RESEARCH ARTICLE

Direct adhesion force measurements between *E. coli* and human uroepithelial cells in cranberry juice cocktail

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Scope: Atomic force microscopy (AFM) was used to directly measure the nanoscale adhesion forces between P-fimbriated *Escherichia coli* (*E. coli*) and human uroepithelial cells exposed to cranberry juice, in order to reveal the molecular mechanisms by which cranberry juice cocktail (CJC) affects bacterial adhesion.

Methods and results: Bacterial cell probes were created by attaching P-fimbriated *E. coli* HB101pDC1 or non-fimbriated *E. coli* HB101 to AFM tips, and the cellular probes were used to directly measure the adhesion forces between *E. coli* and uroepithelial cells in solutions containing: 0, 2.5, 5, 10, and 27 wt% CJC. Macroscale attachment of *E. coli* to uroepithelial cells was measured and correlated to nanoscale adhesion force measurements. The adhesion forces between *E. coli* HB101pDC1 and uroepithelial cells were dose-dependent, and decreased from 9.32 ± 2.37 nN in the absence of CJC to 0.75 ± 0.19 nN in 27 wt% CJC. Adhesion forces between *E. coli* HB101 and uroepithelial cells were low in buffer (0.74 ± 0.18 nN), and did not change significantly in CJC (0.78 ± 0.18 nN in 27 wt% CJC; P=0.794).

Conclusion: Our study shows that CJC significantly decreases nanoscale adhesion forces between P-fimbriated *E. coli* and uroepithelial cells.

Keywords:
Atomic force microscopy / Bacterial adhesion / Cranberry / Uroepithelial cell

1 Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections in adults today, affecting ~150 million people worldwide, and accounting for ~$6 billion in direct costs *per* year [1, 2]. Gram-negative bacteria are responsible for most community-acquired and nosocomial UTIs [3–5]. Uropathogenic strains of *Escherichia coli* account for 85–95% of cystitis cases and 90% of acute pyelonephritis infections [6]. The presence of type P fimbriae on the surface of *E. coli* makes this uropathogen responsible for over 95% of acute pyelonephritis infections in children and 50–90% of acute pyelonephritis infections in adults [7]. P-fimbriated *E. coli* recognize the receptor Galα1$\rightarrow$4Gal of membrane glycolipids on uroepithelial and red blood cells [8, 9]. The present management of UTIs includes the prescription of short or long-term antibiotics, depending on the history of UTI recurrence for the patient [10]. Currently, first-line treatment for uncomplicated UTIs is trimethoprim-sulfamethoxazole [3]. However, resistance to the trimethoprim-sulfamethoxazole treatment has been seen in ~25% of UTI cases in the US and in up to 60% of UTI cases in Asia [3, 11]. In addition, resistance rates to gentamicin, ampicillin, and amoxicillin-clavulanate potassium are as high as 81% in European countries [12].

Alternative strategies toward prevention of UTIs can be helpful in this regard. The American red cranberry (*Vaccinium macrocarpon* Ait., family Ericaceae) has been empirically

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Abbreviation: AFM, atomic force microscopy; **CJC,** cranberry juice cocktail; **PACs,** proanthocyanidins; **UTI,** urinary tract infection

recognized for its benefits to the urinary tract. Although at one time the acidity of cranberry was believed to provide antibacterial benefits in the urinary tract [13], research has shown that cranberries do not alter the pH of urine [14]. The current understanding is that cranberry juice inhibits the attachment of bacteria to uroepithelial cells [14].

A few clinical studies have been undertaken to determine the efficacy of cranberries in preventing UTIs [14–18]. In 1994, Avorn *et al*. first demonstrated that cranberry juice helped prevent recurrent UTIs in elderly women, through a double-blind, placebo-controlled clinical trial [15]. There has recently been a recognition that there are sufficient studies to justify the claim of urinary health benefits due to cranberry consumption [19].

Numerous *in vitro* studies have evaluated the effects of cranberries on bacterial adhesion to model surfaces and uroepithelial cells [20–25]. While several studies have reported the number of retained bacteria on uroepithelial cells or on a surface after exposure to cranberry [24–27], more recent studies are attempting to unveil the mechanisms of cranberry in preventing UTIs at the molecular level [22, 23, 28]. A group of high molecular compounds in cranberries, known as proanthocyanidins (PACs), has been identified and tested for anti-adhesive activity against uropathogens [26, 29]. Adhesion forces between bacteria and a model surface (silicon nitride probe) were characterized as a function of time of exposure to cranberry juice or PACs, using atomic force microscopy (AFM) [22]. Cranberry juice cocktail (CJC) exposure was previously shown to cause the conformation of P fimbriae to change from an extended to a collapsed form [28]. Furthermore, biofilm formation of P-fimbriated *E. coli* was inhibited upon growth in CJC or cranberry PACs [30]. In another study, the anti-adhesive effect of PACs was observed in Gram-positive and Gram-negative bacteria, and non-biological particles [23]. Based on theoretical predictions of biomaterial–bacterial interaction energies calculated using colloidal models, Eydelnant and Tufenkji determined that PACs are adsorbed on biomaterials and microbial surfaces, and therefore a physical mechanism prevents latex particles from adhering to cranberry-coated biomaterials [23]. Therefore, it is likely that cranberry juice compounds can act in both bio-specific and non-biospecific ways to induce changes in either *E. coli* bacteria or the adhering substrate. The present study focuses on the mechanism of the biospecific interaction of CJC on uropathogenic *E. coli* and uroepithelial cells. Direct measurements of adhesion forces are reported for the first time using AFM. Prior work has addressed the strength of a single pilus-galabiose pair [31–33], but forces have never been reported for intact bacterium-uroepithelial cell pairs.

The aim of this study was to measure the nanoscale adhesion forces between P-fimbriated *E. coli* and uroepithelial cells *via* AFM. By using an AFM tip that was functionalized with a single bacterium, we quantified the nanoscale adhesion between *E. coli* and uroepithelial cells. This experiment can serve as a model to help elucidate the mechanisms by which cranberries prevent bacterial adhesion in the urinary tract.

2 Materials and methods

2.1 Bacterial strains and growth conditions

To evaluate the role of P fimbriae on bacterial adhesion, two *E. coli* strains were chosen. *E. coli* HB101 (American Type Culture Collection; ATCC 33694) is plasmid-less and non-fimbriated [34]. *E. coli* HB101pDC1 was created by inserting a plasmid coding for expression of P fimbriae (maintained under chloramphenicol selection, 20 μg/mL) into HB101 [35]. HB101pDC1 was generously provided by Professor Majlis Svensson (Department of Medical Microbiology, Lund University, Sweden). Bacteria were precultured in Tryptic Soy Broth (TSB, 30 g/L, Sigma, St. Louis, MO, USA) at 37°C overnight and cultured in fresh TSB at 37°C until reaching an absorbance at 600 nm of 0.7–0.8, corresponding to the middle exponential growth phase.

Bacteria were centrifuged and washed three times in 0.01 M PBS with a total solution ionic strength of 0.14 M, according to a previously reported protocol [36, 37]. The P fimbriae of *E. coli* HB101pDC1 were not removed during the washing process, as confirmed by hemagglutination assay and AFM imaging (data not shown). because of the chemical similarity between urine and PBS, a phosphate buffer with similar ionic strength was chosen to represent urine and also to eliminate sample variation that would be present in urine from different volunteers [38].

2.2 Uroepithelial cells and growth conditions

Human uroepithelial cells were purchased from ATCC (CRL 9520 VA) and kept in liquid nitrogen. Cells were cultured as previously reported [37]. Briefly, cells were grown in Kaighn's modification of Ham's F12 medium and supplemented with 10% fetal bovine serum. Tissue culture flasks were kept in a 5% CO_2 in air atmosphere incubator at 37°C for 6–7 days where the media was replaced every other day. For the force measurements, cells were cultured on Petri dishes under the same culture conditions.

2.3 Cranberry juice and treatment

CJC (Ocean Spray Cranberries, Lakeville-Middleboro, MA, USA) was purchased from a local grocery store. It contains 27 wt% cranberry juice and was used to prepare dilutions containing 2.5, 5, and 10 wt% CJC in 0.01 M PBS. Prior to use, CJC was neutralized to pH 7.0 to exclude the influence of low pH. Bacteria were incubated in PBS buffer, or 2.5, 5, 10, and 27 wt% neutralized CJC for 3 h at 37°C. This

exposure time did not cause any loss in viability as detected in our lab and reported by other groups [39].

2.4 AFM force measurements

The interaction forces between *E. coli* and uroepithelial cells were directly measured with AFM (Dimension 3100 with Nanoscope IIIa controller, Veeco Metrology, Santa Barbara, CA, USA) using silicon nitride AFM tips on a triangular cantilever. Spring constants were measured using a thermal technique [40]. They were in the range of 0.032–0.19 N/m, with an error of less than 10% *per* tip.

The AFM tip was carefully coated with a bacterium using a technique developed in our lab [41]. The AFM tip was brought into contact with poly-L-lysine (0.1% w/v in water, Sigma, USA) for 5 min. The bacteria pellet was placed on a section of parafilm on a glass slide, to form a very thin film of bacteria. The poly-L-lysine-treated AFM tip was positioned over the bacterial film with the optical microscope that is part of the AFM. Prior to contact with the bacterial film, AFM software parameters, such as scan size, scan rate, and deflection set point, were adjusted to minimize the lateral movement of AFM tip and the indentation into the bacterial film. The AFM tip was allowed to contact the solution containing bacteria for 1–3 min. For all AFM experiments, the loading rates were between (3.2–38) $\times$ 10^4 pN/s (with the tip velocity between 1 and 2 μm/s).

Confluent uroepithelial cells in the Petri dish were washed with fresh culture media and the media was replaced with PBS or one of the juice samples (2.5, 5, 10, and 27 wt% CJC) immediately prior to force measurements. The optical microscope was used to position the bacterium-functionalized AFM tip over the uroepithelial cells. For each CJC concentration, at least three uroepithelial cells were examined with at least five force measurements on each uroepithelial cell. Three replicates were conducted *per* condition. Prior to and after making force measurements on uroepithelial cells, the quality of the bacterial probes was verified by recording characteristic force curves on bare glass slides. Force cycles containing 512 data points for the approach and retraction portions were collected. The adhesion forces were calculated from the retraction portions of the curves.

2.5 Attachment of *E. coli* to uroepithelial cells

A static bacterial attachment/retention assay was performed to quantify the number of retained bacteria on uroepithelial cells, using identical CJC concentrations as we used for the AFM experiments. The protocol used for measuring bacterial attachment was described previously [37].

Bacteria cultured in pure TSB were suspended in aqueous solutions, diluted in PBS, containing 0, 5, 10, and 27 wt% CJC for 3 h at 37°C with slow shaking. Uroepithelial cells were incubated with equal volumes of the different concentrations of CJC for 3 h at 37°C. After cranberry treatment, bacteria (1×10^9 cells/mL) and uroepithelial cells (1×10^6 cells/mL) were incubated together in tissue culture flasks for 90 min at 37°C, with rotation at 18 rpm. After incubation, loosely attached bacteria were removed by gentle centrifugation ($100 \times g$) for 10 min. Uroepithelial cells were resuspended in 0.01 M PBS.

The number of bacteria attached to uroepithelial cells was counted at a magnification of 100 $\times$ under oil immersion, using phase contrast microscopy (Nikon Eclipse E400; Tokyo, Japan). Images were collected using a camera and stored with SPOT 4.6 advanced software (Diagnostic Instrument, MI). The number of bacteria attached to 20 uroepithelial cells was determined *per* sample condition.

Attachment data were analyzed using SigmaStat 2.03 statistical software. A two-way analysis of variance for repeated measurements was used for statistical analysis between treatment and control groups. A difference was considered significant if $p < 0.05$.

3 Results

3.1 Adhesion forces between *E. coli* bacteria and uroepithelial cells

Scanning electron microscopy imaging (Fig. 1) confirmed that a single bacterium (*E. coli* HB101pDC1) could be attached to the coated AFM tip. Using this bacterial probe, we found that adhesion forces between P-fimbriated *E. coli*

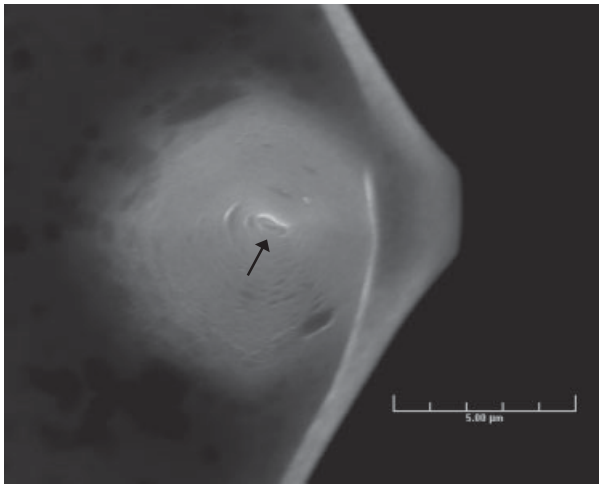

Figure 1. Scanning electron microscopy image of a single *E. coli* functionalized AFM tip. A silicon nitride AFM tip was coated with 0.1% poly-L-lysine and positioned over a thin film of bacteria using an optical microscope. The tip was brought into contact with the bacterial solution at a velocity of 1–2 μm/s and allowed to remain in contact for 1–3 min. By using a low solution concentration of bacteria, we could attach a single *E. coli* bacterium at the apex of the AFM tip (arrow).

HB101pDC1 and uroepithelial cells decreased as a function of increasing concentrations of CJC (Fig. 2). Adhesion forces with the uroepithelial cells were smaller for *E. coli* HB101 and did not depend on the presence or concentration of CJC.

We also observed a difference in the location where the adhesion force breaks off and returns to zero, when comparing *E. coli* HB101pDC1 with *E. coli* HB101. The pull-off distances (points at which the bond breaks between the bacterium and uroepithelial cell) for P-fimbriated *E. coli* HB101pDC1 were longer than for non-fimbriated *E. coli* HB101 (Fig. 2).

The average adhesion forces between *E. coli* and the uroepithelial cells was decreased in CJC for HB101pDC1, but there was no effect of CJC on the adhesion of HB101 and the uroepithelial cells (Fig. 3). In the absence of cranberry treatment, the average adhesion force between *E. coli*

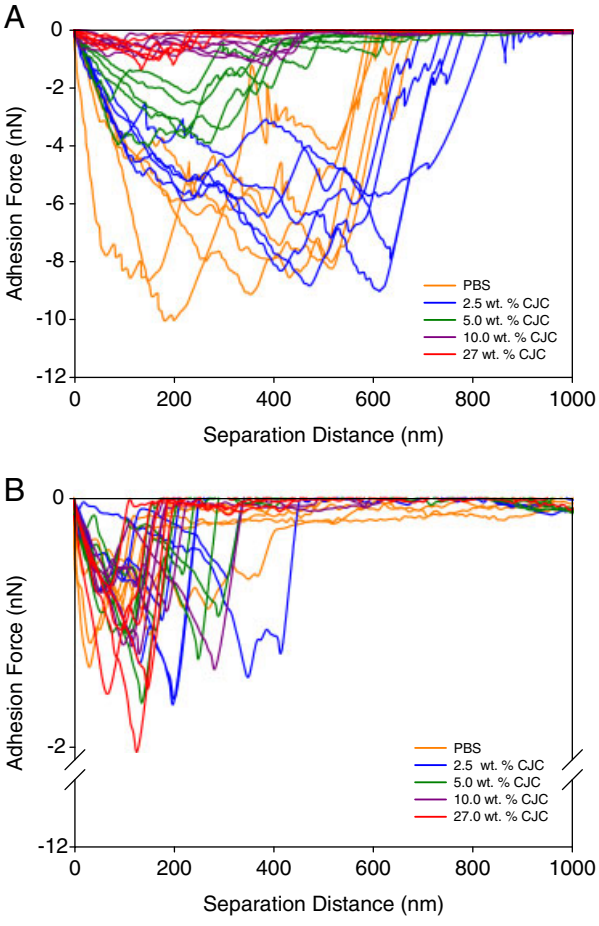

Figure 2. Representative AFM retraction curves. An *E. coli* functionalized tip was brought into contact with a monolayer of uroepithelial cells for 1 μs and retracted to measure the adhesion between the two surfaces. The AFM measurements were done as a function of cranberry juice concentration for (A) *E. coli* HB101pDC1 and uroepithelial cells and (B) *E. coli* HB101 and uroepithelial cells. Three uroepithelial cells were probed with at least five force measurements on each cell, and three replicates were conducted *per* condition ($n = 45$).

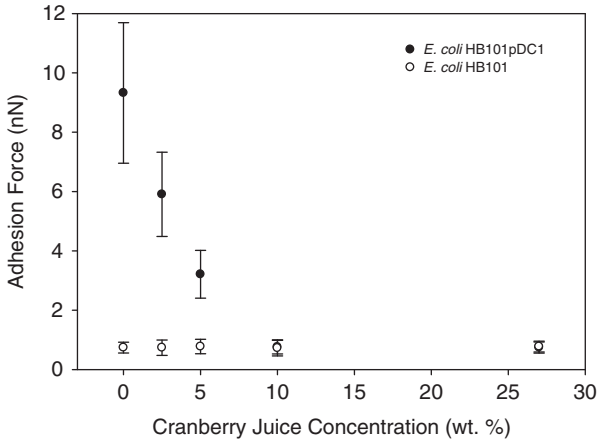

Figure 3. Average adhesion forces between *E. coli* and uroepithelial cells as a function of cranberry juice concentrations for *E. coli* HB101pDC1 (closed circles) and *E. coli* HB101 (open circles). Data are mean ± SD values.

HB101pDC1 and uroepithelial cells was 9.32 ± 2.37 nN, while it was only 0.74 ± 0.18 nN between *E. coli* HB101 and uroepithelial cells (Fig. 3). After CJC treatment, the adhesion forces between *E. coli* HB101pDC1 and uroepithelial cells decreased to 0.75 ± 0.19 nN in 27 wt% CJC. When we compared each condition with one another, adhesion forces in all concentrations of CJC were significantly different from one another ($p < 0.001$) except for the pairwise comparison of adhesion forces in 10 and 27 wt% CJC with one another ($p = 0.893$). The adhesion forces between non-fimbriated *E. coli* HB101 and uroepithelial cells were ~0.74 nN and did not change significantly as a function of cranberry juice treatment ($p = 0.794$).

3.2 Bacterial adhesion assay and correlation with adhesion force measurements

In the absence of CJC, P-fimbriated *E. coli* HB101pDC1 were more retained to uroepithelial cells than non-fimbriated *E. coli*, with an average of 50.2 ± 22.9 *E. coli* HB101pDC1 *per* uroepithelial cell, compared to 8.2 ± 5.5 *E. coli* HB101 *per* uroepithelial cell (Fig. 4). The number of retained P-fimbriated *E. coli* HB101pDC1 decreased as CJC concentration increased (Fig. 4), to a low value of 2.9 ± 1.5 retained bacteria *per* uroepithelial cell, corresponding to 27 wt% CJC. All treatment concentrations resulted in retention numbers that were significantly different from one another ($p < 0.001$). For HB101, the number of retained bacteria was low and insensitive to cranberry treatment (Fig. 4). The retained numbers of *E. coli* HB101 were not significantly different from one another for all CJC concentrations ($p = 0.100$), thus no correlation was made.

AFM adhesion forces and the number of *E. coli* retained on uroepithelial cells were correlated (Fig. 5). For *E. coli* HB101pDC1, higher adhesion forces corresponded with

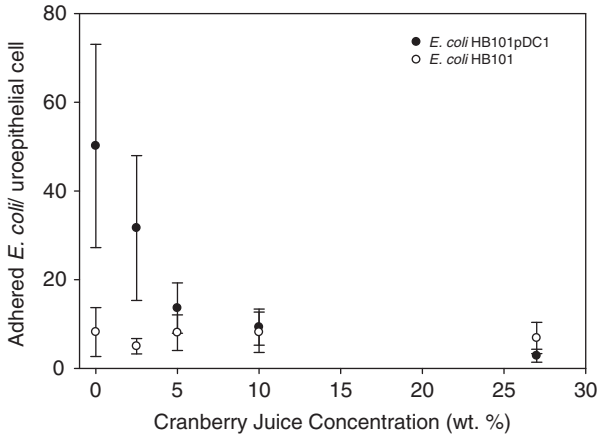

Figure 4. Average number of bacteria attached to uroepithelial cells as a function of cranberry juice concentration for *E. coli* HB101pDC1 (closed circles) and *E. coli* HB101 (open circles). Data are mean ± SD values.

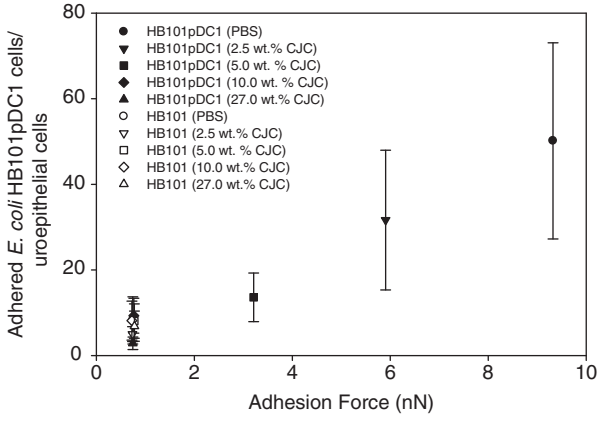

Figure 5. Correlation between adhesion force measurements and bacterial attachment assay as a response to exposure to various concentrations of CJC for P-fimbriated *E. coli* HB101pDC1 (closed symbols) and *E. coli* HB101 (open symbols).

more retained bacteria on uroepithelial cells, and there was generally a linear relationship between the two. For *E. coli* HB101, no correlation could be made since the adhesion forces and the number of retained bacteria were low and did not change significantly with various cranberry juice treatments ($p = 0.794$).

4 Discussion

4.1 Adhesion forces between P-fimbriated *E. coli* and uroepithelial cells

E. coli HB101pDC1 express P fimbriae, which are able to move due to Brownian motion and other intermolecular forces. P fimbriae appeared to form multiple ligand–receptor bonds with uroepithelial cells when the *E. coli*-functionalized AFM force probe was contacted with uroepithelial cells. Fimbriae are the primary adherence factors encoded by uropathogenic strains of *E. coli*, which play an important role in mediating initial bacterium-uroepithelial cell adhesion. Acute pyelonephritis is associated with P-fimbriated *E. coli*, since the adhesin PapG forms a specific bond with the glycolipid galabiose (α-D-galactopyranosyl-(1–4)-β-D-galactopyranoside) present on uroepithelial cells and red blood cells [42]. The function of P fimbriae is mainly to help bacteria overcome host mechanical defenses, such as shear forces created by urine flow. Therefore, the biomechanical properties of P fimbriae are important and have been previously studied [43, 44]. For example, Andersson *et al.* investigated how the PapA subunit of P fimbriae responds to various elongation speeds and the unfolding and refolding mechanism of pili [43, 44]. Bjornham *et al.* attached a single P-fimbriated *E. coli* to a PLL-treated 9.6 μm latex bead and measured the interaction with a 3.2 μm carboxymethyl latex bead that had been functionized with amino-galabiose [31]. From optical tweezers experiments, they showed that the PapG-galabiose bond strengths were 45 and 32 pN at 5.0 and 0.5 μm/s unfolding velocities, respectively [31]. Lugmaier *et al.* covalently bonded amino-galabiose on a silicon nitride AFM tip and P-fimbriated *E. coli* on a glass slide *via* EDC/NHS (1-ethyl-3-[3-(dimethylamino)propyl]carbamide/*N*-hydroxysuccinimide) [32]. Using AFM they found that the specific bond had strength of ~49 pN at a retraction velocity of 0.5 μm/s [32]. Our study is the first to measure the forces between the P-fimbriated *E. coli* and whole uroepithelial cells. We found that the adhesion force between P-fimbriated *E. coli* and a layer of uroepithelial cells in PBS is 9.32 ± 2.37 nN. If we estimate the single bond strength of the PapG-galabiose interaction as 41 pN from previously reported data (average of 32 and 49 pN) measured at a similar velocity, then the adhesion force we found should correspond to roughly 150–259 fimbriae *per* bacterium. This value is in agreement with the number of pili expressed by a typical P-fimbriated *E. coli* [45].

Since the main function of fimbriae is to help bacteria withstand the shear flow in the urinary tract, we can compare the relative magnitude of shear stress and the adhesion forces. For laminar flow *in vivo*, the physiological shear rates in the urinary tract can range from 40 to 2000 S^{-1} (*i.e.* shear stress ranging from 0.16 to 7.62 pN/μm^2) [46]. The urethra is unable to adapt to different pressures and forces of urinary flow and hence avoids turbulence by reducing the Reynolds number [47]. A laminar flow pattern has been observed in children with healthy voiding hygiene [48]. Therefore, we estimated the shear stress in the urinary tract assuming laminar flow conditions. A shear stress of 4 pN/μm^2 corresponds to a total drag force of 130 pN on a bacterium [49]. Hence, the drag force created by the physiological flow can reach up to 247.65 pN at 2000 s^{-1} shear rate, which is one order of magnitude smaller than the adhesion force we measured between P-fimbriated *E. coli* and uroepithelial cell. Our results suggest that P fimbriae can offer bacteria a way to bind very strongly to host cells

and that this bond cannot be disrupted by normal shear from urinary flow. However, for the non-fimbriated *E. coli* HB101, the adhesion force is of the same magnitude as the drag force in the flow. Hence, the non-fimbriated strain is likely to be removed by the flow of urine.

4.2 Role of cranberries in P-fimbriated *E. coli* adhesion

Recently, there has been evidence that the consumption of cranberry juice products aid in the prevention of UTIs [15, 18, 19, 50]. Researchers have focused on understanding the mechanisms of action of cranberries and cranberry compounds and there is evidence that these compounds have a biophysical and a biological effect on bacterial adhesion [20, 21, 23, 28, 37]. In our previous study published in 2008, we investigated P-fimbriated *E. coli* bacterial adhesion under the impact of cranberry juice [37]. Using a macroscale-based thermodynamic modeling approach, we found that the Gibbs free energy of adhesion increased after bacteria and uroepithelial cells were exposed to CJC. The thermodynamic modeling is based on physical interactions, and cannot account for ligand–receptor interactions that also occur between P-fimbriated *E. coli* and uroepithelial cells. Since the model was effective in predicting the adhesion behavior, we speculated that CJC disrupted the ability of P fimbriae to bind with the uroepithelial cell receptor. The single-cell investigation we carried out in the current study was designed to prove this hypothesis. As we measured much lower adhesive forces between P fimbriated *E. coli* and uroepithelial cells with the AFM, we conclude that CJC disrupts ligand-receptor bonds at the single-cell level.

Many *in vitro* bacterial adhesion assays incubated P-fimbriated *E. coli* with cranberry juice or urine after consumption of cranberry juice from 20 min [21], 30 min [51], and 90 min [25] to 3 h [37]. During this short period and in the absence of nutrients, the effects on P fimbriae were most likely biophysical, such as passive obstruction of binding sites on P fimbriae. Some studies cultured P-fimbriated bacteria in the presence of cranberry juice or compounds from cranberries. For example, Ahuja *et al.* cultured P-fimbriated *E. coli* on CFA solid medium containing 25% cranberry juice. After the third plating in this media, the bacteria did not appear to be expressing fimbriae in transmission electron microscopy images [52]. Johnson *et al.* cultured P-fimbriated *E. coli* in LB broth with 10% neutralized cranberry juice or 500 μg/mL PACs that were isolated from cranberries [53]. After 230 generations of cells, they could not observe fimbriae on the bacteria that were cultured in cranberry juice or PACs, according to electron microscopy imaging [53]. In another recent study, we tested the adhesion ability of P-fimbriated *E. coli* after culturing in growth medium supplemented with cranberry juice (10 wt% as final concentration) or PACs (128 μg/mL) [22]. Decreased attachment of P-fimbriated *E. coli* to uroepithelial cells was observed for bacteria that had grown in the presence of CJC or PACs [22]. This effect was reversible, since when P-fimbriated bacteria were regrown in CJC-free or PACs-free media, they regained the ability to attach to uroepithelial cells [22]. These studies indicated that cranberry juice and its compounds have biological and biophysical effects on P fimbriae.

While there are several *in vitro* studies that have incubated *E. coli* bacteria in urine after cranberry juice consumption for short periods of time, no study has investigated how the expression of P fimbriae or attachment of bacteria changes in the urine of a volunteer who has consumed cranberry juice. Other studies have focused on the upregulation or downregulation of genes after bacterial growth in urine [54], but no study has shown how consumption of cranberry juice leads to altered regulation of genes or changes the binding forces between P-fimbriated bacteria and epithelial cells.

4.3 Dose dependence of CJC's effects on adhesion

In the current study, we found that the adhesion forces between P-fimbriated *E. coli* and uroepithelial cells were related to cranberry juice concentration in a first order exponential decay manner. The *in vivo* non-linear dose–effect relationship has been previously observed. Zafriri *et al.* incubated Type 1 or P-fimbriated *E. coli* with CJC or fructose solution from 10 min to 90 min before conducting adhesion experiments [25]. They reported a linear relationship between inhibition of type 1 fimbriated bacterial adhesion and the natural logarithm of cranberry juice concentration. For P-fimbriated bacteria, they did not quantify the relationship but reported qualitatively decreased adhesion with increased CJC concentration and with increased incubation from 0, 2, 10, 15, and 30 to 90 min [25]. Howell *et al.* demonstrated that A-type PACs from cranberry juice can cause P-fimbriated *E. coli* to exhibit anti-adhesion properties [18, 20, 21]. In an *in vitro* study, PACs and cranberry metabolites found in urine post-consumption were able to prevent the adhesion of 80% of uropathogenic P-fimbriated *E. coli* isolates to uroepithelial cells, human red blood cells and resin beads coated with isolated P-receptor oligosaccharides [18]. The effects of cranberry juice and cranberry PACs also prevented the adhesion of 79% of antibiotic-resistant isolates in all bioassays, while the urine collected pre-consumption of cranberry juice did not prevent adhesion [18]. This anti-adhesion activity has not been observed in the urine of volunteers consuming other products, such as apple juice, grape juice, green tea, or dark chocolate, presumably since these products do not contain the A-type linkage that is characteristic of cranberries [21]. The nature of the metabolites after cranberry or PACs has been consumed and passed through the body has not yet been reported in the literature.

Concentrations between 5–10 wt% cranberry juice have shown significant bacterial adhesion inhibition activities

in vitro [21, 28, 37], as is also supported by our current studies. According to Howell *et al.*, commercially available CJC (27 wt% cranberry juice) contains 0.346 mg/mL PACs based on MALDI-TOF/MS and direct infusion/ESI-MS quantification methods [21]. We used the same commercial CJC product in our study; hence, the 27 wt% cranberry juice has 346 μg/mL PACs. According to this calculation, the other cranberry juice samples we tested (2.5, 5, and 10 wt%) contained 32.0, 64.1 and 128.0 μg/mL PACs, respectively. Gupta *et al.* reported a linear regression relationship between the natural logarithm of PACs concentration (5–75 μg/mL) and the number of retained P-fimbriated *E. coli*, which was equivalent to an exponential decay correlation [51]. PACs concentrations ranging from 6 to 375 μg/mL have been shown to have a potent *in vitro* anti-adhesion activity against antibiotic resistant strains of *E. coli* [18]. The anti-adhesive effect observed after consumption of cranberry juice persisted for up to 10 h in these *in vitro* studies [18]. However, further *in vivo* studies are needed to evaluate the dose of cranberry juice that would be effective in preventing the adhesion of bacteria to uroepithelia *in situ*.

5 Concluding remarks

Our study is the first to directly show that exposure to CJC decreases the nanoscale adhesion forces between P-fimbriated *E. coli* and uroepithelial cells. These results suggest that P-fimbriated bacteria cannot be removed from uroepithelial cells once they have attached, since the estimated drag force created by urine flow is one order of magnitude smaller than the adhesion force between P-fimbriated *E. coli* and uroepithelial cells.

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The authors have declared no conflict of interest.

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