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A new urinary catheter design reduces in-vitro biofilm formation by influencing hydrodynamics

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SUMMARY

Aim: To evaluate the performance of a new catheter design based on different hydrodynamics aiming to reduce the development of biofilm, and compare it with a conventional Foley catheter (FC).

Methods: The new proposed design (NPD) catheter is a modification of the FC, based on asymmetric positioning of the balloon and additional drainage holes allowing continuous urine drainage and complete voiding of the bladder. A first experiment was undertaken to assess drainage capability, and a second experiment was performed using a bioreactor with a set-up simulating the bladder and using the test catheter as a flow-through system. The biofilm formation of five bacterial species associated with catheter-associated urinary tract infection (CAUTI) was determined after 24 h of incubation using an MTT assay. Morphological evaluation was performed using scanning electron microscopy. In-vitro determination of residual fluid, and quantitative and morphological data on biofilm formation on the intravesical and intraluminal parts of the tested catheters were assessed.

Results: Residual fluid was significantly higher in the FC (5.60 ± 0.43 mL) compared with the NPD catheter (0.2 ± 0.03 mL). The NPD catheter showed significantly less biofilm formation ($P<0.0001$) than the FC. Catheter design had a variable effect on biofilm formation depending on the bacterial strain tested. There was significantly less intraluminal biomass compared with intravesical biomass in both catheters ($P<0.0001$). Multi-layered biofilms that covered the FC surfaces completely were seen for all tested strains, while the NPD catheter surfaces showed reduced biofilm formation.

Conclusions: Modifications of the hydrodynamic characteristics of a catheter can significantly reduce bacterial colonization. Integrated design approaches combining chemical, mechanical and topographical elements can help to reduce the occurrence of CAUTI.

<A>Introduction

Hospital infections are frequently associated with the use of biomaterials and devices such as heart valves, venous catheters and urinary tract catheters [1]. Catheter-associated urinary tract infections (CAUTIs) are the most common hospital-acquired infections. CAUTIs represent 80% of all nosocomial urinary tract infections in European countries. In is estimated that nearly 5–8% of 1.6 million individuals in US nursing homes have an indwelling urinary catheter, and in the UK, there are an estimated 52,086 CAUTIs and 7528 catheter-associated bloodstream infections each year [2,3].

Once an indwelling urinary catheter has been placed, bacterial colonization of its surfaces begins, mainly as a consequence of contamination during catheter positioning. Colonization occurs within 3–4 days in patients with open drainage systems, and within 1 month in patients with closed drainage systems [4]. Furthermore, the presence of an indwelling urinary catheter dramatically increases the possibility of infection with nosocomial bacterial species, such as *Pseudomonas aeruginosa* or *Staphylococcus aureus*, and encrustations [4,5].

The Foley catheter (FC) design is based on a flexible tube with drainage holes on the lateral parts of its tip and an inflatable balloon immediately below (Figure 1). Once the catheter is inserted into the bladder, the balloon is inflated, maintaining the position of the device. As they are simple to use, well tolerated by patients and relatively inexpensive, FCs are used worldwide. Nevertheless, the use of FCs for long-term catheterization is the subject of criticism due to their causal role in the development of CAUTIs [6,7].

<insert Figure 1 near here>

Care in indications, catheter application technique, anti-infective prophylaxis and maximum hygiene measures are proposed for indwelling FCs to reduce CAUTIs [6,8–10]. Furthermore, it is

crucial to improve the characteristics of the catheter, such as the material and its structure [11,12], and surface roughness [13–16].

From a design viewpoint, the structure can dramatically influence the hydrodynamic characteristics of urinary flow. These can affect the physicochemical characteristics of biofilm colonizing the catheter surfaces. Micro-organisms adhering to a surface interact with their environment through the medium that flows over the biofilm. Changes in the velocity of medium flow can significantly affect metabolic activity, morphology and structure of the biofilm, and can also alter the pattern of gene expression of the microbial community [17–21]. This disturbance of the ecology [22,23] can lead to detachment and dissemination of a pathogenic biofilm, and can influence the efficacy of antibiotic treatment [24–26]. Biofilm formation is enhanced by reduced flow and by the existence of periods where flow is close to zero. This condition typically occurs in FCs, where the bladder is never completely emptied and the flow is intermittent. This situation increases the possibility for bacterial cells to adhere to the surface material and form a biofilm [6,16,27–30]. One of the most important criticisms of the FC is that bladder voiding is not complete as the two drainage holes are placed above the balloon, meaning that a certain amount of urine constantly remains around the balloon itself.

As such, the possibility of influencing biofilm formation by designing a catheter with novel hydrodynamic properties is of interest. Similar to the FC, the new proposed design (NPD) catheter includes an anchoring system consisting of an inflatable balloon placed near the tip. However, the balloon design is different from the FC. In the NPD catheter, the balloon is asymmetric with respect to the major axis of the device. Once inflated, it extends to cover only 180° of the circumference of the catheter, unlike the FC which extends to cover 360°. Furthermore, in the NPD catheter, two drainage holes are made on opposite sides of the inflating balloon, one near the upper boundary and one near the lower boundary. In addition, there is a through-hole on the tip of the device according to the conventional design of the FC (Figure 1). The specific position of the drainage holes allows complete voiding of residual urine from the bladder, and keeps the surface of the balloon free from

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urine. The aim of this study was to evaluate, *in vitro*, if a modified self-retaining drainage system can influence biofilm formation of different pathogenic species and reduce CAUTIs compared with the FC.

Methods

In-vitro determination of residual urine

A simple preliminary test was performed to assess the drainage ability of the FC and NPD catheter (Figure 1). In total, five FCs and five NPD sterile silicon catheters (Medicoplast International GmbH, Illingen, Germany) were used. The luer lock parts of 10 60-mL syringes were drilled, and a catheter was passed through the hole and press-fitted, positioning its intravesical part inside the syringe barrel (Figure 1a). The balloons of the catheters were filled with 10 mL of light-blue coloured water. Balloons were pulled to the bottom of the syringes, and catheters were temporarily clamped. Forty millilitres of yellow-coloured distilled water was inserted in the syringes and clamps were removed, allowing the liquid to flow. Next, the amount of liquid remaining was measured. Food colouring dyes were used (yellow: tartrazine E102; blue: Brilliant Blue FCF, E 133) at a concentration of 0.8 mg/mL.

In-vitro determination of biofilm formation

Specimen preparation

In total, 35 FCs, 35 NPD catheters and 70 vials (Falcon 50 mL, Fischer Scientific, Hampton, NH, USA) were used to produce an in-vitro bladder model. Each vial was drilled in the centre of the lid and the conical bottom, and a catheter was passed through the lower hole, positioning its intravesical part inside the vial. As the diameter of the hole (5.5 mm) was slightly narrower than that of the catheter (6.0 mm), a tight leakproof connection was obtained. The system was sterilized using a plasma peroxide chemiclave (Sterrad; ASP, Irvine, CA, USA). This sterilization process was used to prevent modification of the catheter surfaces, reaching a maximum temperature of 45 °C. The holes in the lid were used to connect the device via silicon tubing to a computer-controlled peristaltic pump (RP-1; Rainin, Emeryville, CA, USA) that provided a constant flow of fluid through the system (Figure 1b–d).

Bacterial strains

All reagents and culture media were obtained from Becton-Dickinson (BD Diagnostics-Difco, Franklin Lakes, NJ, USA). *Escherichia coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 29213) and *Staphylococcus epidermidis* (ATCC 149909) strains were cultured on Columbia CNA agar with 5% sheep blood plates, while *Proteus mirabilis* (ATCC 7002) was cultured on cystine-lactose-electrolyte-deficient agar plates to prevent swarming. Suspensions of each strain in brain heart infusion broth were obtained after overnight incubation at 37 °C in a 5% CO_2-supplemented atmosphere. Cells were harvested by centrifugation (2200 rpm, 19 °C, 5 min), washed twice with sterile phosphate-buffered saline (PBS) and resuspended in the same buffer. The suspensions were then sonicated (Sonifier model B-15; Branson, Danbury, CT, USA; 7W for 30 s) and the bacterial suspensions were adjusted to an optical density (OD) of 0.3 at 550 nm that corresponds to approximately 6.0 x 108 as confirmed by colony-forming unit plate count.

Urine

Human urine was used in this study. It was collected from the experimenters themselves, being three healthy non-smoking donors that had not used any drugs in the 2 weeks preceding collection. Written, informed consent was obtained and no ethical approval was needed by the institutional review board for the experimenters to collect the urine themselves. The collected urine was immediately filter-sterilized using a bottle-top vacuum filter system equipped with 0.45-µm filter discs.

Continuous flow bioreactors

The bioreactors used in this study were based on the in-vitro model proposed by Wang *et al.* [30]. Briefly, the vials described above simulate the bladder in which the tested catheters were introduced. The bioreactors were assembled in a sterile hood, the balloon of each catheter was filled with 10 mL of PBS and the whole system was transferred into an incubator (Figure 1b–d). Each vial

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was inoculated with 10 mL of one of the previously described bacterial suspensions. The suspensions were incubated in batches for 2 h to allow bacterial adherence, and then the peristaltic pump was turned on to provide a flow of urine through the bioreactor. The flow was set to simulate 1.5 L of urine/24 h; after 48 h, the pump was stopped. The lids of the vials were unscrewed and the balloons of the catheters were emptied. Each catheter was removed carefully from the vial by cutting at the level of the bottom hole using a sterile scalpel. From each catheter, a total of 15 sections (length = 10 mm) were obtained using a sterile scalpel, five from the intravesical part and ten equally spaced from the remaining intraluminal length of the catheter (Figure 2a). Fragments were gently rinsed twice with sterile PBS to remove non-adherent bacterial cells. The third (intravesical), seventh and 12th (intraluminal) sections were selected for morphological analysis using scanning electron microscopy (SEM), and the other sections were subjected to viable biomass quantification using an MTT assay. All experiments were performed in triplicate, each in a different month.

<insert Figure 2 near here>

<C>Adherent biomass evaluation (MTT assay)

The assay was conducted as described previously [27]. Briefly, two stock solutions were prepared by dissolving 5 mg/mL of 3-(4,5)-dimethylthiazol-2-yl-2,5- diphenyltetrazolium bromide (MTT) in sterile PBS, and 0.3 mg/mL of N-methylphenazinium methyl sulphate (PMS) in sterile PBS. The solutions were stored at 2 °C in lightproof vials until the day of the experiment, when a fresh measurement solution (FMS) was made by mixing 1 mL of MTT stock solution, 1 mL of PMS stock solution and 8 mL of sterile PBS. A lysing solution (LS) was prepared by dissolving 10% v/v of sodium dodecyl sulfate and 50% v/v of dimethylformamide in distilled water. Both FMS and LS were warmed at 37°C for 2 h before use. Each section was placed inside a well of a 24-well plate and 1250 µL of FMS was added (Figure 2b). The plates were incubated for 3 h at 37 °C in

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lightproof conditions. During incubation, electron transport across the microbial plasma membrane and, to a lesser extent, microbial redox systems converted the yellow MTT salt to insoluble purple formazan; the conversion was facilitated by the intermediate electron acceptor (PMS). The unreacted FMS was gently removed from the wells by aspiration, the formazan crystals were dissolved by adding 1250 µL of LS into each well, and the plates were further incubated for 1 h at room temperature in lightproof conditions. In total, 200 µL of the suspension was then removed from each well, transferred to a 96-well plate, and OD (550 nm) was measured with a spectrophotometer (Genesys 10-S). Results were expressed as OD units, which are proportional to the number of viable and metabolically active bacterial cells adherent to the catheter surfaces. The OD values corresponding to the intravesical portion of the catheters were halved to correct the results for the increase in adherence surface (bacterial colonization of both the inner and outer parts of the catheter).

Morphological evaluation (SEM)

The catheter sections that were selected for SEM analysis were placed in a 2% cacodylate- buffered glutaraldehyde solution (pH=7.4) for 24 h. After that, sections were cut open carefully with a sterile scalpel along the longitudinal axis in order to expose the inner surface. The sections were then passed through graded ethanol solutions (35, 50, 70, 80, 90, 95 and 100 v/v %, 10 min each). Next, they were dried using a critical point dryer (TOP Critical Point 30, Pabisch, Milan, Italy), mounted on a stub using conductive tape, sputter-coated (LTD E5100 cool sputter coater, Polaron, Watford, UK) and observed using SEM (JSM - 840A, JEOL, Tokyo, Japan). Four fields were recorded for each catheter section at 2000 x magnification.

Statistical analysis

Data were analysed using JMP 10.0 (SAS Institute, Inc, Cary, NC, USA). The normality of distribution of the dataset was preliminarily checked using Shapiro–Wilk's test, and

homoscedasticity was verified using Levène's test. Analysis of residual urine data was performed using the Kruskal–Wallis test. For analysis of viable biomass, data were log-transformed to approximate a normal distribution. Three-way analysis of variance (ANOVA) was used, considering catheter design (FC vs NPD catheter), bacterial strain and position (intravesical vs intraluminal) as fixed factors. Student's t-test was used as a post-hoc test. The significance level was set for both tests to $\alpha < 0.05$.

Results

In-vitro determination of residual urine

The data distribution was not normal and variances were not homogeneous. Therefore, a non-parametric test was used. At the end of the emptying phase, a mean of 5.60 ± 0.43 mL of coloured fluid remained under the holes of the catheter tip in the syringes with FCs; in comparison, a mean of 0.2 ± 0.03 mL of coloured fluid remained in the syringes with NDP catheters. This difference was significant ($P=0.009$).

Adherent biomass evaluation (MTT assay)

Adherent biomass evaluation

When considering ANOVA results, the 'catheter design' factor showed significant differences. NPD catheters showed lower biofilm formation than FCs (Fig. 2c). Bacterial strains showed a different level of biofilm formation. In terms of interaction of factors, ANOVA showed a significant interaction between catheter design and bacterial strain ($P=0.014$), meaning that the catheter design had a different effect on biofilm formation depending on the bacterial strain tested. Additionally, in both types of catheter, the amount of intraluminal biomass was significantly lower compared with the amount of intravesical biomass ($P<0.0001$). In the intravesical part, the NPD catheter showed significantly lower formation of *E. coli*, *P. aeruginosa* and *S. aureus* biofilm compared with the FC. In the intraluminal part, the NPD catheter showed significantly lower

formation of *P. aeruginosa, S. aureus* and *P. mirabilis* biofilm compared with the FC. No significant differences in biofilm formation were found for *P. mirabilis* and *S. epidermidis* in the intravesical part, or between *E. coli* and *S. epidermidis* in the intraluminal part.

Morphological evaluation (SEM)

The results of morphological evaluation with SEM are shown in Figures 3 and 4. FCs showed the formation of a multi-layered biofilm that was often able to cover the catheter surfaces completely in all tested strains. The intravesical part showed confluent bacterial growth, while slightly lower colonization was found in the intraluminal part. NPD catheters showed reduced biofilm formation for *P. aeruginosa, S. aureus*, S. *epidermidis* and *P. mirabilis*. For the latter strains, the intraluminal part only showed bacterial cell clusters adherent to the catheter surfaces, while the intravesical part, infected with *P. aeruginosa* and *P. mirabilis*, was predominantly characterized by non-confluent microcolonies and bacterial microcolonies. One exception was *E. coli,* which showed intense biofilm formation in FCs and NPD catheters in both the intravesical and intraluminal parts. The areas free of bacterial colonization had a very rough surface microtexture. The microtexture was mainly oriented in a longitudinal direction, parallel to the long axis of the catheter.

<insert Figures 3 and 4 near here>

Discussion

The first step in CAUTI pathogenesis is the formation of biofilm on the surfaces of catheters. Biofilms are bacterial communities that adhere to a substrate surface with cells growing while protected by an extracellular polysaccharide matrix. This structure enables bacteria to rapidly colonize both natural and artificial surfaces of devices placed in the human body [18,19,23,26]. Biofilm cells may show differences from their planktonic counterparts in terms of virulence and antibiotic susceptibility. This phenomenon can explain why antimicrobial therapies that are

effective against planktonic cells often fail to remove bacterial biofilms from catheter surfaces. Micro-organisms that adhere to a surface interact with their environment through the water flowing over the biofilm. This interaction involves complex hydrodynamic and coupled convection–diffusion phenomena. The flow velocity can have a major effect on the behaviour of the microbial community towards the organism and the material to which it adheres, and alter the pattern of gene expression in a research system or metabolic activity leading to the detachment and dissemination of bacterial cells [16,27].

In this study, a biofilm reactor was used to simulate the environmental characteristics of a catheterized urinary tract as closely as possible to clinical conditions [1]. These conditions include important parameters strictly related to the development of CAUTIs, as human urine was used as the flowing medium, with flow adjusted to clinical values (1.05 mL/min corresponding to 1.50 L/24 h). Starting from this idea, a bioreactor that replicated the bladder was used to approximate the set-up in the clinical setting, with favourable conditions for bacterial adherence and biofilm formation. The NPD catheter showed significantly lower biofilm formation than the FC. This result was found in the intravesical and intraluminal parts of the tested catheters. Taking into account the determination of residual urine, the NPD catheter allowed for almost complete emptying of the bladder model; this physical behaviour can provide a possible explanation for the microbiological behaviour of the tested catheters. The third and fourth holes of the NPD catheter – at the upper and lower edges of the balloon – allowed continuous flow of urine through the catheter. In contrast, the hole on the tip of the FC allowed intermittent flow of urine, continually leaving a volume of residual urine inside the bladder model. This difference in flow characteristics (intensity and timing) inside the two catheters leads to very distinct environments for microbial adherence and subsequent biofilm formation. The suggestion to improve drainage through multiple holes on a catheter was first proposed by Thurner-Warwick in 1973 [28]. This concept was developed further by others in the postradical prostatectomy setting or after urethral surgery [29,30]. However, to the authors' knowledge, attempting to ensure complete emptying of the bladder and the prevention of

CAUTI have not been investigated previously; noticeably, the balloon itself has never been modified in any of the mentioned self-retaining catheters.

The effect of hydrodynamics on bacterial adherence cannot be underestimated [14,22,25,27,31–33]. Hydrodynamic forces deeply influence the behaviour of biofilm in terms of structure, metabolism and dissemination possibilities. Thus, the hydrodynamic characteristics of the environment under study have to be considered and simulated *in vitro* in any biofilm formation experiment. In the present study, modification of the catheter's drainage holes probably resulted in a critical change of hydrodynamics in both the intravesical and intraluminal parts. Biofilm formation could be favoured by periods of reduced flow that were made possible with the design of the FC. The NPD catheter, allowing almost continuous flow, may have had less favourable conditions for biofilm formation. Surface topography is another crucial factor to consider when evaluating biofilm formation in such studies. Microfluidic conditions and strain–stress distribution near the surface are heavily influenced by surface topography [13,33]. Morphological observation showed a characteristic surface pattern in both tested catheters, likely due to manufacturing processes (Figure 5). It is not easy to determine if this pattern is positive or detrimental in terms of microbial adherence to the surfaces. The literature suggests that microtopography alters bacterial attachment patterns by triggering variation in local flow. Halder *et al.* [13,33] investigated the near-surface microfluidic environment developed on a patterned surface and its influence on the adherence of *E. coli* cells. Data demonstrated increased velocity of cells compared with that over a flat surface, and an increase in surface coverage by the micro-organism. On the contrary, Ionescu *et al.*, investigating microbial colonization of laser-micropatterned surfaces, found that this characteristic can reduce both bacterial adherence and biofilm formation significantly [15].

Five of the most common species isolated in CAUTI were tested in this experiment. The strains were ATCC-certified, therefore allowing better standardization of the test procedures and their repeatability by different research groups. This is particularly important when a bioreactor model is developed, the operational envelope of which has to be controlled. Reviews of the

literature show that uropathogenic *E. coli* accounts for 23.9% of cases of CAUTI, *P. aeruginosa* accounts for 10.3%, *Proteus* spp. account for 4%, and *S. aureus* accounts for 1.6% [34]. Other reports have indicated that *S. aureus* is the most common pathogen in CAUTI after *E. coli* [35]. Notably, the catheterized bladder environment lowers the threshold for uropathogens that might not be successful otherwise, and provides a platform for other opportunistic microbes to infect. Among these, *P. aeruginosa* and *P. mirabilis* showed the highest biofilm formation in the present study. Nevertheless, each species showed a characteristic behaviour regarding interaction with the two catheter designs, and in terms of intravesical or intraluminal biofilm formation. Bacterial species colonized different parts of the device with different intensity, except for *S. epidermidis* which did not seem to be influenced by the design of the catheter. A possible explanation for this phenomenon could be that each of the tested species prefers specific environmental conditions depending on its own assortment of virulence factors. As such, the strains mainly colonize the specific catheter sections that recreate the micro-environmental conditions that better fit their growth and biofilm formation capacity. These differences are likely related to different hydrodynamic conditions in the different sections, as well as the metabolic characteristics of each species.

For instance, *E. coli* is known to express virulence factors such as type 1 fimbriae. These promote bacterial persistence by enhancing adherence to the surface. The fact that *E. coli* showed intense biofilm formation in both FCs and NPD catheters in both intravesical and intraluminal parts means that its colonization capacity is able to overcome very different hydrodynamic conditions. Indeed, the production of the exopolysaccharide colanic acid is essential for the final aspect of *E. coli* biofilms. It acts as a physical barrier with a negative charge that allows this bacterium to resist hugely different environmental conditions, including changes in osmotic stress, oxidative stress and changes in temperature. It is known that *P. aeruginosa* produces high amounts of extracellular matrix mainly composed of alginica acid, which confers resistance to mechanical stresses as well as protection against biocidal compounds. The formation of *S. aureus* biofilm is generally mediated by production of the extracellular polysaccharide polysaccharide intercellular adhesin or poly-N-

acetylglucosamine catheter surfaces. All these features are virulence factors that allow the strains to colonize catheter surfaces successfully.

The choice of experimental bioreactor set-up was based on the analysis of published papers regarding the bioreactors used to reproduce CAUTI *in vitro*. Three papers were found to approach clinical conditions as closely as possible using catheters as flow systems. In 2011, Dohnt *et al.* used a continuous flow-through reactor in which the main component of the system was a silicon catheter, with a similar flow rate as used in the present study [1]. However, the system did not simulate a bladder section. The latter was present in the set-up used by Wang *et al.* to evaluate the release of antimicrobial compounds [36]. The authors employed an artificial simulated bladder where the bladder–catheter system was set in a horizontal position, and the flow was set to 0.50 mL/min. These conditions are similar to those in patients who are bedridden and whose urine flow is lower than normal. In 2016, Nzakizwanayo *et al.* tested possible strategies against bioencrustation caused by *P. mirabilis* [37]. Their set-up included a bladder–catheter system set in a vertical position with a flow rate of 0.75 mL/min. Their set-up was comparable to that used in the present study; however, only the bladder section was thermostated.

This study had a few limitations, including the in-vitro set-up and the use of single, ATCC-certified bacterial species instead of clinical isolates. In a second phase, clinical strains with different virulence will possibly provide a more detailed response, closer to clinical conditions. Also, the results of this study do not enable the influence of the catheter position on biofilm formation to be ascertained (e.g. when a patient is bedridden for an extended amount of time). It can be speculated that the amount of urine retention in this case would be lower. Future studies should test the impact of different hydrodynamic conditions on colonization by different strains of the same species expressing different virulence factors.

In conclusion, as CAUTI is a substantial problem related to indwelling devices, biomaterials and design innovation is a critical task. This study found that even simple modifications of the hydrodynamic characteristics of the FC design can have a significant impact on bacterial

colonization. The development of biofilm-resistant devices will likely require integrated approaches in a design that combines chemical, mechanical and topographical elements. This approach could be a step forward in the reduction of the occurrence of CAUTI.

Conflict of interest statement

None declared.

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Figure 1. Tested catheter designs. Similar to the Foley catheter, the new proposed design (NPD) catheter includes an anchoring system consisting of an inflatable balloon placed near the tip of the probe. The balloon is shaped to be asymmetrically oriented considering the major axis of the catheter. In total, two drainage holes are made near the upper and lower limits of the balloon, corresponding with the less inflated part of the balloon. A through-hole is made on the tip of the catheter, according to the usual Foley design. The specific position of the drainage holes keeps the surface of the balloon free from surrounding urine. As such, the surrounding urine is almost completely removed, maximizing bladder emptying, once positioned. Arrows indicate the position of the upper through-hole in the Foley catheter and the additional holes in the NPD catheter. (A) Representation of the model for in-vitro determination of residual urine. The balloons of the catheters were filled with light-blue coloured water, whereas the syringe simulating the bladder was filled with yellow water for better visual representation. (B) The bioreactor model was assembled inside the incubator, shown here with six catheter–vial systems connected to a peristaltic pump through the distribution system. The sterile human urine tank is seen in the background After passing through the catheter, the urine is discarded out of the incubator through exiting tubes (lower right). (C and D) The NPD catheter was able to achieve almost complete emptying of the liquid from the simulated bladder, in contrast to the control Foley catheter, confirming the findings of the simplified model in (A). The fluid-dynamic conditions inside the catheters were also different. While the Foley catheter was characterized by intermittent emptying as soon as the urine level in the simulated bladder reached the upper part of the catheter hole, the NPD catheter emptied the bladder contents continuously through hole #3 that was positioned under the balloon. The other two holes were almost not used.
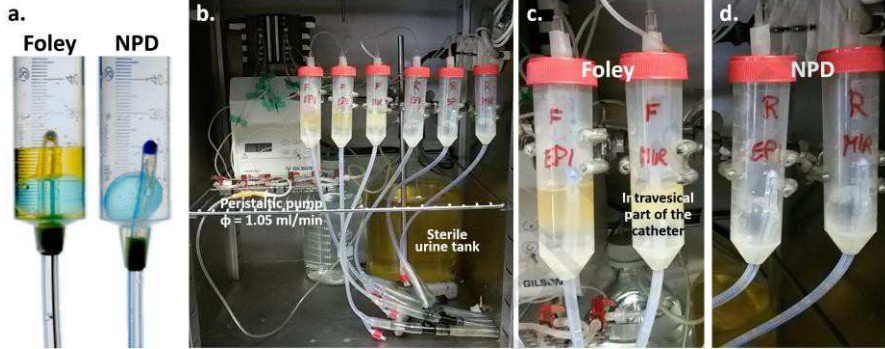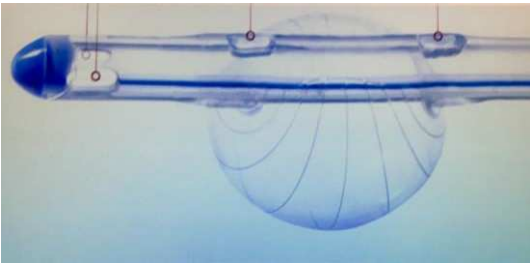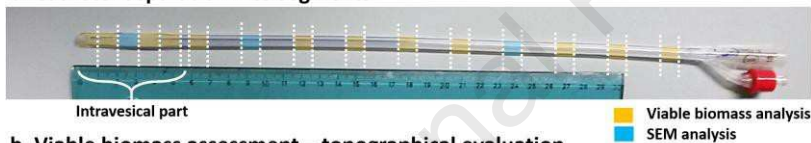
Figure 2. Viable biomass results. (A) Each catheter, after incubation, was sectioned into 15 segments measuring 10 mm in length. The three sections depicted in blue were analysed using scanning electron microscopy (SEM), while the orange sections were subjected to viable biomass assessment. (B) Positioning of the ordered sections in the 24-well plates is shown, where the reacted formazan coloured the lysing solution. The intensity of the colour is proportional to the number of viable and metabolically active cells. Examples of biofilm formation are given for *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. The new proposed design (NPD) catheter was able to reduce biofilm formation. It is clear that catheter design had a differing effect on biofilm formation depending on the bacterial strain tested. OD, optical density.
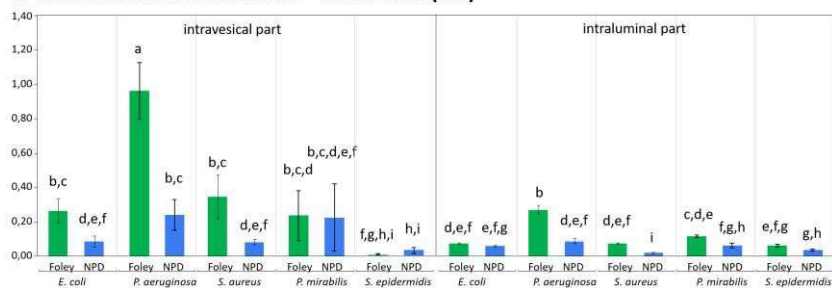
Figure 3. For visual macroscopic representation, a number of catheters were subjected to MTT assay without being sectioned. (A) Intravesical portion of the tested catheters after biofilm formation by *Proteus mirabilis.* The site showing highest biofilm formation was the through-hole of the Foley catheter and the third (lowest hole) of the new proposed design (NPD) catheter. This finding opens interesting possibilities to protect the whole catheter against biofilm formation by targeting the release of biocidal and antibiofilm compounds at specific sites rather than the whole catheter. (B) Macroscopic differences in biofilm formation between catheter designs. Unlike the Foley catheter, the NPD catheter was able to prevent *Pseudomonas aeruginosa* from forming a mature, seamless biofilm along the whole catheter surface. Only non-confluent microcolonies could be identified. One might speculate that this form of bacterial cell aggregation into single microcolonies could be much more sensitive to the activity of a biocidal compound than a fully-grown multi-layered biofilm.

a. Biofilm formation on the intravesical part
P. mirabilis, Foley catheter (control)

1 cm

hole

P. mirabilis, NPD catheter (test)

hole #1   hole #2   hole #3

b. Biofilm formation on the intraluminal part
P. aeruginosa, Foley catheter (control)

P. aeruginosa, NPD catheter (test)

1 cm

2

Figure 4. Representative scanning electron microscope micrographs of the surfaces of the tested catheters, grouped by position and catheter design, after biofilm formation by the tested strains. Biofilm formation is in keeping with the viable biomass results. Foley catheters generally showed multi-layered biofilms that were often able to cover catheter surfaces completely in all tested strains. New proposed design (NPD) catheters showed a reduced degree of colonization. The intravesical part was predominantly characterized by non-confluent microcolonies and bacterial microcolonies, while the intraluminal part only showed bacterial cell clusters adherent to the catheter surfaces. One exception was *Escherichia coli,* which showed intense biofilm formation in both the intravesical and intraluminal parts of both the Foley and NPD catheters. The areas free of bacterial colonization had a very rough surface microtexture. The microtexture was mainly oriented in a longitudinal direction, parallel to the long axis of the catheter.
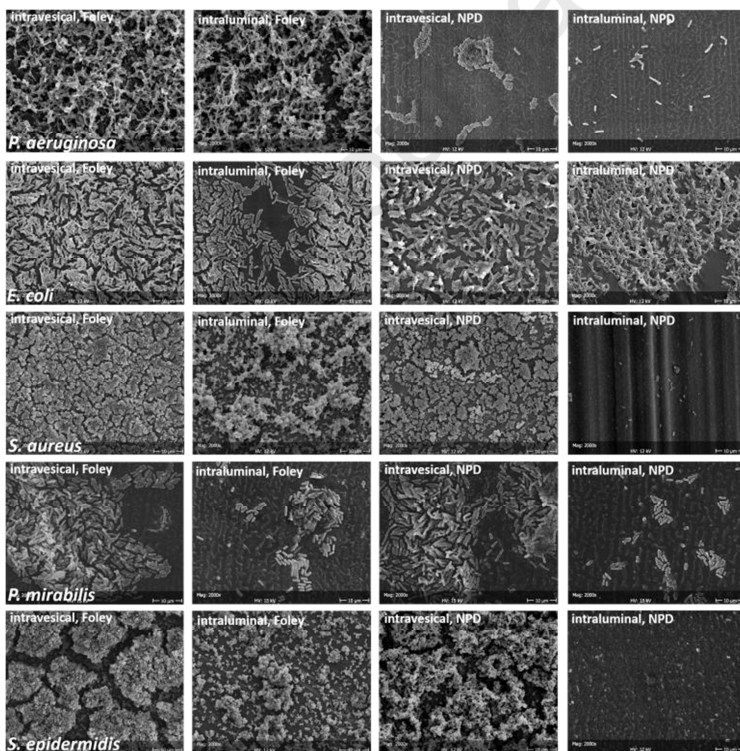
Figure instructions

Fig 1 – reproduce 14 cm wide, colour

Fig 2 – reproduce 14 cm wide, colour

Fig 3 – reproduce 14 cm wide, colour

Fig 4 – reproduce 18 cm wide, black and white