Biofilm prevention potential of 0.02% polyhexanide irrigation solution in urethral catheters under practice-like in vitro conditions

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Abstract

Background

Long-term use of indwelling urethral catheters is associated with high risk of urinary tract infection (UTI) and blockage. Microbial biofilms are a common cause of catheter blockage, reduce their lifetime and significantly increase morbidity of UTIs. A 0.02% polyhexanide irrigation solution developed for routine mechanical rinsing shows potential for bacterial decolonization of suprapubic and indwelling urethral catheters and has the potential to reduce or prevent biofilm formation.

Methods

Using a practice-like *in vitro* assay and standard silicon catheters, artificially contaminated with clinically relevant bacteria, assays were carried out to evaluate the biofilm reduction and prevention potential of polyhexanide vs. no intervention (standard approach) and irrigation with saline solution (NaCl 0.9%). The biofilm mass was measured by crystal violet staining and fluorescence microscopy.

Results

Irrigation with a 0.02% polyhexanide solution reduced the biofilm mass by approx. 85% vs. non-treated catheters. Standard 0.9% saline solution was able to reduce the biofilm mass by approx. 50%. Fluorescence microscopy showed that polyhexanide is able to destroy bacteria in the biofilm, albeit only those cells on the upper layers.

Conclusions

The polyhexanide and standard saline solutions are able to reduce bacterial biofilm from urinary catheters, showing a combination of mechanical and antibacterial effects. The data supports a prevention strategy to avoid the formation of a thick biofilm, which is characteristically difficult to be efficiently removed. Further research is required to evaluate the long-term tolerability and efficacy of polyhexanide in clinical practice.

Background

Urinary tract infections are among the most common nosocomial infections. In Germany, for instance, it was estimated that approximately 155,000 nosocomial urinary tract infections occur every year, and the majority of these cases are catheter-associated (1). In this context, suprapubic and indwelling catheters are considered a good alternative to short-term transurethral catheters due to a lower risk of associated bacteriuria (2). In fact, their long-term use for the treatment of intractable urinary incontinence and retention is frequent in clinical settings and particularly in community healthcare settings (3).

Catheters, however, as many inserted medical devices, are heavily prone to microbial biofilm formation (2). A variety of pathogens are able to colonize catheters: bacteria from the gastrointestinal tract or ascending from the bladder, usually commensal species, or bacteria transferred from the insertion site (2, 4). In a biofilm, microbes are attached to the catheter surfaces in a manner that prevents their removal with gentle rinsing and would require mechanical removal. In fact, biofilms formed in catheters often lead to catheter encrustation and obstruction (5).

Pathogens such as *Escherichia coli*, *Enterobacter* spp., *Pseudomonas* spp., *Enterococcus* spp., *Staphylococcus aureus*, coagulase-negative staphylococci and yeasts are common causes of urinary tract infections and catheter blockage (4, 6) and the most commonly reported species forming biofilms on urethral catheters are *Candida* spp., *Pseudomonas aeruginosa*, *Proteus mirabilis, E. faecalis*, and *S. aureus* (2, 4). Scanning electron microscopy performed on biofilms formed on indwelling catheters has shown depths ranging from 3 to 490 µm and up to 400 visible bacterial cells deep (5).

Biofilms in suprapubic and indwelling catheters have important implications for health as antibiotics are rarely able to penetrate the superficial layers of the biofilm, complicating treatment (5). In fact, microbial biofilms are known to be up to 1500 times more resistant to antibiotic therapy compared to planktonic, free-living bacteria (4, 7). Biofilms on catheters, can lead to significant complications and unfavorable outcomes for the patients' health (4) and for this reason, the development of effective methods and compounds for the prevention of biofilm formation or their reduction is of great importance (2, 4, 8).

Polyhexanide (polyhexamethylene biguanide or PHMB) is a polymer frequently used as an antiseptic with broad antibacterial activity, good tissue tolerability and, to date, shows no development of bacterial resistance (9). Polyhexanide has been used for mechanical rinsing and removal of biofilms across a range of applications (6, 8, 10). In this study, we investigated the potential of a polyhexanide solution to reduce and prevent biofilm formation under practice-like *in vitro* conditions in artificially colonized catheters.

Methods

Test microorganisms

The experiments were performed using the following reference bacterial strains: *Escherichia coli* (ATCC® 11229), *Proteus mirabilis* (ATCC® 14153, DSM 778), methicillin-resistant *Staphylococcus aureus subsp. aureus* (MRSA, ATCC® 33592) and *Pseudomonas aeruginosa* PAO1 (ATCC® 47085, DSM 22644).

Solutions

The following solutions/suspensions were used in this study:

Artificial urine

Used to simulate the pH, salinity, and urea concentration in human urine. It consisted of 25 g/L urea, 9 g/L NaCl, 2.5 g/L potassium hydrogen orthophosphate, dipotassium hydrogen orthophosphate, 3 g/L ammonium chloride 2 g/L creatinine, 3 g/L sodium sulphite, 3 g/L bovine serum albumin in 1000 mL sterile bi-distilled water. All reagents were supplied from Carl Roth (Germany).

Saline solution

A 0.09% NaCl (Carl Roth, Germany) solution.

Polyhexanide solution

Uro-Tainer® 0.02% Polyhexanide solution (B. Braun Medical, Switzerland)

Suspensions of test organisms

An overnight culture plate (Nutrient agar, OXOID, Germany) of each bacterium was washed away in 10 mL NaCl peptone and transferred to a sterile flask with glass beads. This suspension was homogenized for 2 min at 1500 rpm on a mechanical shaker and adjusted to 10^8 cells per mL using standard plate count methods (data not shown).

Practice-like in vitro **test system for decolonization of catheters**

An *in vitro* test system was developed to examine catheters in a model that simulated the three-day use of a permanent catheter system by a patient with a urinary tract infection. We used 10 catheters per treatment and three runs were performed. The Cystofix® silicon catheters (CH10, 65 cm, B. Braun Medical, Switzerland) were filled with 5 mL of a mixed suspension from three test organisms (*E. coli*, *P. mirabilis* and *S. aureus*) each morning for three days and incubated with a clamp for four hours at 37 °C. After this adhesion phase, the catheters were rinsed with a syringe containing 400 mL artificial urine to simulate the patient's metabolism. They were then incubated at 37 °C for at least one more hour before being rinsed again with 400 mL artificial urine. The catheters were then incubated at 37 °C until the next morning. After three days, the catheters were used for further attempts at decolonization.

For the decolonization assays, two solutions were used: 100 mL Uro-Tainer® with 0.02% PHMB and 100 mL 0.9% NaCl. The solutions were connected to different catheters and closed with a clamp after the catheter was filled with liquid. After an exposure time of 5 min, the clamp was opened, and the remaining liquid was flushed through the catheter. Each experiment had a treated catheter and one control, untreated catheter. All catheters were then thoroughly rinsed with 10 ml of sterile, bi-distilled water to remove planktonic or detached cells and filled with 1% crystal violet (Merck, Germany). After an incubation period of 15 min at room temperature, the catheters were again thoroughly rinsed with 10 mL sterile, bi-distilled water. Afterwards, 2 mL 70% ethanol were carefully poured through the catheter into an acrylic cuvette (Sarstedt, Germany) in order to remove the crystal violet from the biofilms still present. The absorbance at 595 nm of the obtained solutions was measured in comparison with a blank cuvette with 2 mL 70% ethanol using a SPECTRAmax PLUS384 Microplate Spectrophotometer (Molecular Devices,

United States). By subtracting the absorbance of the blank value from the measured absorbance of the samples, it was possible to make indirect comparative statements on the layer thickness of the biofilm.

Evaluation of microbial viability using fluorescence microscopy

P. aeruginosa cell cultures were grown overnight and the suspension was adjusted to 10^7 cells per mL in minimal Alginate-promoting medium containing 10 mM sodium gluconate, 10 mM KNO_3, 1.25 mM $NaH_2PO_4 \cdot H2O$, 2.8 mM K_2HPO_4 and 1 mM $MgSO_4 \cdot 7H_2O$ in H_2O (modified from Ohman & Chakrabarty, 1981 (11). These cells were then used for the cultivation of biofilms on glass slides in a 2-channel flow cell following the protocol described by Bijtenhoorn et al., 2011 (12).

The flow cells were subsequently stained using 1 µM SYTO® 9 (Invitrogen, Germany) to stain the DNA of dead and alive bacteria green fluorescent, and 5 µM Propidium iodide (PI) (Thermo Fisher, United Kingdom), which is only able to penetrate damaged cell membranes and stain the DNA of dead cells red fluorescent. The biofilms were analyzed by fluorescence microscopy using a Zeiss LD LCI Plan-Apochromat 25x/0.8 (Carl Zeiss AG, Germany) on a Zeiss Axio Imager 2 fluorescence microscope (Carl Zeiss AG, Germany). Images were taken by layering three-dimensional image stacks of the 72 h old biofilms taken at a wavelength of 470 nM and 490 nM (12). Digital image acquisition, analysis and three-dimensional reconstructions were done using the Zeiss AxioVison Software (version 4.8.1).

Results

Practice-like in vitro **test system for decolonization of catheters**

The smallest amount of crystal violet was retained from biofilms after treatment with polyhexanide, indicating a biofilm mass reduction of 80–90% compared to untreated controls. Treatment with physiological saline still achieved a reduction in biofilm mass of approximately 50%. The largest amount of crystal violet could be recovered from the untreated control catheters (Fig. 1).

Evaluation of microbial viability using fluorescence microscopy

After 72 h growth mature biofilms of 50–70 µm were formed on the glass slides and fluorescence microscopy revealed major differences between treatments (Fig. 2). Treatment of biofilms with polyhexanide solution was able to kill bacteria in the upper layers of the artificial biofilms, in comparison to biofilm treatment with saline solution which had no observable effect.

Discussion

In natural, clinical, and industrial environments the formation of biofilms is a basic microbial survival strategy. Medical devices such as suprapubic and indwelling catheters used in clinical settings are

frequently colonized by biofilms of a variety of microbial species with detrimental consequences for the patients (13). Research is focused on decolonization of urethral catheters via treatment with a range of systemic antibiotic regimens (14) even though antibiotic resistance and drug adsorption are heavily modified in biofilms (4, 6, 13). In fact, antimicrobial compounds are usually not able to penetrate the full depth of the microbial biofilms, reducing the available options for effective therapy (15). Alternative methods and compounds for reduction and prevention of biofilms in catheters are necessary as altered catheter surfaces have proven ineffective at inhibiting microbial attachment (4, 15, 16).

Polyhexanide is considered one of the “most promising substances available” for clinical applications (17). Mechanical rinsing with 0.9% NaCl solution and a 0.02% solution of polyhexanide has been observed to significantly and consistently reduce bacterial colonization, providing an effective, non-systemic approach to biofilm formation on urinary catheters (6). Other studies have also confirmed the antiseptic efficacy and antibacterial effect of polyhexanide in the treatment of skin wounds, as an ingredient in mouthwash solutions or as a supplement of cleansing solutions (17).

Here, decolonization of catheters using a 0.02% polyhexanide solution was tested in an *in vitro* system in silicon catheters by crystal violet staining. Our results showed that rinsing the catheters reduces colonization by microorganisms. The treatment with polyhexanide was more effective compared to rinsing with saline solution or no rinsing at all. We demonstrated, both by fluorescence microscopy and via the practice-like *in vitro*-model, that mechanical rinsing has a considerable effect on the biofilm layer thickness and might aid in removing biofilms from suprapubic or indwelling catheter systems.

The present study goes in accordance to previous reports of the usefulness of polyhexanide (17 and references therein) and confirms the efficacy of a 0.02% polyhexanide solution to reduce and prevent the formation of biofilms in catheter systems *in vitro*. Moreover, our study achieves these results through experiments designed to replicate practice-like conditions as closely as possible.

The catheter material (non-flat, opaque) prevented their direct use for the viability evaluation of the biofilms with microscopy. Further experiments are necessary to extrapolate the behavior of biofilms on silicon catheters compared to glass flow cells as observed here. Biofilms formed in stagnant or stationary conditions are porous and develop mostly in height whereas those formed under continuous flow or with regular shaking develop thinner layers with clumped structures and patterns (18). Consequently, different morphologies have characteristic outcomes which should be considered as part of a comprehensive treatment strategy.

Conclusion

Our experiments show that both the polyhexanide and standard saline solutions are able to reduce bacterial biofilms in urinary catheters, through a combination of mechanical and antibacterial effects. The treatment with polyhexanide was more effective compared to rinsing with saline solution or no rinsing at all, constituting a prevention strategy to avoid the formation of thick bacterial biofilms in

urinary catheters. Additional research is needed to investigate whether the presented results can be transferred into practice and lead to a reduction in urinary tract infections in clinical settings.

Abbreviations

used
UTI
urinary tract infection
PHMB
Polyhexanide (polyhexamethylene biguanide)
MRSA
methicillin-resistant *Staphylococcus aureus*
spp.
species
mL
milliliter
nm
nanometer
µm
micrometer
h
hour(s)
min
minutes
g/L
grams per liter
rpm
revolutions per minute

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The study was partially financially supported by B. Braun Medical Ltd. FHHB was an employee for B. Braun Medical Ltd. between 2006 and 2010. AA is an employee of B. Braun Medical Ltd.

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Authors' contributions

FHHB, CU, HG, J-HK, JS, AA were involved in the development of the methods and the planning of the laboratory tests. JH, CU carried out the experiments. HG and J-HK were responsible for data analysis. DM drafted the original manuscript with input from all authors. All authors reviewed, edited, and approved the final version of the manuscript.

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Figures

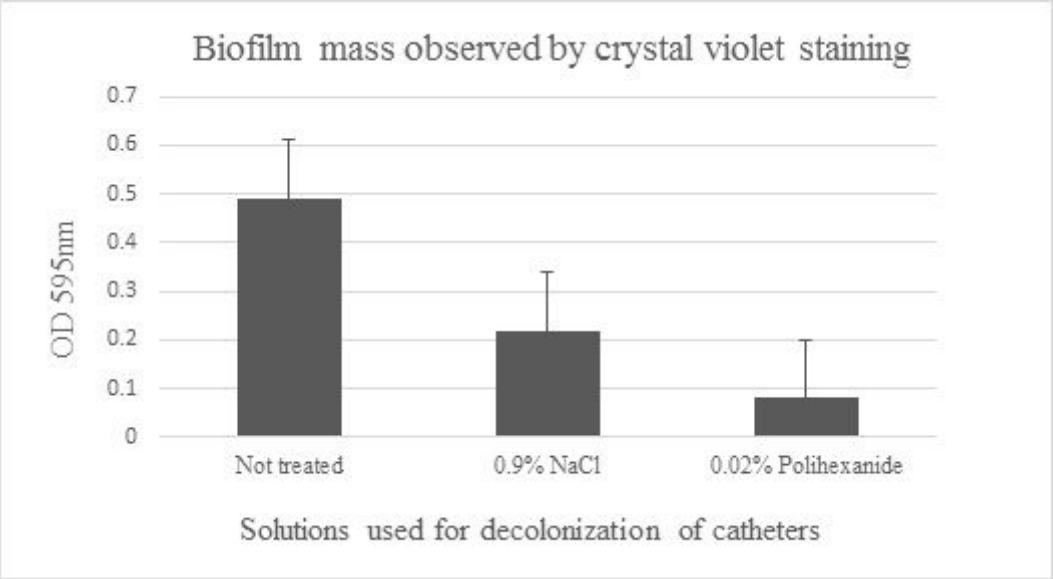

Figure 1

Rinsing of artificially colonized silicone catheters with 0.02% polyhexanide solution resulted in drastic depletion of biofilm mass in the in vitro model by crystal violet staining, compared to rinsing with a 0.9% NaCl solution or no rinsing.

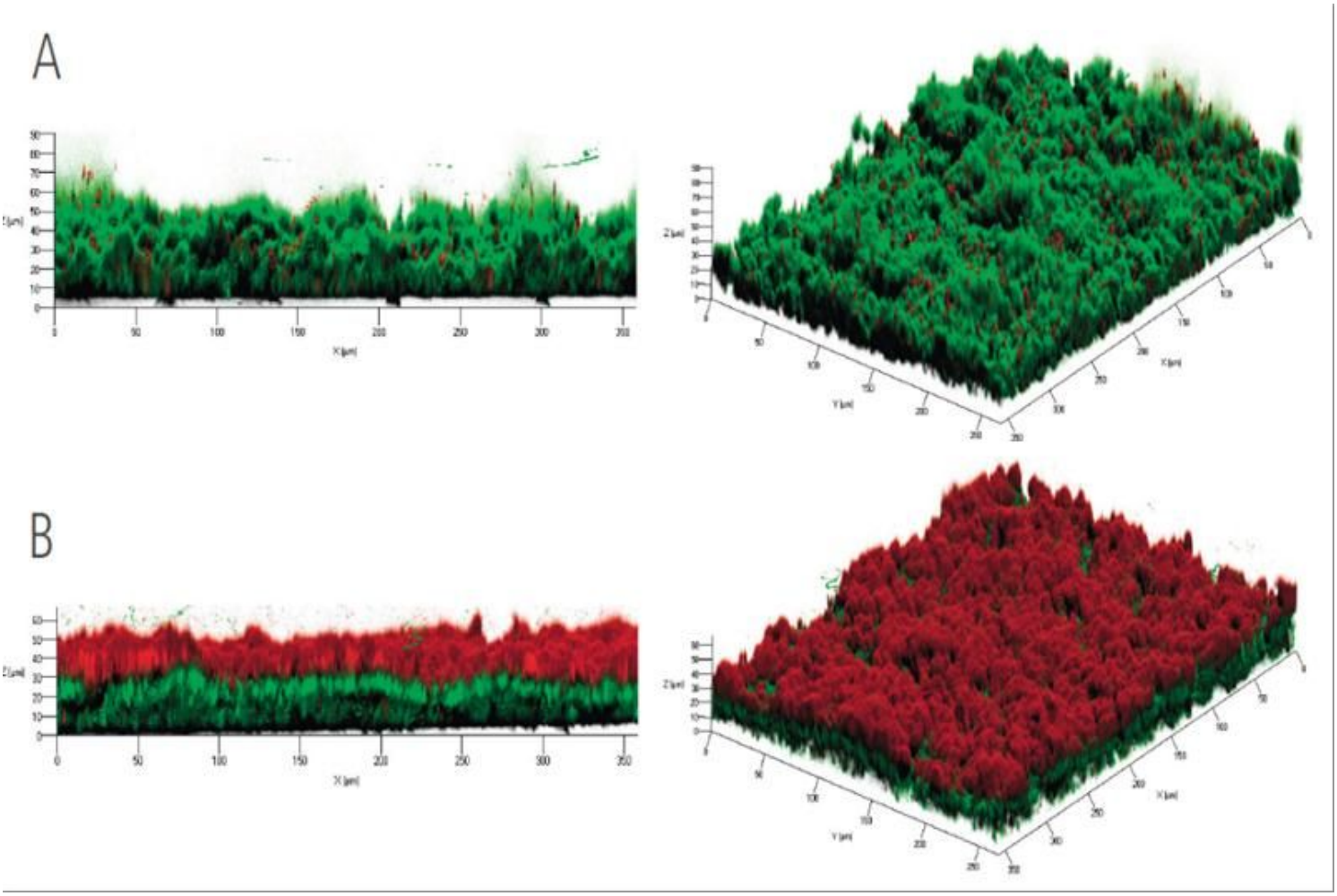

Figure 2

Reduction of biofilm mass and viability was observed for biofilms grown on glass slides under laminar flow. The P. aeruginosa biofilms were treated with A) a 0.9% NaCl solution and B) a 0.02% polyhexanide solution. Right panels show 3D view and left panels show a cross-section of the biofilms. SYTO 9-stained are shown in green, PI-positive, dead cells are shown in red.