PREFACE

Research on bacterial adhesion and its significance is a major field involving many different aspects of nature and human life, such as marine science, soil and plant ecology, the food industry, and most importantly, the biomedical field. The adhesion of bacteria to human tissue surfaces and implanted biomaterial surfaces is an important step in the pathogenesis of infection.

Handbook of Bacterial Adhesion: Principles, Methods, and Applications is an outgrowth of the editors' own quest for information on laboratory techniques for studying bacterial adhesion to biomaterials, bone, and other tissues and, more importantly, a response to significant needs in the research community.

This book is designed to be an experimental guide for biomedical scientists, biomaterials scientists, students, laboratory technicians, or anyone who plans to conduct bacterial adhesion studies. More specifically, it is intended for all those researchers facing the challenge of implant infections in such devices as orthopedic prostheses, cardiovascular devices or catheters, cerebrospinal fluid shunts or extradural catheters, thoracic or abdominal catheters, portosystemic shunts or bile stents, urological catheters or stents, plastic surgical implants, oral or maxillofacial implants, contraceptive implants, or even contact lenses. It also covers research methods for the study of bacterial adhesion to tissues such as teeth, respiratory mucosa, intestinal mucosa, and the urinary tract. In short, it constitutes a handbook for biomechanical and bioengineering researchers and students at all levels.

Handbook of Bacterial Adhesion: Principles, Methods, and Applications is the first inclusive and organized reference book on how to conduct studies on bacterial adhesion to biomaterials and tissues, a topic that has not been covered adequately by existing works. The book also complements other reference titles on bacterial adhesion. The book has six parts: Part I (6 chapters), Mechanisms of Microbial Adhesion and Biofilm Formation; Part II (6 chapters), General Considerations for Studying Microbial Adhesion and Biofilm; Part III (7 chapters), Techniques for Studying Microbial Adhesion and Biofilm; Part IV (7 chapters), Studying Microbial Adhesion to Biomaterials; Part V (8 chapters), Studying Microbial Adhesion to Host Tissue; and Part VI (5 chapters), Strategies for Prevention of Microbial Adhesion. Since yeasts are also a major factor in implant and/or tissue infections, the book includes a chapter covering Candida adhesion and related infections (Chapter 33).

Handbook of Bacterial Adhesion: Principles, Methods, and Applications is designed to be concise as well as inclusive, and more practical than theoretical. The text is simple and straightforward. A large number of diagrams, tables, line drawings, and photographs is used to help readers better understand the content. Full bibliographies at the end of each chapter guide readers to more detailed information. Although a work of this length cannot discuss every aspect of bacterial adhesion that has been studied over the years, it is hoped that all the major principles, methods, and applications have been included.

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Molecular Basis of Bacterial Adhesion

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I. INTRODUCTION

Bacterial infections are responsible for a broad spectrum of human illnesses and medical device complications. For example, urinary tract infections caused by *Escherichia coli* affect over 7 million people annually and are among the most common infectious diseases acquired by humans.[39] Enteropathogenic *E. coli* (EPEC) and shiga toxin-producing *E. coli* (STIC) are diarrhoegenic pathogens causing serious health problems in both industrialized and developing countries.[26,15] *Helicobacter pylori* have been found to be a main factor in the development of gastric and duodenal ulcers and are believed to be a causitive factor of gastic cancer.[34] *Staphylococcus aureus* and *Staphylococcus epidermidis* are major causes of infections associated with wounds, indwelling catheters, and cardiovascular and orthopedic implant devices.[1,19,24,25,35,49,56,59]

Bacteria have a strong tendency to attach to surfaces. Attached cells will form a colony (biofilm) consisting of prokaryotic cells, surrounded by a matrix of biomolecules secreted by the cells. Although the structure and functions of biofilms are as varying as the type of bacteria, the same four step process is always followed in the creation of the biofilm.[21,57]

During the first step, a series of small molecules (initially water and salt ions) will adsorb to the surface. Subsequently, the substrate surface will be covered with a single layer of small organic molecules or proteins that are present in the medium. The mixture of water, ions and proteins is called conditioning film and is always present before the first microorganisms arrive at the surface.[17,50]

The second step is characterized by the initially reversible adsorption of microorganisms to the conditioning film. The microbes arrive by Brownian motion, gravitation, diffusion, or intrinsic motility. They may also adhere to each other forming microbial aggregates before adsorbing to the conditioning film. Since the microorganisms adhere to the conditioning film and not the surface itself, the strength of the initial biofilm depends on the structure of the conditioning film.[8,29]

The initially reversible adsorption becomes irreversible, mainly through the secretion of exopolymeric substances by the adsorbed microorganisms in step three.[18,40] These substances will incorporate in the conditioning film and strengthen its cohesiveness. In a

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few cases, an entirely microbially derived conditioning film has been observed.[7] Once bound to the surface, many bacterial strains, including *S. aureus* and *S. epidermidis*, additionally form a polysaccharide based biofilm surrounding the bacterial colony. Biofilm effectively inhibits phagocytosis and makes the contained bacteria impervious to antibiotics, thus making implant removal an essential part of treatment once an infection is established.[12,24,25,35,56]

Finally, the number of microorganisms in the biofilm accumulates mainly through *in situ* cell growth. The final structure and composition of the biofilm is determined by these initial events. Other aspects such as the influence of surface active compounds secreted by the microorganisms,[41] the hydrodynamic environment,[48,56] the surface roughness,[5,46] the available nutrients,[6,22] and the attraction and adhesion to other microorganisms from the surrounding medium[4,20,32,33] are thought to be of secondary importance regarding the final outcome of the biofilm.[9]

Bacterial adhesion leading to infection can be divided into three distinct categories: specific adhesion to host cell surface molecules, specific adhesion to extracellular matrix and blood plasma derived molecules, and adhesion to biomaterial surfaces of medical devices. In this chapter, an overview of the current understanding of the molecular basis of bacterial adhesion as it pertains to each of the three categories of bacterial adhesion is presented, followed by modeling of bacterial adhesion based upon the general principles governing molecular adhesion. Particular emphasis is given to interactions between the initially arriving microorganisms and the conditioning film at the molecular level.

II. MICROBIAL ADHESION TO EXTRACELLULAR MATRIX MOLECULES

The interactions of arriving microorganisms with a conditioning film on a surface are usually mediated by specific binding events between adhesins on the microbe surface and receptors of the extracellular matrix (ECM). Receptor binding may subsequently activate a series of complex signal transduction cascades in the host cell, which may be either inhibitive or beneficial to bacterial invasion. In several bacterial species, including *E. coli*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, and *Salmonella enteritidis*, adhesins are presented at the tips of complex cell-surface structures which extend from the outer cell membrane called pili or fimbriae.[52] Pili are classified as P, type 1, type IV, and curli, each with distinct structural organization and assembly mechanisms. Alternatively, nonpilus adhesins may be directly presented from the bacterial cell surface as well.[52]

The molecular basis for bacterial adhesion to ECM molecules has been widely studied, and found to occur through specific binding mechanisms involving both piliated and nonpilated bacterial adhesins. These processes involve integrins which are a family of heterodimeric ($\alpha\beta$) cell-surface receptors that recognize specific ECM submolecular structures.[43] While much remains to be learned of the specific molecular mechanisms involved, bacterial cells have been found to utilize many of these same cell wall receptors to specifically adhere to ECM molecules, including fibronectin, collagens, laminin, vitronectin, thrombospodin, elastin, bone sialoprotein and GAGs like heparin, heparan sulphate, and chondroitin sulphate.[34] Bacterial adhesins, which bind with ECM, are termed microbial surface components recognizing adhesive matrix molecules (MSCRAMM).[37,43,44]

One of the most widely studied systems of bacteria-ECM interaction is *S. aureus* binding to fibrinogen. Fibrinogen is specifically recognized by several host cell integrins,

including the platelet integrin $\alpha_{IIb}\beta_3$.[43] Two of the most widely understood fibrinogen binding proteins expressed by *S. aureus* strains are called clumping factors A and B (ClfA and ClfB). Studies have indicated that ClfA binds exclusively with the γ-chain of fibrinogen, while ClfB binds to both α- and β-chains.[19]

ClfA is a 933 residue protein which includes a 520 residue region that contains its fibrinogen binding domain. This binding domain is preceded by a very interesting molecular structure consisting of 154 repeats of a serine-aspartate dipeptide sequence.[19] At a physiologic pH of 7.4, the carboxylic acid side groups of the aspartic acid residues will be deprotonated (thus carrying a single negative charge on each residue) and the interdispersed hydroxyl side groups of the serine residues will be strongly hydrophilic. The repulsion of the sequential aspartic acid residues coupled with the hydrophilicity of the serine residues should thus provide a large electrostatic driving force to extend the adhesin outward from the bacterial cell surface, much like hairs standing up on one's head when electrostatically charged.

Studies suggest that ClfA binds to two distinct sites in the γ-chain of fibrinogen by molecular structures very similar to the fibrinogen-binding integrin, $\alpha_{IIb}\beta_3$. ClfA and the platelet receptor $\alpha_{IIb}\beta_3$ have been found to recognize the same 400-411 residue section at the extreme C-terminus of the fibrinogen γ-chain (residue sequence …HHLGGA-KQAGDV), and studies have shown that alteration of only the last four residues (... AGDV) is sufficient to inhibit ClfA binding.[37,19] The γ-chain binding site of ClfA has been mapped to a region of the α_{IIb} polypeptide of the $\alpha_{IIb}\beta_3$ integrin and both contain the Ca^{2+} binding EF-hand motif found in many eukaryotic calcium ion binding proteins. The EF-hand motif consists of about 12 residues, with the proposed ClfA sequence being **D**S**D**G**N**VIY**T**FT**D**, which represents residue numbers 310-321 in the protein. The sequence letters indicated in bold are the residues specifically involved with cation binding. These residues form a coordination sphere for the divalent cation and are flanked by α-helices which provide support structure for this motif to maintain proper functional conformation.

Studies have shown that high concentrations of calcium ions inhibits ClfA-fibrinogen binding in a manner similar to that observed for integrin-ligand binding.[19,43] Thus, calcium ion concentration serves as an important regulator of *S. aureus* binding to fibrinogen. The second fibrinogen binding site of ClfA, which is also the $\alpha_M\beta_2$ integrin binding site, involves fibrinogen γ-chain residues 190-202. Similar to $\alpha_M\beta_2$, this ClfA binding site also includes a cation-binding metal ion-dependent adhesion site (MIDAS) motif.[19] The ClfB protein does not have an EF hand-like tertiary structure, however, it does possess a MIDAS-like motif, such that the binding of both ClfA and ClfB to their fibrinogen ligand sites are regulated by Ca^{2+} concentration. The specific binding sequences in ClfB and their role in ligand binding have not yet been elucidated to the extent of ClfA.[19]

S. aureus also has been found to express MSCRAMM adhesins for both fibronectin (FnbpA and FnbpB) and collagen.[13,34] Studies have shown that there are at least two binding sites on fibronectin, one occurring in its N-terminus domain, and the other in its C-terminus domain.[34] Although the molecular details of these interactions have not been as widely reported in the literature as the fibinogen binding system, the actual structure of an *S. aureus* collagen-binding adhesin has been determined and is available in the Brookhaven Protein Data Base under protein code 1AMX.

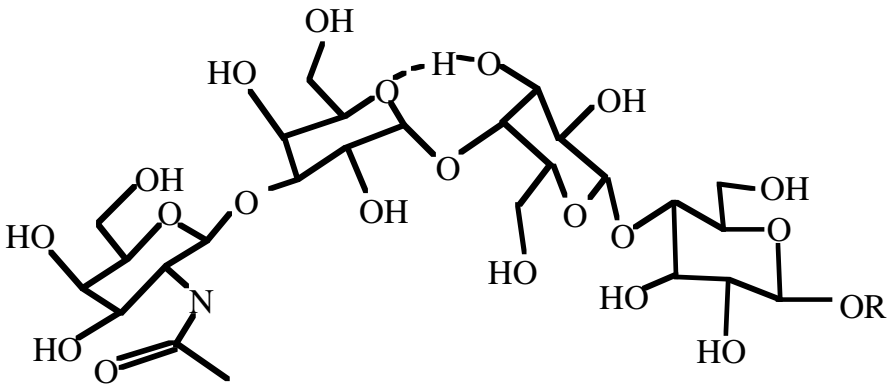


Figure 1. Molecular structure of the globotetraose-binding site of a class II PapG. Oxygen and hydroxyl groups indicated in bold are believed to form hydrogen bonds with the adhesin. Adaped from Striker et al.[53]

III. HOST CELL ADHESION MECHANISMS

Bacterial adhesion to host cells of the urinary tract has been found to occur by specific molecular interactions between adhesins located on the distal tip of pili extending from the bacterial outer membrane and receptor molecular structures present on the host cell outer surface. Although the exact molecular structures involved for many of these interaction are yet to be determined, the specific binding mechanisms involved in a few systems have been relatively well characterized.

Uropathogenic *E. coli* has been shown to adhere to erythrocytes and uroepithelial cells of the kidney and urinary tract.[28,53] Studies have revealed that this specific binding event occurs between PapG adhesins located at the tip of P pili and Galα(1-4)Gal saccharide epitopes in the globo series of glycolipids. This saccharide structure has been determined to be linked by a β-glucose residue to a ceramide group anchoring the receptor in the host cell membrane. The receptor-binding domain of PapG has been determined to lie in the N-terminus of the protein. An example of the globotetraose-binding site of a class II PapG is presented in Figure 1. Studies involving sequential functional group replacement in Galα(1-4)Gal have revealed that PapG binds to this receptor, in part, by hydrogen binding with a series of five oxygen atoms located along the edge of the disaccharide surrounding a central hydrophobic core.[28]

Uropathogenic *E. coli* expressing type-1 pili tipped with the FimH adhesin (a 30-kDa protein[51]) have been shown to specifically bind to mannosylated integral membrane glycoproteins (uroplakins) presented from the luminal surface of bladder epithelial cells using a murine cystitis model.[39] This bacteria exhibits a very interesting but not yet fully understood mechanism to facilitate close bacteria–host cell interactions. Host cell contacting pili were found to be only 0.12 μm in length in contrast to the typical 1-2 μm length for non-contacting pili. This suggests a possible pili retraction mechanism to enhance tight bacterial–host cell apposition, with subsequent possible host-cell internalization of the *E. coli*[39] Sokurenko and coworkers[51] investigated the differences in the 300 residue sequence of FimH and their respective adhesive characteristics for

fourteen *E. coli* strains. This study revealed that the FimH sequences where essentially homologous to one another except for 2 specific residue changes involving a swapping of arginine and serine residues at positions 70 and 78. The exchange of these two residues was found to result in distinct differences in bacterial adhesion. Although not well understood, this alteration is believed to influence the structure of the saccharide binding pocket in FimH for mannose binding.[51]

A different bacterial mechanism has been found to occur for the mucosal lining of the intestine leading to microvilli effacement and diarrhea. A four stage infection process has been suggested involving initial attachment of enteropathogenic *E. coli* bacteria (EPEC) to the microvilli enterocyte cell surface:

1. A nonpiliated adhesin mechanism;
2. Type III bacterial secretion of 80kDa proteins (*E. coli* secreted protein, EspE) which mediate cytoskeleton disruption and the formation of tyrosine-phosphorylated translocated intimin receptors (TIR) on the host-cell surface for intimin binding;
3. Intimin-binding mediated bacterial attachment to the intestinal mucosa; and
4. Bundle-forming pili (BFP) mediated bacterial colonization.[15,26]

Others have suggested that BFP serves as the adhesin controlling initial host cell contact as well as bacterial colonization.[2]

S. aureus can bind to endothelial cells through its fibrinogen binding clumping factors ClfA and ClfB. Adhesion studies found that the preferential attachment of *S. aureus* to umbilical vein endothelial cells is mediated by fibrinogen adsorbed from plasma. Antifibrinogen antibodies could block the binding, indicating the specificity. Cheung et al. found that fibrinogen acts as a bridging molecule, attaching to both endothelial and *S. aureus* cell-wall integrins with each of its two γ-chains.[11]

Finally, some bacteria use the integrin on endothelial cells to invade the host. Filamentous hemagglutinin (FHA), an adhesin formed as a 50 nm monomeric rigid rod of *Bordetella pertussis,* interacts with two classes of molecules on macrophages, galactose containing glycoconjugates and the $\alpha_M\beta_2$ integrin which binds to the Arg-Gly-Asp (RGD) sequence in FHA.[47] Intimin, the outer membrane protein of *E. coli* also binds specifically to $\alpha_M\beta_2$ integrins and is inhibited by RGD containing peptides.[23]

IV. ADHESION TO BIOPOLYMERS AND BIOMATERIALS

A. Natural Biopolymers

In natural heterogeneous microbial ecosystems (such as soil or an aquatic environment), adherence of a cell to a solid surface confers several competitive advantages. The ability to bind to a solid biopolymer (such as the cellulose fiber to which the filamentous bacterium, *Thermomonospora curvata*, has bound itself in Figure 2) provides the cells with a reliable constant carbon and energy source.[27] The adhesion not only brings its surface bound enzymes (cellulosomes[31]) into intimate contact with the substrate, but also affords it prime access to whatever soluble depolymerization products are released by their catalytic action. Cellulosomal organization and molecular structure of its complex components has been most extensively studied in the mesophilic anaerobe, *Clostridium cellulovorans*. Its cellulosome is composed of three major subunits: as caffolding protein, an endoglucanase, and an exoglucanase.[16] The binding of cellulolytic microbes such as *Clostridium cellulovorans* to cellulose-containing substrate surfaces is

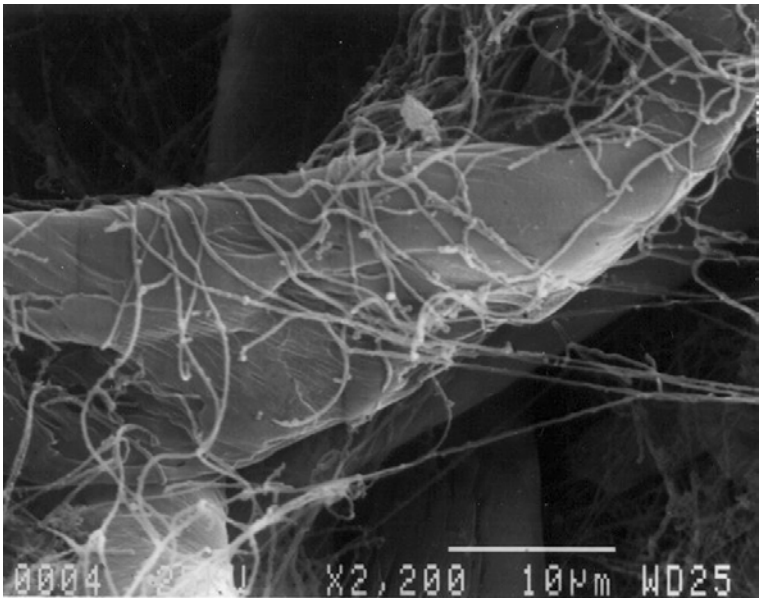


Figure 2. Filamentous bacterium *Thermonospora curvata* bound to cellulose fiber.

mediated by one or more of a heterologous group of cell surface-bound proteins containing cellulose binding domains (CBD). These CBDs have been classified into 10 families (I-X) on the basis of amino acid sequence homology.[55] The amino acid sequences of CBDs in *C. cellulovorans* and *C. josui* show high homology with those from other cellulolytic genera such as *Bacillus*. CBDs in this family contain several highly conserved amino acid sequences:[30]

1. Tryptophane-asparate-phenylalanine-asparagine-asparate-glycine-threonine
2. Isoleucine-alanine-alanine-isoleucine-proline-glutamine
3. Isoleucine-leucine-phenylalanine-valine-glycine

The cell surface-bound cellulosome in *Clostridium* species and in others is a complex of adherence and catalytic proteins. A major cellulosomal subunit (EngE) has been recently characterized.[54] EngE is anchored via a protein having triple-repeated surface layer homology (SLH) domains at the N-terminus; these domains appear to integrate into the lattice of the cell surface peptidoglycan-surface protein complex; they also bind with hydrophobic domains of the EngE. Therefore the cellulosic surface adhesion architecture in *C. cellulovorans* consists of catalytic units which have specific cellulose-binding domains, hydrophobic domains which act as linkers between the catalytic units and the SLH domains, and the SLH which integrates into the peptidoglycan-teichoic acid-lipoteichoic acid lattice which is the major structural component of the bacterial cell wall.[45] This attachment structure appears similar to that complexed with other surface-bound exoenzymes in related bacteria.[38]

B. Synthetic Biomaterials

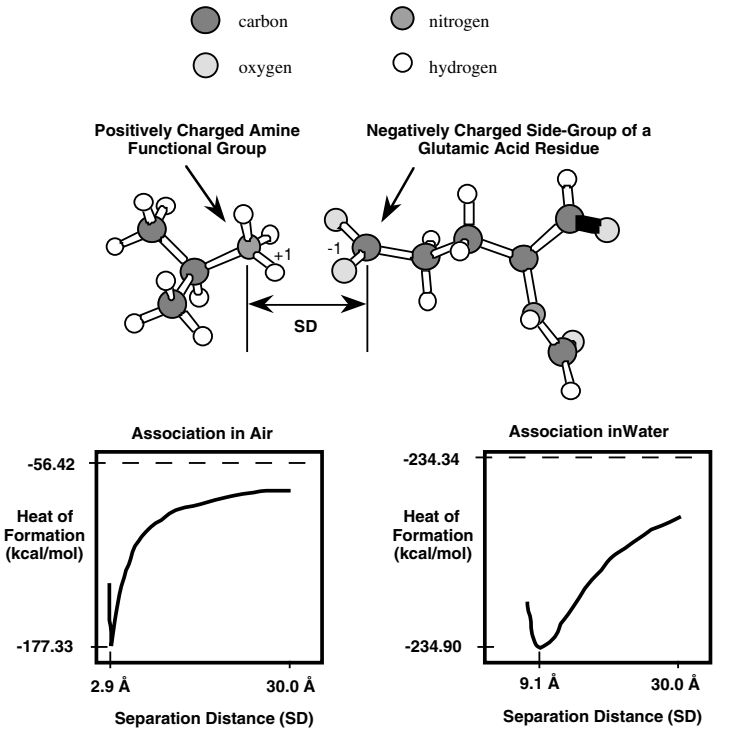
In vivo, coagulase-negative staphylococcal (CNS) (in particular *S. epidermidis*) and *S. aureus* are the leading causes of infection for body fluid-contacting medical devices,

including intravascular catheters, cerebrospinal fluid shunts, prosthetic artificial heart valves, orthopedic devices, cardiac pacemakers, chronic peritoneal dialysis catheters, and vascular grafts.[19,25,35,49,58,59]

Serum proteins, such as albumin, fibronectin, fibrinogen and laminin, have been found to readily adsorb nonspecificially to biomaterial surfaces following body fluid exposure.[41] These adsorbed proteins form the conditioning film onto which specific bacterial adhesion takes place in mechanisms very similar to those that govern bacterial adhesion to extracellular matrix components.[1,34] An additional factor unique to biomaterials, however, is that the serum protein adsorption process may abnormally denaturate the proteins structure, thus potentially exposing binding sites not normally present in either the soluble or ECM form of the proteins. Fibrinogen is one of the major plasma proteins adsorbed onto implanted biomaterials and the adhesion of *S. aureus* to adsorbed fibrinogen and fibrin is an important initiator of infection[13,19,24] The specific adhesion mechanisms between bacteria and surface bound fibrinogen/fibrin are believed to be essentially the same as those which occur for bacteria adhesion to fibrinogen as an ECM component and have been addressed in the previous section.

Fibronectin is another predominant glycoprotein component of plasma which is incorporated into the fibrin matrix during blood clot formation and which also readily adsorbs to implant surfaces following blood contact.[59] This macromolecule is a dimeric glycoprotein composed of a series of domains comprising different combinations of type I, type II, and type III modules.[24] *S. aureus* has been shown to specifically bind to both soluble and adsorbed fibronectin, but has little affinity for fibrinogen incorporated into thrombi by itself without preexposure of the bacteria to soluble fibronectin.[59] Binding has been found to involve at least two *S. aureus* MSCRAMM fibronectin-binding proteins, known as FnbpA and FnbpB, and at least two binding sites on fibronectin involving both the N- and C-termini.[24,34] FnbpA and FnbpB are identical to one another apart from their N-terminal regions which is only 45% homologous.[13,24] In contrast to this, coagulase negative staphylococcus (CNS) does not bind soluble fibronectin; however, it exhibits significant affinity for the N-terminus domain of fibronectin incorporated into fibrin thrombi or immobilized on plastic surfaces.[34,59] These differences between soluble and bound fibronectin are believed to reflect conformational changes induced by adsorption; although soluble fibronectin has a globular structure, the two arms of the dimer are believed to unfold upon binding to expose previously hidden binding sites.[59]

Another pathway for bacterial infection involves the secretion of exopolymeric substances following initial bacterial adhesion to implant surfaces. *S. epidermidis*, for example, colonizes within a self-generated viscous biofilm largely composed of polysaccharides. This amorphous extracellular matrix substance enhances bacterial adhesion to biomaterials surfaces and provides the bacterial colony with protection from antibiotics and phagocytoses.[1,35,49,53] The process of colonization and biofilm production by *S. epidermidis* involves several key molecular interactions. *S. epidermidis* strains produce two similar insoluble polysaccharides, termed polysaccharide adhesins (PS/A) and polylsaccharide intercellular adhesins (PIA). PS/A production is generally correlated with bacterial adherence to biomaterial surfaces, apparently by nonspecific adsorption mechanisms, with subsequent biofilm formation and bacterial colonizaton mediated by PIA.[38] PS/A is a high-molecular-weight variably N-succinylated (65-100%) β-1,6-linked N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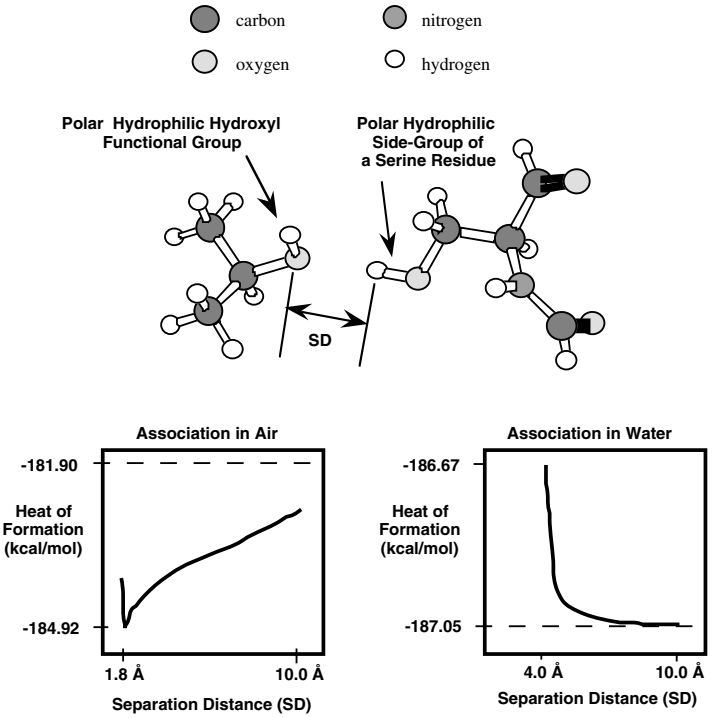
(A)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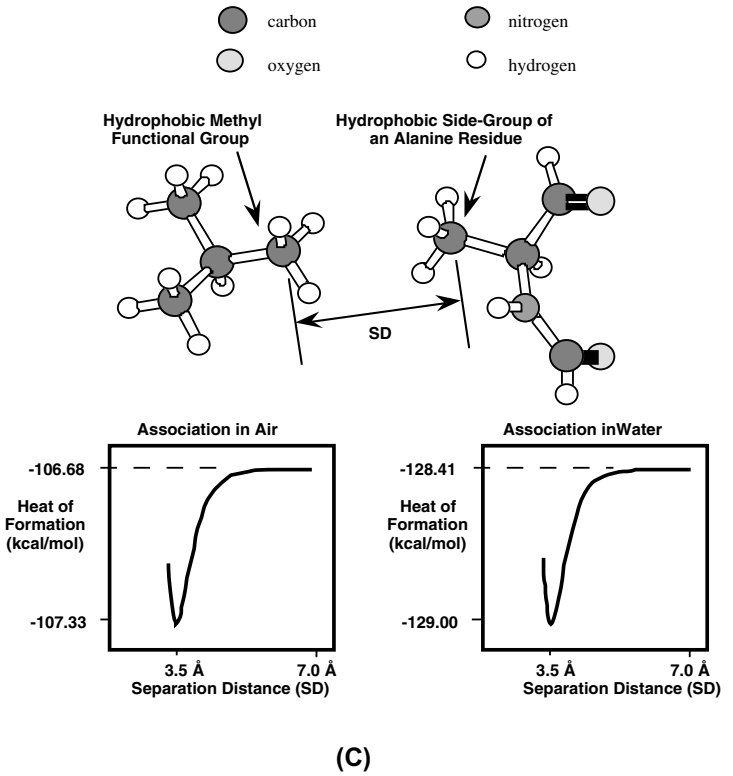

Figure 3. Molecular models illustrating intermolecular interactions as a function of chemical structure and environment. Plots present calculated heats of formation versus functional group separation distance (SD) in air and water. Dotted lines indicate energy at infinite separation. A) Ionic interaction between a positively charged amine functional group and a negatively charged carboxylic acid side-group of a glutamic acid residue. B) Polar interaction (hydrogen bonding) between a hydrophilic hydroxyl functional group and a hydrophilic hydroxyl side-group of a serine residue. C) Nonpolar interaction between a hydrophobic methyl functional group and a hydrophobic methyl side-group of an alanine residue. (Calculations conducted using semi-empirical quantum mechanical theory using MOPAC/PM3 with COSMO water simulation; CAChe Software, Oxford Molecular, Beaverton, OR.)

acetylated glucosamine.[38] NMR data suggests that PIA has an unbranched structure with β-1,6 interglycosidic linkages containing at least 130 residues. PIA is reportedly made up two primary types of polysaccharides, termed PS I (80%) and PS II (20%). PS I has 80 to 85% *N*-acetyl-D-glucosaminyl residues, with the remainder being non-*N*-acetylated positively charged D-glucosaminyl residues which are apparently randomly distributed along the chain. PS II is similar to PS I, however, it contains fewer non-*N*-acetylated side groups and a small number of phosphate and ester-linked succinyl residues, giving the overall chain a moderately anionic character.[25,35,49] The unbranched, long-chain structure of PS I and PS II, combined with their distributed anionic and cationic charges, is believed to be important for both intercellular adhesion and biofilm adherence, with van der Waals, electrostatic, and hydrogen bonding mechanisms providing molecular attraction between polysaccharide chains.[35]

Recent studies have found some evidence for cell-to-cell signaling in bacterial biofilms adsorbed to surfaces such as in the growth of *Pseudomonas aeruginosa* adsorbed onto glass.[14] Cell-to-cell signaling in bacterial biofilms may alter antibiotic resistance or adherence characteristics of other pathogens on medical implant surfaces.

V. MODELING MICROBIAL ADHESION

Molecular adhesion involves noncovalent intermolecular interactions governed by either secondary bonding between induced dipolar and dipolar functional groups (also referred to as physical bonding or van der Waals bonding) or electrostatic interactions between charged functional groups.[10] Although individual secondary bonds and electrostatic interactions within an electrolyte solvent (i.e., physiologic saline) are relatively weak compared to primary chemical bonds, their combined effects can result in very strong binding between macromolecules. The reader is referred to Chapter 1 for a review of intermolecular interactions.

These three basic types of secondary bonding and ionic interactions are graphically presented in Figure 3, which presents molecular models of 3 sets of molecules illustrating hydrophobic, neutral hydrophilic and ionic interactions. Two energy *vs* distance plots are presented for each model; one representing the calculated system heat of formation versus molecular separation in a solvent free environment (i.e., air) and the other in an aqueous solution. The net adsorption enthalpy for each set of molecules represents the difference in the heats of formation between the molecules at infinite separation compared to the system heat of formation when they are closely associated. From these energy plots, the hydrophobic functional groups are shown to only weakly attract one another over a short range of only about 6 Å in both the air and aqueous environments. In an aqueous environment, however, additional effects due to the entropy associated with the orientation of water molecules surrounding the functional groups provide an even larger thermodynamic driving force to minimize the solvent accessible surface, thus tending to strongly bind hydrophobic groups together. In contrast to this, interaction between the polar hydrophilic hydroxyl groups is shown to provide a relatively strong short-range molecular attraction in air but an effective energy barrier to binding in an aqueous environment due to preferential adsorption of water. Finally, interactions between positive and negative charges in an ionic system show very long-range strong attraction in air with close ion group association in the bound state. However, in an aqueous environment the ionic functional groups show long-range but relatively weak attraction with the low energy binding state, predicting that the functional groups are separated by a distance approximately equal to two water molecules, thus representing a solvation layer about each charged group.

These results have recently been measured using atomic force microscopy (AFM). The interactions between uncharged glycolipids with varying head-groups and the organic surfaces of self-assembled monolayers (SAMs) were measured in deionized water. It was found that in the absence of ions, the water structure surrounding the organic layers influences the adhesion. The presence of Ca^{2+} ions produced an enhanced attraction between sialic acid and the ganglioside GM1.[3]

VI. CONCLUDING REMARKS

As discussed above, the critical first step in the development of bacterial infections of tissues and medical devices involves bacterial adhesion to host cells, ECM glycoproteins,

or biomaterials surfaces. These binding mechanisms most often involve very specific submolecular interactions, many of which are still not well understood. Therapies which can prevent or disrupt these molecular mechanisms have great potential for development as alternatives to antibiotics for the prevention and treatment of infection, thus emphasizing the importance of research directed toward the elucidation of the molecular basis for bacterial adhesion.

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