JOURNAL OF ENDOUROLOGY
Volume 25, Number 9, September 2011
© Mary Ann Liebert, Inc.
Pp. 1547–1552
DOI: 10.1089/end.2010.0611

Micropatterned Surfaces for Reducing the Risk of Catheter-Associated Urinary Tract Infection: An *In Vitro* Study on the Effect of Sharklet Micropatterned Surfaces to Inhibit Bacterial Colonization and Migration of Uropathogenic *Escherichia coli*

Shravanthi T. Reddy, Ph.D.,[1] Kenneth K. Chung, M.S.E.,[1] Clinton J. McDaniel, M.P.H.,[1] Rabih O. Darouiche, M.D.,[2] Jaime Landman, M.D.,[3] and Anthony B. Brennan, Ph.D.[4]

Abstract

Background and Purpose: Catheter-associated urinary tract infection (CAUTI) is the most common device-associated infection and can result in serious medical consequences. We studied the efficacy of a novel microscopic physical surface modification (Sharklet) for preventing bacterial colonization and migration of uropathogenic *Escherichia coli* on silicone elastomer.

Materials and Methods: *In vitro* growth assays evaluated *E coli* colonization using three variations of micropatterned silicone surfaces *vs* a smooth silicone control. Enumeration techniques included quantification of colonies on surfaces and analysis of bacterial area coverage and colony size. *In vitro* migration assays involved placement of micropatterned and smooth silicone rod segments between two agar islands to measure incidence of migration.

Results: All three variations of the Sharklet micropattern outperformed the control surfaces in inhibiting *E coli* colonization. On average, 47% reduction in colony-forming units (CFUs) and bacterial area coverage plus 77% reduction in colony size were achieved with the Sharklet surfaces in tryptic soy broth and artificial urine compared with the control nonpatterned surfaces. The incidence of *E coli* migration over the rod segments was reduced by more than 80% for the Sharklet transverse patterned rods compared with the unpatterned control rods.

Conclusion: The Sharklet micropattern is effective at inhibiting colonization and migration of a common uropathogen. This performance is achieved through a physical surface modification without the use of any antimicrobial agents. Because deterrence of bacterial colonization and migration is a critical step to prevent CAUTI, the Sharklet micropattern offers a novel concept in addressing this important problem.

Introduction

Increasing rates of nosocomial infection and escalating trends of bacterial resistance to antimicrobials are among the biggest problems the medical community is facing. Approximately 30 million urinary catheters are inserted into more than 5 million patients each year, and catheter-associated urinary tract infection (CAUTI) is the most common nosocomial infection.[1,2] About 95% of urinary tract infections (UTIs) are associated with the use of a urinary catheter, with an average cost of treatment of $44,043 for each hospital stay from CAUTI and a negative impact on patient recovery and quality of life.[3,4]

The current approach for preventing catheter-associated infections has been either systemic antibiotic prophylaxis or antimicrobial coating of the device surface to reduce the concentration of bacteria.[3] While antimicrobial agents can suppress UTI temporarily (for only days), colonization and

[1]Sharklet Technologies Inc, Aurora, Colorado.
[2]Center for Prostheses Infection and Infectious Disease Section, Michael E. DeBakey Veterans Affairs Medical Center and Baylor College of Medicine, Houston, Texas.
[3]Department of Urology, University of California, Irvine, Irvine, California.
[4]Department of Materials Science Engineering and J. Crayton Pruitt Family Department of Biomedical Engineering, College of Engineering, University of Florida, Gainesville, Florida.

infection of the urinary tract with resistant bacteria will eventually occur.[5] Once a biofilm forms, the biofilm-embedded bacteria become protected from antimicrobial agents.[6] In fact, when treated with antibiotics, these biofilms are not fully eradicated and begin to harbor antibiotic-resistant bacteria, which may compromise the effectiveness of these agents for even nonbiofilm-mediated infections.

A catheter that does not rely on surface chemistries or antimicrobial agents while inhibiting bacterial colonization will obviate the need for toxic or harmful chemicals in patients or for broad-spectrum antibiotics. Minimizing the application of antibiotics in this setting would offer significant advantage by minimizing the development of antibiotic-resistant strains of bacteria, which has become a widespread problem.[7]

The purpose of our study was to investigate the efficacy of a novel surface technology to prevent bacterial colonization and migration of the most common uropathogen, *Escherichia coli*. The Sharklet micropattern is based on biofilm resistance seen in nature.[8] It has been shown that this biomimetic micropatterned surface inhibits bacterial biofilm growth without the use of antimicrobial agents.[9] This study focuses on the feasibility of using a Sharklet micropattern on a silicone Foley catheter to offer a new solution to CAUTI.

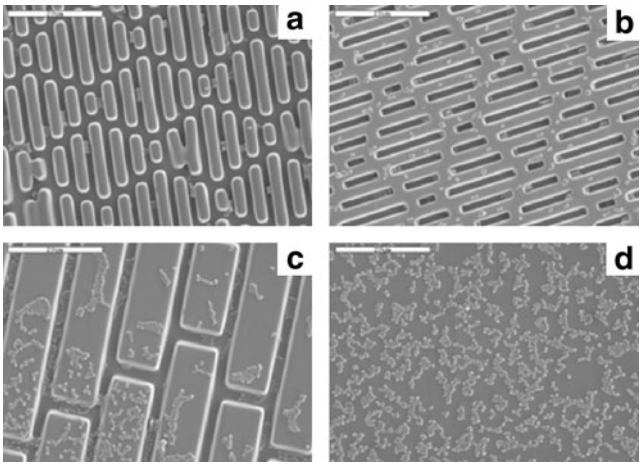

FIG. 1. Representative scanning electron microscope micrographs from one growth assay in artificial urine media after 24 hours: **(a)** positive Sharklet, **(b)** inverse Sharklet, **(c)** Sharklet 10×2, **(d)** smooth. Scale bar = 20 μm in all images; *Escherichia coli* strain ATCC #700336 is visible as individual cells or as colonies on the surfaces.

Materials and Methods

In vitro *colonization assays*

Materials: Silicone elastomer coupons, 12-mm diameter and 0.4-mm thickness, were used for all surface types. Surface types included three variations of the Sharklet pattern and smooth. Assays were carried out in two types of growth media: Tryptic soy broth (TSB) made per manufacturer's instructions or artificial urine (AU) made using common techniques for *in vitro* assays with uropathogens. Uropathogenic *E coli* was purchased from ATCC (#700336).

Methods: For each assay, 10 coupons per surface type (see statistical methods below) were challenged with *E coli* in growth media to assess the extent of colonization on each surface type, which was quantified using two measurement parameters—colony forming unit (CFU) enumeration and surface area coverage. Three variations of the Sharklet pattern were assessed: Positive Sharklet 2×2 (protruding features with 2 micron width and 2 micron spacing), inverse Sharklet (ISK) 2×2 (recessed features with 2 micron width and 2 micron spacing), and positive Sharklet 10×2 (protruding features with 10 micron width and 2 micron spacing) (Fig. 1). The micropatterned silicone film was created as described previously.[10] Coupons were rinsed with ethanol and cohesively bonded to petri dishes, and sterilized with ethylene oxide gas. *E coli* inoculum was grown to a concentration of 10^6 CFU/mL in log phase in either TSB or AU sterile growth media, prepared daily using a well-established method.[11,12] Coupons were immersed in inoculum suspension and incubated at 37°C. Dishes were replenished with fresh media daily up to 7 days. One dish was removed for enumeration at each time point: 24 hours, 48 hours, 3 days, and 7 days. The experiment was repeated three times for each growth media.

The enumeration procedure began by rinsing coupons in the dish with sterile NaCl with shaking at 100 revolutions per minute for 5 minutes. Rinse procedure was repeated three times to adequately remove nonadherent cells. Coupons were then punched using 8-mm sterile biopsy punches (to avoid counting bacterial colonies on unpatterned coupon edges) and placed in sterile NaCl in conical tubes. Tubes were sonicated for 1 minute on and 1 minute off for a total of 10 minutes, with 30 seconds vortexing before and after sonication. Cell solution was serially diluted and plated onto tryptic soy agar (TSA), incubated at 37°C for 24 hours, and subsequently CFUs were enumerated. Coupons for scanning electron microscopy (SEM) were taken out after the rinse procedure and exposed to osmium tetroxide for 30 minutes to fix cells, followed by graded ethyl alcohol series dehydration.[9] SEM analysis was at six predetermined locations on each coupon. Micrographs were analyzed for area coverage and average colony size using ImageJ software with techniques described previously.[9]

In vitro *migration assays*

The migration assay was performed to assess the incidence of migration over Sharklet-patterned *vs* smooth silicone rods, based on a model previously used to examine incidence of migration over hydrogel-coated catheter segments.[13] Silicone elastomer rods, 1-cm long and 16F (5.3 mm) outer diameter, were used for all surface types. Surface types included two variations of the Sharklet pattern, each in two orientations (parallel or perpendicular to the long axis of the rod) and smooth. The same *E coli* strain was used as for the colonization assays. These assays were performed in TSA plates (Fig. 2). Migration was measured as a plus or minus occurrence—plus meaning that the bacteria traveled from the inoculation site to the end of the catheter segment within a 24-hour incubation period, and minus meaning that no visible evidence of migration occurred.

The experimental procedure for the migration experiment was described in detail previously.[13] Our interest was to examine the difference in incidence of migration over the external surface of tubes, for which we made plugged tubes (rods) with and without the Sharklet pattern. Sharklet features were either parallel or perpendicular to the axis of radial

symmetry. All rods were made in silicone elastomer, segmented into 1-cm sections, and autoclave sterilized.

The bacterial inoculum was prepared in log phase growth at 10^8 CFU/mL. The rod surface types were randomly assigned among dishes, with two rods per dish and a positive control (inoculation without a rod) (Fig. 2). *E coli* migration was inferred by growth of colonies on the agar adjacent to the end of a given silicone rod. Two experimental repetitions were carried out, with 30 replicates of each surface type used for each experiment.

Statistical methods

Statistically powered *in vitro* colonization assays were carried out to reject the null hypothesis that bacterial attachment is the same on all surface types. Sample size was calculated for a two-sample *t* test comparing CFU counts on Sharklet surfaces with smooth; assuming a power of 0.9, $p \leq 0.05$, difference of means of 0.5 log and variance of 0.3 log, sample size is 9 coupons per surface type. CFU counts were transformed to percent reduction of colonization on each of the Sharklet surface types relative to the control surface using the formula $(CFU_{Smooth} - CFU_{Sharklet})/(CFU_{Smooth})$. The same calculation was performed for SEM area coverage measurements. Percent reductions were compiled over the repeated experimental runs and analyzed for statistical significance via pair-wise Student *t* tests for $p \leq 0.05$. To assess whether a particular surface type performed best, a one-way analysis of variance (ANOVA) was computed by comparing average percent reductions relative to the control for each Sharklet surface type in a given media. A two-way ANOVA for average percent reductions at each time-point for each surface type was calculated for CFU and SEM area coverage data in both media types.

Statistically powered *in vitro* migration assays were performed to validate rejection of the null hypothesis that bacterial migration occurs at the same frequency on all surface types. For a test of equality of two proportions, where p_1 is the proportion of smooth rods with positive migration, p_2 is the proportion of Sharklet rods with positive migration, and assuming $p_1 = 0.3$, $p_2 = 0.1$, at a power of 0.8 and $\alpha = 0.05$, a sample size of $n = 60$ rods per surface type was calculated. For feasibility of performing the experiment, $n = 30$ rods were used per migration experiment, and the experiment was repeated twice. For each experiment, percent reduction was computed using $(\%Migration_{Smooth} - \%Migration_{Sharklet})/(\%Migration_{Smooth})$.

Results

Colonization of this uropathogenic strain of *E coli* was inhibited by all three Sharklet micropatterns (Fig. 1), which were tested in a static environment with both types of growth media. Percent reduction of both average bacterial counts and area coverage were calculated for each Sharklet micropattern compared with smooth for each experimental time point. These values were then compiled across all time points for all experiments conducted, separated by type of media and measurement method (Figs. 3 and 4). SEM area coverage data showed statistically significant percent reductions in bacterial area coverage consistently for all Sharklet patterns relative to smooth control surfaces. CFU enumeration data showed higher variability, which is addressed in the Discussion section. All three Sharklet surface types performed equally well based on ANOVA analysis of the percent reduction of either CFU counts or the SEM area coverage at each measurement time point in each type of media. The Sharklet micropatterns also caused a statistically significant reduction in the colony size of *E coli* attached to surfaces (Fig. 5), with an average 76% reduction in colony size for the various Sharklet patterns and

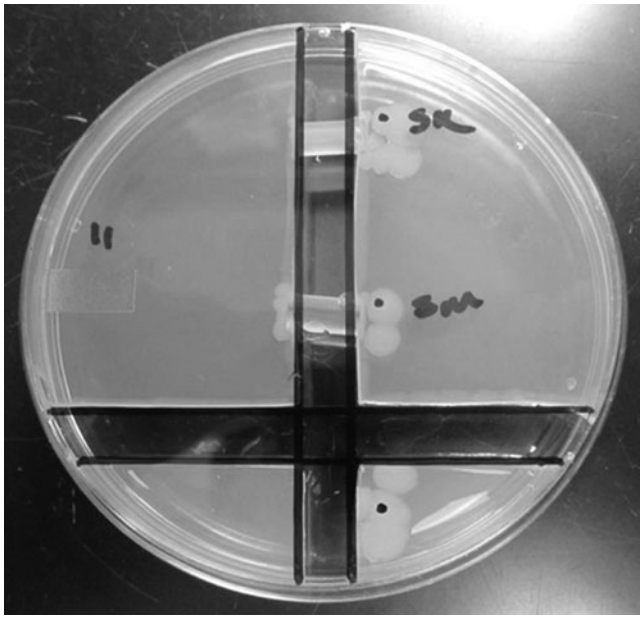

FIG. 2. Example of an agar plate from a migration experiment, in which a Sharklet-patterned rod is at the top of the vertical tract and a smooth control rod in the middle of the tract. Inoculation sites are marked by dots (right side), with a positive control isolated at the bottom of the plate to ensure migration is not occurring on the plate surface. In this example, migration is observed across the smooth rod based on visible colonies at the other end of the rod (left side), but not across the Sharklet-patterned rod.

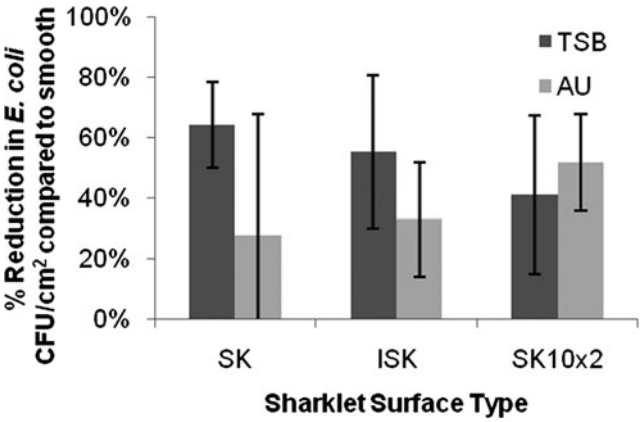

FIG. 3. Percent reduction in colony-forming unit (CFU) counts on the Sharklet micropatterned surfaces compared with a smooth control surface. Error bars represent standard error of the mean. TSB = tryptic soy broth; AU = artificial urine; SK = Sharklet; ISK = inverse Sharklet.

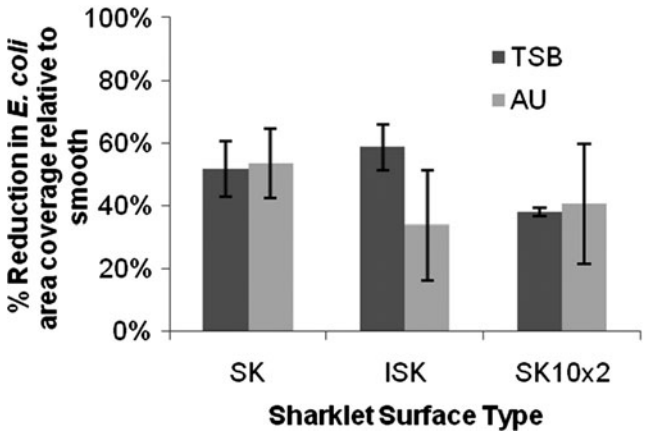

FIG. 4. Percent reduction of bacterial coverage area on the Sharklet micropatterned surfaces compared with a smooth control surface. Error bars represent standard error of the mean. TSB = tryptic soy broth; AU = artificial urine; SK = Sharklet; ISK = inverse Sharklet.

as much as 80% reduction on the ISK surface compared with the smooth surface.

The Sharklet-patterned rods demonstrated significant inhibition in the migration of *E coli*. Rods with the Sharklet feature orientation transverse to the long axis of the rod (SK T) allowed migration to occur over only 5% of the rods compared with migration occurring on 13% of the Sharklet rods with feature orientation parallel to the long axis of the rod (SK ||) and on 26.7% of the smooth rods (Table 1). Thus, an 81% reduction ($P = 0.028$) and 50% reduction ($P = 0.216$) in incidence of migration was achieved for the SK T and SK || rods, respectively, compared with smooth rods (Fig. 6).

Discussion

Significant reduction of colony number, bacterial area coverage, colony size, and bacterial migration were demonstrated on Sharklet surfaces compared with smooth control surfaces regardless of time point, type of growth media, or Sharklet pattern type (Fig. 7). The SEM bacterial area coverage

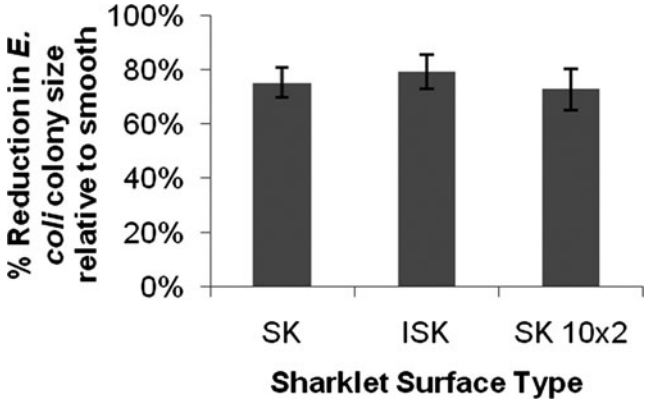

FIG. 5. Percent reduction of colony size on the Sharklet micropatterned surfaces compared with a smooth control surface. Error bars represent standard error of the mean. SK = Sharklet; ISK = inverse Sharklet.

Table 1. Percent of Rods Allowing Migration of *Escherichia Coli* to Occur After 24 Hours

Rod surface type	*Incidence of migration (#rods with positive migration/#replicates*	*95% confidence interval*	p *value for comparison with smooth*
Smooth	26.7% (16/60)	20.1%–33.2%	
SK ||	13.3% (8/60)	0.3%–26.4%	0.216
SK T	5.0% (3/60)	1.7%–8.3%	0.028

Sixty replicates of each surface type were included over the course of two experiment repetitions.
SK || = Sharklet parallel; SK T = Sharklet transverse.

analysis offered a more consistent measurement method for evaluating bacterial load on sample surfaces compared with the colony enumeration method. Although a similar result in degree of reduction of bacterial load was obtained for the colony enumeration method, the variances were larger. The incalculable variability between results of the viable plate count technique has been well documented and is likely because of the inconsistency in disaggregating biofilm.[14] Biofilm harvesting and disaggregation remain imprecise and biased techniques, despite the use of sonication and vortex mixing methods for removal of surface-attached populations.[15,16] This remains a critical area of research as debate among biofilm experts continues.[14,17] Imaging of biofilms avoids many of the limitations of colony dispersal and enumeration.[17] The unique nature of the Sharklet technology, which has no active kill mechanism and includes no biocidal agents, needs new techniques to quantify the antimicrobial effect.

The reduction in colony size on the Sharklet surfaces could be an indicator for the inhibition of colony proliferation. The exact mechanism of the Sharklet inhibitory effect on bacterial colonization and proliferation continues to be explored.[18,19] Biofilm communities generally exist as large groups of attached cells, forming an exopolysaccharide matrix to house the community,[20] which are not observed on the Sharklet micropatterns. It is reasonable that limiting bacterial colony

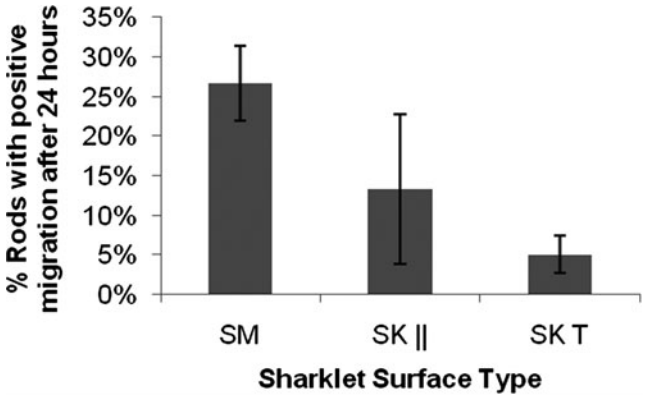

FIG. 6. Percent of rods with observed migration of *Escherichia coli* after 24 hours of incubation. SK|| indicates that feature orientation was parallel to the long axis of symmetry and SK T indicates that feature orientation was perpendicular to the long axis of symmetry. Error bars represent standard error of the mean. SM = smooth.

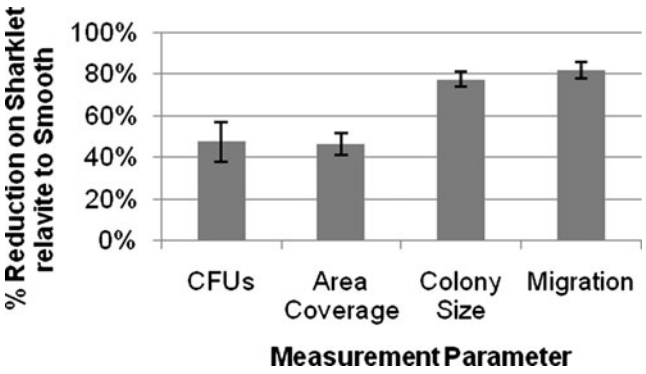

FIG. 7. Compiled percent reductions for all measurement parameters comparing Sharklet surfaces with smooth. Chart represents compiled data for all Sharklet surface types at various time points for both artificial urine and tryptic soy broth. Data are compiled for several sets of experiments; analysis for each measurement parameter was performed for specific subsets of the experiments. The migration experiments were carried out using a different protocol compared with the other measurement methods, as described in the Methods section; result shown is for the transverse feature orientation. Error bars represent standard error of the mean. CFU = colony-forming unit.

size would inhibit biofilm expression. This observed biofilm phenomenon is important because of its relevance to the pathogenesis of device-related infections and needs further investigation.

Interestingly, the Sharklet micropatterns also effectively inhibit bacterial migration compared with smooth surfaces. This effect has important implications for preventing CAUTI, because the mode of bacterial access into the bladder is primarily via extralumenal migration of bacteria that originate from the interface between the urethral meatus and catheter external surface.[3] An important result from this experiment was the indication that the transverse Sharklet feature orientation offered a more significant reduction in migration than the parallel orientation. This behavior may be related to the physical barrier created by the features of the Sharklet micropattern when oriented perpendicular to the overall direction of migration. It is worth noting that this species of *E coli* did not have any swimming or swarming motility and, thus, no apparent mechanistic drive for migration, possibly explaining the low incidence of migration for even the smooth control rods. Future migration studies will be conducted using motile species of other uropathogens in an *in vitro* migration model that more closely resembles the *in vivo* environment.[21,22]

The Sharklet micropattern represents a paradigm shift in the current strategy using chemical modifications to surfaces of medical devices, instead relying on a microscopic texture to provide an inhospitable surface for microorganism colonization. Existing Foley catheters aimed at inhibiting bacterial colonization of the catheter surface all rely on antimicrobial agents incorporated into the catheter material or applied onto the catheter surface. These formulations have not been shown to produce statistically significant reductions in the incidence of symptomatic CAUTI. Although some antimicrobial-based formulations have been associated with reduction in bacteriuria, the use of certain agents could eventually lead to emergence of antimicrobial resistance in the presence of exceedingly high bacterial counts in the catheterized bladder. The unique micropatterning approach of the Sharklet design offers an antimicrobial function that does not rely on antimicrobial agents.

While previous studies have examined the use of microscopic surface structures to direct microorganism attachment,[23,24] the Sharklet pattern is the first microscopic surface texture designed to inhibit bacterial colonization and migration. Explanation of the mechanism behind this unique effect to prevent microorganism colonization through microscopic texture alone has led to an "Engineered Roughness Index" and other models of material properties that affect microorganism behavior.[18,25,26]

Conclusion

The Sharklet micropatterned surfaces demonstrated the ability to inhibit colonization and migration of uropathogenic *E coli* under growth conditions *in vitro*. This effect was from the shape modification of the surface alone, without the use of chemical agents. The results of this *in vitro* study suggest that a Sharklet-patterned Foley catheter would likely allow significantly less bacterial colonization and lower rate of bacterial migration over the device surface than the standard of care smooth surface Foley catheters. Further studies are needed to assess the ability of this surface technology to inhibit colonization and migration of other pathogenic microorganisms and under *in vivo* conditions. Ultimately, clinical testing will be needed to confirm the ability of the Sharklet-patterned surface to reduce CAUTI. A urinary catheter with the Sharklet modification may offer a much needed alternative to antimicrobial agents in the effort to prevent CAUTI.

Acknowledgments

This work was funded by NIH National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Small Business Innovation Research (SBIR) grant number 1R43DK084590-01. The authors would like to thank Donald E. Dean, Jeremy O'Neal and Dr. Kenneth Rand for their contributions to this work.

Disclosure Statement

Authors are affiliated with Sharklet Technologies Inc, Aurora, CO, either as full-time employees or consultants.

References

1. Jacobsen SM. Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clin Microb Rev 2008;21:26–59.
2. Wald HL, Kramer AM. Nonpayment for harms resulting from medical care: Catheter-associated urinary tract infections. JAMA 2007;298:2782–2784.
3. Darouiche RO. Antimicrobial coating of devices for prevention of infection: Principles and protection. Int J Artif Organs 2007;30:820–827.
4. Centers for Medicare & Medicaid Services. Proposed Changes to the Hospital IPPS and FY2009 rates. http://edocket.access.gpo.gov/2008/pdf/08-1135.pdf 2008.

5. Trautner BW, Darouiche RO. Catheter-associated infections: Pathogenesis affects prevention. Arch Intern Med 2004; 164:842–850.
6. Stickler DJ. Bacterial biofilms in patients with indwelling urinary catheters. Nat Clin Pract Urol 2008;5:598–608.
7. Nordmann P, Naas T, Fortineau N, Poirel L. Superbugs in the coming new decade; multidrug resistance and prospects for treatment of Staphylococcus aureus, Enterococcus spp. and Pseudomonas aeruginosa in 2010. Curr Opin Microbiol 2007;10:436–440.
8. Schumacher JF, Carman ML, Estes TG, et al. Engineered antifouling microtopographies—effect of feature size, geometry, and roughness on settlement of zoospores of the green alga Ulva. Biofouling 2007;23:55–62.
9. Chung KK, Schumacher JF, Sampson EM, et al. Impact of engineered surface microtopography on biofilm formation of Staphylococcus aureus. Biointerphases 2007;2:89–94.
10. Carman ML, Estes TG, Feinberg AW, et al. Engineered antifouling microtopographies—correlating wettability with cell attachment. Biofouling 2006;22:11–21.
11. Brooks T, Keevil CW, A simple artificial urine for the growth of urinary pathogens. Lett Appl Microbiol 1997;24:203–206.
12. Lange D, Elwood CN, Choi K, et al. Uropathogen interaction with the surface of urological stents using different surface properties. J Urol 2009;182:1194–1200.
13. Sabbuba N, Hughes G, Stickler DJ. The migration of Proteus mirabilis and other urinary tract pathogens over Foley catheters. BJU Int 2002;89:55–60.
14. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: From the natural environment to infectious diseases. Nat Rev Microbiol 2004;2:95–108.
15. Kobayashi H, Oethinger M, Tuohy MJ, et al. Improved detection of biofilm-formative bacteria by vortexing and sonication: A pilot study. Clin Orthop Relat Res 2009;467:1360–1364.
16. Bjerkan G, Witso E, Bergh K. Sonication is superior to scraping for retrieval of bacteria in biofilm on titanium and steel surfaces in vitro. Acta Orthop 2009;80:245–250.
17. Hamilton MA. Checking the validity of the harvesting and disaggregating steps in laboratory tests of surface disinfectants. J AOAC Int 2009;92:1755–1762.
18. Long CJ, Schumacher JF, Robinson PA II, et al. A model that predicts the attachment behavior of Ulva linza zoospores on surface topography. Biofouling 2010;26:411–419.
19. Schumacher JF, Long CJ, Callow ME, et al. Engineered nanoforce gradients for inhibition of settlement (attachment) of swimming algal spores. Langmuir 2008;24:4931–4937.
20. O'Toole GA, Stewart PS. Biofilms strike back. Nat Biotechnol 2005;23:1378–1379.
21. Darouiche RO, Safar H, Raad II. In vitro efficacy of antimicrobial-coated bladder catheters in inhibiting bacterial migration along catheter surface. J Infect Dis 1997;176: 1109–1112.
22. Jones BV, Young R, Mahenthiralingam E, Stickler DJ. Ultrastructure of Proteus mirabilis swarmer cell rafts and role of swarming in catheter-associated urinary tract infection. Infect Immun 2004;72:3941–3950.
23. Allion A, Baron JP, Boulange-Petermann L. Impact of surface energy and roughness on cell distribution and viability. Biofouling 2006;22:269–278.
24. Whitehead K, Colligon J, Verran J. Retention of microbial cells in substratum surface features of micrometer and submicrometer dimensions. Colloids Surf B Biointerfaces 2005; 41:129–138.
25. Magin CM, Long CJ, Cooper SP, et al. Engineered antifouling microtopographies: The role of Reynolds number in a model that predicts attachment of zoospores of Ulva and cells of Cobetia marina. Biofouling 2010;26:719–727.
26. Long CJ, Finlay JA, Callow ME, et al. Engineered antifouling microtopographies: Mapping preferential and inhibitory microenvironments for zoospore attachment. Biofouling 2010;26:941–952.

Address correspondence to:
Shravanthi T. Reddy, Ph.D.
Sharklet Technologies, Inc.
12635 E. Montview Blvd., Suite 160
Aurora, CO 80045

E-mail: sreddy@sharklet.com

Abbreviations

ANOVA = analysis of variance
AU = artificial urine
CAUTI = catheter-associated urinary tract infection
CFU = colony forming unit
ISK = inverse Sharklet
SEM = scanning electron microscopy
SK = Sharklet
SK || = Sharklet parallel
SK T = Sharklet transverse
TSA = tryptic soy agar
TSB = tryptic soy broth
UTI = urinary tract infection