

Biosensors in the Body

Continuous *in vivo* Monitoring

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Biocompatibility of Active Implantable Devices

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5.1 INTRODUCTION

This century has seen an explosion in the number and the varieties of implantable biomaterials used in the treatment of various medical problems. Excluding dental implants and contact lenses, more than 3 million people in

the USA have long-term implants (Tang & Eaton, 1995). The market for biomaterials applied in biomedical devices exceeds currently \$1.5 billion worldwide, with the tendency to increase further (Braybrook, 1994). These devices range from sutures at the one end of the spectrum to complicated electronic devices at the other, and include breast prostheses, joint replacements, vascular grafts, pacemakers, catheters, biosensors and others. All of them are made from biomaterials, which by definition are systemically and pharmacologically inert substances designed for implantation or incorporation within the human body. As such they include synthetic and natural polymers, metals, ceramics and combinations of these materials. Biomaterials are used in contact with living tissue, resulting in an interface between living and non-living substances (Szycher, 1992). Biomaterials should not cause (i) a direct injury to living tissues, (ii) any adverse immune response, (iii) other adverse systemic effects or (iv) delayed adverse effects (Wallin, 1995a).

Although medical science has great experience with synthetic biomaterials, very few (or some might say none) have proved to be both safe and effective over a long time. Materials that initially seemed to be appropriate and successful became dangerous after a long implantation time and further, more detailed scientific investigation. The recent withdrawal of specific silicones, polyurethanes, polyesters, fluorinated hydrocarbon polymers and other commercially available polymers sold for use in implantable medical devices reflects the current critical situation in the field of biomaterials (Gould *et al.*, 1993). In this context it should be underlined that, at present, there is no biomaterial available which can be said to be completely inert when implanted in the body (Williams, 1994a). So indeed, the clinical application of biomaterials has exceeded, in both quality and quantity, our knowledge about the complex interactions between the biomaterial and the biological system.

Biocompatibility, which concerns the relationship between a biomaterial and its host, traditionally used only to be equated with a lack of toxicity. However, the difficulties with this concept have led to a different definition termed as "the ability of a material to perform with an appropriate host response in a specific application" (Williams, 1989). So in fact, biocompatibility should be concerned with all aspects of material-tissue reactions of the body but must concentrate on eliciting an appropriate host response. It includes the following:

- (1) The energetics and kinetics of all physical, chemical and biochemical processes at the interface between the biomaterial and the biological system.
- (2) Direct reactions of the biological system provoked by processes at the interface.
- (3) All changes in the physical and chemical properties of the material (surface composition, corrosion, biodegradation, surface free energy and so on).

- (4) The sum of changes of the biological system outside the interface, triggered or induced by the interaction (toxic or immune reactions, cancer etc.).

Returning to the variety of medical devices, the "state of the art" concerns active implantable medical devices such as pacemakers, neurostimulators, drug delivery systems and, of course, biosensors. Implantable biosensors consisting of a biocomponent (e.g. an enzyme or an antibody) and a sensor element have been presented as tools to improve patient care, to reduce costs in health care, and to be a potentially huge consumer market for home-care and point-of-care medicine. *In vivo* chemical sensing would benefit diagnosis and treatment of serious clinical problems such as diabetes mellitus, followed by monitoring of vital functions in the intensive care unit or operating theatre to monitor the effectiveness of surgical intervention or dialysis. However, it is widely agreed amongst clinicians that the measurement of glucose in diabetic patients by means of *in vivo* biosensors could be important in optimising insulin therapy thus avoiding or at least delaying diabetic complications (Pickup & Alcock, 1991).

To understand fully the behaviour of an *in vivo* sensor, it is necessary to consider both the actions of the biological system as well as the complex cumulative effects of sample constituents on sensor function. Even in the aqueous fraction of body fluids, one finds a great variety of low molecular-weight inorganic and organic solutes as well as macromolecular components, which may all affect the reactivity of the biocomponent of the sensor or the surface of the transducer element (Vadgama & Réach, 1993). However, the most complex effects *in vivo* are the cell-mediated actions induced by local inflammatory cells or the associated adverse processes in the surrounding tissue. In addition to these effects and the uncertainty of the sensor behaviour with prolonged implantation time, the individuality of sensor recipients and therefore the individuality of responses against the foreign body (sensor) has been taken into consideration.

So it is not surprising that despite considerable research effort since the first publication of a glucose sensor based on an enzyme electrode (Clark & Lyons, 1962), at present there is no reliable *in vivo* sensor for continuous, long period measurement available for clinical application (Fischer, 1995). In contrast to the excellent stability and sensitivity of sensor function *in vitro*, a reduction in sensitivity occurs after implantation, with a resulting rapid decrease in the *in vivo* sensor signal followed by complete sensor failure within hours or days. The bio(in)compatibility of biomaterials used for sensor preparation, namely the outer membranes, seems to be a major cause for the loss of sensitivity, resulting finally in a loss of sensor function. The degree and the nature of the *in vivo* response and its effect on biomaterial/biomembrane function—and therefore the sensing function—serve as markers of biocompatibility.

5.2 BIOMATERIAL-HOST INTERACTIONS AND THE HOST RESPONSE

When evaluating the biocompatibility of active implantable devices, it is important to note that biocompatibility must refer to a specific situation, i.e. the biocompatibility characteristics of a given material/device in one situation may be quite different to those of the same material/device in another. So biocompatibility describes a changing phenomenon or various characteristics, and it is not justified to use uniquely the term "biocompatible" to describe a material/device (Williams, 1989).

There are two main variables which influence the biocompatibility of an implantable device: the material response and the host response. The possible heterogeneity of the chemical nature of the biomaterial, in relation to bulk and surface chemistry, electrical properties and resistance to corrosion/biodegradation, is an important factor which has to be taken into account because the critical events in interfacial reactions may be controlled at a microstructural level by minor constituents of the device. Furthermore, in addition to the implant chemistry and structure, the surface topography, the implant size and shape are also important. Significant differences in both material stability and cellular responses have been demonstrated when comparing ultra-smooth surfaces and porous structures. So surface roughness will control (along with implantation site and duration) many of the parameters of biocompatibility (Inoue *et al.*, 1987).

The size of the implant influences the biological response. On one hand, larger devices cause greater tissue injury upon implantation, which can lead to greater inflammation and thicker fibrous capsule formation around the device. On the other hand, implants of small size (particles) may induce a phagocytic response by stimulating and activating macrophages and foreign body giant cells. Regarding the shape of an implant, it can be demonstrated that the local tissue response was generally greater around the corners, e.g. shape contours of a device (this is referred as "clubbing"), suggesting that a component of the tissue reaction around implants is probably also due to mechanical trauma produced by the presence of an implant (Wood *et al.*, 1970). So triangular implants induce a greater degree of tissue reaction than specimens with pentagonal or circular structures as measured by histochemical techniques (Matlaga *et al.*, 1976; Woodward, 1982). Material-centred responses have traditionally been considered to be most relevant for biocompatibility, however, it is now becoming clear that the host reaction can be at least equally important.

Among the various components of the host response, inflammation seems to play a central role (Williams, 1989; Anderson, 1993; Tang & Eaton, 1995). Many mediators of inflammation, each of them influenced by a variety of physiological and pharmaceutical variables, act within the local host response, which thus represents a balance between inflammatory and repair processes.

In a clinical context, these processes will further be influenced by the age, sex, health, immunological and pharmaceutical status, implantation site and, of course, the individuality of the organism and the individuality of the response. For example, as shown by various immunisation experiments with defined immunogens in in-bred animals, a variability in immune response could be demonstrated, including high-, low- and non-responders. However, the human is not in-bred and furthermore, when trying to assess biocompatibility characteristics from animal experiments, there will be inevitable species differences when compared to humans.

Inflammation, wound healing and foreign body responses are generally considered to be parts of the tissue or cellular host responses to the injury. From the view of implantable biosensors, the placement of the device in the *in vivo* environment involves injection, insertion, or surgical implantation, all of these injuring the tissues or organs involved, and contacting blood. After the implantation procedure, complex mechanisms, which result in wound healing as a response to the injury, are activated. The extent of these mechanisms for any particular device determine its biocompatibility and its functional biostability. However, the inflammatory response to implanted biomaterials is difficult to understand in terms of the classical view of the "non-toxic", "inert", and "non-immunogenic" nature of biomaterials, and therefore one must assume that the surface of the implanted material alone cannot be the proximate cause of inflammation.

Although it is convenient—especially when discussing biocompatibility of implantable biosensors—to consider blood-material interactions separately from tissue-material interactions, it should be kept in mind that many of the cells and the mechanisms involved in either case are common to blood and tissue, and are closely related (Cotran *et al.*, 1989; Anderson, 1993). Regardless of the tissue or organ into which a biomaterial is implanted, the initial inflammatory response is activated by the injury to vascularised connective tissue, and therefore blood and its components are involved primarily. In the following sections, the possible interactions between a medical device (for example, an implantable biosensor) and the host are discussed.

5.2.1 THE INITIAL EVENTS: THE BLOOD-DEVICE INTERFACE

Aqueous soluble components present in biological fluids may be subdivided into low-molecular-weight solutes such as ions, small organic molecules and gases, and high molecular composites such as proteins and enzymes. Most biological fluids have a near-neutral pH, but the concentration of low molecular components can vary by up to 20% (Vadgama & Réach, 1993), so that altered ionic strength (and therefore the biological variability of buffer capacity) may be the cause of analytical imprecision in some electrochemical measurements. Furthermore, it has been shown that the surface deposition of low molecular solutes on noble metallic electrodes such as Pt can diminish

the electrode response to the analyte, and can also promote premature degradation/corrosion of the metallic component (Williams, 1994a). These problems—the vulnerability of electrodes or “biometals” to hydration, oxidation or passivation—can be partly avoided by coating electrodes with polymers.

The main components of solutes present in biological fluids are a variety of macromolecular proteins, which occur in high concentrations for example at around 80 g/l in blood plasma. The adsorption of proteins at the surface of implantable devices—and this is in fact the initial step of interaction—differs from those of low molecular weight components, mainly in that proteins are large and chemically heterogeneous: they contain hydrophilic and hydrophobic regions, and electrically positive and negative charged regions. Biomaterial surfaces are also heterogeneous and contain a similar variety of surface chemical domains, so that a number of different protein–surface interactions are possible. The initial contact between a biomaterial and a protein solution results in a coating of the surface within seconds/minutes, followed by a competitive exchange between different proteins, especially albumin, immunoglobulins and fibrinogen, which were found to be adsorbed at high levels on the biomaterial surface (Anderson *et al.*, 1990; Tang & Eaton, 1993; Tang *et al.*, 1993). Furthermore, high molecular weight kininogen (HMWK), Hageman factor (FXII), von Willebrand factor (FVIII), fibronectin, complement components and other species have been detected and quantified on biomaterials (Ziats *et al.*, 1990). The coating depends on biomaterial characteristics such as surface topography, charge density, distribution and mobility, surface groups (chain length, hydrophobicity, hydrophilicity), structural ordering (crystallinity, soft- and hard segment distribution, amorphous domains, polymer chain mobility) and the extent of hydration. Therefore the composition of an adsorbed layer of protein may differ from one biomaterial to another. So it is extremely important to characterise comprehensively the biomaterials used for medical devices.

The relatively large size of proteins means that adsorption of a single molecule may involve several types of binding sites, resulting in a different strength of binding of different proteins (high and low surface-protein affinity) to different biomaterials. Many investigators studying the protein–biomaterial interface in blood or plasma *in vitro*, *ex vivo* and *in vivo* have demonstrated that adsorbed proteins may already be desorbed and exchanged by the time that others adsorb, and may be reoriented or denatured as a consequence (Vroman, 1988; Anderson *et al.*, 1990).

Despite the many questions that remain unanswered, it is generally accepted that when a biomaterial is implanted, there is no steady state in protein adsorption. Since native soluble proteins have a well defined, but asymmetric conformation, they can exist after adsorption in distinct orientations and conformations. Recently, it has been demonstrated by infrared spectroscopic analysis that conformational changes of two human plasma proteins, albumin

and fibrinogen, occur after adsorption to both hydrophobic and cross-linked hydrophilic polyurethanes, and that changes of conformation could be completely prevented by surface heparinisation (Barbucci & Magnani, 1994). Thus it has to be taken into account that by changing their conformation, native proteins may become recognised as "non-self" conformational epitopes by the host immune system, and that once adsorbed, they may induce and promote the host's inflammatory/immune response more aggressively. Following protein adsorption, cellular interactions occur with the protein-coated biomaterial surface. These interactions lead to adhesion and activation of cells: events which then characterise the inflammatory response.

5.2.2 THE CENTRAL EVENT: THE INFLAMMATORY RESPONSE TO BIOMATERIALS

Inflammation is generally defined as the reaction of the vascularised living tissue to the local injury which serves to destroy or dilute the injurious agent/medical device and the injured tissue. As mentioned earlier, the size, shape, and the chemical and physical properties of the biomaterial and therefore the dimensions of the injury are responsible for variations in the intensity and the time duration of the inflammatory and the wound healing processes (Williams, 1989). Furthermore, bacterial infection due to implantation/injury can amplify these inflammatory processes. Therefore, the miniaturisation of the device as well as the appropriate and effective sterilisation method are also goals of the present efforts of scientists investigating *in vivo* biosensors.

5.2.2.1 The "Normal" Process of Wound Healing

If tissue and blood vessels are damaged by local injury, well-defined mechanisms resulting in healing will be initiated. These mechanisms involve two distinct phases, which do, however, overlap in time. The first phase, acute inflammation, is the initial reaction of the body, and involves changes to the microvasculature and cellular composition of the tissue. In the second phase, the repair phase, the tissue attempts structurally and functionally to restore the damage caused by the injury.

Immediately after injury, the blood vessels in the direct vicinity dilate: changes in vascular flow, vessel diameter and permeability occur, thus allowing the diffusion of white blood cells (especially neutrophils), and an extra-cellular exudate containing plasma proteins and other mediators of biochemical reactions, through the vessel walls into the damaged tissue. The immediate response to the injury is bleeding and then thrombus formation which involves the activation of the extrinsic and intrinsic coagulation systems, the complement system, the fibrinolytic system, platelets and the generation of kinins. Those cells with phagocytic activity act on the injury by removing cellular and other debris. Concomitant with this process, the repair

process is initiated by vascular regeneration, i.e. new capillaries grow into the wound area, and fibroblasts generate new collagen, the essential structural protein of this connective tissue. In very simple cases the resultant layer of fibrous connective scar tissue may differ only slightly from the original tissue. In cases of extensive injury, a more extensive inflammatory response will take place, which results in both the delay of repair phase and an increased formation of reparative tissue.

If the injury is followed by an implantation of a medical device consisting of an "inert", "non-toxic" and "non-degradable" biomaterial, the initial event of the interaction between blood and the material is the "controlled" adsorption of plasma proteins. The first phase (acute inflammation) will be comparable to those of a simple injury including dilated vessels, activated coagulation cascade and complement factors, and recruitment of neutrophils, macrophages and fibroblasts. However, regeneration of a network of blood vessels and the morphology of generated collagen are disrupted by the implant. The presence of the implant will probably inhibit and therefore prolong the inflammatory and the repair process, and cellular infiltration will persist for a longer time when compared to a normal incision case. However, this view of the response is too simplistic because absolute inertness of medical devices/biomaterials implanted in the body does not exist (Williams, 1989; Black, 1995). Furthermore, implants which are ignored by the body may be not really incorporated in it and cannot be said to have achieved their functional biostability (Williams 1989, 1994b).

5.2.2.2 Wound Healing after Implantation of Biomaterials: the Central Role of Phagocytic Cells

The predominant cell type present in the phases of inflammatory response varies with the age of the injury. Whereas neutrophils (half-life of 6–9 h) disappear after 1–2 days, monocytes (which differentiate into macrophages) are very long-lived, and their emigration may continue for days to weeks depending on the implanted biomaterial (Ward, 1994). The inflammatory response is a series of complex reactions involving different proteins, either adsorbed or present in body fluids, and various cell types such as neutrophils, monocytes/macrophages, platelets, fibroblasts, B and T lymphocytes, foreign body giant cells, epithelial cells etc. The sequence of local events following implantation of biomaterial may be subdivided into acute inflammation, chronic inflammation, foreign body reaction and granulation of tissue, and fibrosis (Anderson, 1993).

Acute inflammation after implantation of a biomaterial is of relatively short duration depending on the extent of injury—commonly from minutes to a few days. The main characteristics are the exudation of fluid and plasma proteins, followed by their immediate physical adsorption on the biomaterial (in the

case of plasma proteins) or covalent binding of complement proteins (e.g. C3b and iC3b). Also initiated are the emigration and accumulation of leukocytes, especially neutrophils and monocytes (Colton *et al.*, 1994; Ward, 1994). Studies by Marchant *et al.* (1983) using the cage implant system have shown that monocytes and macrophages are present at high concentrations when neutrophils are also at their highest concentrations, i.e. already in the first phase of acute inflammatory response. Furthermore, B and T lymphocytes have been detected at increased levels at the implantation site (Gong *et al.*, 1993; Holgers *et al.*, 1995). The protein-coated surfaces interact with the coagulation and the complement systems as well as with platelets and immune cells.

Nearly all biomaterial surfaces can activate the complement system to some degree, notably via biomaterial-bound C3b triggering the alternative pathway (Chenoweth *et al.*, 1988; Brett, 1992). Platelets and immune cells can adhere to proteins on the biomaterial surface. For example, phagocytic cells, red cells, B cells and some T cells have CR1 receptors that bind to C3b whereas phagocytes with CR3 receptors bind to iC3b. The CD3 receptor (also designated CD11b/CD18) is a member of the β_2 -integrin family of cell adhesion receptors, and interacts with the endothelial cell ICAM-1 counter receptor (Anderson, 1993; Colton *et al.*, 1994). The complement fragment generation causes a C5a-induced increase in expression of adhesion receptors by phagocytes. The anaphylatoxins mediate other functions of phagocytes and also activate other cells including eosinophils, basophils and mast cells. The complement cascade is completed by the production of C5b-9 complex, and there is evidence that this complex can bind to a variety of cells including neutrophils and platelets potentially leading to pathophysiological consequences.

In addition to the alternative pathway of complement activation, the classical pathway has also to be taken into account (Brett, 1992). The classical pathway is based on the reaction of circulating C1 with any Ig molecules which might be initially adsorbed onto biomaterial surfaces. After activation of C1, the decomposition of C2 and C4 generates C3 convertase, which in turn degrades C3 into C3a and C3b. The C3b can either bind to its receptor on various cells (monocytes, leukocytes, B lymphocytes) or adsorb to the biomaterial surface to complete the complement cascade as shown for the alternative pathway.

Proinflammatory cytokines that may be relevant in biocompatibility include IL-1 and TNF. These have received the most attention because they are released by immune and inflammatory effector cells (Dinarello, 1990; Anderson, 1993). Furthermore, IL-6 generated by macrophages induces the proliferation and differentiation of B cells, the proliferation of fibroblasts and also the production of IL-8 (a chemotactic peptide synthesised by macrophages, endothelial cells, and fibroblasts) which stimulates neutrophil migration and degranulation (Dinarello, 1990). A variety of stimuli can activate

monocytes by inducing gene expression for one or more cytokines. These stimuli include adhesion to foreign surfaces, the non-physiological chemical environment caused by biomaterial leachables, the presence of complement fragments, or the secretion of cytokines by other cells during the immune response, as has been shown for IL-1 synthesis due to sequential treatment with interferon followed by IL-2 (Ward, 1994).

At this point, it is interesting to speculate as to which component might be the initiator of the inflammatory response to a biomaterial. As noted earlier, the initial event in the biomaterial-host interaction is the significant adsorption of albumin, immunoglobulins, and fibrinogen. Whereas albumin is unlikely to mediate inflammatory response because of passivation of surfaces, immobilised IgG together with activated complement components might be powerful mediators of phagocytic activation and the inflammatory response. However, Tang *et al.* (1993) showed that the acute inflammatory response to a biomaterial (polyester terephthalate), as measured by phagocyte accumulation, is normal in both severely hypogammaglobulinaemic mice and complement-depleted mice, suggesting that neither surface-bound IgG nor complement activation is required to initiate acute inflammatory response. Recently, the same group has shown that materials pre-coated with fibrinogen or plasma (but not with serum or albumin) elicit large numbers of phagocytes when biomaterials are implanted subcutaneously or intraperitoneally (Tang & Eaton, 1993). Most interestingly, afibrinogenaemic mice fail to show inflammatory response to implants whereas plasma or fibrinogen coated materials elicit a large number of phagocytes in these animals. These phagocytes (probably together with fibroblasts) may initiate the long-term inflammatory and fibrotic response which is seen around implants (Tang & Eaton, 1995). Thus, fibrinogen adsorption *per se* or conformational changes of fibrinogen structure (Barbucci & Magnani, 1994) might be of great importance in the inflammatory response to implanted biomaterials.

Although biomaterials in the non-degraded state are unlikely to be phagocytosed by neutrophils or macrophages because of their size, phagocytosis *after* biodegradation may occur. First, neutrophils and macrophages become highly activated in their attempt to digest and to degrade the biomaterial. The release of large quantities of lysosomal, hydrolytic enzymes by direct extrusion or exocytosis from the cells may lead to biodegradation of biomaterials (Henson, 1971; Weiss *et al.*, 1989). Furthermore, activated macrophages can release large quantities of oxidants (HO and O₂ radicals, H₂O₂) which may attack polymers because all organic polymers are susceptible to auto-oxidation, the autocatalytic reaction with oxygen (Stokes, 1993). Second, biomaterials are not homogeneous: polymers contain various functional groups; the constituent polymer chains have a range of molecular weights; some polymer chains possess "hard" and "soft" segments depending on the constituent monomers; some contain leachables including solvents and microparticles which can be phagocytosed (Hill, 1995; Ruyter, 1995). Third,

the biological host contains a large amount of water in intracellular and extracellular fluids (interstitial fluids, blood plasma, cerebrospinal fluid, intraocular fluid etc.), an average of 57% of the body weight. Water is, of course, the primary agent for hydrolytic degradation—particularly biopolymers such as cellulosic esters, aliphatic esters and nylons, i.e. those with ester or amide links (Stokes, 1993). Fourth, mechanical deterioration has been observed in some total hip replacement systems (for example, those made from ultrahigh density polyethylene (HDP)) because of wear and loading. It is suggested that the average wear rate for HDP of 0.1 mm per year generates 20 million particles per day, corresponding to 7 billion particles per year (Tang & Eaton, 1995).

When biodegradation is initiated, the inflammatory response is steadily catalysed. The persistent inflammatory stimuli initiate chronic inflammation, characterised by the presence of phagocytes, foreign body giant cells, monocytes and lymphocytes, with the proliferation of blood vessels and connective tissue (Anderson, 1993; Tang & Eaton, 1995). Phagocytic cells, present in all regions of the body, are the most important cell population in chronic inflammation because of their wide spectrum of biologically-active products, which include chemotactic factors, neutral proteases, arachidonic acid metabolites, reactive oxygen metabolites, complement components, coagulation factors, growth-promoting factors and cytokines (Anderson *et al.*, 1990; see Figure 5.1, lower part).

In particular, growth factors such as platelet derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor (TGF) and epithelial growth factor (EGF) are released by activated cells, and can stimulate a variety of other cells. Growth factors are involved in wound healing and are important in the proliferation of fibroblasts, the growth of blood vessels and the generation of epithelial cells (Anderson, 1993). Monocytes/macrophages have other important functions in the specific immune response, in addition to their phagocytic function as a non-specific reaction during the chronic inflammatory response. They express human leukocyte antigen (HLA) Class II molecules, which are essential for the presentation of foreign antigens to lymphoid immunocompetent cells (Unanue & Allen, 1987). Lymphocytes and plasma cells involved in immune reactions are the key mediators of antibody production and cellular immunity. Laboratory and clinical evidence indicates that components of some implanted biomaterials—either leachables after release into the biological system, or microparticles released after biodegradation—can migrate and then be phagocytosed and carried by macrophages and lymphocytes to distant areas. Thus Teflon, silicone, polyurethane, and other biopolymers have been found in regional lymph nodes and in other tissues and may be presented as antigens to immunocompetent cells (Brunstedt *et al.*, 1990; Glaser, 1993; Kaiser, 1993).

The foreign body reaction to biomaterials also involves the formation of foreign body giant cells by fusion of monocytes/macrophages in an attempt to

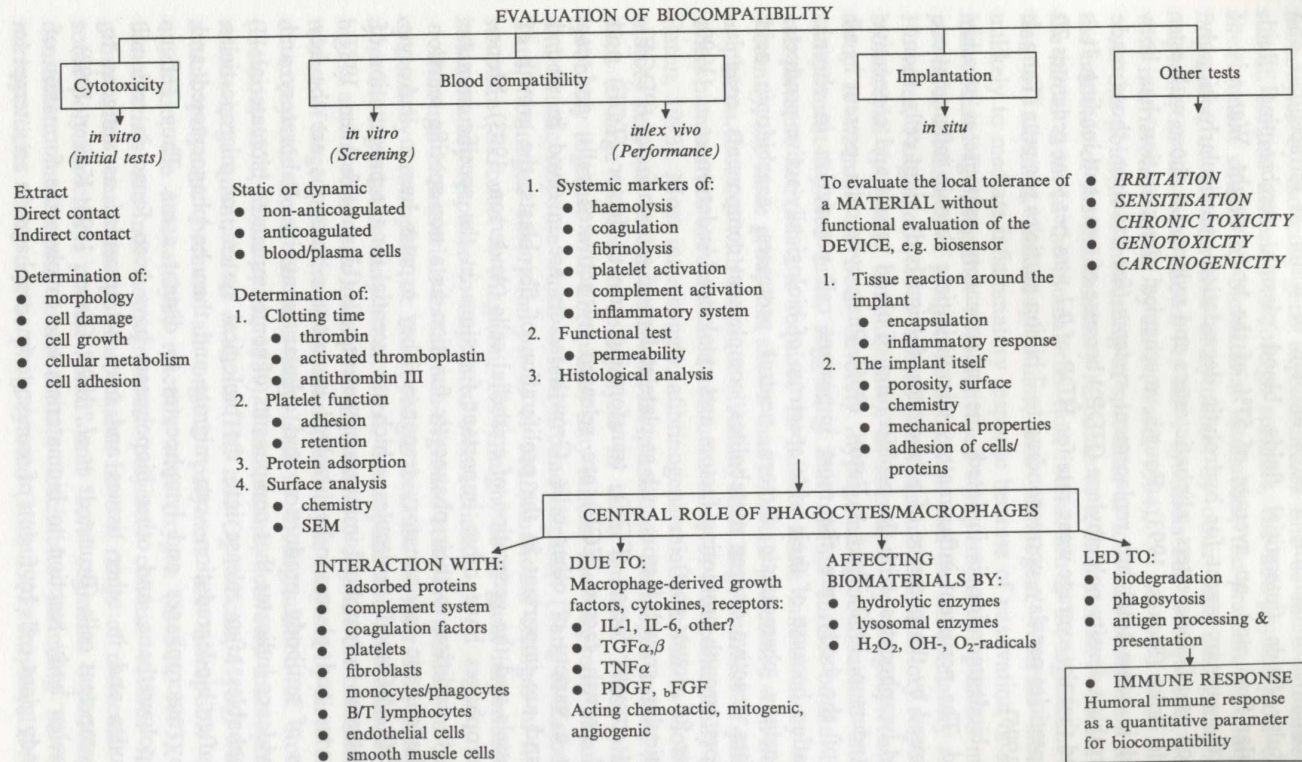


Figure 5.1 The complexity of biocompatibility evaluation of biomaterials used for implantation according to ISO 10993 (upper part). The central role of phagocytic cells in the inflammatory/immunologically response to biomaterials urgently requires new concepts and new test methods to consider the individual host responses (lower part)

phagocytose the material. In parallel, the granulation of tissue initiates the healing process by action of monocytes/macrophages followed by proliferation of fibroblasts, which synthesise collagen and proteoglycans to form a fibrous capsule around the implant, and endothelial cells, which are involved in vascularisation at the implant site (Williams, 1989; Anderson, 1993).

Immunological reactions within a foreign body response are thought to result from the attempts of the biological host to distinguish between self and non-self. Many complicated mechanisms exist by which the biological system recognises and resists the foreign material: however, these self-recognition mechanisms are imperfect and can sometimes break down. Such an event can possibly be initiated by denaturation of proteins after initial adsorption on biomaterials, which may then lead to an autoimmune reaction. In the subsequent interaction between plasma cells and antigenic or immunogenic molecules (as has been shown for silicone implants), cytotoxic T cells and B cells participate in the complex immunoregulatory network. In the case of silicone implants, four basic mechanisms have been suggested: (i) cell-mediated cytotoxicity, (ii) antigen-antibody interaction, (iii) complement activation and (iv) hypersensitivity (Glaser, 1993).

There are numerous reports suggesting significant immunological and related responses to certain implanted polymers including scleroderma, connective tissue disease, human adjuvant disease, lymphadenopathy, systemic sclerosis, rheumatic disease, granulomas, neoplastic transformations, sarcomas, carcinomas and others (Kircher, 1980; Endo *et al.*, 1987; Kossovsky *et al.*, 1987; Snow & Kossovsky, 1989; Varga *et al.*, 1989; Thomsen *et al.*, 1990; Nakamura *et al.*, 1992; Rowley *et al.*, 1994; Tsuchiya *et al.*, 1995; Bar-Meir *et al.*, 1995; Smith, 1995). There is an individual potential for chronic and systemic effects observed after implantation of biomaterials, and susceptibility to hypersensitivity might be strongly controlled by the genetic characteristics of the host (Williams, 1994b). However, the typical situation after implantation is the lack of an early marker that can indicate development of a latent complication or a bioincompatibility. In addition, it is noted that the individual immune response to a biomaterial shows a broad variability and depends on the age and the health of an individual.

However, as shown by our group and others, and as far as the humoral component of a specific immune reaction is concerned, the detection of specific antibodies directed to biomaterial components might be an earlier marker for the degree of individual chronic inflammatory response, which might then induce the implant failure (Goldblum *et al.*, 1992; Shirakawa *et al.*, 1992; Schlosser *et al.*, 1994a; Yang & Merritt, 1994; Ziegler *et al.*, 1994). Additionally, the detection of specific antibodies directed against those biomaterials potentially suitable for application in medical devices might be an important tool and new parameter for evaluation of their biocompatibility.

There are numerous unanswered questions concerning the complex process of inflammation with biomaterials. The estimation of future complications of

implant recipients and implant failure on one hand, and the search for the "right" biomaterial or surface modification on the other hand, might be the challenges of the future.

5.2.2.3 Consequences of Biomaterial-Host Interactions for *in vivo* Biosensors

Among the variety of implantable devices used in therapeutic medicine, biosensors must fulfil the highest requirements regarding biocompatibility and functional biostability because bioincompatibility of sensor materials ultimately results in the loss of sensor function, which may have dramatic consequences for the patient. Although the technology of construction has reached an advanced state of development, in contrast to the high functional stability of sensors *in vitro*, there is a lack of functional stability after implantation *in vivo*, and sensor lifetime is usually restricted to several hours or several days (Fischer, 1995). This is in fact a problem for all implantable biosensors but will be discussed below for glucose sensors in particular.

Some of the most important data for explaining the loss of sensitivity after subcutaneous implantation in dogs came from Rebrin *et al.* (1992), who have demonstrated that despite loss of sensor function *in vivo*, the sensors' functional stability *in vitro* after explantation was unaffected. This indicates that the loss of sensitivity of implanted sensors might not exclusively be due to changes at the underlying electrode. Furthermore, it could be demonstrated that after implantation of biosensors into rats, no glucose oxidase antibodies (GOD-ab) were detectable, in contrast to the control animals receiving GOD by injection, which developed high GOD-ab titres (Schlosser *et al.*, 1994a; Ziegler *et al.*, 1994). In principle, these findings also demonstrate functional biostability because no leakage of GOD was detectable. So the loss of functional biostability might be caused mainly by alterations of the microenvironment of the sensor surface, which then might influence the diffusion conditions and the true availability of the two substrates, glucose and oxygen.

In the case of amperometric glucose sensors, where reproducible mass transfer of the substrates is needed, the initial step of protein adsorption may immediately result in an unstable, ill-defined protein layer, which may alter or totally block the substrates' flux, and therefore diminishes radically the sensitivity (Vadgama & Réach, 1993; Fraser, 1994). Once a protein layer has formed, it undergoes continuous change and remodelling, including simultaneous desorptions and denaturations of proteins resulting in multiple layers of different compositions (Anderson *et al.*, 1990). Regardless of whether the implantation site is intravascular or subcutaneous, a blood-device interaction occurs which involves formation of microthrombi of blood cells by coagulation, and activation of complement factors as well as phagocytic cells.

Especially when sensing in blood (and over and above the dangers caused by clot propagation and embolism), the covering clot and the presence of phagocytic cells on a biosensor creates a microenvironment which distorts the ambient biochemistry (Ertefai & Gough, 1989). It is difficult, therefore, to distinguish true changes in the circulating analytes from artefacts.

The main cause of loss of sensitivity after implantation (confirmed by clinical and histological findings) resembles the inflammation that occurs in response to injury, with the recruitment of inflammatory cells, i.e. neutrophils and phagocytes, which are present in both blood and intracellular space of tissue (Ertefai & Gough, 1989; Rebrin *et al.*, 1992). Owing to the exudative reaction, the diffusion distance for substrates is greatly increased and the true glucose concentration in the exudate at the implantation site was up to four-fold lower than that in non-inflamed subcutaneous tissue or in blood (Rebrin *et al.*, 1992). The explanation for this might be the glucose uptake by the inflammatory cells in all kinds of injured tissues (Forster *et al.*, 1989). In a longitudinal study, Daley *et al.* (1990) analysed the glucose metabolism in injured tissue and showed that the glucose uptake does not differ from control up to 12 h after injury. However, this initial period is followed by a significant increase in glucose uptake up to the third day. Taking into account the short half-life time of neutrophils during acute inflammation (Ward, 1994), it becomes clear that macrophages and foreign body giant cells may be responsible for the glucose utilisation.

As noted earlier, the degree of inflammation is further influenced by the size and the geometry of the biosensor, with triangular implants producing greater response than circular implants (Woodward, 1982). Furthermore, smooth solid structures apparently become surrounded by a fibrous capsule, whereas spongy surfaces continue to be supplied by the vascular tissue (Spector *et al.*, 1989). The fibrous capsule surrounding the sensor presents a structurally distinct barrier of collagen fibrils, which might have an effect on the substrate mass transport, resulting in a delay in sensor response and an underestimation of analytes.

In addition, to date there is no completely satisfactory solution for sterilisation of intracorporal sensors using chemical or ionising radiation, i.e. a method that neither influences the enzymatic activity of GOD nor affects the properties of the outer membrane, as this may further influence the biocompatibility/inflammatory response (von Woedtke *et al.*, 1994). Bacterial infections, due to non-sterilised sensors or to permanent skin penetration at the injury site, may drastically influence the inflammatory process and therefore the sensor function (Fischer, 1995).

Furthermore, the individuality of the inflammatory/immune response seems to be the greatest barrier for controlled, long-term, *in vivo* sensing. In this context it should be remembered that the potential recipients for glucose sensors, i.e. insulin-dependent diabetics, often have a poor peripheral circulation, a quite differential tissue sensitivity to insulin or a modified immune

system, and therefore probably a different response to the implant than the healthy individuals in whom most biosensors have been evaluated (Award *et al.*, 1989; Fraser, 1994).

Once the inflammatory response becomes chronic, the process autocatalyses itself by ongoing biodegradation of the outer sensor membrane. Then a breakdown of the sensor membrane may occur followed by a leakage of the detector enzyme. The biochemical attack, and in particular the inflammatory attack on biomaterials used for sensor preparation, is a cumulative process which involves different cell populations and their products and results. At the present stage of sensor development, these attacks almost inevitably result in breakdown of sensing soon after implantation in the body (Fraser, 1994; Fischer, 1995).

5.3 FURTHER STRATEGIES FOR BIOSENSOR IMPROVEMENT

5.3.1 MATERIAL SELECTION

A correct and well-informed selection of materials (e.g. for the sensor's outer membrane) is crucial to the manufacture of safe and successful medical devices/biosensors. Recently, international standards requiring characterisation of biomaterials have been introduced. The general standard ISO 9001: 1994, *Quality systems—model for quality assurance in design, development, production, installation and servicing*, together with EN 46001: 1993, *Quality systems—Medical devices—particular requirements for the application of EN 29001*, regulate to a certain extent the requirements regarding product specifications, safety of medical devices, traceability and removal of process agents from the products. The European Council Directive 93/42/EEC concerning medical devices postulates in its essential requirements that "the device must be designated and manufactured in such a way as to reduce to a minimum the risks posed by substances leaking from the device". It is therefore essential to not only know the biomaterial *per se*, i.e. its rough elemental composition and its chemical properties, but the origin of the biomaterials used. It is essential to have detailed information about the polymerisation process, the raw materials, i.e. the monomers, and the additives used (which may be potential leachables). These include plasticisers, initiators, accelerators, fillers, chain-transfer agents, inhibitors, antioxidants, catalysts, processing aids, emulsifying agents, colorants and UV-stabilisers (Bruck, 1990). Once implanted in the body, additives can leach from the biomaterial; this may have significant effects on the polymers themselves as a result of generating voids within the polymer matrix and/or interactions with particular chemical groups on the polymer chain which can lead to initiation of biodegradation (Stokes, 1993). Additionally, leachables may have drastic

biological effects including cytotoxicity and other deleterious effects on platelets and leukocytes, as shown for the plasticiser di-(2-ethylhexyl phthalate) used for most PVC preparations (Jaeger & Rubin, 1979), or the formation of a potential carcinogen (4,4'-methylenedianiline), formed in thermoplastic polyurethanes after sterilisation (Shintani & Nakamura, 1991; Daka & Chawla, 1993).

Different methods are used to characterise biomedical polymers (Ruyter, 1995). The molecular weight and molecular weight distribution of a polymer, as analysed by size exclusion chromatography or gel permeation chromatography, is of great importance in determining the exact pathway for the inflammatory process and subsequent biodegradation and elimination from the organism. Furthermore, several methods such as differential scanning calorimetry, dynamic mechanical thermal analysis, ^{13}C -nuclear magnetic resonance spectroscopy are used to study selected and structural properties of biopolymers. Methods based on infrared spectroscopy and Fourier transform infrared spectrometry are used to analyse polymer materials and their hexane extracts, and thus allow determination of the degree of conversion after polymerisation. Gas chromatography and HPLC are used extensively to determine additives, residual monomers, other leachables and degradation products. Further important characterisation methods for polymers are the measurement of specific gravity, assessment of hardness and tensile properties, assessment of metal content, analysis of thermal properties, and surface analysis using scanning electron microscopy (Wallin, 1995b).

In the case of the implantable glucose sensor, a variety of biomaterials have been used as outer membranes, and with varying success. The most popular have been cellulose (Clark *et al.*, 1988), cellulose acetate (Fischer *et al.*, 1987), various polyurethanes (Pickup, 1993; Updike *et al.*, 1994), polycarbonate (Higson & Vadgama, 1993), polyvinylalcohol (Brinkmann *et al.*, 1991), alginate-polylysine-alginate (Shichiri *et al.*, 1989), NafionTM (Turner *et al.*, 1991), PTFE (Vaidya & Wilkins, 1993) and PVC (Cha *et al.*, 1991). Possibly more promising than the sensor materials mentioned above are those using polyethylene glycol (PEG, also known as poly(ethylene oxide), PEO) coatings (Bergström *et al.*, 1992) or PEG-containing copolymers (Quinn *et al.*, 1995). Protein adsorption studies have shown that fibrinogen adsorption was significantly reduced after coating polymers with either linear or branched PEG (Bergström *et al.*, 1992). Recently, using photo-crosslinked copolymers containing PEG as outer glucose sensor membrane it could be demonstrated that these sensors induce a much less fibrous encapsulation after subcutaneous implantation in rats compared to the same copolymers containing no PEG (Quinn *et al.*, 1995).

However, in summary, a thorough material characterisation and standardisation will be an important milestone in the development of active implantable medical devices, which may then allow more reproducible assessment of implantable biosensors.

5.3.2 MODIFICATION OF BIOMATERIALS TO IMPROVE THEIR BIOCOMPATIBILITY

Since a biosensor (even one covered with a biomaterial that has been well-studied *in vitro*) is placed within the body, it has been recognised that initial interactions with blood may be of great importance for both device performance and the patient's safety. As already noted, a universally biocompatible or indeed blood- and/or tissue-compatible material does not exist (Williams, 1989; Black, 1995). Furthermore, it is very difficult to develop a material which shows simultaneously the required mechanical/physicochemical characteristics and the desired biocompatibility.

Thus, after selection of the best material in terms of device performance, it becomes necessary to seek alternative solutions for improvement of biocompatibility. One of the most attractive methods involves surface modifications. There are at least three general approaches in surface modification which may be relevant for improved biocompatibility of implantable biosensors. First, one can provide the polymer surface with biologically active components which inhibit blood-material interactions, for example with heparin, the commonly used anticoagulant. A second approach is to mask the biomaterial surface in order to prevent it being recognised as a foreign body; this includes phospholipid-mimicking surfaces. Finally, surfaces can be coated with very hard substances such as diamond-like carbons, which improve biocompatibility by maximising inertness.

5.3.2.1 Surface Heparinisation

The concept of coating the anticoagulant activity of heparin onto biomaterials is not new (Gott *et al.*, 1963) and was further encouraged following the important discovery of active, structurally and functionally heparin-like molecules localised on the natural vascular wall (Marcum *et al.*, 1984). Heparin, chemically a polysaccharide, prevents clotting by suppressing the activity of the coagulation system. In response to contact with a biomaterial, the activation occurs by the intrinsic pathway triggered by the conversion of Hageman factor (FXII) into an active enzyme, FXIIa, followed by the activation of the subsequent factors. Finally, FXa converts prothrombin to enzymatically active thrombin which precipitates the soluble plasma protein fibrinogen into a solid fibrin clot. The inhibitory force of heparin is due to its ability to interact with plasma prothrombin III to form a heparin-antithrombin III complex which then inactivates thrombin by forming the inactive heparin-antithrombin III-thrombin complex. Thus, heparin acts as a cofactor or catalyst that accelerates the inactivation of thrombin by antithrombin III. Only about one-third of heparin molecules contain the specific sequence of saccharides which form the binding sites to which antithrombin III binds with high affinity. When bound to this sequence, antithrombin III inhibits the

coagulation enzymes at a much higher rate than in the absence of heparin (Hubbell, 1993; Riesenfeld *et al.*, 1995).

In addition to the anticoagulant effect after heparinisation of biomaterials, their ability to activate complement can also be significantly diminished. This has been demonstrated for PVC, silicone and Tecoflex (a polyurethane) which all caused a substantial complement activation without heparinisation. Furthermore, using end-point immobilised heparin commercialised by Carmeda AB and several partners (Larm *et al.*, 1986), activation of complement system was completely eliminated for all materials tested (Riesenfeld *et al.*, 1995). This underlines the importance of surface immobilisation of heparin, i.e. the functional activity of the antithrombin III binding site must be preserved.

A successful technique is the covalent end-point immobilisation, which involves the coupling of the reducing part of the molecule by a single covalent linkage to an amino group of the biomaterial surface (Larm *et al.*, 1986). Other immobilisation methods include electrostatic interactions using the high negative charge density of the heparin molecule (Salzman *et al.*, 1969). Furthermore, heparin can be immobilised via a hydrophilic spacer arm, such as polyethylene glycol (Grainger *et al.*, 1990). Polyethylene glycol spacers of molecular weight 2000–4000 appear to be optimal for preservation of activity of heparin, even to the extent of increasing activity ten-fold.

Complete retention of heparin activity has been observed through many cycles *in vitro* and after periods of implantation of up to four months (Arnander *et al.*, 1987). However, a critical factor is the catalytic activity of heparin in long-term experiments. Multiple factors may be altered by the attachment of heparin to biomaterial, e.g. the physicochemical nature of the surface, which may result in a change in biological behaviour independent of the anticoagulant activity of heparin. In addition to heparin, other anti-coagulants or antiplatelet agents such as hirudin, plasmin and plasminogen activators, prostaglandins and antibacterial agents may be useful for surface modification (Hubbell, 1993).

5.3.2.2 Phospholipid-based Surfaces

The rational basis for this coating procedure is to make biomaterials biomimics, in order to render the surface more biological (Williams, 1995). Many biological cell membranes have an asymmetric lipid distribution, containing typically negatively charged phospholipids such as phosphatidylserine on the inner cytoplasmic side and neutral phosphorylcholine (PC) molecules outside the membrane. PC head groups are found on 90% of the lipid components of the outer erythrocyte membrane (Campbell, 1994). Zwaal & Hemker (1982) observed that the cytoplasmic side of erythrocyte membrane is thrombogenic *in vitro* whereas the extracellular side had no effect on the haemostatic mechanism. On this basis, coating of PC-containing materials onto biomaterials was proposed in order to reproduce these lipid characteristics and mimic the

cell surface membrane, transferring the non-thrombogenicity to the biomaterial.

The coating procedure uses the ability of specially synthesised PC-containing materials to subsequently polymerise, which can be induced by UV-light or gamma irradiation, and to form stable crosslinked layers on a variety of surfaces. In 24-h stability tests, less than 0.01% leaching was detected from various polymers (Campbell, 1994). If PC is attached to a surface with the appropriate orientation, it renders the surface extremely hydrophilic so that plasma proteins may only adsorb to the biomaterial with difficulty because of the layer of bound water. Therefore, these surfaces might be non-thrombogenic. In the case of PC-coated PVC, platelet adhesion and activation *in vitro* was reduced by 99%. Contact activation by PVC, polycarbonate and polyethylene (as measured by the presence of FXIIa) was also drastically diminished. Furthermore, PC-coating results in reduced complement activation.

With many polymers, it could be demonstrated that fibrinogen adsorption was reduced by 90% when compared to non-coated materials (Campbell *et al.*, 1992; Campbell, 1994). This fact might be of great importance for the acute inflammatory response to biomaterials, i.e. the initial event being the adsorption of abundant proteins such as albumin, immunoglobulins, and especially fibrinogen. As demonstrated by Tang & Eaton (1995), fibrinogen adsorbed to biomaterials (polyester terephthalate) is a powerful mediator of phagocytic activation and inflammatory response, in contrast to albumin and IgG. So phagocytes, probably with fibroblasts, may initiate the long-term inflammatory and fibrotic response which is seen around chronic implants.

Thus, PC-coating of biomaterials might result in both anticoagulant and anti-inflammatory surfaces. Recently, new biocompatible polymers (2-methacryloyloxyethylphosphorylcholine (MPC) copolymers with *n*-butylmethacrylate (BMA)) were synthesised and showed good biocompatibility. They were subsequently used as the outer membrane for glucose sensors, which were able to measure the glucose concentration up to 14 days after implantation in subcutaneous tissue in a human (Ishihara *et al.*, 1994).

5.3.2.3 Diamond-like Carbons

The use of very hard diamond-like carbon (DLC) surfaces is dependent on their extreme inertness and marked lack of adverse physiological effects. Depending on the techniques used, the properties of the resultant coatings can vary. Thus the DLC film can retain the essential crystalline structure of diamond, it can be amorphous, or can be both crystalline and amorphous. Depending on the nature of the carbon-containing substances used for coating, the resulting DLC film may be of pure carbon or it may contain other elements, in particular hydrogen (Evans, 1994; Williams, 1993).

The commonest method used to modify biomaterials with DLC is the plasma-assisted chemical-vapour deposition (CVD), although the high

temperature needed excludes its use with organic polymers. However, other low-temperature techniques using evaporated oil which condenses on materials have been introduced. In the case of biosensors, after cleaning of the outer membranes by fast argon atoms, DLC coating was achieved in a neutral saddle field source (Franks, 1989; Higson & Vadgama, 1993). The advantage of DLC coating is the fine control of membrane permeability, resulting in a significant extension in linearity to glucose response.

Biocompatibility studies have shown no cytotoxic effects and a reduced material-tissue interface. This must not be confused with an enhancement of biocompatibility because the biomaterial is ignored and therefore not truly incorporated in the body. However, DLC and other inert coatings will play an important role as outer membranes, due to enhancement of the dynamic range of sensors, especially those destined for repeated use in the body (Higson & Vadgama, 1993).

5.3.3 THE ISSUE OF BIOCOMPATIBILITY TESTING

Once biomaterials are judged to be suitable in a specific situation with respect to their physicochemical properties, and before they are used in commercial medical devices, it must be demonstrated by testing that there are no local or systemic adverse effects on the patient. Adverse effects are generally classified as cytotoxic, irritant, sensitising, systemic, genotoxic, carcinogenic (Wallin, 1995b).

Especially in the last decades, many different biocompatibility tests have been published, but there is still no universal test available for objective evaluation of the biological safety of medical devices. Although the main principles applied in testing device materials have changed very little over the last 20 years, the standards, specific laboratory methods, regulatory views towards testing, interpretation of results, and availability of new materials have changed greatly. Basic problems such as a failure of standardisation (including "real" standards as well as a broad interlaboratory variability) still exist. The main unsolved problems in this context are different test results and interpretations of *in vitro*- vs. *in vivo*-based methods.

The European Commission has issued the Medical Device Directive (MDD, operational as of 01.01.1995) and the Active Implantable Medical Device Directive (AIMDD) for evaluation of biomaterials and medical devices. Biosensors will either be dealt with by the MDD or, if an energy source is applied, by the AIMDD. The general principles of biological evaluation according to ISO 10993-1 (*Biological Evaluation of Medical Devices, Part 1: Guidance on Selection of Tests*) (Eloy, 1994) are as follows:

- (1) The assays must be performed on the final product "biosensor" or on representative samples of the biomaterial.

- (2) The test selection must be performed according to:
- (a) the nature, duration, frequency and exposure conditions of devices;
 - (b) the toxicological data of the components of the device;
 - (c) the physicochemical nature of the final product;
 - (d) the ratio between the surface of the device and the volume of the implantation site;
 - (e) new literature and experimental data.

Preliminary *in vitro* cytotoxicity tests such as extract tests, and direct and indirect contact tests, are acceptable for material screening but they are not sufficient for devices. The tests are performed using different cell lines such as mouse fibroblasts L-929, baby hamster kidney cells BHK-21 and others (Zhang *et al.*, 1991). Cell lines present many advantages, notably that they are easy to grow and can be subcultured indefinitely; however, a serious concern is that these will not be the types of cells which are most likely to come in contact with the biomaterial (or with substances released from it) in real use. If the cells are directly seeded on polymer discs, the effectiveness in promoting cell adhesion can be tested. Determination of cytotoxicity is generally performed by morphological assessment of cell damage, measurement of cell damage, cell growth and cellular metabolism (see Figure 5.1).

More complex but closely-connected issues are those of blood-material and/or tissue-material interactions involving red blood cell damage, the activation of coagulation, platelets and the immune system, the activation/inhibition of fibrinolysis, and, after implantation in subcutaneous, muscular or bone tissue, the evaluation of the tissue reaction around the implant. Assays to be performed *in vitro* (under either static or dynamic conditions), *ex vivo* or *in vivo*, are listed in Figure 5.1. Genotoxicity evaluation involves *in vitro* assays performed on biomaterial extracts including gene mutation, chromosomal observations and DNA effects (Eloy, 1994). For evaluation of carcinogenicity, it should be pointed out that there is no acceptable test currently available which defines the clinical risk for cancer. It is well known that most biomaterials can cause tumours in certain circumstances in laboratory animals (especially rodents), and components of many biomaterials are known to be chemical carcinogens. However, in clinical use of biomaterials in humans, malignant tumours are rare (Eloy, 1994; Wallin, 1995b).

Among the risks associated with clinical use of medical devices, only cytotoxicity can be measured and assayed by means of established standardised protocols. However, other risks, involving more complex biological events, i.e. blood-device and/or tissue-device interactions have to be evaluated according to test protocols with lower precision. In addition, prolonged clinical use or use in some specific situations may involve additional risks (based on the individuality of host response) that are not currently amenable to investigation by established biocompatibility testing protocols. Therefore, new testing models involving evaluation of carcinogenesis, immunogenicity,

thromboresistance, wound-healing, blood compatibility and chronic toxicity are urgently needed (Williams, 1991; Wallin, 1995b).

5.3.4 NEW STRATEGIES IN BIOCOMPATIBILITY TESTING

In particular, immune reactions seem to play a central role in the inflammatory process that follows implantation of a foreign "non-self" body, and this process also involves the following:

- (1) Complement activation (Brett, 1992).
- (2) Phagocytic cell function (quantification of the number of circulating phagocytes, their expression of adhesion receptors, and their products, the cytokines (Ward, 1994)).
- (3) Hypersensitivity (approximately two-thirds of patients who experience dialysis anaphylaxis on first-use of an ethylene oxide (ETO)-sterilised dialyser have IgE against ETO-protein conjugates, compared to less than 5% of patients without anaphylaxis (Grammer & Patterson, 1987).
- (4) The role of basophilic leukocytes and mast cells (IgE-specific antibodies, anaphylatoxin-dependent reactions).
- (5) Activation of histamine-releasing factors (Götze, 1994).
- (6) Production of cytokines and cytokine-inhibitory proteins (Pereira & Dinarello, 1994).

From our point of view, and in accordance with findings by others (Kossovsky *et al.*, 1987; Goldblum *et al.*, 1992; Yang & Merritt, 1994), evaluation of immunogenicity (i.e. cellular and humoral immune response to components of implants) might be a new, important tool for evaluating biocompatibility after implantation. Because cellular immunity is difficult to quantify and to standardise, humoral immune reactions by means of quantification of specific antibodies possibly reflect better the material-host interaction in general. In particular cases (and this might be a new aspect in biocompatibility testing) the individual host response/humoral immune response to an implant might therefore be an early sign of functional biostability and therefore implant failure.

The rationale of this approach is that, in the inflammatory response, large quantities of phagocytes at the implantation site can affect the biostability of biomaterial and may initiate the process of biodegradation (Vince *et al.*, 1991). Even microparticles, monomers, or oligomers can be "extracted" by the highly aggressive environment surrounding the implant; and they may then be phagocytosed. This, in fact, has been demonstrated for many polymers including silicone, Teflon (PTFE), L-lactic acid/glycolic acid homo- and copolymer, poly(L-lactic acid) and others (Glaser, 1993; Kaiser, 1993; Tang &

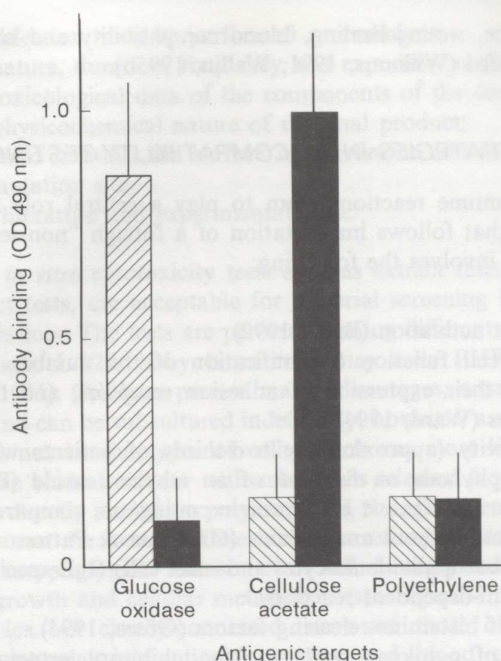


Figure 5.2 Humoral immune response against components of glucose sensor on day 23 after subcutaneous implantation in rats ($n = 5$) on days 1 and 8; the symbol ■ marks cases where IgG antibodies were only detectable against the outer membrane (cellulose acetate) but not to the inner membrane (polyethylene) in comparison with control animals ($n = 5$) injected with saline only ▨. No antibodies were detectable against glucose oxidase (GOD) in the sensor group ■ in contrast to control rats which received $100 \mu\text{g}$ GOD/rat ($n = 2$) ▨. From Ziegler *et al.* (1994), with permission

Eaton, 1995). Since T and B lymphocytes are also present at increased levels at the implantation site (Gong *et al.*, 1993; Holgers *et al.*, 1995) phagocytes may act as antigen-presenting cells to induce a specific immune response.

In our first study, we have detected specific IgG antibodies against the outer biomembrane (cellulose acetate) 23 days after subcutaneous implantation of glucose sensors into Lewis rats—and this without cross-reaction with the inner polyethylene membrane (Ziegler *et al.*, 1994). However, no anti-GOD antibodies could be detected, as might be expected upon release of GOD provoked by breakdown of the external membrane. This indicates in principle the functional biostability of the sensor (Figure 5.2). In recent studies, we have implanted different biomembranes suitable for sensor preparations in order to investigate the time course of immune response of polymers in rats (Schlosser *et al.*, 1994b). Surprisingly, we obtained the highest antibody formation against

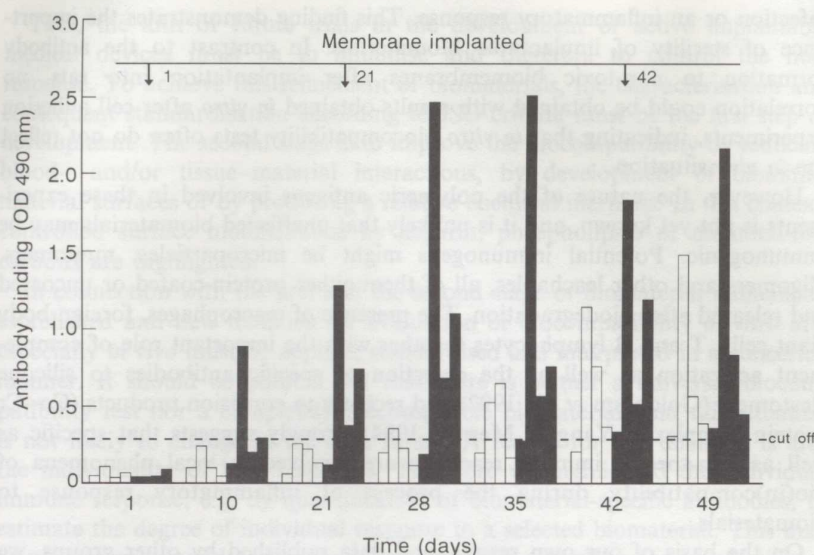


Figure 5.3 Time course of IgG antibody formation against PTFE (Teflon) in individual rats induced after subcutaneous implantation of PTFE sheets (1 cm²) alone on experimental days 1, 17 and 38 (□), or in combination with one additional application of complete Freund's adjuvant (CFA) on day 1 (■). The cut off for antibody positivity was calculated from the mean antibody binding + 2 SD of control rats which received CFA in combination with saline on day 1. From Schlosser *et al.* (1994b), with permission

Teflon (PTFE) which is well-known to be chemically inert. Polycarbonate and Pellethane have shown only a moderate immunogenicity.

Although inbred rats were used, the individual antibody formation of rats to the different polymers has shown a broad variability (Figure 5.3). However, the formation of anti-polymer antibody was independent of the (age-dependent) time course of the serum IgG concentration of individual rats (Schlosser, unpublished results). Thus it becomes possible to evaluate the individual biocompatibility, i.e. the individual host response to a biomaterial which is likely to vary more in humans compared to inbred animals. The detection and quantification of specific antibodies to implants probably enables the quantification of the degree of bioincompatibility/host immune response to an implant.

As shown in Figure 5.3, a second implantation in the same animal results in an increase of antibody formation, resembling a booster effect. Furthermore, the immune response could be significantly enhanced by the polyclonal activation of the immune system using one application of complete Freund's adjuvant which contains heat-killed mycobacteria, i.e. simulating a bacterial

infection or an inflammatory response. This finding demonstrates the importance of sterility of implantable biomaterials. In contrast to the antibody formation to non-toxic biomembranes after implantation into rats, no correlation could be obtained with results obtained *in vitro* after cell adhesion experiments, indicating that *in vitro* biocompatibility tests often do not reflect the *in vivo* situation.

However, the nature of the polymeric antigens involved in these experiments is not yet known, and it is unlikely that unaffected biomaterials may be immunogenic. Potential immunogens might be microparticles, monomers, oligomers and other leachables, all of them either protein-coated or uncoated and released after biodegradation. The presence of macrophages, foreign body giant cells, T and B lymphocytes together with the important role of complement activation as well as the detection of specific antibodies to silicone elastomers (Goldblum *et al.*, 1992) and recently to corrosion products (Co-Cr protein complexes; Yang & Merritt, 1994) strongly suggests that specific as well as non-specific immune reactions are involved in local phenomena of bio(in)compatibility during the process of inflammatory response to biomaterials.

On the basis of our own results, and data published by other groups, we suggest the inclusion of immunogenicity as a standardisable, quantitative parameter in the "puzzle" of biocompatibility evaluation of implantable medical devices, and particularly in the case of implantable biosensors.

5.4 CONCLUSIONS

Although the technology of conception and construction of active implantable medical devices such as biosensors has reached an advanced state of development, *in vivo* sensing of important clinical parameters is still limited by the host's reactions against the implant as a foreign body, especially against the outer biomembrane.

In general, the implantation of medical devices or biomaterials results in injury of tissue and initiates an inflammatory response. The inflammatory response, of which the major components are acute and chronic inflammation, involves a variety of different cell-biomaterial and cell-cell interactions in both blood and tissue, and leads to a highly aggressive microenvironment which may then affect the medical device by initiation of biodegradation. The complete range of non-specific and specific host defence mechanisms may be involved, including the coagulation system, the complement cascade, phagocytosis, and the immune system. Although the complexity of interaction is mainly uncharted and therefore hypothetical, the activated phagocyte seems to play a central and important role in the response to an implant because of the great spectrum of its various cellular and humoral interactions, and its effects on biomaterials.

Thus, the aim of future trials in the development of active implantable medical devices must be to minimise and therefore to control the host response. To achieve this refinement of biomaterials, the characterisation and consequent standardisation according to ISO criteria must be the first step of development. The second stage is to improve the biocompatibility by reducing blood- and/or tissue-material interactions, by development of biomimic material surfaces or by producing a relative chemical inertness. In this context, controlled surface modifications by heparin, phospholipids or diamond-like carbons are highlighted.

In connection with the first and the second stage of biomaterial refinement, established and new methods for evaluation of biocompatibility *in vitro* and especially *in vivo* must be applied, standardised and interpreted in a concerted manner. It should be pointed out that there is neither a universal biocompatibility test nor a completely biocompatible biomaterial, and this situation is not likely to change in the near future. A reason for this dilemma is also the individuality of host responses. Therefore we highlighted the individual immune response, e.g. by quantification of biomaterial-specific antibodies, to estimate the degree of individual response to a selected biomaterial. This may be necessary to avoid or to reduce complications as well as to aid selection of the "biomaterial of choice" for an application in medical devices.

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