Published in final edited form as:

J Biomed Mater Res A. 2012 August ; 100(8): 1990–1997. doi:10.1002/jbm.a.34036.

Adhesion of *Staphylococcus epidermidis* to biomaterials is inhibited by fibronectin and albumin

J.C. Linnes, K. Mikhova, and J.D. Bryers*

Department of Bioengineering, University of Washington, Seattle, WA, USA 98195-5061

Abstract

Decades of contradictory results have obscured the exact role of adsorbed fibronectin in the adhesion of the bacterium, *Staphylococcus epidermidis* (*S. epidermidis*), to biomaterials. Here, the ability of adsorbed fibronectin (FN) or bovine serum albumin (BSA) to modulate *S. epidermidis* adhesion to various biomaterials is reported. FN or BSA were adsorbed in increasing surface densities up to saturated monolayer coverage onto various common biomaterials, including poly(ethylene terephthalate) (PET), fluorinated ethylene propylene (FEP), poly(ether urethane) (PEU), silicone, and borosilicate glass. Despite the wide range of surface characteristics represented, adsorption isotherms varied only subtly between materials for the two proteins considered. *S. epidermidis* adhesion to the various protein-coated biomaterials was quantified in a static-fluid batch adhesion assay. While slight differences in overall adherent cell numbers were observed between the various protein-coated substrata, all materials exhibited significant dose-dependent *decreases* in *S. epidermidis* adhesion with increasing adsorption of either protein (FN, BSA) to all surfaces. Results here indicate that *S. epidermidis* adhesion to FN-coated surfaces is not a specific adhesion (i.e., receptor:ligand) mediated process, as no significant difference in adhesion was found between FN- and BSA-coated materials. Rather, results indicate that increasing surface density of either FN or BSA actually inhibited *S. epidermidis* adhesion to all biomaterials examined.

Keywords

Fibronectin; Bacterial adhesion; Infection; Adsorption; Biomaterials

1. Introduction

Nearly one in every ten American hospital patients will incur an infection related to their health care treatment, a problem that costs more than \$30 billion per year and is the fifth leading cause of death in the United States.^{1,2} Over 60–70% of these nosocomial (hospital acquired) infections are associated with some type of biomedical device. Nosocomial infections are overwhelmingly caused by *Staphylococcus epidermidis*, a bacterium that can infect patients who have indwelling medical devices (such as intravascular catheters) and those with weakened immune systems.^{1,3}

S. epidermidis are Gram-positive cocci that normally reside on the human skin and mucosal membranes. At the device surface (substratum), cells can attach through either non-specific adhesion mechanisms (i.e., electrostatic forces between the hydrophobic cell wall and the surface) or specific adhesion of cell surface receptors binding to proteins adsorbed to the implant (ligands).

*Corresponding Author: jbryers@uw.edu.

Specific adhesion acts through stereospecific bond interactions between cell surface receptors and ligand molecules adsorbed to the substratum. The force of this specific binding is due to both the bond strength of the individual receptor-ligand interaction (affinity) and the total number of interactions (avidity) occurring.⁴ *S. epidermidis* has the ability to bind specifically to many proteins found in the blood and extracellular matrix including fibrinogen⁵, collagen⁶, vitronectin^{7,8}, and allegedly FN⁹. *S. epidermidis* specific adhesion to these various ligands is mediated by bacterial cell wall receptors such as: Fbe binding to fibrinogen, SdrG to collagen, autolysins AtlE and Aae to vitronectin, and supposedly Embp binding to FN.⁵⁻⁹

Determining the adhesion mechanisms of *S. epidermidis* is an important first step in preventing *S. epidermidis* colonization of biomedical devices. However, *S. epidermidis* adhesion to adsorbed blood plasma proteins, such as FN, is highly variable and even the most basic mechanisms of this binding are as yet unknown. Analysis of past studies of *S. epidermidis* specific adhesion to an immobilized ligand can be obfuscated by differences in substrata materials, substrata preparations, immobilized ligand concentration and orientation, and system hydrodynamics (stagnant fluid, laminar or turbulent flow). For example, considering only *S. epidermidis* specific adhesion to FN-coated substrata, reports in the literature state that *S. epidermidis* does specifically bind to FN¹⁰⁻¹³, FN inhibits *S. epidermidis* adhesion¹⁴⁻¹⁶, or FN has no effect on *S. epidermidis* adhesion¹⁷⁻¹⁹. Galliani *et al.* found a significant impairment in the adhesion of twenty various *S. epidermidis* strains when polystyrene plates were coated with FN or albumin.¹⁵ In contrast, experiments by Hussain *et al.* indicated that five *S. epidermidis* strains not only bound to FN-coated polymethylmethacrylate and polystyrene plates but that this binding was increased in the presence of teichoic acid.²⁰ Finally, Russel *et al.* observed significant increases in adhesion of three *S. epidermidis* strains to FN-coated heparin-bonded polyurethane but not to FN-coated polyvinyl chloride catheter segments.¹⁸ The present study examines the role of chemical and physical properties of biomaterials on *S. epidermidis* adhesion to FN with the goal of resolving these numerous conflicting accounts.

2. Materials and Methods

2.1. Biomaterial sample preparations

Biomaterials employed in this study were poly(ethylene terephthalate) (PET), fluorinated ethylene propylene (FEP), poly(ether urethane) (PEU), silicone, and borosilicate glass controls. Eight (8) mm diameter circular glass coverslips (ProSciTech, Australia) were cleaned by immersion in piranha solution (70% H₂SO₄, 30% H₂O₂) for 10 minutes to remove any organic contaminants and rinsed three times with de-ionized water (DI water) before use.

PET (Lux catalog # 5414) was purchased from Nalge Nunc International (Rochester, NY). FEP (CF(CF₃)-CF₂(CF₂-CF₂)_n)_m) was a gift from Du Pont de Nemours & Co. (Circleville, OH). Medical grade silicone was manufactured by Specialty Silicone Fabricators (Paso Robles, CA) from MED-4770 silicone elastomer (Nusil, Carpinteria, CA). PEU films were prepared by mixing biomedical-grade Biospan™ (DSM PTG, Berkeley, CA) with N,N-dimethylacetamide (DMAc) in a ratio of 6:19 w/v, Biospan™:DMAc, overnight. The solution was then cast into a 8.5 cm x 4 cm poly(tetrafluoroethylene) (PTFE) mold and heated to 60°C for 3 days alongside a dessicant to absorb moisture. A vacuum was then applied for 24 hours at 60°C before the polymerized film was removed from the PTFE mold. FEP, PET, PEU, and silicone samples were manually punched into 8-mm diameter discs with an arch punch. Silicone surfaces were cleaned by sonication in Liquinox detergent alone and rinsed in DI water. The remaining polymer surfaces were cleaned by sonicating sequentially in dimethylene chloride, acetone, and methanol.

2.2. Contact angle measurements

Static contact angle measurements were performed on an FTÅ 200 goniometer system (FTÅ, Portsmouth, VA) with 5 μ L DI water pipetted onto the sample from the vertical position and imaged with a CCD video camera. Contact angles were then analyzed via FTÅ32 software provided by the manufacturer using an edge finder algorithm and non-spherical curve fitting mode. Measurements were done in quadruplicate and two or more droplets were measured on each surface.

2.3. Surface roughness measurements

Tapping mode Atomic Force Microscopy (AFM) was performed with a Veeco IconTM AFM (Santa Barbara, CA) using a TESPAC cantilever tip with a 5 N/m spring constant and 100KHz resonant frequency in tapping mode. Image processing was performed using Nanoscope software 8.10. Prior to roughness calculations, all images were 0th order flattened. The average roughness of each image was calculated as well as the average roughness of a representative smooth area was calculated for each sample.

2.4. Protein preparations and protein radiolabeling

Commercial human serum FN was obtained from Sigma (St. Louis, MO). High purity BSA was purchased from Sigma (St. Louis, MO). FN was reconstituted and frozen in 100 μ L aliquots of 1mg/mL for up to 6 months. BSA was dissolved in PBS at 1mg/mL before each new set of experiments. Protein solutions were stored for up to 1 month at 4°C and each new batch was sampled to ensure protein purity greater than 96% and absence of fragments or aggregates by SDS-PAGE gel electrophoresis.

FN and BSA were radiolabeled with Iodine-125 (¹²⁵I) using the Iodobead method as outlined elsewhere.²¹ Briefly, FN lyophilized from Tris-buffered saline was reconstituted to 1 mg/mL using DI water. BSA was dissolved at 1 mg/mL in phosphate buffered saline, pH 7.2 (PBS, Pierce, Rockford, IL). Two IodobeadsTM were washed with 500 μ L of PBS and dried on a Whatman Nr. 1 filter paper (GE Healthcare, Piscataway, NJ). Next, the beads were placed in a 1.5 mL siliconized Eppendorf tube containing carrier-free sodium-¹²⁵I (Perkin Elmer, Waltham, MA), diluted with reaction buffer and allowed to react for 5 min at room temperature. Then, 100 μ g of unlabeled protein solution was added to the bead mixture, and allowed to react for 15 min. Beads were separated from the reaction solution and washed with PBS to recover any non-adsorbed protein. Finally, the iodinated protein solution was applied to an Econo-PacTM 10DG chromatography column (BioRad, Hercules, CA) to separate ¹²⁵I-protein from the free iodine based on size. Following the elution of initial radioactive fractions of low molecular weight free ¹²⁵I from the column, a second set of higher MW radioactive fractions consisting of protein bound ¹²⁵I and were collected and eluted again on a new column. Resulting ¹²⁵I-protein fractions were pooled and stored at -20°C until needed for adsorption studies. The specific activity of ¹²⁵I-FN was measured to be 0.70 μ Ci/ μ g.

2.5. FN and BSA adsorption studies

Unlabeled protein was dissolved in degassed PBS at a concentration of 200 μ g/mL. The specific activity of the ¹²⁵I-protein was determined via scintillation counting (0.70 μ Ci/ μ g) and a known concentration was added to the unlabeled protein solution, such that the stock FN solution was 10% labeled. Serial dilutions of the spiked protein mixture were made at 200, 20, 2, and 0.2 μ g/mL. Four sets of each biomaterial sample substrata (total substratum surface area was 1 cm² per sample) were submerged in 100 μ L PBS in scintillation tubes that had been blocked with protein-free blocking buffer (Pierce, Rockford, IL). Then, 100 μ L of protein solution was added to the scintillation tubes for a total volume of 200 μ L.

Biomaterial samples were then incubated in the spiked protein solution at 4°C. After 16 hours, the sample substrata were rinsed three times with degassed PBS before radioactivity counts were read on a Cobra II 5005 Series Auto-GammaTM Counter (Packard Instrument Company, Meriden, CT). The total amount of protein adsorbed on a biomaterial sample was calculated from the protein's specific activity and the radioactive counts of each surface. Surfaces were tested for any preferential adsorption of ¹²⁵I-protein and for free ¹²⁵I in solution by varying the proportion of labeled to unlabeled protein while maintaining a constant total protein concentration.

2.6. Bacterial strains and culture conditions

S. epidermidis strain 1457, a biofilm forming bacterial strain isolated from a patient with catheter-related sepsis, was kindly provided by Dr. Michael Otto (NIH, Bethesda MD).²² *S. epidermidis* 12228, *S. epidermidis* RP62a, and *S. aureus* Cowan III (strain 12600) were purchased from ATTC. *S. epidermidis* strains SCH1.2 and SCH1.3 were cultured from intravascular catheter infections and generously provided by Dr. Xuan Qin at Seattle Children's Hospital. Prior to experiments, bacteria were grown overnight in Trypticase Soy Broth (TSB) at 37°C, 180 rpm. Cultures were then diluted 1:50 into fresh TSB and cultured for an additional 4 hours until mid-exponential phase at which the optical density at 600 nm (OD₆₀₀) was approximately 2.0. Cells were rinsed twice and re-suspended in PBS to an OD₆₀₀ of 0.5 (~5 X 10⁸ cells/mL).

2.7. Static fluid bacterial adhesion assays

One hundred (100) µL of unlabeled human serum FN or BSA at concentrations ranging from 0–100 µg/mL in PBS, were added to custom machined microwell plates in which the various biomaterial samples served as the bottom surface. (Figure 1) Protein solutions remained in contact with the materials in the wells overnight at 4°C. Wells were then rinsed three times with PBS. No subsequent blocking of substratum surfaces was employed in the FN-only or BSA-only trials, in order to illustrate the effect of the single protein on bacterial adhesion. An additional set of samples was prepared in which FN-coated substrata were subsequently blocked with 1% w/v solution of BSA in PBS. Then, 100 µL of staphylococcal cells in PBS were placed in each well and incubated for 2 hours at 37°C. (Preliminary assays had been performed to ensure that no appreciable cell death occurred due to incubation in PBS.) Each well was then rinsed twice in PBS, the apparatus was carefully disassembled, and biomaterials samples were placed in conventional 24-well plates and covered with 1mL of sterile-filtered PBS. Adherent cells were then removed from the samples by sonicating three times for 10 seconds each at 30% power with a micro ultrasonic cell disruptor with an ASI tip (Kontes, Vineland, NJ). Resultant cell suspensions were stained with Syto9 nucleic acid stain (Invitrogen, Carlsbad, CA) and filtered onto a 0.10 µm pore size black Cyclopore polycarbonate membrane (GE Healthcare, Piscataway, NJ). Membranes were imaged by fluorescence microscopy at five random locations and cell numbers quantified using Image J software (NIH, Bethesda, MD). Adhesion was calculated from the average of three separate experiments, each with three replicates for a total of 9 samples of each protein concentration.

2.8. Statistical analysis

Experimental results were analyzed using Microsoft Excel software (Redmond, WA). Paired Student's T-tests were used to assess the statistical significance of adhesion studies,

3. Results

3.1 Materials characterization

Biomaterial substrates were chosen based on their current use in catheter and blood contacting devices and for their variable surface characteristics. As expected, the surfaces exhibited a wide range of contact angles, from the very hydrophobic silicone samples to the extremely hydrophilic glass samples (Table 1). These contact angles are consistent with previous literature.^{23–25} Additionally, surface roughness of random 10 μm x 10 μm areas of the biomaterial samples was highly variable since samples contained irregularities resulting in large standard deviations (Table 1). Silicone had the largest surface roughness and variability while PET surfaces were the smoothest of the polymer substrata. When smooth regions of interest on the polymers were selected from each AFM sample image (available upon request), the roughness estimates decreased considerably although the trends remained the same. Silicone samples did not contain a smooth region and therefore no region of interest was examined.

3.3 Protein adsorption to surfaces

A similar trend in protein adsorption was observed on all surfaces. As seen in Figure 2, adsorbed protein surface concentrations increased in a dose dependent manner with adsorption isotherms primarily exhibiting Langmuir Blodgett behavior, wherein increases in adsorbed protein asymptotically level off at a monolayer concentration of protein on all surfaces. According to Rocco *et al.*, an extended conformation of FN has a hydrodynamic radius of 14.5 nm yielding a closely packed monolayer of FN at a density of 110 ng/cm².²⁶ For BSA, this hydrodynamic radius has been calculated at 7 nm resulting in a monolayer surface density of 71 ng/cm².²⁷ At 100 $\mu\text{g/mL}$ bulk solution concentrations, the quantity of FN adsorbed was similar on all surfaces, ranging between 90 and 110 ng/cm². BSA adsorption was more variable; at a 100 $\mu\text{g/mL}$ maximum BSA in the coating solution, the PEU surface exhibited ~ 1000 ng/cm², while other polymer surfaces adsorbed amounts clustered between 50–100 ng/cm². From these results, it can be inferred that both FN and BSA adopt a closely packed monolayer on the surfaces.

3.4 *S. epidermidis* adhesion to FN and BSA-coated surfaces

S. epidermidis 1457 bacterial adhesion after 2 hours to uncoated biomaterial surfaces showed high variability between substrates, ranging from $\sim 2.5 \times 10^7$ cells/cm² on FEP to 4.5×10^7 cells/cm² on PET. As seen in Figure 3, incubation of surfaces with proteins prior to *S. epidermidis* adhesion resulted in a dose-dependent decrease in adhesion on all surfaces for both FN (Figure 4A) and BSA (4B). On all surfaces except for glass, a monolayer of FN, achieved with a 100 $\mu\text{g/mL}$ bulk solution coating concentration, resulted in minimal cell adhesion between 1.0×10^7 to 1.5×10^7 cells/cm². On glass, this adhesion was 1.9×10^7 cells/cm² for BSA pre-incubation and 2.5×10^7 cells/cm² for FN pre-incubation. The greatest decrease in cell adhesion compared to bare substratum alone was seen on PET surfaces for both FN and BSA, where cell numbers dropped from a maximum of 5.0×10^7 cells/cm² at 0.1 $\mu\text{g/mL}$ protein pre-incubation to 1.0×10^7 cells/cm² at 100 $\mu\text{g/mL}$ protein pre-incubation.

Comparing *S. epidermidis* adhesion onto similar substrates, pre-incubated with FN and BSA resulted in no statistical differences in cell adhesion at any concentration, except on FEP surfaces. On FEP surfaces, BSA coatings at 1 and 10 $\mu\text{g/mL}$ solution concentrations yielded a two-fold increase in *S. epidermidis* adhesion compared to the corresponding cell adhesion to FN-coated surfaces at the same coating concentrations. However, cell adhesion to BSA-coated FEP was not statistically different than cell adhesion to uncoated FEP. Conversely, a four-fold decrease in cell adhesion to 100 $\mu\text{g/mL}$ protein coatings with either FN or BSA was seen compared to adhesion on uncoated PET surfaces.

3.5 Effect of BSA blocking on *S. epidermidis* adhesion to FN-coated surfaces

S. epidermidis 1457 adhesion to FN-coated surfaces was also carried out using the currently accepted protocol, where a ligand of interest (here, FN) is adsorbed to substrata at various concentrations followed by blocking of any open (*i.e.*, not occupied by FN) substratum surface with an “inert protein” (here, BSA) in order to eliminate non-specific adhesion of the cells. Figure 4 illustrates adhesion of *S. epidermidis* 1457 to BSA-blocked FN-coated glass substrata as an example. Using BSA as a blocking agent totally obscures any trends in cell adhesion (or its inhibition) by the FN. As FN surface concentrations drop, BSA surface concentrations increase, resulting in the erroneous conclusion that FN may slightly increase cell adhesion. The number of *S. epidermidis* cells attached to BSA blocked glass did not increase significantly at any concentration of immobilized FN. Instead, *S. epidermidis* cell adhesion remained at the background level of adhesion to BSA alone, $\sim 3.3 \times 10^6$ cells/cm².

This currently accepted adhesion protocol was repeated with *S. epidermidis* strains SCH1.2 and SCH1.3 clinical strains and laboratory test strains RP62a and 12228. None of these strains showed increased adhesion to FN over background BSA binding (data not shown). In contrast to the various *S. epidermidis* strains tested, *Staphylococcus aureus* Cowan III strain is known to be a strong FN binding strain containing genes for the fibronectin binding proteins FnbpA and FnbpB.²⁸ When the specific adhesion studies were repeated with this strain, *S. aureus* bound to FN in a dose dependent manner, indicating that the FN itself was functional (data not shown).

Discussion

In an attempt to uncover the influence of various surface properties on bacterial adhesion, we measured both contact angle and surface roughness of the various biomaterial samples. However, neither of these properties correlated well with bacterial adhesion. Only the presence or absence of protein appeared to influence bacterial adhesion. While substratum hydrophobicity has been shown to affect protein conformation and orientation, which can in turn impact bacterial specific adhesion, no specific adhesion to either BSA or FN was observed in the present study.²⁹

A custom designed adhesion apparatus allowed for carefully controlled experimental adhesion parameters and comparison of many different biomaterials. Under these static adhesion conditions, bacterial adhesion to FN-coated surfaces appears to be a non-specific phenomenon as adhesion levels were not significantly different from those seen on BSA only-coated substrata. At monolayer protein densities, a minimum background of *S. epidermidis* adhesion ($\sim 1.5 \times 10^7$ cells/cm²) was observed for surfaces coated with either protein.

Numbers of bacteria adhered to uncoated samples did correlate with previous literature.³⁰ Unexpectedly, however, no preferential increase in *S. epidermidis* adhesion to FN-coated surfaces was seen when compared to uncoated bare substrata. In fact, cell adhesion decreased with increasing adsorbed amounts of FN (or BSA). *S. epidermidis* 1457 did not appear to distinguish between BSA- and FN-coated surfaces, which would suggest that *S. epidermidis* adhesion to adsorbed FN occurs via the same non-specific adhesion mechanism as to BSA. Additionally, blocking the FN-coated substrata with BSA resulted in no preferential binding of *S. epidermidis* to FN-coated surfaces over BSA blocked surfaces. To confirm that this result was universal among blocking agents, many additional blocking buffers were examined in place of BSA. These included human serum albumin, fetal bovine serum (Invitrogen, Carlsbad, CA), protein-free blocking buffer (Pierce, Rockford, IL), and non-fat dry milk. In each case, bacterial adhesion to FN did not vary significantly from the baseline of the blocking agents alone. To further examine the potential effects of blocking

residual non-specific binding, we suspended cells in either BSA or FN during the FN-specific adhesion experiments to saturate any potential specific binding sites. However, neither suspension in FN nor in BSA appreciably altered the adherent cell numbers counted during the specific adhesion experiments.

Finally, both well characterized laboratory strains, SERP62A and SE12228, and clinical strains isolated from catheter infections (SCH1.2 and SCH1.3) were further probed for potential specific adhesion to glass. None of these strains exhibited an increase in adhesion to FN-coatings over BSA-blocking alone. Thus, indicating that the lack of specific adhesion is not restricted to strain 1457 but is widely experienced among diverse *S. epidermidis* isolates.

The absence of specific binding evidence is in contrast with previous results from our group by Jarvis *et al.*, wherein *S. epidermidis* adhesion to oriented FN on non-fouling PEG backgrounds illustrated a specific receptor:ligand adhesion.¹² However, this discrepancy could be related to the random orientation assumed by FN and BSA molecules when passively adsorbed to surfaces, as described in this report, versus the controlled FN orientation bound to antibodies in the previous study. Also, FN surface immobilized densities in the Jarvis *et al.* previous study were comparable only to the lower FN adsorbed concentrations reported here. In those prior controlled experiments, increases in *S. epidermidis* adhesion over the background non-fouling surface, while statistically different, were slight (< 6x). In the present study, FN was randomly adsorbed onto various biomaterial surfaces currently used in medical devices as opposed to decreasing the background binding with an experimental non-fouling surface. Additionally, the random adsorption utilized, allowed for numerous orientations and conformations of the FN as would occur in the body.

Atomic force microscopy studies performed by other groups have also shown high strength bonds forming between *S. epidermidis* and FN.^{10,31} These researchers used a clinical staphylococcal strain selected specifically for its ability to bind FN. While this strain was characterized to ensure that it was not *Staphylococcus aureus*, the authors state that it did contain genes for the *S. aureus* FN binding proteins, FnbpA and FnbpB.¹⁰ However, the genes encoding FnbpA or B are not contained in the *S. epidermidis* 1457 strain utilized in this study, nor are they contained in *S. epidermidis* RP62A or 12228, the two most common laboratory strains of *S. epidermidis*. Furthermore, Xu *et al.*³² have found that FN coated onto AFM probes interacts with *Staphylococcus aureus* FN binding proteins deep within the cell wall and that despite their high interaction strength, these proteins are most likely not involved in initial bacterial adhesion to substrates.

Here, we have shown a dose-dependent decrease in adhesion to adsorbed FN on various substrata and no increase in adhesion compared with BSA-coated substrata. These results suggest not only passivation of substrata by FN and BSA proteins but also a complete lack of specific adhesion interactions. This is in contrast to findings by a number of other researchers over the years. A wide variety of growth conditions, substrata, and *S. epidermidis* strains have been used in past reports and these differences could potentially lead to discrepancies in specific adhesion. The research contained herein has examined five separate substrata (silicone, FEP, PEU, PET, and glass), five diverse *S. epidermidis* strains (1457, RP62A, 12228, SCH1.2 and SCH1.3), and five alternative blocking agents (BSA, human serum albumin, fetal bovine serum, protein free blocking buffer and non-fat dry milk), to ensure that results are, in fact, representative of *S. epidermidis* interactions with both substrata and FN.

Neither biomaterial surface roughness nor contact angle measurements of hydrophobicity appear to correlate directly with *S. epidermidis* adhesion to uncoated biomaterial surfaces.

While PEU and PET exhibited very similar contact angles, *S. epidermidis* adhesion to the bare surfaces differed significantly. Unlike findings by Cerca *et al.*³³, hydrophobic surfaces did not exhibit increased adhesion compared to hydrophilic surfaces. Bacterial adhesion to silicon, the most irregular surface ($R_A = 193\text{nm}$), exhibited similar *S. epidermidis* adhesion compared to PET, the smoothest surface ($R_A = 1.4\text{nm}$). While binding of *S. epidermidis* and other bacteria may be influenced by such properties, it would appear that the major factor in *S. epidermidis* adhesion to surfaces *in vitro* is the presence or absence of pacifying proteins, such as FN and BSA.

Our findings have significant ramifications for anyone carrying out specific bacterial adhesion assays (or using protein micro-arrays to detect selected pathogenic bacterial contaminants). Typically, such assays first determine the adsorption isotherm of the adhesion ligand of interest (here FN). Then, bacterial adhesion via receptors specific to the immobilized ligand are carried out at increasing ligand surface densities, but not before any bare substratum surface is blocked by an inert blocking agent, typically BSA. As seen in comparing results from Figure 4 to Figure 5, use of BSA totally obscured the inhibitory role of immobilized FN. In order to ensure physiological relevance, bacterial adhesion to surfaces designed to inhibit infection should be examined in conditions that include pre-coating with blood proteins, as this adhesion is significantly altered compared to bare surfaces alone.

While differences in overall adherent cell numbers were seen between substrata, significant dose dependent decreases in *S. epidermidis* adhesion were found with increasing adsorption of either protein to all surfaces. Results reported here contradict recent literature claiming that *S. epidermidis* bacteria express an Embp cell-associated protein that acts as a receptor to specifically bind FN.³⁴ Our group is currently performing an in-depth molecular and genetic based analysis of the role of the Embp protein that further substantiates the results reported here.

Conclusions

While surface chemical and physical characteristics may play a role in bacterial adhesion to biomaterials, we found that passivation of these surfaces occurred with the adsorption of either FN or BSA protein. Numerous strains of *S. epidermidis* appear to adhere to biomaterials through non-specific mechanisms and are unable to differentiate between FN- and BSA-coated surfaces. The addition of FN to biomaterial surfaces resulted in inhibition of cell adhesion, producing the same trend as compared to cell adhesion to BSA-coated surfaces. Our results indicate that *S. epidermidis* adhesion to FN may actually be a non-specific phenomenon only.

Acknowledgments

This work was funded by NIH/NIAID grant (1R03AI079461-01) and NSF/BES (0102139). Additional graduate support was provided by the NSF Graduate Research Fellowship Program. AFM studies were performed and funded by NESAC/BIO (grant EB-002027). The authors wish to thank Dr. James Hull and Dr. Lizzy Mayorga-Szott for their assistance in acquiring the AFM and protein adsorption data.

References

1. Klevens, RM.; Edwards, JR.; Richards, CL.; Horan, TC.; Gaynes, RC.; Pollok, DA.; Cardo, DM. Center for Disease Control and Prevention Public Health Reports. 2007. Estimating Healthcare-Associated Infection and Deaths in U.S. Hospitals, 2002; p. 122
2. Scott, RDI. Center for Disease Control and Prevention Public Health Reports. 2009. The Direct Medical Costs of Healthcare-Associated Infections in U.S. Hospitals and the Benefits of Prevention.

3. Vuong C, Otto M. Staphylococcus epidermidis infections. *Microbes Infect.* 2002; 4(4):481–9. [PubMed: 11932199]
4. Mascari L, Ross JM. Hydrodynamic shear and collagen receptor density determine the adhesion capacity of *S. aureus* to collagen. *Ann Biomed Eng.* 2001; 29(11):956–62. [PubMed: 11791678]
5. Ponnuraj K, Bowden MG, Davis S, Gurusiddappa S, Moore D, Choe D, Xu Y, Hook M, Narayana SV. A "dock, lock, and latch" structural model for a staphylococcal adhesin binding to fibrinogen. *Cell.* 2003; 115(2):217–28. [PubMed: 14567919]
6. Bowden MG, Visai L, Longshaw CM, Holland KT, Speziale P, Hook M. Is the GehD lipase from *Staphylococcus epidermidis* a collagen binding adhesin? *J Biol Chem.* 2002; 277(45):43017–23. [PubMed: 12218064]
7. Heilmann C, Thumm G, Chhatwal GS, Hartleib J, Uekotter A, Peters G. Identification and characterization of a novel autolysin (Aae) with adhesive properties from *Staphylococcus epidermidis*. *Microbiology.* 2003; 149(Pt 10):2769–78. [PubMed: 14523110]
8. Heilmann C, Hussain M, Peters G, Gotz F. Evidence for autolysin-mediated primary attachment of *Staphylococcus epidermidis* to a polystyrene surface. *Mol Microbiol.* 1997; 24(5):1013–24. [PubMed: 9220008]
9. Williams RJ, Henderson B, Sharp LJ, Nair SP. Identification of a fibronectin-binding protein from *Staphylococcus epidermidis*. *Infect Immun.* 2002; 70(12):6805–10. [PubMed: 12438356]
10. Arciola CR, Bustanji Y, Conti M, Campoccia D, Baldassarri L, Samori B, Montanaro L. *Staphylococcus epidermidis*-fibronectin binding and its inhibition by heparin. *Biomaterials.* 2003; 24(18):3013–9. [PubMed: 12895573]
11. Francois P, Letourneur D, Lew DP, Jozefonwicz J, Vaudaux P. Inhibition by heparin and derivatized dextrans of *Staphylococcus epidermidis* adhesion to in vitro fibronectin-coated or explanted polymer surfaces. *J Biomater Sci Polym Ed.* 1999; 10(12):1207–21. [PubMed: 10673017]
12. Jarvis RA, Bryers JD. Effects of controlled fibronectin surface orientation on subsequent *Staphylococcus epidermidis* adhesion. *J Biomed Mater Res A.* 2005; 75(1):41–55. [PubMed: 16059880]
13. Liu Y, Strauss J, Camesano TA. Adhesion forces between *Staphylococcus epidermidis* and surfaces bearing self-assembled monolayers in the presence of model proteins. *Biomaterials.* 2008; 29(33):4374–82. [PubMed: 18760835]
14. Dunne WM, Burd EM. Fibronectin and proteolytic fragments of fibronectin interfere with the adhesion of *Staphylococcus epidermidis* to plastic. *J Appl Bacteriol.* 1993; 74(4):411–6. [PubMed: 8486548]
15. Galliani S, Viot M, Cremieux A, Van der Auwera P. Early adhesion of bacteremic strains of *Staphylococcus epidermidis* to polystyrene: influence of hydrophobicity, slime production, plasma, albumin, fibrinogen, and fibronectin. *J Lab Clin Med.* 1994; 123(5):685–92. [PubMed: 8195675]
16. Zdanowski Z, Ribbe E, Schalen C. Influence of some plasma proteins on in vitro bacterial adherence to PTFE and Dacron vascular prostheses. *APMIS.* 1993; 101(12):926–32. [PubMed: 8110449]
17. Holmes SD, May K, Johansson V, Markey F, Critchley IA. Studies on the interaction of staphylococcus aureus and staphylococcus epidermidis with fibronectin using surface plasmon resonance (BIAcore). *Journal of Microbiological Methods.* 1997; 28:77–84.
18. Russell PB, Kline J, Yoder MC, Polin RA. Staphylococcal adherence to polyvinyl chloride and heparin-bonded polyurethane catheters is species dependent and enhanced by fibronectin. *J Clin Microbiol.* 1987; 25(6):1083–7. [PubMed: 3597751]
19. Schroeder AC, Schmidbauer JM, Sobke A, Seitz B, Ruprecht KW, Herrmann M. Influence of fibronectin on the adherence of *Staphylococcus epidermidis* to coated and uncoated intraocular lenses. *J Cataract Refract Surg.* 2008; 34(3):497–504. [PubMed: 18299078]
20. Hussain M, Heilmann C, Peters G, Herrmann M. Teichoic acid enhances adhesion of *Staphylococcus epidermidis* to immobilized fibronectin. *Microb Pathog.* 2001; 31(6):261–70. [PubMed: 11747374]

21. Valdes TI, Ciridon W, Ratner BD, Bryers JD. Surface modification of a perfluorinated ionomer using a glow discharge deposition method to control protein adsorption. *Biomaterials*. 2008; 29(10):1356–66. [PubMed: 18155292]
22. Mack D, Siemssen N, Laufs R. Parallel induction by glucose of adherence and a polysaccharide antigen specific for plastic-adherent *Staphylococcus epidermidis*: evidence for functional relation to intercellular adhesion. *Infect Immun*. 1992; 60(5):2048–57. [PubMed: 1314224]
23. Dann J. Forces involved in the adhesive process : I. Critical surface tensions of polymeric solids as determined with polar liquids. *Journal of Colloid Interface Science*. 1970; 32(2):302–320.
24. Westerdaal CAL, Hall JR, Schramm EC, Levi DW. Gas Plasma Effects on Polymer Surfaces. *Journal of Colloid Interface Science*. 1974; 47(3):610–620.
25. Sousa C, Teixeira P, Oliveira R. Influence of Surface Properties on the Adhesion of *Staphylococcus epidermidis* to Acrylic and Silicone. *Int J Biomater*. 2009; 2009:718017. [PubMed: 20126579]
26. Rocco M, Carson M, Hantgan R, McDonagh J, Hermans J. Dependence of the shape of the plasma fibronectin molecule on solvent composition. Ionic strength and glycerol content. *J Biol Chem*. 1983; 258(23):14545–9. [PubMed: 6643501]
27. Yohannesa G, Wiedmera SK, Elomaab M, Jussilaa M, Aseyevb V, Riekkola M-L. Thermal aggregation of bovine serum albumin studied by asymmetrical flow field-flow fractionation. *Analytica Chimica Acta*. 2010; 675(2):191–198. [PubMed: 20800732]
28. Mongodin E, Bajolet O, Cutrona J, Bonnet N, Dupuit F, Puchelle E, de Bentzmann S. Fibronectin-binding proteins of *Staphylococcus aureus* are involved in adherence to human airway epithelium. *Infect Immun*. 2002; 70(2):620–30. [PubMed: 11796591]
29. Koike N. Adherence ability of coagulase-negative staphylococci to catheter materials. *Kansenshogaku Zasshi*. 1990; 64(12):1542–9. [PubMed: 2074372]
30. MacKintosh EE, Patel JD, Marchant RE, Anderson JM. Effects of biomaterial surface chemistry on the adhesion and biofilm formation of *Staphylococcus epidermidis* in vitro. *J Biomed Mater Res A*. 2006; 78(4):836–42. [PubMed: 16817192]
31. Bustanji Y, Arciola CR, Conti M, Mandello E, Montanaro L, Samori B. Dynamics of the interaction between a fibronectin molecule and a living bacterium under mechanical force. *Proc Natl Acad Sci U S A*. 2003; 100(23):13292–7. [PubMed: 14573699]
32. Xu CP, Boks NP, de Vries J, Kaper HJ, Norde W, Busscher HJ, van der Mei HC. *Staphylococcus aureus*-fibronectin interactions with and without fibronectin-binding proteins and their role in adhesion and desorption. *Appl Environ Microbiol*. 2008; 74(24):7522–8. [PubMed: 18952882]
33. Cerca N, Pier GB, Vilanova M, Oliveira R, Azeredo J. Quantitative analysis of adhesion and biofilm formation on hydrophilic and hydrophobic surfaces of clinical isolates of *Staphylococcus epidermidis*. *Res Microbiol*. 2005; 156(4):506–14. [PubMed: 15862449]
34. Christner M, Franke GC, Schommer NN, Wendt U, Wegert K, Pehle P, Kroll G, Schulze C, Buck F, Mack D, et al. The giant extracellular matrix-binding protein of *Staphylococcus epidermidis* mediates biofilm accumulation and attachment to fibronectin. *Mol Microbiol*. 2009; 75(1):187–207. [PubMed: 19943904]

Figure 1.
Custom Microwell Plate Apparatus

Figure 2. Adsorption of (A) BSA adsorption and (B) FN adsorption to various biomaterials as function of protein liquid concentrations. Error bars shown are standard deviation of the mean (n = 4)

Figure 3. Comparison of *S. epidermidis* strain 1457 adhesion to (A) non-blocked FN-coated and (B) BSA-coated biomaterial substrates. Proteins were coated onto substrates at 100, 10, 1, and 0.1 $\mu\text{g/mL}$ solutions in PBS and included a no protein control (0). *indicates significance compared to uncoated controls ($p < 0.05$). Error bars shown are standard deviations of the mean ($n = 3$)

Figure 4. *S.epidermidis* 1457 adhesion to FN-coated glass surfaces blocked with BSA. FN was coated onto glass at 100, 10, 1, and 0.1 μg/mL solutions in PBS and was subsequently blocked with 1% BSA. Error bars shown are standard deviations of the mean (n = 3). No significant differences existed between the FN-coated samples and the control.

Table 1

Hydrophobicity and surface roughness characteristics of material surfaces as measured by water contact angle (Θ_W) and average roughness (R_A), as well as average roughness of a smooth region of interest ($R_{A, ROI}$).

Averages are presented $\pm$ one standard deviation.

Biomaterial Sample	Θ_W (degrees)	R_A (nm)	$R_{A, ROI}$ (nm)
Silicone	106 ± 3	193 ± 249	- - -
FEP	97 ± 3	13.0 ± 19.0	5.9 ± 7.4
PEU	70 ± 7	13.2 ± 16.3	4.6 ± 5.7
PET	69 ± 7	1.4 ± 1.9	0.7 ± 0.9
Glass	3.9 ± 3	0.4 ± 1.0	- - -