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Review
Tissue–biomaterial interactions

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A wide variety of materials is being increasingly used in medical practice for the treatment of patients in which the materials come into direct and often sustained contact with the tissues of the body. The commonly-known examples of hip replacements, contact and intraocular lenses and pace-makers serve to emphasize the importance of this subject. Because the body is so well equipped to reject any intruding object, whether that is a bacterium or a splinter of wood, the materials which are to stand any chance of success within this hostile yet sensitive milieu must be chosen very carefully. The subject of biomaterials represents an almost unique blend of physical and biological sciences, and it is becoming increasingly important that these aspects are drawn together to help in the development of quality biomaterials that are able to perform optimally in this environment. The key to this subject lies in the interactions that take place between biomaterials and these tissues and this review is aimed at providing, for the materials scientist, an understanding of the mechanisms of these interactions.

1. Introduction

Biomaterials are those materials which are used in medical, dental, veterinary or pharmaceutical applications and which come into intimate and sustained contact with tissues of the body, generally (although not exclusively) being implanted within these tissues. Put a little more succinctly, and as defined by a recent conference devoted to defining such terms [1], a biomaterial is a non-viable material used in a medical device, intended to interact with biological systems. They may be distinguished from other materials in that they possess a combination of properties, including chemical, mechanical, physical and biological properties that render them suitable for safe, effective and reliable use within a physiological environment, an environment that is both extremely hostile and yet sensitive to and unforgiving of irritating foreign bodies.

It is not uncommon, in journals such as this, which relate to materials science, to find papers dealing with the subject of biomaterials. Nor is it unusual for an industry that is involved with the development of new materials, and academia that is concerned with the basic properties of these materials, to turn their attention to biomaterials for commercial or intellectual reasons. It is both relevant and timely, therefore, to review some of the important aspects that are concerned with the transformation of an ordinary material into a biomaterial.

In order to familiarize the reader with the types of situation that are involved here, a few examples may be cited. Total joint replacements [2], such as the hip and knee, are well known and represent some of the most successful surgical innovations of this century. Hundreds of thousands of patients receive new joints worldwide each year; there is a multiplicity of designs and a selection of materials ranging from ultrahigh molecular weight polyethylene to austenitic stainless steel and alumina. At the other end of the spectrum is the total artificial heart [3], so far used in little more than a handful of patients, and largely constructed of polyurethane elastomer. In between these extremes we find the replacement of the lens in the eye in the treatment of cataracts [4], kidney machines [5], maxillofacial prostheses [6], neurological stimulators [7], drug depots [8], heart pacemakers [9] and many others. The reader may refer to recent reviews for further information [10–15]. The materials utilized in these devices cover as wide a spectrum as found in any industry, with examples of metals, ceramics, glasses, plastics, fibres, elastomers, composites and tissues themselves [16].

Although there are many factors which play a part in determining the fate and effectiveness of a material used in this way, including the mechanical and physical properties, it is the interactions between the material and the tissues that dominate the discussions. In all those applications referred to above, it is relatively easy to find materials that are able to satisfy the functional requirements (e.g. strength, fatigue strength, rigidity, optical transparency, electrical conductivity). It is very difficult, however, to find materials which are able to continue performing these functions for a long period of time (more than twenty years in some cases) without deterioration of the material itself or undesirable effects being induced in the tissues of the body.

This review is concerned with the nature of these tissue–biomaterial interactions and is specifically aimed at the reader who has a materials science background but who is interested in the fate of materials when interfaced with tissues. These interactions are,

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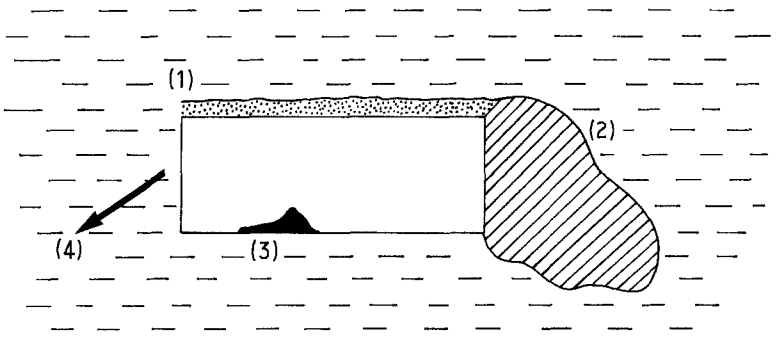


Figure 1 Aspects of biocompatibility: (1) initial events at the surface, (2) local host response, (3) corrosion or degradation of material, (4) systemic host response.

of course, complex, and, it should be admitted, largely unpredictable. There are many components and sequelae of the interactions, but for the purposes of introduction we may identify four features. These, as shown in Fig. 1, are

(1) the initial events that take place at the interface, largely concerned with the physicochemical phenomena that take place in times measured in seconds or minutes following contact between biomaterial and tissues;

(2) the effect that the presence of a foreign body has on the tissue surrounding the implant, which may be seen at any time ranging from minutes to years;

(3) the changes seen in the material as a result of its presence in the tissues, usually described under the headings of corrosion or degradation; and

(4) the sequelae of the interfacial reaction that are seen systemically (that is, throughout the body) or at some specific but remote site.

These four phenomena collectively constitute the subject of biocompatibility. They may be treated independently and, indeed, since the mechanism and principles may be so different, it is often convenient to do so. On the other hand, they are all interrelated and there is logic in the argument that they should be considered together. It is known, for example, that the corrosion of metals is influenced by pH and oxygen potential. Since the presence of an implant in tissues can induce changes that on a very localized and microscopic scale are concerned with such variables, the rate of, or indeed mechanism of corrosion may vary. As the rate of corrosion varies, so the tissue response, which as we shall see is partly mediated by the release of corrosion products, varies. Both processes are, therefore, dependent on each other.

It is, however, impossible in a review of this nature to cover all four of these aspects in any great depth. Instead, the review will concentrate on the second of those listed above, the localized effects on the tissues, since this is most relevant to the objectives of the review. This will be preceded by brief sections on the initial events and corrosion and degradation phenomena in order to provide the basis for understanding the way in which these phenomena may control the tissue response. The subject of systemic biocompatibility will not be addressed in this review.

It should be emphasized that the review will not attempt to catalogue the responses seen with specific materials but rather will describe the type of phenomena that may be observed and review the mechanisms that are involved. This paper is, in fact, attempting to outline a hypothesis for the mechanism of tissue–biomaterial interactions.

2. Initial events at the tissue–biomaterial interface

For applications in the body, the duration of which may be measured in tens of years, the events which occur within a few seconds of contact with tissues might appear irrelevant. Indeed, for many such applications there is little evidence that these initial events are important. In some other situations, however, it is clear that the nature of this initial interaction plays a significant role and it is necessary to review briefly the state of our knowledge.

The biomaterial–tissue interface that is established on implantation is, almost inevitably, a biomaterial–blood interface and the initial events are dominated by the adsorption of proteins from the blood on to the surface. Baier and Dutton [17] demonstrated the ubiquitous and inevitable nature of this process a number of years ago and Gendreau *et al.* [18] and Vroman *et al.* [19] determined that proteins were already present on polymer surfaces within seconds of the exposure. At least three different driving forces are at play here [20]. First, thermodynamically either enthalpy or entropy changes may be sufficiently great to provide a negative free energy change for adsorption of proteins on to polymers under physiological conditions [21, 22]. Secondly, the ambivalent polar/non-polar characteristics of proteins favour a concentration of proteins at interfaces; and thirdly, proteins are usually only sparingly soluble and adsorption increases as the solubility decreases.

The type of binding of proteins to foreign surfaces depends on the nature of the surfaces with hydrophilicity being the most frequently discussed parameter. It is widely assumed that no specific covalent attachment occurs between proteins and polymers so that all binding is secondary in nature, for example hydrogen bonding. The amount that adsorbs similarly varies; Brash and Uniyal [23] report variations from 0.02 to 0.57 $\mu g\,cm^{-2}$ for albumin and 0.034 to 1.09 $\mu g\,cm^{-2}$ for fibrinogen adsorbed under steady state conditions on to polymers ranging from hydrophilic to hydrophobic in character. *In vivo*, the situation will probably be different for there may be an initial maximum amount of protein adsorbed and a subsequent decline as desorption takes place. There will almost certainly be conformational changes as the proteins organize themselves on the polymer surface.

In a very extensive study of the mechanisms of protein adsorption on to hydrophilic polymer surfaces (conducted in relation to soft contact lenses) Costillo and co-workers [24–27] have discussed the kinetics of adsorption of a range of proteins on to hydroxyethylmethacrylate–methacrylic acid copolymers. They have shown structural changes in the adsorbed proteins, especially the α-helix content of the protein reducing and the β-sheet conformation increasing with time. When exposed to γ-globulins, these materials rapidly adsorb the protein, charge and hydrophobic interactions as well as hydrogen bonding resulting in conformational changes. It was suggested, and indeed seems very likely, that any protein or glycoprotein will experience a conformational change in order to maximize these interactions.

The vast majority of work on protein adsorption has involved polymer surfaces and the reader is referred to further review articles on this subject [28–31]. Very little has been performed in relation to metals, but the author has shown here that whilst many pure metals adsorb proteins to the same extent as those polymers described above, other metals, such as gold, silver and copper, adsorb far greater amounts, suggesting far stronger binding forces [32]. The significance of this is not yet clear.

3. The corrosion and degradation of biomaterials

A considerable amount has been written on the subject of the corrosion and degradation of biomaterials and it is not intended to review all the information here. The reader is referred to a number of publications on this subject for this detail [33–42].

3.1. Metals

In the context of metals it is now appreciated that the physiological environment is extremely hostile, consisting of an aqueous solution of various anions, cations and biological macromolecules. Corrosion phenomena in clinical practice are well known [43–47] and only the noblest of metals, such as gold and certain platinum group metals, or the most passive, such as titanium or chromium, stand any chance of keeping the corrosion rates within apparently acceptable levels. Whereas it has for long been assumed, however, that this corrosion is attributable to the chloride ion (which is unsurprising in view of the known aggressiveness of saline solutions), it has recently become apparent that the biological macromolecules, and specifically the proteins in extracellular fluids, are able to influence this corrosion considerably. This aspect has been reviewed by the author [48] and in several other papers published in the last year or so [49, 50].

In most engineering applications of metals and alloys, the importance of corrosion lies in the effect it has on structural integrity; corroding structures fail because they cannot support stresses, or they cause physical malfunctions. This is not the case with biomaterials, for only exceptionally is a mechanical failure clearly associated with corrosion, excepting those cases where corrosion fatigue is important [51]. Instead, the significance is on the release of corrosion products into the surrounding tissue, where even minute amounts can have far-reaching consequences. In passivated metals it is not necessary for breakdown of passivity to occur for metal ions will be slowly released through oxide layers and cause an accumulation of metal in the tissue. The effect of this corrosion on the tissue response will be discussed in the next section, but it is sufficient here to note that even with commercially pure titanium, demonstrable amounts of the metal can be found in the surrounding tissue after relatively short periods of time [52].

3.2. Polymers

In theory, polymers should have an advantage over metals since the isotonic saline and protein solution that comprises the extracellular fluid is not normally associated with the degradation of synthetic high molecular weight polymers. Although all polymers are susceptible to degradation, the majority of degradation processes involve the absorption of some kind of energy that is able to cause disruption of primary covalent bonds to form free radicals, which then cause the propagation of molecular degradation by secondary reactions. The conditions under which these processes take place include elevated temperatures, especially in the presence of oxygen, electromagnetic radiation, mechanical stress at elevated temperatures and ultrasonic vibration. Clearly the physiological environment within the human body does not offer any of these conditions to an implanted polymer, and thus high polymers such as certain polyolefins, acrylics and halogenated hydrocarbon polymers should be very stable. As reviewed in this journal a few years ago, such is the case in practice [37].

On the other hand, some polymers contain linkages that are susceptible to hydrolysis. If such a material is hydrophilic and capable of absorbing water, then degradation is possible within the physiological environment. This is the rationale for the choice of many intentionally degradable polymer systems (for drug delivery implants, for example) where aliphatic polyesters, poly(orthoesters) and poly(amino acids) may be selected [53–55]. Other polymers have been used for more permanent applications but suffered, albeit more slowly, from this type of hydrolytic degradation, including some polyamides [56] and poly(ester urethanes) [57].

One interesting and relevant observation in this respect concerns the effect that tissue enzymes may have on this hydrolytic process. Enzymes are catalysts for specific biochemical reactions, many of which are hydrolytic in nature. There is now some evidence that certain enzymes are able to accelerate or induce hydrolytic degradation of polymers such as poly(ether urethanes) and aromatic polyesters [58–61] which, although containing hydrolysable ester bonds, are not hydrophilic and not associated with hydrolytic degradation at low temperatures. It is also possible that certain cells of the tissue response could be involved with degradative processes, either by their attachment to polymer surfaces and the release of destructive enzymes on to the surface [62] or by the ingestion of fragments of the polymer.

3.3. Ceramics and glasses

Ceramics, glasses and glass–ceramics present a spectrum of materials with wide-ranging characteristics as far as stability in physiological environments is concerned. As discussed by Hench [36] and Hench and Etheridge [63] these materials may be divided into those which are essentially inert, those which are soluble, and those which display limited or controlled surface reactivity. Into the former category come a number of oxide ceramics, typically alumina [64] and certain hydroxylapatites, especially dense calcium hydroxylapatite [65], the naturally occurring mineral phase of bones and teeth that can now be made synthetically. A close relative of this hydroxylapatite is tricalcium phosphate but rather than being essentially inert, this is totally degradable, or soluble in the body [66]. Naturally the essentially inert ceramics are used when permanency is required and the minimal or undetectable degradation rate seen with oxide ceramics such as alumina is a very desirable property. The totally degradable ceramics have potential use for short-term applications, especially where the implant is being used as a matrix for new tissue regeneration. The rate of degradation will depend on local conditions and may involve either cellular processes or direct solution, or both. Obviously this rate of degradation and the nature of the degradation products have to be such that any deleterious effect of the degradation on the tissue response is minimized.

The concept of ceramics or glasses with controlled surface activity [67] arises from the desire to achieve a degree of bonding to the tissues, by mechanisms which are described later. The surface reactivity is achieved by the preferential leaching of certain components from the glass, which is comprised of SiO_2, P_2O_5, CaO and Na_2O and other species, so that dissolution leaves a stable silica-rich surface. The reaction that does take place, involving the release of calcium and phosphate, results in the formation of new bone at the implant surface.

4. The localized response of tissues to biomaterials

It has to be said at this stage that there is no simple, well-understood and universally applicable mechanism by which tissues respond to the presence of an implanted device. The response, instead, is a dynamic phenomenon, a sequence of events each of which may be triggered by its predecessor but which may also be influenced by the environmental features created by the implant. These events centre around the activity of different cell types, this activity being mediated by a variety of biochemical substances which in turn are influenced by the physical or chemical presence of the implant. We can, in fact, consider an implant as a source of irritation, or a stimulus to the tissue. This stimulus provided by the implant is not a great deal different to that provided by other insults to the tissue (for example trauma or infection) in terms of the response that it incites, and most features of the tissue response to an implant will bear a close similarity to the classical features of wound repair after trauma or of the cellular and humoral response to invading bacteria.

It is most instructive, therefore, when considering the mechanisms by which implanted biomaterials influence the localized tissues, to consider the sequence of events that is seen in normal wound healing and then to consider how this sequence is modified by the presence of an implant. Naturally, the precise features of the wound healing process depend on the nature of the tissues in question. For present purposes, we may consider the situation of wound healing in soft connective tissue (for example muscle) and the response of this tissue to implants. In real clinical use, implants may find themselves in situations other than those where they are encased in soft connective tissues and, indeed, the most important clinical areas in which biomaterials are prominent involve hard tissues (orthopaedic surgery and dentistry) and blood (cardiovascular surgery). Some of the specific questions concerning the response of bone and blood to implants will, therefore, be covered later. In addition, we have to consider separately the influence of other variables, such as infection, on the response, and the development of some very specific responses such as the formation of tumours around implants.

4.1. Normal wound healing

The immediate response to injury, whatever its cause, is inflammation. This involves vascular, neurological, humoral and cellular responses and is essentially the same whether it is induced by mechanical trauma or microbiological infection, or by electrical, chemical or radiological energy [68]. This arises because the response is mediated by the same substances within the tissue in each case. The inflammatory process is aimed at eliminating, or at least containing, the causative agent so that the tissue can be subsequently repaired. The process of repair involves the replacement of lost or destroyed cells by vital cells and of damaged tissue by new tissue, and is, therefore, the second component of wound healing. Although the functions of the inflammatory and repair processes are quite distinct and chronologically the containment of the injurious agent has to precede the repair, the repair process itself can begin during the inflammatory phase and the two are very much closely interwoven. This becomes an important factor when the modification of this tissue response by implants is considered, since the implant represents an injurious agent that cannot normally be eliminated and often cannot be contained.

The nature and timing of this transition from inflammation to repair, and the extent of the clinical manifestation of the inflammation, will depend on the nature and severity of the injury, even though qualitatively the processes that are involved are non-specific to the causative agent. Thus, if the injury is minimal and the cause transient, the process of inflammation does not have to deal with the containment of a harmful agent and the repair process can proceed rapidly. With more extensive injuries, the inflammatory process will be intense and may not be confined to the locality of the injury, while the repair process may result in varying degrees of scarring and possibly the loss of specialized functions.

In those cases where the injurious agent is persistent and cannot be eliminated, or where the inflammatory response cannot effectively deal with a harmful agent because of interference to normal mechanisms, the inflammatory–reparative process becomes considerably more complex, with prolonged or chronic inflammation and excessive fibroblastic repair. It is in this context that we have to consider implanted devices. The situation becomes especially complex if the implant, instead of acting as an immovable but otherwise non-irritant foreign body, becomes a persistent source of irritation due to the release of corrosion or degradation products or leachables, in which case the inflammatory response may never subside and the repair process may never proceed to completion.

For convenience, the sequence of events may be divided into acute inflammation, chronic inflammation and repair, although there is obviously a considerable overlap between them.

4.2. Acute inflammation

This, by definition, is the immediate response to injury. Associated with the acute inflammation are the mechanisms by which the two principal forces in the body's defence capability, the leucocytes and antibodies, are brought into prominence and action. Since both of these are blood-borne, changes in the vasculature are the main features of acute inflammation.

4.2.1. Vascular changes

Immediately after localized injury, the microvasculature undergoes considerable change, the vessels becoming dilated and filling with excess blood [69]. This is rapidly followed by a stagnation of flow since the blood becomes more viscous as water is lost into the surrounding tissue through the now abnormally permeable capillaries, yielding a situation of stasis and increased pressure. In this way, far more blood than normal is available to the injured tissue. The blood rapidly starts to clot, while leucocytes (the white blood cells) and plasma proteins pass through the capillary wall into the surrounding tissue. These changes in the permeability that allow such processes to occur are mediated by histamine, a substance that is rapidly released into the tissue upon injury.

4.2.2. White cell activity

During this dilation of the blood vessels, the red cells tend to occupy central positions within the flowing blood, while the leucocytes (white cells) accumulate at the periphery [70]. There are many different types of white cell, the most prominent being the neutrophils, monocytes and lymphocytes [71]. The immediate contact between them and the endothelial surfaces allows them to stick to the latter. Emigration of the white cells through the vessel walls then takes place, with neutrophils and monocytes being more active in this respect than the slower lymphocytes. The neutrophils [72] tend to dominate the extravascular spaces in the early stages since they are also the most numerous white cells in the blood. The monocytes [73], which are known as macrophages or histiocytes when extravascular, are also reasonably mobile but are initially present in only small numbers. However, they are attracted to the area in increasing numbers and have a longer lifespan so they soon outnumber the neutrophils in the tissue.

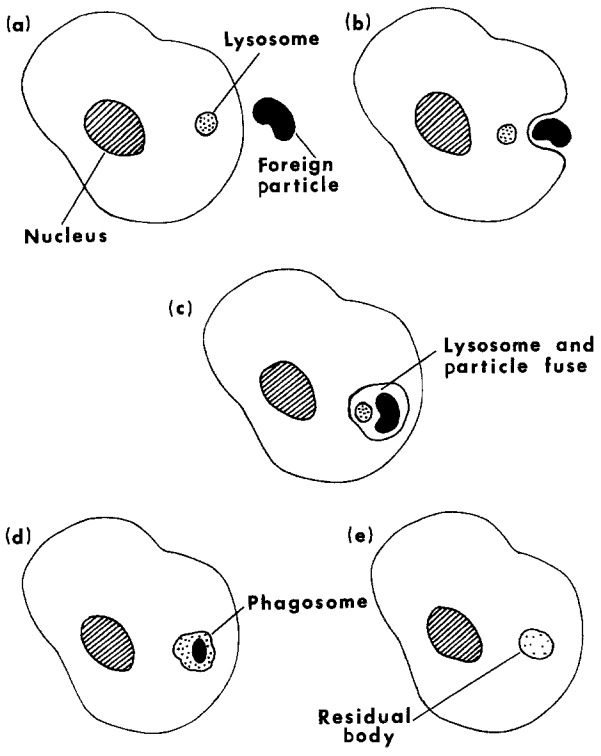


Figure 2 Process of phagocytosis: (a, b) the cell ingests a foreign particle; (c) the particle and lysosome fuse; (d) the phagosome that is thereby created digests the particle if possible, leaving (e) a residual body.

Once in the extravascular tissue, these cells have to migrate to the precise locations where they carry out their functions. This migration is directional and is mediated by a chemical process known as chemotaxis [74].

One of the most important functions of the cells is that of phagocytosis, a defence against invading microbes and particles; both neutrophils and macrophages are phagocytic. The process of phagocytosis [75] (Fig. 2) is initiated by the attachment of the particle to the surface of the cell, and this is facilitated if the particle becomes coated (in the tissues) with one of the substances (such as the immunoglobulin IgG) for which the cell surface has receptors. Since bacteria may be coated with IgG antibodies from the serum, this receptor-mediated attachment, or recognition, may result in a far more effective clearance rate than with, for example, the biologically unrecognizable degradation products of a biomaterial.

After attachment, the cell engulfs the object. This may be easy in the case of bacterium, which becomes enclosed within a membrane that was hitherto part of the cell membrane, producing a phagosome. The cytoplasmic granules of the cell then fuse with the phagosome and shed their collection of powerful enzymes to kill and cause the disintegration of the entrapped bacterium.

The release of enzymes can have a number of effects since they may find their way into the surrounding tissues and cause destruction there. This may be particularly important in the genesis of prolonged

responses to inert foreign bodies such as implanted materials. In such cases, the offending particles, unlike bacteria, may be of varying dimensions and not amenable to complete and effective engulfment. This is especially significant if phagocytes attempt to engulf particles but, while failing to do so completely, get close enough to cause degranulation, with much of the released enzyme finding its way into the surrounding tissue, thereby mediating and promoting inflammatory responses.

4.2.3. Chemical mediators of inflammation

The development of an inflammation following an injury is dependent upon chemical mediators [71, 76]. These are substances derived from either the plasma or directly from the tissue, which are able to activate one or more of the processes described above and which are then deactivated, by enzymes or antagonists, once their function has been performed. These mediators may be divided into those which control vascular permeability and those which have chemotactic capability. Histamine [77, 78], serotonin, complement [79] and prostaglandins [80] are particularly effective mediators of dilation and increased permeability of blood vessels.

4.3. Chronic inflammation

The vascular and exudative changes that constitute the acute inflammation will subside and lead directly to the repair stage if the injurious agent is mild and rapidly eliminated. If, however, the agent is not transient but persistent, or very severe, the acute inflammation will be followed by a chronic inflammation, which will often occur simultaneously with the repair process. The chronic inflammation is a proliferative rather than exudative response and the tissue is characterized by fibroblasts associated with repair and an accumulation of leucocytes that attempt to carry on the work of the cells of the acute response. These cells of the chronic response largely consist of the macrophages, plasma cells [81] and lymphocytes [82].

Macrophages are able to transform to several derivatives, the most important of which in this situation is the multinucleated foreign body giant cell. They are derived, in fact, from macrophages fusing together in an attempt to increase their effectiveness against larger and more resistant foreign bodies [83].

The lymphocytes and plasma cells may be seen in large numbers in chronic responses, especially when the immune system is involved. They may therefore be seen in the reaction to implants which, although outwardly non-immunogenic, may still be capable of eliciting an immune response, which is discussed briefly later.

4.3.1. Granulomas

When the stimulus to inflammation is particularly resistant to degradation by phagocytic cells, a specialized form of inflammatory tissue may develop, which is known as granulation tissue or a granuloma [84]. This is defined as a focus of cells in a chronic inflammatory response that predominantly consists of macrophages and their derivatives, the epithelioid and giant cells. The term is frequently used to describe the response to biomaterials and indeed, several specific responses have been given distinctive terms in this context. For example, the release of particulate polytetrafluoroethylene from the first generation of total hip prostheses gave rise to a very severe chronic response, similar to that induced by particulate PTFE in other situations, now referred to as a Teflon granuloma [85].

4.4. Reparative processes

The extent and nature of tissue regeneration is dependent upon the ability of the cells within that tissue to replicate. Labile cells have a very large regenerative capacity, being able to multiply at any time throughout life in order to replace those lost during normal physiological processes, an example being the cells of the epithelial surface that give rise to the continual replacement of skin. Stable cells may not actively replicate in the same way because there is not normally any need for them to do so, but they do have the potential for replication should the need arise. Thus mesenchymal cells may differentiate (or transform) into chondroblasts (cartilage-producing cells) or osteoblasts (bone-producing) should either of these tissues be involved in order to bring about their repair [86]. Fibroblasts are, perhaps, the best examples of mesenchymal cells that are extensively involved in repair processes [87].

Permanent cells have no replicating ability at all, their destruction representing permanent loss. Repair here merely involved unspecialized connective tissue formation and scarring. Neurons in nerve tissue and skeletal muscle cells are good examples, the repair of these tissues being effected by fibroblasts which merely lay down collagen instead of the original type of tissue [88].

4.5. Incisional wound healing

Having considered the general mechanisms by which tissue responds to injury, we shall not consider the specific situation in which a surgical incision is made into the soft connective tissue, this being the normal precursor to the implantation of a biomaterial. If the wound margins can be accurately joined without any tissue loss, healing is said to take place by primary union, or by first intention. If there is some degree of tissue loss, secondary union takes place. With primary union (Fig. 3) the incision will initially fill with blood, which will clot. The acute inflammatory response is rapidly initiated with the release of a neutrophil exudate. The fibrin meshwork of the blood clot provides a basis for both re-epithelialization of the surface and sub-epithelial fibrous tissue growth to occur. Capillary buds start to migrate through the clot and fibroblasts at the edge start to become active within a day or so. Cells at the epithelial margin extend their processes across the wound surface and a thin epithelial surface, possibly only one cell thick, is established.

The acute inflammation will start to subside after about three days, macrophages taking over from neutrophils to clear the area of dead cells and the remaining fibrin. Blood vessel sprouts grow into

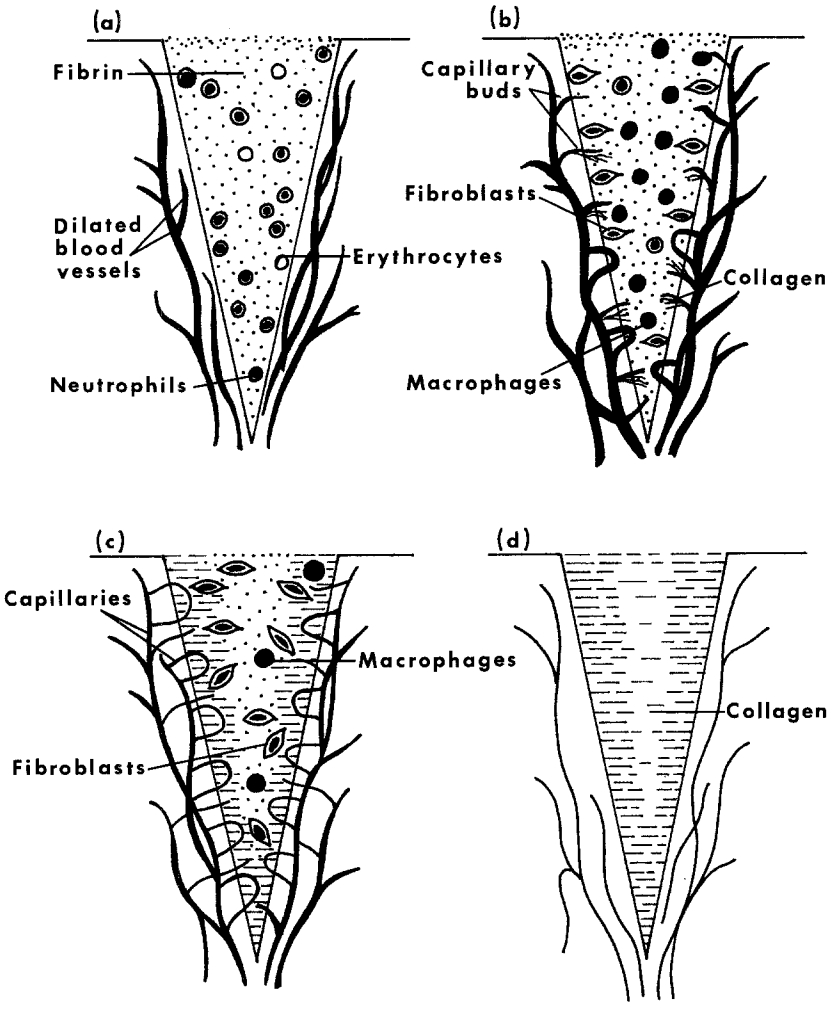


Figure 3 (a–d) Process of wound healing, in the case of a simple incisional wound.

subepithelial space at a rapid rate such that by the fifth day there is a richly vascularized fibroblastic connective tissue within the wound. Fibroblasts lay down more and more collagen, which will be immature and weak initially. By the end of the second week, the inflammation will have subsided, as will the intense vascularity. Maturation of the collagen will continue for many weeks as it slowly increases in strength.

During secondary union, a more extensive repair process is necessary because of the greater amount of dead tissue requiring removal and the greater amount of tissue that needs replacement. The edges of a large wound are filled with granulation tissue and it is this which forms a base for epithelialization to take place with the migration of the epithelial cells over its surface. New blood vessels grow in from the edges and scar tissue starts to form as fibroblast proliferation is also initiated.

4.6. The response of soft connective tissue to implantation

The above discussion has shown that there are many cells and chemical mediators involved in the process of inflammation and repair that constitute the response to trauma and irritation in tissues. If a surgical implant is placed within an incisional wound, then the response may be considered as a modification to one of these processes. There are many points at which the normal sequence of events may be modified by variations in the nature of the irritant. It should be emphasized that the same mechanisms exist whatever the irritant, but different components of the response may take on greater or lesser significance with variations in the conditions.

4.6.1. Minimal fibrosis

In the case of a monolithic solid consisting of a single material that is neither toxic to the host, in the normal sense of that word, nor degraded by the tissues, the inflammatory response and repair processes may take place virtually unaffected. The site of implantation fills with blood and a fibrin network forms a basis for subsequent fibrous tissue growth to occur. There is an initial interaction between the blood and the implant, as indicated in Section 2, with plasma proteins being adsorbed on the implant surface, the features of which depend to a certain extent on the chemistry and surface morphology.

An acute inflammatory response is initiated and it is unlikely that the exudate will be different to that produced by the incision in the absence of the implant. Macrophages take over from the neutrophils fairly rapidly, as before. Capillary buds migrate through the clot, but their passage across the wound is disrupted by the implant and the blood vessel network will inevitably be different. Fibroblasts will also become active, laying down collagen as the fibrin clot is resorbed, although this will not be able to traverse the incision in the region of the implant, resulting in an altered morphology. It is probable that the presence of the implant will prolong the inflammation and repair processes and the cellular infiltrate will persist for a

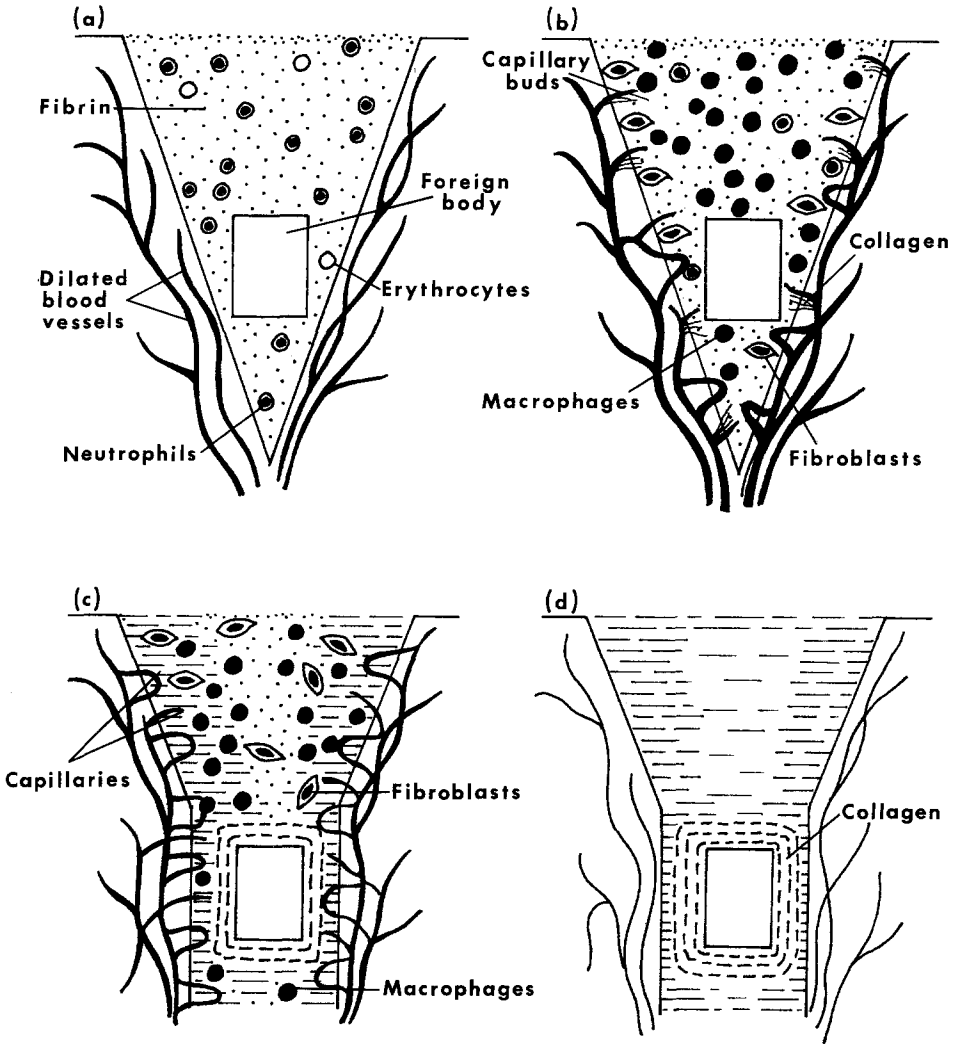


Figure 4 (a–d) Wound healing in the presence of a foreign body, such as an implant.

longer time than in the normal incisional case. Within four to eight weeks, however, the tissue response should have stabilized, leaving a zone of tissue that is rather similar to the normal scar tissue, with perhaps differing patterns of vasculature and collagen fibres running parellel to the implant surface. This sequence is depicted in Fig. 4.

This response is, however, rarely seen. It is described as the classical fibrous encapsulation of implants, but this term is too simplistic. Absolute inertness of a biomaterial, as already described, is rare and other material and device characteristics can be superimposed on inertness criteria to modify this response. Of the currently used biomaterials, pure titanium [89], high purity alumina [90] and some special grades of polymers such as polytetrafluoroethylene [91], ultra-high molecular weight high density polyethylene [92] and silicone rubber [93] may elicit this minimal fibrous encapsulation under some conditions, with examples being shown in Fig. 5.

4.6.2. Deviations from the minimal response

There are many ways in which the minimal fibrous response may be modified by the presence of an implant.

First, the initial reaction between the implant and the tissue may be more pronounced than that seen with a "totally inert" material such that the acute response is somewhat more severe and slightly prolonged (Fig. 6a); the fibrous capsule that forms is slightly thicker and takes longer to stabilize.

Secondly, the reaction may be more extensive again such that the acute response is severe and progresses to a significant chronic response. The repair process may be initiated at an early stage but requires more time to deal with the extensively damaged tissue; it results in a fibrous capsule that is different in size and characteristics (Fig. 6b) with variations in cell population, blood capillary density, tissue destruction and so on. It may be that the capsule takes several months to settle down.

Thirdly, the acute response may be minimal such that the repair process is quickly effected but then long-term interactions (corrosion, degradation, abrasion) take place such that the implant becomes a persistent stimulus to the tissue and chronic inflammation ensues. Cells typical of both acute and chronic inflammation may be seen, often in association with reaction products derived from the implant which they are trying to eliminate (for example by phagocytosis). Fibroblasts will be active, attempting to make good the damage, but they may be fighting a losing battle if the implant provides a continuum of irritants. The long-term result is a granuloma (Fig. 6c), especially with numerous foreign body giant cells, which may visibly be associated with oedema (swelling) and in the

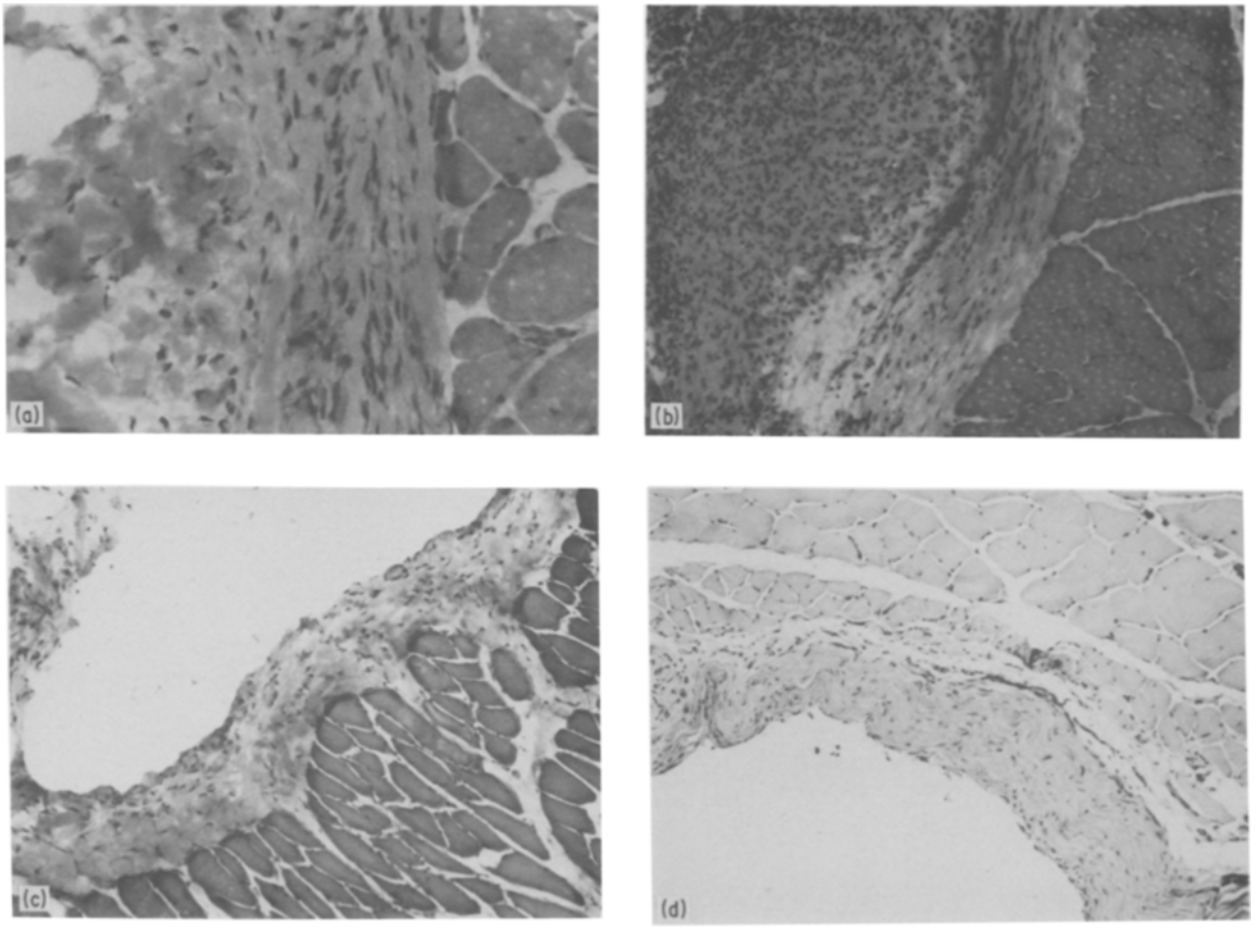


Figure 5 Stages in the development of a fibrous capsule: (a) after 4 weeks, with implant of titanium (normal muscle to the left, implant to the right, relatively acellular capsule developing between implant and muscle); (b) more cellular response at 9 weeks to nickel–titanium implant; (c) thin capsule developed with titanium at 10 weeks; (d) thin capsule developed with thermoplastic material, polyetheretherketone, at 10 weeks. × 200.

clinical sense will result in pain. If the reaction is sufficiently severe, cells, and indeed the tissue, will die (become necrotic), a process which further aggravates the situation.

This discussion implies that there is a progression of tissue responses, from the minimal to the necrotic, which are seen in the context of implanted biomaterials and indeed, we shall see below some examples of how different materials in different forms initiate these various responses. It should not be assumed at this stage, however, that the ideal biomaterial in every clinical situation is that which produces the minimal response described above and that all the others are getting progressively less desirable as far as clinical devices are concerned. It may be said that the prolonged chronic response with granuloma, or worse, is universally undesirable. On the other hand, minimal fibrosis, which effectively means that the implant is being ignored by the tissue, is not necessarily a good thing because it does not lend itself to full incorporation and acceptance of the biomaterial into the tissue. We shall see later how a modified tissue response may be beneficial in terms of the full integration of the implant into the body.

Since there are so many mediators of these tissue responses, it is not surprising that numerous factors may be involved in their modification in the presence of an implant. We may consider these under three broad headings. First, and probably, foremost, is the influence of implant chemistry. Secondly, there is the role of physical factors, including size and shape of the implanted device, its surface morphology and texture and its mechanical relationship with the tissues. Thirdly, there is the influence of biological variations such as implant size, host species, age and sex, the state of health of the host and pharmacological status.

4.6.3. The effect of surface chemistry

There are two ways in which the implant surface chemistry can influence the tissue response. First, by the processes we have already discussed in Section 2, the nature of the surface chemistry, by virtue of the properties such as surface energy, dielectric constant or equilibrium potential that are dependent on this chemistry, will influence the initial events at the implant–tissue surface; that is, the characteristics of protein adsorption will depend on the chemical nature of the surface. If there were no subsequent chemical interaction between biomaterial and tissue, the extent to which the wound healing process would be modified by the implant would be entirely dependent (for constant physical and biological conditions) on the nature of this adsorbed layer. However, as we have seen in Section 3, the prospect of zero chemical interaction within the human body is remote and thus the second way in which the chemistry influences the tissue

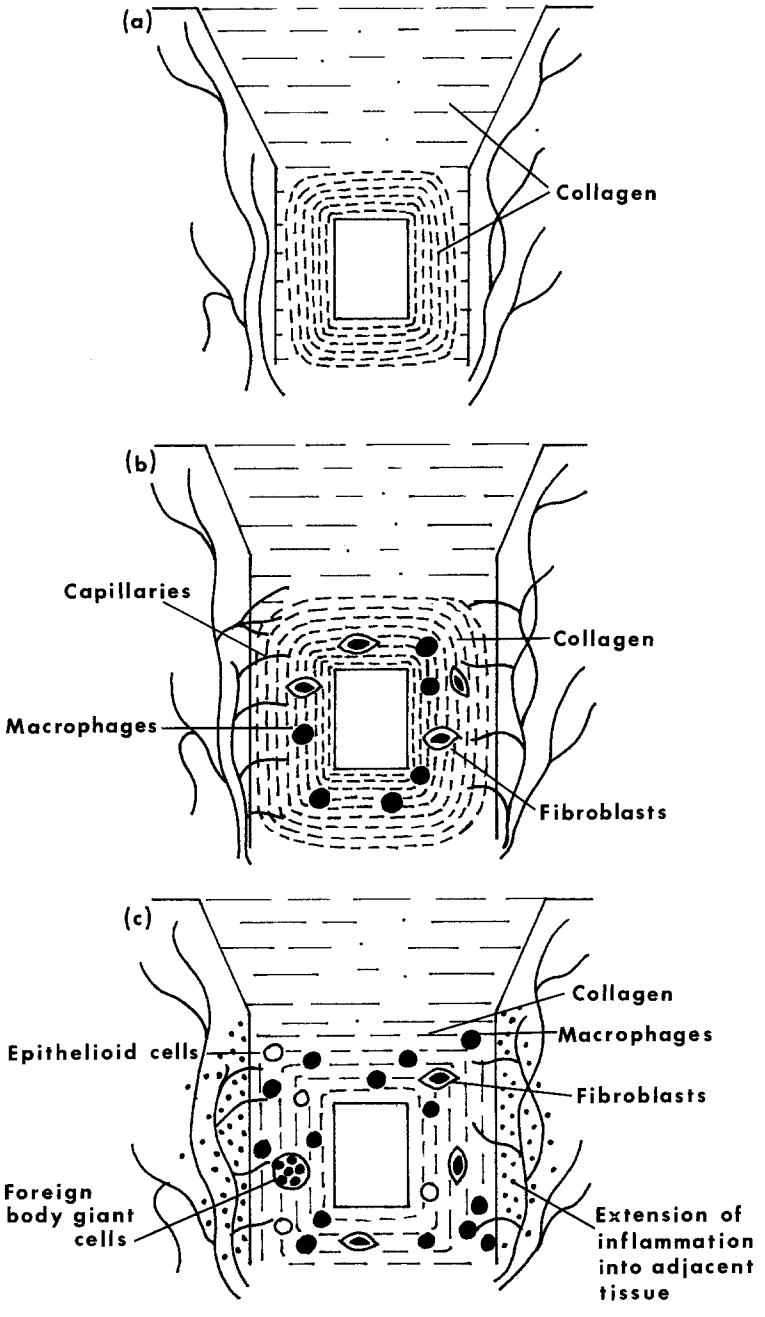


Figure 6 More severe responses at implants (compared to Fig. 4d): (a) more extensive capsule; (b) more extensive and cellular capsule, with persistent macrophagic response alongside fibroblastic response; (c) extensive chromic inflammation (including foreign body giant cells and epithelioid cells) with granulation tissue.

response is through the nature (amount, chemical nature, physical form, solubility, toxicity, etc.) of the reaction products. Naturally, there are many mechanisms by which these reaction products can exert this influence.

In spite of its obvious importance, very little is known about the extent of the effect of adsorbed proteins on the soft-tissue response. In a later section the vast amount of information on the effect on interactions with blood will be reviewed but, apart from a few speculative papers [94, 95] the mediation of connective tissue reactions by this layer remains unclear. It is certainly known that in many *in vivo* conditions, the very important phenomenon of cell adhesion to foreign surfaces is controlled by the nature of the intervening protein layer [96, 97] and this must be assumed to be important *in vivo*. On the other hand, the adsorbed protein layer *in vivo* will not remain unchanged for long and it is difficult to see how a layer that is probably constantly undergoing metallic turnover maintains a long-term influence on the tissue response.

A far greater amount of information is available concerning the role of the interaction between materials and tissues on this response, although the complexity and multiplicity of events still makes a rational and universal understanding of the phenomenon rather difficult.

Let us first of all consider the morphology of the tissue response and use metals as an example. McNamara and Williams [98–100] have reported studies in which the local tissue response induced by discs of some ultra-pure metals (5 mm diameter, 2 mm thick) implanted intramuscularly in rats, was examined. Pure cobalt, nickel, lead, aluminium, copper and titanium were studied. A morphologically distinct pattern of response developed for each metal, although all within a general framework. In the intramuscular site a reaction zone, analogous to the fibrous capsule described in the minimal response situation, develops and separates the implant from the normal surrounding muscle. This capsule, which obviously matures with time, varies in thickness, in its organization and its relationship to the adjacent tissue. In general, as

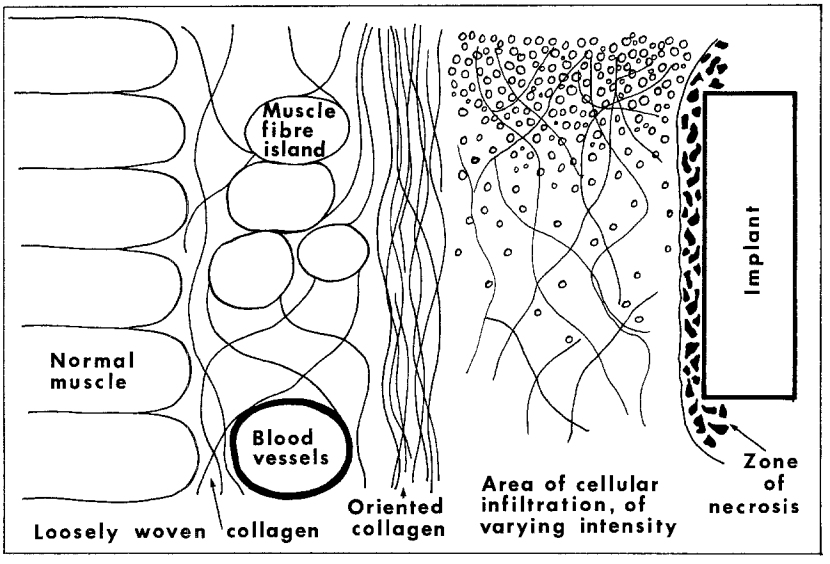


Figure 7 Generalized fibrous capsule forming in response to intramuscularly implanted metal. Some or all of these features may be present depending on the metal.

shown in Fig. 7, the zone may contain an area of necrosis adjacent to the implant, surrounded by a region of chronic cellular infiltration. Often this is adjacent to a band of densely packed oriented collagen, which itself will be surrounded by a more loosely packed collagen zone, which may contain blood vessels, vacuoles, fatty tissue, muscle fragments and discrete cellular populations. In some cases the capsule has a well-defined boundary but in other cases it extends irregularly and diffusely into the surrounding muscle.

With aluminium, for example, there was a distinct necrotic region adjacent to the implant, but the capsule was very compact and only a short distance away the muscle was healthy (Fig. 8a). There were a few layers of collagen but these were not extensive. There was little neutrophil infiltration after the acute stage, but many macrophages were present, actively secreting hydrolytic enzymes. The capsule was poorly vascularized.

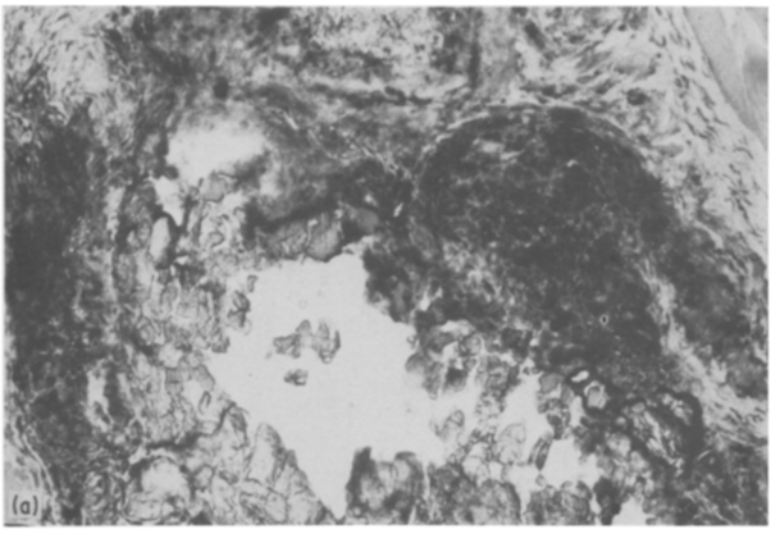
The response to lead, in spite of its known toxicity in other situations, was much less severe. Some fibroblastic proliferation was seen and a dense collagenous layer developed relatively quickly. However, cellular infiltration was minimal and no necrosis was observed. This comparison between aluminium and lead is interesting and important. Aluminium is normally thought to be relatively non-toxic [101] but such a conclusion has been derived from classical toxicological studies where the test substance is administered to animals by the oral route. Since aluminium is so poorly absorbed within the gastrointestinal tract, very little gets into the bloodstream and hence it appears non-toxic. If, on the other hand, the metal is presented directly to the tissues (and by implication, the blood) it is able to exert strong biological activity, which is seen here resulting in tissue and cell death. In recent years the complacency over the safety of aluminium has also been brought into question with several problems of toxicity, leading to a variety of symptoms and occasional mortality in patients undergoing kidney dialysis, due to the traces of aluminium in the water that is used and which gains direct access to the bloodstream [102].

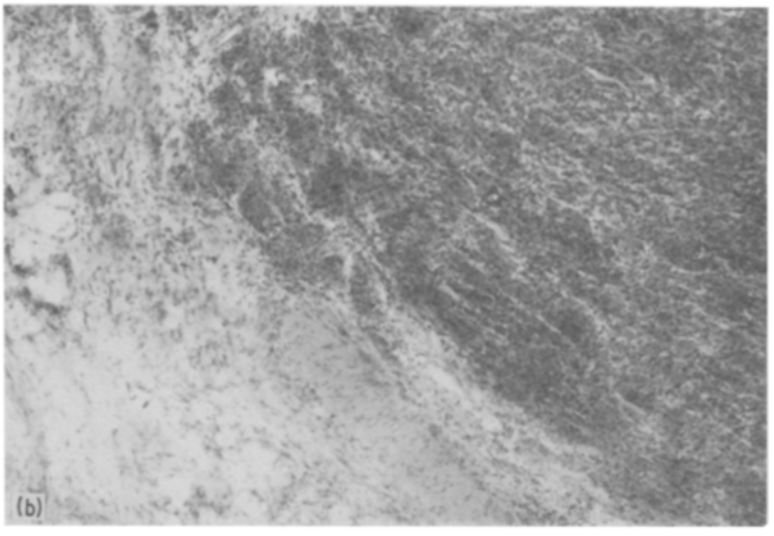
The most noticeable feature of the response to copper was the very high degree of vascularity, which may correlate with the speed of systemic distribution of the metal ions. A sterile exudate was normally present adjacent to the implant. Haemosiderin-laden macrophages were always in evidence, as well as cells containing black pigmented material (Fig. 8b). Large areas of yellow-brown pigmented tissue were also seen, a feature unique to copper. With time, progressive vacuolization and resorption of the muscle fibres occurs within the affected parts.

The reaction to the cobalt was most interesting. There was no clear capsular edge but rather a gradual change in appearance of the tissue. The most noticeable feature was the presence of significant areas of lymphoid tissue (Fig. 8c), consisting principally of plasma cells, which indicate some form of immunological activity. It is likely that proteins, not in themselves antigenic, are rendered "foreign" by contact with the metal. Cobalt may corrode fairly freely in the tissues, such that corrosion products intermingle with the cells in the reaction zone (Fig. 8d).

There are probably many factors involved in the development of this range of responses. The rate of the interfacial reaction inevitably is important, and the extent and longevity of the chronic responses to copper and nickel are in part associated with these kinetics. Reaction products can take many forms. In the case of passivated metals, discrete and discernible corrosion products may not be observed, but rather the process involves the diffusion of metal ions through an oxide film, these ions immediately being bound to some organic or inorganic species in the surrounding milieu. Some such products could precipitate locally, whilst others are easily transported away in vascular or lymphatic systems. With titanium, for example, although the corrosion rate is almost immeasurably small, titanium complexes are precipitated locally and give a distinct discoloration to the tissue (Fig. 8e) [103]. This question of the binding of metal ions to tissue components has been addressed in a number of recent papers [104–6].

Bearing in mind that some of the metals involved are essential trace elements in animal tissues and are naturally present at levels often in the p.p.b. or p.p.m. range [107], whilst others have no physiological func-





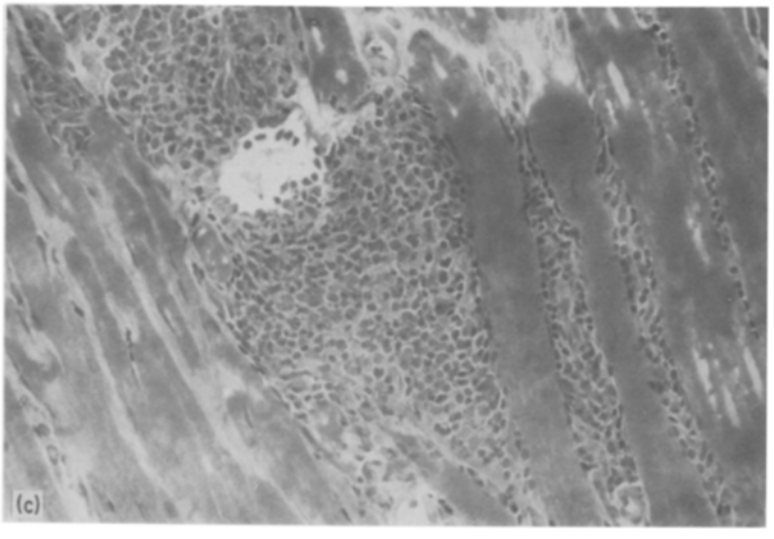
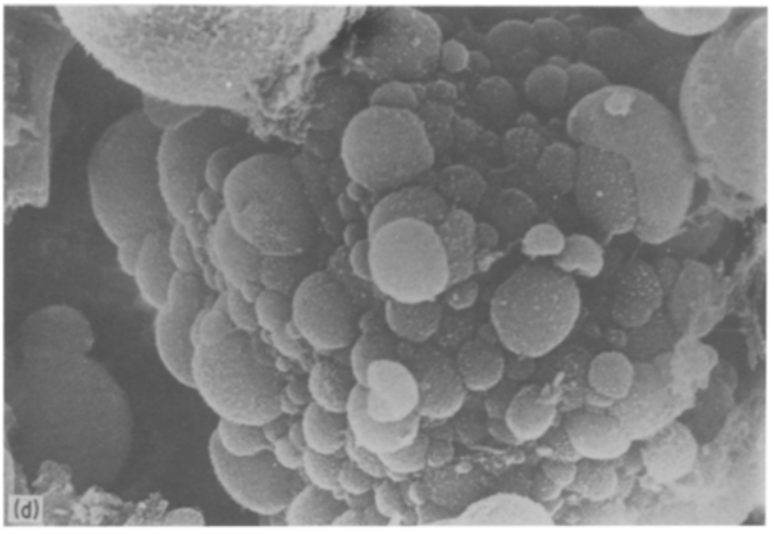


Figure 8 Some examples of the response to metals: (a) necrosis seen adjacent to aluminium ×100, (b) pigmented tissue around copper implant ×100; (c) plasma cells and lymphocytes in response to cobalt ×200; (d) cells, corrosion products and proteins on metal surface SEM ×3000; (e) discoloration of tissue adjacent to titanium ×150.

tion and cannot normally be detected therein, a multiplicity of mechanisms exist whereby these metals derived from implants can influence biochemical and physiological reactions. The metals may, for example, compete with the normal cations present for the binding sites on proteins. If these proteins are enzymes then these catalysts may readily be inactivated, a classical mechanism for toxicity [108]. Williams and Crowley [109] have recently shown that very small amounts of metals such as vanadium can radically alter the kinetics of enzyme activity associated with cells of the inflammatory response. These metals may also influence the chemotactic mechanisms that are involved in the attraction of cells to the area; aluminium is found to be strongly positively chemotactic whilst cobalt is negatively chemotactic (repels cells), possibly explaining the narrow but densely packed reaction zone to aluminium compared



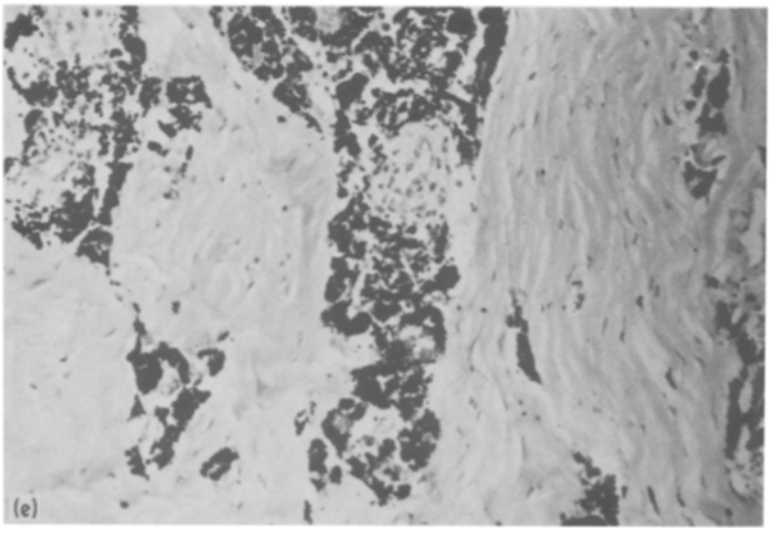


Figure 8 Continued.

with the wide invasive capsule around cobalt [98]. If discrete particles are derived from the corrosion process, as indeed is seen in clinical practice in the case of stainless steel [110, 111], then the tissue response may be modified by the physical effects as well as chemical effects, as explained in a later section.

When working with pure metals, it is possible to interpret the biological response in terms of single cation species only. When working with alloy systems, as indeed is the practice in the surgical application of these materials, the interpretation is much more difficult. References of the soft-tissue response to many different metallic systems have been published including stainless steel [112], titanium and its alloys [52, 113], cobalt–chromium alloys [114], nickel-based alloys [115], noble and platinum group metals [116] and dental amalgam [117].

In the case of polymers, a somewhat different story emerges for several reasons. On the one hand it is far more practical to prepare polymers of minimal reactivity, as we have seen. At the same time, however, the chemical and physicochemical features of the surfaces can be varied more extensively with ranges of hydrophilicity/hydrophobicity, crystallinity, surface charge, reactive groups and so on. As indicated above, there are several polymers which can be prepared with sufficient inertness to allow the formation of the thin fibrous capsule, examples being PTFE [91], polyethylene [92], silicone rubber [93], polymethylmethacrylate [118] and polyetherurethane [119]. Few systematic studies of the influence of these surface variables on the response have been reported, however.

Some years ago Gilding *et al.* [120] presented a series of abstracts on work of this type, describing the tissue response to a series of polymers with controlled hydrophilicity and surface charge, but the full results appear not to have been published. Some other studies have involved far fewer polymers or variables, or have used quantifiable *in vitro* techniques rather than the more descriptive *in vivo* methods. For example, Lentz *et al.* [121] have studied macrophage adhesion to hydroxyethylmethacrylate–ethylmethacrylate copolymers and hydroxystyrene–styrene copolymers. The data showed that there was a time delay between the contact and adhesion of cells to surfaces that varied with hydrophilicity within the former group, but not in the latter. The results were explained in terms of exclusion volumes relating to the swelling of these hydrophilic polymers.

The general scheme of the tissue response to implanted materials as outlined above has been discussed in detail with respect to some polymers by Anderson and co-workers [122, 123], who have designed an experimental model for the quantitative

study of certain parameters of this interaction. A stainless steel cage containing the polymer of interest is implanted subcutaneously in rats and exudate that forms within the cage as part of the inflammatory response is aspirated and analysed for protein content, enzyme activity and white cell content. The surfaces of the polymers are also examined for cell attachment. In an extensive study with the polyetherurethane Biomer® the total white cell count in the exudate decreased with time (4, 7 and 21 days), as would be expected from the resolution of the acute inflammatory response, while the differential cell count showed a decrease in the proportion of neutrophils and an increase in macrophages and lymphocytes, again as expected. Those cells attached to the polymer surface were largely macrophages, with a slow increase in the number of foreign body giant cells.

4.6.4. The effect of surface topography

Not all implants, either experimental or clinical, have smooth surfaces and attention must be given to the influence of surface topography on the development of the tissue response. Leaving aside the separate question of tissue ingrowth into porous materials, it is now known that minor variations in surface texture can influence this response [124–126]. Perhaps the most interesting observations are those of Gibbons and co-workers [127, 128], who have studied the tissue response to polymers given different textures by ion-beam milling. Using PTFE and a texture containing conical projections of height 12 μm and base width 4 μm, they have been able to show increased cell adhesion and increased lysosomal enzyme activity in the cells of the response. The macrophages in the response, compared to that for smooth surfaces, had differing structural characteristics, especially increased cytoplasmic-to-nuclear ratios and vacuolization. The rate at which the capsule developed also changed: at 8 weeks, capsules associated with textured surfaces were some 30% smaller than those around smooth-surfaced implants, possibly because of reduced fibroblast proliferation. Further experiments showed that protein adsorption also differed between smooth and textured surfaces.

In addition to the surface topography, the actual shape of the implant may influence the response. Little is known of this phenomenon, although Matlaga *et al.* [129] have demonstrated clear differences in the fibrous capsule that develops in response to various cross-sectional shapes of polymeric implants.

4.6.5. The effect of physiological variables

The general scheme described above for the development of the tissue response assumes a uniform and consistent host site. In addition, most of the information on the tissue–biomaterial interaction has been derived from studies on young healthy animals. For us to be able to extrapolate from these animal studies to the human clinical situation, we should ideally take into account variations of the tissue response that arise with differences of species, sex, age, state of health, pharmacological status and other factors. Unfortunately, very little is known of these effects and we can do no more than bear them in mind at this time.

4.7. The response of hard tissues to implantation

Bone responds to the presence of implants in several different ways, the nature of which depend on the type of bone, the previous history of the bone, the geometrical and morphological relationships between the bone and implant, the method of attachment of one to the other, the mechanical stress system acting on the implant–bone system and the material chemistry.

Let us consider the two most important aspects of the bone–implant interaction, the effect of the material on the response of bone to damage or to loss of tissue, and the influence of an implant on the stress system within the bone.

4.7.1. Biomaterials and bone growth or remodelling

Bone will normally come into contact with a biomaterial in one of three ways. First there is the situation where a bone fractures and an implant is used, as a plate, nail or other device, to hold fragments together while bone healing takes place. In this situation, the healing process is controlled by the mechanics of fixation and the material has little direct effect on the bone since it is not normally placed within the healing zone. Secondly there may be some defect within a bone which requires repair. This could be a bacterially-derived resorptive process, an ageing process, the result of trauma or surgical intervention, and here the implant is used to facilitate the healing of bone within this defect. In this case the nature of the material is vitally important. Thirdly the implant may be inserted into a bone for the purpose of reconstruction in adjacent tissues, the bone itself acting as a secure fixation point. Examples here include the intramedullary fixation of joint prostheses, bone screws, and the location of dental implants in mandible or maxilla. Here again the material is an important factor in determining the response of the bone to the implant.

We may consider these latter two together and Fig. 9 indicates the general scheme. In Fig. 9a we see the progression of healing in a bony defect without the aid of any biomaterial, where bridging the defect can occur spontaneously and completely. Initially an exudate will form within the defect (for example a blood clot), which will slowly reorganize. In the process of osteogenesis, new bone may grow, either directly from the existing bony walls (osteoconduction) or from isolated areas within the reorganizing tissue should the appropriate bone cells (osteoblasts) and growth factors be available (osteoinduction). Ultimately new solid bone may form, although there will inevitably be intervening periods where there are both areas of bone and of unmineralized soft connective tissue present. There is an upper limit to the size of defect which can be bridged in this way, above which the defect will merely fill with unmineralized tissue.

If now a solid object is placed in the bony cavity

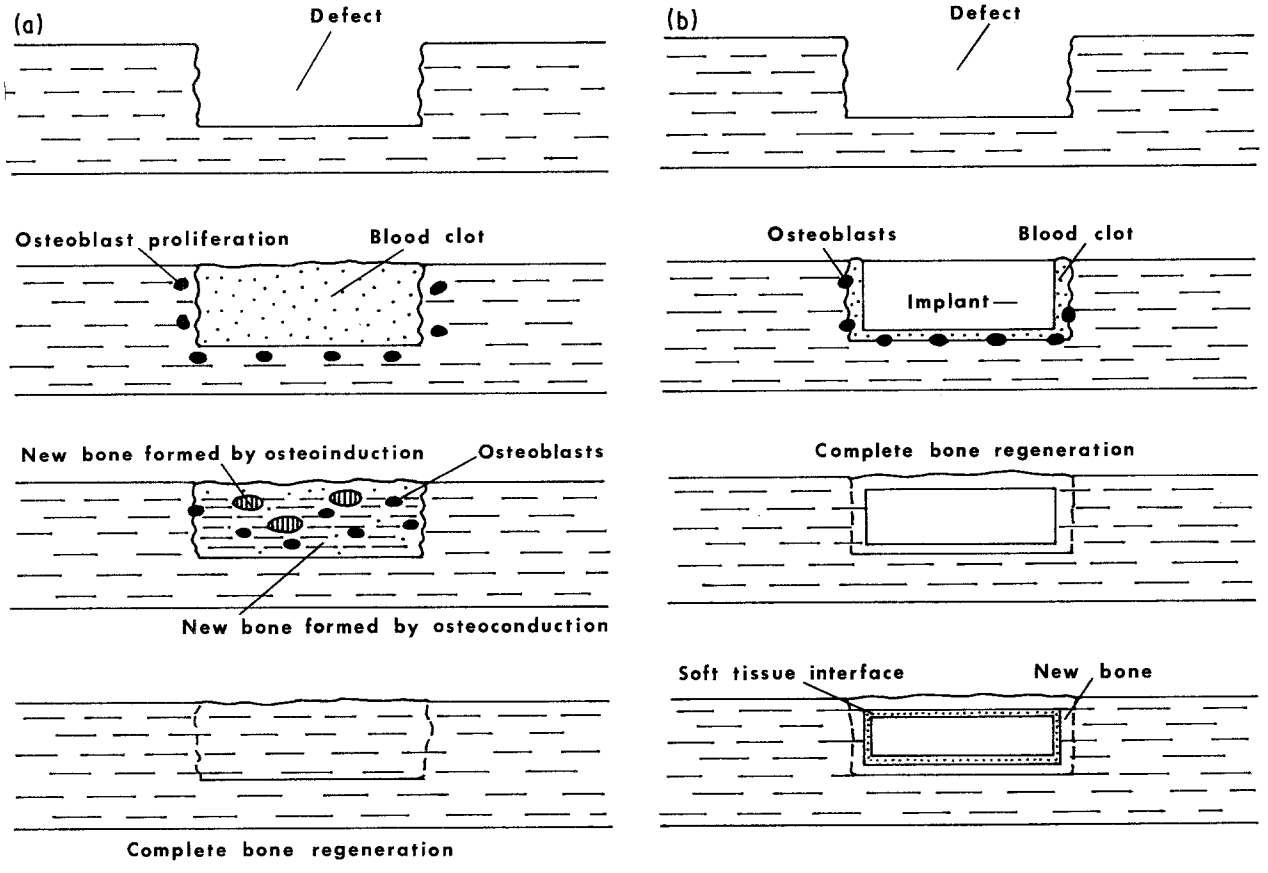


Figure 9 Scheme of healing in bone: (a) sequence in absence of any implant, leading to complete bone regeneration; (b) response in presence of monolithic implant, leading either to complete bone regeneration or a soft tissue interface; (c) response in the presence of particulate implant, leading either to complete bone regeneration or a zone of soft fibrous tissue around the particles.

(Fig. 9b) the healing will take place in the space between the implant and the bone. If this gap is small there will be a tendency for new bone to form, but this process can be modified by mechanical and chemical influences. Again there will be a time when this space is filled, partly with new bone and partly with fibrous unmineralized tissue. Under some circumstances the defect will completely fill with bone such that there will be intimate bone–implant contact. This is a matter of considerable current controversy, for it appears that most materials will not allow such contact but instead a soft fibrous tissue interface will exist between bone and implant. This, of course, has considerable functional consequences if the objective is for the implant to remain secure in the bone. The evidence for and against direct bone contact will be reviewed below.

If, instead of placing a monolithic solid within the bone, some particulate matter is used (Fig. 9c), then a combination of the two processes seen in Figs 9a and b may take place. Several permutations are, in fact, possible; the particles may actively encourage osteogenesis such that the defect fills faster, or larger defects will fill with bone rather than unmineralized tissue. Alternatively the particles may have a neutral effect such that bone growth occurs around them, while it is also possible for the particles to prevent the total conversion to new bone and instead promote the formation of fibrous tissue envelopes around each of them. Again, the chemical nature of the material has been assumed critical in determining which event will occur.

4.7.2. The biomaterial–bone interface

As noted above, the question of whether any bio-

material can allow the formation of new bone up to and contiguous with its surface is highly controversial. Several terms have been used in this context to describe the performance of materials and it is important that the ideas behind these are understood, even if their definitions have not been widely accepted. First we have to recognize that there are materials which are indifferent as far as bone is concerned; they may be essentially inert in the body but have no positive or negative effects on bone growth. These are sometimes referred to as bioinert, although the mention of that term here does not imply endorsement of its use. Secondly, there are materials which appear to actually encourage the formation of new bone at their surface; that is, osteogenesis takes place right at the implant–tissue interface without necessarily any bridging to pre-existing bone. Such materials are sometimes described as bioactive. Thirdly, there are materials which allow sustained growth from the surrounding bone up to their surfaces with direct bone–material contact. Such a process has been described as osseointegration.

In spite of many attempts to do so, it has not yet proved possible to define the conditions under which this bone–material contact will occur. Much early comment was related to the use of orthopaedic devices such as bone screws and intramedullary stems [130], but the complex interrelationship between mechanical stresses, surgical technique and implant material made sensible interpretation of the data very difficult. In some cases bone could be seen forming right up to (say) screws inserted into cortical bone, whilst in other cases, the bone would actually resorb away. More recently attention has been directed towards the role of the material and especially on the claim that one metal in particular, titanium, is highly conducive to this direct bone–implant contact. Most of this work has been reported by Branemark, Albrektsson and co-workers [131–133] and is reviewed briefly.

Titanium implants placed within bone have been shown to become surrounded by bone tissue without interposed fibrous structure and without chronic inflammatory reactions. Although the structure of the bone distant from the implants was normal, the arrangement of bone lamellae changed near the surface such that they became oriented parallel to the implant surface. At a distance of 0.5 μm from the implant surface, as reviewed by transmission electron microscopy, the collagen bundle arrangement gave way to randomly arranged collagen filaments, which could be observed down to 20 nm from the surface. There was a partly calcified amorphous ground substance, consisting of proteoglycans and glycosaminoglycans within the 20 nm layer at the surface. Although in places the degree of calcification was less than normal, bone was observed up to the limit of 3 nm detection. Cellular processes (from osteoblasts) approached the surface but were separated from them by a 20 to 30 nm thick proteoglycan layer.

Other metals were evaluated using the same technique and were found to have proteoglycan layers of 50 to 500 nm. Other materials, such as polymethylmethacrylate or certain glass–ceramics, were surrounded by proteoglycan layers of up to 2000 and 300 nm, respectively. Since a layer of proteoglycans up to 20 nm thick may be seen between individual collagen filaments or at cell–collagen interfaces, it has been argued that titanium, and no other biomaterial, is behaving as a natural tissue. It is not clear why titanium should behave uniquely in this way. It is claimed that titanium is binding by irreversible electrostatic forces to the bone and that the physical characteristics of the titanium oxide surface are important but such hypotheses have yet to be proved.

There is no suggestion that new bone growth occurs spontaneously on the surface of these materials in the absence of growth from adjacent, pre-existing bone. A few materials have, however, been described as bioactive, that is, having this property of inducing osteogenesis in their vicinity. Although many such claims have been made, we shall refer to three such situations, involving calcium hydroxylapatite ceramics, certain glasses or glass–ceramics of controlled surface activity, and certain porous surfaces.

Calcium hydroxylapatite is an interesting biomaterial that owes its use to the fact that it is the synthetic analogue to the mineral phase of bone itself. It has been prepared, under a number of trade names and in various physical forms, as a bone reconstructive material and, along with its near neighbours in the calcium phosphate group of ceramic structures, it has been the subject of a number of recent reviews [134–136]. Of special interest here is the observation that when certain samples of calcium hydroxylapatite are placed within a bony environment, they may become well incorporated into the bone, without intervening inflammatory or fibrous tissue elements. Whilst this is not a universally recognized phenomenon, and the precise conditions under which this can occur are not understood, there is no doubt that new bone can intimately merge with the ceramic, with individual crystals of biological apatites epitaxially deposited on the surface of the synthetic material [137].

Glasses or glass–ceramics of controlled surface activity appear to provide a different mechanism. These glasses, first developed by Hench *et al.* [138], are typically based on the SiO_2–CaO–Na_2O phase diagram and contain a small quantity of P_2O_5. The significance is that, within a narrow compositional range, these glasses have a reactive surface that initially leaches out calcium and phosphate ions. These are able to promote the formation of new bone at their surface, since once again this calcium phosphate is chemically equivalent to the mineral phase of bone. This surface reaction is self-limiting because of the protective effect of the silica-rich surface layer that is left after the preliminary leaching, but the transition zone is of such a character that it is able to incorporate the molecules of the organic matrix of new bone as it is forming, hence providing for a chemical bond between the glass and the new bone [67, 139, 140].

It has been known for some time that if a porous-surfaced material is placed within tissues, tissues can grow into the pores and provide for some

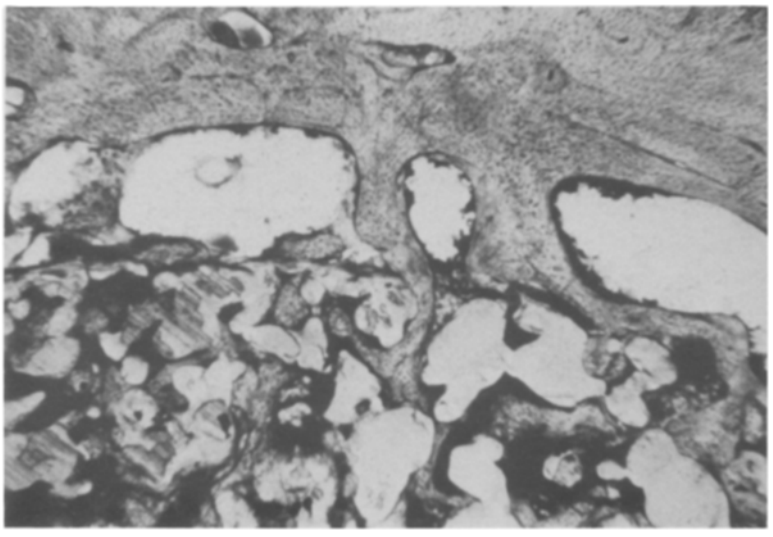


Figure 10 Tissue ingrowth into porous polyethylene. × 100.

attachment. Generally, and as indicated previously, porous materials placed within soft connective tissue will act as hosts for ingrowth of soft tissue, although there have been occasions when they will initiate the spontaneous formation of bone under unusual circumstances [141]. Placed adjacent to existing bone, porous surfaces may promote the ingrowth of new bone. This process is largely independent of chemistry, but is, instead, controlled by the morphology of the pores. If the porosity is interconnecting, and the minimum pore size is in the region of 150 μm, then bone can grow into the surface area and promote attachment [142–145] (Fig. 10). There is no inference that the materials are acting in any osteogenic manner here, but rather the open porosity allows for a conduction of new bone growth emanating from the surrounding bone.

4.7.3. Biomechanical compatibility

Although all tissues are subjected to mechanical stresses and each behaves in a different but always complex way, the reaction of bone is perhaps the most significant. The mechanical properties of bone itself have been reviewed on many occasions (e.g. [146]), but it is of most relevance to note here that the structure and indeed viability of bone are determined by the stress system to which it is subjected. The magnitude and nature of this stress system determines the degree of mineralization and the extent of any porosity, and alterations in stress can, by a feedback mechanism which influences cellular activity, produce changes in the bone structure, either of a growth or resorptive nature [147].

The significance of this is that implanted devices which are integrated into bone (e.g. by bone screws or other fixation) will usually have quite different elastic properties and will, therefore, totally alter the stress distribution [148]. In particular, since metals and ceramics are of substantially higher elastic modulus, their attachment to bone must inevitably result, under constant strain conditions, in reductions in stress levels within the adjacent bone. This results in a remodelling which is most likely to be resorptive in nature, giving a porous, less richly mineralized tissue with grossly inferior mechanical properties [149, 150]. This problem of disuse atrophy or osteoporosis is of considerable clinical importance and is likely to have an influence on the response to any material within bony tissue.

4.8. The response of blood to biomaterials

Although the history of using biomaterials and devices within the cardiovascular system does not stretch as far back as that involved with some soft and hard tissue applications, it is probably by now the most extensive in terms of the allocation of resources to tackle the very significant problems inherent within this system. The first serious attempts to replace blood vessels can be traced to the mid 1940s, but most significant developments did not take place until open-heart surgical techniques evolved during the 1950s. Since that time numerous materials and designs have been introduced into areas such as heart valve replacement [151], circulatory assist devices [152], the total artificial heart [153], oxygenators [154], liver support systems [155], renal dialysis [156], blood vessel replacement [157] and so on. With these devices the difficulties, and the constraints on progress, are largely related to the interactions between the blood and the devices. Blood compatibility is, therefore, of the utmost importance in determining the performance of devices within the cardiovascular system and has to be considered somewhat separately from other aspects of biocompatibility.

It is possible to discuss this subject at a number of levels, including specific reactions associated with different materials and different devices. In this review we shall only discuss, and briefly at that, the basic principles of the interactions between blood and synthetic materials. This will be done under the headings of the essential characteristics of blood, the mechanisms of blood clotting, the effects of materials on clotting proteins, material–platelet interactions and mechanical damage to blood. In addition to the references quoted in the sections that follow, the reader is referred to some general texts on blood compatibility [158–162].

4.8.1. Essential characteristics of blood

Blood is a suspension of cells in plasma, an aqueous solution containing a variety of organic and inorganic

TABLE I The composition of human plasma (major components only)

Surface	Concentration ($g\,dl^{-1}$)	Molecular weight
Water	90 to 92	
Proteins		
Serum albumin	3.3 to 4.0	69 000
Fibrinogen	0.34 to 0.43	340 000
α_1-globulins	0.31 to 0.32	44 000 to 200 000
α_2-globulins	0.48 to 0.52	150 000 to 300 000
β-globulins	0.78 to 0.81	90 000 to 1 300 000
γ-globulins	0.66 to 0.74	160 000 to 320 000
Cations		
Na^+	0.31 to 0.34	
K^+	0.016 to 0.021	
Ca^{2+}	0.009 to 0.011	
Mg^{2+}	0.002 to 0.003	
Anions		
Chloride	0.36 to 0.39	
Bicarbonate	0.20 to 0.24	
Phosphate	0.003 to 0.004	

molecules, the major components being shown Table I. Thare are three groups of blood cell, as indicated in Table II.

The erythrocytes or red cells are clearly the most numerous. They have a lifespan of 105 to 120 days, becoming more fragile as they age. The main effect of blood–material interactions in relation to the red cell is that of accelerated ageing, or premature mechanical destruction. This is discussed in Section 4.8.5.

There are far fewer white blood cells than red cells per unit volume. Unlike red cells or platelets, these are not confined to blood and, as we have seen in Section 4.2, are released into the tissue where they carry out certain specific functions. These cells are not of great significance in blood compatibility.

Platelets, on the other hand, are of enormous significance. When resting, they are small discoid cells of diameter 2 to 3 μm, with a very complex cell membrane [163] which has numerous receptors for interaction with key proteins contained in the plasma. The interior of the platelet consists of a number of granules that contain a variety of proteins which control the ability of the platelets to aggregate and interact with other structures.

Under certain conditions, platelets are activated, resulting in significant functional, biochemical and structural alterations to the cell, compared to its resting state. The most noticeable effects of the activation are adhesion of the cells to sites of blood vessel wall injury, their aggregation and the fusion of granules with the plasma membrane to facilitate the release of granular contents. This is particularly significant in the blood clotting process.

As indicated in Table I, the plasma is a very complex mixture of proteins, anions and cations. The plasma proteins [164] include those which provide nutrients to the cells, principally albumin and lipoproteins, those which are involved in the transport of hormones and other chemicals, such as transferrin (carrying iron), ceruloplasmin (copper), vitamin-binding proteins and steroid-binding proteins, and those which are involved in defence, especially the immunoglobins, complement, and, of special relevance to this discussion, the proteins of the clotting process.

4.8.2. Mechanisms of blood clotting

In normal healthy humans, blood flows through the vascular system and does not clot. Blood does, however, have the ability to form clots when a blood vessel is injured, so that bleeding can be arrested. The mechanism by which this haemostasis takes place is very complex and there are many opportunities for interference. Thus, the process may be initiated in patients when physiological parameters change for reasons other than the arrest of bleeding, or when the vascular system is interrupted by some foreign surface.

Haemostasis is achieved by the formation of a mass of platelets and fibrin that is deposited in such a way that it is impervious to the flow of blood. There are two crucial events in this process, involving first the cellular components of blood and secondly the plasma proteins. Injury to a vessel that involves disruption of the endothelium, initiates a sequence of events which allow platelets, normally non-adherent to endothelium, to adhere to the damaged surface. The first stage is the platelet–collagen interaction, in which contact with collagen causes the platelet membrane to undergo a series of changes. This stimulated platelet releases a number of substances, principally adenosine diphosphate (ADP), which causes the platelets to aggregate and, ultimately, form a platelet mass [165].

The other crucial event is the activation of the sequence of events known as the coagulation cascade [166] in the plasma proteins, that leads to the formation of a thrombus. The blood coagulation proteins are a series of enzymes that function sequentially,

TABLE II The cells of circulating blood

Cell	Concentration (Number per mm^3)	Normal shape	Volume (%) in blood
Erythrocytes	4 to 6 $\times 10^6$	Biconcave disc, 8 μm $\times$ 1 to 3 μm	45
Leukocytes			
Neutrophils	1.5 to 7.5 $\times 10^3$	Spherical, 7 to 22 μm diameter	1
Eosinophils	0 to 4 $\times 10^2$		
Basophils	0 to 2 $\times 10^2$		
Lymphocytes	1 to 4.5 $\times 10^3$		
Monocytes	0 to 8 $\times 10^2$		
Platelets	250 to 500 $\times 10^3$	Rounded or oval, 2 to 4 μm	

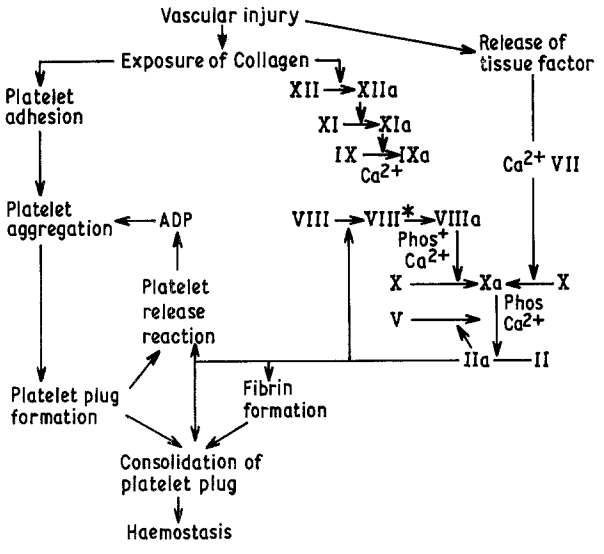


Figure 11 The coagulation cascade in relation to blood clotting.

with the final event being the polymerization of the fibrinogen monomer, brought about by the action of thrombin, to form a cross-linked biological macromolecule, fibrin. The fibrin strands begin to reinforce the primary platelet plug, consolidating it into an impermeable mass. Several substances released during degranulation are involved in this sequence. The fibrin is important in the platelet plug since it stabilizes the platelets irreversibly; without the fibrin, the platelets would soon disintegrate.

The coagulation cascade may be initiated by one of two mechanisms [167]. The first of these involves the presence of the so-called tissue factor, a glycoprotein associated with phospholipid, and the activation of the extrinsic pathway. The second involves the exposure of a non-endothelial surface, such as collagen, and the activation of the intrinsic pathway. In either case, there is a localized conversion of inactive molecules to proteolytic enzymes, in a sequential pattern that culminates in the conversion of prothrombin to thrombin, which catalyses the polymerization of fibrinogen to fibrin. As shown in Fig. 11, the two pathways come together with the conversion of Factor X to Xa and thereafter they follow a common pathway to the formation of fibrin.

Initiation of the extrinsic pathway is a relatively straightforward matter [168]. Tissue factor is an enzymatically inert glycoprotein that is present on the surface of many cells, but not in plasma proteins. It is released when tissue is damaged, interacts with Factor VII, and together they catalyse the conversion of Factor X. The intrinsic pathway is more complex and more relevant to the use of biomaterials. The enzyme central to this intrinsic, surface-initiated coagulation pathway is Factor XII, the Hageman factor. This is activated by the presence of one or more co-factors and some non-endothelial surfaces, including collagen and the synthetic surfaces of biomaterials [169].

4.8.3. The effect of materials on the clotting proteins and other plasma proteins

The precise nature of the mechanism by which foreign surfaces (e.g. those of biomaterials) initiate coagulation is not clear, but a considerable amount of evidence has been obtained. We may first of all refer briefly to the proposed mechanisms by which surfaces may influence the factors of the intrinsic pathway, and then briefly describe the known interactions between surfaces and plasma proteins in general.

The available evidence suggests that it is negatively charged surfaces which possess the ability to initiate coagulation, and that these surfaces perform three vital functions. First, they introduce a structural change in Factor XII such that the surface-bound Factor XII becomes susceptible to proteolytic activation. Secondly, the surface promotes an interaction between Factor XII and the inactive molecule prekallikrein which results in the reciprocal activation of each. Thirdly, the surface promotes the activation of Factor XI by surface-bound Factor XIIa.

Although a great deal is now known about the biochemical changes which are taking place here, little is known of the way in which different biomaterials are able to influence these events. On the other hand, much experimental data has been accumulated concerning the ability of foreign surfaces to adsorb and interact with plasma proteins in general [170–173]. Most available information concerns the adsorption of single isolated proteins on to polymer surfaces in the absence of the competition that would arise in plasma. It is generally believed that the Langmuir model of adsorption applies and that surfaces which adsorb albumin are generally thromboresistant, while those which adsorb fibrinogen, gamma globulin and fibronectin tend to be more thrombogenic [174, 175].

4.8.4. Material–platelet interactions

In spite of the obvious importance of material–platelet interactions in the development of thrombogenesis, and the vast amount of information about the adhesion of platelets to foreign surfaces, the surface characteristics which are responsible for the attraction of platelets have not really been identified. Several theories have been proposed but the literature shows that it is quite possible to argue that opposing characteristics are equally important. Thus, it has been shown on the one hand that platelet adhesion decreases with decreasing interfacial free energy [176], while on the other hand that it decreases with increasing interfacial free energy [177], the role of adsorbed proteins apparently being crucial here.

This subject has been reviewed at great length recently by Anderson and Kottke-Marchant [178] from which it is concluded that no one theory is able to explain the interaction of platelets with foreign surfaces. One point of interest, however, is that since natural blood vessels do not themselves cause platelet adhesion until damaged, there must be substances within the vessel walls that repel the platelets. Several such substances have been isolated, including prostacyclin, one of the prostaglandins. One of the many attempts to prepare non-thrombogenic materials has involved the attachment of prostacyclin or prostacyclin-like substances to the surface and this has indeed shown some success in respect of anti-platelet activity [179].

4.8.5. Mechanical damage to blood

As noted above, red cells, which are responsible for carrying the haemoglobin in blood, have a finite life span. Their eventual removal from the system involves a disintegration of the cell membrane; new cells are continually produced to offset this controlled removal. The red cell membrane is, in fact, a highly deformable material, since it needs to change its discoid shape as it passes through narrow vessels, especially in the spleen, and it is quite susceptible to damage induced by shear stresses [180].

The interposition of hard or rigid materials in the vascular system usually perturbs the haemodynamic regime and high shear stresses are easily generated. It is not well appreciated that the interaction of flowing blood with a variety of devices leads to premature ageing of the cells and, therefore the development of a haemolytic anaemia [181–183]. The nature of the material is not particularly important, but rather the design and location of the device.

4.8.6. Blood-compatible materials

It is clear that the future of biomaterials within the cardiovascular system depends on the understanding of their interactions with the blood, especially those leading to the resistance to clot formation. For many years attempts have been made to define the ideal thromboresistant surface in terms of physicochemical parameters. Some theories have, for example, suggested that negatively charged surfaces are ideal [184], others have defined surface energy parameters [185] and yet others have suggested that water-containing surfaces of hydrogels are most appropriate [186]. For many years, however, the choice of the best blood-compatible surfaces has been dominated by the perceived need for inertness so that carbon, titanium, PTFE, silicone rubber and similar materials have been extensively used.

More recently, serious attempts have been made to tackle this problem from a more logical biological view. As well as the anti-platelet substances such as described above, there have been attempts to coat polymer surfaces with phospholipids that mimic the cell membrane [187], or with heparin or heparin-like substances that are themselves known to be anti-thrombogenic [188–190]. More natural materials in their own right are also being used in some applications, such as the glutaraldehyde-treated pericardial tissue derived from cows or pigs used in some heart valves [191].

5. Variations in biocompatibility phenomena

Having described in some detail the various mechanisms by which biomaterials may interact with the tissues of the body, and giving the impression that some unified theory of biocompatibility, however complex, may be possible, it is necessary at this stage to introduce a few other phenomena which may be superimposed on the whole process and which make interpretation of the overall response very difficult. Three particular phenomena come into this category: the initiation of tumours by implanted biomaterials, the development of allergies to biomaterials (the immunological response), and the influence of infection.

5.1. Tumour induction by biomaterials

Tumours are the result of excessive and uncontrolled proliferations of cells, which may be benign, in which case they are confined to their site of origin, or malignant, which grow locally by infiltration and expansion and have the ability to spread via body fluids. Tumours occur at a wide variety of anatomical sites and show a wide variation in type. Although for many tumours the cause is not known, for others, the agents responsible may be identified. Such agents include radiation, viruses, hormones and exogenous chemical substances.

It has been known for some time that implantable biomaterials may cause tumours under certain circumstances [192]. It is logical to assume that the chemical nature of the biomaterial may be important in this context (i.e. the material may act as a chemical carcinogen) but it is also clear that other factors, such as physical factors, may also be important.

The experimental data on tumour induction is derived mainly from laboratory animals. As reported by Pedley *et al.* [192] very many materials, including examples of polymers, metals, ceramics and minerals have been shown to be carcinogenic under some conditions in experimental animals, especially rodents. It is known that a latent period for induction of the tumours is generally applicable in these animals (that is there is a time lapse between implantation and the occurrence of the tumour, measured in months in the rat, for example) and that considerable variations are seen with different species of animal.

The experiments also indicate that both physical and chemical variables influence the extent of tumour induction. With polymers which are relatively inert chemically, it is likely that chemical carcinogenesis is playing a significant part and the most important variable appears to be the size of the implant. Large monolithic objects tend to be the most effective agents, whereas small particles are generally less active tumorigenic agents. With metals the same situation does not necessarily prevail, since small particles, with their much enhanced surface area, are able to act as a source of metal ions, some of which (e.g. nickel) are known to be chemical carcinogens, so that chemical effects dominate particle size effects.

Two questions dominate the discussion of "solid-state carcinogenesis". First, is it possible to predict which materials will be carcinogenic when implanted? Secondly, are materials which appear to be carcinogenic in rodents also carcinogenic in humans? Clear answers can be given to neither question. It is likely that tumours can be induced under some conditions with any material on implantation, although rates of induction and species specificity may vary. However, although some recent journals have borne some evidence of tumours associated with implants in humans [193], the chances of this being a significant problem are quite low.

In studying the biocompatibility of potential

biomaterials, it is clearly important to consider the carcinogenicity aspect. It remains, however, difficult to interpret the data that are obtained.

5.2. Immunological response

Even more difficult to assess and understand is the possibility of the host developing a hypersensitivity response to a biomaterial.

The inflammatory response to the presence of a foreign body (described earlier) has to be considered as the first line of defence, but it lacks specificitv in relation to the nature of the material and has no built-in memory facility. In addition to this response, however, there is a second line of defence which possesses both specificity and memory; this is the immunological response. Specificity arises because the response is not activated by all materials; indeed, only certain types of material can elicit an immune response and the extent of this response will depend on the nature of the material. The memory is involved because on first exposure the tissues develop a recognition pattern, and at this time may react only minimally, but this recognition can be fully brought into play when the tissues are exposed a second or subsequent times, a very considerable response being called into action.

Thus, the immunological defence mechanism (which may differ in detail in different animal species) is characterized by two important features. First, the reaction is directed against one specific foreign invader at a time. Following exposure to an invader (e.g. micro-organism or foreign particle) the host will react against that invader and will be left with a state of immunity to that body. That particular immune state will be ineffective against subsequent challenge with a different organism or material. Secondly, the immunological memory for the invader results in a far more rapid generation of immunological effectors on the second exposure.

The type of immune response generated against any particular foreign body will depend to a large extent on the nature of the body, which is referred to as the antigen. Generally it is proteinaceous substances that are the most potent antigens, synthetic, man-made substances themselves being unlikely antigens. The reaction itself may be either a humoral or a cell-mediated response, or both. A humoral response is one in which antibodies are developed by the host which react with (i.e. bind to and inactivate) the invading foreign body. A cell-mediated response is one involving special lymphocytes, specifically endowed with receptors or recognition facilities for (and the capacity to kill) the particular invading substance.

The relevance of these immunological responses to biomaterials lies in a number of points. First, although antigens are usually complex organic molecules of relatively high molecular weight, some other substances may become antigenic when coupled to other molecules even if they themselves are non-antigenic. Thus, the vast majority of biomaterials are non-antigenic but they may activate the immune response because of the binding of products released from their interaction with the environment of the body with some appropriate carrier molecules in the tissue. Secondly, although it is not usual to continually re-expose the body to repeated challenges from biomaterials, in the same way as tissues are exposed to the micro-organisms of contagious or infectious diseases for example, the continued presence of a material in the tissues, leading to a persistent source of reaction products, can provide a continuum in which this immunological memory can be developed.

It should be borne in mind that the development of immunological reactions is idiosyncratic, as witnessed by the great variability in the susceptibility of the population to allergens, such as in hay fever and asthma, so that the testing for (and predictability of) the immunological properties of biomaterials is a very difficult matter. Nevertheless, there is increasing evidence that biomaterials are involved in eliciting a response from the immune system and this aspect has to be taken very seriously in their evaluation. The reader is referred to several recent reviews on this subject for further information [194–196].

5.3. Biomaterial–bacterial interactions

It will be recalled from Section 4 that the response of tissues to injury follows the same general pathway irrespective of the causative agent. One of the most frequent causes of tissue damage is infection by micro-organisms (bacteria, fungi, viruses) and the ability of tissues to deal with such an invasion is well documented. It should be apparent, however, that micro-organisms (and especially bacteria) and biomaterials have a number of similarities, especially as both may be considered as persistent sources of tissue irritation, so that the mechanisms and features of the tissue response to them will have some common features. This implies that there is some scope for interaction between bacteria–tissue and biomaterial–tissue phenomena; this is indeed the case and such an interaction can very significantly influence the course of events.

We may consider this subject from two different points of view. First there is the prospect that the stability of materials in the physiological environment may be comprised by the presence of bacteria in an infection. This subject has been discussed at some length recently by the author [197]. It is relevant here merely to point out that microbiological corrosion and degradation are phenomena directly relevant to biomaterials usage, where the rates and indeed mechanisms of degradative processes may be either enhanced or reduced by the activity of bacteria, especially by the changes in the microenvironment, such as pH, oxygen and enzyme activity, introduced by the bacteria.

Secondly, and as reviewed recently by Sugarman and Young [198], biomaterials or prosthetic devices are associated with increased susceptibility to infection. At its simplest, it is known that the presence of a foreign material in a wound can reduce the number of bacteria necessary to produce a clinical infection by orders of magnitude [199]. It also appears that implanted devices can alter the pathogenicity or virulence of micro-organisms such that bacteria which are normally non-pathogenic may become pathogenic

in the presence of materials. Furthermore, and of considerable clinical significance, implanted devices may act as host sites for infection years after the operative procedure to place the device and there is evidence that such late infections can occasionally arise from the spread of bacteria from other, possibly harmless transient infections. It is difficult to assess the significance of this, and the assumption that infections arising from dental treatment may be involved has recently been challenged [200].

6. Concluding comments

The biomaterials field, as measured by any parameter, is large and is growing fast. New materials are constantly being introduced into various medical and surgical applications. Their success depends upon the manner with which they interact with the tissues of the body. This review has attempted to define the basic mechanisms by which this takes place and to indicate the variables which influence the course of the reactions observed.

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