

Use of a Ceragenin-Based Coating to Prevent Bacterial Colonization of Urinary Catheters

P. B. Savage,¹ J. E. Pollard,¹ Y. Feng,¹ L. K. Reddy,¹ C. Genberg²
¹Brigham Young University, Provo, UT; ²Ceragenix Pharmaceuticals, Denver, CO

Paul B. Savage, Ph.D.
 Department of Chemistry and Biochemistry
 Brigham Young University
 C100 BNSN
 Provo, UT 84602
 paul_savage@byu.edu
 801-422-4020

AMMENDED ABSTRACT

Background: Hospital acquired infections are a major source of increased morbidity, mortality and added costs, with urinary tract infections associated with catheter use the most common occurrence. Bacterial colonization of medical devices, in the form of biofilms, plays a key role in these infections. Ceragenins are bile acid-based mimics of antimicrobial peptides and are potent, broad-spectrum bactericidal agents. Under physiological conditions, the ceragenins are polycationic and associate with polyanionic materials. Thin films of high-acid-content polyurethanes on medical devices effectively sequester ceragenins and provide a means of controlled release of the antimicrobial agents over extended periods.

Methods: Thin films (20 microns) containing ceragenin CSA-13 in polyurethane were deposited on urinary catheter segments via dip coating. Segments were immersed in artificial urine and inoculated with *Escherichia coli* (10^3 CFU/ml). Samples were incubated with agitation for 24 h. The growth medium was replaced with fresh broth and samples were reinoculated every 24 h. Biofilm formation was characterized by removing the segments, washing with PBS, immersing in neutralizing broth, sonicating, plating samples on agar, incubating, and counting colonies.

Days of Incubation	CFU (log) of <i>E. coli</i> Adhered to Urinary Catheter Segments (5 mm) after Incubation for the Indicated Period				
	Control	Silver-Releasing (1)	Silver-Releasing (2)	Nitrofurazone-Releasing	Ceragenin-Releasing
14	5.8	5.6	6.8	6.5	0
21	6.8	6.7	7.5	7.4	1.4

Results: Ceragenin-releasing segments were compared to urinary catheter segments that release silver or nitrofurazone. Comparators were colonized after four days, while the ceragenin-releasing segments were uncolonized at day 14, and at day 21 the amounts of adhered bacteria were decreased by more than five log units as compared to controls.

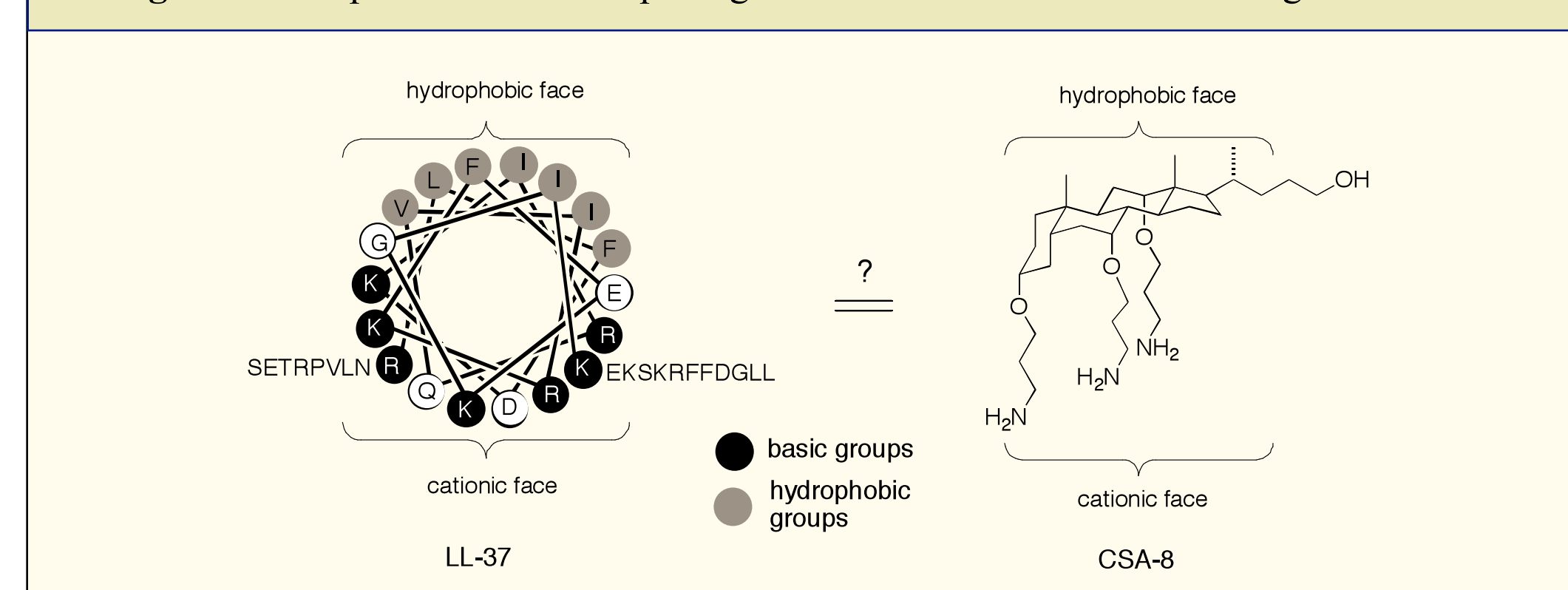
Conclusions: The cationic character of ceragenin CSA-13 provides an effective means for sustained release of the antimicrobial. A ceragenin-releasing coating may prove useful in preventing or lowering the incidence of catheter-associated urinary tract infections.

INTRODUCTION

Endogenous antimicrobial peptides, such as the human cathelicidin LL-37, play key roles in innate immunity.¹ For example, deficiencies in cathelicidins have been correlated with an increase in urinary tract infections in mice.² The ubiquity of antimicrobial peptides has been used as evidence that these compounds do not readily engender bacterial resistance. In addition, considering the varied sequences of antimicrobial peptides among diverse organisms, it is apparent that they have evolved independently multiple times. Thus, antimicrobial peptides appear to be one of "Nature's" primary means of controlling bacterial growth. However, clinical use of antimicrobial peptides presents significant issues including the relatively high cost of producing peptide-based therapeutics and the susceptibility of peptides to proteases generated by the host and by bacterial pathogens.

Ceragenins are small molecules, derived from a common bile acid, that were developed as mimics of antimicrobial peptides.³ Antimicrobial peptides generally adopt a facially amphiphilic conformation (as alpha helices or beta sheets), and the ceragenins mimic this morphology (Figure 1). However, unlike antimicrobial peptides, ceragenins are relatively easy to prepare and purify on a large scale. Furthermore, because ceragenins are not peptide-based, they are not substrates for proteases that limit the lifetimes of antimicrobial peptides. Ceragenins display high bactericidal activity against Gram-negative and positive organisms, and this activity has been correlated to that of antimicrobial peptides. Consequently, it is anticipated that even with wide-spread use, there is little likelihood of generation of resistance to ceragenins.

Figure 1. Comparison of the morphologies of cathelicidin LL-37 and ceragenin CSA-8.



Under physiological conditions, ceragenins are cationic. Consequently, they associate strongly with anionic substances, including bacterial cell membrane components. Anionic polyurethanes are routinely used as medical device coatings, and their charge complementarities with ceragenins makes them well suited for controlling the release of ceragenins from thin films. Generation of thin films derived from anionic polymers and ceragenins provides a means of preventing biofilm formation on coated devices for extended periods even in situations in which devices are exposed to relatively large volumes and/or changing fluids. The prevalence of urinary tract infections associated with catheter use⁴ has prompted the development of antimicrobial releasing catheters, including examples that elute silver or nitrofurazone. We compared the abilities of these catheters to prevent biofilm formation with *E. coli* to those coated with a thin film derived from an anionic polymer and CSA-13, a lead ceragenin. We also challenged ceragenin coated catheter segments with other organisms to verify that the activity of the coating was not limited to one organism. Of particular importance is that assays were performed in artificial urine with inoculation repeated daily with fresh media. This approach has been suggested to be representative of in vivo conditions.⁵

MATERIALS AND METHODS

Generation of Thin Film

Ceragenin CSA-13 and an acidic, water-insoluble polyurethane (acid number = 19) were dissolved in a solution of methanol and tetrahydrofuran (1:9 vol:vol). Segments of silicone urinary catheters (5 mm) were repeatedly dipped in the polyurethane-CSA-13 solution until a weight increase of 5% was measured. This weight difference corresponded to a film thickness of ca. 20 μ m. Coated tube segments were exposed to high vacuum in order to remove remaining solvent.

Assay Conditions

Assays were performed based upon a method developed by Cook and Costerton⁵ that reflects conditions found in urinary catheters. That is, exposure to urine and an exchange of urine over time. Assayed urinary catheter segments were immersed in artificial urine (0.8 ml) containing 10^3 - 10^4 CFU/mL of *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 29245) or *Klebsiella pneumoniae* (ATCC 13883). Samples were incubated in 48-well plates for 24 h on a gyratory shaker (50 rpm) at 37° C. Following incubation, urinary catheter segments were transferred to a new well containing new inocula (10^3 - 10^4 CFU/mL) in fresh media. Aliquots from the used media were subsequently plated on nutrient agar containing 0.2% glucose to monitor bacterial growth. The procedure was repeated daily. All assays were performed in triplicate.

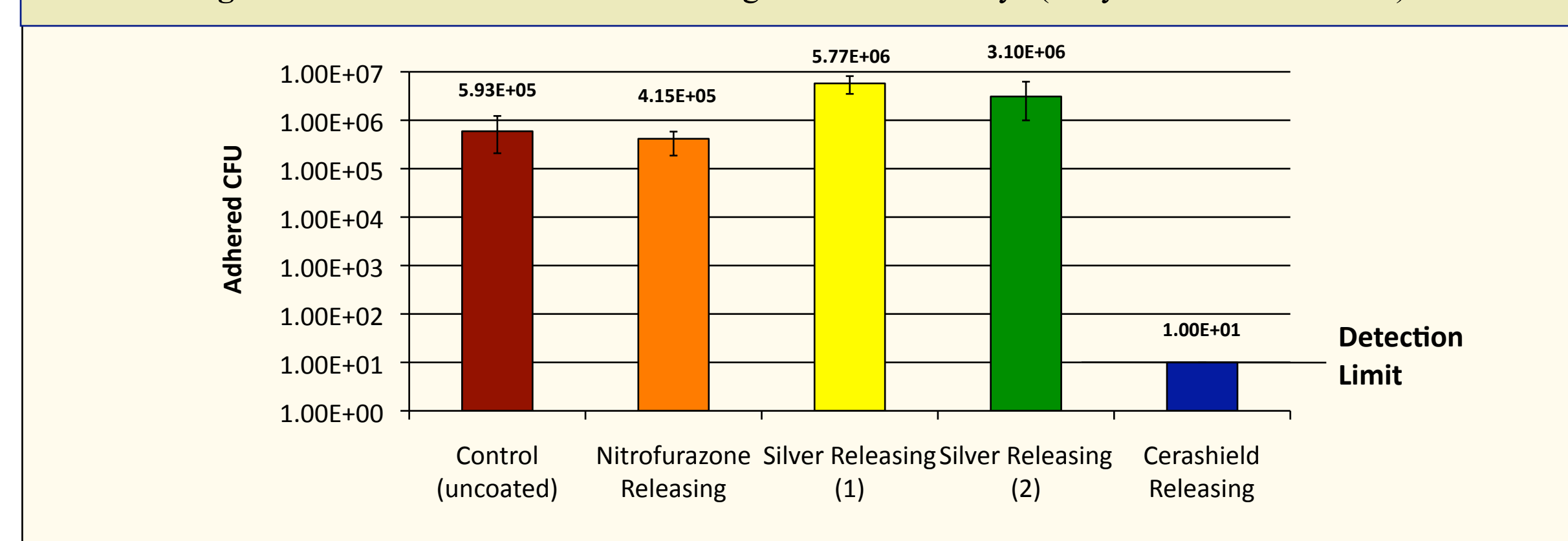
Quantification of Biofilm Formation

After specified time periods, incubated urinary catheter segments were removed from the artificial urine media and gently rinsed with sterile PBS to remove planktonic bacteria. Segments were then placed in a neutralizing broth to prevent any further antibacterial activity of eluting antimicrobial agents. Adhered bacteria were removed by sonication (bath sonicator) for 10 min followed by high-speed vortexing for 30 sec. Bacterial counts were determined by 10-fold serial dilutions plated on nutrient agar containing 0.2 % glucose. Following incubation for 18-24 h, plates were removed and colonies were counted to determine the extent of biofilm formation. The detection limit for performed serial dilutions was 10 CFU.

RESULTS

For comparison purposes, segments from commercially available urinary catheters that release silver ion (two types) or nitrofurazone were assayed vs. *E. coli* (ATCC 25922) as described above. Testing was conducted to 21 days. After 14- and 21-day periods, catheter segments were removed and biofilms (adhered bacteria) were quantified. Silver and nitrofurazone coated urinary catheter segments sustained heavy biofilm colonization (comparable to an uncoated control), while ceragenin-coated segments remained completely free of biofilm colonization (Figure 2).

Figure 2. Adhered *E. coli* on catheter segments after 14 days (daily inocula of 10^3 CFU).



Within four days the growth medium of the silver- and nitrofurazone-releasing segments sustained bacterial growth. In contrast, the growth medium containing ceragenin-coated urinary catheter segments sustained no bacterial growth for a 21-day period (Figure 3). Because the assays were performed in artificial urine that was exchanged every 24 h, the antimicrobial agents were free to elute from the surfaces of the catheter segments and were removed with the medium exchange. This approach was designed to replicate the conditions found in the interior lumen of urinary catheters. These results suggest that the amounts silver and nitrofurazone released were sufficient to eliminate the inocula for four days under the conditions of this assay while controlled release of the ceragenin was sufficient to eliminate the inocula for more than 21 days.

Ceragenin-releasing urinary catheter segments were further tested against isolates of *P. aeruginosa*, *P. mirabilis* and *K. pneumoniae* for periods of 14 and 21 days. These organisms are causative agents of urinary tract infections. After 21 days, controls supported high counts of adhered bacteria (Figure 4). Ceragenin releasing catheters resisted biofilm formation from all three organisms for a 21-day period (Figure 4). The experiment was terminated after day 21. At this time point there were few CFUs adhered to the segments challenged with *P. mirabilis*; nevertheless, a reduction of over five logs in adhered bacteria was observed. With the other two organisms, the log reduction was greater than six.

Figure 3. Comparison of silver, nitrofurazone, and ceragenin-releasing catheter segments challenged daily with *E. coli* (10^3 CFU/ml). The trial was stopped at day 21.

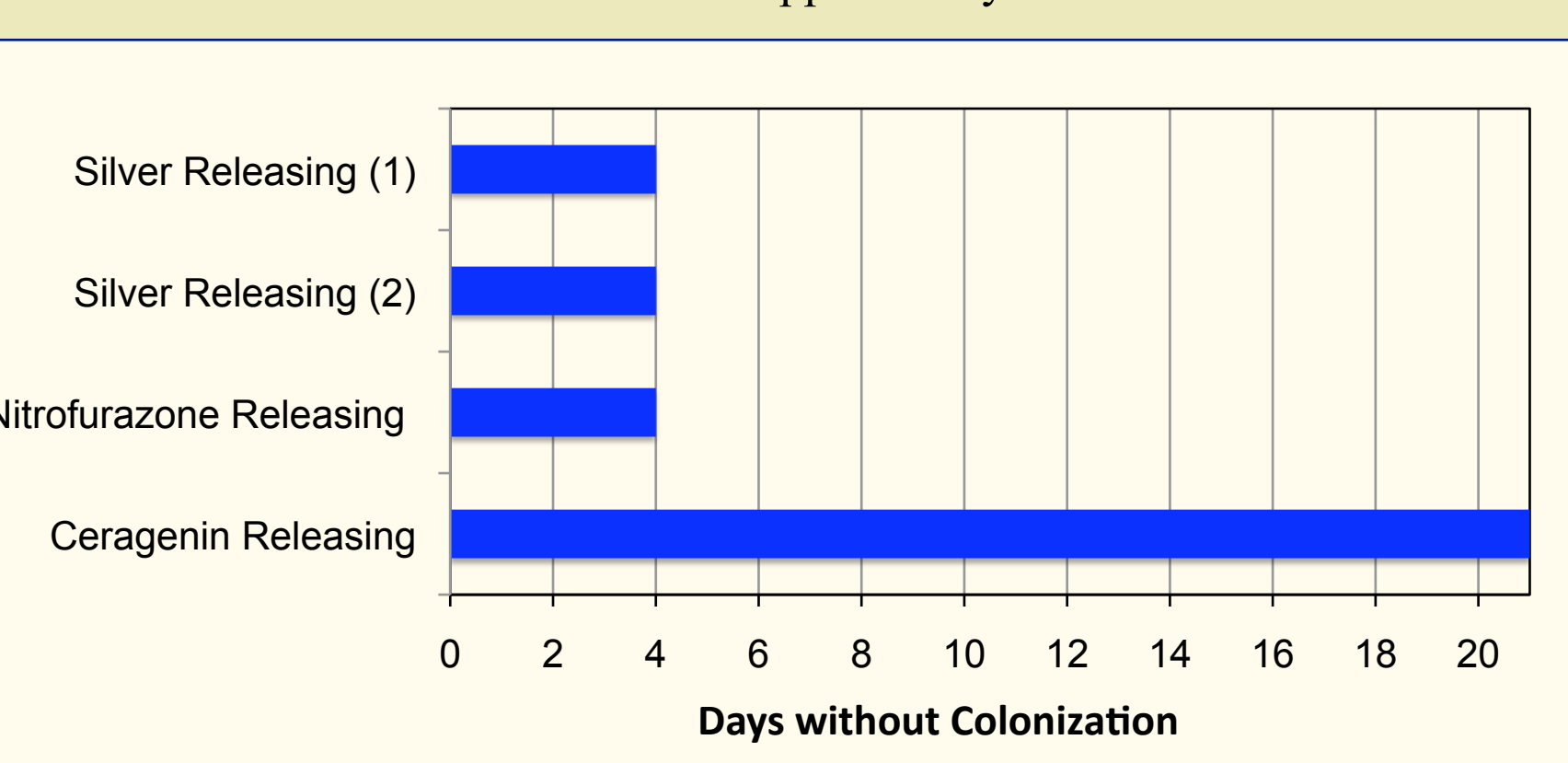
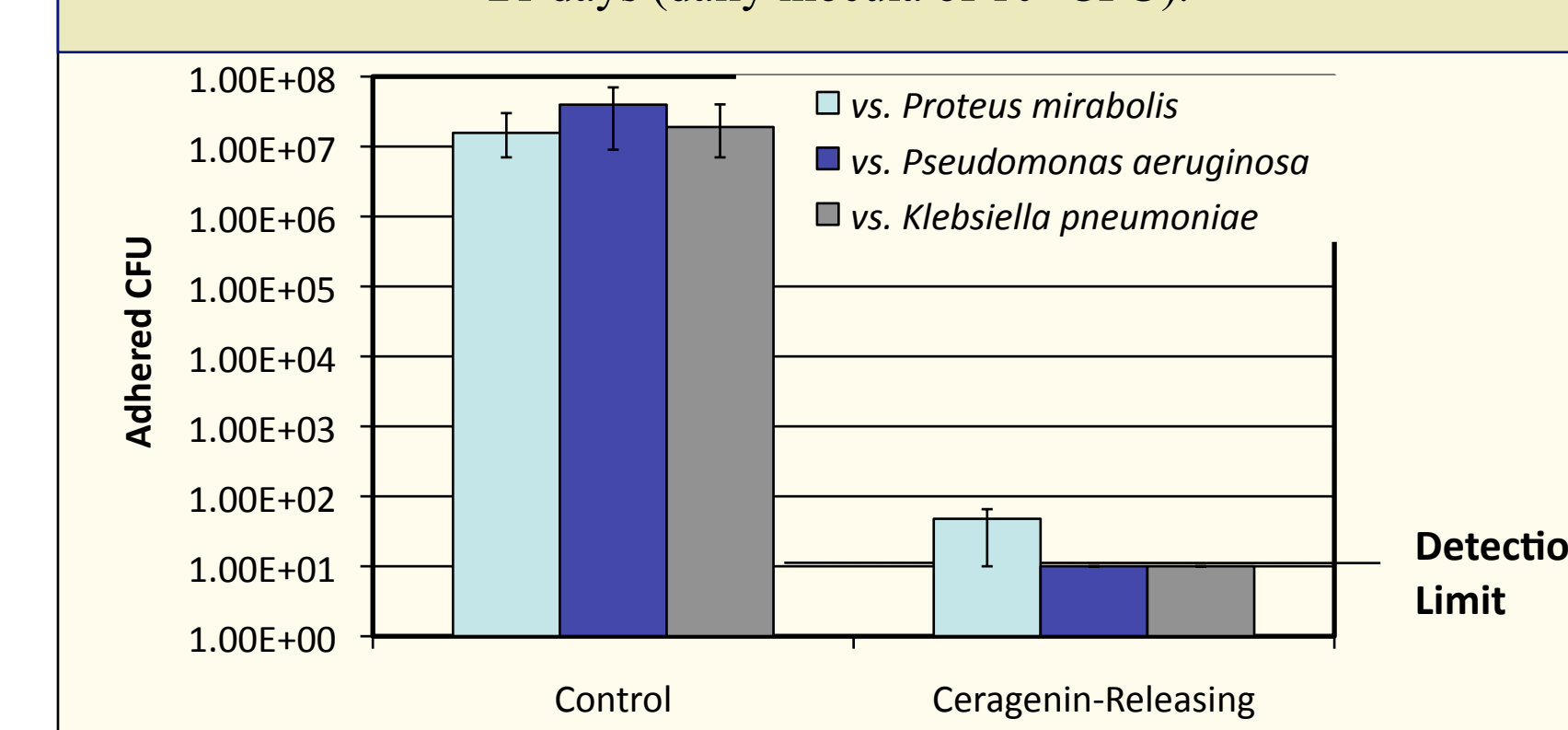


Figure 4. Quantities of the indicated bacterial strains adhered to ceragenin-releasing catheter segments after 21 days (daily inocula of 10^3 CFU).



CONCLUSIONS

As potent and broad-spectrum bactericidal agents, ceragenins are well suited for wide-spread use in preventing bacterial colonization of medical devices. Because they mimic the activity of antimicrobial peptides, it is anticipated that use of ceragenins will not readily engender resistance. In addition, the ceragenins are relatively simple to prepare and purify on a large scale. Though ceragenins are highly water soluble, due to their cationic character, their release from thin films can be controlled. Thin films derived from anionic polymers regulate the release of ceragenins, and sufficient quantities of a lead compound, CSA-13, are released even after 21 days to eradicate inocula of a variety of pathogens associated with catheter-associated urinary tract infections.

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