Bacterial adhesion to orthopedic implant polymers

Alan J. Barton,[1] Richard D. Sagers,[2] and William G. Pitt[1,*]
Departments of [1]Chemical Engineering and [2]Microbiology, Brigham Young University, Provo, Utah 84602

The degradable polymers poly(orthoester) (POE), poly(L-lactic acid) (PLA), and the nondegradable polymers polysulfone (PSF), polyethylene (PE), and poly(ether ether ketone) (PEEK) were exposed to cultures of *Staphylococcus epidermidis, Pseudomonas aeruginosa,* or *Escherichia coli.* Bacteria washed and resuspended in phosphate buffered saline (PBS) adhered to polymers in amounts nearly twice those of bacteria that were left in their growth medium, tryptic soy broth (TSB). In TSB, there was variation in adhesion from species to species, but no significant variation from polymer to polymer within one species. In PBS there were significant differences in the amounts of bacteria adhering to the various polymers with the exception of *S. epidermidis,* which had similar adhesion to all polymers. As a whole, *P. aeruginosa* was the most adherent while *S. epidermidis* was the least adherent. The estimated values of the free energy of adhesion (ΔF_{adh}) correlated with the amount of adherent *P. aeruginosa.* When POE, PLA, and PSF were exposed to hyaluronic acid (HA) before exposure to the bacteria, there was 50% more adhesion of *E. coli* and *P. aeruginosa* on POE and PLA. With respect to bacterial adhesion, the biodegradable polymers (POE and PLA) in general were not significantly different from the nondegradable polymers. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Persistent infections associated with implant devices are a major clinical problem. These infections persist despite the use of antibiotics, thereby limiting the usefulness of many prosthetic devices.[1–3] Bacterial infections are a significant problem because they frequently cannot be eradicated without surgical revision or implant removal, and infections can result in morbidity, amputation, or death. The initial event in the development of such infections is the adhesion of bacteria to the implant surface, and therefore materials that are less adherent to bacteria would be preferred.

The purpose of this research was to study the adhesion of bacteria to several polymers that show promise as orthopedic materials based on material strength and biocompatibility, but that have been characterized as to their propensity to adhere bacteria. The group listed below includes two biodegradable polymers in order to determine if this class of polymers invokes a different adhesive response from bacteria.

(1) *Poly(orthoester)* (POE) is a bioerodible amorphous polymer being used in implantable drug delivery devices and is currently being studied for use in bone fixation devices.[4–8]
(2) *Poly(L-lactic acid)* (PLA) is a biodegradable polymer used in a host of medical applications such as degradable sutures and drug delivery devices.[9] PLA is also being studied for use in degradable orthopedic implants.
(3) *Poly(ether ether ketone)* (PEEK) is a semicrystalline, nondegradable, high temperature thermoplastic material that has initially shown promise as a bone fixation material because it can be heat contoured to fit the shape of a bone.[10–13]
(4) *Polysulfone* (PSF) is another nondegradable material frequently used in implant devices. This polymer is also being reinforced with carbon fiber to increase its strength in orthopedic applications.[10,11,14,15]
(5) High molecular weight polyethylene (PE) has been studied extensively in the past, and was included as a control.

The bacteria selected for this study, *Staphylococcus epidermidis, Pseudomonas aeruginosa,* and *Escherichia coli,* have often been implicated in implant infections.[16–18] It was of interest to determine the adhesion rate of the bacteria under the following conditions:

(1) while the bacteria were still in tryptic soy broth (TSB), their growth medium;

*To whom correspondence should be addressed.

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(2) when the bacteria were washed and resuspended in phosphate buffered saline (PBS); and
(3) when the polymers were preexposed to hyaluronic acid (HA).

Previous studies have shown that a much different adhesive response is observed when bacteria are adsorbed from TSB compared to adsorption from PBS,[19] although it is unknown whether the response is due to the abundance or lack of nutrient. *In vivo* the bacteria are not bathed in either TSB or PBS. HA is the major constituent of synovial fluid that bathes the surfaces of bones and joints; therefore, studying bacterial adhesion in the presence of HA more closely models actual implant conditions.

METHODS AND MATERIALS

Polymer preparation and characterization

POE obtained from the lab of Dr. A. U. Daniels at the University of Utah had a molecular weight of 82,000. PLA was obtained from Boehringer–Ingelheim. The polysulfone was Udel™ obtained from Amoco (Naperville, IL). High density PE film was obtained from Union Carbide (Charleston, WV). The PEEK was STABAR™ film obtained from ICI (Wilmington, DE). Glass slides were cleaned and primed by dipping in a 1% solution of aminopropyl-triethoxysilane. These slides were then dipped in a 1% solution of POE, PLA, or PSF in methylene chloride and dried for 48 h in a vacuum oven operating at 70°C and 21 kPa. The extruded PE and PEEK films were adhered to glass slides using an adhesive.

Microscopic analysis of the dip coated polymers verified that they had smooth and homogeneous surfaces. The extruded films were much rougher, as evidenced by grooves and bumps of a micrometer size scale. In addition, the polymers were analyzed using X-ray photoelectron spectroscopy (XPS) on a Hewlett–Packard 5950 XPS. The surface energies of the polymers were calculated from underwater contact angles of octane and air on the polymers using the procedure described by King et al.[20]

Bacteria preparation

The day before the experiment, TSB without dextrose was inoculated with one colony of *P. aeruginosa* (GNRNF-Ps-1), *S. epidermidis* (ATCC 12228), or *E. coli* (ATCC 10798) that had been maintained on sheep-blood agar. One milliliter of an overnight culture was pipetted into 100 mL of TSB and the culture was incubated 6 h at 37°C.

Washed bacteria experiments

Washed bacterial cells were obtained by growing the culture for 7 (rather than 6) h, and filtering through 0.45-μm filter papers. Each mat of bacteria was washed twice with 5 mL of PBS before the bacteria were resuspended in PBS by vortexing. A 7-h growth provided a similar bacterial concentration as the 6-h unwashed culture that continued to grow during the subsequent 1-h experimental procedure.

The average concentrations of the cultures used in the experiments are shown in Table I, including the concentrations of the bacteria at the end of the experiment. Each concentration was determined by averaging eight counts obtained with a hemacytometer.

Bacteria surface energy

The bacterial surface energy was calculated using the method described by Cook and colleagues.[19] Briefly, the contact angle values of the water and methylene iodide were used in to determine the surface energies of the bacteria. The overall bacterial surface energy was calculated using the equations reported by Wu[21] that relate the surface energy of test liquids and their contact angles to the polar and dispersive components of the bacteria surface energy (γ_B). From the surface energy components of the polymers, bacteria, and water, the free energy of adhesion was calculated by the method of Absolom et al.[22]

TABLE I
Bacterial Concentrations

Media	Culture Age (h)	*P. aeruginosa* (Cells/mL)	*E. coli* (Cells/mL)	*S. epidermidis* (Cells/mL)
TSB	6	$6.1 \pm 1.3 \times 10^7$	$3.4 \pm 0.6 \times 10^8$	$2.7 \pm 0.3 \times 10^7$
TSB	7	$7.9 \pm 0.7 \times 10^7$	$4.0 \pm 0.7 \times 10^8$	$3.8 \pm 0.4 \times 10^7$
PBS	7	$6.9 \pm 0.9 \times 10^7$	$3.8 \pm 1.2 \times 10^8$	$3.6 \pm 0.4 \times 10^7$

Values are mean ± SD.

Adhesion procedure

The 6-h unwashed or the 7-h washed culture was continuously fed for 1 h to four parallel flow cells that were 37 mm wide, 1.65 mm high, and 86 mm long in the flow direction.[23] The flow rate was 1.0 mL/min, which produces a Reynolds number of 1.6 and a wall shear rate of 1.9 s^{-1}. The flow of the culture was vertical (bottom to top) to avoid density-induced instabilities when the culture initially displaced PBS in the flow cell.

At the end of the 1-h experiment, the flow cells were inverted and rinsed (top to bottom) with 3 mL of PBS and then with 3 mL of 95% ethanol. The rinse served to remove any loosely adherent cells and to lower the tension at the air/water interface. This procedure prevents cell displacement during the rinsing procedure.[24]

After the rinses, the polymer-coated slides were removed from the flow cell and the cells were heat fixed, stained with methylene blue, and examined under a light microscope. Each slide was counted at six positions in specific increments along the lengthwise centerline of the slide. Experiments were repeated three or four times.

Adsorption of HA

Adsorption of HA was measured using the ATR-FTIR techniques described previously.[25] A germanium crystal was coated with PSF and mounted into a small flow cell and placed inside an FTIR spectrometer (Nicolet, Madison, WI). First, a background spectrum was collected of the polymer-coated crystal surface. PBS was injected into the flow cell 2 h before the reference spectrum was collected. Following this collection, 10 mL (about five times the cell volume) of 0.1 mg/mL HA solution were injected into the flow cell, and five sequential spectra were collected during the next 60 min. After these collections, the flow cell was flushed with 10 mL of PBS, and an additional five spectra were collected. The amount of adsorbed HA was quantified as the height of the 1075-cm^{-1} peak.

Bacterial adhesion with HA

Some bacterial adhesion measurements were done on surfaces precoated with HA. Typically, following the prerinse with PBS, three of the four flow cells were filled with a 0.1 mg/mL HA in PBS solution. The three flow cells were exposed for 1 h to the HA solution, rinsed with 3 mL PBS, and subsequently exposed to the bacterial suspensions for 1 h. The flow cell not exposed to HA received washed bacteria resuspended in PBS, while the other three received washed bacteria resuspended in 0.1 mg/mL HA in PBS.

RESULTS AND DISCUSSION

Polymer characterization

Table II shows the atomic composition revealed by XPS analysis as well as the theoretical compositions of each polymer. Because the PLA, PSF, and POE were cast onto glass slides coated with a silane coupling agent, the slight silicon contamination is not unexpected. The excellent agreement of theoretical and experimental compositions indicates that there was little contamination, and that the polymer compositions were consistent with published formulations.

The water contact angles on dry polymers are shown in Table III. The high water contact angles show that all of these polymers are fairly hydrophobic. The surface energies of the hydrated polymers were calculated from underwater contact angles of air and octane as shown in Table IV. The air/water contact angle of PLA decreased considerably on the hydrated polymer, indicating that some surface rearrangement or hydrolysis may have occurred upon hydration. The air/water contact angles of hydrated POE, PSF, and PE are similar to those on dry polymers, indicative of surface stability in an aqueous environment.

There is a fair amount of variation in surface energies of the polymers. The PLA has a fairly high γ_S^p

TABLE II
Polymer Composition

Polymer	XPS Analysis (%)				Theoretical Composition (%)		
	Carbon	Oxygen	Sulfur	Silicon	Carbon	Oxygen	Sulfur
PLA	63	36	0	1	60	40	0
PSF	90	9	1	1	84	13	3
POE	76	23	0	1	75	25	0
PE	99	1	0	0	100	0	0
PEEK	87	12	0	0.3	86	14	0

TABLE III
Water Contact Angles on Dry Polymers

Polymer	Water Contact Angle (°)
PLA	84 ± 1
PSF	84 ± 2
POE	78 ± 2
PE	106 ± 2
PEEK	90 ± 2

Values are mean ± 95% confidence interval.

which may indicate that the ester groups are hydrolyzing to acid and hydroxyl groups. The POE has extremely high γ_S^d and fairly low γ_S^p, suggesting that any degradation occurring upon hydration does not greatly change the surface energy.

Bacteria characterization

Table V shows the average contact angles of the bacteria as well as the calculated bacterial surface energies. *P. aeruginosa* has the lowest and *S. epidermidis* has the highest surface energy, although the range of surface energies among this set of organisms is fairly small.

Free energy of adhesion

The free energies of adhesion (ΔF_{adh}) for the bacteria on the various surfaces are reported in Table VI. The negative values for ΔF_{adh} indicate that adhesion is thermodynamically favorable for all of the bacteria/polymer systems in this study. The value of ΔF_{adh} changes significantly from bacteria to bacteria and from polymer to polymer. In general, *P. aeruginosa* has the most negative ΔF_{adh} and *S. epidermidis* has the least negative value.

TABLE IV
Underwater Contact Angles and Calculated Surface Energies

Polymer	θ_{air}* (°)	θ_{octane} (°)	$\gamma_S^{d\dagger}$ (dyn/cm)	$\gamma_S^{p\dagger}$ (dyn/cm)	$\gamma_S^{\dagger}$ (dyn/cm)
PLA	57 ± 2	70 ± 4	22 ± 2	25 ± 2	47 ± 3
PSF	87 ± 2	123 ± 2	35 ± 5	6.5 ± 0.5	41 ± 5
POE	73 ± 2	127 ± 2	114 ± 23	5.6 ± 0.5	120 ± 28
PE	109 ± 4	154 ± 4	23 ± 5	1.3 ± 0.4	24 ± 5
PEEK	75 ± 3	126 ± 3	93 ± 23	5.8 ± 0.6	99 ± 26

Values are mean ± 95% confidence interval.
*Underwater contact angles of air and octane.
†Dispersive, polar, and total components of surface energy, respectively.

TABLE V
Bacteria Surface Energy

Bacteria	θ_{H_2O}* (°)	θ_{MI} (°)	$\gamma_B^{d\dagger}$ (dyn/cm)	$\gamma_B^{p\dagger}$ (dyn/cm)	$\gamma_B^{\dagger}$ (dyn/cm)
E. coli	31 ± 3	52 ± 2	28 ± 1	37 ± 1	64 ± 1
P. aeruginosa	38 ± 3	44 ± 1	30 ± 1	32 ± 1	62 ± 1
S. epidermidis	24 ± 2	52 ± 2	27 ± 1	41 ± 1	68 ± 1

Values are mean ± 95% confidence interval.
*Underwater contact angles of air and octane.
†Dispersive, polar, and total components of surface energy, respectively.

Bacterial adhesion to polymers

Figures 1–3 summarize the results of the adhesion experiments and show interesting trends regarding the effect of TSB, the general adhesiveness of the polymers (compared to PE), and the significance of biodegradability and roughness. The most obvious feature of the data is that for all three bacteria, the adhesion of bacteria in the presence of TSB is significantly less than the adhesion of bacteria in PBS. This has been observed previously and will be discussed later.

Washed bacteria

Turning our attention to the washed bacteria (in PBS), Figures 1–3 show that none of these polymers appear to be significantly worse than PE in terms of bacterial adhesion (with the possible exception of *P. aeruginosa* on PEEK). For *P. aeruginosa*, only the adhesion on PEEK was statistically greater ($p < 0.05$) than on PE, and adhesion on the other polymers was not significantly different ($p > 0.05$) than on PE. For *E. coli*, the other polymers had lower adhesion than was found on PE, indicating that these polymers are no more susceptible to *E. coli* colonization than is PE. Similarly for *S. epidermidis*, the adhesion to PE was not significantly different from that on the other polymers. Previous studies in these flow cells[26] have demonstrated that the adhesion of *S. epidermidis* is trans-

TABLE VI
Free Energy of Adhesion

Polymer	*P. aeruginosa*	*E. coli*	*S. epidermidis*
PLA	−11.7 ± 2.4	−8.5 ± 1.9	−5.9 ± 1.5
PSF	−25.0 ± 2.5	−17.9 ± 2.2	−13.0 ± 1.6
POE	−36.5 ± 3.3	−26.3 ± 2.8	−20.4 ± 2.1
PE	−22.6 ± 2.7	−16.1 ± 2.3	−11.1 ± 1.7
PEEK	−34.6 ± 3.7	−25.0 ± 3.0	−19.2 ± 2.33

Values are mean ± 95% confidence interval of ΔF_{adh} (dyn/cm).

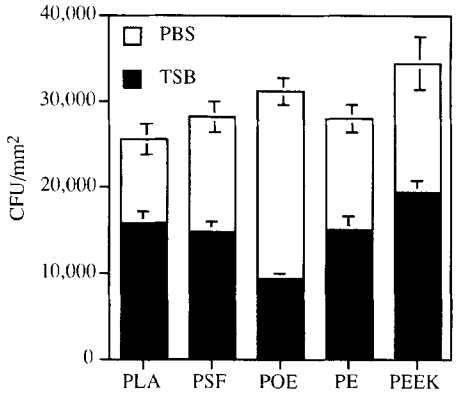

Figure 1. The amount of adhesion of *P. aeruginosa* after 1 h of exposure to the polymers in TSB and PBS. The white bars represent the adhesion of bacteria that have been washed and resuspended in PBS, and the dark bars indicate the amounts of adhesion in the experiments in which the bacteria were left in their growth media. The error bars represent the 95% confidence intervals. Where error bars are not shown, the 95% confidence intervals are too small to be represented.

port limited at flow rates below 1 mL/min; therefore it appears that the adhesion of *S. epidermidis* is either insensitive to polymer chemistry or is possibly limited by mass transport to the surface.

Although it is impossible to predict how these data would compare to an actual *in vivo* situation, these results suggest that the amount of bacterial adhesion on these polymers would be comparable to or less than that on PE.

Erodible polymers

When one compares adhesion on the degradable polymers (POE, PLA) to the nondegradable polymers (PE, PEEK, PSF), there is no consistent difference in the amount of adhesion. Consequently it appears that degradability is not a significant factor in bacterial adhesion. This seems to indicate that in a clinical

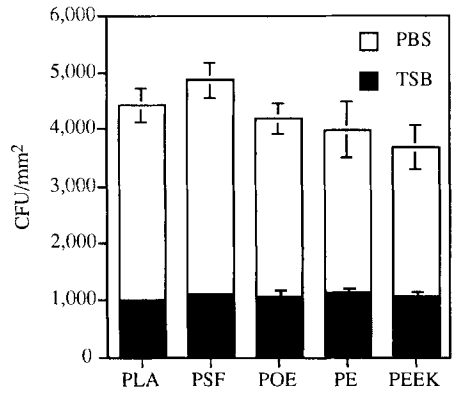

Figure 2. The amount of adhesion of *S. epidermidis* after 1 h of exposure to the polymers in TSB and PBS. See Figure 1 caption for details.

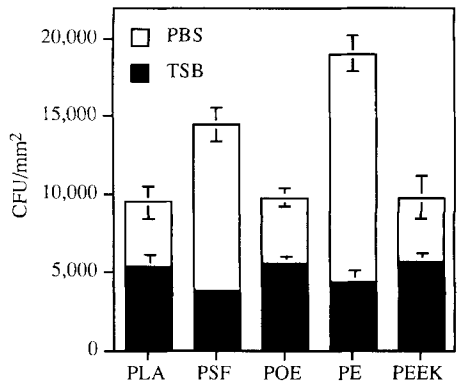

Figure 3. The amount of adhesion of *E. coli* after 1 h of exposure to the polymers in TSB and PBS. See Figure 1 caption for details.

setting, degradable polymers (such as POE and PLA) would be colonized by bacteria to a similar extent as nondegradable polymers.

Roughness

The roughness of these polymers does not appear to influence adhesion as significantly as does the polymer chemistry; the three cast films (PLA, PSF, and POE) are much smoother than the two extruded films (PE and PEEK), and yet the amounts of adhesion are similar. Other reports have shown that rougher surfaces can lead to higher amounts of adhesion.[27]

Adhesion in TSB

With one exception, the amounts of adhesion in TSB of a given bacterial species in TSB on all five polymers are about the same. This exception is the adhesion of *P. aeruginosa* on POE which is significantly lower ($p < 0.05$) than *P. aeruginosa* adhesion on the other four polymers.

TSB contains amino acids, polypeptides, and polysaccharides, and after 6 h of growth, cell metabolic by-products are also present. It is hypothesized that the components of TSB coat the polymer surfaces and mask the differences in polymer surface chemistries. Subsequently, similar bacterial interactions occur with all of the TSB-coated surfaces. This coating may also be responsible for significantly lower levels of adhesion in TSB. As the surface becomes qualitatively more similar to the bulk fluid, the attraction of the bacteria to the surface is reduced. This can qualitatively be described from a thermodynamic perspective by saying that the free energies of the coated polymer and that of the suspension liquid are similar enough that there is little driving force for adhesion.

Another possible explanation for decreased adhe-

sion in the presence of TSB was proposed by Costerton et al. who postulated that the bacteria in TSB are somewhat adhesive, but not as adhesive as the bacteria in PBS that lack the necessary nutrients to grow and divide. Under starvation conditions, they may undergo a physiologic or metabolic change, sequester themselves in a biofilm, and wait until nutrients are more plentiful before they begin growing again.[28]

Bacterial adhesion to HA-coated polymers

Figures 4 and 5 show the results of the bacterial adhesion to the HA-coated polymers. The amount of *P. aeruginosa* and *E. coli* adhering to PLA and POE increased significantly ($p < 0.05$) in the presence of HA (compared to no HA); the amount adhering to PSF was significantly different. Additionally, there was no significant difference in the amount of bacteria adhering to the various polymers coated with HA, suggesting that the HA coats the polymers sufficiently that the surfaces appear similar to the bacteria. There are, however, significant differences in adhesion between the bacterial strains; for example, the amount of *P. aeruginosa* adhesion was nearly 3 times the adhesion of *E. coli* on the HA-coated surfaces. These data suggest that HA increases adhesion of *P. aeruginosa* and *E. coli*, possibly by providing biological binding sites that are not modulated by the underlying polymer.

Thermodynamics of bacterial adhesion

Figure 6 compares the adhesion amounts of bacteria in PBS with the free energy of adhesion ($-\Delta F_{adh}$) on all of the surfaces. Overall there is no general trend if all surfaces are examined together. It is notable that *S. epidermidis*, which had the least negative values of ΔF_{adh} (ranging from -7 to -21 dyn/cm), also showed the lowest amount of adhesion. *P. aeruginosa* had both the most negative values of ΔF_{adh} (-12 to -37 dyn/cm) and the greatest amount of adhesion. Examination of the amount of adhesion versus ΔF_{adh} for each bacterial species showed a positive correlation for *P. aeruginosa* but no correlation for *S. epidermidis* or *E. coli*. In a previous study, Reid et al. found no correlation between the adhesion of *S. epidermidis* or *E. coli* and the surface tension of various polymers, but they did show a positive correlation for another species (*Lactobacillus acidophilus*).[27,29] Apparently *S. epidermidis* or *E. coli* do not correlate with surface properties as well as other species.

Figure 7 shows the correlation between the amounts of adhesion of *P. aeruginosa* (in PBS) and the $-\Delta F_{adh}$. For all polymers except the highly aromatic PEEK, this correlation is fairly strong ($r = 0.855$ without PEEK). Busscher et al. showed similar correlations with several oral bacteria on various surfaces,[30,31] but also showed that not all bacteria or all surfaces follow the correlation exactly.

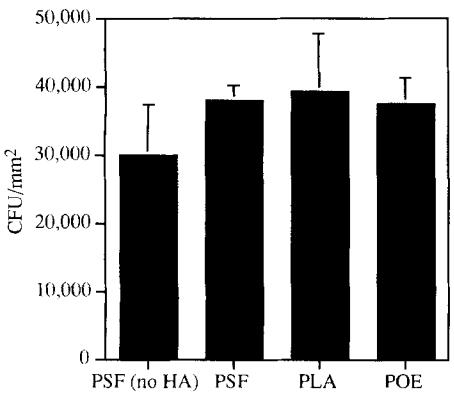

Figure 4. Adhesion of *P. aeruginosa* on surfaces preexposed to hyaluronic acid.

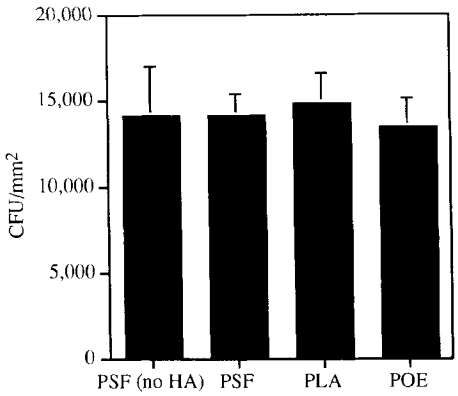

Figure 5. Adhesion of *E. coli* on surfaces preexposed to hyaluronic acid.

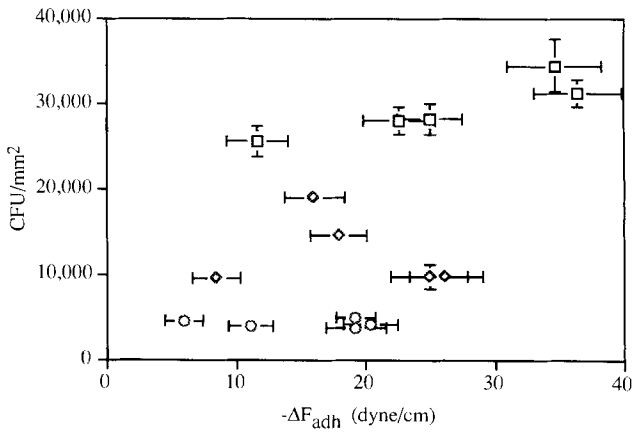

Figure 6. The amount of adhesion versus ΔF_{adh}. (□) *P. aeruginosa*, (◇) *E. coli*, and (○) *S. epidermidis*. Error bars indicate the 95% confidence intervals.

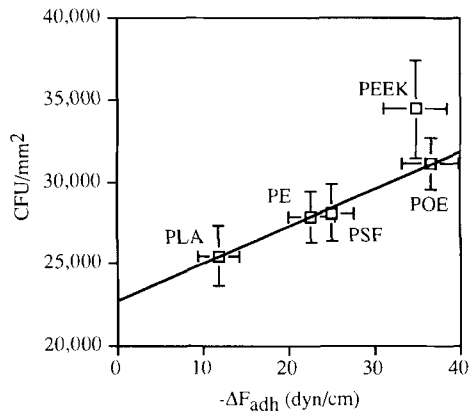

Figure 7. The amount of *P. aeruginosa* adhesion versus ΔF_{adh}. Error bars indicate the 95% confidence intervals. The line is the linear regression of the data excluding PEEK.

CONCLUSIONS

Several important observations and conclusions can be drawn from this study. Foremost is the observation that the liquid environment surrounding the bacteria and the surfaces has a significant impact on adhesion, nearly as, or equivalently as important as the surface chemistry of the particular bacteria and substrate pair. At this point we cannot determine if the liquid environment alters the bacterial physiology, just coats the surface, or coats (blocks) adhesive biomolecules on the bacterial surface. Compared to PBS, the TBS has properties that significantly decrease adhesion, while HA increases adhesion.

The new orthopedic materials appear to be satisfactory in terms of bacterial adhesion (at least with the present bacteria). The amount of adhesion on these polymers is not significantly greater than on PE, which has been in use for decades.

The biodegradability of the polymer does not significantly change the amount of adhesion. This suggests that the dissolution of substrate to which the bacteria are attached does not cause the bacteria to release. We suspect that multiple adhesive contacts keep the bacteria on the surface while individual contact points may dissolve and then reform on freshly exposed polymer.

Large-scale, integrated macroscopic surface properties, such as the free energy of adhesion, may play a significant role in the adhesion of some, but not all species of bacteria. Other factors involving specific molecular interactions between the surface proteins of the bacteria and the molecules on the surface (substrate polymer or adsorbed biomolecules) may have much more influence in determining the extent of adhesion. Bacterial–polymer interactions appear to be very complex and not easily generalized by simple rules or schemes.

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