononuclear cells does not necessarily denote a rejection pathogenesis.
In order to invoke an immunological reaction to a biomaterial as the cause of a device failure, an immunological variant of the classical Koch’s postulates, which are the objective criteria for concluding that a disease is infectious and caused by a specific microbiologic agent, would be appropriate. The classic Koch’s postulates state that:

1. A suspected infectious agent should be recoverable from the pathologic lesions of the human host.

2. The agent should cause the pathologic lesions when inoculated into an animal host.

3. The agent should then be recoverable from the pathologic lesions in the animal.
• **Most biomaterials** of potential clinical interest typically elicit the **foreign body reaction (FBR)**, a special form of **non-specific inflammation**.

• **The most prominent cells** in the FBR are **macrophages**, which attempt to **phagocytose** the material and are variably successful, but complete engulfment and degradation area often difficult.

• **The macrophages**, **activated in the process of interacting with a biomaterial**, may **elaborate** cytokines that stimulate inflammation or **fibrosis**.

• **Multinucleated giant cells** in the vicinity of a foreign body area generally considered evidence of a more **severe FBR**.

• The more “**biocompatible**” the implant, the more **quiescent** (less inflammation) in the ultimate response.
When the implant is a **source of particles** not easily controlled, such as **wear debris** from articulating joint surfaces, the inability of **inflammatory cells** to adhere to but not **phagocytose** particles **larger than a critical size** (“frustrated phagocytosis”) can lead to release of enzymes (exocytosis) and **cytokines and other chemical mediators** (e.g., prostaglandin, tumor necrosis factor-alpha, and interleukin-1) and cause **harm to the extracellular environment**.

Thus, inflammatory cell products that are critical in killing microorganisms in typical inflammation can **damage tissue adjacent** to foreign bodies.

The nature of the reaction is largely **dependent on the chemical and physical characteristics of the implant**.
For most inert biomaterials, the late tissue reaction is encapsulation by a relatively thin fibrous tissue capsule (composed of collagen and fibroblasts).

Tissue interactions can be modified by changing the chemistry of the surface (e.g., by adding specific chemical groupings to stimulate adhesion or bone formation in orthopedic implants), inducing roughness or porosity to enhance physical binding to the surrounding tissues, incorporating a surface-active agent to chemically bond the tissue, or using a bioresorbable component to allow slow replacement by tissue to simulate natural healing properties.
Biomaterials-related systemic toxicity and hypersensitivity reaction (through lymphatics and the bloodstream) in animals and patients with either stainless steel or cobalt-base orthopedic total joint replacement components, elevations of metallic components occur in tissue (at both local and remote sites) and in serum and urine.

Transport of particulates over large distances by macrophages to regional lymph nodes and to the lungs has been considered a systemic and remote effect.

As a consequence of silicone migrated through lymphatic vessels to lymph nodes, an enlarged, hard axillary lymph node in a woman who received a silicone-gel breast prosthesis for reconstruction following mastectomy for a carcinoma can be misdiagnosed as tumor.
“Metal allergy” is well-recognized and is frequently associated in women with the use of cheap, high-nickel-alloy costume jewelry or earrings and can occur in association with metallic implants.

By themselves, metal ions lack the structural complexity required to challenge the immune system. However, when combined with proteins, such as those available in the skin, connective tissues, and blood, a wide variety of metals induce immune responses and this can have clinical effects.

Cobalt, chromium, and nickel are included in this category, with nickel perhaps the most potent; at least 10% of a normal population will be sensitive by skin test to one or more of these metals, at some threshold level.
Exposure of blood to an artificial surface can induce thrombosis, embolization, and consumption of platelets and plasma coagulation factors, as well as the systemic effects of activated coagulation and complement products, and platelet activation.

It is clear that no synthetic or modified biological surface generated by man is as resistant to thrombosis (thromboresistant) as normal unperturbed endothelium (the cellular lining of the circulatory system).

However, it is important to understand that under some circumstances endothelial cells can be “dysfunctional” and although physically intact can express prothrombotic molecules that can induce thrombosis.

Thromboembolic complications are a major cause of mortality and morbidity with cardiovascular devices.
• Both fibrin (red) thrombus and platelet (white) thrombus form in association with valves and other cardiovascular devices.

• As in the cardiovascular system in general, Virchow’s triad (i.e., the conditions of surface thrombogenicity, hypercoagulability, and locally static blood flow) largely predicts the relative propensity toward thrombus formation and often the location of thrombotic deposits with cardiovascular prostheses.

• However, despite over a quarter century of intense research, the physical and chemical characteristics of materials that control the outcome of blood-surface interaction are incompletely understood.

• When non-physiologic surfaces contact blood, three events comprise thrombotic interactions: 1) plasma protein deposition, 2) adhesion of platelets and leukocytes, and 3) bulk fibrin formation (blood coagulation).
• All foreign materials exposed to blood spontaneously and rapidly (seconds) absorb a film of plasma protein, largely fibrinogen. This is followed by cellular thrombogenesis (beginning with platelet adhesion to the first adsorbed plasma proteins).

• If conditions of relatively static flow are present, the fiber-forming steps of the coagulation process occur, and macroscopic thrombus ensues. Considerable evidence implicates a primary regulatory role for blood platelets in the thrombogenic response to artificial surfaces.

• Platelet adhesion to artificial surfaces strongly resembles that of adhesion to the vascular subendothelium that has been exposed by injury.

• Nevertheless, the major clinical approach to controlling thrombosis in cardiovascular devices is the use of systemic anticoagulants, particularly Coumadin (warfarin), which inhibits thrombin and fibrin formation but does not inhibit platelet-mediated thrombosis.
The Structure of an Artery Wall

- Tunica media
- Tunica externa
- Tunica intima
- Smooth muscle
- Endothelium
- External elastic membrane
- Internal elastic membrane
Although animals frequently have sarcomas at the site of an experimental biomaterial implant, neoplasms in humans occurring at the site of implanted medical devices are rare, despite the large numbers of implants used clinically over an extended duration.

Moreover, the presence of a neoplasm at an implant site does not prove that the implant had a causal role. Cancers associated with foreign bodies can appear at any postoperative interval but tend to occur many years postoperatively.

The pathogenesis of implant-induced tumors is not well understood; most experimental data indicate that the physical rather than chemical characteristics of the foreign body primarily determine tumorigenicity.
Infection occurs in as many as **5 to 10% of patients with implanted prosthetic devices** and is a major source of morbidity and mortality.

Infections associated with medical devices are often **resistant** to antibiotics and host defenses, often **persisting until** the devices are removed.

**Early implant infections** (less than approximately 1 to 2 months postoperatively) are most likely **due to intraoperative contamination** from airborne sources or non-sterile surgical technique, or to early postoperative complications such as wound infection.

In contrast, **late infections** likely occur by a **hematogenous** (blood-borne) **route** and are often initiated by **bacteremia** induced by therapeutic dental or genitourinary procedures.
• **Perioperative prophylactic antibiotics** and periodic antibiotic prophylaxis given shortly **before diagnostic and therapeutic procedures** protect against implant infection.

• Infections associated with foreign bodies are characterized microbiologically by a high prevalence of *Staphylococcus epidermidis* and other staphylococci, especially *S. aureus*.

• Ordinarily, *S. epidermidis* is an organism **with low virulence** and thus an infrequent cause of **non-prosthesis-associated deep infections**. This emphasizes **the unique environment** in the vicinity of a foreign body.

• The presence of a foreign body **per se potentiates infection**.

• A classic experiment indicated that the staphylococcal bacterial **inoculum** required to cause infection in the presence of foreign implant was **less than that when no foreign body** was present.
Devices could facilitate infection in several ways.

Microorganisms are provided access to the circulation and to deeper tissue by damage to natural barriers against infection during implantation or subsequent function of a prosthetic device.

Moreover, an implanted foreign body could
- (1) limit phagocyte migration into infected tissue or
- (2) interfere with inflammatory cell phagocytic mechanisms,
- through release of soluble implant components or surface-mediated interactions, thus allowing bacteria to survive adjacent to the implant.

Adhesion of bacteria to the prosthetic surface and the formation of micro-colonies within an adherent biofilm are fundamental steps in the pathogenesis of clinical and experimental infections associated with foreign bodies.
2.2 INFLAMMATION, WOUND HEALING, AND THE FOREIGN-BODY RESPONSE

- Inflammation, wound healing, and foreign body reaction are generally considered as parts of the tissue or cellular host responses to injury.
- Table 1 lists the sequence/continuum of these events following injury.
- Overlap and simultaneous occurrence of these events should be considered; e.g., the foreign body reaction at the implant interface may be initiated with the onset of acute and chronic inflammation.
- From a biomaterials perspective, placing a biomaterial in the in vivo environment requires injection, insertion, or surgical implantation, all of which injure the tissues or organs involved.
- The placement procedure initiates a response to injury by the tissue, organ, or body and mechanisms are activated to maintain homeostasis.
• The **degrees** to which the **homeostatic mechanisms** are perturbed and the **extent** to which **pathophysiologic conditions** are created and undergo resolution are a **measure of the host reactions** to the biomaterial and may ultimately **determine its biocompatibility**.

• Although it is convenient **to separate** homeostatic mechanisms into **blood-material or tissue-material interactions**, it must be remembered that **the various components or mechanisms involved in homeostasis** are present in both blood and tissue and are a **part of the physiologic continuum**.

• Furthermore, it must be noted that host reactions may be **tissue-dependent**, **organ-dependent**, and **species-dependent**.

• Obviously, **the extent of injury** varies with the **implantation procedure**.
Inflammation is generally defined as the reaction of vascularized living tissue to local injury.

Inflammation serves to contain, neutralize, dilute, or wall off the injurious agent or process.

In addition, it sets into motion a series of events that may heal and reconstitute the implant site through replacement of the injured tissue by regeneration of native parenchymal cells, formation of fibroblastic scar tissue, or a combination of these two processes.

Immediately following injury, there are changes in vascular flow, caliber, and permeability.

Fluid, proteins, and blood cells escape from the vascular system into the injured tissue in a process called exudation.

Following changes in the vascular system, which also include changes induced in blood and its components, cellular events occur and characterize the inflammatory response.
The effect of the injury and/or biomaterial in situ on plasma or cells can produce chemical factors that mediate many of the vascular and cellular responses of inflammation.

Blood-material interactions and the inflammatory response are intimately linked, and in fact, early responses to injury involve mainly blood and vasculature.

Regardless of the tissue or organ into which a biomaterial is implanted, the initial inflammatory response is activated by injury to vascularized connective tissue (Ch.4.2: Table 2).

Since blood and its components are involved in the initial inflammatory responses, Blood clot formation and/or thrombosis also occur.

Blood coagulation and thrombosis are generally considered humoral responses and may be influenced by other homeostatic mechanisms such as the extrinsic and intrinsic coagulation systems, the complement system, the fibrinolytic system, the kinin-generating system, and platelets.
• Thrombus or blood clot formation on the surface of a biomaterial is related to the well-known Vroman effect (see Chapter 3.2), in which hierarchical and dynamic series of collision, absorption, and exchange processes, determined by protein mobility and concentration, regulate early time-dependent changes in blood protein adsorption.

• From a wound-healing perspective, blood protein deposition on a biomaterial surface is described as provisional matrix formation. Blood interactions with biomaterials are generally considered under the category of hematocompatibility and are discussed elsewhere in this book.

• Injury to vascularized tissue in the implantation procedure leads to immediate development of the provisional matrix at the implant site.

• This provisional matrix consists of fibrin, produced by activation of the coagulation and thrombosis systems, and inflammatory products released by the complement system, activated platelets, inflammatory cells, and endothelial cells.
The **Vroman effect**

is exhibited **by protein adsorption** to a surface by blood serum proteins.

- The **highest mobility** proteins generally arrive first and are later **replaced** by **less motile proteins** that have a **higher affinity** for the surface.
- The process is **delayed** in narrow spaces and on hydrophobic surfaces that fibrinogen is usually not displaced.
- Under stagnant conditions initial protein **deposition** takes place in the **sequence**: albumin; globulin; fibrinogen; fibronectin; factor XII, and HMWK.
• These events occur **early, within minutes to hours following implantation** of a medical device.

• **Components** within or released from the provisional matrix, i.e., fibrin network (thrombosis or clot), initiate **the resolution, reorganization, and repair processes** such as inflammatory cell and fibroblast recruitment.

• The **provisional matrix** appears **to provide both structural and biochemical components** to the process of wound healing.

• The **complex three-dimensional structure of the fibrin network** with attached adhesive proteins provides a **substrate for cell adhesion and migration**.

• **The presence of** mitogens, chemo-attractants, cytokines, and growth factors within the provisional matrix provides for a rich milieu of **activating and inhibiting substances** for various cellular proliferative and synthetic processes.

• The **provisional matrix** may be viewed as a **naturally derived, biodegradable, sustained release system** in which mitogens, chemo-attractants, cytokines, and growth factors are released to control **subsequent wound-healing processes**.
• In spite of the increase in our knowledge of the provisional matrix and its capabilities, our knowledge of the control of the formation of the provisional matrix and its effect on subsequent wound healing events is poor.

• In part, this lack of knowledge is due to the fact that much of our knowledge regarding the provisional matrix has been derived from in vitro studies, and there is a paucity of in vivo studies that provide for a more complex perspective.

• Little is known regarding the provisional matrix which forms at biomaterial and medical device interfaces in vivo, although attractive hypotheses have been presented regarding the presumed ability of materials and protein adsorbed in materials to modulate cellular interactions through their interactions with adhesive molecules and cells.

• The predominant cell type present in the inflammatory response varies with the age of the inflammatory injury (Ch.4.2: Fig. 1).
• In general, neutrophils predominate during the first several days following injury and then are replaced by monocytes as the predominant cell type.

• Three factors account for this change in cell type:
  – neutrophils are short lived and disintegrate and disappear after 24-48 hour;
  – neutrophil emigration from the vasculature to the tissues is of short duration;
  – chemotactic factors for neutrophil migration are activated early in the inflammatory response.

• Following emigration from the vasculature, monocytes differentiate into macrophages and these cells are very long-lived (up to months).

• Monocyte emigration may continue for day to weeks, depending on the injury and implanted biomaterial, and chemotactic factors for monocytes are activated over longer periods of time.

• The temporal sequence of events following implantation of a biomaterial is illustrated in (Ch.4.2: Fig. 1).
• The size, shape, and chemical and physical properties of the biomaterial may be responsible for variations in the intensity and duration of the inflammatory or wound-healing process.

• Thus, intensity and/or time duration of the inflammatory reaction may characterize the biocompatibility of a biomaterial.

• While injury initiated the inflammatory response, the chemicals released from plasma, cells, or injured tissue mediate the inflammatory response.

• Important classes of chemical mediators of inflammation are presented in Table 3.

• Several points must be noted in order to understand the inflammatory response and how it relates to biomaterials.
  – First, although chemical mediators are classified on a structural or functional basis, different mediator systems interact and provide a system of checks and balances regarding their respective activities and functions.
Second, chemical mediators are quickly inactivated or destroyed, suggesting that their action is predominantly local (i.e., at the implant site).

Third, generally the lysosomal proteases and the oxygen-derived free radicals produce the most significant damage or injury.

These chemical mediators are also important in the degradation of biomaterials. (Ch.4.2: Table 3)
• Acute inflammation is of relatively short duration, lasting for minutes to hours to days, depending on the extent of injury.
• Its main characteristics are the exudation of fluid and plasma proteins (edema) and the emigration of leukocytes (predominantly neutrophils).
• Neutrophils (polymorphonuclear leukocytes, PMNs) and other motile white cells emigrate or move from the blood vessels to the perivascular tissues and the injury (implant) site.
• Leukocyte emigration is assisted by “adhesion molecules” present on leukocyte and endothelial surfaces. The surface expression of these adhesion molecules can be induced, enhanced, or altered by inflammatory agents and chemical mediators.
• White cell emigration is controlled, in part, by chemotaxis, which is the unidirectional migration of cells along a chemical gradient.
• A wide variety of exogenous and endogenous substances have been identified as chemotactic agents.
Specific receptors for chemotactic agents on the cell membranes of leukocytes are important in the emigration or movement of leukocytes. These and other receptors also play a role in the transmigration of white cells across the endothelial lining of vessels and activation of leukocytes.

Following localization of leukocytes at the injury (implant) site, phagocytosis and the release of enzymes occur following activation of neutrophils and macrophages.

The major role of the neutrophil in acute inflammation is to phagocytose microorganisms and foreign materials.

Phagocytosis is seen as a three-step process in which the injurious agent undergoes recognition and neutrophil attachment, engulfment, and killing or degradation.

In regard to biomaterials, engulfment and degradation may or may not occur, depending on the properties of the biomaterial.
Although biomaterials are not generally phagocytosed by neutrophils or macrophages because of the disparity in size (i.e., the surface of the biomaterial is greater than the size of the cell), certain events in phagocytosis may occur.

- The process of recognition and attachment is expedited when the injurious agent is coated by naturally occurring serum factors called “opsonins.”
- The two major opsonins are immunoglobulin G (IgG) and the complement-activated fragment, C3b.
- Both of these plasma-derived proteins are known to adsorb to biomaterials, and neutrophils and macrophages have corresponding cell-membrane receptors for these opsonization proteins.
- These receptors may also play a role in the activation of the attached neutrophil or macrophage.
• Other blood proteins such as **fibrinogen, fibronectin, and vitronectin** may also facilitate **cell adhesion** to biomaterial surfaces.

• Owing to the **disparity in size** between the biomaterial surface and the attached cell, frustrated phagocytosis may occur.

• This process does not involve engulfment of the biomaterial but does cause the **extracellular release of leukocyte products** in an attempt to degrade the biomaterial.

• **Neutrophils** adherent to complement-coated and immunoglobulin-coated nonphagocytosable surfaces may release enzymes by direct extrusion or exocytosis from the cell.

• The **amount of enzyme** released during this process depends on the **size of the polymer particle**, with larger particles inducing greater amounts of enzyme release.
This suggests that the specific mode of cell activation in the inflammatory response in tissue depends upon the size of the implant and that a material in a phagocytosable form (i.e., powder or particulate) may provoke a different degree of inflammatory response than the same material in a nonphagocytosable form (i.e., film).

Acute inflammation normally resolves quickly, usually less than 1 week, depending on the extent of injury at the implant site.

However, the presence of acute inflammation (i.e., PMNs) at the tissue/implant interface at time periods beyond 1 week (i.e., weeks, months, or years) suggests the presence of an infection (Ch.4.2: Fig. 2).