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BIOLOGICAL RESPONSES TO MATERIALS

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■ **Abstract** All materials intended for application in humans as biomaterials, medical devices, or prostheses undergo tissue responses when implanted into living tissue. This review first describes fundamental aspects of tissue responses to materials, which are commonly described as the tissue response continuum. These actions involve fundamental aspects of tissue responses including injury, inflammatory and wound healing responses, foreign body reactions, and fibrous encapsulation of the biomaterial, medical device, or prosthesis. The second part of this review describes the in vivo evaluation of tissue responses to biomaterials, medical devices, and prostheses to determine intended performance characteristics and safety or biocompatibility considerations. While fundamental aspects of tissue responses to materials are important from research and development perspectives, the in vivo evaluation of tissue responses to these materials is important for performance, safety, and regulatory reasons.

INTRODUCTION

The goal of this review is to provide material scientists and engineers with an appreciation of the fundamental aspects of tissue responses to materials, as well as the in vivo evaluation of tissue responses to materials. Fundamental aspects of tissue responses to materials include the tissue response continuum, which is initiated when a material (biomaterial), medical device, or prosthesis is implanted in living tissue. The tissue response continuum is the series of responses that are initiated by the implantation procedure, as well as by the presence of the biomaterial, medical device, or prosthesis. The fundamental aspects of the tissue response continuum are viewed from the classical medical perspective of the pathologist. It includes our current understanding of inflammatory and wound healing responses, foreign body reactions, and ultimately fibrous encapsulation (scar formation) of the biomaterial, medical device, or prosthesis.

The second part of this review addresses the in vivo evaluation of tissue responses to materials. From a practical perspective, i.e. manufacturing, clinical, and regulatory, the in vivo evaluation of prostheses and medical devices, i.e. biomaterials in their ready-to-use form, is necessary to determine their biocompatibility.

Biocompatibility is generally defined as the ability of a biomaterial, prosthesis, or medical device to perform with an appropriate host response in a specific application, and biocompatibility assessment, i.e. evaluation of biological responses, is a measure of the magnitude and duration of the adverse alterations in homeostatic mechanisms that determine the host response. Practically speaking, the evaluation of biological responses to a medical device is carried out to determine that the medical device performs as intended and presents no significant harm to the patient or user. Thus the goal of biological response evaluation is to predict whether a biomaterial, medical device, or prosthesis presents potential harm to the patient or user by evaluating conditions that simulate clinical use.

FUNDAMENTAL ASPECTS OF TISSUE RESPONSES TO MATERIALS

Injury

The process of implantation of a biomaterial, prosthesis, or medical device results in injury to tissues or organs (1, 2). It is this injury and the subsequent perturbation of homeostatic mechanisms that lead to the cellular cascades of wound healing. The response to injury is dependent on multiple factors including the extent of injury, the loss of basement membrane structures, blood-material interactions, provisional matrix formation, the extent or degree of cellular necrosis, and the extent of the inflammatory response. These events, in turn, may affect the extent or degree of granulation tissue formation, foreign body reaction, and fibrosis or fibrous capsule development. These events are summarized in Table 1. The host reactions are considered to be tissue, organ, and species dependent. In addition, it is important to recognize that these reactions occur very early, i.e. within 2 to 3 weeks of the time of implantation.

TABLE 1 Sequence of host reactions following implantation of medical devices

Injury
Blood-material interactions
Provisional matrix formation
Acute inflammation
Chronic inflammation
Granulation tissue
Foreign body reaction
Fibrosis/fibrous capsule development

In considering these early host reactions following injury, it is important to consider whether tissue resolution or organization occurs within the injured tissue or organ. In situations where injury has occurred and exudative inflammation is present, but no cellular necrosis or loss of basement membrane structures has occurred, the process of resolution occurs. Resolution is the restitution of the pre-existing architecture of the tissue or organ. On the other hand, with necrosis, granulation tissue grows into the inflammatory exudate and the process of organization with development of fibrous tissue occurs. With implants, the process of organization with development of fibrous tissue leads to the well-known fibrous capsule formation at the tissue/material interface. The proliferative capacity of cells within the tissue or organ also plays a role in determining whether resolution or organization occurs. In general, the process of implantation in vascularized tissues leads to organization with fibrous tissue development and fibrous encapsulation.

Blood-Material Interactions and Initiation of the Inflammatory Response

Blood-material interactions and the inflammatory response are intimately linked and, in fact, early responses to injury involve mainly blood and the vasculature (1–4). Regardless of the tissue or organ into which a biomaterial is implanted, the initial inflammatory response is activated by injury to vascularized connective tissue (Table 2). Because blood and its components are involved in the initial inflammatory responses, thrombi and/or blood clots also form. Thrombus formation involves activation of 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 of protein adsorption. From a wound-healing perspective, blood protein deposition on a biomaterial surface is described as provisional matrix formation.

Immediately following injury, changes occur 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 (3–6). 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. Although injury initiates the inflammatory response, released chemicals from plasma, cells, and injured tissue mediate the response. Important classes of chemical mediators of inflammation are presented in Table 3. Several important 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

TABLE 2 Cells and components of vascularized connective tissue

Intravascular (blood) cells
Erythryocytes (RBC)
Neutrophils
Monocytes
Eosinophils
Lymphocytes
Basophils
Platelets
Connective tissue cells
Mast cells
Fibroblasts
Macrophages
Lymphocytes
Extracellular matrix components
Collagens
Elastin
Proteoglycans
Fibronectin
Laminin

are quickly inactivated or destroyed, suggesting that their action is predominantly local (i.e. at the implant site). Third, generally the lysosomal proteases and oxygen-derived free radicals produce the most significant damage or injury. These chemical mediators are also important in the degradation of biomaterials.

The predominant cell type present in the inflammatory response varies with the age of the injury. 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: (*a*) Neutrophils are short-lived and disintegrate and disappear after 24 to 48 h; neutrophil emigration is of short duration because chemotactic factors for neutrophil migration are activated early in the inflammatory response. (*b*) Following emigration from the vasculature, monocytes differentiate into macrophages, and these cells are very long-lived (up to months). (*c*) Monocyte emigration may continue for days to weeks, depending on the injury and implanted biomaterial, and chemotactic factors for monocytes are activated over longer periods of time.

Provisional Matrix Formation

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

TABLE 3 Important chemical mediators of inflammation derived from plasma, cells or injured tissue

Mediators	Examples
Vasoactive agents	Histamines, serotonin, adenosine, endothelial-derived relaxing factor (EDRF), prostacyclin, endothelin, thromboxane a_2
Plasma proteases	
Kinin system	Bradykinin, kallikrein
Complement system	C3a, C5a, C3b, C5b-C9
Coagulation/fibrinolytic system	Fibrin degradation products, activated Hageman factor (FXIIA), tissue plasminogen activator (tPA)
Leukotrienes	Leukotriene B_4 (LTB_4), hydroxyeicosa-tetraenoic acid (HETE)
Lysosomal proteases	Collagenase, elastase
Oxygen-derived free radicals	H_2O_2, superoxide anion
Platelet activating factors	Cell membrane lipids
Cytokines	Interleukin 1 (IL-1), tumor necrosis factor (TNF)
Growth factors	Platelet derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor (TGF-α or TGF-β), epithelial growth factor (EGF)

matrix consists of fibrin, produced by activation of the coagulative and thrombosis systems, and inflammatory products, released by the complement system, activated platelets, inflammatory cells, and endothelial cells (7–9). 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. Platelets, activated during the fibrin network formation, release platelet factor 4, platelet-derived growth factor (PDGF), and transforming growth factor β (TGF-β), which contribute to fibroblast recruitment (10, 11). Upon activation, monocytes and lymphocytes generate additional chemotactic factors, including LTB_4, PDGF, and TGF-β, to recruit fibroblasts.

Fibrin, the major component of the provisional matrix, has been shown to play a key role in the development of neovascularization, i.e. angiogenesis. Implanted porous surfaces filled with fibrin exhibit new vessel growth within four days. The intensity of this angiogenic response is enhanced when zymosan-activated serum or PDGF is incorporated in the fibrin matrix (12).

The provisional matrix is composed of adhesive molecules such as fibronectin and thrombospondin bound to fibrin, as well as platelet granule components

released during platelet aggregation. Platelet granule components include thrombospondin, released from the platelet α-granule, and cytokines, including TGF-α, TGF-β, PDGF, platelet factor 4, and platelet-derived endothelial cell growth factor. The provisional matrix is stabilized by the cross-linking of fibrin by factor XIIIa.

The provisional matrix appears to furnish 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, chemoattractants, cytokines, and growth factors within the provisional matrix supplies 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, chemoattractants, cytokines, and growth factors are released to control subsequent wound healing processes (13–18). In spite of the rapid 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 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 that forms at biomaterial and medical device interfaces in vivo. Attractive hypotheses have been presented regarding the presumed ability of materials and protein adsorbed materials to modulate cellular interactions through their interactions with adhesive molecules and cells.

Temporal Sequence of Inflammation and Wound Healing

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 (3, 4).

The sequence of events following implantation of a biomaterial is illustrated in Figure 1. The size, shape, and chemical and physical properties of the biomaterial and the physical dimensions and properties of the prosthesis or device may be responsible for variations in the intensity and time duration of the inflammatory and wound healing processes. Thus intensity and/or time duration of inflammatory reaction may characterize the biocompatibility of a biomaterial, prosthesis, or device.

In general, the biocompatibility of a material with tissue has been described in terms of the acute and chronic inflammatory responses and of the fibrous capsule formation that is seen over various time periods following implantation (19, 20).

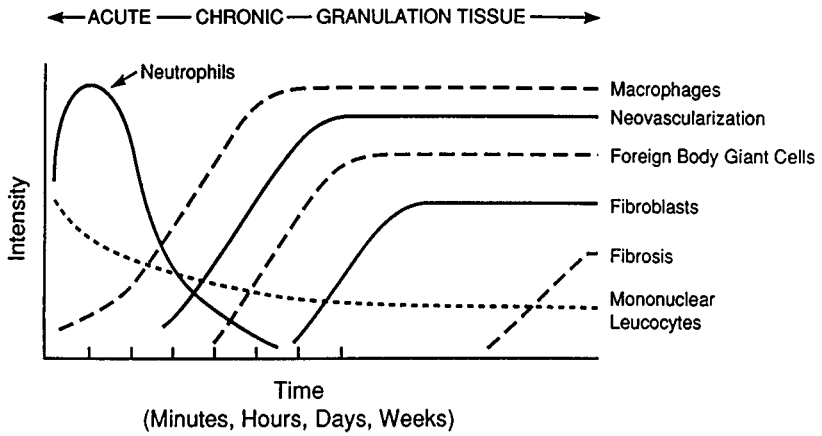

Figure 1 The temporal variation in the acute inflammatory response, chronic inflammatory response, granulation tissue development, and foreign body reaction to implanted biomaterials. The intensity and time variables are dependent upon the extent of injury created in the implantation and the size, shape, topography, and chemical and physical properties of the biomaterial.

Histological evaluation of tissue adjacent to implanted materials as a function of implant time has been the most commonly used method of evaluating the biocompatibility. Classically, the biocompatibility of an implanted material has been described in terms of the morphological appearance of the inflammatory reaction to the material; however, the inflammatory response is a series of complex reactions involving various types of cells, the densities, activities, and functions of which are controlled by various endogenous and autocoid mediators. The simplistic view of the acute inflammatory response progressing to the chronic inflammatory response may be misleading with respect to biocompatibility studies and the inflammatory response to implants. Studies using the cage implant system show that monocytes and macrophages are present in highest concentrations when neutrophils are also at their highest concentrations, i.e. the acute inflammatory response (21, 22). Neutrophils have short lifetimes—hours to days—and disappear from the exudate more rapidly than do macrophages, which have lifetimes of days to weeks to months. Eventually macrophages become the predominant cell type in the exudate, resulting in a chronic inflammatory response. Monocytes rapidly differentiate into macrophages, the cells principally responsible for normal wound healing in the foreign body reaction. Classically, the development of granulation tissue has been considered to be a part of chronic inflammation, but because of unique tissue-material interactions, it is preferable to differentiate the foreign body reaction—with its varying degree of granulation tissue development, including macrophages, fibroblasts, and capillary formation–from chronic inflammation.

Acute Inflammation

Acute inflammation is of relatively short duration, lasting from minutes to days, depending on the extent of injury. The main characteristics of acute inflammation are the exudation of fluid and plasma proteins (edema) and the emigration of leukocytes (predominantly neutrophils). Neutrophils and other motile white cells emigrate or move from the blood vessels to the perivascular tissues and the injury (implant) site (23–25).

The accumulation of leukocytes, in particular neutrophils and monocytes, is the most important feature of the inflammatory reaction. Leukocytes accumulate through a series of processes including margination, adhesion, emigration, phagocytosis, and extracellular release of leukocyte products (26). Increased leukocytic adhesion in inflammation involves specific interactions between complementary adhesion molecules present on the leukocyte and endothelial surfaces (27, 28). The surface expression of these adhesion molecules is modulated by inflammatory agents; mechanisms of interaction include stimulation of leukocyte adhesion molecules (C5a, LTB_4), stimulation of endothelial adhesion molecules (IL-1), or both effects, i.e. tumor necrosis factor (TNF). Integrins make up a family of transmembrane glycoproteins that modulate cell-matrix and cell-cell relationships by acting as receptors to extracellular protein ligands and also as direct adhesion molecules (29). An important group of integrins (adhesion molecules) on leukocytes include the CD11/CD18 family of adhesion molecules. These integrins have identical beta (CD18) subunits but different alpha (CD11a, b, c) subunits. Inflammatory mediators, i.e. cytokines, stimulate a rapid increase in these adhesion molecules on the leukocyte surface, as well as increased leukocyte adhesion to endothelium. Leukocyte-endothelial cell interactions are also controlled by endothelial-leukocyte adhesion molecules (ELAMs, E-selectins) or intracellular adhesion molecules (ICAM-1, ICAM-2, and VCAMs) on endothelial cells (30).

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 (5, 23–34). Important to the emigration or movement of leukocytes is the presence of specific receptors for chemotactic agents on the cell membranes of leukocytes. These and other receptors may also play a role in the 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 neutrophils 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. With 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 size disparity (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 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. Because of the size disparity between the biomaterial surface and the attached cell, frustrated phagocytosis may occur (31, 32). 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 non-phagocytosable surfaces may release enzymes by direct extrusion or exocytosis from the cell (31, 32). 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 is dependent upon the size of the implant and that a material in a phagocytosable form (e.g. powder or particulate) may provoke a degree of inflammatory response different from that of the same material in a non-phagocytosable form (e.g. film).

Chronic Inflammation

Chronic inflammation is less uniform histologically than is 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 (3, 4, 35, 36). It must be noted that many factors modify the course and histological appearance of chronic inflammation.

Persistent inflammatory stimuli lead to chronic inflammation. Although the chemical and physical properties of the biomaterial may lead to chronic inflammation, motion in the implant site by the biomaterial may also produce chronic inflammation. The chronic inflammatory response to biomaterials is confined to the implant site. Inflammation with the presence of mononuclear cells, including lymphocytes and plasma cells, is given the designation chronic inflammation, whereas the foreign body reaction with granulation tissue development is considered the normal wound healing response to implanted biomaterials (i.e. the normal foreign body reaction).

Lymphocytes and plasma cells are involved principally in immune reactions and are key mediators of antibody production and delayed hypersensitive responses. Their roles in non-immunologic injuries and inflammation 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 immunocompetent cells and thus are key mediators in the development of immune reactions.

The macrophage is probably the most important cell in chronic inflammation because of the great number of biologically active products it produces (35). 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 PDGF, FGF, TFG-β, TGF-α/EGF, and IL-1 or TNF are important to the growth of fibroblasts and blood vessels and the regeneration of epithelial cells. Growth factors, released by activated cells, stimulate production of a wide variety of cells; they initiate cell migration, differentiation, and tissue remodeling and may be involved in various stages of wound healing (37–42). It is clear that there is a lack of information regarding interaction and synergy among various cytokines and growth factors and their abilities to exhibit chemotactic, mitogenic, and angiogenic properties.

Granulation Tissue

Within one day following implantation of a biomaterial (i.e. injury), the healing response is initiated by the action of monocytes and macrophages, followed by proliferation of fibroblasts and vascular endothelial cells at the implant site, leading to the formation of granulation tissue, 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 histological features include the proliferation of new small blood vessels and fibroblasts. Depending on the extent of injury, granulation tissue may be seen as early as three to five 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 (43–45). This process involves proliferation, maturation, and organization of endothelial cells into capillary tubes. 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; later, however, collagen, especially type I collagen, predominates and forms the fibrous capsule. Some fibroblasts in developing granulation tissue may have features of smooth muscle cells. These cells are called myofibroblasts and are considered to be responsible for the wound contraction seen during the development of granulation tissue.

The wound healing response is generally dependent on the extent or degree of injury or defect created by the implantation procedure. Wound healing by primary union (or first intention) is the healing of clean, surgical incisions in which the wound edges have been approximated by surgical sutures. Healing under these conditions 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 where there is extensive loss of cells and tissue. In wound healing by second intention, regeneration of

parenchymal cells cannot completely reconstitute the original architecture, and much more granulation tissue is formed, resulting in larger areas of fibrosis or scar formation.

Granulation tissue is distinctly different from granulomas, which are small collections of modified macrophages called epithelioid cells. Foreign body giant cells may surround non-phagocytosable particulate materials in granulomas. Foreign body giant cells are formed by the fusion of monocytes/macrophages in an attempt to phagocytose the material.

Foreign Body Reaction

The foreign body reaction is composed of foreign body giant cells and the components of granulation tissue, which consist of macrophages, fibroblasts, and capillaries in varying amounts, depending upon the form and topography of the implanted material. Relatively flat and smooth surfaces, such as those 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) 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.

As previously discussed, the form and topography of the surface of the biomaterial determines 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 or porous materials will have higher ratios of macrophages and foreign body giant cells in the implant site than will 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 (1, 2, 46–48). 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 nature of the subsequent events regarding the activity of macrophages at the surface is not clear. Tissue macrophages, derived from circulating blood monocytes, may coalesce to form multinucleated foreign body giant cells. Very large foreign body giant cells containing large numbers of nuclei are typically present on the surface of biomaterials. Although 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.

Efforts in our laboratory have focused on differential lymphokine regulation of macrophage fusion, which leads to morphological variants of multinucleated giant cells, and the role played by the surface chemistry and other properties of the foreign material in facilitating monocyte adhesion, macrophage development, and giant cell formation. Foreign body giant cells are observed at the tissue/material interface of medical devices implanted in soft and hard tissue and remain at the implant/tissue interface for the lifetime of the device in vivo, which in some cases may extend beyond 20 years. In addition, foreign body giant cells have been implicated in the biodegradation of polymeric medical devices. Foreign body giant cells and macrophages constituting the foreign body reaction at the tissue/device interface are surface-area dependent. Fabrics utilized as vascular grafts show high densities of foreign body giant cells, whereas flat surfaces such as those found on breast implants exhibit only a one- to two-cell layer of macrophages and foreign body giant cells at the tissue/material interface. For these and other reasons, we have sought to identify the mechanism of induction of foreign body giant cells on biomaterials and the physiological and material bases for their formation.

Early studies utilizing lymphokines in the induction of foreign body giant cell formation employed a wide variety of experimental conditions that resulted in both positive and negative modulation of these cells' formation. A number of these studies utilized conditioned media or supernatants. To provide a clearer identification of cell-derived agents that produce foreign body giant cells, we used recombinant human lymphokines with freshly isolated human monocytes in our culture systems. We believe that these conditions provide greater insight into foreign body giant cell formation and obviate unidentified problems that may result from the use of transformed cell lines and conditioned media and supernatants.

In our studies, human interleukin-4 (IL-4) induced the formation of foreign body giant cells from human monocyte-derived macrophages, an effect that was optimized with either GM-CSF or IL-3, dependent on the concentration of IL-4, and specifically prevented by anti-IL-4 (49, 50). Very large foreign body giant cells with randomly arranged nuclei and extensive cytoplasmic spreading (285 $\pm$ 121 nuclei and 1.151 $\pm$ 0.303 mm^2 per cell) were consistently obtained. Rates of macrophage fusion in this system were high, 72 $\pm$ 5%.

Figure 2 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 considered to play an important role in foreign body giant cell development. Möst and colleagues have shown that the fusion rates of monocytes/macrophages decrease with advancing differentiation, and almost no giant cell formation was observed with 8-day-old macrophages that were derived from freshly isolated monocytes stimulated with cytokine-containing supernatants (51). A distinct difference in differentiation was seen in our studies when IL-4 was added to freshly adherent (2 h) monocytes. IL-4 under these conditions resulted in a detachment of adherent cells and an

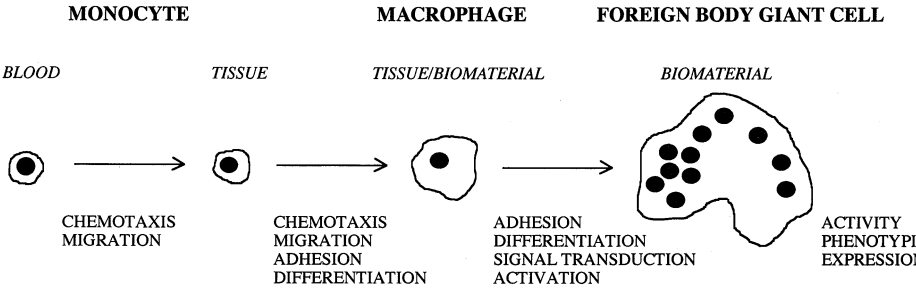

Figure 2 In vivo transition from blood-borne monocyte to biomaterial adherent monocyte/macrophage to foreign body giant cell at the tissue/biomaterial interface. Little is known regarding the indicated biological responses that are considered to play important roles in the transition to foreign body giant cell development.

inhibition of initial monocyte adhesion by IL-4. To accelerate the development of macrophage morphology, we added GM-CSF initially and at 3 days added IL-4 to induce macrophage fusion and foreign body giant cell formation. Although positive effects may result from the use of conditioned media or inflammatory cell-derived supernatants, it is also possible that negative autocrine or paracrine effects with down-regulation of biological interactions important to macrophage differentiation and foreign body giant cell development may occur. It is obvious that the utilization of human cells, together with appropriate recombinant human cytokines and antibodies, provides for cleaner and more relevant systems in mechanistic studies of macrophage differentiation and fusion with foreign body giant cell formation.

Figure 3 demonstrates the sequence of events involved in inflammation and wound healing when medical devices are implanted. In general, the polymorphonuclear neutrophil 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 implant. Studies utilizing IL-4 by ourselves and others demonstrate the role for Th2 helper lymphocytes in the development of the foreign body reaction at the tissue/material interface. Th2 helper lymphocytes have been described as anti-inflammatory based on their cytokine profile of which IL-4 is a significant component. Th2 helper lymphocytes also produce IL-13, and we have utilized this to demonstrate its similar effect to IL-4 on foreign body giant cell formation (52). In this regard, it is noteworthy that anti-IL-4 does not inhibit IL-13-induced foreign body giant cell formation nor does anti-IL-13 inhibit IL-4-induced foreign body giant cell formation. In our IL-4 and IL-13 foreign body giant cell culture systems, the macrophage mannose receptor (MMR) has been identified as critical to the fusion of macrophages in the formation of foreign body giant cells (52, 53). This formation can be prevented by competitive inhibitors of MMR activity, i.e. α-mannan, or inhibitors of glycoprotein processing that restrict MMR surface expression.

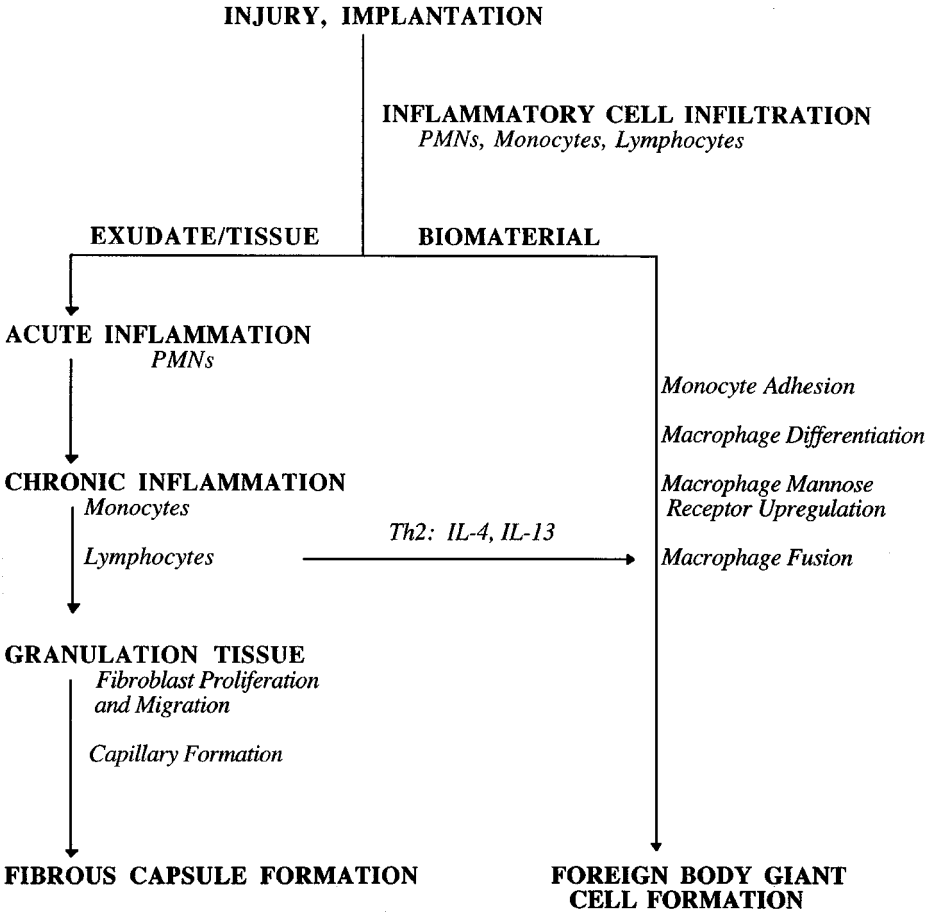

Figure 3 Sequence of events involved in inflammatory and wound healing responses leading to foreign body giant cell formation. This shows the importance of Th2 lymphocytes in the transient chronic inflammatory phase with the production of IL-4 and IL-13, which can induce monocyte/macrophage fusion to form foreign body giant cells.

Two factors that may play a role in multinucleated giant cell studies are the surface chemistry of the substrate onto which the cells adhere and the protein adsorption that occurs before cell adhesion. These two factors have been hypothesized to have significant roles in the inflammatory and wound healing responses to biomaterials and medical devices in vivo.

We have extensively investigated the effect of substrate surface chemistry on monocyte/macrophage adhesion, macrophage fusion, and foreign body giant cell development (54–58). The overall goal of these studies is to identify surfaces that do not permit monocyte/macrophage adhesion and/or macrophage fusion to form foreign body giant cells. Long-chain hydrocarbon groups on glass surfaces

markedly reduce macrophage adhesion and nearly eliminate IL-4-induced foreign body giant cells (58). In contrast, polyethylene oxide (PEO) chains on glass surfaces do permit macrophage adhesion, but the level of IL-4-induced foreign body giant cell formation is markedly reduced (56). In the case of clean glass surfaces, adherent macrophage densities are high enough to allow maximal levels of foreign body giant cell formation; however, negligible formation is observed. A comparison of these three different types of surfaces supports the hypothesis that the composition and conformation of proteins adsorbed on surfaces provide signals or ligands for the adhesion of monocytes/macrophages as well as the macrophage fusion process itself. Thus long-term macrophage adhesion and IL-4- or IL-13-induced foreign body giant cell formation are surface-dependent phenomena.

Cytoskeletal and adhesive structure studies of in vitro FBGC formation have demonstrated that podosomal structures, and not focal contacts, are the major adhesive structures present within macrophages and foreign body giant cells on surfaces (59, 60). The podosomal structures found at the ventral periphery of the foreign body giant cells contain vinculin, talin, and paxillin in a ring-like structure surrounding an F-actin core. These podosomal adhesion structures are similar to those identified for osteoclast adhesion, and their presence at the ventral and peripheral surface implies a functional polarization and suggests frustrated phagocytosis via the formation of a closed compartment between the foreign body giant cells and the underlying substrate where degradative enzymes, reactive oxygen intermediates, and/or other products are secreted.

The lifetime of foreign body giant cells at tissue/material interfaces is still unknown. Early publications had suggested that they were relatively short-lived, lasting for several days. This is probably not true as clinical specimens show the presence of foreign body giant cells for years and, in some cases, decades. Honma & Hamasaki have reported on the ultrastructure of multinucleated giant cell apoptosis in a collagen sponge granuloma (61). They noted the disappearance of giant cells coincident with the resorption of the collagen sponge, which is most probably accurate because once the inciting agent for giant cell formation is no longer present, the presence of giant cells is no longer necessary.

The osteoclast, the multinucleated giant cell responsible for bone resorption, is the most widely studied of all types of giant cells. Unlike other types of giant cells, which are found with pathological conditions, the osteoclast is found at bone surfaces where it participates in the constant process of bone remodeling. Excessive osteoclast activity in bone resorption has been implicated in pathological processes such as the advanced stages of multiple myeloma, with lytic lesions in bone, and post-menopausal osteoporosis. The majority of studies suggest that the CFU-GM, the granulocyte-macrophage progenitor, a cell in the monocyte-macrophage lineage, is the earliest osteoclast precursor. While the osteoclast, like the Langhans giant cell and the foreign body giant cell, may have a hematopoietic precursor, molecular and cell biology studies have shown that the osteoclast has distinctly different functional and phenotypic characteristics (62, 63).

The calcitonin receptor is the best marker for distinguishing mammalian osteoclasts because this receptor is not expressed on monocyte/macrophage-derived giant cells. A wide variety of factors that influence osteoclast formation and function include systemic hormones, cytokines, and growth factors. It is noteworthy that neither IL-4 (FBGC formation) nor γ-interferon (Langhans giant cell formation) is described as a significant factor in the formation or activation of osteoclasts. These findings suggest that although the CFU-GM progenitor is of monocytic lineage, its differentiation does not include expression of IL-4 or IFN-γ receptors or, perhaps, even a common signal transduction pathway. This is somewhat surprising as both foreign body giant cells and osteoclasts adhere to substrates through podosomal structures.

Recent studies demonstrate the ability of IL-1 and TNF-α to induce both osteoclast formation and bone-resorbing activity (64–66). These studies suggest that activated macrophages may facilitate bone resorption by participating in osteoclast formation and activation. The role of TNF-α in regulating osteoclastic bone resorption continues to be elucidated with studies demonstrating that osteoblasts/stromal cells express a new member of the TNF-ligand family—osteoclast differentiation factor (ODF)/osteoprotegerin (OPGL)/TNF-related activation-induced cytokine (TRANCE)/receptor activation of NF-κB ligand (RANKL)—as a membrane associated factor (66–68).

Fibrosis and Fibrous Encapsulation

The end-stage healing response to biomaterials is generally fibrosis or fibrous encapsulation. However, there may be exceptions to this general statement (e.g. porous materials inoculated with parenchymal cells or porous materials implanted into bone).

Repair of implant sites involves 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 (3, 69, 70). These processes are generally controlled by either (*a*) 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 (*b*) persistence of the tissue framework of the implant site. The regenerative capacity of cells permits classification into three groups: labile, stable (or expanding), and permanent (or static) cells. Labile cells continue to proliferate throughout life, stable cells retain this capacity but do not normally replicate, and permanent cells cannot reproduce themselves after birth. Perfect repair with restitution of normal structure theoretically occurs 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, skeletal muscle cells, and cardiac muscle cells) most commonly undergo an organization of the inflammatory exudate, leading to fibrosis. Tissues composed of stable cells (e.g. parenchymal cells of the liver, kidney, and pancreas), mesenchymal cells (e.g. fibroblasts, smooth muscle

cells, osteoblasts, and chrondroblasts), 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 may lead to restitution of the normal tissue structure, 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 in which cells stop producing one family of proteins and start producing another (phenotypic change) or begin a 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), as well as 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, hematological and immunological derangements, glucocortical steroids, and preexisting diseases such as atherosclerosis, diabetes, and infection.

IN VIVO EVALUATION OF TISSUE RESPONSES TO MATERIALS

From a practical perspective, the in vivo assessment of tissue compatibility of medical devices is carried out to determine that the device performs as intended and presents no significant harm to the patient or user. Thus, the goal of the in vivo assessment of tissue compatibility of medical devices is to determine and predict whether such devices present potential harm to the patient or user by evaluations under conditions simulating clinical use.

Recently, extensive efforts have been made by government agencies, i.e. FDA and regulatory bodies, i.e. ASTM, ISO, USP, to provide procedures, protocols, guidelines, and standards that may be used in the in vivo assessment of tissue compatibility of medical devices (71–76). This chapter draws heavily on the ISO 10,993 standard, Biological Evaluation of Medical Devices, in presenting a systematic approach to such assessments (71).

TABLE 4 Biomaterials and components relevant to in vivo assessment of tissue compatibility

The material(s) of manufacture
Intended additives, process contaminants and residues
Leachable substances
Degradation products
Other components and their interactions in the final product
The properties and characteristics of the final product

In the selection of biomaterials to be used in device design and manufacture, the first consideration should be fitness for purpose with regard to characteristics and properties of the biomaterial(s), which include chemical, toxicological, physical, electrical, morphological, and mechanical properties. Relevant to the overall in vivo assessment of tissue compatibility of a biomaterial or device is a knowledge of the chemical composition of the materials, including the conditions of tissue exposure, as well as the nature, degree, frequency, and duration of exposure of the device and its constituents to the intended tissues into which it will be utilized. Table 4 presents a list of biomaterial components and characteristics that may impact the overall biological responses of the medical device. Therefore, knowledge of these components in the medical device, i.e. final product, is necessary. The range of potential biological hazards is broad and may include short-term effects, long-term effects, or specific toxic effects, which should be considered for every material and medical device. However, this does not imply that testing for all potential hazards is necessary or practical.

Selection of In Vivo Tests According to Intended Use

In vivo tests for assessment of tissue compatibility are chosen to simulate end-use applications. To facilitate the selection of appropriate tests, medical devices with their component biomaterials can be categorized by the nature of body contact of the medical device and by the duration of contact of the medical device. Table 5 presents medical device categorization by body contact and contact duration. The tissue contact categories and subcategories, as well as the contact duration categories, have been derived from standards, protocols, and guidelines utilized in the past for safety evaluation of medical devices. Certain devices may fall into more than one category, in which case testing appropriate to each category should be considered.

The ISO 10,993 standard and the FDA guidance document present a structured program for biocompatibility evaluation in which matrices are presented that indicate required tests according to specific types of tissue contact and contact duration. These matrices are not presented here but the in vivo tests are indicated in Table 6.

TABLE 5 Medical device categorization by tissue contact and contact duration

Tissue contact	
Surface devices	Skin Mucosal membranes Breached or compromised surfaces
External communicating devices	Blood path, indirect Tissue/bone/dentin communicating Circulating blood
Implant devices	Tissue/bone Blood
Contact duration	Limited, $\leq$24 h Prolonged, $>$24 h and $<$30 days Permanent, $>$30 days

Significant Issues in In Vivo Testing

Two perspectives may be considered in the in vivo assessment of tissue compatibility of biomaterials and medical devices. The first perspective involves the utilization of in vivo tests to determine the general biocompatibility of newly developed biomaterials for which some knowledge of the tissue compatibility is necessary for further research and development. In this type of situation, manufacturing and other processes necessary to the development of a final product,

TABLE 6 In vivo tests for tissue compatibility

Sensitization
Irritation
Intracutaneous reactivity
Systemic toxicity (acute toxicity)
Subchronic toxicity (subacute toxicity)
Genotoxicity
Implantation
Hemocompatibility
Chronic toxicity
Carcinogenicity
Reproductive and developmental toxicity
Biodegradation
Immune response

i.e. medical device, have not been carried out. However, the in vivo assessment of tissue compatibility at this early stage of development can be used to evaluate the general tissue responses of the biomaterial, as well as provide additional information relating to the proposed design criteria in the production of a medical device. While it is generally recommended that the identification and quantification of extractable chemical entities of a medical device should precede biological evaluation, it is quite common to carry out preliminary in vivo assessments to determine if there may be unknown chemical entities that produce adverse biological reactions. Utilized in this fashion, early in vivo assessment of the tissue compatibility of a biomaterial may provide insight into its biocompatibility and may permit further development of this material into a medical device. Obviously, adverse reactions observed at this stage of development require further efforts to improve the biocompatibility of the biomaterial and to identify the agents responsible for the adverse reactions. As the in vivo assessment of tissue compatibility of a biomaterial or medical device is focused on the end-use application, it must be appreciated that a biomaterial considered compatible for one application may not be compatible for another.

The second perspective regarding the in vivo assessment of tissue compatibility of medical devices focuses on the biocompatibility of the final product, that is, the medical device and its component materials in the condition in which it is implanted. Although medical devices in their final form and condition are commonly implanted in carefully selected animal models to determine function as well as biocompatibility, it may be not appropriate to carry out all of the recommended tests necessary for regulatory approval on the final device. In these situations, some tests may be carried out on biomaterial components of devices that have been prepared under manufacturing and sterilization conditions and other processes utilized in the final product development.

Specific Biological Properties Assessed by In Vivo Tests

In this section, brief perspectives on the general types of in vivo tests are presented. Details regarding these tests are found in the references. The selection of tests for in vivo biocompatibility assessment is based on the characteristics and end-use application of the device or biomaterial under consideration.

SENSITIZATION, IRRITATION, AND INTRACUTANEOUS (INTRADERMAL) REACTIVITY

Exposure to or contact with even minute amounts of potential leachable agents in medical devices or biomaterials can result in allergic or sensitization reactions. Sensitization tests that estimate the potential for contact sensitization of medical devices, materials and/or their extracts are usually carried out in guinea pigs, and should reflect the intended route (skin, eye, mucosa) and nature, degree, frequency, duration, and conditions of exposure of the biomaterial in its intended clinical use. Emphasis is placed on utilizing extracts of the biomaterials to determine the irritant effects of potential leachables. Intracutaneous (intradermal) reactivity tests determine the localized reaction of tissue to extracts of medical devices, biomaterials,

or prostheses in the final product form. Irritation and intracutaneous tests may be applicable where determination of irritation by dermal or mucosal irritation tests are not appropriate, for example, albino rabbits are most commonly used.

Because these tests focus on determining the biological response of leachable agents that may be present in biomaterials, their extracts in various solvents are utilized to prepare the injection solutions. Critical to the conduct of these tests is the preparation of the test material and/or extract solution and the choice of solvents, which must have physiological relevance.

SYSTEMIC TOXICITY (ACUTE TOXICITY) AND SUBACUTE AND SUBCHRONIC TOXICITY Systemic toxicity tests estimate the potential harmful effects of either single or multiple exposures, during a period of less than 24 h, to medical devices, biomaterials and/or their extracts. These tests evaluate the systemic toxicity potential of medical devices, which release constituents into the body. These tests also include pyrogenicity testing.

In these tests, the form and area of the material, the thickness, and the surface area to extraction vehicle volume are critical considerations in the testing protocol. Appropriate extraction vehicles, i.e. solvents, should be chosen to yield a maximum extraction of leachable materials to conduct the testing. Mice, rats, or rabbits are the usual animals of choice for these tests and, depending on the intended application of the biomaterial, oral, dermal, inhalation, intravenous, intraperitoneal, or subcutaneous application of the test substance may be used. Acute toxicity is considered to be the adverse effect, which occurs after administration of a single dose or multiple doses of a test sample given within 24 h. Subacute toxicity (repeat dose toxicity) focuses on adverse effects occurring after administration of a single dose or multiple doses of a test sample per day given during a period of from 14 to 28 days. Subchronic toxicity is considered to be the adverse effects occurring after administration of a single dose or multiple doses of a test sample per day given during a part of the life span, usually 90 days but not exceeding 10% of the life span of the animal.

Pyrogenicity (fever-producing) tests are also included in the systemic toxicity category to detect material-mediated pyrogenic reactions of extracts of medical devices or materials. It is noteworthy that no single test can differentiate pyrogenic reactions that are material-mediated from those due to endotoxin contamination.

GENOTOXICITY In vivo genotoxicity tests are carried out if indicated by the chemistry and/or composition of the biomaterial (see Table 4) or if in vitro test results indicate potential genotoxicity. Initially, at least three in vitro assays should be used, and two of these assays should utilize mammalian cells. The initial in vitro assays should cover the three levels of genotoxic effects: DNA effects, gene mutations, and chromosomal aberrations. In vivo genotoxicity tests include the micronucleus test, the in vivo mammalian bone marrow cytogenetic tests, chromosomal analysis, the rodent dominant lethal tests, the mammalian germ cell cytogenetic assay, the mouse spot test, and the mouse heritable translocation assay. Not all of the in vivo genotoxicity tests need be performed, and the most common test is the rodent

micronucleus test. Genotoxicity tests are performed with appropriate extracts or dissolved materials using media as suggested by the known composition of the biomaterial.

IMPLANTATION Implantation tests assess the local pathological effects on living tissue of a sample of a material or final product that is surgically implanted or placed into an implant site or tissue appropriate to the intended application of the device. Evaluation of the local pathological effects is carried out at both the gross level and the microscopic level. Histological (microscopic) evaluation is utilized to characterize various biological response parameters. For short-term implantation evaluation out to 12 weeks, mice, rats, guinea pigs, or rabbits are the usual animals utilized in these studies. For longer-term testing in subcutaneous tissue, muscle or bone, animals such as rats, guinea pigs, rabbits, dogs, sheep, goats, pigs and other animals with relatively long-life expectancy are suitable. If a medical device is to be evaluated, larger species may be utilized. For example, substitute heart valves are usually tested in sheep, whereas calves are usually the animal of choice for ventricular assist devices and total artificial hearts.

HEMOCOMPATIBILITY Hemocompatibility tests evaluate effects on blood and/or blood components by blood-contacting medical devices or materials. In vivo hemocompatibility tests are usually designed to simulate the geometry, contact conditions, and flow dynamics of the device or material in its clinical application. From the ISO standards perspective, five test categories are indicated for hemocompatibility evaluation: thrombosis, coagulation, platelets, hematology, and immunology (complement and leukocytes).

Two levels of evaluation are indicated: Level 1 (required) and Level 2 (optional). Regardless of blood contact duration or time, hemocompatibility testing is indicated for external communicating devices:blood path, indirect; external communicating devices, circulating blood; and blood-contacting implant devices.

Several issues are important in the selection of tests for hemocompatibility of medical devices or biomaterials. While in vivo testing in animals may be convenient, species differences in blood reactivity must be considered, and these differences may limit the predictability of any given test in the human clinical situation. Although blood values and reactivity between humans and non-human primates are similar, European community law prohibits the use of non-human primates for blood compatibility and medical device testing. Hemocompatibility evaluation in animals is complicated by the lack of appropriate and adequate test materials; for example, appropriate antibodies for immunoassays. Use of human blood in hemocompatibility evaluation implies in vitro testing, which usually requires the use of anticoagulants, which are not usually present with the device in the clinical situation, except for perhaps the earliest implantation period. Although species differences may complicate hemocompatibility evaluation, the utilization of animals in short- and long-term testing is considered to be appropriate for evaluating thrombosis and tissue interaction.

CHRONIC TOXICITY Chronic toxicity tests determine the effects of either single or multiple exposures to medical devices, materials, and/or their extracts during a period of at least 10% of the life span of the test animal, e.g. over 90 days in rats. Chronic toxicity tests may be considered an extension of subchronic (subacute) toxicity testing, and both may be evaluated in an appropriate experimental protocol or study.

CARCINOGENICITY Carcinogenicity tests determine the tumorigenic potential of medical devices, materials, and/or their extracts from either single or multiple exposures or contacts over a period of the major portion of the life span of the test animal. Carcinogenicity tests should be conducted only if data from other sources suggest a tendency for tumor induction. In addition, both carcinogenicity (tumorigenicity) and chronic toxicity may be studied in a single experimental study. With biomaterials, carcinogenicity studies focus on the potential for solid-state carcinogenicity, i.e. the Oppenheimer effect. In carcinogenicity testing, controls of a comparable form and shape should be included; polyethylene implants are a commonly used control material. The use of appropriate controls is imperative since animals may spontaneously develop tumors, and statistical comparison between the test biomaterial/device and the controls is necessary.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY These tests evaluate the potential effects of medical devices, materials, and/or their extracts on reproductive function, embryonic development (teratogenicity), and prenatal and early postnatal development. The application site of the device must be considered, and tests and/or bioassays should only be conducted when the device has potential impact on the reproductive potential of the subject.

BIODEGRADATION Biodegradation tests determine the effects of a biodegradable material and its biodegradation products on the tissue response. They focus on the amount of degradation during a given period of time (the kinetics of biodegradation), the nature of the degradation products, the origin of the degradation products (e.g. impurities, additives, corrosion products, bulk polymer, etc), and the qualitative and quantitative assessment of degradation products and leachable agents in adjacent tissues and in distant organs. The biodegradation of biomaterials may occur through a wide variety of mechanisms that, in part, are biomaterial dependent, and all pertinent mechanisms related to the device and the end-use application of the device must be considered. Test materials comparable to degradation products may be prepared and studied to determine the anticipated biological response of these products in long-term implants. An example of this approach is the study of metallic and polymeric wear particles that may be present with long-term orthopedic joint prostheses.

IMMUNE RESPONSES Immune response evaluation is not a component of the standards currently available for in vivo tissue compatibility assessment. However,

TABLE 7 Potential immunological effects and responses

Effects
Hypersensitivity
Type I-anaphylactic
Type II-cytotoxic
Type III-immune complex
Type IV-cell-mediated (delayed)
Chronic inflammation
Immunosuppression
Immunostimulation
Autoimmunity
Responses
Histopathological changes
Humoral responses
Host resistance
Clinical symptoms
Cellular responses
T cells
Natural killer cells
Macrophages
Granulocytes

ASTM, ISO, and the FDA currently have working groups developing guidance documents for immune response evaluation where pertinent. An example of the need for immune response evaluation is with modified natural tissue implants such as collagen, which has been utilized in a number of different types of implants. The Center for Devices and Radiological Health of the FDA has released a draft immunotoxicity testing guidance document whose purpose is to provide a systematic approach for evaluating potential adverse immunological effects of medical devices and constituent materials (73). Immunotoxicity is any adverse effect on the function or structure of the immune system or other systems as a result of an immune system dysfunction. Adverse or immunotoxic effects occur when humoral or cellular immunity needed by the host to defend itself against infections or neoplastic disease (immunosuppression) or unnecessary tissue damage (chronic inflammation, hypersensitivity, or autoimmunity) is compromised. Potential immunological effects and responses that may be associated with one or more of these effects are presented in Table 7.

Selection of Animal Models for In Vivo Tests

Animal models are used to predict the clinical behavior, safety, and biocompatibility of medical devices in humans (Table 8). The selection of animal models

TABLE 8 Animal models for the in vivo assessment of medical devices

Device classification	Animal
Cardiovascular	
Heart valves	Sheep
Vascular grafts	Dog, pig
Stents	Pig, dog
Ventricular assist devices	Calf
Artificial hearts	Calf
Ex-vivo shunts	Baboon, dog
Orthopedic/bone	
Bone regeneration/substitutes	Rabbit, dog, pig, mouse, rat
Total joints—hips, knees	Dog, goat, non-human primate
Vertebral implants	Sheep, goat, baboon
Craniofacial implants	Rabbit, pig, dog, non-human primate
Cartilage	Rabbit, dog
Tendon and ligament substitutes	Dog, sheep
Neurological	
Peripheral nerve regeneration	Rat, cat, non-human primate
Electrical stimulation	Rat, cat, non-human primate
Ophthalmological	
Contact lens	Rabbit
Intraocular lens	Rabbit, monkey

for the in vivo assessment of tissue compatibility must consider the advantages and disadvantages of the animal model for human clinical application. Below, several examples demonstrate the advantages and disadvantages of animal models in predicting clinical behavior in humans.

As described above, sheep are commonly used for the evaluation of heart valves. This is based on size considerations and also the propensity for calves to calcify tissue components of bioprosthetic heart valves. The choice of this animal model for bioprosthetic heart valve evaluation is made on the basis of accelerated calcification in rapidly growing animals, which has its clinical correlation in young and adolescent humans.

The in vivo assessment of tissue responses to vascular graft materials is an example in which animal models present a false picture of what generally occurs in humans. Virtually all animal models, including non-human primates, heal rapidly and completely with an endothelial blood-contacting surface. Humans, on the other hand, do not show extensive endothelialization of vascular graft materials, and the resultant pseudo-intima from the healing response in humans is potentially thrombogenetic. Consequently, despite favorable results in animals, small-diameter vascular grafts (less than 4 mm in internal diameter) yield early thrombosis in humans, the major mechanism of failure, which

is secondary to the lack of endothelialization in the luminal surface healing response.

The use of appropriate animal models is an important consideration in the safety evaluation of medical devices that may contain potential immunoreactive materials. The in vivo evaluation of recombinant human growth hormone in poly(lactic-co-glycolic acid)(PLGA) microspheres demonstrates the appropriate use of various animal models to evaluate biological responses and the potential for immunotoxicity. Utilizing biodegradable PLGA microspheres containing recombinant human growth hormone (rhGH), Cleland et al used Rhesus monkeys, transgenic mice expressing hGH, and normal control (Balb/C) mice in their in vivo evaluation studies (77). Rhesus monkeys were utilized for serum assays in the pharmacokinetic studies of rhGH release as well as tissue responses to the injected microcapsule formulation. Placebo injection sites were also utilized, and a comparison of the injection sites from rhGH PLGA microspheres and placebo PLGA microspheres demonstrated a normal inflammatory and wound healing response with a normal focal foreign body reaction. To further examine the tissue response, transgenic mice were utilized to assess the immunogenicity of the rhGH PLGA formulation. Transgenic mice expressing a heterologous protein have been previously used for assessing the immunogenicity of structural mutant proteins. With the transgenic animals, no detectable antibody response to rhGH was found. In contrast, the Balb/C control mice had a rapid onset of high titer antibody response to the rhGH PLGA formulation. This study points out the appropriate utilization of animal models not only to evaluate biological responses but also to evaluate one type of immunotoxicity (immunogenicity).

Future Perspectives on In Vivo Medical Device Testing

As presented above, the in vivo assessment of tissue compatibility of biomaterials and medical devices is dependent on the end-use application of the device under consideration. In this sense, the development and utilization of new biomaterials and medical devices will dictate the development of new test protocols and procedures for evaluating them. Furthermore, it must be understood that the in vivo assessment of tissue compatibility of biomaterials and medical devices is open-ended and new end-use applications will require new tests.

Over the past half-century, medical devices and biomaterials have generally been passive in their tissue interactions. That is, a mechanistic approach to biomaterials/tissue interactions has rarely been used in the development of biomaterials or medical devices. Heparinized biomaterials are an exception to this statement, but considering the five subcategories of hemocompatibility, these approaches have minimal impact on the development of blood-compatible materials.

In the past decade, increased emphasis has been placed on tissue engineering in the development of biomaterials and medical devices for potential clinical application. Rather than a passive approach to tissue interactions, tissue-engineered devices have focused on an active approach in which biological or tissue components,

i.e. growth factors, cytokines, drugs, enzymes, proteins, extracellular matrix components, and cells that may or may not be genetically modified, are used in combinations with synthetic, i.e. passive, materials to produce devices that control or modulate a desired tissue response. Obviously, in vivo assessment of the targeted biological response of a tissue-engineered device will play a significant role in the research and development of that device as well as in its safety assessment. It is clear that scientists working on the development of tissue-engineered devices will contribute significantly to the development of in vivo tests for biocompatibility assessment as these tests will also be utilized to study the targeted biological responses in the research phase of the device development.

Regarding tissue-engineered devices, it must be appreciated that biological components may induce varied effects upon tissue in the in vivo setting. Simply put, cell types in the implant site may react differently to the presence of an extrinsic growth factor. Autocrine, paracrine, and endocrine signaling may be different between the same cell types and different cell types in the implant site. Signal transduction systems may be variable depending on the different cells that are present within the implant site. The presence of a growth factor may result in markedly different cell proliferation, differentiation, protein synthesis, attachment, migration, shape change, etc, which would be cell type-dependent. Thus different cell type-dependent responses in an implant site, reacting to the presence of a single exogenous growth factor, may result in inappropriate, inadequate, or adverse tissue responses. These perspectives must be integrated into the planned program for in vivo assessment of tissue compatibility of tissue-engineered devices. Finally, a major challenge to the in vivo assessment of tissue compatibility of tissue-engineered devices is the use of animal tissue components in the early phase of device development, whereas the ultimate goal is the utilization of human tissue components in the final device for end-use application. Novel and innovative approaches in the in vivo tissue compatibility of tissue-engineered devices must be developed to address these significant issues.

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