CHAPTER 2

Host reactions to Biomaterials and Their Evaluation
Chronic inflammation is less uniform histologically than acute inflammation. In general, chronic inflammation is characterized by the presence of macrophages, monocytes, and lymphocytes, with the proliferation of blood vessels and connective tissue.

Many factors can modify the course and histologic appearance of chronic inflammation.

Persistent inflammatory stimuli lead to chronic inflammation.

While the chemical and physical properties of the biomaterial in themselves may lead to chronic inflammation, motion in the implant site by the biomaterial or infection may also produce chronic inflammation.

The chronic inflammatory response to biomaterials is usually of short duration and is confined to the implant site.
The presence of mononuclear cells, including lymphocytes and plasma cells, is considered chronic inflammation, whereas the foreign-body reaction with the development of granulation tissue is considered the normal wound healing response to implanted biomaterials (i.e., the normal foreign-body reaction).

Chronic inflammation with the presence of collections of lymphocytes and monocytes at extended implant times (weeks, months, years) also may suggest the presence of a long-standing infection (Ch.4.2: Fig. 3A, B).

Lymphocytes and plasma cells are involved principally in immune reactions and are key mediators of antibody production and delayed hypersensitivity responses. Although they may be present in nonimmunologic injuries and inflammation, their roles in such circumstances are largely unknown.

Little is known regarding humoral immune responses and cell-mediated immunity to synthetic biomaterials.
• The role of macrophages must be considered in the possible development of immune responses to synthetic biomaterials.

• Macrophages process and present the antigen to immuno-competent cells and thus are key mediators in the development of immune reactions.

• Monocytes and macrophages belong to the mononuclear phagocytic system (MPS), also known as the reticuloendothelial system (RES).

• These systems consist of cells in the bone marrow, peripheral blood, and specialized tissues.

• Table 4 lists the tissues that contain cells belonging to the MPS or RES.

• The specialized cells in these tissues may be responsible for systemic effects in organs or tissues secondary to the release of components or products from implants through various tissue-material interactions (e.g., corrosion products, wear debris, degradation products) or the presence of implants (e.g., microcapsule or nanoparticle drug-delivery systems).
The **macrophage** is probable the most important cell in chronic inflammation because of **the great number of** biologically **active products** it can produce.

Important classes of products produced and secreted by macrophages include **neutral proteases**, **chemotactic factors**, **arachidonic acid metabolites**, **reactive oxygen metabolites**, **complement components**, **coagulation factors**, **growth-promoting factors**, and **cytokines**.

Growth factors such as **platelet-derived growth factor (PDGF)**, **fibroblast growth factor (FGF)**, **transforming growth factor (TGF-α, TGF-β)**, **epidermal growth factor (EGF)**, and **interleukin-1 (IL-1) or tumor necrosis factor (TNF-α)** are important **to the growth of fibroblasts and blood vessels and the regeneration of epithelial cells**.

Growth factors released by activated cells can **stimulate production** of a wide variety of cells; initiate cell migration, differentiation, and tissue remodeling; and may be involved in various stages of wound healing.
Within **1 day** following implantation of a biomaterial (i.e., injury), the healing response is initiated by the action of monocytes and macrophages. Fibroblasts and vascular endothelial cells in the implant site proliferate and begin to form granulation tissue, which is the specialized type of tissue that is the hallmark of healing inflammation. Granulation tissue derives its name from the pink, soft granular appearance on the surface of healing wounds, and its characteristic histologic features include the proliferation of new small blood vessels and fibroblasts (*Ch.4.2: Fig. 4*).

Depending on the extent of injury, granulation tissue may be seen as early as **3-5 days** following implantation of a biomaterial. The new small blood vessels are formed by budding or sprouting of preexisting vessels in a process known as neovascularization or angiogenesis. This process involves proliferation, maturation, and organization of endothelial cells into capillary vessels.
• Fibroblasts also proliferate in developing granulation tissue and are active in synthesizing collagen and proteoglycans.

• In the early stages of granulation tissue development, proteoglycans predominate but later collagen, especially type III collagen, predominates and forms the fibrous capsule.

• Some fibroblasts in developing granulation tissue may have the features of smooth muscle cells, i.e., actin microfilaments. These cells are called myofibroblasts and area considered to be responsible for the wound contraction seen during the development of granulation tissue.

• Macrophages are almost always present in granulation tissue. Other cells may also be present if chemotactic stimuli are generated.

• The wound-healing response is generally dependent on the extent or degree of injury or defect created by the implantation procedure.

• Healing of clean surgical incisions by primary union or first intention occurs without significant bacterial contamination and with a minimal loss of tissue.
• Wound healing by secondary union or second intention occurs when there is a large tissue defect that must be filled or there is extensive loss of cells and tissue.

• In wound healing by secondary intention, regeneration of parenchymal cells cannot completely reconstitute the original architecture and much larger amounts of granulation tissue are formed that result in larger areas of fibrosis or scar formation.

• Under these conditions, different regions of tissue may show different stages of the wound-healing process simultaneously. Granulation tissue is distinctly different from granulomas, which are small collections of modified macrophages called epithelioid cells.

• Langhans’ or foreign-body-type giant cells may surround nonphagocytosable particulate materials in granulomas. Foreign-body giant cells are formed by the fusion of monocytes and macrophages in an attempt to phagocytose the material (Ch.4.2: Fig. 5).
The foreign-body reaction to biomaterials is composed of foreign-body giant cells (FBGCs) and the components of granulation tissue (e.g., macrophages, fibroblasts, and capillaries in varying amounts, depending upon the form and topography of the implanted material; *(Ch.4.2: Fig. 6).*

- Relatively flat and smooth surfaces such as that found on breast prostheses have a foreign-body reaction that is composed of a layer of macrophages one to two cells in thickness.
- Relatively rough surfaces such as those found on the outer surfaces of expanded poly tetrafluoroethylene (ePTFE) or Dacron vascular prostheses have a foreign-body reaction composed of macrophages and foreign-body giant cells at the surface.
• Fabric materials generally have a surface response composed of macrophages and foreign body giant cells, with varying degrees of granulation tissue subjacent to the surface response (Ch.4.2: Fig. 7).

• As previously discussed, the form and topography of the surface of the biomaterial determine the composition of the foreign-body reaction.

• With biocompatible materials, the composition of the foreign-body reaction in the implant site may be controlled by the surface properties of the biomaterial, the form of the implant, and the relationship between the surface area of the biomaterial and the volume of the implant.
• For example, **high-surface-to-volume implants** such as fabrics, porous materials, particulate, or microspheres will have higher ratios of macrophages and foreign-body giant cells in the implant site than smooth-surface implants, which will have **fibrosis** as a significant component of the implant site.

• The **foreign-body reaction** consisting mainly of macrophages and/or foreign-body giant cells may persist at the tissue-implant interface for the lifetime of the implant (Fig. 1).

• Generally, **fibrosis** (i.e., fibrous encapsulation) surrounds the biomaterial or implant with its interfacial foreign-body reaction, **isolating** the implant and foreign-body reaction from the local tissue environment.

• **Early in the inflammatory** and wound-healing response, the **macrophages** are activated upon **adherence** to the material surface. Although it is generally considered that the **chemical and physical properties of the biomaterial** are responsible for macrophage activation, the subsequent events regarding the activity of macrophages at the surface are **not clear**.
• Tissue macrophages, derived from circulating **blood monocytes**, may coalesce to form **multinucleated foreign-body giant cells** containing large numbers of nuclei on the surface of biomaterials.

• While these **foreign-body giant cells** may persist for the lifetime of the implant, it is **not known** if they remain **activated**, releasing their lysosomal constituents, or become quiescent.

• Figure 5 demonstrates the **progression from circulating blood monocyte to tissue macrophage to foreign-body giant cell development** that is most commonly observed. Indicated in the figure are important biological responses that are considered **to play an important role** in FBGC development.

• **Material surface chemistry** may control **adherent macrophage apoptosis** (i.e., programmed cell death) (see Chapter 3.3) that tenders potentially harmful macrophages nonfunctional, while the surrounding environment of the implant remains unaffected.
• The level of adherent macrophage apoptosis appears to be inversely related to the surface’s ability to promote diffusion of macrophages into FBGCs, suggesting a mechanism for macrophages to escape apoptosis.

• Figure 8 demonstrates the sequence of events involved in inflammation and wound healing when medical devices are implanted.

• In general, the PMN predominant acute inflammatory response and the lymphocyte/monocyte predominant chronic inflammatory response resolve quickly (i.e., within 2 weeks) depending on the type and location of the implant.
The end-stage healing response to biomaterials is generally fibrosis or fibrous encapsulation (*Ch.4.2: Fig. 9*).

However, there may be exceptions to this general statement.

(e.g., porous materials inoculated with parenchymal cells or porous materials implanted into bone) (*Ch.4.2: Fig. 10*).

As previously stated, the tissue response to implants is in part dependent upon the extent of injury or defect created in the implantation procedure and the amount of provisional matrix.
• Repair of implant sites can involve **two distinct processes**: regeneration, which is the replacement of injured tissue by parenchymal cells of the same type, or replacement by connective tissue that constitutes the fibrous capsule.

• **These processes** are generally controlled by either
  – (1) **the proliferative capacity of the cells** in the tissue or organ receiving the implant and the extent of injury as it relates to the destruction, or
  – (2) **persistence of the tissue framework** of the implant site. (organ parenchyma and stroma).

• The **regenerative capacity of cells** allows them to be classified into three groups: labile, stable (or expanding), and permanent (or static) cells.
• Perfect repair with restitution of normal structure can theoretically occur only in tissues consisting of stable and labile cells, whereas all injuries to tissues composed of permanent cells may give rise to fibrosis and fibrous capsule formation with very little restitution of the normal tissue or organ structure.

• Tissues composed of permanent cells (e.g., nerve cells and cardiac muscle cells) most commonly undergo an organization of the inflammatory exudate, leading to fibrosis.

• Tissues of stable cells (e.g., parenchymal cells of the liver, kidney, and pancreas); mesenchymal cells (e.g., fibroblasts, smooth muscle cells, osteoblasts, and chondroblasts); and vascular endothelial and labile cells (e.g., epithelial cells and lymphoid and hematopoietic cells) may also follow this pathway to fibrosis or may undergo resolution of the inflammatory exudate, leading to restitution of the normal tissue structure.
• The **condition** of the underlying framework or supporting stroma of the parenchymal cells following an injury plays an **important role** in the **restoration** of normal tissue structure.

• **Retention of the framework** with injury may lead to **restitution** of the normal tissue stricture, whereas **destruction of the framework** most commonly leads to **fibrosis**.

• It is important to consider the **species dependent nature** of the regenerative capacity of cells. For example, **cells** from the same organ or tissue but from **different species** may exhibit **different regenerative capacities** and/or connective tissue repair.

• Following injury, **cells** may undergo **adaptations** of growth and differentiation. Important cellular adaptations are **atrophy** (decrease in cell size or function), **hypertrophy** (increase in cell size), **hyperplasia** (increase in cell number), and **metaplasia** (change in cell type).
- Other adaptations include a change by cells from producing one family of proteins to another (phenotypic change), or marked overproduction of protein.
  - This may be the case in cells producing various types of collagens and extracellular matrix proteins in chronic inflammation and fibrosis.
- Causes of atrophy may include decreased workload (e.g., stress-shielding by implants), and diminished blood supply and inadequate nutrition (e.g., fibrous capsules surrounding implants).
- Local and systemic factors may play a role in the wound-healing response to biomaterials or implants.
  - **Local factors** include the site (tissue or organ) of implantation, the adequacy of blood supply, and the potential for infection.
  - **Systemic factors** may include nutrition, hematologic derangements, gluco-cortical steroids, and preexisting diseases such as atherosclerosis, diabetes, and infection.
Finally, the implantation of biomaterials or medical devices may be best viewed at present from the perspective that the implant provides an impediment or hindrance to appropriate tissue or organ regeneration and healing.

Given our current inability to control the sequence of events following injury in the implantation procedure, restitution of normal tissue structures with function is rare.

Current studies directed toward developing a better understanding of the modification of the inflammatory response, stimuli providing for appropriate proliferation of permanent and stable cells, and the appropriate application of growth factors may provide keys to the control of inflammation, wound healing, and fibrous encapsulation of biomaterials.
2.3 SYSTEMIC TOXICITY AND HYPERSENSITIVITY

- Artificial implant devices comprise a variety of **metallic alloys, polymers, ceramics, hydrogels, or composites** for a large number of purposes and with widely different properties.

- With the exception of drug delivery systems, sutures, and other degradable biomaterial systems, **the implant devices are intended to resist** chemical and biochemical degradation and to have minimal leaching of structural components or additives.

- However, synthetic devices **are influenced by chemical** and in some cases enzymatic processes resulting in the **release** of biomaterials-associated components.

- With the exception of pathologic calcification of certain polymer implants, the **surface changes may not be significant for the mechanical strength** of the implant, whereas in contrast the **released substances** very often have **biological effects** on the surrounding tissues or, possibly, at other **remote locations**.

- The following discussion is concerned with **the possibility of systemic toxic reactions and/or hypersensitive reactions** caused by biomaterials-derived **xenobiotics**
• Xenobiotic components derived from in vivo medical devices have parenteral contact with connective tissue or other specialized tissues such as bone, dentin, and vascular or ocular tissue, whereas leachables from skin-and mucosa-contacting devices have to pass the epithelial lining of the oral mucosa, the skin, the gastrointestinal tract, or-for volatiles-the lung alveoli to get “inside” the body.

• In either case, further distribution of foreign substances to other tissues and organs is dependent on membrane diffusion into blood capillaries and lymph vessels.

• The transport may be facilitated by reversible binding to plasma proteins, globulins (metal, metal compounds), and chylomicrons (lipophilic substances).

• Storage-and later release-may take place for certain components in tissues such as fat and bone.

• In addition to particulate matter the released components consist of chemical substances of different atomic and molecular size, solubility, and other chemical characteristics depending on the mother material.
• Examples are **metal ions** such as cobalt, chromium, nickel, molybdenum, and titanium from metallic orthopedic implants or prosthodontic materials, or **residual monomers, chemical initiators, inhibitors, plasticizers, antioxidants, etc.**, from polymer implants and dental materials.

• Other **degradation products** from inorganic, organic, and composite devices also “rub off” to the surrounding tissues.

• The **kinetic mechanisms** for biomaterials components are **in part the same** as those of xenobiotics introduced **by food or environmental exposure**, i.e., the released components are subject to oxidation, reduction, and hydrolysis followed by conjugation mechanisms.

• All **metabolic changes** are in their nature intended to **eliminate** them by way of the urine, bile, lungs, and to a certain degree in salivary, sweat, and mammary glands and hair.

• A **key question** is, do the **released components or their metabolites** have any **systemic toxic effect** on the host and/or could they **induce unwanted immunological reactions**?
Systemic toxicity depends on toxic substances hitting a target organ with high sensitivity to a specific toxicant.

Target organs are the central nervous system, the hematopoietic system, the circulatory system, and visceral organs such as liver, kidney, and lungs, in that order.

The toxicity is based on interference with key cell functions and depends on the dose and the duration of the exposure.

Serious effects may be incompatible with continued life, but most effects are local and reversible cell damage.

However, some sublethal effects may include somatic cell mutation expressed as carcinogenesis, or germinal cell mutation, resulting in reproductive toxicity.

The key word in the evaluation of general toxicity is the dose, defined as the amount of a substance an organism is exposed to, usually expressed as mg per kg body weight.
Adverse effects of foreign substances are often the result of repeated, chronic exposure to small doses that over a prolonged period of time may have deleterious effects similar to one large, short time exposure, provided that the repeated doses exceed a certain threshold level.

This level is determined by the capacity of metabolism and elimination.

Another important factor is the possibility of synergistic potentiating effects when several toxicants are present simultaneously.

Whatever mechanism is involved, the principle of systemic toxicity presupposes a dose dependent reaction that may be measured and described, and that may be explained by specific reactions at distinct molecular sites.

The components derived from biomaterials represent a large series of widely different foreign substances with few characteristics in common and with a largely unknown concentration.
Most of them have to be characterized as toxic per se, with large variations regarding their place on a ranking list of potential toxicity.

Metal ions and salts derived from biomaterials devices, such as mercury, nickel, and chromium, are classified as toxicants.

A similar statement could be made for components associated with polymeric materials.

However, clinically relevant data on the concentration of degradation products are scarce, e.g., phthalate additives and degradation products from chemical additives derived from poly (methyl methacrylate) dental prostheses have been quantified in saliva.

In vitro experiments have shown that chromium and nickel are released from base metal orthodontic appliances, although the amounts are not comparable with the amounts calculated in food intake. In addition, the proportion of uptake by mucosa is unknown.
The presence of leachable substances has also been demonstrated in the surrounding tissues of implants, but quantification is difficult.

Information is available on the release and uptake of mercury derived from dental amalgam.

– For example a series of studies has shown the presence of mercury in plasma and urine after inhalation of metallic mercury released from dental amalgam.

– Accumulation of mercury in tissues belonging to the central nervous system has been shown after occupational exposure.

Reproductive toxicity has been of specific concern. However, similar to other metals such as chromium and nickel, mercury exposure also takes place through food and through respiratory air.

Careful scrutiny of the large number of partly controversial data by national and international scientific committees has not resulted in a consensus conclusion that the application of mercury amalgam should be discontinued as a dental biomaterial, although mercury is a significant environmental concern.
When occupational exposure is disregarded, the possibility of systemic toxicity or reproductive toxicity has not been seriously considered for other biomaterial components or metabolites, because of their low concentration as compared with their toxic potential.

A fair conclusion at this point would be that there are no data indicating any systemic toxicity caused by biomaterials-derived xenobiotics.

However, this field of interest is characterized by the increasing number of synthetic biomaterials on the market.

Despite the premarketing testing programs it is difficult to predict single or synergistic toxic effects of leachable components and degradation products in the future.
• **The low probability** of direct *systemic adverse effects* on target organs caused by biomaterial products does *not rule out deleterious effects* by other, *dose-independent mechanisms*.

• **All substances** not recognized as natural components of the tissues are *subject to possible clearance* by several mechanisms, e.g., phagocytic cells such as polymorphonuclear leukocytes, macrophages, and monocytes attempt to degrade and export the components.

• **Larger foreign components** are subject to *more aggressive reactions* by giant cells causing an inflammatory foreign-body reaction. *Enzymes and other bioactive molecules* associated with the phagocytosis and foreign-body reaction may cause *severe local tissue damage*.

• In addition, **phagocytic cell** contact and the contact with the circulatory system of lymph and blood opens up another way of neutralizing foreign substances by way of the immune system, introducing a *biologic memory of previously encountered foreign substances* and an enhancement system for their neutralization.
The immune system is an indispensable biologic mechanism to fight potentially adverse invaders, most commonly of microbial origin. However, the immune system occasionally strikes invading molecules-adverse or not-with an intensity that stands in contrast to the sometimes minute amounts of foreign substances, and with the ability to cause host tissue damage. This phenomenon is called hypersensitivity. The resulting injury is part of a group of adverse reactions classified as immunotoxic. In principle, immunologic hypersensitivity comprises two different mechanisms:

- Allergy is a acquired condition resulting in an overreaction upon contact with a foreign substance, given a genetic disposition and previous exposure to the substance. Allergic reactions may include asthma, rhinitis, urticarial, intraoral and systemic symptoms, and eczema.

- Intolerance is an inherited reaction that resembles allergy and has common mediators and potentiating factors, such as complement activation, and histamine release, but is not dependent on a previous sensitization process. The intolerance reactions have been associated with drugs such as acetylsalicylic acid, whereas intolerance to leachable biomaterial components such as benzoic acid is conceivable but not known.
A foreign substance able to induce an allergic reaction is called an allergen.

There is no acceptable way of predicting whether a substance or a compound is potentially allergenic only on the basis of its chemical composition and/or structure.

However, experimental evidence and years of empirical results after testing substances causing allergic reactions have given some leads, e.g., large foreign molecules such as proteins and nucleoproteins are strong allergens, whereas lipids are not.

However, the strongest chemical allergens associated with biomaterials are often chemically active substances of low molecular weight, often less than 500 Da, such as lipid soluble organic substances derived from polymer materials or metal ions and metal salts.

These are called haptens, i.e., they become full allergens only after reaction or combination with proteins that may be present in macrophages and Langerhans cells of the host.
The allergies are most often categorized into four main groups (type-I-IV) according to the reaction mechanisms.

- The types I to III are associated with humoral antibodies initiated by B-lymphocytes that develop to immunoglobulin-producing plasma cells.
- The immunoglobulins are classified into five different classes, Ig E, A, D, G, and K, according to their basic structure and size. A variable portion of the immunoglobulin is specific for the antigen that induced its production.

- The type IV reaction is a cell-mediated reaction caused by T-lymphocytes.
- The types II and III allergies comprise antigen/antibody encounters including complement activation, cell lysis, release of vasoactive substances, inflammatory reaction, and tissue damage.

- Necrosis of peri-implant tissue with histologic appearance and serum complement analyses consistent with Type III hypersensitivity has been observed in cases of atypical loosening of total hip prostheses.

- However, an FDA document omits the type II and III reactions for reasons of being “relatively rare and less likely to occur with medical devices/materials” leaving the types I and IV as relevant in the present context.
The type I reaction is based on an interaction between an intruding allergen and IgE immunoglobulins located in mast cells, basophils, eosinophils, and platelets, resulting in release of active mediators such as histamine and other vasoactive substances.

The results are local or systemic reactions seen within a short time (minutes).

The symptoms depend on the tissue or organ subject to sensitization, e.g.,

- (1) inhaled allergens such as pollen or residual proteins associated with surgical latex gloves or other natural latex products that may result in asthmatic seizures, swelling of the mucosa of the throat, or worse; or

- (2) decreased blood pressure and anaphylactic shock. Food allergies may also give systemic symptoms.

This type of host reaction is usually associated with full antigens.

Since the potential allergens associated with biomaterials are small molecular haptens, the probability of IgE-based allergic reactions is low, although IgE antibodies to chromium and nickel have been reported.
The cell-mediated hypersensitivity is referred to as “delayed” because it takes more than 12 hours to develop, often 24-72 hours.

Prolonged challenges of macrophage-resistant allergens, usually of microbial origin, may result in persistent immunological granuloma formation.

The T-lymphocytes producing the response have been sensitized by a previous encounter with an allergen and act in concert with other lymphocytes and mononuclear phagocytes to create four histologically different types characterized by skin-related tissue reactions. The reactions are elicited by interaction of cells and mediators that comprise

- (1) swelling (the Jones-Mote type);
- (2) induration (the granulomatous type);
- (3) swelling and induration and possibly fever (the tuberculin type); and
- (4) eczema (the contact type).

The latter form of delayed hypersensitivity has been of specific importance in relation to biomaterials.
• **Most information on this reaction** has been obtained by studying the reaction patterns following **exposure to external environmental and occupation-related chemicals**.

• **Allergic contact dermatitis** is acquired through **previous sensitization** with a foreign substance.
  
  – The **hapten** is absorbed by the skin or mucosa and binds to certain proteins associated with the **Langerhans cells**, forming a complete **antigen**.
  
  – The **antigen** is brought in contact with **the regional lymph nodes**, resulting in the formation of activated, specialized **T cells** that are brought into circulation.

• **Upon new exposure**, the allergen may again be transported from the site of entrance. The new **contact** between the **allergen** and the activated, specialized **T cells** releases **inflammatory mediators**, resulting in further **production and attraction of T cells causing tissue damage**.
• The presence of **allergic contact dermatitis** is evaluated by allergologists or dermatologists by applying the **suspected haptens** using **epidermal or intradermal skin tests** and reading the dermal or epidermal reaction after specified amounts of time.

• **Commercial test kits** for epidermal testing are available for a series of chemical substances related to different occupations. A **vast amount of information** on the allergenic characteristics of **biomaterials-related substances** has been obtained in this way, especially as regards dental materials.

• Many biomaterials employed in dentistry such as **metal alloys** and **resin-based materials** have medical counterparts, and both categories of biomaterials have materials counterparts **met with in everyday life**.

• The **sensitization process** therefore often has **taken place before** the biomaterials contact.
Atopic individuals have a constitutional predisposition for IgE-based hypersensitive reactions caused by environmental and food allergens. The reactions include histamine-mediated hay fever, asthma, gastrointestinal symptoms, or skin rashes and are more pronounced at an early age.

Atopics have an increased risk of acquiring irritant contact dermatitis to external biomaterial devices such as orthodontic appliances.

The relation to allergic contact dermatitis is unclear; so also is the relationship between atopy and allergens or haptens from biomaterials exposed parenterally.