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Biomaterial-Centered Infection: Microbial Adhesion Versus Tissue Integration

ANTHONY G. GRISTINA

Biomaterials are being used with increasing frequency for tissue substitution. Complex devices such as total joint replacements and the total artificial heart represent combinations of polymers and metal alloys for system and organ replacement. The major barriers to the extended use of these devices are the possibility of bacterial adhesion to biomaterials, which causes biomaterial-centered infection, and the lack of successful tissue integration or compatibility with biomaterial surfaces. Interactions of biomaterials with bacteria and tissue cells are directed not only by specific receptors and outer membrane molecules on the cell surface, but also by the atomic geometry and electronic state of the biomaterial surface. An understanding of these mechanisms is important to all fields of medicine and is derived from and relevant to studies in microbiology, biochemistry, and physics. Modifications to biomaterial surfaces at an atomic level will allow the programming of cell-to-substratum events, thereby diminishing infection by enhancing tissue compatibility or integration, or by directly inhibiting bacterial adhesion.

THE TWO MAIN BARRIERS TO THE EXTENDED USE OF IMplanted biomaterials and complex artificial organ devices are the possibility of biomaterial-centered infection and the lack of successful tissue integration of biomaterial surfaces. These seemingly disparate phenomena are actually similar expressions of cell-to-substratum surface interactions. "Foreign body" (biomaterial)–centered infections are causally related to the highly adaptive ability of bacteria to colonize the surfaces of "inert" biomaterials or of adjacent, damaged tissue cells (*1–9*). Successful tissue integration of biomaterials depends on the ability of tissue cells to arrive at an intimate, possibly chemically bonded relation between their membrane molecular entities and the biomaterial surface (*6, 10, 11*).

Interactions between bacteria or tissue cells and a substratum surface depend largely on the surface and near-surface atomic structure and composition of implanted biomaterials (*6, 10, 12*). This article reviews recent studies in the composite science of cells and surfaces and outlines the significance of and relation between microbial colonization of biomaterials and tissue cell integration of those surfaces in the "race for the surface."

It is suggested that the fate of an available surface may be conceptualized as a race for the surface, which is a contest between tissue cell integration and bacterial adhesion to that same surface. Host defense systems that are perturbed by biomaterials are a vital factor. If the race is won by tissue, then the surface is occupied and defended and is thus less available for bacterial colonization.

Numerous biomaterial components are permanently or temporarily implanted in humans, including the artificial heart, joint replacements, contact lenses, heart valves, vascular prostheses, dental implants, fabrics and sutures, and intravascular catheters. Ultimately, almost every human in technologically advanced societies will host a biomaterial. Resistant, recurrent, often catastrophic, and always costly infection is a frequent complication of the use of these materials. Infection of a vascular or total joint prosthesis will almost always result in reoperation, osteomyelitis, amputation, or death. Combined rates of death or amputation from infected cardiac, abdominal, and extremity vascular prostheses may exceed 30% (*13–15*). Transcutaneous or transmucosal devices such as intravenous catheters, peritoneal dialysis catheters, and urologic devices rarely escape infection if left indwelling for any length of time (*13, 14*).

Pathogenic Sequence in Substratum-Induced Infection

When nonliving substrata (the artificial heart, biomaterial implants, and some tissue transplants) are introduced into mammalian hosts, they may become favored sites for adhesive bacterial colonization, especially in the immunocompromised host. Adhesion-mediated infections develop that are notoriously resistant to antibiotics and host defenses and that tend to persist until the biomaterial or foreign body is removed (*4, 5, 8, 15*). The pathogenesis of adherent infections is related, in part, to preferential colonization of inert substrata whose surfaces are not integrated with healthy tissues composed of living cells and intact extracellular polymers (*5, 7, 16–19*).

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Progression to clinical infection in biomaterial-related disease in normal or immunosuppressed patients involves the maturation of an inoculum of known pathogens (for example, *Staphylococcus aureus* or *Pseudomonas aeruginosa*) or the transformation of nonpathogens (*Staphylococcus epidermidis*) to a septic focus of adhesive, "slime-producing," virulent organisms. This transformation occurs in the presence of, and is potentiated by, the surface of the biomaterial (*6, 15*).

A major organ device such as the total artificial heart (TAH), which interacts with hemodynamic and solid tissue systems and traverses body cavities to the external environment, is particularly susceptible to infection. Recent experience indicates that biomaterial-centered infection of the TAH restricts its use to that of a bridging device to heart transplants for periods of less than 100 days. The massive surface area and systemic placement of the TAH create a locus for colonization and a source of seeding, causing abscess of distant organs and death by massive sepsis (*20*). Patients with a TAH are also particularly at risk because of their depressed host defense status. Even in healthy patients, host defense mechanisms may be perturbed by the presence of biomaterials.

Microbial Adhesion and Tissue Integration

Microbial adhesion, aggregation, and disaggregation (dispersion) involve interactions between cells and substrata surfaces in an ambient fluid milieu. The surface may be inorganic or organic, inert or reactive, devitalized or living; the organisms may be of the same or different species; the cell types may be prokaryocytes or eukaryocytes; and the environment may be any that supports life (*3, 21–23*).

Bacteria in natural habitats, certain diseases, infections related to biomaterials or damaged tissue, and osteomyelitis have a common survival mode based on the adhesive colonization of substrata (*3, 21–23*). In biomaterial infections or osteomyelitis, compromised tissue, bone, or prosthetic devices provide those substrata. Interaction of physical and biological factors then allows bacterial attachment and adhesion. Proteinaceous adhesins (fimbriae in Gram-negative bacteria), polysaccharide polymers, and surface and milieu substances interact and intermix to form an aggregate of bacteria, elemental substances, glycoproteins, and polysaccharides in a biofilm (*3, 21–23*). Additional symbiotic species may join in consortia and present as a polymicrobial infection. Characteristically, these infections do not respond to treatment until the substratum is removed. Thus, bacterial adhesion and its denominators direct the pathogenesis of damaged tissue and biomaterial infections (*3, 6, 21, 23*).

Tissue integration is a desired phenomenon for the biocompatibility of certain implants and biomaterials. Tissue integration requires a form of eukaryocytic adhesion or compatibility with possible chemical integration to an implant surface (*10, 11*). In effect, this is the goal of the science of biomaterials for solid tissue system implants. For hemodynamic systems, a biocompatible, nonadhesive, luminal surface is desirable to prevent thrombus formation or infection. Many of the fundamental principles of interfacial science apply to microbial adhesion, tissue integration, and biocompatibility and are general to and independent of the substratum materials involved (*2, 10, 21, 23*).

Bacteria in Biomaterial Sepsis

The ability to adhere to surfaces is a general property of almost all bacteria and depends on an intricate and sometimes exquisitely specific series of events. These events are based on determinants and characteristics of the bacteria, the substratum surface to be colonized, and the ambient fluid milieu.

Studies have most frequently resulted in isolation of *Staphylococcus epidermidis* and *Staphylococcus aureus* from infected biomaterial surfaces (*3, 6, 15*). Additional organisms isolated include *Escherichia coli,* peptococci, *Pseudomonas aeruginosa, Proteus mirabilis*, and beta hemolytic *Streptococcus* (*3, 6*). The variety of organisms recovered is expanding as awareness increases and methodology improves. *Staphylococcus epidermidis*, usually thought of as a nonpathogenic, commensal, human skin saprophyte, has emerged as a serious pathogen in biomaterial-related infections and is a leading cause of infections of vascular prostheses, neurosurgical shunts, and orthopedic implants (*13–15, 24*).

Staphylococcus epidermidis is frequently involved when the biomaterial surface is a polymer or when a polymer is a component of a complex device, such as in extended-wear contact lenses, vascular prostheses, the TAH, and total joints (*6*). *Staphylococcus aureus* is often the major pathogen in biometal, bone and joint, and soft tissue infections; however, it is less frequently associated with polymer-sited infections than *Staphylococcus epidermidis* (*6*). *Staphylococcus aureus* is the most common pathogen isolated in osteomyelitis when damaged or dead bone acts as a substratum (*5, 6, 25*).

Our studies in vitro verify preferential adhesion to the surfaces of polymers for *Staphylococcus epidermidis* and to metals for *Staphylococcus aureus* (*6*). Similar findings have been presented by other investigators (*2, 26*). *Staphylococcus epidermidis* is also a factor in polymicrobial infections associated with other substrata, such as metals, compromised bone, and tissue. However, in those cases it is less frequently seen and tends to augment or be augmented by other organisms (*6, 27*).

Pseudomonas aeruginosa is the most frequently identified cause of bacterial keratitis associated with the use of extended-wear contact lenses (*7*). It may prove to be the ultimate problem bacterium as the use of polymers in special sites expands [for example, extended-wear contact lenses (*7*) and the TAH (*20*)].

Studies of adult osteomyelitis have shown polymicrobial infection in more than two-thirds of the cases (*5, 25*). The most common pathogens isolated were *Staphylococcus aureus* and *Staphylococcus epidermidis* and *Pseudomonas, Enterococcus, Streptococcus, Bacillus,* and *Proteus* species. Polymicrobial infections, therefore, appear to be an important feature of substratum-induced infections, are probably present more often than is realized, and should be regarded as a poor prognostic sign for revision surgery (*27*).

Extracellular Polymers

The extracellular polysaccharide substance of slime-producing bacteria is a loose amorphous material composed of a range of low and high molecular weight polymers associated in large part through ionic interactions. In general, exopolysaccharides are composed of neutral monosaccharides such as D-glucose, D-galactose, D-mannose, L-fucose, and L-rhamnose and contain amino sugars, uronic acid, and polyols (ribitol and glycerol) (*28*). Extracellular slime may be an important factor in the development and persistence of biomaterial-centered infections. This complex exopolysaccharide is believed to act as an ion-exchange resin for enhanced nutrition, to interfere with phagocytosis, to influence response to antibodies, and to function in later stages of surface adhesion, aggregation, and polymicrobial interaction (*2, 5, 29*).

Currently, only the monomeric carbohydrate moieties and several amino acids in the exopolysaccharide slime of *Staphylococcus epidermidis* have been described. Glucose, galactose, glycerol, hexosamine, phosphorus, glycine, alanine, and phenylalanine have been found to

be major components of the slime produced by *Staphylococcus epidermidis* (*30*). These *Staphylococcus epidermidis* strains were randomly selected from clinical specimens unrelated to biomaterial infections. Recent studies of a *Staphylococcus epidermidis* strain (SE-360 Yoshida) indicated the presence of mannose (*7*). These and other constituents of the slime vary between and within species. Polysaccharide composition and therefore aggregation or dispersion of bacteria may vary with nutritional and antagonist qualities of the environment (*29, 31*).

Conditioning Films

Glycoproteinaceous conditioning films, derived from fluid or matrix phases containing fibronectin, fibrinogen, collagen, and other proteins, almost immediately coat a biomaterial or tissue substratum and provide receptor sites for bacterial or tissue adhesion (*2, 32*). The specific role of each of the macromolecular constituents of this layer will differ for each organism or type of tissue cell. The sequence of protein deposition and layering is directed by biomaterial surface properties. Animal studies with specific preparations of glycoproteinaceous films on a biomaterial surface have shown inhibition of osseointegration to titanium and glass ceramic implants (*33*). Thick (more than 1000 Å) proteoglycan films also form on the surface of fluoride glass ceramics in bone and apparently inhibit osseointegration (*11, 33, 34*). *Staphylococcus aureus* has discrete binding sites for collagen and fibronectin; this implies a role for fibronectin in mediating adhesion and possibly infection (although it may not necessarily cause infection) (*3, 35, 36*).

Molecular Mechanisms

Initial attachment (reversible nonspecific adhesion) depends on the general long-range physical characteristics of the bacterium, the fluid interface, and the substratum. Specific irreversible adhesion, which occurs after initial attachment, suggests time-dependent biosynthetic chemical processes that in part depend on specific protein adhesin-receptor interactions, as well as on carbohydrate polymer synthesis (*15, 22, 29, 37*).

The delineation of complex natural processes into discrete categories such as reversible or irreversible is useful but may not be representative of actual dynamic or specific events. Bacteria may arrive randomly near the surface of a biomaterial, foreign body, or tissue substrata by direct contamination, contiguous spread (as from adjacent epithelial cells or by means of transcutaneous drive lines as in the TAH), or by hematogenous seeding (for example, heart valves, joint replacements, or osteomyelitis).

By virtue of their atomic structures, surfaces tend to present energy profiles or available binding sites for environmental interactions (*38*). For metallic alloys, a thin (100 to 200 Å) oxide layer forms almost immediately and represents the true interface (*10*). For polymers and metals, binding sites are further modified by surface texture, manufacturing processes, trace chemicals, and debris, and by ionic and glycoproteinaceous constituents from the host environment. The atomic structure, electronic state, oxidation layer, contamination level, and glycoprotein-coating sequence or dynamics in a human host have not been defined for even a few biomaterials, but these factors can be assumed to be specific for the material, host environment, and the bacterium or tissue cell attempting adhesion or integration (*2, 10, 32*). An interesting concept, in addition to specificity of interaction, is that surfaces may also act as catalytic arenas for molecular and cellular activities that occur at close range (*6, 10, 39*).

The cell surface charge is negative, as is that of most substratum surfaces; however, isoelectric points of materials at the surface-liquid interface can vary with *p*H, inflammation, and tissue damage caused by surgery, trauma, and infection (*40, 41*). Corrosion of the biomaterial also alters *p*H and charge (*41*). The common charges of the microbe and substratum tend to repel each other, but van der Waals forces at the secondary minimum (approximately 10 nm) effectively position a particle or bacterium near the surface (*2, 29, 37*). At closer ranges repulsion occurs until the primary minimum is entered (less than 2 to 3 nm) where attraction occurs (Fig. 1) (*2, 29, 37, 42*).

Studies indicate that hydrophobic forces are exerted at distances as great as 15 nm, and at 8 to 10 nm are 10 to 100 times as great as van der Waals forces (*42*). Some degree of hydrophobicity exists for many bacteria and most surfaces (*2, 37*). Attractive hydrophobic interactions tend to overcome repulsion and position bacteria at the primary minimum. When a bacterium or tissue cell is within 1 nm or less, or is proximate to the surface, it is conceivable that short-range chemical interactions (ionic, hydrogen, and covalent bonding) occur with extracellular moieties.

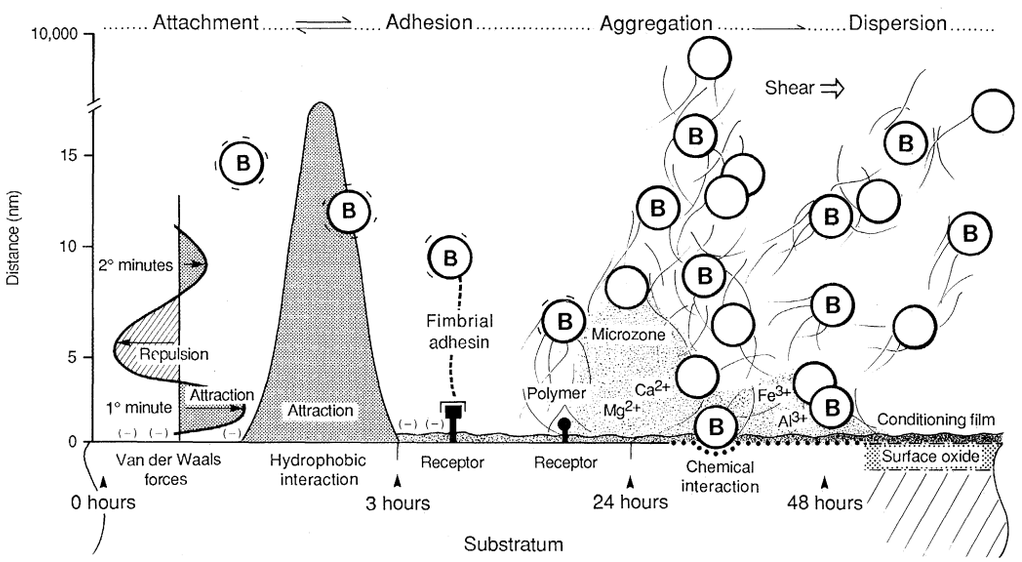

Fig. 1. Molecular sequence in bacterial (B) attachment, adhesion, aggregation, and dispersion at substratum surface. A number of possible interactions may occur depending on the specificities of the bacteria or substratum system (graphics, nutrients, contaminants, macromolecules, species, and materials) (*37, 42*).

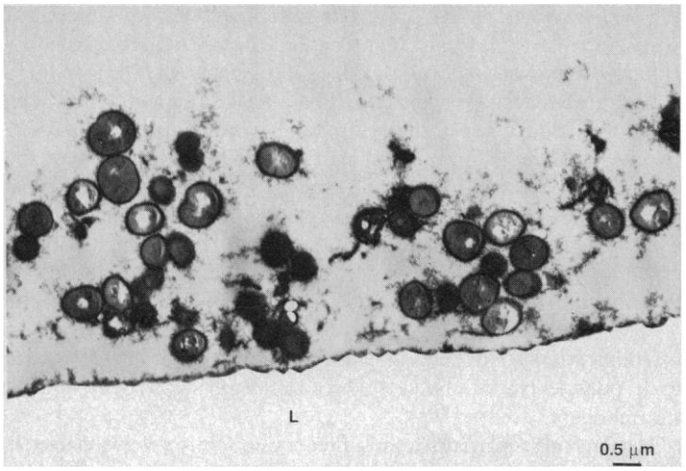

Fig. 2. *Staphylococcus epidermidis* on surface of extended-wear contact lens (L). Ruthenium-red–stained material encompasses bacteria within clusters, and flocculent material appears to provide continuity with lens surface. A decrease in peripheral colony density is shown.

Subsequent to or concomitant with initial attachment, specific fimbrial adhesins and substratum receptors may interact if they are present in the particular biological system, as in bacteria-to-tissue cell pathogenesis or glycoproteinaceous conditioning films that immediately coat implants (*3*). Fimbriae may also react nonspecifically (by charged or hydrophobic interaction) with inorganic substratum elements (*43*).

Bacterial exopolysaccharides may also bind to surfaces or to surface adsorbates and may function in cell-to-cell aggregation, further consolidating adhesion and microcolony formation (*23*). If environmental conditions, such as temperature, nutrient substrates, antagonists, and cation balance are favorable, bacterial propagation occurs.

Disaggregation or detachment. Subsequent to aggregation and colony maturation, cells on the periphery of the expanding biomass may detach or disaggregate and disperse. Disaggregation is extraordinarily relevant in the pathogenesis of the septic process that surrounds substratum-centered biomaterial infection. In natural environments it is a survival and propagation strategy, but it has seldom been considered as a factor in human infection (except under the general heading of septic emboli). At a cellular and molecular level, disaggregation becomes a function of growth phase, colony size, nutrient conditions, and graphics such as hemodynamic or mechanical shear forces (hemodynamic, ocular, or total joint systems). Changes in extracellular polysaccharide polymer production and composition may play an important role in detachment or disaggregation (*31, 44*).

Aggregation, dispersion, and fractal growth processes. Bacterial cell aggregations or clusters of aggregates created by random particle settling or after cell division and colony growth appear in some cases to resemble fractal geometry. Fractal progressions are nonequilibrium, usually diffusion-limited processes with a sprawling or tenuous pattern that may be used to describe the spatial characteristics of natural phenomena such as particle accumulation or cell growth (*45–47*). Examples of colonization that resemble fractal dimensionality are the radial and spherical arrangements of soil and coryneform bacteria and the rosette formation by *Pseudomonas* species (*48*). The random aggregate arrangement of *Staphylococcus epidermidis* on the surface of an extended-wear contact lens also suggests a fractal form (Fig. 2) (*7*).

For some species, as aggregation (assuming a continuing or periodic increment) or colony growth occurs, cells tend to move or accumulate outward to form projections from the biomass rather than to be distributed in available spaces within the colony, thereby resulting in a decrease in mean peripheral density.

An understanding of these processes may be relevant in clinical infection. Disaggregation (assuming dispersion from peripheral areas) simulates a reverse fractal progression, dispersing decreasing numbers of cells as colony diameter diminishes. In a dynamic environment when a colonized surface area approaches theoretical maximum or when aggregational factors decrease, large numbers of bacteria or aggregate fragments from projections are available for dispersion. This sequence may explain the relatively intermittent or short-term phenomena of "bacterial showers" or disseminated bacterial emboli.

The Substrata

Many implants are composed of one or more metals or polymers. Biomaterials, foreign bodies, and devitalized tissue and bone in a biological environment are passive and susceptible substrata because they are inanimate and do not resist infection. In fact, regardless of inertness, they are physiochemically active and may directly modulate adhesion or interact with host defenses (*4, 7, 8, 49, 50*).

Surface effects of metal alloys and catalysis. Metal surfaces represent planar cuts through crystalline structures and generally exhibit moderate to high surface energies (exceeding 40 dynes per centimeter), which are believed to be adhesive for tissue cells (*32*). The geometric arrangement of metal atoms at the exposed surface plane, and thus the number of unsatisfied bonds, depends on surface cut orientation (*38, 51, 52*). A clean metallic surface yields high surface-free energy and is therefore reactive and potentially capable of catalyzing chemical reactions (*52, 53*). Surface diffusion plays an additional role in catalytic activity (*52, 53*). Molecules adsorbed to clean surfaces diffuse about freely as the energy to perform random movement is directly acquired from thermal vibrations of the underlying lattice (*52*). Therefore, molecular fragments encounter other molecules and interact more frequently than in free solution. Adsorption at specific sites may also lower the activation energy barrier for specific chemical reactions so that the reaction may proceed at a much reduced temperature (*52*).

Clean metallic surfaces, especially those of stainless steel and chrome cobalt and titanium alloys, are resistant to corrosion by virtue of their elemental composition, crystalline homogeneity, and surface oxides that form spontaneously or are created by an accelerated nitric acid passivation process during their production (*41*). These surface oxides form the reactive interface with glycoproteinaceous molecules of the conditioning film and possibly directly with the surfaces of bacterial cells. Surgical alloys have relatively high surface energy values that encourage tenacious binding of intermediary glycoproteins and colonizing cells (*10, 32, 54*). Specific environmental proteins will interact as coapting agents for prokaryocytes and eukaryocytes. Titanium and chrome cobalt alloys appear to allow closer and stronger tissue cell binding than polymers or bioglass (*33, 54*). However, wollastonite glass ceramics, which rapidly form an apatite surface layer in vivo, show high bone-bonding strength (*55*).

Polymer substrata. Most medical polymers are amorphous. Three are in part crystalline: polytetrafluoroethylene, polyethylene, and polypropylene (*41*). Crystalline zones (spherulites) confer rigidity, whereas amorphous zones confer toughness.

Solid polymers are nonequilibrium structures for which adsorbates tend to satisfy the residual binding capacity, resulting in decreased surface energies (*56*). The hierarchies that result are not as complex as those of higher energy surfaces such as metals or

ceramics but are of biologic relevance (*57*). Increased rates of reaction may occur even on a noncatalytic, two-dimensional planar surface because molecular contact is more likely in a planar or membrane system than in a three-dimensional one (*58*).

Although high molecular weight medical polymers in general are thought to be resistant to deterioration by bacteria, polyvinyl chloride, for example, contains low molecular weight plasticizers (polypropylene sebacate) that are vulnerable to attack by *Pseudomonas aeruginosa* and *Serratia marcescens* (*41*). Methyl methacrylate has a noncrystalline, porous structure that provides increased surface area for diffusion and molecular interaction.

Staphylococcus epidermidis has a higher rate of adhesion to polymers than does *Staphylococcus aureus* (*59*). Tissue adhesion to polymers such as methyl methacrylate is poor and is often characterized by an inflammatory interface, especially after wear (*11, 60*). Polymers of high hydrophobicity are adhesive for many bacterial pathogens (*2*). It may be assumed that contamination and adsorption tend to be specific to each polymer and its manufacturing process. This assumption suggests specificity of bacterial interaction.

Ceramics and glass ceramics. Most ceramics are crystalline structures, as are metals, and are used as dental and orthopedic implants. Bioactive glass ceramics are composites of crystals dispersed in an amorphous glass phase.

Bioactive glass ceramics are brittle and possess poor mechanical properties. They are postulated to react chemically with biological tissue, providing integration, but at the same time to be bioinert, that is, to be nonreactive with foreign bodies and nonbiodegradable by tissues (*61*). The main constituent of these materials is amorphous, glassy, silicon dioxide interspersed with various crystalline substances.

Bacterial adhesion to ceramics and glass ceramics probably occurs, but the clinical significance and microbiological characteristics of infections of these surfaces require further study before any conclusions can be drawn. Studies of the comparable colonization of metals, polymers, and ceramics by *Staphylococcus epidermidis* suggest that ceramic substrata behave like metals and are colonized less than polymers (*62*).

Damaged tissue as substrata. Traumatized soft tissue is represented by amorphous organic fragments of cellular tissue and matrices, is rich in microbial nutrient material, ligands and adhesins, and provides a surface for colonization by bacteria that possess the appropriate receptors. Inanimate, passive, and fertile damaged tissues are unable to resist colonization. Bone allografts represent a particularly large mass of dead tissue, bone, and cartilage that have a clinically demonstrated rate of infection of 5 to 14% (*63*).

Endothelial cells are surrounded by a well-developed glycocalyx. When this outer polysaccharide margin is traumatized by viruses, toxins, or inflammation, receptor sites and fibronectin may be exposed (*17, 19, 64*). Fibronectin may then be available for bacterial adhesion, leading to the development of vascular infection (*16, 19*). This, as well as the presence of a biomaterial, may explain in part the colonization and infection of aortofemoral graft vascular junctions by staphylococci and other bacteria. Endothelial damage may also be a factor in site localization by trauma or by septic emboli in osteomyelitis. Healthy endothelial cell cultures seeded over vascular graft polymer surfaces may protect against bacterial adhesion or thrombogenic events (*16, 17*).

Platelets also participate in infection. Gram-positive bacteria, *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus mutans*, and *Streptococcus sanguis*, which are common causes of bacterial endocarditis, bind to fibronectin, fibrin, or platelets. Trauma to natural heart valves or conditioning of plastic valves by fibronectin, fibrin, and platelet vegetations may be an initial step in the colonization of synthetic heart valves. Bridging of platelets may allow bacterial binding to damaged tissues (*3, 19, 65*). This mechanism is also possible in trauma-induced osteomyelitis.

Bone is a composite structure composed of calcium hydroxyapatite crystals and a collagen matrix grossly similar to synthetic composites or to partially crystalline polymers. Devitalized bone provides a passive substratum for bacterial colonization (some staphylococci have collagen receptors) and the ultimate incorporation of its proteinaceous and mineral constituents as bacterial metabolites (*5, 6, 35*).

Atomic Structure of Surfaces

Biomaterial surfaces present geometric configurations that represent active or unsatisfied binding sites (dangling bonds) and elemental segregations that are only indirectly related to the crystalline or amorphous bulk state (*10, 38, 51, 53*). Properties such as surface segregation and surface oxidation can be understood by considering the thermodynamic driving forces and kinetic limitations of the system. However, because of the complex and ill-defined interac-

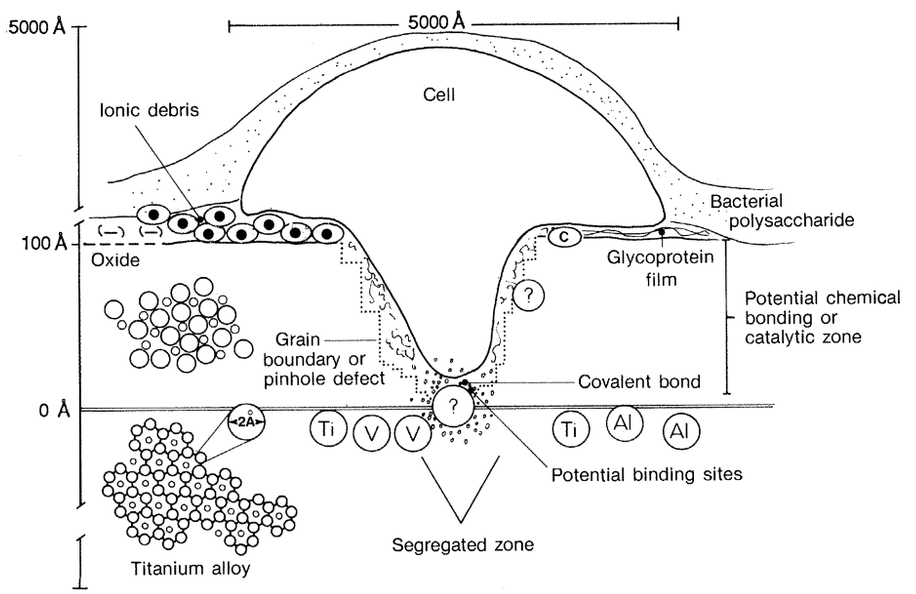

Fig. 3. Schematic representation of suggested interaction between cell and metal alloy substratum at any atomic scale. Bacterial cell (or tissue cell) is shown at defect or irregularity in Ti_6Al_4V alloy oxide surface. Atomic geometry and electronic state direct accumulation of ionic contaminants and result in catalytic processes. Ionic debris, shown by area with ovals encircling dots, includes Cl^-, Mg^{2+}, K^+, Ca^{2+}, S^{3+}, C, Fe^{2+}, and unknown ions. Bacterial receptors or extracapsular polysaccharides and glycoprotein conditioning films of host origin may interact at the substratum surface. Unsatisfied or dangling bonds are potential binding sites. Cell and surface perimeters are exaggerated because of the nonlinear scale.

tions between biomaterials and their environment, surface configurations are not easily predicted from bulk phase properties (*38, 51*).

Elemental composition of metallic alloy surfaces (Ti_6Al_4V) may be significantly different for surface atomic layers (up to a thickness of 0 to 10 Å) than for bulk phase composition because of segregation of specific elements at the surface (*10*). These surfaces are covered by oxide layers 100 to 200 Å thick, depending on preparation and biologic atmosphere. Surface oxide layers may be incomplete and perturbed by grain boundaries and pinhole defects.

The primary status (atomic geometry and electron energy distribution) of the clean biomaterial (metal alloy or polymer) surface will affect the sequence, distribution, and content of initial adsorbates from the host environment. The surface geometry and energy states of nonmetallic crystalline or amorphous polymers are also subject to rearrangements based on molecular composition or crystal structure and size. Even rigid polymers such as polymethyl methacrylate reorient their surface molecules because of relaxation mechanisms (*56*). Surface molecular arrangements and energy states of polymers vary in air, vacuums, and water, with interface domination by high-energy phases in aqueous systems. It has been suggested that adsorption of biologic macromolecules at the surface in an aqueous environment both restructures polymer surface properties and is directed by the primary structure and surface energy of the polymer (*56*).

Initial Events in Adhesion or Integration to Surfaces

Cell behavior on surfaces. As bacteria or tissue cells (bone, endothelial cells, or fibroblasts) approach or contact a substratum surface, their envelope and outer membranes are exposed to increased molecular activity at the substratum surface and to adsorbed macromolecules on the substratum surface. Pioneer colonizing tissue cells or bacteria (if present) then bind more or less directly, and with varying degrees of physiochemical integration, to the substratum by means of this complex macromolecular layer. There is a potentially high degree of sensitivity and selectivity to these interactions. Increased chemical reactivity (catalysis), the ready formation of new molecules at surfaces, and the presence of free ions released from the biomaterial, or as contaminants, may explain the acceleration of bacterial metabolic processes that result in growth, polysaccharide production, and colony and biofilm formation on specific substrata after they are contaminated by bacteria (Fig. 3) (*6*). These same phenomena interact in tissue compatibility or attempted tissue integration. Therefore, the physical and chemical qualities of biomaterial surfaces, especially of metals or crystalline substances, may be the triggers for increased metabolic activity and growth phase changes in some bacteria and possibly in eukaryocytes (*6, 50, 52, 53*). For metals, cations (Fe^{3+}, Mg^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, K^+, and Ni^{2+}) released or accumulated at the surface and involved in corrosion may serve as cofactors for enzymes engaged in protein and sugar metabolism and DNA replication (*39, 66*).

Tissue cell integration. Covalent, ionic, or hydrogen bonding may occur at the boundary between the bone tissue and a titanium oxide surface (*10*). It is the chemical qualities of the surface oxide with which tissue cell (for example, osteoblasts, endothelial cells, and fibrocytes) biomolecules interact. The exact composition of the first monolayer has not been identified. However, it is believed that molecular interactions are taking place at distances that approximate chemical bonding. The sequences may involve the same forces as those involved with reversible and irreversible bacterial adhesion. These interactions and affinities are both specific and dynamic (*6, 10*). For titanium alloys a form of direct bone-implant contact is seen even at the "ultrastructural" level.

Intermediary conditioning films act as molecular bridges between cells and substrata, and integrity or strength of binding or integration is a function of the proteinaceous conditioning film (*32*). Studies indicating that collagen filaments approach to within 200 Å of the titanium oxide surface suggest that chemical bonding to bone tissue may be occurring without intervening fibroinflammatory tissue (*11*).

Substratum Disruption, the Biofilm, and Microzones

Substratum disruption caused by trauma, wear, corrosion, toxins, viral effects, bacterial mechanisms, or biosystem chemical degradation establishes appropriate environmental conditions for opportunistic microorganisms. Surfaces provide an interface for the concentration of charged particles, molecules, and nutrients from mineral or organic sources, or they themselves may be metabolized.

The microzone is an environmental, metabolic microclimate that exists at a colonized surface and within a complex of microcolonies and bacterial biofilm (*67*). This concept may be applied to biomaterial surfaces when adhesive, possibly polymicrobial, colonization creates a microclimate within which optimal conditions are created and from which antagonistic environmental factors are excluded. It is possible that polymer, metal, and compromised tissue fractions may be used directly within the microzone and are available to the bacterial envelope or tissue cell membranes. The microzone may sequester iron from binding with host protein complexes (lactoferrin and transferrin) that normally lower iron concentration levels

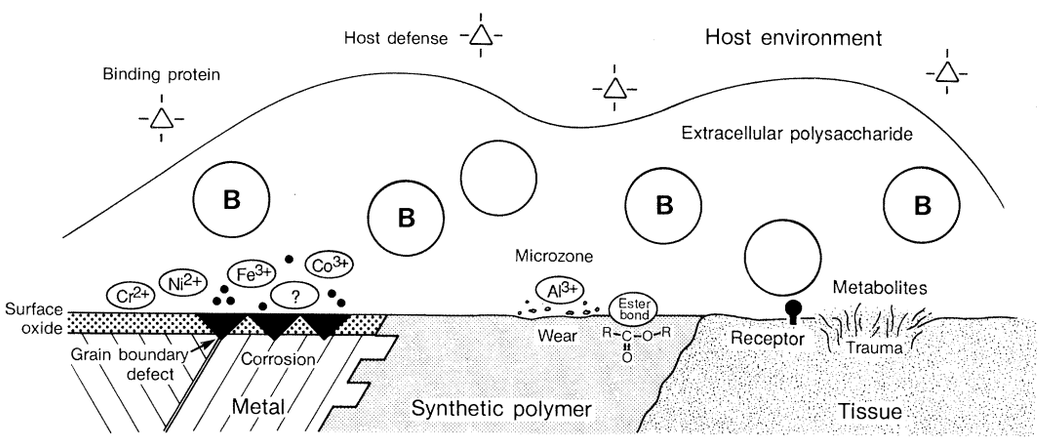

Fig. 4. Surface disruption by wear, corrosion, trauma, or bacterial mechanisms frees metabolites or ions, which are then available to bacteria (B) within a biofilm microenvironnment. At microzones, metal ions required by pathogenic bacteria are not lost by diffusion and may be shielded from host protein-binding complexes. Bacteria are also protected by biofilms and may metabolize polymer or tissue components. Interactions occur between exposed receptors on bacteria or surfaces.

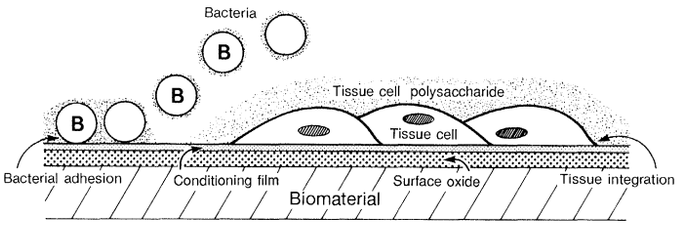

Fig. 5. At the instant of insertion, a biomaterial represents a ready surface for colonization. It is the nature of biomaterial surfaces that their outer atomic layers must interact instantly with the juxtaposed biologic environment. Macromolecules, bacteria, and tissue cells compete for surface domains at the reactive interface. The destiny of an implant will be determined by the conditioning macromolecules and cells that dominate its surface. If the race is won by tissue and a stable integrated relation is achieved, then the surface is less available for bacterial colonization.

below those required by pathogenic bacteria (*68, 69*). Accumulation of iron, rather than loss of iron by diffusion, may occur in microcolonies by localization of siderophores and acid metabolites.

Excesses of iron in a low *p*H or inflammatory environment may also lead to saturation of transferrin and an increased iron supply to bacteria (*69*). Iron has been linked to virulence for *Staphylococcus aureus, Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* and to adhesiveness and virulence for *Escherichia coli* strains (*70*). In excess quantities, iron may inhibit macrophage function (*68*).

Metal ions (such as Fe^{3+}, Mg^{2+}, Cr^{2+}, and Co^{3+}) are available in trace or greater amounts, especially after corrosion. Even with more stable alloys such as stainless steel, some degree of corrosion (especially at grain boundaries) may occur when damage is incurred during implantation or wear or upon chemical interaction with hostile biologic environments (Fig. 4). As indicated by energy-dispersive x-ray analysis, traces of iron, aluminum, and other substances may also be present as a result of polymer manufacturing processes or contamination (*6*). Trace ions, such as Mg^{2+}, Ca^{2+}, and others, may function to stabilize (by means of acidic groups) complex exopolysaccharides in gel formation, enhancing both cell-to-cell and cell-to-surface adhesion and increasing resistance to external antagonists (*2, 29*).

It is also likely that unstable polymers may be directly metabolized or may provide remnants of plasticizers, monomers, antioxidants, and stabilizers. Some synthetic polymers, such as polyester urethane and methyl methacrylate contain ester bonds that may be hydrolyzed by staphylococci (*1*).

Design, Function, Adhesion, and Integration

The design of many implants requires, in addition to general biocompatibility, that a sector (usually metallic for orthopedic and dental implants and polymeric for vascular devices) of the prosthesis or artificial organ be colonized by host cells to provide secure fixation or integration (for example, a total joint replacement requires fixation in bone and should be adhesive for tissue). Another portion (usually polymer) remains intra-articular or intravascular and, by mechanical design, of a low coefficient of friction and antiadhesive for tissue or blood elements. For cemented orthopedic implants, a third portion (methyl methacrylate) is designed to bridge the gap between metal and bone, providing fixation to both and acting as a cement. This latter sector should be adhesive to both metal and bone. Methyl methacrylate, however, is not adhesive for tissue or bone cells; it may provoke an inflammatory response (*11, 60*), inhibit host defenses, and provide an excellent substratum for bacterial colonization.

Vascular prostheses have similar general design characteristics. Although they may be composed completely of polymers, preferably the hemodynamic portion remains nonadhesive for blood fractions whereas the peripheral regions are designed for theoretical tissue ingrowth and integration. Practically, however, it is coaptation that occurs rather than chemical bonding, often through a transitional inflammatory zone.

The TAH presents special problems as it is a composite of many materials, including metals and polymers, and involves compatibility both among the materials and between materials and adjacent tissues. Complexity is added by the need for adhesive (solid system tissue integration) and antiadhesive (fluid environment or hemodynamic system compatibility) sectors. Furthermore, there is usually a power conduit (drive line) that traverses organ space, body cavities, and skin to the external ambient and microbial environment. The conduit represents a communications pathway not only for the power source but for microbes as well. The surfaces of the artificial heart represent colonization sites for bacteria, with each biomaterial favoring a particular colonizing species. The hemodynamic interactions required within the device create fluid eddies and tissue damage that are favorable to clotting cascades and the initial events of microbial adhesion. The attempted integration of synthetic vessel and natural vasculature creates a site of intimal perturbation, inflammation, and endothelial damage, exposing potential receptor sites for bacterial adhesion (*20*). The pumping of hemodynamic elements provides shear forces sufficient to dislodge septic or thrombotic aggregates that may have accumulated on luminal biomaterial or damaged tissue surfaces.

Conclusions

Observations based on the use of implanted biomaterials in human hosts suggest that adhesive or integrative phenomena involving bacteria or tissue cells and substratum surfaces are critical, interrelated, and based on similar molecular mechanisms. Biomaterials present available unsatisfied energy bonds or potential receptors for bacteria or tissue. At the instant of insertion, an implant represents a ready surface for colonization. Vacant binding sites await satisfaction by the first available elements, macromolecules, or cells. In the case of metals or polymers, if the first colonizing cells are tissue and a secure bond is established, then subsequent arrivals are confronted by living, integrated cells. If it is not traumatized or altered, this integrated surface is basically resistant to bacterial colonization by virtue of its viability, intact cell membranes, polysaccharides, and functioning host defense mechanisms (Fig. 5). In vivo, bacteria may defeat host tissue cells in the race for the surface and thus cause infection instead of tissue integration. Once bacterial adhesion has occurred, it is unlikely that tissue cells will be able to displace these primary colonizers to occupy and integrate the surface. Biomaterials are in part susceptible to infection because they are usually not well integrated or, if hemodynamic, not optimally biocompatible or antiadhesive.

Antiadhesive surfaces are neutralized by conditioning films and have in effect already been defeated by an infinite number of experiments in nature. Even in a theoretically antiadhesive system, colonization will probably be accomplished by a few pioneer bacteria that have optimal attachment abilities and use one of the several determinants of adhesion. These initial colonizers provide a foundation for propagation or for subsequent colonization, as their surfaces and polysaccharides are adhesive for other bacteria.

Therefore, a biomaterial or biometal surface that is adhesive for appropriate tissue cells and that encourages rapid eukaryocytic colonization or integration may be the best strategy for decreasing bacterial colonization. The implant environment can be maintained

in a state resistant to bacterial colonization and favorable for tissue cells by appropriate surface constituents, energy state, sterility, and antibiotics, or by precolonization with eukaryotic cells. During the initially vulnerable period before the surface is stabilized and when random colonization by bacteria might occur, antibiotics may be used protectively. Antibiotics directed against known biomaterial pathogens that interfere with bacterial polymer synthesis and adhesive mechanisms, that penetrate biofilm, or that are delivered from the substratum should be effective. Blocking or saturating analogs are an appropriately sophisticated approach that may provide an effective countermeasure. A better understanding is required of the atomic geometry and quantum energy states of substrata surfaces and their interactions with ionic or organic conditioning macromolecules, elements, bacteria, and tissue cells before biomaterials designed for specific tasks can be perfected.

Bacteria are ancient and highly adaptive organisms. Biomaterials are new, but they imitate basic substrata for which bacteria, but not tissue cells, have already evolved colonization and survival strategies. Biomaterial surfaces must be modified to improve compatibility and tissue integration and to resist microbial colonization in the race for the surface.

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