Biofilm reduction potential of 0.02% polyhexanide irrigation solution in several types of urethral catheters

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Abstract

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

**Methods** Using an *in vitro* assay with standard market-leading types of catheters artificially contaminated with clinically relevant bacteria, assays were carried out to evaluate the biofilm reduction and prevention potential of a 0.02% polyhexanide solution vs. no intervention (standard approach) and irrigation with saline solution (NaCl 0.9%). The efficiency of decolonization was measured through microbial plate count and membrane filtration.

**Results** Irrigation using a 0.02% polyhexanide solution is suitable for the decolonization of a variety of transurethral catheters. The effect observed is significant compared to irrigation with 0.9% saline solution ($p = 0.001$) or no treatment ($p = 0.018$). No significant difference was found between irrigation with 0.9% saline solution and no treatment ($p = 0.94$).

**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 reduce thick biofilms, which are 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). Catheters, as many inserted medical devices, are heavily prone to microbial biofilm formation (2). A variety of pathogens are able to colonize catheters: commensal species of bacteria from the gastrointestinal tract or ascending from the bladder, or bacteria transferred from the insertion site (2,3).

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 (3,4) and the most commonly reported species forming biofilms on urethral catheters are *Candida* spp., *Pseudomonas aeruginosa*, *Proteus mirabilis*, *E. faecalis*, and *S. aureus* (2,3). Scanning electron microscopy performed on biofilms formed on indwelling catheters has shown depths ranging from 3 to 490µm and up to 400 visible bacterial cells deep (5).
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). Biofilms in 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 (3,6). Biofilms on catheters can lead to significant complications and unfavorable outcomes for the patients’ health (3) and for this reason, the development of effective methods and compounds for the prevention of biofilm formation or their reduction is of great importance (2,3,7).

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 (8). Polyhexanide has been used for mechanical rinsing and removal of biofilms across a range of applications (4,7,9). In this study, we investigated the potential of a polyhexanide solution to reduce and prevent biofilm formation under *in vitro* conditions in a variety of artificially colonized catheters.

**Methods**

All experiments were performed using a mixed culture of the following bacterial strains: *Escherichia coli* (ATCC® 11229), *Proteus mirabilis* (ATCC® 14153, DSM 778) and *Staphylococcus aureus* (ATCC® 6538). 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 $10^9$ CFU per mL using standard plate count methods (data not shown).

Six different types of catheters were used, as shown in Table 1. These different catheters were selected to cover a broad range of commercially available products and observe the performance of the treatments in all of them.

Table 1. Characteristics of the catheters used in this study
Thirty (30) catheters of each type were used for the decolonization test. The catheters were incubated with 5 ml of the mixed bacterial suspension for 4 h at 37°C, after which the catheters were irrigated 2 x 400 ml of an organic load suspension (0.3 % bovine albumin + 3.0 % urea, reagents from Carl Roth Germany) per day to simulate the process of contamination with urine and organic materials. After 72 h, ten of the catheters were irrigated with 100 mL Uro-Tainer 0.02 % PHMB (B. Braun Medical, Switzerland) with 5 minutes exposure time, ten catheters were irrigated with 100 mL Uro-Tainer 0.9% NaCl (B. Braun Medical, Switzerland) with an exposure time of 5 minutes and 10 catheters were not treated (controls).

After treatment, the microbial count was determined by irrigation of the catheters with 100 ml of a TLH-SDS neutralizer solution (0.1 % polysorbat 80, 0.1 % g/L lecithin, 0.1 % histidine, 0.2 % SDS, all reagents from Carl Roth Germany) and membrane filtration (0.45 µm pore size, MF-Millipore USA) of 50 mL via serial dilution method on trypticase soy bean agar (TSA, Oxoid Germany). No measurements of pH were made for the rinsed filtrates as the slightly acidic pH of the Uro-Tainer 0.02% PHMB (pH at 20 ºC of 5.5) was neutralized with the use of the TLH-SDS solution. We anticipated that the pH of the filtrates did not modify the pH of the culture media used in this study and therefore did not affect the growth of the surviving bacteria.

In addition, all catheters were cut and the material in the lumen was extracted with a sterile cotton swab. The swab was suspended in 0.9% NaCl solution (Carl Roth Germany) and the microbial count was determined via serial dilution method on TSA plates.

All nutrient media were incubated at 37 ºC for 2 days. Mean values of microbial count ($\log_{10}$ CFU) after the different treatments were calculated as well as the reduction factors for the Uro-Tainer 0.02% PHMB solution compared to Uro-Tainer 0.9% NaCl and no treatment. Statistical analyses were done using the two-tailed Student’s ‘t’ test and p values of (* $\leq$ 0.05) were considered as significant.

After negative reduction factors were observed for the type E catheters, an estimation of the biofilm mass was performed on them. Thirty new E catheters were artificially colonized as described before. For the decolonization, two solutions were used: 100 mL Uro-Tainer® with 0.02% PHMB and 100 mL Uro-Tainer
0.9% NaCl. Each of the solutions was connected to ten catheters and closed with a clamp after the catheter was filled with liquid with ten catheters left untreated as controls. After an exposure time of 5 min, the clamp was opened, and the remaining liquid was flushed through the 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 of 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 of 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 in the type E catheters.

**Results**

We observed that treatment with the Uro-Tainer 0.02% PHMB solution effectively reduced the biofilms artificially formed in the different catheter types as measured in after rinsing/membrane filtration (Fig. 1, a) as well as in the swab samples (Fig. 1, b). In general, the effect of irrigation with Uro-Tainer 0.02% PHMB solution is superior compared to the untreated catheters ($p = 0.018$) as well as compared to those treated with Uro-Tainer 0.9% NaCl solution ($p = 0.0016$) while there was no significant effect of irrigation with 0.9% NaCl compared with no treatment at all ($p = 0.94$) (Table S1). Reductions factors (Table 2) ranged from 0.3 in the case of the type B catheter with no treatment to 4.6 for the type C catheter treated with Uro-Tainer 0.9% NaCl.

**Table 2. Biocidal activity of Uro-Tainer 0.02 % PHMB compared to Uro-Tainer 0.9 % NaCl and no treatment represented by Reduction factors of log$_{10}$ CFU**

| Catheter | Reduction factors using Uro-Tainer 0.02 % PHMB compared to |  |
|----------|-------------------------------------------------------------|---|
|          | Uro-Tainer 0.9 % NaCl no treatment | Uro-Tainer 0.9 % NaCl no treatment |
|          | Cell counts after filtration | Cell counts after swab sampling |
| Catheter A | 3.2 | 3.7 | 3.4 | 3.5 |
| Catheter B | 1.3 | 0.3 | 1.8 | 1.9 |
| Catheter C | 3.6 | 3.9 | 4.6 | 4.1 |
| Catheter D | 1.2 | 1.6 | 1.5 | 1.2 |
| Catheter E | 0.6 | -0.3 | 0.5 | -0.4 |
| Catheter F | 2.3 | 3.3 | 2.1 | 2.4 |

In two occasions, we observed an increased log$_{10}$ CFU after treatment, reflected by negative reduction factors (Table 2, in bold). Both cases were of the untreated type E catheters. Through the staining of the biofilms with crystal violet, an estimation of the biofilm mass was obtained to evaluate the effect of the
Uro-Tainer 0.02 % PHMB solution. By comparing the measured absorbance of the samples (data not shown here), the results indicated a significant biofilm mass reduction of up to 90% for the catheters rinsed with 0.02 % PHMB solution compared to the untreated controls ($p = 0.0159$), whereas rinsing with 0.9 % NaCl still achieved a reduction in biofilm mass of approximately 50%. No statistically significant differences were found between rinsing the catheters with Uro-Tainer 0.02 % PHMB and Uro-Tainer 0.9 % NaCl ($p = 0.4102$).

**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 (10). Research is focused on decolonization of urethral catheters via treatment with a range of systemic antibiotic regimens (11) even though antibiotic resistance and drug adsorption are heavily modified in biofilms (3,4,10). In fact, antimicrobial compounds are usually not able to penetrate the full depth of the microbial biofilms, reducing the available options for effective therapy (12). Treatment is further complicated by the fact that biofilms are frequently composed of a variety of species, as demonstrated from urine samples of colonized-catheters (13). 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 (3,12,14).

Polyhexanide is considered one of the “most promising substances available” for clinical applications (15). 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 (4). 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 (15). Furthermore, observational studies in patients with indwelling catheters in which the Uro-Tainer 0.02% PHMB solution was used for rinsing, showed no serious adverse events for the patients (16).

Here, the reduction of biofilms in artificially colonized commercially available catheters using a 0.02% polyhexanide solution was tested in an *in vitro* assay. The study confirms the efficacy of the Uro-Tainer 0.02% PHMB solution on a broad range of catheters types. Our results showed that rinsing the catheters reduces colonization and the treatment with polyhexanide was more effective compared to rinsing with saline solution or no rinsing at all ($p = 0.0016$ and $p = 0.018$, respectively), in all 6 types of catheters tested. These results go in accordance to previous reports of the usefulness of polyhexanide (15 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* (4).

The formation of biofilms in urethral catheters is aided by the deposition of organic molecules from the urinary components, such as proteins and electrolytes (13). The inert materials of catheters make them
susceptible to microbial colonization (13), as they cannot produce the immunological response triggered in the mucosa of living tissue responsible for neutralizing colonizing microbes. While some bacteria have proven to adhere less to certain materials, as is the case of *E. coli* and *Klebsiella pneumoniae* in siliconized-rubber catheters (13,17), with enough time the microflora inevitably finds a way to colonize the catheter. This study provides evidence of the efficacy of Uro-Tainer 0.02% PHMB to reduce biofilms independently of the catheter material, as the results show significant reductions of the log$_{10}$ CFU in all tested catheters. Such efficacy is of great importance as catheter material generally does not diminish the ability of microbes to form biofilms within them, except in the setting of short-term catheterization (13).

In addition, our results showed that rinsing the catheters reduces colonization by microorganisms as observed in the biofilm mass estimation of the type E catheters. Rinsing with 0.02% polyhexanide was still more effective compared to rinsing with saline solution or no rinsing at all, albeit not as noticeably as for the other catheter types. While the mechanical rinsing might explain the effect observed, crystal violet staining did not provide us with a measure of biofilm viability, as both the alive and dead bacterial cells were stained as well as their extracellular matrix. Thus, we concluded that the negative reduction factors observed for the type E catheters might respond to an intrinsic property of these devices to prevent the formation of the bacterial biofilm in the first place.

**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 **Uro-Tainer 0.02 % PHMB** was significantly more effective compared to rinsing with saline solution or no rinsing at all, constituting a strategy for the reduction of the viability of thick bacterial biofilms in a variety of urinary catheters.

Additional research is recommended to investigate whether the presented results can be transferred into practice and lead to a reduction in urinary tract infections in clinical settings. Parameters such as the pH of patients’ urine could be investigated further to determine its effect on the efficacy of polyhexanide

**Abbreviations**

**UTI:** urinary tract infection

**PHMB:** Polyhexanide (polyhexamethylene biguanide)

**spp.:** species

**mL:** milliliter

**nm:** nanometer

**µm:** micrometer
Declarations

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Availability of data and materials
The data supporting the conclusions of this article is included within the article (and its Figure S1 in Supplemental material). Additional raw 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. All authors reviewed, edited, and approved the final version of the manuscript.

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Supplementary Table 1

Table S1. Cell counts of rinsing solutions and of swab samples after different treatments. The values of log_{10} CFU shown are mean of 10 parallel replications.

| Type of catheter | Mean log_{10} CFU after treatment | Cell counts after filtration | Cell counts after swab sampling |
|------------------|----------------------------------|-----------------------------|--------------------------------|
|                  | Uro-Tainer 0.02 % PHMB | Uro-Tainer 0.9 % NaCl | no treatment | Uro-Tainer 0.02 % PHMB | Uro-Tainer 0.9 % NaCl | no treatment |
| Catheter A (Ref. 176818) | 1.6 | 4.8 | 5.3 | 1.4 | 4.8 | 4.9 |
| Catheter B (Ref. AD6518) | 2.3 | 3.6 | 2.6 | 1.4 | 3.2 | 3.3 |
| Catheter C (Ref. 250100) | 1.7 | 5.3 | 5.6 | 1.1 | 5.7 | 5.2 |
| Catheter D (Ref. 171305) | 2.0 | 3.2 | 3.6 | ≤ 1.1 | 2.6 | 2.3 |
| Catheter E (Ref. 4441001) | 2.5 | 3.1 | 2.2 | 2.3 | 2.8 | 1.9 |
| Catheter F (Ref. 04563182) | 2.0 | 4.3 | 5.3 | 1.9 | 4.0 | 4.3 |
| Weighted average | 2.01667 | 4.05 | 4.1 | 1.5333 | 3.85 | 3.65 |
| Standard deviation | 0.343 | 0.896 | 0.1499 | 0.476 | 1.219 | 1.370 |
| Variance | 0.117 | 0.803 | 2.248 | 0.226 | 1.487 | 1.879 |

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.