Influence of Cranberry Derived Materials on the Motility of Uropathogenic *Escherichia coli*

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Abstract

Urinary tract infections (UTIs) are the most common type of infection acquired in hospitals and nursing homes. Uropathogenic *Escherichia coli* (UPEC) is the most common etiological agent of uncomplicated UTIs. Cranberry derived materials (CDMs), including a component in cranberry known as proanthocyanidin, have been linked to UTI prevention because of their anti-microbial, anti-infective, and anti-adhesive properties. In this thesis, the effect of CDMs on flagella-mediated motility of UPEC was investigated. Swimming and swarming motility, both dependent on flagella, are hindered when the bacteria are grown in the presence of CDMs. When *E. coli* are exposed to CDMs, a reporter of flagellin expression as well as qRT-PCR data consistently show a downregulation of the flagellin gene, *fliC*, which precludes flagella synthesis. These data were complemented with images of *E. coli* grown in the presence of CDMs, which revealed fewer flagella than on bacteria grown under control conditions.

CDMs were also incorporated into silicone, which is a common material used in the manufacture of urinary catheters. The bioactivity of the CDMs in the silicone was quantified by its ability to release CDMs into an aqueous environment and decrease *fliC* expression of *E. coli* grown in the presence of the CDM-incorporated silicone. Furthermore, CDM-incorporated silicone inhibited the swarming of *Proteus mirabilis* across the silicone surface.

Flagella-mediated motility has been suggested to enable bacteria to disseminate to the urinary tract, to escape host immune responses and to move to new sites within the urinary tract. Because CDMs inhibit flagella-mediated motility via downregulation of the flagellin gene, *fliC,* these findings could prove to be effective during *in vivo* settings for UTI prevention and for application in medical devices.

Résumé

Les infections urinaires (IU) sont le type le plus commun d'infection acquise dans les hôpitaux et maisons de soins infirmiers. L'uropathogène *Escherichia coli* (UPEC) est l'agent étiologique le plus commun des IU non-compliquées. Les matériaux dérivés des canneberges (MDCs), y compris un composant des canneberges connues sous le nom de proanthocyanidine, ont été liés à la prévention IU en raison d'être anti-microbiens, anti-infectieux, et à leurs propriétés anti-adhésives. Dans cette thèse, nous étudions l'effet des MDCs sur la motilité médiée par les flagelles des UPEC. La natation et la motilité essaimage, deux types de motilité dépendantes sur les flagelles, sont entravées lorsque les bactéries sont cultivées en présence des MDCs. Lorsque *E. coli* est exposés aux MDCs, un reporter d'expression ainsi que qRT-PCR montrent régulièrement une régulation négative du gène flagelline, *fliC*, ce qui empêche la synthèse des flagelles. Ces données ont été complétées par des images de *E. coli* cultivées en présence des MDCs ou dans des conditions de contrôle. Moins de flagelles étés observées sur les bactéries cultivées en présence des MDCs.

Les MDCs ont également été incorporées dans de la silicone, un matériau de cathéter commune. La bioactivité du MDCs dans la silicone a été quantifiée par sa capacité à libérer des MDCs dans un environnement aqueux ainsi que par sa capacité de réduire l'expression du gène flagelline, *fliC*, des *E. coli* cultivées en présence de la silicone intégrée des MDCs. De plus, la silicone intégrée de MDCs a inhibé l'essaimage de *Proteus mirabilis* à travers la surface de silicone.

La motilité médiée par les flagelles a été suggérée de permettre aux bactéries de diffuser à l'appareil urinaire, d'échapper aux réponses immunitaires de l'hôte et de les permettre à se déplacer vers de nouveaux sites au sein de l'appareil urinaire. Puisque les MDCs inhibent la motilité médiée par des flagelles par une régulation négative du gène flagelline, ces conclusions pourraient se révéler efficaces dans les milieux *in vivo* pour la prévention UTI et pour l'application dans les dispositifs médicaux.

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Contribution of Authors

The thesis is divided into two manuscripts, the first manuscript has been accepted by *Applied and Environmental Microbiology* and the second manuscript will be submitted to *Langmuir*. Authorship of the first manuscript is Michelle Chan, Gabriela Hidalgo and Nathalie Tufenkji. Authorship of the second manuscript is Michelle Chan, Gabriela Hidalgo, Sergio Almeida, Naser Muja, Showan N. Nazhat and Nathalie Tufenkji.

Experimental work was conducted by Michelle Chan as well as the majority of data analysis and writing of the manuscripts. Gabriela Hidalgo supported with the RNA extraction and qPCR experiment as well as writing and editing of the manuscript. Sergio Almeida and Naser Muja showed me how to formulate silicone. Nathalie Tufenkji and Showan Nazhat helped throughout in supervision of the experiments and editing the manuscript text.

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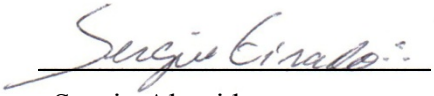

Sergio Almeida

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List of Abbreviations

BG	biomedical grade
CDM	cranberry derived material
CP	cranberry powder
cPAC	cranberry proanthocyanidin
DW	distilled deionized water
IG	industrial grade
LB	Luria-Bertani
OD_{280}	optical density at 280nm
OD_{600}	optical density at 600nm
PAC	proanthocyanidin
UPEC	uropathogenic *Escherichia coli*
UTI	urinary tract infection

Chapter 1: Introduction

1.1 Urinary tract infections and North American Cranberry

Urinary tract infections (UTIs) are an important health concern with approximately 150 million cases of UTIs occurring each year on a global basis [1] and with associated healthcare costs estimated at $1.6 billion [2]. More than 80% of these infections are caused by uropathogenic strains of *Escherichia coli* [3]. Uropathogenic *E. coli* (UPEC) biofilms are responsible for many catheter-associated and chronic UTIs because the biofilm mode-of-life protects the microorganisms from the host immune response and antimicrobials [4]. *E. coli* CFT073 is a uropathogenic strain that was isolated from the blood and urine of a woman with acute pyelonephritis [5]. Its complete genome has been sequenced [6] and has been shown to be particularly rich in genes that encode potential fimbrial adhesins, autotransporters, iron-sequestration systems, and phase-switch recombinases.

The main method to treat UTIs is the prescription of low-dose antibiotic regimes; however, bacteria may acquire antibiotic resistance [7–9]. Therefore, there is a great interest among the scientific and medical communities in developing treatments that will prevent UTIs by inhibiting bacterial adhesion [10], particularly, using dietary inhibitors of adhesion [11]. North American cranberries, *Vaccinium macrocarpon*, have been used for centuries as a common home remedy in UTI prevention and treatment [12]. *In vivo* and *in vitro* studies have shown that the consumption of cranberry juice may inhibit the onset of UTIs

through their anti-adhesion effects on uropathogenic bacteria to uroepithelial cells [13–16]. It has been suggested that proanthocyanidins (PACs), which are condensed tannins found in cranberry, are the group of molecules responsible for its anti-adhesive properties [13]. However, the mechanism by which cranberries or cranberry PACs (cPACs) prevent bacterial infection is not yet well understood.

1.2 Motility

Bacteria can move by five different mechanisms: swarming, swimming, twitching, gliding or sliding, as shown in Figure 1 [17]. The flagellum is the rotating motor used for swarming and swimming motility. The difference between swarming and swimming is that swarming is a coordinated, multicellular movement across a surface whereas swimming occurs when individual cells move in liquid environments. Type IV pilus is the motor that is used for twitching. The pilus extends and retracts to slowly move across the surface [18]. Gliding is the active movement of cells without flagella or pili across a surface and it involves focal-adhesion complexes that bind to a substrate surface [19]. Sliding is a kind of passive surface translocation mediated by cell growth and involves surfactants that enable the colony to spread away from the origin by reducing surface tension [20].

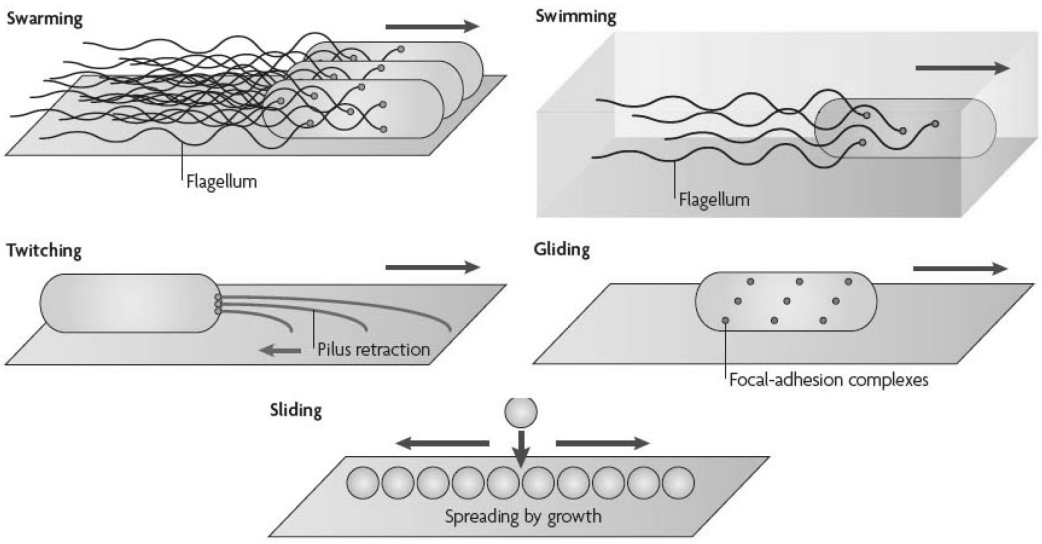

Figure 1 Various mechanisms that bacteria use to move [17]

Motility relates to several virulence factors in pathogenic bacteria and contributes to colonization of host organisms or target organs because it facilitates the spread of the pathogens to favourable environments and is assumed to promote initial cell-to-surface contact [21]. Flagellar-mediated motility is a widespread phenotype in bacteria. The structure and arrangement of flagella are dependent on the environment in which the bacteria reside and are specific for every bacterial species [22,23].

1.2.1 Flagella

Flagella are complex organelles that are encoded by more than 40 genes [24]. They have been associated with motility, which allows bacteria to move to favourable environments for colonization. Information about attractants and repellents is conveyed to the flagellar motors through a signal transduction

network, allowing chemotactic movement in response to external chemical stimuli [25].

Genes for flagellum synthesis form a highly ordered cascade, where the expression of one gene at a given class requires the transcription of another gene at a higher class [26]. There are three classes of flagellar genes in *E. coli*. Class I genes form the *flhDC* master operon, which encode the transcriptional activator of the class II genes. Class II genes encode the flagellar basal body and the hook, FliA and FlgM. The *fliA* gene encodes a sigma factor (σ^{28}) and *flgM* encodes an anti-sigma factor, which are necessary for the transcription of class III flagellar genes [27–29]. The anti-sigma factor ensures that the flagellar basal body and hook are completed before the transcription of class III genes [30]. Class III genes encode the components of the flagellar filament, and other proteins associated with motility and chemotaxis (e.g. MotA, MotB, MotAB, CheW, and CheY) [26].

The *fliC* gene encodes the flagellin subunit, which is the principal component of the flagellar filament [31]. This means that without the transcription of *fliC*, there will be no flagellar filament and the bacteria become nonmotile [32]. Two studies have found that *fliC* and flagellum-mediated motility greatly enhance the persistence and fitness of UPEC during pathogenesis in a mouse cystitis model and allow the bacteria to outcompete strains lacking *fliC* [32,33]. Flagellar motility also assists UPEC in the ascent from the bladder to the kidneys [34,35]. In a study by Hagen *et al* [36], *fliC* gene expression was found to be

downregulated during infection *in vivo* compared to *in vitro* culture, which is the same conclusion reached by Snyder [37] and Lane [32]. A probable explanation for this phenomenon is that, *in vivo*, the flagella and flagellum-mediated motility and chemotaxis are initially expressed within the urinary tract to aid in motility and colonization but then are quickly repressed to avoid the activation of the hosts' immune response [32,34].

1.2 Thesis Objectives

The objective of this thesis was to investigate the role of cranberry derived materials (CDMs) on bacterial behaviour, and in particular, the effect of CDMs on bacterial motility. More specifically, the three main goals were:

1. To evaluate the effect of CDMs on flagella-mediated motility.
2. To evaluate the effect of CDMs on *fliC* expression of an uropathogen.
3. To embed CDMs in a common catheter material and evaluate its bioactivity.

1.3 Thesis Layout

This thesis is manuscript based and has been organized into four chapters. Chapter 1 introduces the topic and outlines the rationale and research objectives. Chapter 2 reports the effect of CDMs on *fliC* expression and flagella-associated motility of the uropathogen *E. coli* CFT073. Chapter 3 focuses on the incorporation of CDMs into silicone and examination of the release and bioactivity of the CDM-incorporated silicone. Chapter 4 is the conclusion of the thesis.

Chapter 2: Cranberry Materials Inhibit Escherichia coli CFT073 *fliC* Expression and Motility

2.1 Introduction

There are more than 150 million cases of UTIs in the world every year with correspondingly significant morbidity and health care costs [1]. Uncomplicated UTIs, *i.e.*, infections that occur in healthy, immunocompetent individuals, are caused over 80% of the time by UPEC [38]. Among the UPEC strains, *E. coli* CFT073, a strain isolated from the blood and urine of a woman diagnosed with acute pyelonephritis [5], is one of the most prevalent UPEC clonal lines [39,40]. The majority of UTIs develop in an ascending manner [38,41] that commences when bacteria inoculate the periurethral area and then the bladder [42–44]. The bacteria may then ascend to the upper urinary tract and kidneys and establish a secondary infection [38]. Once in the kidneys, the bacteria can access the bloodstream causing bacteremia and potentially death [38].

The bacterial flagellum is a molecular machine driven by a motor which rotates a long, curved filament [45]. This filament extends from the basal body outward and is a polymer of flagellin subunits encoded by the *fliC* gene [46,47]. Mutations in *fliC* result in loss of flagellation and motility [47]. Flagellum-mediated motility has been suggested to contribute to virulence by enabling UPEC to disseminate to the urinary tract, to escape host immune responses and to disperse to new sites within the urinary tract [35]. Even though this hypothesis remains to be

demonstrated, several groups have shown that *fliC* mutants are out-competed by motile, wild-type strains during experimental co-challenge of mice [33–35], thereby demonstrating that flagella provide a fitness advantage in the colonization of the urinary tract. Furthermore, flagellar-motility has been shown to be essential for the pathogenesis of other bacteria, including *Proteus mirabilis* [48,49], *Salmonella* species [50,51], *Helicobacter pylori* [52], and enteropathogenic *E. coli* [53].

The standard management approach for uncomplicated UTIs is empiric therapy with antibiotics [54,55]; however, current rising antimicrobial resistance [8,56] has resulted in increasing clinical failure rates [7,9], emphasizing the need to develop alternate options for infection prevention and treatment. North American cranberries (*Vaccinium macrocarpon*) have long been considered to have protective properties against UTIs. Some of the first scientific research conducted on this topic concluded that acidification of the urine following the consumption of cranberries was responsible for the preclusion of UTIs [57]; however, subsequent studies showed that the pH of urine is not significantly altered by the intake of cranberries [8,58]. Several studies have also demonstrated that cranberry products are non-bacteriostatic [59,60]. Current research supports the hypothesis that cranberries, and specifically, the condensed tannins that they contain and that are known as PACs, hinder bacterial attachment to abiotic surfaces [61,62] and uroepithelial and kidney cells [13,63–65]; therefore, preventing infection.

However, a mechanistic understanding of the way in which cranberries influence bacterial behavior is still lacking.

With the purpose of elucidating how bacterial gene expression is affected by cPACs, our laboratory previously examined the transcriptional profile of *E. coli* CFT073 cultures grown in Luria-Bertani (LB) broth and in the presence or absence of cPACs [59]. Among the genes that were found to be downregulated in the presence of cPACs was the flagellin gene, *fliC* [59]. Additional research has shown that, in *Pseudomonas aeruginosa,* swarming motility is blocked by cPACs and other tannins [60]. The main goal of this study was to further investigate if exposure to cranberry materials would result in downregulation of the *fliC* gene in *E. coli* CFT073 and whether this would impact this bacterium's motility.

2.2 Materials and Methods

2.2.1 Bacterial Strains and Media

The strains and plasmids used in this study are detailed in Table 1. *E. coli* strain CFT073 (ATCC 700928) was used as the test bacterium in this study. Cultures were grown in LB medium (10 g/L tryptone, 10 g/L NaCl and 5 g/L yeast extract) or in M9 minimal medium as indicated. M9 medium contained, per liter: 12.8 g $Na_2HPO_4{\cdot}7H_2O$, 3 g KH_2PO_4, 0.5 g NaCl, 1.0 g NH_4Cl, 0.24 g $MgSO_4$, 0.01g $CaCl_2$ and 4 g of glucose as the sole carbon source. The pH of all the M9 growth media used in this study, whether it was supplemented or not with cranberry powder or cPACs, was adjusted to 7.1 with NaOH as required using an Accumet

AR20 pH meter. Planktonic bacterial cultures were incubated at 37°C and rotary shaking at 150 rpm unless otherwise indicated. Ampicillin and kanamycin were supplemented as needed at final concentrations of 100 μg/mL and 50 μg/mL, respectively. Dehydrated, crushed cranberry powder (Canneberges Atoka Cranberries, QC, Canada) and dry cPACs extract purified by HPLC (Marucci Center for Blueberry and Cranberry Research, Rutgers University) were solubilized in distilled, deionized water and sterilized by filtration.

2.2.2 Growth curves

Cultures of *E. coli* CFT073 were grown in the presence or absence of cPACs at 0.1 mg/mL and cranberry powder (CP) at 1, 5, 10, 15 and 20 mg/mL. An overnight culture of CFT073 grown at 37°C with shaking at 200 rpm was diluted 1000 fold with M9 media. This cell suspension, containing approximately 3×10^6 cells/mL, was aliquoted into sterile 96-well plates and incubated at 37°C until stationary phase was reached (~12 hours). Optical density at 600 nm (OD_{600}) was recorded at periodical intervals using a TECAN Infinite M200 Pro (Tecan Group Ltd., Männedorf, Switzerland). Each condition was set up in quadruplicate.

2.2.3 *fliC* downregulation

An overnight culture of *E. coli* strain CFT073 *pfliC-lux* [35], grown as described above, was diluted 1000 fold. Aliquots of the cell suspension (3×10^6 cells/mL) were mixed with CP at 0, 1, 5, 10, 15 and 20 mg/mL or with cPACs at 0.1 mg/mL and the cultures were incubated at 37°C in a 96-well white polystyrene plate with

clear bottom. Luminescence and OD_{600} were measured periodically using a TECAN Infinite M200 Pro (Tecan Group Ltd., Männedorf, Switzerland) for 12 hours. Expression of the flagellar gene *fliC* was quantified by measuring the luminescence and normalizing it to cell concentration, which was calculated by subtracting the initial OD_{600} reading from every OD_{600} time point ($Expression_{fliC}$ = Luminescence/(OD_{600}-$OD_{600\ initial}$)). This was done to account for the variability in cell numbers as well as for the changes in OD_{600} generated by the addition of CP or cPACs.

The response of the *fliC-lux* reporter was also tested by growing the bacteria to mid-exponential phase (OD_{600}-$OD_{600\ initial}$ ≈ 0.25) in M9 media, harvesting the cells and spiking them with cPACs or with CP at the same concentrations described above. Luminescence and optical density were recorded for the following 5 hours and *fliC* expression was quantified as described above.

2.2.4 RNA Extraction, cDNA Synthesis and Comparative qPCR

A culture of *E. coli* CFT073 that was grown overnight was diluted 1000 fold in M9 media. Aliquots of this suspension (approximately 3×10^6 cells/mL) were mixed with CP at 0, 5, 10, and 20 mg/mL or with cPACs at 0.1 mg/mL and incubated at 37°C with agitation to mid-exponential phase (OD_{600}-$OD_{600\ initial}$ ≈ 0.25). Total RNA was extracted using TRIzol® (Invitrogen, Carlsbad, California) following the manufacturer's instructions. After elution, nucleic acid concentrations and quality were determined by spectrophotometry using an

Eppendorf BioPhotometer Plus (Eppendorf, Hamburg, Germany). Expression of target genes (*fliC* and housekeeping gene *gapA*) was quantified using two-step qRT-PCR analysis. Total RNA (200 ng) was mixed with 0.25 ng of random hexamers (Invitrogen, Carlsbad, California) and reverse-transcribed with 200 U of Superscript Reverse Transcriptase II (Invitrogen, Carlsbad, California) and RNasin (Promega). The equivalent of 2 ng of total RNA was loaded with TaqMan Universal PCR Master Mix (Applied Biosystems) per well and qRT-PCR was processed with ABI Prism 7900 HT (Applied Biosystems). Conditions for qRT-PCR were as follows: 50°C for 2 minutes, initial denaturation at 95°C for 10 minutes, and 45 cycles of 15 seconds at 95°C and 1 minute at 60°C. Results were analyzed with SDS software, version 2.2 (Applied Biosystems). Data were normalized to the endogenous reference gene *gapA* and analyzed by the threshold cycle method ($2^{-\Delta\Delta CT}$) [66]. Three independent isolated cDNA samples were analyzed. Sequences of the primers used to amplify *gapA* and *fliC* are detailed in Table 1.

Table 1 *E. coli* strains and plasmids used in this study

Strains	Relevant genotype	Source or reference
CFT073	Wild type pyelonephritis isolate	Laboratory collection
CFT073 Δ*fliC*	CFT073 *fliC*::*aphA*; kan^R	[35]
Plasmids		
P_{fliC}-*lux*	flagellin-transcription reporter vector; amp^R	[35]
Primers		
fliC F	5'-ACAGCCTCTCGCTGATCACTCAAA-3'	[35]
fliC R	5'-GCGCTGTTAATACGCAAGCCAGAA-3'	[35]
gapA F	5'-AAGTTGGTGTTGACGTTGTCGCTG-3'	[35]
gapA R	5'-ATAACCACTTTCTTCGCACCAGCGG-3'	[35]

2.2.5 Electron Microscopy

For transmission electron microscopy, bacteria from the outer motility rings of 0.5% agar LB plates supplemented with 0.5% glucose were cultured in LB with and without CP at 10 mg/mL in a rotary shaker set at 200 rpm and 30°C for 16 h. Next, 5 μL of this bacterial suspension and 5 μL of 2.5% glutaraldehyde were placed on carbon-coated copper grids (SPI Supplies, West Chester, PA) for 2 minutes. Bacteria were stained with 1% uranyl acetate for 1 minute. The grids were examined with a FEI Tecnai 12 transmission electron microscope at an operating voltage of 120kV. Digital images were captured with a Gatan Bioscan CCD camera Model 792.

2.2.6 Motility

Swimming and swarming motility were evaluated using soft-agar plates. For the swimming assays, 0.25% agar, M9 plates with CP added at concentrations ranging from 1 to 20 mg/mL or cPACs at 0.1 mg/mL were allowed to dry at room temperature overnight before use. Swimming plates were seeded with overnight cultures of *E. coli* CFT073 using a sterile inoculating needle. Control plates without CP or cPACs were also set up. Swarming motility was assessed in 0.5% Eiken agar (Eiken Chemical, Tokyo, Japan), LB plates supplemented with 0.5% glucose. CP or cPACs were also added at the concentrations described above. An overnight culture of *E. coli* CFT073 was diluted 1000 fold and incubated until early stationary phase (OD_{600}-$OD_{600\ initial} \approx 0.5$). At that point, 5 μL of the culture were spotted onto the surface of the plates. Swimming and swarming plates were incubated at 30°C or 37°C, respectively, and the motility recorded at various time points.

2.3 Results and Discussion

A whole transcriptome analysis of UPEC CFT073 grown in LB in the presence or absence of 0.1 mg/mL cPACs was previously conducted by our lab [59]. The study revealed that in the culture grown in cPACs, the flagellin gene, *fliC*, was downregulated 2.4 fold [59]. Given that several works have demonstrated that flagella play an important role during ascending UTIs [33–35], we decided to further investigate the downregulation of *fliC* during bacterial growth in cranberry materials.

Our prior work showed that, in LB, concentrations of cPACs as high as 1.6 mg/mL did not inhibit the growth of CFT073 [59]. However, LB is an undefined medium that is rich in essential elements [67]. So, to test whether cPACs or CP added to M9, a defined, minimal medium with no added trace metals, would hinder bacterial growth, CFT073 was grown in cPACs or CP at various concentrations. As may be seen in Figure 2, bacterial growth rates and yields were not significantly altered by the introduction of either cPACs or of CP at any of the working concentrations. This is an important result because conditions that are adverse to growth, such as high temperature, high concentrations of salts or of certain alcohols or the presence of gyrase inhibitors, have been reported to inhibit motility [68,69]. Based on these results, we concluded that the differences in gene expression and phenotype that were observed in the bacterial cultures exposed to cranberry compounds did not result from toxic effects of these compounds.

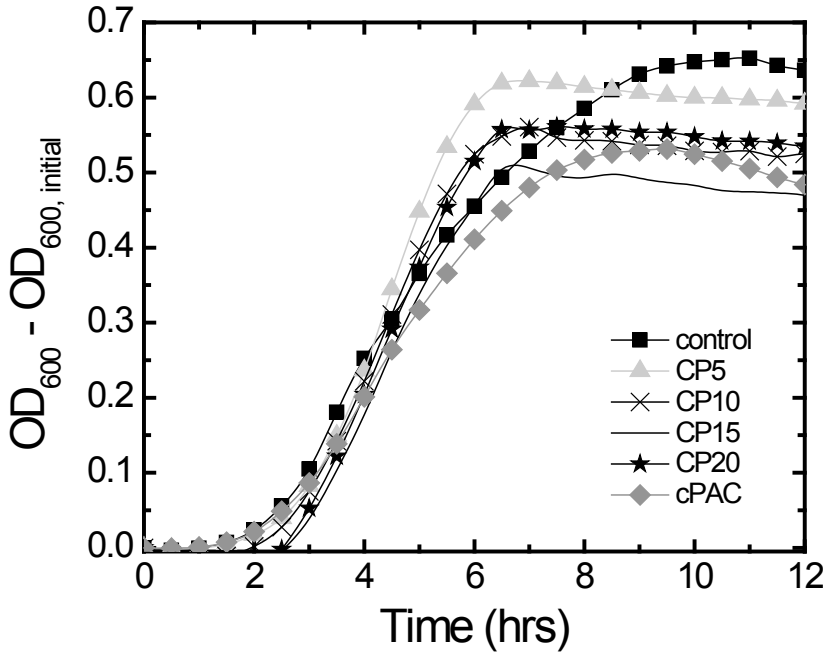

Figure 2 Growth curves of *E. coli* CFT073 in cranberry compounds. OD_{600} - $OD_{600, initial}$ versus time for a culture of *E. coli* CFT073 grown in M9 medium (■), supplemented with cPACs at 0.1 mg/mL (◆), or CP at 5 (▲), 10 (×), 15 (–) or 20 (★) mg/mL. Results are representative of at least three independent experiments. Abbreviations: CP5, cranberry powder at 5 mg/mL (number indicates cranberry concentration in mg/mL); cPAC, cranberry proanthocyanidins at 0.1 mg/mL

The effect of cPACs or CP in the regulation of *fliC* transcription was first assayed using a luminescent *fliC* reporter [35]. A culture of CFT073 harboring the P_{fliC}-*lux* plasmid was grown in M9 and cPACs at 0.1 mg/mL or CP at various concentrations (Figure 3A). Our results show that the normalized luminescence decreased with increasing CP concentration. Also, the normalized luminescence was lower when the bacteria were grown in cPACs relative to growth in M9 alone. Because a decrease in the transcription of *fliC* in the *fliC-lux* reporter correlates with a decrease in the luminescent signal, our data supports the hypothesis that growth in the presence of CP or purified cPACs results in a

reduction of *fliC* expression. These results were further validated by conducting a reverse transcription PCR (RT-PCR) to determine the relative quantities of *fliC* transcripts in the mRNA extracted from UPEC cultures grown in the presence or absence of cPACs or CP (Figure 3B). Relative mRNA expression of *fliC* was normalized to a stable housekeeping gene, *gapA*. The RT-PCR confirmed that in the presence of 0.1 mg/mL of cPACs, *fliC* was downregulated. Also, the flagellin gene was observed to be significantly downregulated when the bacteria were grown in M9 supplemented with CP at the concentrations tested (5, 10 and 20 mg/mL).

To further assess the conditions under which cranberry compounds may reduce *fliC* transcription, CFT073 P_{fliC}-*lux* bacteria were grown in M9, harvested, and then spiked with CP or cPACs. Luminescence and OD_{600} were measured as described above and the normalized luminescence calculated (Figure 3C). The results obtained show that, relative to the control, a spike with cPACs (0.1 mg/mL) or with 5 mg/mL or more of CP reduce the normalized luminescence. Based on this data, we concluded that growth in CP or in cPACs is not necessary to achieve a reduction in the level of expression of the flagellin gene.

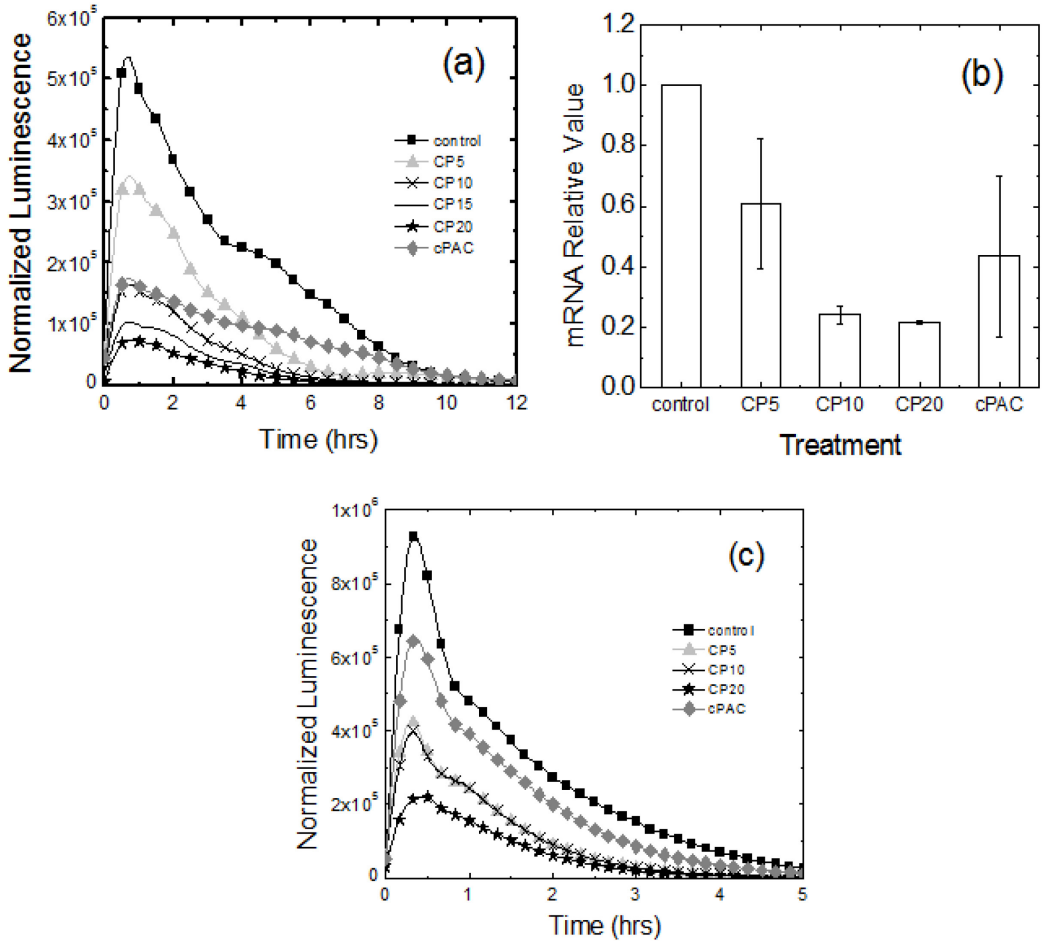

Figure 3 Downregulation of *fliC* expression by cranberry compounds. (a) Planktonic cultures of CFT073 P_{fliC}-*lux* were grown in M9 at 37°C and in the presence (◆) or absence (■) of 0.1 mg/mL cPACs or cranberry powder at 5 (▲), 10 (×), 15 (–) or 20 (★) mg/mL. Induction of the *fliC* gene was calculated as follows: Normalized luminescence = (Luminescence/(OD_{600}-$OD_{600\ initial}$)). Three independent experiments were performed; the results of a typical experiment are presented. (b) Expression of the *fliC* gene was also assessed on the mRNA level using quantitative reverse transcription-polymerase chain reaction. Relative mRNA expression of *fliC* was first normalized by a stable housekeeping gene, *gapA*, and then related to the normalized expression level of the same gene in control treatment. Results represent mean values of three independent experiments and mean values are expressed ± standard deviation (SD). (c) *fliC* induction versus time of CFT073 P_{fliC}-*lux* cultures grown in M9, harvested at mid-log phase and spiked with sterile water (■), 0.1 mg/mL cPACs (◆) or cranberry powder at 5 (▲), 10 (×), 15 (–) or 20 (★) mg/mL. Similar results were obtained in at least three other experiments.

Additional evidence that growth of UPEC in media supplemented with cranberry compounds precludes flagellar synthesis was obtained by using electron microscopy to image bacteria cultivated under conditions that optimize motility as described by Lane *et al*. [70]. Abundant flagella were observed in bacteria that were grown in the control medium (Figure 4A); however, in the samples that were obtained from bacteria grown in CP, few or no flagella were imaged (Figure 4B). This result agreed with our expectations because if exposure to cranberry materials results in a decrease in the transcription of the flagellin gene, less flagellar filaments should be synthesized by the bacteria in the presence of those materials.

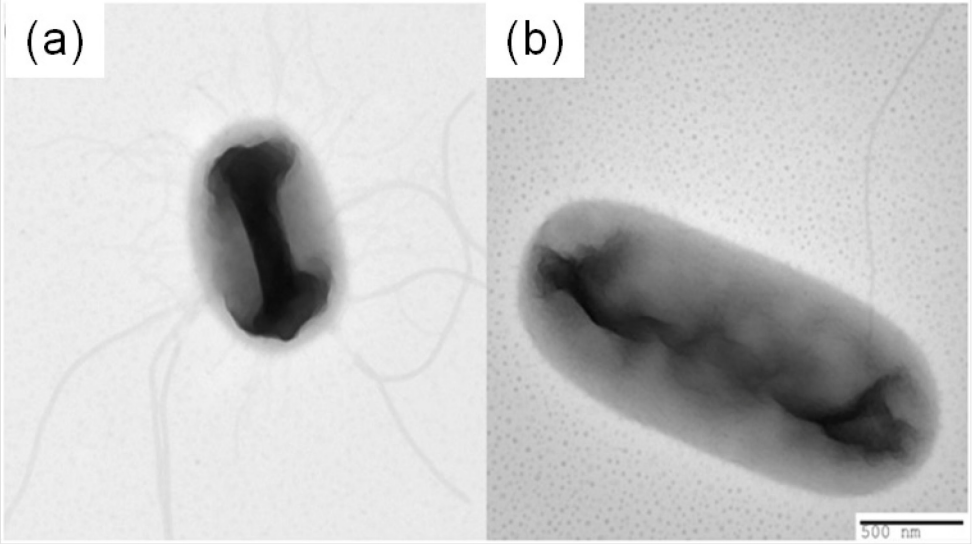

Figure 4 Electron microscope images of CFT073 cells negatively stained with 1% uranyl acetate. Samples were visualized at a magnification of ×43700. Bacteria were cultured from motility agar and grown to early exponential phase in (a) LB broth alone, or (b) supplemented with 10 mg/mL cranberry powder at 30°C with shaking (200 rpm). Bar, 500 nm

Next, we set out to evaluate whether the downregulation of *fliC* that results from growth or exposure to cranberry compounds would impair bacterial motility.

Swimming motility is a type of bacterial movement that is powered by rotating flagella and that takes place as individual cells move in liquid environments [17]. Swarming is also a type of motility that is powered by rotating helical flagella; however, it differs from swimming in that it requires an increase in the number of flagella per cell, the secretion of surfactants to reduce surface tension and allow spreading, and in that the movement occurs in a coordinated manner across a surface [17]. Because flagella are essential for both swimming and swarming, the effect of cranberry compounds on both of these motility phenotypes was tested. Figure 5A shows that concentrations of CP higher than 10 mg/mL completely blocked swimming motility. Moreover, the figure illustrates how, relative to the control, 0.1 mg/mL of cPACs also resulted in a decrease in motility. Figure 5B shows representative images of swimming motility plates of CFT073 in the absence and presence of CP (10 mg/mL) or cPACs (0.1 mg/mL). An image of CFT073 Δ*fliC* grown under control conditions was included to illustrate that CP blocked the swimming motility in a similar manner as a *fliC* gene deletion.

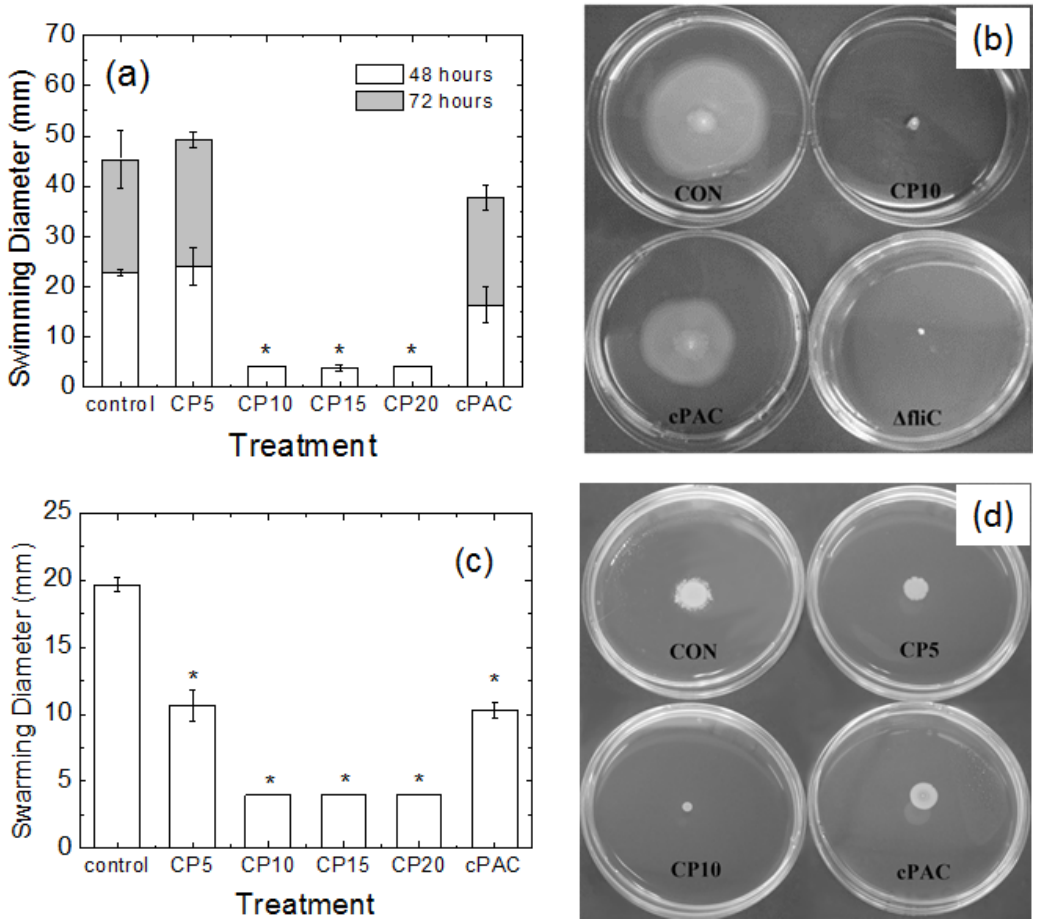

Figure 5 Effect of cranberry compounds on motility. (a) Characterization of the effects of cranberry powder or cPACs on swimming motility in soft agar, M9 plates. Asterisk, $p<0.001$, one-way ANOVA, as compared with the control (M9 alone). (b) Swimming motility plates for the control (CON), cranberry powder at 10 mg/mL (CP10) and cPACs at 0.1 mg/mL (cPAC). An image of the results obtained with a *fliC* mutant is included. (c) Swarming motility of CFT073 cells inoculated onto LB medium containing 0.5% (wt/vol) glucose and 0.5% (wt/vol) Eiken agar and incubated for 18 hours at 37°C. Error bars represent the SD of triplicate samples. (d) Swarming motility plates for the control, cranberry powder at 5 and 10 mg/mL and cPACs at 0.1 mg/mL.

Evaluation of swarming motility was tested in LB plates instead of in M9 because attempts at evaluating swarming motility in M9 were not successful (data not shown). This is not surprising given that swarming motility has been shown to require a glucose-supplemented, energy-rich medium [25,71] and swarming-cell differentiation has been shown to be repressed in some standard laboratory media

[17,71]. The swarming motility tests revealed that the swarming of UPEC CFT073 was impaired by cPACs and by CP in a dose-dependent manner as depicted in Figure 5C. It is noteworthy that the inhibitory effect on swarming motility of both CP and cPACs was more pronounced than on swimming motility, *i.e.* CP at 5 mg/mL was enough to significantly reduce swarming motility but higher concentrations (10 mg/mL) were required for significant reduction of swimming motility. Swarmer cells are hyperflagellated but one flagellum is sufficient for swimming motility [17,25,72]. It is possible that the decrease in expression of *fliC* upon exposure to CP still allows for the synthesis of enough flagellar filaments that enable bacteria to swim but makes swarming prohibitive.

All of our experiments were conducted with HPLC-purified cPACs but also with dried, crushed cranberries. The rationale for testing both sources of cPACs was to test whether highly purified cPACs would elicit the same response as whole cranberries. As our data shows, with regards to *fliC* expression and motility, similar results were achieved whether cPACs or CP were utilized. These results highlight the role that consumption of whole, unpurified cranberries might have in the prevention and treatment of UTIs.

Cranberry ingestion has long been associated with the prevention of UTIs; however, a mechanistic understanding of the way in which the consumption of cranberries results in UTI prevention is achieved remains elusive. Recent research has focused on the inhibitory effect that PACs from cranberries, blueberries and

other *Vaccinium* species have on the adherence of *E. coli* to epithelial cells and surfaces [61,63–65]. The anti-adhesive properties of cranberries have been attributed to inhibition of p-fimbriae expression [62,64,73]. However, a relationship between cranberry compounds and the downregulation of the flagellin gene has not been reported.

In this study, we demonstrate that growth or exposure to cPACs or CP results in the downregulation of the flagellin gene *fliC*. Moreover, we show that the decrease in the level of *fliC* expression precludes the synthesis of flagella which then results in hindered swimming and swarming motility. The cause of the downregulation of *fliC* transcription and the accompanying decrease in motility as a result of the exposure to cranberry compounds remains to be determined. There is compelling evidence that UPEC express flagellin and utilize flagellum-mediated motility to ascend to the upper urinary tract during UTI [33–35]. Therefore, we hypothesize that the prevention of UTIs that can result from the consumption of cranberries is linked with a decreased expression of *fliC*. Before this hypothesis is tested, additional research needs to be conducted to determine whether *fliC* is downregulated by cranberry compounds *in vivo*.

2.4 Acknowledgement

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Chapter 3: Inhibition of bacterial motility via release of cranberry derived materials from silicone substrates

Preface

Cranberry derived materials inhibit flagella-mediated motility of *E. coli* via the downregulation of *fliC* expression, as demonstrated in Chapter 2. Chapter 3 describes the incorporation of cranberry derived materials into silicone and the investigation of the bioactivity of the cranberry derived materials following said incorporation.

3.1 Introduction

Bacteria can colonize a wide variety of surfaces, resulting in the subsequent formation of attached, antibiotic-resistant communities of bacteria called biofilms [74]. In the case of biomedical devices, such as catheters and implants, bacterial colonization of surfaces puts patients at risk for local and systemic infectious complications or device failure [75]. Moreover, the spread of foodborne and waterborne diseases and food spoilage are commonly related to bacterial colonization of polymeric surfaces [76,77]. Bacterial motility plays a key role in the colonization of such surfaces by bacteria and in the subsequent formation of biofilms [78]. Derivatives of cranberry fruit have been reported to interfere with bacterial motility [60,79].

Polymeric materials are used in a wide variety of medical devices, such as catheters, sutures and implants [80]. Urinary catheters are standard medical devices used to relieve urinary retention and incontinence [81]. The most common type of material used for catheter manufacture is silicone, a hydrophobic material with excellent biocompatibility and biodurability properties [82]. However, its surface is prone to bacterial attachment and biofilm formation [83]; therefore, strategies such as antibiotic impregnation [84–86], silver coatings [85,87] and the covalent bonding of antimicrobials (e.g. quaternary ammonium salts) [88,89] are being investigated for the development of modified catheter materials that will inhibit bacterial adhesion and biofilm formation. Nonetheless, important deficiencies are associated with each of these approaches, respectively, the

emergence of antibiotic and antiseptic resistant bacteria, the high cost of silver and the toxicity of antimicrobials towards mammalian cells [84–89]. Furthermore, because bacterial biofilms exhibit increased resistance to host immune systems and antibiotics relative to their planktonic counterparts, there is much interest in the development of catheter materials that deter bacterial attachment and biofilm formation.

North American cranberries (*Vaccinium macrocarpon*) have traditionally been used for the treatment and prevention of UTIs. Recent studies show that CDMs impair a broad range of microbial and host cell behaviors essential in the establishment of chronic infections or illnesses. Namely, CDMs have been shown to hinder bacterial attachment to abiotic [61,64] and biotic [90–92] surfaces, to impair bacterial motility [60,79], to disrupt actin-mediated host cell functions [93], and to exhibit anti-inflammatory properties [94,95]. Based on the demonstrated bioactivity of CDMs, their incorporation into and controlled release from implantable medical devices (such as catheters) may yield considerable benefits to patient health.

In this study, we report the incorporation of CDMs – CP or cPACs – into silicone substrates with the aim of impairing pathogen motility and spreading on the surface. We show that CDMs released from two silicone substrates inhibit swarming motility and downregulate the expression of the flagellin gene, *fliC*, in an uropathogen.

3.2 Materials and Methods

3.2.1 Materials

Industrial grade (IG) silicone was made with Sylgard 184 Silicone Elastomer (Dow Corning Corporation, Michigan) and biomedical grade (BG) silicone was synthesized from LSR30 Implant Grade Silicone (Applied Silicone, California). *E. coli* strain CFT073 (ATCC 700928), one of the most prevalent uropathogenic strains of *E. coli* [39] and *P. mirabilis* strain HI4320, isolated from the urine of an elderly, long-term-catheterized woman, were used in this study. Dried, crushed CP was obtained from Canneberges Atoka Cranberries (QC, Canada). Dry cPACs purified by HPLC were obtained from Marucci Center for Blueberry and Cranberry Research (Rutgers University, New Jersey).

3.2.2 Preparation of CDM-incorporated silicones

Two types of silicone discs, IG and BG, were formulated using the respective curing agent and liquid silicone rubber base that were thoroughly mixed in a 1:10 weight ratio. CP was incorporated into the mixture at 0, 1, 5, 10, or 15% or cPACs were added at 0.5 or 1%. Throughout the manuscript, % refers to concentrations in weight by volume, unless otherwise noted. The mixture was then poured into a mold and cured under pressure for 2 days to form silicone rubber sheets of 1mm thickness. A circular punch cut the sheets into discs having 1 cm diameter.

3.2.3 Measurement of CDMs released from silicone

Standard curves for CP or cPACs dissolved in DW were prepared by measuring the absorbance (Biomate 3, Thermo Scientific) of aqueous solutions of known concentrations at 280 nm (OD_{280}), which is known to be specific for the absorbance of PACs [96]. The release of CP or cPACs from the silicone discs was measured while the discs were soaking in distilled deionized water (DW). IG and BG discs with or without CP or cPACs were sterilized by UV irradiation and placed into a 24-well plate, with one disc in each well. Each well was filled with 750 µL DW and the plate was incubated at 37°C with shaking. The OD_{280} of the supernatant was measured periodically, and the dissolved concentration of CP or cPACs was determined from the standard curves to establish the amount of released CDMs.

3.2.4 *fliC* Expression Assay

E. coli CFT073 *pfliC-lux* was grown in LB broth media with 100 µg/mL ampicillin (Sigma-Aldrich) at 37°C to mid-exponential phase (OD_{600}-$OD_{600,initial}$≈0.25). IG and BG discs with or without CP or cPACs were sterilized by UV irradiation and placed into a 24-well white polystyrene plate with clear bottom, with one disc per well. Each well was filled with 750 µL bacterial culture. The plate was then incubated at 37°C and the luminescence and OD_{600} were measured periodically for 5 hours (TECAN Infinite M200 Pro). Induction of the flagellar gene *fliC* was quantified by measuring the luminescence and normalizing it to cell concentration, which was calculated by subtracting the initial OD_{600}

reading from every OD_{600} time point (Normalized Luminescence=Luminescence/(OD_{600}-$OD_{600,initial}$)). This was done to account for the variability in cell numbers.

3.2.5 Swarming Motility

To assess the migration of bacteria over the silicone discs, *P. mirabilis* HI4320 was used. This bacterium was grown in liquid LB for 2 hours. Plates containing 10mL of 2% LB agar were dried at ambient temperature overnight. Channels were cut across the center of the plate and additional channels cut at right angles. Discs were placed in the central channel. As a control, a silicone disc that did not contain CP or cPACs was placed in each plate. 2 µL aliquots of the bacterial culture were inoculated 1 cm away from the central channel where the discs were placed. The plates were incubated at 37°C for 18 hours. The distance that *P. mirabilis* swarmed across the disc onto the non-inoculated side of the plates was measured.

3.3 Results and Discussion

Custom-made discs of IG or BG silicone having various concentrations of CP or cPACs were prepared and used in subsequent assays aimed at evaluating the release of CDMs from the silicone and their bioactivity against the key uropathogens *E. coli* CFT073 and *P. mirabilis* HI4320. In order to quantify the release of cranberry materials from the silicone, we first generated standard curves for CP and cPACs by measuring the absorbance of aqueous solutions of

known concentrations at 280 nm (Figure 6). The standard curve equation for CP was determined to be OD_{280} = $Concentration_{CP}$ × 0.3473, R^2 = 0.9746 and for cPACs it was OD_{280} = $Concentration_{PACs}$ / (6.366 × 10^{-3}), R^2 = 0.9911. This shows a linear correlation between the absorbance at 280 nm and CP or cPACs concentration, for the range of concentrations tested.

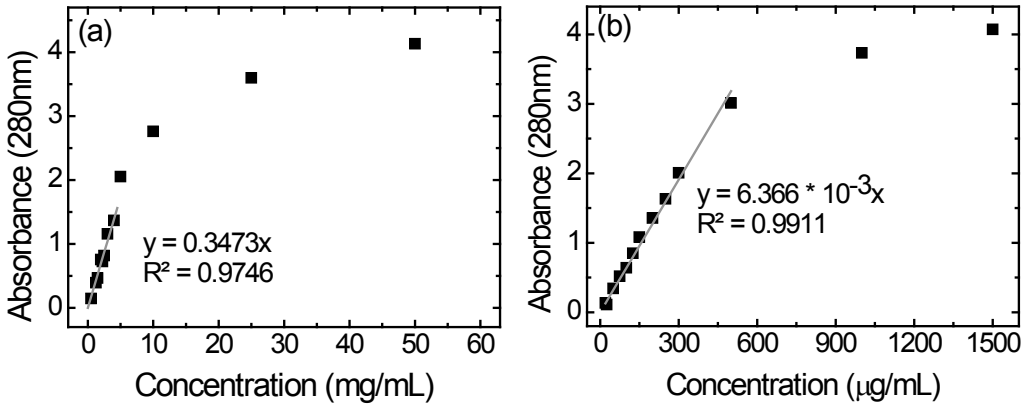

Figure 6 Standard curves of (a) CP and (b) cPACs for CDMs release experiment

The release of CDMs from the silicone discs when soaking in DW was quantified for a period of 24 hours. Figure 7 shows the release profile of the CDMs from the silicone discs plotted as CDMs concentration, whereas Figure 8 shows the release profiles plotted as the percentage of CDMs released from the silicone. Our results show that the IG silicone releases a considerably greater amount of CP and a slightly greater amount of cPACs than the biomedical grade material. The incorporation of the hydrophilic CDMs into silicone causes a significant increase in the water sorption of the polymer, which is likely to lead to the formation of droplets within the matrix. As a consequence of the osmotic potential, these droplets expand, which will exert a strain on the bulk material and lead to the creation of channels thus facilitating the release of CDMs [97]. The observed

difference in release behavior may be due to the greater crosslinking density of BG silicone. Further research is required to understand the difference in the release kinetics of IG and BG silicone.

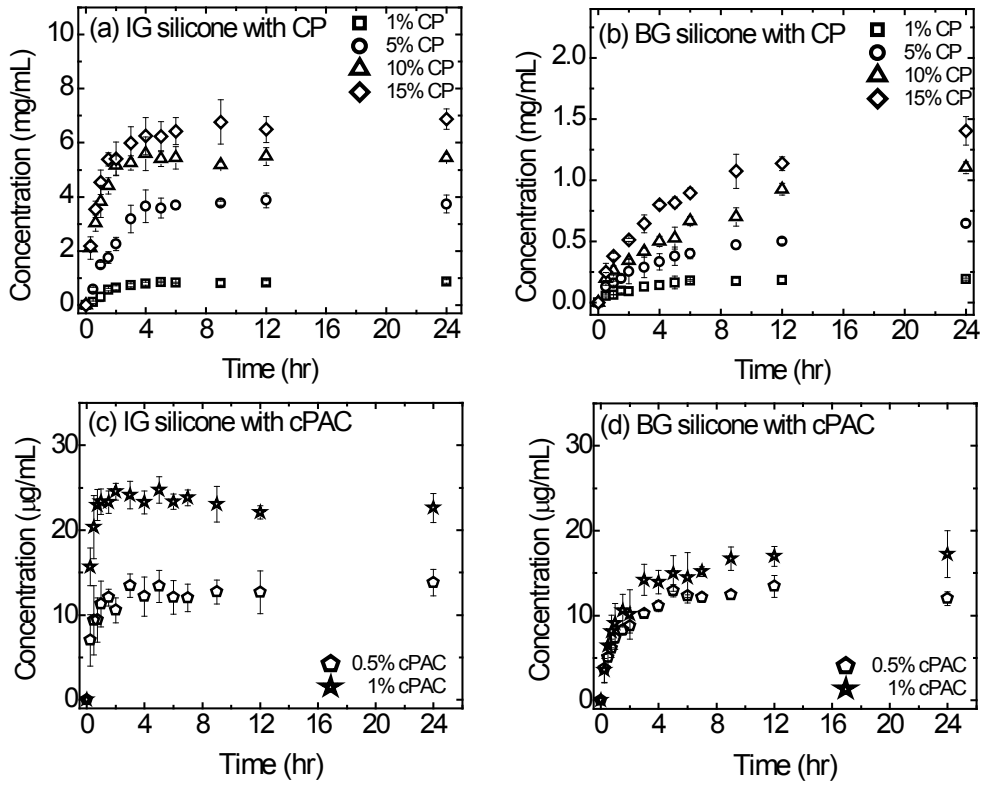

Figure 7 Release profiles of CP in water from (a) industrial grade and (b) biomedical grade silicone discs incorporated with different concentrations of CP. Release profiles of cPACs in water from (c) industrial grade and (d) biomedical grade silicone discs incorporated with two different concentrations of cPACs.

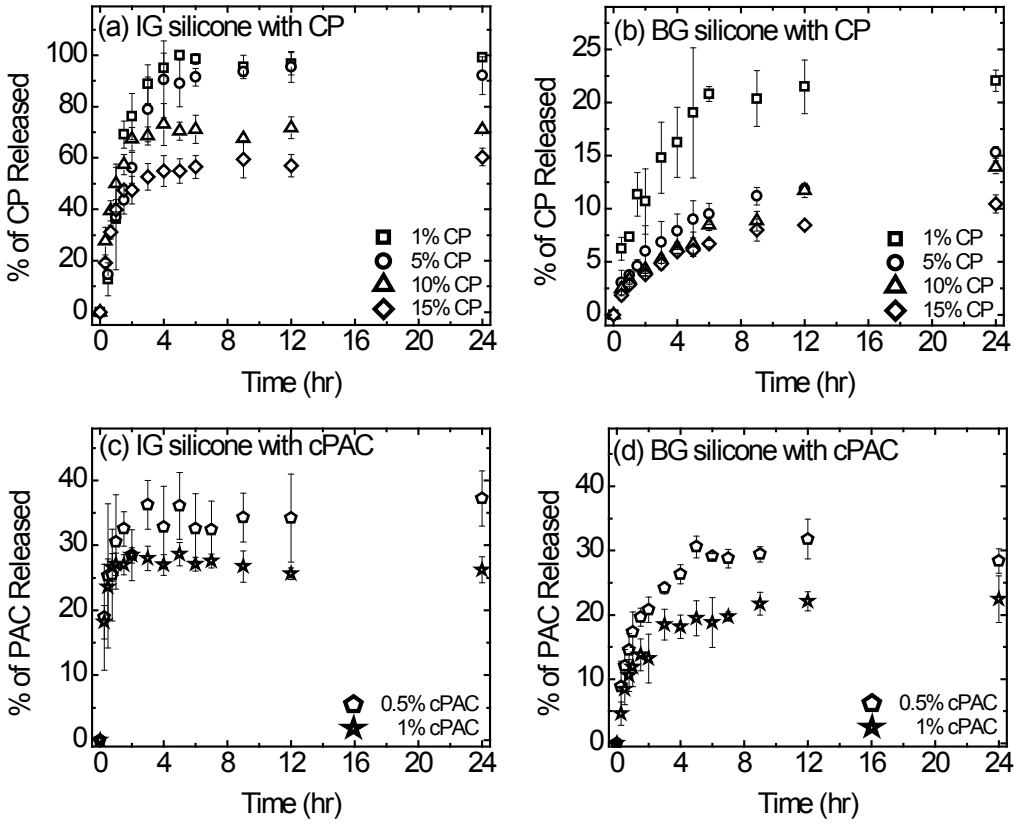

Figure 8 Percentage of CP released into water from (a) industrial grade and (b) biomedical grade silicone discs. Percentage of cPACs released into water from (c) industrial grade and (d) biomedical grade silicone discs incorporated with two different concentrations of cPACs.

As shown in Figure 8, CDMs, especially cPACs, were not completely released from the silicone materials. The CDMs were mixed into the liquid silicone base as a powder. When more CDMs were incorporated into the silicone material, the CDMs in the center of the discs were not released. This may be due to the properties of the silicone materials, such as small pore size or high crosslinking density. To increase the release of CDMs in the silicone materials, further studies are required to understand the impact of mixing different ratios of the silicone base and curing agent. Another way to improve the release of CDMs is to only incorporate CDMs on the outside of the silicone material. This may be achieved

by coating the silicone surface with CDMs or modifying the silicone surface with functional groups that will bond with the CDMs. Even though CDMs were not completely released from the silicone material, the released CDMs were able to effectively inhibit the motility of *E. coli* and *P. Mirabilis*, as shown in the following results.

It has been shown that exposure of the uropathogen *E. coli* CFT073 to CP or cPACs results in the downregulation of the flagellin gene, *fliC*, which is essential for flagella-mediated motility [79]. Therefore, exposure of bacteria to CP or cPACs results in the loss of flagellation and renders the bacteria non-motile, which may impair its ability to colonize a surface. We characterized the bioactivity of cranberry-incorporated silicone discs by quantifying the expression of the flagellin gene, *fliC*, of *E. coli* CFT073 in the presence of the IG and BG silicone discs using a *fliC-lux* reporter strain. Bacteria that harbor the P_{fliC}-*lux* plasmid luminesce proportionately to *fliC* transcription; therefore, luminescence may be used as a surrogate measurement for *fliC* expression. Our results show that, in the cultures grown in the presence of cranberry-embedded silicone discs, there is a significant decrease in luminescence, relative to the cultures grown in the presence of the control discs (untreated silicone) (Figure 9). This decrease in the luminescent signal signifies that, relative to control conditions, *fliC* is downregulated, in a dose-dependent manner. Additionally, we observed that the effectiveness of the IG silicone was slightly greater than the BG silicone, which is in agreement with the greater release of CDMs observed for the IG silicone discs.

This result demonstrates that the CP or cPACs impregnated in IG and BG silicone discs remain bioactive as they are released into the surrounding liquid media and that they effectively decrease bacterial flagellation. UTIs occur in an ascending manner, with bacteria moving from the bladder to kidney, leading to infection and potentially bacteremia [98]. It has been suggested that uropathogenic *E. coli* disseminates to the upper urinary tract via flagellum-mediated motility [35,98]. Therefore, the type of bioactivity demonstrated by the discs could prove to be effective in preventing the development of UTIs following catheterization.

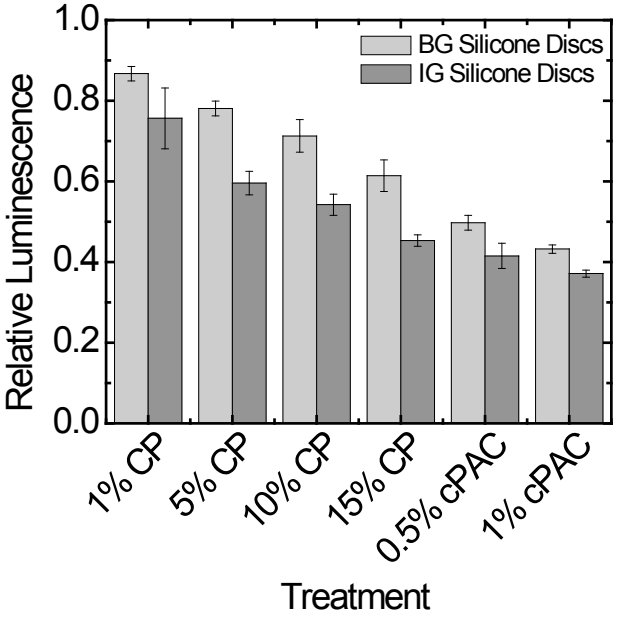

Figure 9 Expression of the *fliC* gene measured as the normalized luminescence (luminescence/(OD600-OD600 initial)) of *E. coli* CFT073 P_{fliC}-*lux* culture grown in LB broth media after 0.5 hours incubation time with (a) industrial grade silicone discs and (b) biomedical grade silicone discs incorporated with CP at 1, 5, 10, 15% w/v or cPACs at 0.5 or 1% w/v.

Previous studies have shown that CDMs added directly into a liquid broth do not inhibit bacterial growth [59,79,99]. To further confirm that the decrease in *fliC* expression did not result from toxic effects of the released CDMs, growth curves

for wild type *E. coli* were measured in the presence of treated and untreated silicone discs (Figure 10). Bacterial growth was not hindered under any of the conditions tested. This result is of importance because it evidences that, as opposed to antibiotics, CDMs are neither bacteriostatic nor bacteriocidal at the concentrations used in this study; therefore, the development of bacterial resistance is precluded.

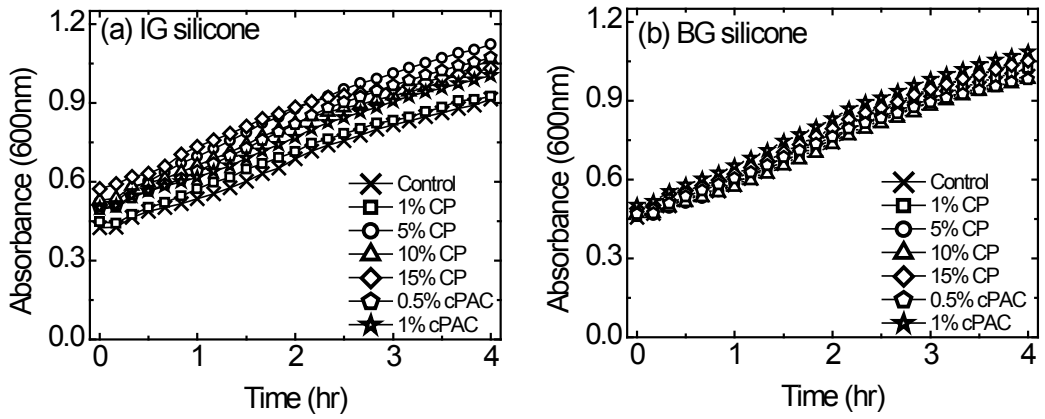

Figure 10 Growth curve of *E. coli* CFT073 in the presence of (a) industrial grade and (b) biomedical grade silicone discs incorporated with various concentrations of CP and cPACs

Additional characterization of the bioactivity of the CDM-incorporated silicone discs was undertaken by testing the efficacy of the discs at inhibiting swarming motility. Swarming motility is a type of bacterial movement that occurs in a coordinated manner across a surface and is powered by rotating helical flagella [17]. The swarming motility assay was adapted from Stickler and Hughes [100] to evaluate the bioactivity of the CDM-incorporated silicone. Briefly, silicone discs with or without CP or cPACs were placed in the central channel of an agar plate and *P. mirabilis* HI4320 was inoculated on one side of the channel (Figure 11A). When bacterial growth appeared on the non-inoculated side of the agar adjacent to

a control disc but not on the agar adjacent to a CDM-incorporated disc, it was concluded that *P. mirabilis* had not swarmed across the silicone disc surface. Figure 11A shows that *P. mirabilis* readily swarms over the surface of the control disc and the disc containing 1% CP; however, this bacterium is unable to swarm across the disc containing a higher (5%) concentration of CP. *P. mirabilis* is an aggressive swarmer that has been shown to swarm on agar plates with up to 3.6% agar [101]. This characteristic makes this bacterium an ideal candidate for this test. *E. coli,* on the other hand, was not tested in the swarming motility assay because it requires a soft, moist surface for swarming [25,102]. Hence, the consistency of the *E. coli* swarm plates is inadequate for cutting and for holding the silicone discs in place. Figure 11B shows that IG and BG silicone discs containing more than 5% CP or 0.5% cPACs were able to block or significantly impair swarming motility.

It has been demonstrated that *P. mirabilis* cells are capable of swarming across the surface of urinary catheters [100,103]. Furthermore, it has been suggested that swarming motility is the mechanism by which *P. mirabilis* gains access to the host, allowing the bacteria to ascend the urinary tract against the flow of urine [104]. Swarming motility is also a phenotype associated with the expression of virulence genes, the ability to invade human cells and increased resistance to antibiotics [105–107]. Thus, swarming motility plays an important role in the initiation of catheter-associated UTI and in the subsequent spread of a biofilm over a catheter surface. For these reasons, the inhibition of *P. mirabilis*' swarming by CDM-incorporated silicone is a result of medical importance. The relevance of

our results is further underscored by the fact that, as opposed to antibiotics, this is accomplished in a non-toxic manner.

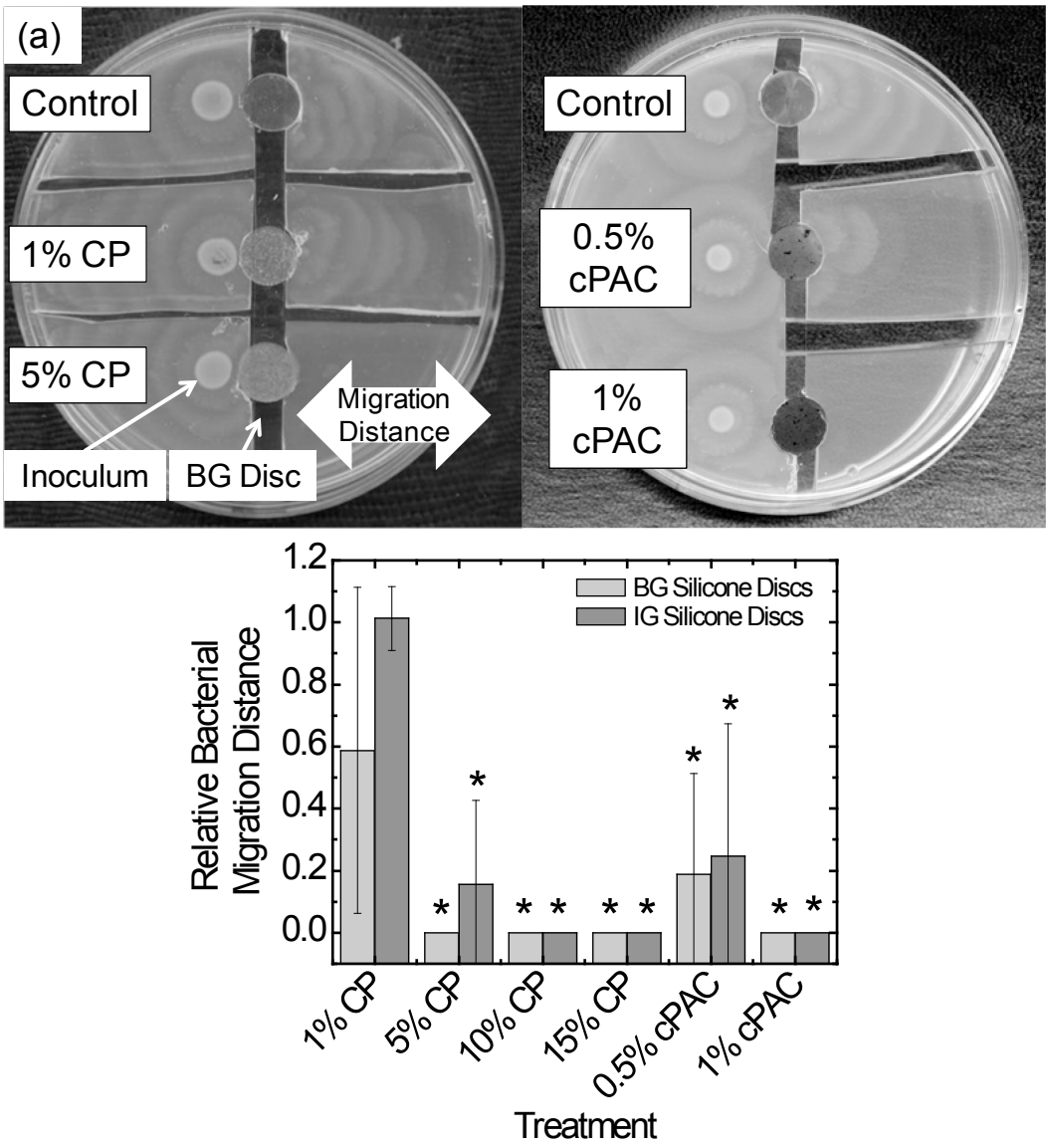

Figure 11 (a) The model in which the migration of *P. mirabilis* HI4320 over 1 cm silicone discs was tested. The agar plate on the left shows BG silicone discs containing 1% or 5% CP and the agar plate on the right shows BG silicone discs containing 0.5% or 1% cPACs inoculated with 2 µL of a 2-hr culture. (b) The migration distance that *P. mirabilis* moved on the agar plate following 18 hours of incubation. The migration distance is measured after bacteria cross the silicone discs containing different concentrations of CP or cPACs. Asterisk, $p<0.05$.

Dehydrated, crushed cranberries and a highly purified cranberry fraction were tested in all of our experiments. The 10% CP discs contained comparable amounts of cPACs as the 1% cPACs discs. As our data show, with regards to *fliC*

expression and swarming motility, similar results were achieved whether cPACs or CP were utilized. This is noteworthy because if CP elicits the same response as the cPACs, it can provide a more economical option for commercial applications.

This is the first report of the incorporation of CDMs into silicone. The observation that the bioactive CDMs are released into an aqueous medium is exciting as it offers the potential to develop bioactive implantable medical devices or food packaging materials that yield significant health benefits. Our results show that the CDM-incorporated silicone discs decrease the expression of the flagellin gene, *fliC*, which is essential for flagellum-mediated motility and has been suggested to prevent uropathogenic *E. coli* to disseminate to the upper urinary tract [33–35]. Finally, we showed that CDM-incorporated silicone can inhibit the swarming motility of *P. mirabilis*, which can colonize the surface of catheters via swarming motility [103]. The type of bioactivity demonstrated by the hybrid discs could prove to be effective if it can be incorporated in various polymeric matrices, which should be of interest for applications in the health, biomedical device and food industries. Cranberry can be a nontoxic and more economical material which will not induce the development of bacterial resistance.

3.4 Acknowledgements

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A. Howell (Rutgers University) for providing cranberry PAC, Canneberges Atoka Cranberries for providing cranberry powder, A. Manges for *E. coli* CFT073 strain, and H. Mobley (University of Michigan) for *P. mirabilis* HI4320 and the P_{fliC}-lux plasmid.

Chapter 4: Conclusions and Future Work

This thesis investigated the effect of the CDMs, CP and cPACs, on the motility of the uropathogen *E. coli* CFT073. Concentrations of CP greater than 10 mg/mL or of cPAC greater than 0.1 mg/mL inhibited swimming and swarming motility. On a transcriptional level, RT-PCR as well as a *fliC-lux* reporter showed that CP and cPACs downregulated the expression of the flagellin gene, *fliC*. Furthermore, TEM images of bacteria grown under control conditions showed relatively more flagella attached to the bacterial cell than of bacteria grown with CP or cPACs.

CDMs were incorporated into silicone and results showed that the CDMs were released into aqueous environments. Additional studies are required to understand the different release kinetics exhibited by the BG and IG silicones. The results also show that the CDM-incorporated silicone discs downregulated *fliC* transcription, which is essential for flagellum-mediated motility. The CDM-incorporated silicone also inhibited the swarming motility of *P. mirabilis*.

Overall, the results suggest that the inhibition of flagella-mediated motility by CDMs might be the mechanism by which cranberries prevent UTI. Additional research needs to be conducted to determine whether the CDMs inhibit motility in *in vivo* settings. If proven to be effective *in vivo*, the type of bioactivity demonstrated by the hybrid discs has the potential to be used in medical devices such as catheters.

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