Cranberry Derived Proanthocyanidins Reduce Bacterial Adhesion to Selected Biomaterials

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Catheter associated urinary tract infections (CAUTI) linked with the uropathogens Escherichia coli (E. coli) and Enterococcus faecalis (E. faecalis) account for the majority of nosocomial infections acquired in the clinical environment. Because these infections develop following initial adhesion of the bacterial pathogens to the catheter surface, there is increased interest in developing effective methods to inhibit attachment of cells to biomaterials used in the manufacture of indwelling devices. High molecular weight proanthocyanidins (PAC) extracted from the North American cranberry (Vaccinium macrocarpon) were examined for their potential to reduce the initial adhesion of uropathogenic bacteria (E. coli CFT073 and E. faecalis 29212) to two model biomaterials, poly (vinyl chloride) (PVC) and polytetrafluoroethylene (PTFE). Well-controlled experiments conducted in a parallel-plate flow chamber (PPFC) demonstrated decreased attachment of both bacteria to PVC and PTFE when either the bacteria, biomaterial or both surfaces were treated with PAC. Most significant inhibition of bacterial adhesion was observed for the condition where both the bacteria and biomaterial surfaces were coated with PAC. Additional experiments conducted with nonbiological model particles demonstrate comparable extents of adhesion inhibition, supporting a nonbiospecific mechanism of PAC action. The results of this study are promising for the implementation of PAC in the clinical milieu for prevention of device associated infection as the proposed functional modification is independent of antibacterial mechanisms that may give rise to resistant strains.

1. Introduction

Microbial contamination of invasive medical devices causes a broad spectrum of nosocomial pathologies (e.g., urinary tract infection, lower respiratory tract infection, etc.). The initial adhesion of pathogens to biomaterials is the first in a series of steps leading to the development of device associated infection (DAI), acting as a major obstacle in the extended use of devices ranging from urinary catheters to cardiac pacemakers.1 Catheter associated urinary tract infections (CAUTI) account for the majority of nosocomial infections acquired in hospitals and nursing homes.2 In 2002, the National Nosocomial Infections Surveillance system in the United States identified approximately 1.7 million cases of nosocomial infection that resulted in nearly 100 000 patient deaths. CAUTI accounted for nearly one-third of all CAUTI infections.3 Colonization of urinary catheters by uropathogenic organisms leads to the formation of ascending infection (UTI).4,5 Yet, current antibiotic regimes are often ineffective at halting biofilm formation and potentiate the development of multidrug resistant bacteria. As a result, alternative treatments are required that inhibit bacterial adhesion processes and shift therapeutic strategies from bacteriocidal to functional modifications.

DAI begins with the initial adhesion of pathogenic microorganisms on biomaterial surfaces. Lifshtiz—van der Waals (LW) forces and electrical double layer (EL) forces have been recognized as the predominant physicochemical forces that control particle or bacteria interactions with solid surfaces in aqueous media.6,7 The Derjaguin—Landau—Verwey—Overbeek (DLVO) theory provides a framework to account for the contributions of these forces in the interpretation of bacteria—surface interactions.8,9 Researchers have used the classical DLVO or “extended” DLVO (XDLVO) approach in attempts to describe microbial adhesion to a broad range of substrates, including glass, sand, and polymers.10–18 In DLVO theory, the total interaction energy between a bacterial cell and a solid surface is considered the sum of the noncovalent LW and EL forces. As the separation distance between the cell and the solid increases, EL forces decay exponentially, whereas LW forces decay more gradually. The

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(9) Roosjen, A.; Busscher, H. J.; Norde, W.; van der Mei, H. C. Microbiology 2006, 152, 2073–2082.
DLVO approach is useful in qualitatively describing cell adhesion onto solid surfaces; however results of bacterial adhesion experiments are not always in agreement with theoretical predictions. Such deviations from DLVO theory have been partly attributed to the complexity and heterogeneity of bacterial cell surfaces. This inherent constitutional complexity of bacterial surfaces, combined with variability across different strains and species, contribute to the difficulty in generalizing findings regarding the mechanisms of microbial adhesion to solid substrata.

An understanding of bacterial interaction with and adherence to mammalian cells is also of significant importance as it is often the first stage in bacterial disease. A broad range of biomolecules and macromolecular structures (i.e., fimbrial and afimbrial adhesins) are involved in controlling bacterial adhesion to mammalian cell surfaces or receptors. For instance, in the development of UTI, E. coli adhere to uroepithelial cells via type 1 and P-fimbriae, which attach to mannose specific receptors and α-D-Gal(1→4)-β-D-Gal, respectively. Several studies have demonstrated how the implementation of bioactive components of the North American cranberry (Vaccinium macrocarpon) or cranberry juice may inhibit the onset of UTI by preventing attachment of uropathogenic bacteria to uroepithelial cells. Ahuja et al. found that cells of E. coli grown in media supplemented with cranberry juice lost expression of P-fimbriae leading to a loss in fimbrial adhesion. Characterization of bacterial surfaces using atomic force microscopy (AFM) in the presence and absence of cranberry juice indicated conformational changes in the macromolecules at the cell surface. Other studies have shown that P-fimbriated E. coli lose the ability to adhere to bladder cells following preincubation with (i) urine of humans who had consumed cranberry juice cocktail, or (iii) proanthocyanidins (PACs) extracted from cranberry fruit. Cranberry derived PACs have been found to have unique A-type linkages which are believed to play a key role in the antiadhesive properties of these compounds. Although previous studies demonstrate that cranberry derived products can prevent adhesion of E. coli to eukaryotic cells, or (iii) proanthocyanidins (PACs) extracted from cranberry fruit. Cranberry derived PACs have been found to have unique A-type linkages which are believed to play a key role in the antiadhesive properties of these compounds.

In this study, we investigate whether bacterial adhesion to common biomaterials can be reduced by treatment with cranberry derived PACs. Due to its prevalence, we have selected catheter associated urinary tract infection (CAUTI) as a model pathology for investigation. Using a parallel-plate flow chamber (PPFC), we examined the influence of PAC on the adhesion of both Gram negative and Gram positive uropathogenic bacteria to two common biomaterials used in the manufacture of urinary catheters; namely, poly(vinyl chloride) (PVC) and polytetrafluoroethylene (PTFE). Our results demonstrate the effectiveness of these high molecular weight tannins in the reduction of bacterial adhesion to common biomaterials independent of antibacterial mechanisms. To elucidate the mechanism of PAC action we investigated changes in cell and biomaterial surface properties following PAC treatment. The experimental data suggests a physical mechanism of steric interference further supported by evidence that PAC treatment significantly reduces the adhesion of nonbiological model colloids to biomaterials.

2. Materials and Methods

2.1. Bacterial Cell Preparation. Uropathogenic E. coli ATCC 700928 (more commonly known as CFT073) and E. faecalis ATCC 29212 were used in this study. E. coli CFT073 is a Gram-negative clinical isolate from the blood and urine of a woman with acute pyelonephritis and its complete genome has been sequenced. E. faecalis ATCC 29212 is a well-characterized Gram-positive uropathogenic bacterium isolated from urine. Pure cultures were maintained at -80°C in Luria–Bertani Lennox broth (20 g/L) supplemented with 30% glycerol. Cultures were streaked onto LB agar plates that were then incubated for 24 h at 37°C. For each experiment, a single colony from a fresh plate was used to inoculate 50 mL of LB broth (in a 150 mL Erlenmeyer flask). Cultures were incubated at 37°C for 17 h at 200 rpm, then harvested by centrifugation at 5860g for 15 min (SS-34 rotor, Kendro) at 4°C. The growth medium was decanted and the pellet was resuspended in phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4). Centrifugation and resuspension were repeated one additional time to remove any traces of growth media and metabolites. The concentration of the cell suspension was determined with a Helber (SV400, Proscitech) bacteria counting chamber and the suspensions were diluted accordingly to the desired final concentration of 1 × 107 CFU/mL in PBS (pH 7.4). Analytical reagent grade chemicals (Fisher) and deionized (DI) (Milli-Q) water were used to prepare all solutions and media.

In selected experiments conducted to determine the effects of PAC on bacterial adhesion and bacterial surface properties, the final cell dilution was prepared using PBS supplemented with cranberry derived solubilized PACs (100 µg/mL). Briefly, dry PAC extract with an average molecular weight of 15 kDa (obtained from B.B. Howell, Rutgers) was solubilized in DI to obtain a PAC stock solution (1.5 mg/mL). PAC supplemented PBS (PAC-PBS, 100 µg/mL) was then prepared by adding an appropriate volume of the PAC stock to PBS.

2.2. Bacterial Cell Characterization. Microelectrophoresis (ZetaSizer Nano ZS, Malvern) was used to characterize the electrokinetic properties of the cells in PBS and PAC-PBS. Electrophoretic mobility (EPM) was measured at 22°C using cell suspensions (1 × 107 cells/mL) prepared in either diluent. These measurements were repeated using at least three different samples (prepared from separate cultures) of each bacterial suspension. Measured EPMs were converted to cell zeta potential using the Smoluchowski equation. Individual components of bacterial surface free energies were determined from contact angle measurements on bacterial films with

(20) Finlay, B. B.; Cossart, P. Science 1997, 276(5313), 718–725.
the polar liquids water and formamide and the apolar liquid diiodomethane. Contact angle measurements (sessile drop technique, OCA 20, Future Digital Scientific) were conducted on bacterial lawns formed on a cellulose acetate membrane filter (0.45 µm, 47 mm diameter, GE Osmonics) using the method described in ref 38. At least four measurements were made on each filter, and at least three filters were used for each condition at ambient temperature 22 °C.

The nominal size of the bacteria was determined by analyzing images taken in an inverted fluorescent microscope (IX-71, Olympus) operating in phase contrast mode. An image processing program (ImageJ, NIH) was used to determine the average lengths of the major and minor axes of the cells and the resulting equivalent spherical diameter.

2.3. Bacterial Cell Viability. Plate counts were used to verify loss of cell viability when suspended in PAC-PBS. Cell suspensions were diluted in High Recovery Diluent (Oxoid), spread plated on R2A agar (Difco; in triplicate) and incubated for 24 h (37 °C) before counting.

2.4. Bacteria and Particle Adhesion Experiments. Initial bacterial adhesion to two common biomaterials was examined using a dual channel parallel plate flow chamber (PPFC) (dimensions, L × W × H = 39.5 × 13 × 0.32 mm, Model FC271, Biosurface Technologies). Clean unplasticized PVC or PTFE disks (diameter 14 mm, Biosurface Technologies) were mounted in the flow cell and the system was equilibrated with PBS using a syringe pump (Model 200, KD Scientific) at 0.5 mL/min for a duration of 10 min. Bacterial suspensions (1 × 10^6 CFU/mL) were then injected into the flow cell (0.5 mL/min, 10 min), followed by an equivalent injection of PBS alone (0.5 mL/min, 10 min) to remove any unbound cells from the system. The PVC or PTFE disks were then carefully removed from the flow cell (avoiding passage through an air–liquid interface that can cause removal of attached cells), stained with DAPI (4’,6-diamidino-2-phenylindole) (100 µL at 100 µg/mL) and incubated in the dark for 10 min. The PVC or PTFE disks were then imaged by fluorescence microscopy (IX-71, Olympus), and the attached bacteria were enumerated with image analysis software (ImagePro). Four separate treatments were used in the adhesion experiments: (i) control: bacteria suspended in PBS were injected into the flow cell; (ii) bacteria only treated with PAC: bacteria were resuspended in PAC-PBS (100 µg/mL) for a duration of 10 min to allow PAC adsorption onto the cell surfaces, the suspension was centrifuged, the supernatant decanted, and cells were resuspended in PBS prior to injection in the flow cell; (iii) biomaterials only treated with PAC: prior to injecting the bacteria into the flow cell, a PAC-PBS solution (100 µg/mL) was injected (0.5 mL/min, 10 min) to allow adsorption of the PAC onto the biomaterial surface, followed by injection of PBS alone (0.5 mL/min, 10 min) and finally a suspension of bacteria in PBS; (iv) biomaterials and bacteria treated with PAC: bacteria resuspended in PAC-PBS (100 µg/mL) were directly injected into the flow cell, allowing the PAC to adsorb both on the bacteria surface and the biomaterial surface. These experiments were conducted with the two bacteria selected (E. coli CFT073 and E. faecalis). To better understand the mechanism by which PAC influences the initial adhesion of the bacteria, an additional set of flow cell experiments was conducted using fluorescent blue sulfate latex microspheres (Invitrogen, Eugene, OR). The microspheres were selected to be similar in size to the bacteria (1.0 µm diam.) and experiments were conducted for the four treatments described above using a microsphere concentration of 1 × 10^7 particles/mL. All bacteria and microsphere adhesion experiments were conducted at ambient temperature (22 °C) and repeated at least 4 times.

2.5. Characterization of Biomaterials. The zeta potential of the PVC and PTFE substrates was determined by streaming potential analysis conducted on thin rectangular coupons made from the same materials (Biosurface Technologies). The rectangular coupons were mounted inside a PPFC (Model FC81, Biosurface Technologies) of comparable dimensions to the one described in section 2.4, but specially designed for thin rectangular coupons (L × W × H = 40.6 × 11.4 × 0.203 mm). After mounting a coupon inside the flow cell, the coupon was equilibrated with PBS (control) or PAC-PBS (to allow adsorption of PAC onto the biomaterial) for 10 min (0.5 mL/min). The treated coupon was then removed from the flow cell and mounted in the clamping cell of a streaming potential analyzer (EKA, Brookhaven Instruments Corp., NY). The streaming potential measurements were conducted by pumping PBS across the biomaterial surface as described in ref 39. The biomaterial zeta potentials were determined from the measured streaming potentials using the analysis presented by Walker et al.30 Individual components of biomaterial surface free energies were determined from contact angle measurements on the biomaterial coupons as described above for bacterial films. Contact angle measurements (sessile drop technique, OCA 20, Future Digital Scientific) were conducted on PVC or PTFE coupons after they had been equilibrated with PBS or PAC-PBS for 10 min as described above. At least four measurements were made on each coupon (at ambient temperature 22 °C), and at least two coupons were used for each condition.

2.6. Adsorption of Cranberry Proanthocyanidins onto Biomaterials. A quartz crystal microbalance with dissipation monitoring (QCM-D) was used to verify adsorption of cranberry derived PACs onto a biomaterial surface (note: only unplasticized PVC coated quartz crystals were available from the manufacturer). The QCM-D E4 unit (Q-Sense AB, Västra Frölunda, Sweden) consists of a measurement chamber platform that can hold 4 sensor flow modules. Each flow module holds a 5 MHz AT-cut quartz sensor crystal with a PVC coated surface and is configured such that the flow is parallel to the crystal surface (as in the parallel plate flow cell). Prior to mounting in the flow modules, the PVC coated crystals were soaked in DI and dried with ultrahigh purity N2 gas. The clean dry crystals were mounted in a flow chamber, and PBS was drawn through the chamber with a peristaltic pump (150 µL/min, Reglo-Digital, Ismatec) until baseline was achieved (i.e., the measured change in frequency was less than 1 Hz/hour). PAC-PBS (100 µg/mL) was then drawn through the chamber for 35 min (150 µL/min), followed by a 15 min injection of PBS (150 µL/min). Finally, a mild detergent (1% Hellmanex in DI, 300 mL/min, 10 min) was drawn through the chamber until the resonance frequency returned to the baseline value. During the QCM-D experiment, the temperature inside the measurement chamber was maintained at 22 °C. The thickness and density of the adsorbed PAC film were assumed to be uniform. Analysis of the QCM-D data was done using the Q-Tools program (Q-Sense AB).

2.7. Statistical Analysis. Results were reported as means ± standard deviation (SD) for all experiments and were analyzed using a Student’s t-test. A p-value <0.01 was considered statistically significant.

3. Results and Discussion

3.1. Influence of PAC on Cell Viability. One motivation for using cranberry derived PAC in the prevention of device associated infection lies in its potential action as an antiadhesive compound independent of antibacterial mechanism that may lead to the development of resistant strains. Antibacterial properties have previously been reported at PAC concentrations greater than 200 µg/mL while antiadhesive effects have been observed at concentrations as low as 5 µg/mL.21 In the present study, a working concentration of 100 µg/mL was selected and verified for any effects on bacterial viability during the time scale of the experiments. When bacteria were grown in LB then resuspended and incubated in PAC-PBS for 30 min, there were no significant differences in viability as determined by plate counting. For E. coli, the plate count after 30 min of incubation in PAC-PBS was 1.4 ± 0.2 × 10^8 CFU/mL, in comparison to 1.4 ± 0.1 × 10^8 CFU/mL when suspended in PBS alone for 30 min. Similarly, for E. faecalis, the plate count after 30 min of incubation in

effects. An increase in cell surface hydrophobicity has often been associated with increased bacterial adhesion to both hydrophobic and hydrophilic surfaces. Contact angle measurements conducted on the surface of untreated and PAC treated biomaterials revealed that both substrates are generally hydrophobic (θw close to or greater than 90°), Exposure to PAC resulted in lower water contact angles for both PVC and PTFE. PAC-PBS was 1.3 ± 0.2 × 10⁷ CFU/mL, in comparison to 1.2 ± 0.2 × 10⁷ CFU/mL when suspended in PBS alone for 30 min. Hence, at the working concentration of PAC used in this study, the cranberry derived compound does not have antibiotic effects, thereby reducing selective pressures for antibiotic resistant bacteria.

### 3.2. Effect of PAC on Bacteria and Biomaterial Properties

Several studies have demonstrated that bacterial surface properties can control the extent of bacterial attachment to inert surfaces. Although the current body of knowledge does not allow generalization of findings regarding the influence of a specific cell property on bacterial adhesion, measurable cell surface characteristics such as hydrophobicity and surface potential (charge) have been linked with adhesion onto surfaces. To examine the potential (charge) have been linked with adhesion onto surfaces such as glass, polymers, and silica. To examine the effects of PAC on the physicochemical properties of the selected bacteria and biomaterials, experiments were conducted to evaluate changes in the relative hydrophobicity of the bacteria and materials, as well as variations in surface potential before and after exposure to cranberry derived PAC.

Sessile drop contact angle measurements conducted on dried microbial lawns are widely used to characterize the relative hydrophobicity of microbial cell surfaces. Contact angle measurements conducted on dried lawns of *E. coli* and *E. faecalis* as well as the two biomaterials were used to determine the various components of surface tension using eqs 4 and 5 (Table 1 and Supporting Information). Both bacterial strains were found to be hydrophilic (θw < 90°), with PAC treatment resulting in an increase in *E. coli* hydrophobicity but no significant effect on *E. faecalis*. Ionic and polar groups on surfaces lead to an increased surface tension components using eqs 4 and 5 (Table 1 and Supporting Information). Both bacterial strains were found to be hydrophilic (θw < 90°), with PAC treatment resulting in an increase in *E. coli* hydrophobicity but no significant effect on *E. faecalis*. Ionic and polar groups on surfaces lead to an increased surface tension components using eqs 4 and 5 (Table 1 and Supporting Information). Both bacterial strains were found to be hydrophilic (θw < 90°), with PAC treatment resulting in an increase in *E. coli* hydrophobicity but no significant effect on *E. faecalis*.

### Table 1. Summary of Bacteria and Biomaterial Surface Properties in the Presence and Absence of PAC

<table>
<thead>
<tr>
<th>Organism/Particle</th>
<th>θw (deg)</th>
<th>θl (deg)</th>
<th>θm (deg)</th>
<th>γf (mJ/m²)</th>
<th>γ&lt;sub&gt;DLVO&lt;/sub&gt; (mJ/m²)</th>
<th>γ&lt;sub&gt;AB&lt;/sub&gt; (mJ/m²)</th>
<th>γ+/γ−</th>
<th>EPM (μm cm/Vs)</th>
<th>ζ (mV)</th>
<th>aP (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>24 (2.6)</td>
<td>22 (2.6)</td>
<td>51 (4.6)</td>
<td>53.7</td>
<td>33.9</td>
<td>9.9</td>
<td>2.0</td>
<td>48.5</td>
<td>−1.96</td>
<td>(0.2)</td>
</tr>
<tr>
<td><em>E. coli</em> + PAC</td>
<td>49 (3.0)</td>
<td>30 (5.6)</td>
<td>49 (4.3)</td>
<td>50.4</td>
<td>35.0</td>
<td>7.7</td>
<td>2.5</td>
<td>24.1</td>
<td>−2.08</td>
<td>(0.1)</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>26 (5.7)</td>
<td>33 (7.7)</td>
<td>49 (2.2)</td>
<td>47.1</td>
<td>34.7</td>
<td>6.2</td>
<td>0.7</td>
<td>54.7</td>
<td>−1.38</td>
<td>(0.1)</td>
</tr>
<tr>
<td><em>E. faecalis</em> + PAC</td>
<td>24 (3.1)</td>
<td>25 (4.3)</td>
<td>50 (1.5)</td>
<td>52.4</td>
<td>34.5</td>
<td>9.0</td>
<td>1.6</td>
<td>30.4</td>
<td>−1.41</td>
<td>(0.1)</td>
</tr>
<tr>
<td>Latex</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Latex + PAC</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Biomaterial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVC</td>
<td>87 (3.9)</td>
<td>79 (3.4)</td>
<td>45 (3.3)</td>
<td>36.7</td>
<td>36.7</td>
<td>0.0</td>
<td>0.0</td>
<td>10.5</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>PVC + PAC</td>
<td>66 (3.4)</td>
<td>55 (5.9)</td>
<td>45 (1.9)</td>
<td>38.7</td>
<td>37.0</td>
<td>0.9</td>
<td>0.0</td>
<td>19.1</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>PTFE</td>
<td>108 (2.5)</td>
<td>92 (4.3)</td>
<td>53 (6.5)</td>
<td>32.3</td>
<td>32.3</td>
<td>0.0</td>
<td>0.0</td>
<td>1.4</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>PTFE + PAC</td>
<td>95 (1.3)</td>
<td>73 (4.7)</td>
<td>92 (4.3)</td>
<td>28.9</td>
<td>27.7</td>
<td>0.6</td>
<td>0.2</td>
<td>1.5</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

θ<sup>b</sup> Contact angles were measured using water (θw), formamide (θl), and diiodomethane (θm). The values in parentheses are standard deviations.

All measurements conducted on bacterial films demonstrated relatively low values for the electron accepting components of surface energy, ranging from 0.7 mJ/m² for *E. faecalis* to 2.5 mJ/m² for PAC treated *E. coli*, yielding total surface tensions dominated by Lifshitz–van der Waals interactions. Similarly, Lifshitz–van der Waals interactions accounted for nearly the entire measured surface tension of the biomaterials where electron donating components were between 0 mJ/m² for PTFE and PVC, and 0.9 mJ/m² for PAC treated PVC. Moreover, all bacterial surfaces were determined to be higher energy than biomaterial surfaces, a property reflective of hydrophilic bacteria and hydrophobic substrata.

Table I also includes a summary of the zeta potentials (ζ) of the two bacteria and the two biomaterials, measured in PBS at pH 7.4. The two strains of bacteria and the two inert substrates are negatively charged in the presence and absence of PAC and the data shows no significant change in bacteria or biomaterial ζ with PAC treatment.

The equivalent spherical radii of the two cell types were determined by image analysis of microscope images taken before and after exposure to PAC (Table 1). The data shows that no significant differences in size exist between the PAC treated and untreated bacteria. *E. coli* were slightly rod shaped and formed some aggregates with an average equivalent spherical radius of 1.76 μm. In contrast, *E. faecalis* were typically cocci generally found in pairs with an average equivalent spherical radius of 0.75 μm. Measured zeta potentials, contact angles, and bacterium sizes are used later to calculate interaction energy profiles for the different bacteria-biomaterial systems using two models of colloid-surface interactions; the DLVO model and the Steric model (details provided in the Supporting Information).

### 3.3. PAC Adsorption onto PVC

To verify whether PAC was readily adsorbed on a biomaterial surface, a QCM installed with PVC coated quartz sensor crystals was used. The QCM measures changes in the resonance frequency of the sensor crystal as mass is added or removed from the crystal surface. As increased mass adsors on the crystal surface, the shift in the resonance frequency increases proportionally, as described by the Sauerbrey relationship. Figure 1a shows the measured frequency shift of the PVC coated crystal as it is equilibrated with PBS (phase I), PAC-PBS is flowed over the crystal surface (phase II), followed by an equivalent injection of PBS alone (phase III). The rapid and substantial change in resonance frequency (f) upon injection of the PAC-PBS suggests that the PVC is readily coated with PAC. In phase IV of the
reductions in the extent of
been treated. In Figure 2a, the results show 1.6 and 1.5-fold
whether only the biomaterial or bacteria or both surfaces had
that PAC reduced bacterial attachment under all conditions,
and bacteria treated with PAC (BOTH). Overall, the data shows
biomaterial only treated with PAC (MAT), and (iv) biomaterials
where the four treatments are indicated as follows: (i) control
bacterial adhesion experiments conducted with both biomaterials,
to biomaterial surfaces. Figure 2 summarizes the results of the
effectivity of PAC in preventing the initial adhesion of bacteria
Parallel plate flow cell experiments were performed to determine
the extent of
PAC-PBS is injected (100 µg/mL); phase II: PAC-PBS is injected; phase IV:
0.1% Hellmanex is injected. (b) The measured frequency shift is converted
to adsorbed mass per unit area of the sensor crystal using the Sauerbrey
relationship.

Figure 1. (a) QCM measurements (i.e., crystal resonance frequency, 3rd overtone) for PAC adsorption onto a PVC coated quartz crystal. Phase I: baseline is obtained by flowing PBS over the crystal surface; phase II: PAC-PBS is injected (100 µg/mL); phase III: PBS is injected; phase IV: 0.1% Hellmanex is injected. (b) The measured frequency shift is converted to adsorbed mass per unit area of the sensor crystal.

3.4. Adhesion of E. coli and E. faecalis onto PVC and PTFE. Parallel plate flow cell experiments were performed to determine the effectivity of PAC in preventing the initial adhesion of bacteria to biomaterial surfaces. Figure 2 summarizes the results of the bacterial adhesion experiments conducted with both biomaterials, where the four treatments are indicated as follows: (i) control (CTRL), (ii) bacteria only treated with PAC (BAC); (iii) biomaterial only treated with PAC (MAT), and (iv) biomaterials and bacteria treated with PAC (BOTH). Overall, the data shows that PAC reduced bacterial attachment under all conditions, whether only the biomaterial or bacteria or both surfaces had been treated. In Figure 2a, the results show 1.6 and 1.5-fold reductions in the extent of E. coli 700928 attached to PVC when either the substrate or the bacteria is treated with PAC, respectively. Similarly, 1.7 and 1.8-fold reductions in attached E. coli cells were observed for experiments conducted with PTFE when either the material or the cells were treated with PAC. When both the bacteria and the biomaterial were PAC treated, the extent of E. coli attachment to PVC and PTFE was 1.8 and

2.1-fold lower, respectively. To our knowledge, this is the first evidence that PAC has the ability to inhibit the adhesion of uropathogenic E. coli to biomaterials.

The effects of PAC were even more pronounced when E. faecalis 29212, a representative Gram-positive microorganism, was tested (Figure 2b). Results of experiments examining the influence of PAC on E. faecalis adhesion reveal 2.0-fold and 1.4-fold reductions in the extent of bacterial adhesion onto PVC when either the biomaterial (MAT) or the microorganism (BAC) is treated with cranberry PAC, respectively (Figure 2b). Experiments conducted with E. faecalis and PTFE show an even greater effect, whereby the extent of bacterial attachment decreases by 56% when the biomaterial is treated with PAC prior to injection of the bacteria into the PFFC. When both the bacteria and the biomaterial were PAC treated, the reduction in E. faecalis adhesion to PVC and PTFE was 90% and 88%, respectively. This is also the first evidence of PAC preventing the adhesion of a Gram-positive microorganism. For both bacteria-biomaterial systems, maximum inhibition of adhesion occurred when both the biomaterial and the bacteria were treated with PAC, suggesting that the compound was acting at both surfaces. This new evidence of PAC functioning independent of the Gram staining of the bacteria is suggestive of nonspecific mechanisms of action, unlike those previously suggested in the literature with respect to adhesion of E. coli to mammalian cells.22,23

3.5. Examining the mechanism(s) by which PAC prevents bioadhesion. To investigate the likelihood of a nonspecific mechanism of action for PAC, PPFC experiments were repeated using latex microspheres instead of bacteria, thereby eliminating any biospecific effects. Experiments were conducted for all four treatments, as was described previously for the bacteria. Interestingly, the attachment behavior observed using the microspheres was very similar to that observed for the cells, particularly, with E. faecalis (Figure 3). Representative microscope images of the biomaterial surface (PTFE) following PPFC
experiments conducted with the latex microspheres are presented in Figure 4. The images reveal clear differences in the number of attached latex microspheres on the PTFE surface in the absence and presence of PAC. The summarized results shown in Figure 3 reveal reductions in the extent of adhesion ranging from 1.2 to 5.8-fold. The observed significant influence of PAC on a nonbiological particle demonstrates that, at least in part, nonbiospecific mechanisms account for its antiadhesive properties on biomaterials. Furthermore, in contrast to the results obtained with the two bacteria, PAC treatment resulted in lower absolute values of zeta potential for the latex particles (Table 1). Within the context of the classical DLVO theory of colloidal stability, a particle with a lower absolute potential will experience a lower extent of electrostatic repulsion upon approach to an oppositely charged surface, thereby leading to a greater extent of attachment. However, the opposite behavior is observed in Figure 3, whereby the degree of particle deposition onto the biomaterial surfaces is lower in the presence of PAC. This observation suggests that PAC effects are not electrostatically mediated at the conditions examined here.

To better understand the mechanisms controlling the observed bacterial deposition behavior, the DLVO and Steric models (described in Supporting Information) were used to calculate bacteria-biomaterial interaction energies. Figure 5a shows representative DLVO interaction energy profiles for the E. coli-PVC system, where the total interaction energy ($V_T$) was evaluated as the sum of eqs 1 and 7. The calculations reveal no energy barrier to deposition in the primary energy minimum for all four treatments (note that the energy profiles all fall on the same curve). Hence, based on the DLVO interaction energy calculations, the E. coli is expected to deposit extensively on the PVC surface under all conditions examined. Despite these DLVO predictions, E. coli adhesion is high in the absence of PAC but much lower when both the biomaterial and the bacteria are treated with PAC. Table 2 summarizes the key parameters estimated using the DLVO model for the four different bacteria-biomaterial systems; namely, the maximum height of the energy barrier ($V_b$), and the Hamaker constant ($A$). The DLVO model predicts no energy barrier to adhesion for all four systems, under all conditions examined. Yet, the data presented in Figure 2 reveal distinct variations in the bacterial adhesion behavior when PAC is used to treat one or both surfaces. Clearly, the observed E. coli and E. faecalis deposition behavior in the presence of the cranberry derived PACs is not well described by the DLVO model alone.

Steric stabilization is a well-known phenomenon in colloid literature.\(^{42-50,53}\) Adsorbed layers of polymer (such as cranberry derived PAC) on the surfaces of biocolloids (e.g., bacteria) or collector surfaces (e.g., biomaterials) can give rise to steric interactions upon approach of the cell to the biomaterial surface. To evaluate the potential role of steric interactions in the four bacteria-biomaterial systems, the Steric model was used to estimate the overall interaction energy profiles for all experimental conditions considered.

Representative interaction energy profiles calculated using the Steric model (sum of eqs 1, 7, and 11) for the *E. coli*-PVC system are presented in Figure 5b. The graph shows significantly different predictions from those evaluated using the DLVO model. Most clearly, calculated heights of the energy barrier \((V_b)\) are extremely high when PAC is used to treat at least one of the surfaces (bacteria or biomaterial). Values of \(V_b\) calculated using the Steric model for the four different bacteria-biomaterial systems are summarized in Table 2. When steric interactions \((\xi^2)\) are considered, the calculated values of the energy barrier \((V_b)\) are significant for PAC conditions whereas no energy barrier is predicted for the control conditions (for all four bacteria-biomaterial systems). These calculations based on the Steric model can be used qualitatively to better understand the observed cell adhesion behavior; namely, higher energy barriers are generally predicted under conditions where less bacterial adhesion is observed (Figure 2). For example, for the case of *E. faecalis*, the Steric model predicts larger barriers to adhesion (on the order of 82 200\(kT\)) for the dual treatment condition ("BOTH") in contrast to the conditions where only one (bacteria or biomass) or no surface was treated with PAC (values range from 77 300 to 81 300\(kT\)). Accordingly, the treatment with the lowest number of attached *E. faecalis* cells is that where both the bacteria and the biomaterial surfaces are treated with PAC (Figure 2b). Likewise, the greatest extent of *E. faecalis* adhesion is observed for the control condition where no energy barrier to deposition is expected (for both biomaterials examined). The two remaining treatments ("BAC" and "MAT") exhibit cell attachment in between that observed for the "CTRL" and "BOTH" conditions, and correspondingly, lower values of \(V_b\) than the "BOTH" treatment. A similar trend is observed in the calculations for the interaction of *E. coli* with both biomaterial; namely, the largest calculated energy barrier is associated with the "BOTH" treatment which exhibits the least amount of adhesion (Table 2 and Figure 2a). Comparison of the experimental adhesion data (Figure 2) with theoretical predictions of bacteria-biomaterial interaction energies based on the two models (Table 2) suggests that steric stabilization may be an important mechanism by which PAC inhibits bacterial adhesion to inert surfaces. Namely, the calculations demonstrate that DLVO interactions alone can not be used to interpret the experimental results, but rather a mechanism of steric hindrance must be invoked to explain the observed trends in bacterial adhesion.

While the measured extent of bacterial adhesion in the presence of PAC is generally in qualitative agreement with estimated heights of the energy barriers based on the Steric model, it should be noted that values of \(V_b\) determined using the Steric model are generally on the order of 77,300\(kT\) or greater (when an energy barrier is present) (Table 2). However, bacteria are not theoretically capable of overcoming repulsive energy barriers of such magnitude. Theoretically, bacteria are expected to deposit on surfaces (i.e., overcome the repulsive energy barrier) where \(V_b\) is on the order of 0 to 25\(kT\). Despite these predictions, however, bacterial adhesion is observed even when both surfaces are treated with PAC and energy barriers are on the order of 77 300\(kT\) or greater. These very high energy barriers to attachment predicted by the theoretical calculations suggest that bacteria must be retained on the biomaterial surface by a mechanism other than deposition in the primary energy minimum of the interaction energy profile.

There is increasing evidence in the literature pointing to the importance of the secondary energy minimum in controlling particle and microbial deposition onto inert surfaces.\(^{1,18,54-56}\) The secondary energy well in the bacteria-surface interaction energy profile is located at a greater separation distance than that of the repulsive energy barrier. In Figure 5c, where the Steric model interaction energy profiles are replotted on a different scale, we note the presence of a secondary energy well for each PAC treatment (note: the calculations for the BAC and MAT

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**Table 2. Summary of Energy Barriers Estimated Using DLVO and Steric Models**

<table>
<thead>
<tr>
<th>bacteria-biomaterial system</th>
<th>treatment</th>
<th>estimated energy barriers ((V_b)) and Hamaker constants ((A))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTRL(^a)</td>
<td>BAC</td>
</tr>
<tr>
<td><em>E. coli</em>-PVC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLVO model(^d)</td>
<td>0(^b)</td>
<td>3.0 \times 10^{-20}</td>
</tr>
<tr>
<td>Steric model(^d)</td>
<td>3.0 \times 10^{-20}</td>
<td>186,200</td>
</tr>
<tr>
<td><em>E. coli</em>-PTFE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLVO model</td>
<td>0</td>
<td>2.9 \times 10^{-20}</td>
</tr>
<tr>
<td>Steric model</td>
<td>2.9 \times 10^{-20}</td>
<td>190,800</td>
</tr>
<tr>
<td><em>E. faecalis</em>-PVC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLVO model</td>
<td>0</td>
<td>3.0 \times 10^{-20}</td>
</tr>
<tr>
<td>Steric model</td>
<td>3.0 \times 10^{-20}</td>
<td>77,500</td>
</tr>
<tr>
<td><em>E. faecalis</em>-PTFE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLVO model</td>
<td>0</td>
<td>2.9 \times 10^{-20}</td>
</tr>
<tr>
<td>Steric model</td>
<td>2.9 \times 10^{-20}</td>
<td>79,400</td>
</tr>
</tbody>
</table>

\(^{a}\) For the control condition (i.e., no PAC), only the DLVO model was used for the interaction energy calculations. \(^{b}\) "0" indicates that no energy barrier is predicted for this condition. \(^{c}\) The DLVO model considers the sum of eqs 1 and 7. \(^{d}\) The Steric model considers the sum of eqs 1, 7 and 11.

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treatments fall on the same curve). Table 3 summarizes the predicted depths of the secondary energy well based on calculations using the Steric model. Because the heights of the repulsive energy barrier are so great, it is much more likely that the bacteria are being first retained in the secondary energy minimum prior to transitioning to the primary energy minimum.\(^{18}\)

Indeed, the observed bacterial adhesion behavior (Figure 3) is generally in qualitative agreement with the predicted depths of the secondary energy minimum, whereby deeper wells are associated with a greater extent of adhesion (for each bacteria-biomaterial system). For instance, in the case of \(E. \text{faecalis}\) interacting with PTFE, the shallowest secondary energy well (39\(kT\)) is linked with the lowest extent of adhesion (“BOTH” treatment), whereas the deepest secondary minimum (111\(kT\)) is associated with a greater number of attached cells (“BAC” treatment). The same trend is followed for the three other bacteria-biomaterial systems. The data in Table 3 also indicate that the deeper energy wells are located at smaller separation distances (\(h\)), thereby facilitating bacterial attachment to the biomaterial surface. An earlier investigation suggests that bacteria retained in a secondary energy well are readily washed away in a flow system because of their freedom to move laterally with respect to the substratum.\(^{14}\) However, in a more recent study, de Kerchove and Elimelech\(^\text{18}\) used a novel technique to provide direct evidence that bacteria are being retained in a secondary energy well where they may transition to the primary energy minimum.

Although the depths of the secondary energy well (and heights of the repulsive energy barrier) are in qualitative agreement with the experimental data, a few inconsistencies can still be observed in the overall trends. These inconsistencies may be attributed to the influence of surface charge heterogeneities on the bacteria or biomaterial surface, or nonhomogeneous coverage of the bacteria and/or biomaterial surfaces by the cranberry derived PAC. In this work, continuum models of biocolloid-surface interactions (e.g., DLVO) based on macroscopic measurements of bacterial and material surface properties are used to better understand the adhesion behavior of bacteria to biomaterials. It should be noted, however, that these comparisons of experimental adhesion data with DLVO and Steric model calculations may be considered mostly qualitative in nature as they do not include the influence of microbe specific properties (e.g., lipopolysaccharides and proteins on the cell surface). Several studies\(^{19,57–59}\) have demonstrated the importance of molecular-scale details on microbial adhesion and these could also account for some of the observed inconsistencies with the theoretical models considered here. Moreover, it can be noted that the models utilized here require assumptions about the values of several parameters (e.g., in eqs 1, 2 and 7) which are not definitively known for the system in question. Nevertheless, the general qualitative trends in the model calculations provide useful information for better understanding of the role of cranberry derived PACs in bacterial adhesion to biomaterials.

### 4. Conclusions

Cranberry derived proanthocyanidins are shown to be effective in the reduction of initial adhesion of uropathogens to the biomaterials PTFE and PVC, commonly used in the manufacture of urinary catheters. We have demonstrated, for the first time, the action of cranberry PACs against a Gram-positive organism and a nonbiological particle (latex microsphere). These results suggest that a nonbiospecific physical mechanism prevents the bacteria and microspheres from adhering to the biomaterial surfaces. Comparison of the experimental data with theoretical predictions of bacteria-biomaterial interaction energies calculated using the DLVO and Steric models further supports a mechanism of steric stabilization due to adsorption of PAC on both biomaterial and microbe surfaces. Importantly, we have shown that PAC acts in such a way that viability of the microorganisms is not negatively affected, thereby reducing selective pressures for resistant bacteria. Although the application of cranberry proanthocyanidin at the dose used in this study does not completely eliminate bacterial adhesion, the simplicity of the adsorption process to biomaterial surfaces and the nonspecificity of the proposed mechanism make the use of PAC in the treatment of medical devices quite promising. The clinical implementation of this natural system has the potential to decrease financial expenditures related to device associated infection and improve patient care. Further research is being pursued in our laboratory.

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**Table 3. Summary of the Estimated Depths of the Secondary Energy Minimum**

<table>
<thead>
<tr>
<th>bacteria-biomaterial system</th>
<th>BAC</th>
<th>MAT</th>
<th>BOTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E. \text{coli-PVC})</td>
<td>-279</td>
<td>-276</td>
<td>-98</td>
</tr>
<tr>
<td>depth of secondary minimum ((kT))</td>
<td>4.6</td>
<td>4.6</td>
<td>10</td>
</tr>
<tr>
<td>(h) (nm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E. \text{coli-PTFE})</td>
<td>-267</td>
<td>-250</td>
<td>-89</td>
</tr>
<tr>
<td>depth of secondary minimum ((kT))</td>
<td>4.6</td>
<td>4.6</td>
<td>10</td>
</tr>
<tr>
<td>(h) (nm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E. \text{faecalis-PVC})</td>
<td>-116</td>
<td>-117</td>
<td>-41</td>
</tr>
<tr>
<td>depth of secondary minimum ((kT))</td>
<td>4.6</td>
<td>4.6</td>
<td>10</td>
</tr>
<tr>
<td>(h) (nm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E. \text{faecalis-PTFE})</td>
<td>-111</td>
<td>-106</td>
<td>-37</td>
</tr>
<tr>
<td>depth of secondary minimum ((kT))</td>
<td>4.6</td>
<td>4.6</td>
<td>10</td>
</tr>
<tr>
<td>(h) (nm)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) For the control condition (i.e., no PAC), the DLVO model was used for the interaction energy calculations and predicts no energy barrier to deposition and therefore no secondary energy minimum. \(^{b}\) The separation distance (\(h\)) where the secondary energy minimum is located.

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to explore the use of PAC in preventing biofilm formation on various materials.

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Supporting Information Available: Details regarding theoretical models used in the analysis. This material is available free of charge via the Internet at http://pubs.acs.org.