



# Evaluating Efficacy of Antimicrobial and Antifouling Materials for Urinary Tract Medical Devices: Challenges and Recommendations

Madeleine Ramstedt,\* Isabel A. C. Ribeiro, Helena Bujdakova, Filipe J. M. Mergulhão, Luisa Jordao, Peter Thomsen, Martin Alm, Mette Burmølle, Todorka Vladkova, Fusun Can, Meital Reches, Martijn Riool, Alexandre Barros, Rui L. Reis, Emilio Meaurio, Judith Kikhney, Annette Moter, Sebastian A. J. Zaat, and Jelmer Sjollem


In Europe, the mean incidence of urinary tract infections in intensive care units is 1.1 per 1000 patient-days. Of these cases, catheter-associated urinary tract infections (CAUTI) account for 98%. In total, CAUTI in hospitals is estimated to give additional health-care costs of £1–2.5 billion in the United Kingdom alone. This is in sharp contrast to the low cost of urinary catheters and emphasizes the need for innovative products that reduce the incidence rate of CAUTI. Ureteral stents and other urinary-tract devices suffer similar problems. Antimicrobial strategies are being developed, however, the evaluation of their efficacy is very challenging. This review aims to provide considerations and recommendations covering all relevant aspects of antimicrobial material testing, including surface characterization, biocompatibility, cytotoxicity, in vitro and in vivo tests, microbial strain selection, and hydrodynamic conditions, all in the perspective of complying to the complex pathology of device-associated urinary tract infection. The recommendations should be on the basis of standard assays to be developed which would enable comparisons of results obtained in different research labs both in industry and in academia, as well as provide industry and academia with tools to assess the antimicrobial properties for urinary tract devices in a reliable way.

## 1. Introduction

Healthcare-associated infections (HAI) have been estimated to affect more than 4 million patients every year in Europe, causing 16 million extra days in hospital and leading to approximately €7 billion in direct costs.<sup>[1]</sup> The majority of HAI are associated with sometimes invasive devices such as ventilators, central lines and urinary catheters. Thus, 83% of patients with a hospital acquired pneumonia were being ventilated, 87% of patients with a blood stream infection had a device in their blood stream, and 97% of patients with a urinary tract infection had a urinary tract device in place.<sup>[1]</sup> The risk for acquiring HAI varies between sectors of healthcare and type of patient, with patients having burn wounds or transplants as well as neonates and intensive care unit (ICU) patients being considered high-risk populations.<sup>[1]</sup> Numbers from

Dr. M. Ramstedt  
Department of Chemistry  
Umeå University  
901 87, Umeå, Sweden  
E-mail: madeleine.ramstedt@umu.se

Dr. I. A. C. Ribeiro  
Research Institute for Medicines (iMed.Ulisboa)  
Faculty of Pharmacy  
Universidade de Lisboa  
1649-004 Lisbon, Portugal

 The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/mabi.201800384>.

© 2019 The Authors. Published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

The copyright line for this article was changed on 14 January 2020 after original online publication.

DOI: 10.1002/mabi.201800384

Prof. H. Bujdakova  
Department of Microbiology and Virology  
Faculty of Natural Sciences  
Comenius University in Bratislava  
81499 Bratislava 1, Slovakia

Dr. F. J. M. Mergulhão  
Laboratory for Process Engineering, Environment, Biotechnology and Energy (LEPABE)  
Faculty of Engineering  
University of Porto  
Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

Dr. L. Jordao  
Department of Environmental Health, Research and Development Unit  
National Institute of Health Dr. Ricardo Jorge (INSA)  
Avenida Padre Cruz 1649-016, Lisbon, Portugal

Dr. P. Thomsen, Dr. M. Alm  
BioModics ApS  
Stengårds Alle 31A DK-2800, Lyngby, Denmark

Dr. M. Burmølle  
Department of Biology  
University of Copenhagen  
2100 Copenhagen, Denmark



2015 solely from ICUs in Europe show that 8% of ICU patients develop at least one HAI, 6% acquired pneumonia, 4% bloodstream infections, and 2% urinary tract infections (UTI). Of these, 97% of the pneumonia cases, 43% of blood stream infections, and 97% of UTIs were associated to invasive devices.<sup>[2]</sup> Moreover, these numbers only relate to patients in hospital care, and do not include patients in residential homes, who each have one to three infections per year, mainly pneumonia and UTI.<sup>[1]</sup>

A further complication with HAI is the growing resistance of pathogenic microorganisms against antimicrobial agents. A survey of strains isolated from HAIs in the United States between the years 2011 and 2014 showed that resistant strains appear more frequently in device-associated infections than in surgical-site infections.<sup>[3]</sup> Resistance to  $\beta$ -lactam antibiotics (e.g., oxacillin, methicillin, and ceftioxin) was found in approximately half of the *Staphylococcus aureus* strains tested, and multidrug resistance (i.e., non-susceptible to at least one agent in three or more antimicrobial categories<sup>[4]</sup>) was found in 60–75% of *Acinetobacter baumannii* strains and approximately 20% of *Pseudomonas aeruginosa* strains tested. Carbapenem-resistant

strains of these species are indicated as priority 1, that is, “critical,” strains on the recent World Health Organization’s (WHO) priority pathogen list, stressing the urgent need for new alternative treatment methods beside the traditional antibiotics.<sup>[5]</sup> This calls for alternative and complementary methods for combating device-associated infections. One popular route explored is to produce antimicrobial or antifouling materials that prevent microbial colonization of the surface. The following antimicrobial strategies are typically employed: i) development of anti-/low adhesion materials (also referred to as antifouling); ii) systems that release antimicrobial agents; iii) contact-killing materials; or iv) a combination of these approaches.<sup>[6]</sup> To assess the efficacy of such surfaces, a plethora of methods have been described in the scientific literature.<sup>[7,8]</sup> However, due to a lack of standardization, the results are very difficult to compare between studies and research labs, which delays and limits the progress in this field as well as the translation of results to device manufacturing and to final clinical use. In other words, in order to increase the correlation between in vitro studies of microbial response and what can be expected in vivo, it is of great importance that the in vitro testing is performed in such a way that the results can have clinical significance. Thus, a truly interdisciplinary approach is required where material properties, host biocompatibility, as well as microbial responses are all evaluated and optimized during device development and testing.

This perspective review has been written to provide guidance in this context and is a result of work conducted as part of a multidisciplinary trans-domain European initiative in the framework of the COST (European Cooperation in Science and Technology) Action TD1305 iPROMEDAI (improved protection of medical devices against infection).<sup>[9]</sup> Through this review, we aim to provide researchers from the fields of materials science, chemistry, physics, and related areas, sufficient background to oversee the elements important for clinically relevant microbiological assessment of their antimicrobial materials or devices. Furthermore, we aim to provide researchers from the biological and medical sciences guidance concerning what types of materials characterization can be employed to obtain increased reproducibility of the biological testing and to ensure a solid base for interpreting biological assessment of materials and devices. To illustrate how experiments can be designed with increased clinical predictability we use the case of device-associated UTI. However, similar reasoning can be employed to design clinically relevant testing of medical devices used in other areas of the body, taking into account specifics connected to each device group.

## 2. Device-Associated Urinary Tract Infections

Urological devices are divided into several different market segments managing, for example, urinary incontinence, urinary stones, treatment of prostate hyperplasia or cancer, and erectile dysfunction. Devices aimed to manage urinary incontinence or maintain the ureter or urethra open and unobstructed, include ureteral stents for the upper urinary tract, urethral stents for the lower urinary tract, and urinary catheters. The focus of this paper is on catheter- and ureteral stent-associated UTI (Figure 1) as these are the major device groups and give rise to large numbers of infections worldwide.<sup>[10]</sup> In this review,

Prof. T. Vladkova  
Department of Polymers  
University of Chemical Technology and Metallurgy (UCTM)  
8 Kliment Ohridski Blvd 1756, Sofia, Bulgaria

Dr. F. Can  
Department of Medical Microbiology  
School of Medicine  
Koc University  
34450 Sariyer, Istanbul, Turkey

Prof. M. Reches  
Institute of Chemistry and the Center for Nanoscience and Nanotechnology  
The Hebrew University of Jerusalem  
Jerusalem 91904, Israel

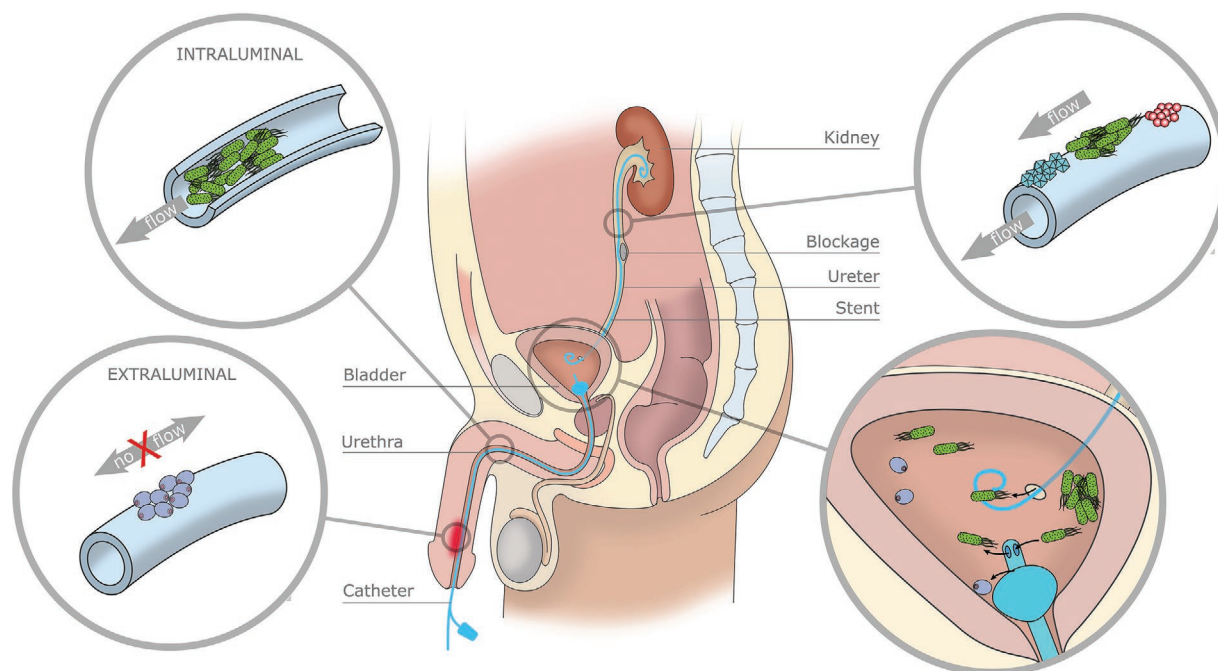
Dr. M. Riool, Dr. S. A. J. Zaat  
Department of Medical Microbiology  
Amsterdam UMC  
Amsterdam Infection and Immunity Institute  
University of Amsterdam  
Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands

Dr. A. Barros, Prof. R. L. Reis  
3B's Research Group  
I3Bs Research Institute on Biomaterials  
Biodegradables and Biomimetics  
University of Minho  
Guimarães, 4710-057 Braga, Portugal

Dr. E. Meaurio  
Department of Mining-Metallurgy Engineering and Materials Science POLYMAT  
School of Engineering  
University of the Basque Country  
48940 Leina, Bizkaia, Bilbao, Spain

Dr. J. Kikhney, Dr. A. Moter  
Biofilmcenter, Department of Microbiology, Infectious Diseases and Immunology  
Charité University Medicine Berlin  
Hindenburgdamm 30, 12203 Berlin, Germany

Dr. J. Sjollega  
University of Groningen  
University Medical Center Groningen  
Department of Biomedical Engineering  
Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands



**Figure 1.** Anatomical cross section of the renal system in a male showing the position of a *urinary catheter* in the lower urinary tract (left) and a *ureteral stent* in the upper urinary tract (right). Flow conditions around the devices may vary between the upper and lower urinary tract as well as on the intraluminal and extraluminal part of a device. Microorganisms (here exemplified as the fungi *C. albicans* in purple and the bacterium *E. coli* in green) have been reported to reach the bladder following both extraluminal and intraluminal routes where the extraluminal colonization will have a more pronounced contact with the host epithelium in the urethra and may have access to other types of nutrients compared to bacteria residing intraluminally. Furthermore, the device may give rise to an inflammatory response in surrounding tissue that further changes the local growth conditions for microorganisms at the device surface. Ureteral stents and urinary catheters are generally colonized both on extraluminal and intraluminal sides and often by mixed colonies of microorganisms (not shown in image for simplicity). When bacteria reside on the device for long periods of time (as shown on the extraluminal side of the stent above to the right) encrustations may arise due to bacteria-induced precipitation of different types of salts from the urine (illustrated by blue and red hexagons).

the term device-associated UTI designates both these types of devices. The following clinical needs have been identified: a) better methods for diagnosing and quantifying microbial colonization, b) development of antimicrobial agents that can disrupt and/or prevent formation of biofilms including crystalline biofilms, c) development of surface coatings that resist and/or reduce microbial adhesion and colonization, as well as d) reducing the use of conventional antimicrobial agents in order to reduce development of resistance.<sup>[11,12]</sup>

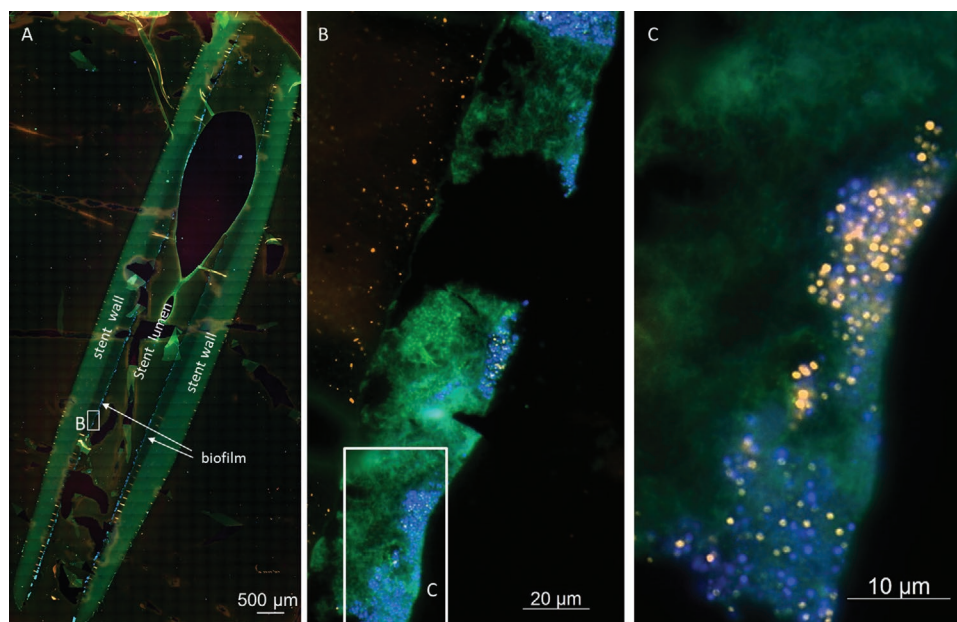
## 2.1. Clinical Perspectives

Clinically, the presence of microorganisms (fungi and/or bacteria) in urine is diagnosed as UTI only if other symptoms are present at the same time (i.e., symptomatic).<sup>[13]</sup> If no symptoms are occurring (asymptomatic) the diagnosis is limited to candiduria or bacteriuria if the fungi *Candida* or bacteria, respectively, are present in the urine. These conditions are not to be treated if the patient has no symptoms and does not meet the criteria of predisposition for UTI according to clinical guidelines.<sup>[11,14]</sup> The distinction between UTI and bacteriuria or candiduria is made in order to reduce the development and spread of drug resistance. If more than  $10^5$  colony forming units of bacteria per mL (CFU mL<sup>-1</sup>) are cultured from urine

with no other symptoms, the patient is given the diagnosis of asymptomatic bacteriuria.<sup>[11]</sup> Asymptomatic candiduria in adult patients is considered if more than  $10^3$  CFU mL<sup>-1</sup> of *Candida* are present in the urine without symptoms.<sup>[15]</sup> Criteria for catheter-associated UTI (CAUTI) consist of signs or symptoms (such as fever, rigors, and pain) related to UTI and more than  $10^3$  CFU mL<sup>-1</sup> of one or several bacterial species detected in a urine sample taken from the catheter or up to 48 h after catheter removal<sup>[11]</sup> in a midstream urine sample.

### 2.1.1. Pathology of Device-Associated Urinary Tract Infections

Indwelling devices, such as urinary catheters, disturb the local host defense mechanisms thereby facilitating the access of bacteria to vital organs such as the urinary tract.<sup>[16,17]</sup> An indwelling device may introduce bacteria by transferring the patient's own fecal and/or skin microbiota during insertion, or colonization may occur at a later stage through microbial migration along the device along extraluminal and/or intraluminal routes.<sup>[17,18]</sup> Microorganisms attached to the surface of the device can form a biofilm consisting of cells encapsulated in an extracellular matrix of substances that they secrete (Figure 2).<sup>[19,20]</sup> Biofilms are naturally occurring communities of microorganisms, and in patients, they protect the microorganisms from the host



**Figure 2.** Presence of biofilm inside a pigtail stent from a 2-year-old patient visualized by fluorescence in situ hybridization (FISH). A) Overview of a longitudinal section of the device (green autofluorescence) shows that its inside is covered by a continuous layer (dark green) with a blue lining toward the lumen. B) At higher resolution of the insert, biofilms embedded in a green autofluorescent matrix are visible, partly stained blue with the nucleic acid stain DAPI. C) At high resolution of the insert marked in B, FISH detects cocci with the *Staphylococcus aureus*-specific FISH probe (orange). Note the differential signal intensity of the bright orange cocci, indicating a high ribosomal content and therefore activity, versus the blue, resting cells.

immune system as well as from antibiotics. It has been reported that bacteria within a biofilm can tolerate more than 1000-fold higher antibiotic concentrations than free swimming or suspended (planktonic) bacteria.<sup>[21]</sup> Bacteria can subsequently be released from the biofilm, perhaps stimulated by device movements, and have been estimated to ascend from a catheter to the bladder within 1–3 days<sup>[11,22]</sup> or colonize other parts of the device. In both routes of infection (intraluminal and extraluminal), bacteria move to the bladder, from where they may (re) inoculate the intraluminal area of the catheter forming biofilms that serve as a persistent and continuous source for recurrent bacteriuria. It has been concluded from a prospective clinical study, in both men and women, that CAUTIs are predominantly caused by microorganisms that gain access extraluminally.<sup>[23]</sup> However, another postinsertion route of infection for catheters is when the collecting tube or drainage bag becomes contaminated by bacteria, which thereafter migrate and to form biofilm intraluminally, and then detach to infect the bladder.<sup>[17,18,24]</sup> After catheter removal, remnants of extraluminal biofilm are generally cleared by the immune system and the restored urine flow, leading to gradual disappearance of UTI.<sup>[11]</sup> For ureteral stents, the colonization is expected to follow the same or very similar routes as for catheters but starting with bacterial contamination of urine in the bladder. Therefore, preventive measures should ideally not only inhibit bacterial colonization on the intraluminal surface of devices, but should prevent colonization and migration of organisms via all possible routes along the device, that is, both intraluminally and extraluminally. In addition to this, a very important consideration for materials and devices residing in a patient for time periods longer than a week, is prevention of encrustation and blockage of ureteral stents and urinary catheters. It results from the colonization of

a particular species of bacteria in the urinary tract (e.g., *Proteus mirabilis*) that give rise to crystalline biofilms.<sup>[25,26]</sup>

The patient group with device-associated UTI is very heterogeneous, and as there is no optimal one-solution-fits-all, various device solutions exist. Infections of catheters and stents share a number of characteristics. Although literature on device-associated UTI mainly addresses CAUTI and its causative microorganisms (e.g., *Escherichia coli*, *Enterococcus* species, *P. aeruginosa*, and *Candida* species), the same species also cause stent-associated UTI.<sup>[27]</sup> Furthermore, environmental factors, such as fluid composition around the different devices, are the same or very similar. There can, however, be flow differences around the devices depending on their placement in either the upper or lower urinary tract, in the bladder, or between the intraluminal and extraluminal sides of the devices (Figure 1). Furthermore, it can be assumed that the fluid composition may be influenced by the surrounding tissue on the extraluminal sides, for example, through substances secreted following irritation or damage to the epithelium by the catheter. However, if such processes occur within the bladder, similar substances will also be present intraluminally. Both the differences in flow and fluid composition may influence the microbial composition, colonization rate, and biofilm formation on the device. The microbiota may, therefore, differ in composition and level of tolerance to antimicrobial agents depending on the local conditions. Intraluminal flow conditions in the lower urinary tract in presence of a catheter will depend on the way the catheter is placed (i.e., urethra or suprapubic) and “operated,” that is, constant drainage or with a closing tap allowing the bladder to fill before voiding. The latter allows for higher flow rates of urine through the catheter at voiding. Since the flow through the ureters is considered more uniform and constant than

through the urethra, ureteral stents are expected to be exposed to a lower flow rate than devices in the lower urinary tract.<sup>[28]</sup> An additional complication of device-associated UTI is that uropathogenic *E. coli* have been shown to invade epithelial cells and cause recurrent infections, which may indicate the need for devices that can release antimicrobial agents specifically targeting intracellular microorganisms.<sup>[29,30]</sup>

### 2.1.2. Device-Associated UTI in Numbers

UTIs are the most frequently occurring type of HAI in Europe (27% of HAI) and the United States (36–40% of HAI) followed by respiratory tract infections, surgical-site infections, and blood stream infections.<sup>[1,31]</sup> The number of patients with a urinary catheter varies between hospitals as well as between units within the same hospital. The reported percentage of patients with an indwelling catheter in hospitals and in elderly homes varies between studies performed in Europe and United States. The percentages also vary between European countries.<sup>[32]</sup> Literature suggests that 5–10% of patients in elderly homes are catheterized,<sup>[11,24]</sup> although percentages as high as 40% have been reported.<sup>[33]</sup> A surveillance report from the European Centre for Disease Prevention and Control (ECDC) from 2013 claims an average incidence of UTI of 31% in long-term care facilities in Europe, the majority of these cases are likely being related to CAUTI.<sup>[34]</sup> In Europe, the mean incidence of UTI in ICUs was reported as 1.1 per 1000 patient-days<sup>[2]</sup> and UTIs occurred in 1.9% of the patients staying in an ICU for more than 2 days. Of these UTI cases, CAUTI accounted for 97.4%.<sup>[2]</sup> In general hospitals, 15–25% of patients have been estimated to have a urethral catheter at some time of their stay.<sup>[11]</sup> In the United States, 30 million urinary catheters are inserted every year (number based on market reports<sup>[35]</sup>) and they have an infection rate of 10–30%.<sup>[26,35]</sup> Starting on the first day of catheterization, a patient has a 3–8% increased risk of acquiring CAUTI for each day the urinary catheter remains in place,<sup>[11]</sup> and these infections are reported to increase hospital stay with up to four days.<sup>[11]</sup> One fourth of patients with short term (<7 days) catheterization and all patients who have a catheter in place for more than 1 month develop bacteriuria.<sup>[36]</sup> Not only catheterized patients suffer from device-associated UTI, in patients with ureteral stents, 38% develop UTI,<sup>[37]</sup> and 45–100% of patients with ureteral stents have bacteriuria.<sup>[38]</sup>

A cost estimate from 2015 suggests that the annual total additional costs for treating CAUTI in the United Kingdom is £1–2.5 billion.<sup>[16]</sup> A rough estimate of total direct hospital costs connected to CAUTI in the United States gives a figure of \$36 billion.<sup>[16]</sup> An assessment of the economic burden of ureteral-stent-related problems published in 2016 illustrates that while the ureteral stent is in the price range of one hundred USD, the total cost including drug therapy, healthcare professional consultation, hospitalization, and work incapacity amounts to a fourfold higher costs per patient (median of \$455 in a span of \$113–\$11948).<sup>[39]</sup> However, the reported range shows that in some cases the total cost can increase by a factor of 100. The average fourfold increase in costs reported for stents in the study by Staubli et al.<sup>[39]</sup> appears less than the general estimates of increased costs for urinary catheters. This indicates

that more research is needed that can clearly show the full increase of health-care costs from device-associated infections in these device groups. However, the estimates still illustrate that even though device-associated UTIs have been described to account for low mortality, they increase morbidity, resource utilization, hospitalization periods, and health care costs. Furthermore, these infections cause increased use of antimicrobials and thereby promote the emergence of antimicrobial resistance.<sup>[40]</sup>

Hospital-acquired CAUTIs are frequently caused by bacterial species of *Enterobacteriaceae* among which extended-spectrum  $\beta$ -lactamase-producing isolates are prevailing, such as *Enterobacter aerogenes*, *Enterobacter cloacae*, *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Morganella morganii*, *P. mirabilis*, and *Serratia marcescens*.<sup>[31]</sup> However, the presence of fungi, especially *Candida* species is of importance and may occur simultaneously with a bacterial infection.<sup>[15]</sup> Indeed, infections by *Candida* species have been reported for 20–30% of CAUTI cases.<sup>[11,38,41]</sup> In Europe, the most frequently isolated organisms are *E. coli*, *Enterococcus* species, *P. aeruginosa* and *Klebsiella* species.<sup>[2]</sup> The relative ratios between different species of pathogens isolated in Europe have been reported to remain similar over time (between 2012 and 2014) but display geographical variations.<sup>[2]</sup> Isolates resistant to carbapenems (e.g., imipenem and meropenem) were identified in high percentages among *P. aeruginosa* (21–25%), *A. baumannii* (58–69%), and *K. pneumoniae* (9–11%).<sup>[2,3]</sup> Multidrug resistance in CAUTI isolates was identified in 5–8% of *E. coli*, 9–11% of *Enterobacter* species, 16–19% of *P. aeruginosa*, and 65–76% of *A. baumannii* strains tested.<sup>[3]</sup> Up to 10% of ureteral stents have been reported to be colonized by *Candida* species resulting in candiduria in about 40% of these cases.<sup>[20,27]</sup> Colonization by these different types of microbes is often related to the formation of biofilms, routinely observed on urinary catheters and stents, and play a major role in device-associated UTI.<sup>[42]</sup> In patients with long-term catheterization, the infections are often caused by a combination of different microorganisms (i.e., polymicrobial) with *P. mirabilis* as a major pathogen.<sup>[11,24,41]</sup> This bacterium, from the patient's own fecal flora, colonizes indwelling catheters and produces urease, an enzyme that degrades urea thereby increasing urinary pH. This in turn causes stone formation, precipitation of salts such as calcium phosphate and struvite, formation of crystalline biofilms (described above), and leads to recurrent catheter or stent blockages.<sup>[25,26]</sup>

### 2.1.3. Devices or Materials Aimed to Reduce UTI

Currently, coatings on urinary-tract devices that release antimicrobial compounds have only been reported to yield positive results for short-term devices (e.g., catheters).<sup>[43]</sup> Furthermore, reviews of the clinical effect of catheters currently on the market have pointed out that many clinical studies are too limited to provide a solid basis for clinical recommendations.<sup>[43]</sup> A review from 2017 of commercially available catheters identified two main antimicrobial strategies used: silver-coated (with a range of different forms of silver) and antibiotic-coated (including nitrofurantoin, sparfloxacin, rifampicin, or minocycline) catheters.<sup>[44]</sup> Several solutions are currently at research stage

and have not yet been clinically tested, such as chlorhexidine- or triclosan-loaded catheters, or catheters with antimicrobial peptides, enzymes, bacteriophages, nitric oxide or zwitterionic coatings, other polymer coatings, or liposomes.<sup>[44,45]</sup> In some limited patient groups, the use of antimicrobial catheters has been reported to reduce the incidence of CAUTI, for example, in diabetic insulin-dependent patients, a high-risk group for this type of infections.<sup>[46]</sup> However, the same study did not observe a reduction in CAUTI for patients with acute cerebral infarction.<sup>[46]</sup> A parallel multicenter clinical trial (over 7100 patients) compared two leading antimicrobial urinary catheters (Bardex IC silver alloy catheter and ReleaseNF nitrofurantoin catheter) with a standard non-antimicrobial urinary catheter (standard polytetrafluoroethylene (PTFE)-coated latex catheter) for efficacy in preventing CAUTI, but found no clinically important reduction in the rate of symptomatic CAUTI.<sup>[47]</sup> This was found despite results from an earlier study with 180 patients finding a significant delay in the onset of bacteriuria for the silver-coated Bardex IC catheter with respect to non-coated catheters.<sup>[48]</sup> In vitro assays in a bladder model have shown that none of the catheters on the market prevent encrustation and blockage when exposed (1–2 days) to the urease-producing *P. mirabilis*.<sup>[42,49]</sup>

Several approaches have been studied for reducing the risk of infection of ureteral stents: i) the use of antiadhesive surfaces by modifying, for instance, the surface charge, hydrophilicity, or roughness; ii) elution of antimicrobials (silver, antibiotics, and others), and iii) through a dynamic stent surface (biodegradable ureteral stents).<sup>[50]</sup> The results of a study with triclosan-eluting stents showed inhibition of the growth of *E. faecalis*, *P. mirabilis*, *S. aureus*, and *K. pneumoniae*, both in preclinical in vitro and in vivo models.<sup>[51]</sup> However, in clinical trials, the results showed limited success.<sup>[51–53]</sup> Another strategy includes antibiotic loading in hydrogel-coated stents.<sup>[54]</sup> Studies using combinations of silver nitrate,<sup>[55,56]</sup> rifampicin,<sup>[57]</sup> and ciprofloxacin<sup>[56]</sup> have been performed in animal models while stents with gentamicin, ceftazolin, ceftriaxone, or tobramycin have only been tested in vitro.<sup>[58]</sup> The key challenge in this type of device-modification strategies is to preserve and reproduce an efficient release of antibiotic during the entire residence time of a device in vivo. Furthermore, the release must be large enough to efficiently kill the microorganisms in order to prevent bacterial colonization and avoid development of resistance, without inducing cytotoxicity. Polymeric coatings have also been investigated for stents, and negatively charged heparin coatings and zwitterionic coatings have shown some promise in reducing stent encrustation.<sup>[59]</sup> Physical mechanisms for removing or preventing encrustation are also being tested, for example, ultrasound<sup>[60]</sup> and photodynamic therapy.<sup>[61]</sup>

## 2.2. Overview of the Catheter Market in 2018

Urological devices such as catheters are very cheap consumer products that sell in large quantities by large multinational industries. The scenario for ureteral stents is similar, thus, in this section, we will only focus on the urinary catheter market. The top three industry challenges that impact the market are: i) intense competition, pressuring prices, ii) stringent infection control regulations affecting the market, and iii) lack of

patient awareness about various urological devices and their success rate.<sup>[62]</sup> In Europe, currently more than 20 companies are active. To remain competitive in this market, participants must continue to develop and acquire cost-effective products, information technology platforms, and services. The ability to maintain a competitive edge depends on a combination of factors, including regulatory approvals, brand placement, product quality, safety, cost effectiveness, and continuous documentation of clinical performance. The urinary catheter market is projected to have an annual growth rate of 8.5% and reach \$2.19 billion by 2022, based on the volume of \$1.33 billion in 2016.<sup>[63]</sup> Growth in the market can mostly be attributed to factors such as the rising prevalence of urinary incontinence, increasing number of surgical procedures across the world, and the positive reimbursement scenario for urinary catheters in the United States and the Asian countries. The prevalence of urinary incontinence is increasing with the increasing obesity and rising geriatric population.<sup>[63]</sup> Vendors offer a wide range of devices at competitive prices (**Box 1**<sup>[64]</sup>). The dominant version of indwelling catheter on the market is the Foley catheter named after its inventor Dr. Frederic Foley, who in 1929, designed a catheter with a retaining balloon similar to the one still in use today.<sup>[16]</sup>

Key factors for introduction of new medical devices are market acceptance, price level, and regulations. The market for clinical care products is especially conservative due to the high-level evidence demands of many medical practitioners. Furthermore, these products are often sold through tenders, thus, introducing the risk that the product cost prevails over healthcare costs related to device-associated infections. Moreover, companies may often be reluctant to deviate from standard working practice with dramatically different new technologies. When it comes to pricing, EU countries currently face financial pressure on healthcare budgets. This increased control of costs and the desire to improve efficiency, tends to shift focus toward the cheapest product according to preset specifications. Finally, legal requirements for medical devices need to be implemented by manufacturers following regulations on, for example, national and European levels.

Thus, it can be expected that in order to be able to bring innovative products to the market, high reduction in infection rates need to be validated to motivate industry to add such a device to their portfolio. Furthermore, any change in price level of the products must be justified by prolongation of the infection-free indwelling time. However, factors such as quality of life and overall health care costs will have to be rated higher than the pure manufacturing price of devices for any change to occur in the market.

## 3. Materials Characterization for Evaluation of Antimicrobial or Antifouling Activity

For accurate and reproducible determination of antimicrobial and antifouling effects of materials or devices, it is of great importance that the material/device is well characterized so that the antimicrobial assays do not give rise to false positive and false negative outcomes due to factors like surface heterogeneities and presence of contamination. Thus, a basic level of

### Box 1 Leading vendors of urinary catheters in 2018.

- Bard Medical has the largest portfolio of urinary catheter products globally, including Foley urinary catheters, collection systems, irrigation trays, and intermittent urinary catheters. They got the first patents rights for Foley catheters in the 1930s. For Foley urinary catheters, they offer silver alloy coating technology (licensed from Bactiguard, including an alloy with silver, palladium, and gold), latex hydrogel coating, latex-free silicone urinary catheter with hydrogel coating, and a silicone-elastomer coated latex moisture-repellent Foley urinary catheter. Bard acquired the company Rochester Medical in 2013 adding the Rochesters nitrofurazone coated antimicrobial Foley catheter to their portfolio.
- B. Braun offers the Urimed Cath indwelling Foley urinary catheter for both regular and postoperative bladder drainage and irrigation. This is made of pure uncoated silicone with a relatively large lumen that reduces the tendency to encrust and block.
- Boston Scientific have developed a balloon urinary catheter technology, the UroMax Ultra High Pressure Balloon urinary catheter. It is designed with specially treated polymers and processes to provide maximum strength to maintain its outer diameter during inflation, and ureteral catheters designed with a variety of tip configurations and sizes to facilitate access.
- Coloplast produces the Soft Latex Foley urinary catheter and a silicone Foley urinary catheter. They also produce Folsyl, a long-term all-silicone indwelling catheter with up to 12 weeks indwelling time.
- Medtronic offers hydrogel-coated latex Foley urinary catheters, silicone-coated latex Foley urinary catheters, and silicone elastomer-coated latex Foley urinary catheters. Medtronic recently acquired Covidien adding the antimicrobial Dover silver-coated catheter to their portfolio. They also offer catheters with temperature sensing aimed at ICUs.
- Teleflex sells Foley catheters under the brand name Rüsçh.

material characterization is crucial for obtaining reliable data on the biological effects of a material.

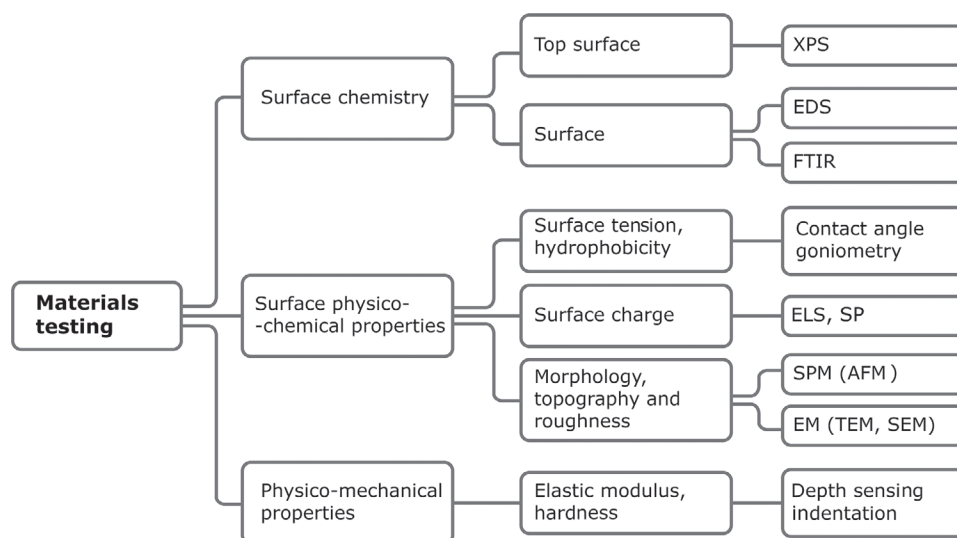
Factors that can play a large role in bacterial adhesion to materials and their antimicrobial performance include the chemical composition, physicochemical properties (e.g., surface charge, hydrophobicity/hydrophilicity, topography, and roughness), and physicomechanical parameters (e.g., elastic modulus and hardness) of the surface.<sup>[65–68]</sup> As the biological testing is influenced mainly by surface properties, surface sensitive methods should be chosen that can show if there are surface contaminations, heterogeneities, or alterations of surface characteristics caused for example by the presence of antimicrobial agent(s), leachable components of the bulk material, or by preconditioning of the tested materials. Methods that are commonly used in this context are summarized in **Figure 3** and will shortly be described in this section with supporting references

for further reading. Many materials are preconditioned by dissolved constituents (e.g., proteins) in biological fluids and this may affect the outcome of the testing. Thus, characterization of a material or device should be performed both before and after exposure to relevant culture medium or biological solutions (e.g., urine). Furthermore, control samples relating to untreated or uncoated materials should be included in all material testing steps. As materials and devices will have to be sterilized prior to use, it is also highly advisable that suitable sterilization methods are investigated early on in the research and development process, as well as their effect(s) on the produced material/device. Standardized procedures exist for several methods concerning materials characterization (some examples listed in **Table 1**).

### 3.1. Materials Used in Urinary Tract Devices

The majority of materials used in urinary tract devices are polymers and the predominant material is high temperature vulcanized polydimethyl siloxane (PDMS), also known as silicone rubber. Materials such as natural rubber and polyurethane (PU) are also used<sup>[44,69]</sup> as well as metals or metal alloys for ureteral stents.<sup>[70]</sup> The chemical nature of the bulk material, composition and processing technologies differ between these materials. Generally, additives are incorporated to different extent in order to improve processing and product properties. These additives include crosslinker/vulcanizing agents, catalysts for the vulcanization, reinforcing fillers, oils, and antiaging agents. Thus, on the surface of a polymeric material, low molecular weight ingredients and additives may be present, as well as degradation products formed during the processing of the polymer.<sup>[71]</sup> On the surface of metal ureteral stents, substances protecting against corrosion may be present. For antimicrobial materials, leachable antimicrobial agents can also be present at the surface<sup>[72]</sup> and be released from the material during testing or usage.

The quantity of leachable substances (including antimicrobial agents) can be estimated after extraction with a suitable solvent and analysis by chromatography, spectroscopy, and other techniques as described in, for example, the ISO 10993-18:2005 standard (Table 1).<sup>[73]</sup> Other standards related to chemical characterization of materials are the ASTM F2038-00(2011) standard<sup>[74]</sup> dealing with the chemical requirements of raw and medical-grade silicone materials, and the ASTM F2042-00(2011) standard<sup>[75]</sup> that describes the requirements for fabrication and testing of medical-grade silicone materials. A general difference between medical-grade silicone and industrial-grade silicone is a lower level of extractable substances in the low-molecular weight range in the former.<sup>[76]</sup> These extractables include impurities from the raw material, residual monomer catalyst, and leachable additives. The amount of residual monomer and catalyst can be reduced during postcuring completing the crosslinking of the polymer, and thereby reducing amounts of residues that may induce toxicity.<sup>[69]</sup> As medical-grade silicone contains lower amounts of extractables, and therefore is expected to give rise to more reproducible data this grade of silicone may be better suitable as a general reference material during testing than industrial-grade silicone.



**Figure 3.** Scheme outlining commonly used methods for materials characterization before biological testing. The following abbreviations are used in the figure and text: XPS, X-ray photoelectron spectroscopy; FTIR, Fourier-transform infrared spectroscopy; EDS, energy dispersive spectroscopy (often used in combination with EM); ELS, electrophoretic light scattering; SP, streaming potential; SPM, scanning probe microscopy; AFM, atomic force microscopy; EM, electron microscopy; TEM, transmission electron microscopy; SEM, scanning electron microscopy. Top surface relates to outermost nm (10 nm or less). Surface relates to outermost  $\mu\text{m}$  of a surface.

### 3.2. Preconditioning and Characterization of Preconditioned Surfaces

When materials are in contact with biological (body) fluids, a layer of adsorbed biological substances generally forms at their surfaces. These substances may completely change surface properties such as chemistry, roughness and topography, (Figure 4).<sup>[70,77,78]</sup> This layer is called a preconditioning layer as it preconditions the surface for cells and microorganisms affecting their ability to anchor and colonize.<sup>[77,79]</sup> Thus, the biological response obtained from testing will often be of the preconditioned surface rather than the bare/raw material.<sup>[80]</sup> To account for such processes, a comparative characterization of the material surface before and after preconditioning is required. This is one important reason why material testing should be performed in standardized solutions that have clinical relevance, as the preconditioning from synthetic growth media may be very different from the same process in body fluids, and may vary between different body fluids. Furthermore, preconditioning from different body fluids may influence microbial species and strains in different ways. As an example, fibrinogen deposition following pretreatment with blood plasma has been shown to increase the attachment of some bacterial species (e.g., *S. aureus* and some *Staphylococcus epidermidis* strains) but not others (Gram-negatives and other *S. epidermidis* strains).<sup>[81,82]</sup>

### 3.3. Surface Chemical Characterization

The interactions between the material and mammalian cells, fungal cells and bacterial cells investigated during biological testing will, to a large extent, be influenced by the surface chemistry and how this chemistry is altered during surface

preconditioning.<sup>[67,78,83]</sup> Thus, data showing the overall chemical composition of the surface may enable more detailed understanding of the mechanisms governing the colonization processes, as well as serve as a quality control for the material processing. Analytical methods generally used for this purpose include attenuated-total-reflectance Fourier-transform infrared spectroscopy (ATR-FTIR, giving information regarding the top few  $\mu\text{m}$  of a surface), grazing-angle FTIR, and X-ray photoelectron spectroscopy (XPS, information depth of a few nm).

XPS is used for elemental and functional group analysis of top atomic layers of a surface, qualitative and semiquantitative, thus enabling detection of surface contamination, leached substances, preconditioning layers, as well as changes in the surface chemical composition as a result of oxidation, chemical interaction with antimicrobial agents, etc. Conventional XPS instruments operate at ultra-high vacuum. Consequently, samples will be exposed to vacuum during analysis, which may result in disappearance of volatile substances from the surface. To reduce such evaporation the samples may be analyzed at low temperatures (e.g., frozen using liquid nitrogen) or using ambient pressure XPS, which enables the analysis of, for example, highly hydrated materials such as hydrogels. For more information about XPS, the reader is suggested to consult previously published reviews.<sup>[84]</sup>

ATR-FTIR or grazing angle FTIR (the latter for reflective surfaces) can be used to characterize the surface composition with respect to chemical functional groups. However, it is important to note that FTIR measurements will in general exhibit a larger analysis depth than XPS and generally include signal from bulk layers below the surface or below a surface coating (depending on coating thickness). Thus, it may not be possible to distinguish differences in surface chemical composition from bulk material unless the coating gives rise to unique vibrations at high enough intensity to be detected unambiguously. The use



**Table 1.** Summary of standards mentioned in the review (please note that they are regularly revised and updated).

Standard	Title
ASTM F2038-00(2011)	Standard guide for silicone elastomers, gels, and foams used in medical applications—Part I: Formulations and uncured materials
ASTM F2042-00(2011)	Standard guide for silicone elastomers, gels, and foams used in medical applications—Part II: Crosslinking and fabrication
ISO/DTS 19278	Plastics: Instrumented micro-indentation test for hardness measurement of plastics materials
ASTM D395-16e1	Standard test methods for rubber property: Compression set
ISO 37:2017	Rubber, vulcanized or thermoplastic: Determination of tensile stress–strain properties
ISO 7619-1:2010	Rubber, vulcanized or thermoplastic: Determination of indentation hardness—Part 1: Durometer method (Shore hardness)
ISO 527-1:2012	Plastics: Determination of tensile properties—Part 1: General principles
ISO 527-2:2012	Plastics: Determination of tensile properties—Part 2: Test conditions for molding and extrusion plastics
ISO 868:2003	Plastics and ebonite: Determination of indentation hardness by means of a durometer (Shore hardness)
ISO 604:2002	Plastics: Determination of compressive properties
ISO 14630	Non-active surgical implants: General requirements
ISO 20696	Sterile urethral catheters for single use
EN 1616	Sterile urethral catheters for single use
ASTM F623-99(2013)	Standard performance specification for Foley catheter
ASTM F1828-17	Standard specification for ureteral stents
FDA	Analytical procedures and methods validation for drugs and biologics: Guidance for industry
ISO 10993-1:2018	Biological evaluation of medical devices—Part 1: Evaluation and testing within a risk management process
ISO 10993-2:2006	Biological evaluation of medical devices—Part 2: Animal welfare requirements
ISO 10993-3:2014	Biological evaluation of medical devices—Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity
ISO 10993-4:2017	Biological evaluation of medical devices—Part 4: Selection of tests for interactions with blood
ISO 10993-5:2009	Biological evaluation of medical devices—Part 5: Tests for in vitro cytotoxicity
ISO 10993-6:2016	Biological evaluation of medical devices—Part 6: Tests for local effects after implantation
ISO 10993-7:2008	Biological evaluation of medical devices—Part 7: Ethylene oxide sterilization residuals
ISO 10993-8:2000	Biological evaluation of medical devices—Part 8: Selection and qualification of reference materials for biological tests
ISO 10993-9:2009	Biological evaluation of medical devices—Part 9: Framework for identification and quantification of potential degradation products
ISO 10993-10:2010	Biological evaluation of medical devices—Part 10: Tests for irritation and skin sensitization
ISO 10993-11:2017	Biological evaluation of medical devices—Part 11: Tests for systemic toxicity
ISO 10993-12:2012	Biological evaluation of medical devices—Part 12: Sample preparation and reference materials
ISO 10993-13:2010	Biological evaluation of medical devices—Part 13: Identification and quantification of degradation products from polymeric medical devices
ISO 10993-14:2001	Biological evaluation of medical devices—Part 14: Identification and quantification of degradation products from ceramics
ISO 10993-15:2000	Biological evaluation of medical devices—Part 15: Identification and quantification of degradation products from metals and alloys
ISO 10993-16:2017	Biological evaluation of medical devices—Part 16: Toxicokinetic study design for degradation products and leachables
ISO 10993-17:2002	Biological evaluation of medical devices—Part 17: Establishment of allowable limits for leachable substances
ISO 10993-18:2005	Biological evaluation of medical devices—Part 18: Chemical characterization of materials
ISO/TS 10993-19:2006	Biological evaluation of medical devices—Part 19: Physico-chemical, morphological and topographical characterization of materials
ISO/TS 10993-20:2006	Biological evaluation of medical devices—Part 20: Principles and methods for immunotoxicology testing of medical devices

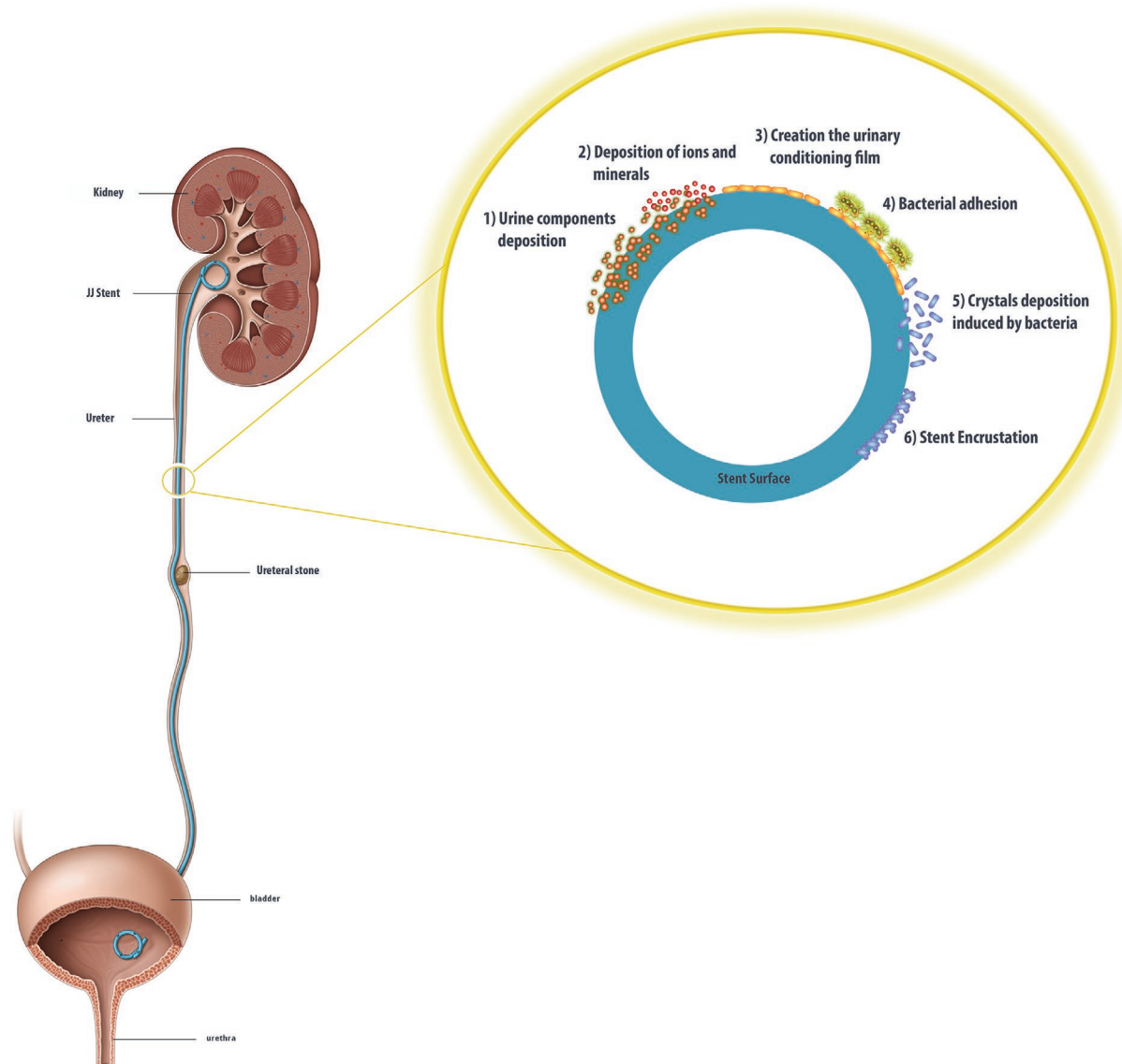
of FTIR to study materials has previously been well described by several authors.<sup>[85]</sup>

Complementary methods that can be used in addition to XPS and FTIR are, for example, ellipsometry, to determine the layer thickness of a coating on a reflective surface; dynamic secondary-ion mass spectrometry (DSIMS), to determine the elemental composition of the surface of materials in the ppm–ppb range; time-of-flight secondary-ion mass spectrometry (ToF-SIMS), to obtain both elemental and molecular surface information with low detection limits; nuclear magnetic reso-

nance (NMR) spectroscopy, for chemical information; as well as X-ray diffraction (XRD), for identification and information about crystallinity for crystalline or partly crystalline materials.

### 3.4. Surface Topography and Roughness

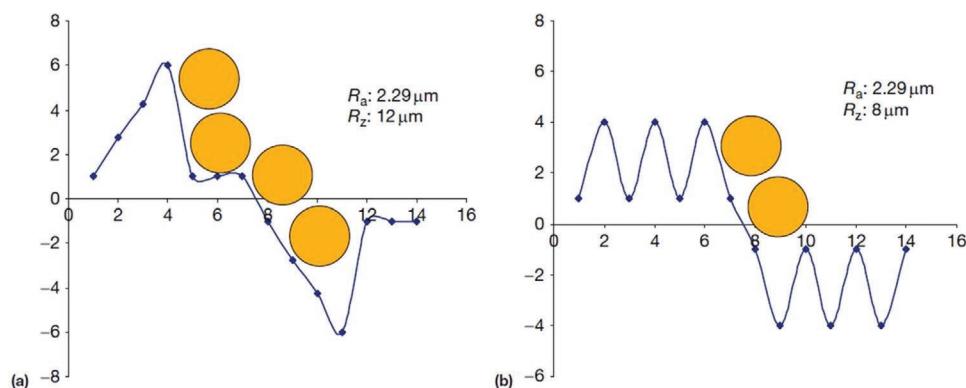
Surface roughness and topography are parameters that can significantly influence attachment of microorganisms and subsequent biofilm formation. For example, if the roughness



**Figure 4.** Illustration of a process forming a surface conditioning film exemplified on the surface of a ureteral stent. These preconditioning films can also include proteins, lipids, and other biomolecules. Reproduced with permission.<sup>[70]</sup> Copyright 2017, Elsevier.

and topography are in the same length scale as a bacterium, this may enable it to adhere, protected “inside” the topography (**Figure 5**) with increased numbers of sites of interaction with the material.<sup>[86,87]</sup> Thus, analysis techniques that can give information about surface morphology are valuable tools for interpreting and understanding microbial interactions with a surface. Furthermore, microorganisms may alter the roughness and topography at contact with the surface by secreting extracellular polymeric substances (EPS) that may penetrate and smoothen surface topographies.<sup>[88]</sup> Preconditioning from culture medium may induce similar alterations of surface morphology. Scanning probe microscopy (SPM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM) are techniques that beneficially can be used to observe the surface morphology and topography.<sup>[89]</sup>

Scanning probe microscopy such as scanning force microscopy (SFM)/atomic force microscopy (AFM) can be used to observe and measure surface roughness. These techniques can also show presence of pinholes in surface coatings, scratches, or other irregularities. AFM has the advantage that it can be used to analyze surfaces in fluid. SEM is a complementary method that can be used in addition to AFM enabling a larger span of magnifications to be used than what is routinely obtained using AFM. If the electron microscope holds energy dispersive spectroscopy (EDS) analysis capabilities, this can be used in parallel to investigate the presence and distribution of specific elements such as silver, zinc, and titanium within the material. For some materials, it may also be advantageous to perform thin sectioning and analyze the material using TEM. Such sectioning may give information about surface roughness, coating thickness, and distribution of, for example, metal



**Figure 5.** Illustration of how both surface roughness and topography can influence interactions between microorganisms and a surface. The blue curve illustrates the roughness of a surface in the  $x$ - $z$  plane (with length scales shown on the two coordinate axes in  $\mu\text{m}$ ) while the yellow spheres represent spherical microorganisms.  $R_a$  represents average roughness and  $R_z$  represents average difference between highest and lowest points. (a) and (b) have identical average roughness but the topographical differences would enable the illustrated organisms to more easily interact with surface (a) compared to surface (b). Reproduced with permission.<sup>[86]</sup> Copyright 2011, Elsevier.

particles within the depth of a coating/material. Both SEM and TEM are vacuum techniques although instrumentation exists that can analyze samples at more ambient conditions or at cryogenic temperatures.<sup>[90]</sup>

### 3.5. Surface Charge

Surface charge of a material may produce repulsive or attractive forces between microorganisms and a material. In general, microorganisms exhibit negative surface charges resulting, for example, from functional groups such as carboxylates and phosphoryl groups.<sup>[91,92]</sup> Thus, microorganisms are often to some extent repelled by negatively charged surfaces, although exceptions exist. Charge interaction between a microorganism and a surface may increase or decrease adhesion depending on both the amount of charges, their nature, as well as the ionic strength of the surrounding liquid between the microorganisms and the material.<sup>[93]</sup> At higher ionic strength, electrostatic forces are reduced due to increased screening of surface charges from ions in the electric double layer forming around a charged particle and/or on top of the substrate material.<sup>[93]</sup> The charge of particulate materials or of microbial cell surfaces can be studied in particular by microelectrophoresis techniques<sup>[93]</sup> such as electrophoretic light scattering (ELS) in which charged particles move in an applied electric field while their movement is monitored through dynamic light scattering techniques.<sup>[91]</sup> The charge of macroscopic surfaces (in particular capillaries) is assessed by means of streaming current or streaming potential measurements. For further reading, please consult previously published reviews on this topic.<sup>[91,93,94]</sup>

### 3.6. Hydrophilic–Hydrophobic Surface Properties and Surface Energy

The hydrophilic/hydrophobic property of a surface has been identified as an important factor controlling microbial adhesion in a number of publications.<sup>[65,95]</sup> Furthermore, the major route of catheter colonization, that is, transmission of bacteria from

the urinary tract to the extraluminal surface of the catheter, has been suggested to be governed by a thermodynamic transfer balance that determines whether it is thermodynamically favorable for bacteria to transfer from the tissue to the catheter surface or not. This balance is based on surface tension of bacteria, tissue and the catheter surface.<sup>[96]</sup>

To analyze the hydrophobicity or hydrophilicity of a surface, several methods exist but the most commonly used is contact angle goniometry. A small drop of liquid is carefully placed at the surface of the material and the angle between the liquid drop and the material is measured. This angle will be influenced by the free energy of the surface as well as the surface roughness, relative humidity and temperature. For this reason, it is of great importance that contact angle data are interpreted together with information on these parameters. Equilibrium contact angle measurement, that is, analysis of the contact angles of a static drop at the surface, are often performed using at least three liquids with known surface tension such as water, ethylene glycol, and hexadecane. For a more detailed interpretation of the surface free energy, dynamic contact angles such as advancing and receding angles are measured.<sup>[97]</sup> Surface free energy can be calculated from these contact angles together with information about the surface tension of the liquids according to, for example, Fowkes' equation or more accurately from the Lifshitz van der Waals/acid base approach that includes both polar and non-polar components.<sup>[98]</sup> The surface tensions can also be calculated based on Hansen solubility parameters.<sup>[99]</sup> Further information about contact angle measurements and determination of surface free energy of materials can for example be found in review articles by Marmur,<sup>[97]</sup> and for bacterial cells by van der Mei et al.<sup>[100]</sup>

### 3.7. Elasticity and Hardness

The effect of physicomechanical properties such as material hardness on microbial adhesion has recently emerged as a topic of increased interest.<sup>[68,101]</sup> Depth-sensing indentation provides detailed information about physicomechanical characteristics, such as indentation elastic modulus, indentation

hardness, dynamic hardness, of a material with probing depth of  $\mu\text{m}$  to  $\text{nm}$ .<sup>[102]</sup> A dynamic ultramicrohardness meter can be used to test surface mechanical parameters under different test conditions, such as loading force and speed, as well as depth of penetration.<sup>[103]</sup> Regarding this technique, an ISO standard is under development.<sup>[104]</sup>

Additional physicochemical testing that may be of interest for antimicrobial materials are measures of tensile strength, compression resistance, and hardness.<sup>[105]</sup> These parameters may be important to monitor in cases where the antimicrobial agents are included in the bulk of the material and can influence its mechanical properties. Standard ISO tests exist for such testing and should include identical material/device without antimicrobial agents as a control and are device specific; EN 1616, DS/EN ISO 20696, and ASTM F623-99(2013) are used for urinary catheters and ASTM F1828-97, ASTM F1828-17, and ISO 14630 for ureteral stents.<sup>[106]</sup>

### 3.8. Pharmaceutical Characterization

Pharmaceutical characterization of drug-releasing antimicrobial agents is made by studying *in vitro* release kinetics.<sup>[107]</sup> This pharmaceutical characterization is compound-dependent and the methods selected should allow for assessment of the amount of active compound released over time. Thus, the antimicrobial mode of action of the test material, such as release, surface killing and non-adhesiveness, should be known beforehand, as well as pharmaceutical information of the active molecules regarding the mechanism of action, antimicrobial spectrum, and effective concentrations (dose response). The background information should also include information about local or systemic effects, toxic concentrations, and half-life of the biological effect.

To study the *in vitro* release kinetics of an active compound from a urinary medical device, release assays are predominantly carried out under sink conditions, that is, with sufficient solution to allow for complete dissolution or release, usually under shaking. Nevertheless, testing catheter specimens connected to flow systems is also an important approach.<sup>[108]</sup> Under sink conditions, the test device or device segment is immersed in a fluid with daily replacement of the fluid. If the device or its coating is intended to dissolve slowly then dissolution should also be assessed in the fluid. Another important aspect is that the *in vitro* drug release/elution kinetics should be evaluated under appropriate conditions, such as media, pH, agitation, flow and temperature, based on the mechanism of drug release and it should mimic hydrodynamic aspects of the location of catheter/stent used. The amount of drug released should be assessed periodically by removal and analysis of aliquots. Usually, the drug can be quantified using high-performance liquid chromatography (HPLC) using Pharmacopeia methods. A suitable method validation should also be included, for example, using Food and Drug Administration (FDA) guidelines from the United States.<sup>[109]</sup> As elution studies should be designed to reach a plateau or reach at least 80% of release of the expected drug content, the total content of the drug needs to be known beforehand. The elution data should be reported as cumulative percentage of total drug content eluted over time from at least

six samples per testing variable.<sup>[110]</sup> Different mathematical models exist to predict release kinetics following different paths such as dissolution, diffusion, partitioning, osmosis, swelling and erosion.<sup>[111]</sup> Examples of how different models have been used to describe release kinetics can, for example, be found in Malcolm et al. that described the release kinetics of metronidazole from self-lubricating silicone using  $t^{1/2}$  kinetics (Higuchi kinetics).<sup>[112]</sup> This was also used by Fong et al. to describe chlorhexidine released from polyurethane nanocomposites,<sup>[113]</sup> while Irwin et al. described release of nalidixic acid from nalidixic acid-loaded polymers (poly methyl methacrylate-*co*-2-hydroxyethylmethacrylate, p(MMA-*co*-HEMA)) by the Korsmeyer-Peppas model equation.<sup>[114]</sup>

## 4. Biocompatibility and Cytotoxicity Testing

Biocompatibility, is in general, assessed through biological evaluation tests included in existing well-established standards for medical devices such as ISO 10993. When performing these tests, all the aspects in the standard that are raised regarding antifouling or antimicrobial materials should be considered. Cytotoxicity, sensitization, and irritation or intracutaneous reactivity tests are recommended to evaluate biological effects of devices in contact with skin or mucosa. In addition, if the device is in contact with mucosa and needs to be used for more than 30 days, genotoxicity evaluation is necessary.

The ISO 10993 standard consists of 20 parts (Table 1). The most relevant parts for cytotoxicity testing of medical devices such as urinary catheter and ureteral stents are Part 1: evaluation and testing within a risk management process,<sup>[115]</sup> Part 3: tests for genotoxicity, carcinogenicity, and reproductive toxicity,<sup>[116]</sup> Part 5: tests for *in vitro* cytotoxicity,<sup>[117]</sup> Part 10: tests for irritation and skin sensitization,<sup>[118]</sup> and Part 12: sample preparation and reference materials.<sup>[119]</sup> Here we will only give a brief overview of information included in some of these parts.

Part 3 is used to evaluate the potential genotoxicity, carcinogenicity, or reproductive and development toxicity of a medical device. These tests must be performed if the biomaterial or medical device in some way may interact with genetic material, or if the composition of the biomaterial is not known. Testing for genotoxicity is required for medical devices with long-term (>30 days) contact time. A series of *in vitro* tests can be selected (at least two tests with different end-points) and mammalian cells must be used. If any of the *in vitro* toxicity tests yields a positive result, *in vivo* mutagenicity tests are required as well.<sup>[116]</sup>

In Part 5, *in vitro* cytotoxicity test methods for medical devices in contact with or close vicinity to mammalian cells are described with information on experimental conditions. For evaluation of cytotoxicity, three different approaches can be followed: i) extract test; ii) direct contact test, and iii) indirect contact test. The choice of one or more approaches depends on the nature of the sample and the nature of the use and implantation site. For example, if an active compound does not leach from the sample, the extraction test can be excluded. Cytotoxicity can be assessed with different endpoints such as by evaluating cell morphology, cell damage, cell growth, or measuring cell metabolic activity. Experimental protocols for these assays are provided in this ISO standard. The test sample can be the



material or the extract (obtained under sterile conditions). Positive and negative controls should be selected based on materials that are known to induce cytotoxic and non-cytotoxic responses, respectively. For the extracts, the blank control (i.e., extraction medium) must also be tested.<sup>[117]</sup> When evaluating cytotoxicity of materials aimed to be used in the urinary tract, suitable cell lines from the urinary tract should be used including epithelial cell lines.

The way to assess if the medical device/material causes irritation or skin sensitization is described in Part 10. The following aspects are considered: pretest considerations for irritation (including *in silico* and *in vitro* methods for dermal exposure), details of *in vivo* (irritation and sensitization) test procedures, and key factors for the interpretation of the results. The *in vitro* skin model irritation assay is based on the premise that irritant chemicals can penetrate the stratum corneum by diffusion and are cytotoxic to the underlying cells, as detected by microscopy/histology. If the cytotoxic effect is only weak or absent and cannot be detected, released inflammatory mediators may be evaluated in addition.<sup>[118]</sup>

Sample preparation and choice of suitable references are described in Part 12, which addresses the following topics: test sample selection; selection of representative portions from a device; test sample preparation; experimental controls; selection of, and requirements for reference materials; and preparation of extracts. It also highlights that biological evaluation must be performed on the final product (e.g., representative samples, materials processed similarly or adequate extracts). A solvent extraction can be used to extract the chemicals that could possibly leach from the biomaterial and the extract should be obtained under conditions that mimic the physiological reality and are appropriate for the sample being tested. The solvents to be used and extraction conditions (e.g., time, temperature) are also described in Part 12.<sup>[119]</sup> For comparison between bacterial exposure and cell exposure, culture medium with similar chemical composition should be used. For example, if compounds such as silver are used as active ingredients diffusing out from the material, the silver ions will be detoxified by serum used in cell experiments. If comparisons are to be made with bacterial effect, serum at the same concentration as used in the cell experiments should be added in the bacterial experiments.<sup>[120]</sup>

## 5. Microbiological Testing In Vitro and In Vivo

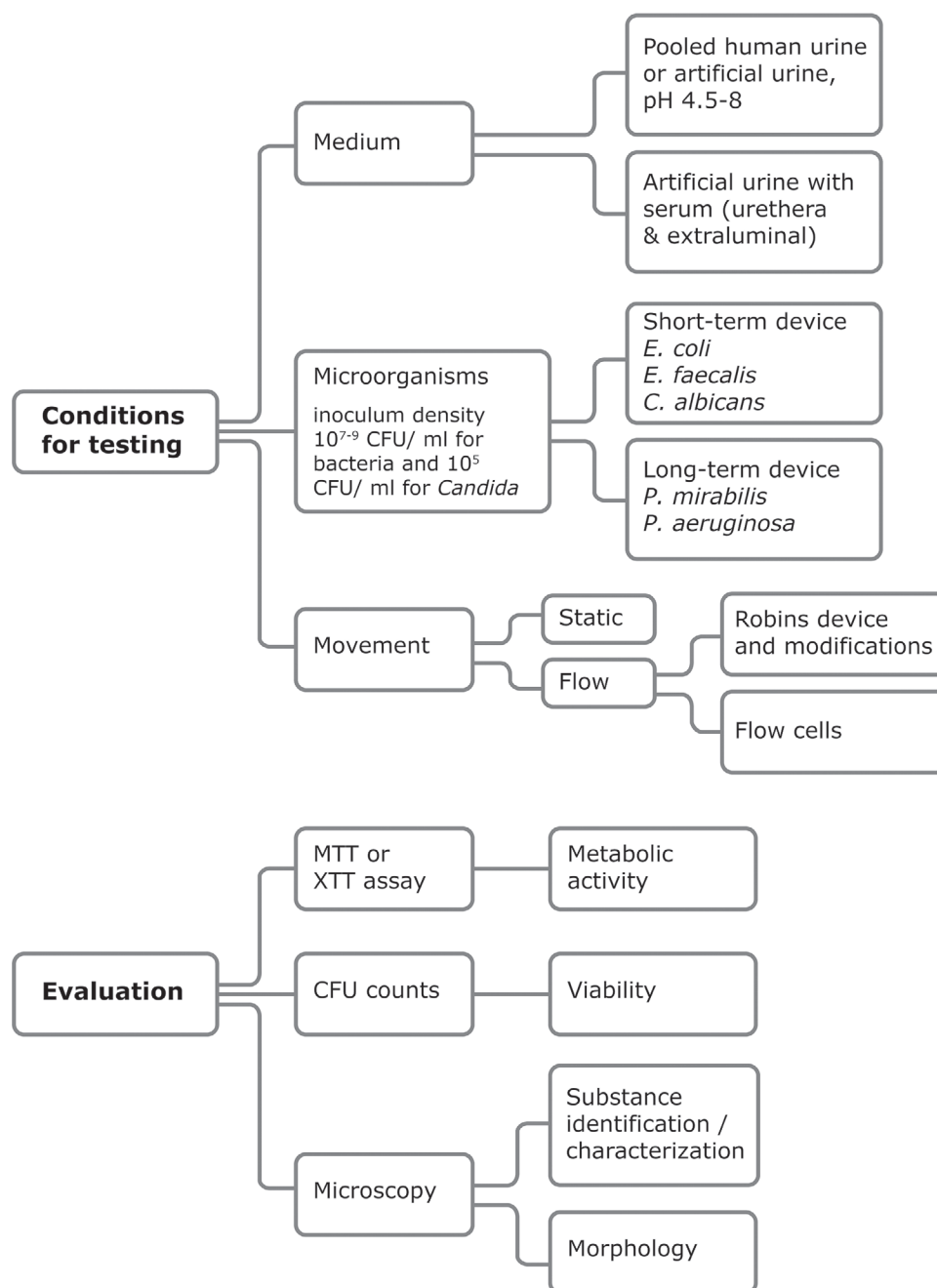
Contrary to biocompatibility testing (ISO 10993) no formal regulations exist for the assessment of devices with regard to infection risk. Thus, there is no formal guidance for medical device industries how to evaluate their products with regard to antimicrobial properties. Still several testing and validation methods of antimicrobial designs exist, some of them are standardized and others are dedicated tests.<sup>[121]</sup> However, there are no standard tests available for testing the antimicrobial efficacy of urinary catheters.

For *in vitro* microbiological testing, several parameters should be considered that all might significantly affect the obtained data. These include parameters of the experimental setup such as medium conditions, choice of microorganism

(species and strain), inoculum size, and flow conditions. Ideally, experiments should be designed so that they can predict the outcome of *in vivo* tests, in order to reduce the number of *in vivo* experiments. A range of *in vitro* methods can be used to evaluate the efficacy of different types of antifouling or antibacterial surfaces (Figure 6). It is important that samples are sterilized and kept sterile until microbial exposure, and care needs to be taken to avoid contamination during microbial testing. As the variability may be high, sufficient numbers of replicates (both repeats of experiment and with different microbial inoculum/cultures) should be performed, in general at least three biological replicates should be tested. Negative controls, positive controls, standard (benchmark) materials, and untreated material controls should ideally all be included in every experiment to enable quality control and to allow for better comparisons between studies.

### 5.1. Medium Conditions

Medium conditions should mimic the conditions found in the host. For the evaluation of (coatings/materials for) ureteral stents, intraluminal surfaces of urinary catheters, or parts of devices in the bladder, (artificial) urine should be used as incubation medium. When testing extraluminal surfaces of (materials for) urinary catheters the authors suggest the culture medium should be supplemented with small amounts of serum, from which various proteins may adsorb onto the material surface<sup>[122]</sup> as an approximation of the extraluminal fluid. Not much is known about the constituents of the extraluminal fluid but in addition to expressed proteins from urothelial cells, it is likely that small damages in the urethral lining as a result of catheter passage will lead to low concentrations of serum proteins.<sup>[123]</sup> Serum is chosen, in this application, over blood plasma since many of the model organisms for UTI are Gram-negatives and their adhesion has been reported to decrease after blood plasma pretreatment but not after whole blood treatment.<sup>[81]</sup> Human urine is a fluid with a variable pH in the range of 4.5–8.0, it has both intra- and interpersonal variable compositions.<sup>[124,151]</sup> It is hypertonic and despite having antimicrobial properties<sup>[125]</sup> is able to support bacterial growth up to  $10^8$  CFU mL<sup>-1</sup>.<sup>[126]</sup> To overcome urine variability, studies on uropathogens could be performed in large batches of pooled human urine<sup>[127]</sup> or in artificial urine.<sup>[125]</sup> However, when using pooled urine samples it is very difficult to obtain a normalized fluid composition over time (between batches) due to the intrinsic high variability. For this reason, it may be better to use an artificial urine that resembles the composition and physical properties of human urine as closely as possible, as outlined and reviewed in the literature.<sup>[49,125,126,128]</sup> For testing of materials aimed for long-term use in the urinary tract with the pathogen *P. mirabilis*, an alkaline version of the artificial urine can be chosen to accurately mimic the conditions for formation of crystalline biofilm also during short experimental time spans. For experiments designed to run for long time intervals, these urease-positive bacteria will by themselves induce this alkalization, as was described for cultivation of *P. mirabilis* and *P. aeruginosa* in artificial urine.<sup>[126]</sup>



**Figure 6.** Schematic box diagram outlining choices of conditions for testing which need to be decided when designing in vitro microbial testing of antimicrobial materials for application in the urinary tract (at the top of the figure), and commonly used evaluation methods after bacterial exposure (bottom of the figure). Substance identification/characterization in microscopy can for example relate to the use of fluorescent probes identifying specific strains of microorganisms in a sample.

## 5.2. Microorganisms and Microbial Challenge Dose

The microorganisms used should be representative to a clinical situation, in order to have predictive value. Furthermore, the microbial profile for infections of short-term urinary devices differs from that of long-term devices. For short-term catheterization, *S. epidermidis*, *E. coli*, and *E. faecalis* have been described as the most common pathogens<sup>[18]</sup> while in long-

term catheterization *P. aeruginosa*, *P. mirabilis*, *Providencia stuartii*, *M. morgani*, and *K. pneumoniae* were most prevalent.<sup>[18]</sup> Although there is no consensus between different studies for the topmost prevalent etiological agents of these infections,<sup>[2,129]</sup> the most common bacteria isolated were *E. coli* followed by *Enterococcus* species, which also dominate in short-term devices. Furthermore, bacterial strains should be selected that are good biofilm producers in the relevant medium, thus

in artificial urine for testing of materials for the urinary tract. In this context, it is important to remember that different strains from the same bacterial or fungal species may exhibit very different phenotypes significantly influencing their ability to form biofilm on surfaces<sup>[130,131]</sup> and their suitability as model organisms for testing of materials surfaces. However, at this time, information about identified suitable clinically significant model strains for testing antimicrobial materials in the urinary tract is lacking in the scientific literature.

In 3–32% of patients with short-term catheters, fungi were also detected<sup>[11]</sup> and the majority of these cases included *Candida albicans*.<sup>[132,133]</sup> Surveillance data from U.S. National Nosocomial Infections Surveillance (NNIS) reported *C. albicans* to be the fourth-most important nosocomial pathogen in UTI.<sup>[134]</sup> The highest risk for *Candida* infection was found for patients with immune suppression and long-term catheterization. Additionally, *Candida* strains frequently exhibit drug resistance.<sup>[133]</sup> Both monospecies and multispecies biofilms have been reported including bacteria and/or fungi, and for long-term devices, multispecies biofilms are very common<sup>[18,135]</sup> including pathogens promoting device encrustation, as previously described.<sup>[136]</sup>

Besides choosing microorganism used to test an antimicrobial or antifouling surface, an appropriate dose also has to be decided in a testing protocol. Bacterial numbers in the urogenital area can be very high and bacterial titers of up to  $10^8$  CFU mL<sup>-1</sup> have been described in the bladder.<sup>[137]</sup> This is an important consideration during materials/device testing as devices may be placed into an already infected patient, thereby encountering very high microbial densities. Standard testing of microbicidal activity generally minimally requires evidence of three or even four log units reduction of viability or growth in comparison to a control. If 10  $\mu$ L of a sample aliquot is plated and one cell counted on this plate, it corresponds to 100 CFU mL<sup>-1</sup>. Thus, in order to accurately assess a log-4 reduction on a culture plate, at least  $10^6$  CFU mL<sup>-1</sup> should be used. If 100  $\mu$ L sample aliquots are used, at least  $10^5$  CFU mL<sup>-1</sup> is needed as starting inoculum. Following this, testing of urological devices should be performed using bacterial densities of at least  $10^5$  CFU mL<sup>-1</sup> and ideally in the range of  $10^7$ – $10^9$  CFU mL<sup>-1</sup>, with the possibility to adjust the densities with respect to microorganism tested. For fungi such as *C. albicans*, a density of  $10^5$  CFU mL<sup>-1</sup> is suggested following the European Committee on Antibiotic Susceptibility Testing (EUCAST) recommendations.<sup>[138]</sup> For antifouling surfaces, higher microbial densities may be used in the tests. Furthermore, suitable controls should be included to ensure that sample treatment steps such as sonication do not affect the viability of microorganisms.<sup>[139]</sup>

Although separate methods are seldom applied for testing efficacy of extraluminal sides of urological devices of the lower urinary tract, lower doses could be required here. The reason is that bacterial concentrations found on the epithelial linings facing the extraluminal surface of the devices are presumably much lower than in the bladder. Moreover, catheter contamination by bacteria in this scenario is due to bacterial transmission between surfaces making close contact, rather than via mass transport from a liquid volume. Thus, relatively low bacterial concentrations are here suggested in order to avoid that potentially adequate preventive designs fail in the evaluation process. For this limited case,  $10^3$ – $10^4$  CFU cm<sup>-2</sup> may be used thereby mimicking an assumed lower number of pathogens on the urethral surface

exposed to the extraluminal surface of the catheter, bearing in mind that this set-up may not allow a 3–4 log reduction to be proven. Since methods used in these cases are typical systems with a high area to volume ratio, such as in the JIS Z2801 test (see also Section 5.3.1), challenge numbers of bacteria may be defined per area as these values can be more relevant than bacterial concentrations per volume.<sup>[7]</sup> However, these extraluminal tests may not provide a microbial challenge suitable for intraluminal parts of a catheter or devices in the ureter or bladder.

### 5.2.1. Multispecies Interactions

Interactions occurring between bacteria of different species are important to consider when designing CAUTI model test systems. As stated above, biofilms on urinary devices are often composed of multispecies consortia, especially in long-term catheters.<sup>[140]</sup> Experimental data show that interspecies interactions in biofilms bring out synergistic effects, also described as community intrinsic properties, which are traits expressed only in the presence of other species. Obviously, these characteristics cannot be studied in monocultures.<sup>[141]</sup> With relevance to UTIs, multispecies biofilms have been reported to be more virulent and have higher tolerance to antimicrobial agents, than would have been expected from assessment of monospecies biofilms studied individually.<sup>[142]</sup> Importantly, some uropathogen species are only able to colonize the catheter surface in the presence of others species.<sup>[143]</sup> In line with this, it was recently suggested that interspecies interactions between multidrug-resistant organisms should be considered when treating CAUTI by antibiotic therapy, as applications of antibiotics would select for specific resistant organisms that would then positively select for other multidrug-resistant organisms through complex network interactions.<sup>[144]</sup> Interactions resulting in enhanced virulence and antibiotic tolerance are not restricted to the bacterial kingdom; examples of bacteria–fungi interactions have also been described, mainly focusing on the interactions between *S. aureus* and *C. albicans*, where extended *S. aureus* invasion has been reported due to bacterial association to fungal hyphae.<sup>[145]</sup> Therefore, we recommend that these interactions are also considered in microbiological assessments of materials and antimicrobials. This should be at a later stage, after monoculture assessment, and may include testing of defined multispecies combinations (representing a CAUTI community). Only by stepwise enhancement of complexity, we will enable truly relevant evaluation of catheters and ureteral stents capable of preventing colonization by relevant microbial communities causing infection.

### 5.3. Clinical Flow Conditions

Antimicrobial coating designs are usually developed to prevent device-associated UTI<sup>[79,146]</sup> and, in order to do so, all routes of infection should be considered and preferably blocked<sup>[17]</sup> including those that may occur on replacement devices in previously infected patients. In previously non-colonized patients receiving a catheter, the main route along the extraluminal side of a urinary catheter, is established under predominantly stationary conditions in a situation with relatively low liquid volume. However, in patients where a colonized

catheter is replaced, the main route of infection may differ depending on where the microorganisms are residing in the urinary tract of the colonized patient. Intraluminal colonization can be established in very different conditions of flow and these depend on the way the catheter is operated. Indwelling urinary catheters can be directly connected to a drainage bag (free-flow) or they can be fitted with a valve at the end of the tube to allow, for instance, voiding into a toilet. There are several medical conditions (e.g., people with severe cognitive impairment or high pressure chronic retention, ureteric reflux or obstructive renal impairment/failure) where the use of a catheter valve is not recommended.<sup>[147]</sup> However, it has been shown that using a catheter valve is beneficial to decrease the risk of infection,<sup>[147]</sup> reduce catheter blocking<sup>[148]</sup> and is a more cost-effective solution<sup>[147]</sup> increasing patient comfort.<sup>[149]</sup> Additionally, it has been shown that using a free-flow drainage bag for 6 months can lead to bladder shrinkage and compromised function<sup>[150]</sup> whereas the use of a valve enables the operation in conditions that mimic the normal bladder function<sup>[147]</sup> maintaining its functionality. Since the way the catheter is operated will affect intraluminal flow, with reduced flow in free drainage mode or with interrupted and varying flow when using a valve, it is difficult to propose a standardized condition for in vitro testing. Given the different routes of infection and the operation modes of urinary catheters, the evaluation of the efficacy of antimicrobial coatings typically requires testing under static and flow conditions.

### 5.3.1. Static Conditions

In general, standardized microbial testing methods for static conditions are more developed than those for flow conditions.<sup>[7]</sup> Among the most applied evaluation methods are “agar zone of inhibition” tests, in which antimicrobials released from a functionalized material positioned upside down on an agar plate diffuse into the agar to form a zone of growth inhibition during incubation. The diameter of this zone of inhibition indicates the amount or efficacy of the released antimicrobial toward the applied bacterial or fungal species. Other methods applicable to antimicrobial releasing designs are executed in a nutrient suspension. Suspended bacteria exposed to these samples may be growth-inhibited or killed in the medium with an efficacy measured by the numbers of viable bacteria in the suspension or left on the sample after incubation. Typical “high area to volume” tests apply to situations encountered in the narrow space between the catheter and the urethral epithelial layer, which create an intimate contact between the microorganisms and the sample. Examples of these tests are the standard JIS-Z 2801 and “all in one” plating systems (Petrifilm, 3M, St. Paul, MN, USA).<sup>[7]</sup> Since an intimate contact between bacteria and the sample is created, these type of tests are also applicable to contact killing designs<sup>[152]</sup> but may be less valuable for repellent systems.

### 5.3.2. Flow

Under flow conditions, the fluid mechanics of the liquid in contact with, for example, an attaching bacterial cell will affect its

subsequent adherence, biofilm formation, and biofilm structure.<sup>[153,154]</sup> Furthermore, the antimicrobial substrate and its interaction with the microbial cell may be highly influenced by flow. Contact killing surfaces that are adhesive for microbial cells may be covered in microbial debris and loose (part of their) activity,<sup>[8,131]</sup> and this process may be highly influenced by the fluid flow around the surface. Surfaces releasing antimicrobial substances exposed to flow may be depleted at shorter lengths of time compared to the same set up under static conditions.<sup>[155]</sup> Therefore, it is important that an experimental set-up used to perform assays of biofilm formation is able to mimic the flow conditions occurring, for example, in the urinary tract. In fact, hydrodynamics determine the rate of transport of cells, oxygen, and nutrients to the device surface, as well as the magnitude of the shear forces acting on a developing biofilm.<sup>[156]</sup> Because the flow rate by itself provides little information about shear without taking into account the geometry of the applied in vitro flow system it is useful to mimic the flow conditions in a catheter by using either the wall shear stress or the shear rate (See also **Box 2**).<sup>[157,158]</sup>

For performing in vitro assays on biofilm formation under flow conditions, biofilm reactors are widely used. Although standardized methods exist, such as ASTM E2871-13 and ASTM 2562-12 for the Center for Disease Control (CDC) biofilm reactor, the operation of some of these reactors have specific limitations. For example, some of them cannot be used to test different materials, some of them have reduced sampling areas or, since most biofilm reactors are basically stirred containers, the flow pattern changes within the coupon surface and does not mimic the plug flow characteristics of a urinary catheter. For a more detailed discussion on these platforms, the reader is referred to comprehensive reviews on this matter.<sup>[157,159]</sup> Unfortunately, there are no standardized methods for specifically testing materials for the urinary tract. Platforms often used to perform in vitro assays for the urinary tract are flow systems that mimic flow conditions as well as transport of nutrients and bacteria in a device such as a urinary catheter or ureteral stent. These can be generally divided into two broad categories: those that are designated as bioreactors such as the Robbins device, used to study the efficacy of sample surface functionalization on biofilm formation and those that are essentially built for microscopic observation of individually adhering bacteria, designated as flow chambers or flow cells.<sup>[160]</sup> Additional methods exist that study larger sections of devices under flow exemplified by Fisher et al.,<sup>[161]</sup> who designed an in vitro catheter challenge model by placing a section of a urinary catheter in a liquid and exposing the intraluminal side with pulses of bacterial suspensions under flow. Methods utilizing intact catheters have been described by Chua et al.<sup>[162]</sup> and Stickler et al.,<sup>[49,163]</sup> allowing for flow and bacterial exposure of the intraluminal side of urinary catheters, as well as the formation of a stagnant urine pool around the inflated balloon.

**Robbins Device and Modifications:** The Robbins device is mainly operated as a biofilm reactor allowing a continuous flow of fresh growth medium and is based on the designs of Jim Robbins and Bill McCoy that were later patented by the Shell Oil Company.<sup>[164]</sup> They are essentially pipes containing holes in which screws are fixed. These screws, which are aligned parallel to the fluid flow direction, contain coupons that can



**Box 2 Equations useful for mimicking flow conditions of catheters in parallel plate flow cells.**

The main characteristics of flow in a tube are the wall shear rate and shear stress. The wall shear rate  $\sigma$  (with unit  $s^{-1}$ ) is a measure of change of the fluid velocity near the wall of the tube in the radial direction toward the center of the tube. The shear rate is related to the force which the fluid flow exerts on the wall, expressed as the shear stress  $\tau$  ( $N\ m^{-2}$ ), according to  $\tau = \mu \times \sigma$  with  $\mu$  the dynamic viscosity of the fluid (for water around  $10^{-3}\ Ns\ m^{-2}$ ).

The wall shear rate ( $\sigma$ ) for a cylindrical geometry, assuming that the fluid velocity is zero at the surface and flow is laminar in the bulk, is given by:

$$\sigma = \frac{4Q}{\pi r^3}$$

With  $Q$  the flow rate ( $m^3\ s^{-1}$ ) and  $r$  the radius of the catheter (m).

The wall shear rate ( $\sigma$ ) for a rectangular channel geometry of a parallel plate flow cell is:

$$\sigma = \frac{3Q}{2\left(\frac{h_0}{2}\right)^2 \cdot w_0}$$

With  $h_0$  the height of the channel (m) and  $w_0$  the width of the channel (m).

In order to mimic the flow conditions of a urinary catheter in a parallel plate flow cell, the dimensions of the flow cell and volume flow should relate to the ones in the catheter according to

$$\sigma = \frac{Q_{catheter}}{\pi r^3} = \frac{3 * Q_{flow\ chamber}}{2 * h_0^2 w_0}$$

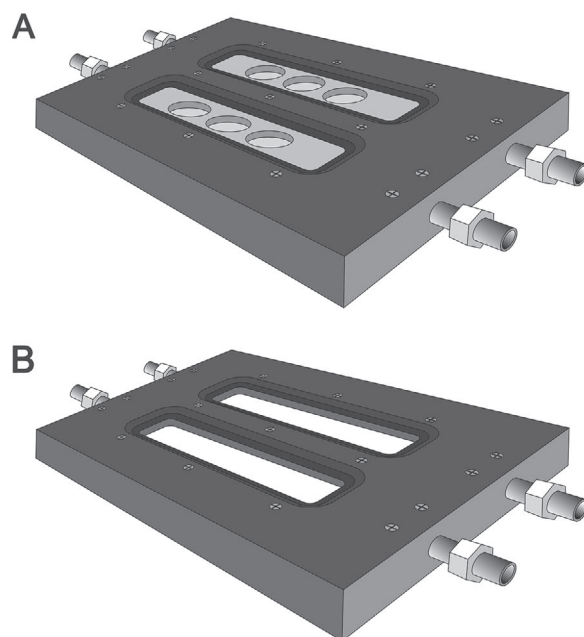
Assuming that the flow rate in the parallel plate flow chamber is taken equal to the one in the catheter, a flow chamber with a channel height 0.5 mm and channel width of 20 mm would mimic the flow characteristics of a catheter with an inner diameter of 2 mm.

be independently removed.<sup>[165]</sup> The modified Robbins device (MRD) is basically a square-channel pipe with coupons fixed to sampling plugs that can be unscrewed from the walls.<sup>[164]</sup> These plugs are flush (even) with the inner surface and therefore the coupon surfaces do not disturb the flow characteristics.<sup>[164]</sup> Other designs include a half-pipe geometry that more closely resembles the circular section of a tube.<sup>[154]</sup> It has been shown that the shear stress field is approximately the same in the curved and flat walls of these devices<sup>[166]</sup> so that coupons can be placed on the flat wall for convenience and still be subjected to the same shear forces acting on the curved wall. The MRD can be operated either in laminar or turbulent regime<sup>[166–168]</sup> but it is important to make sure that, particularly in turbulent

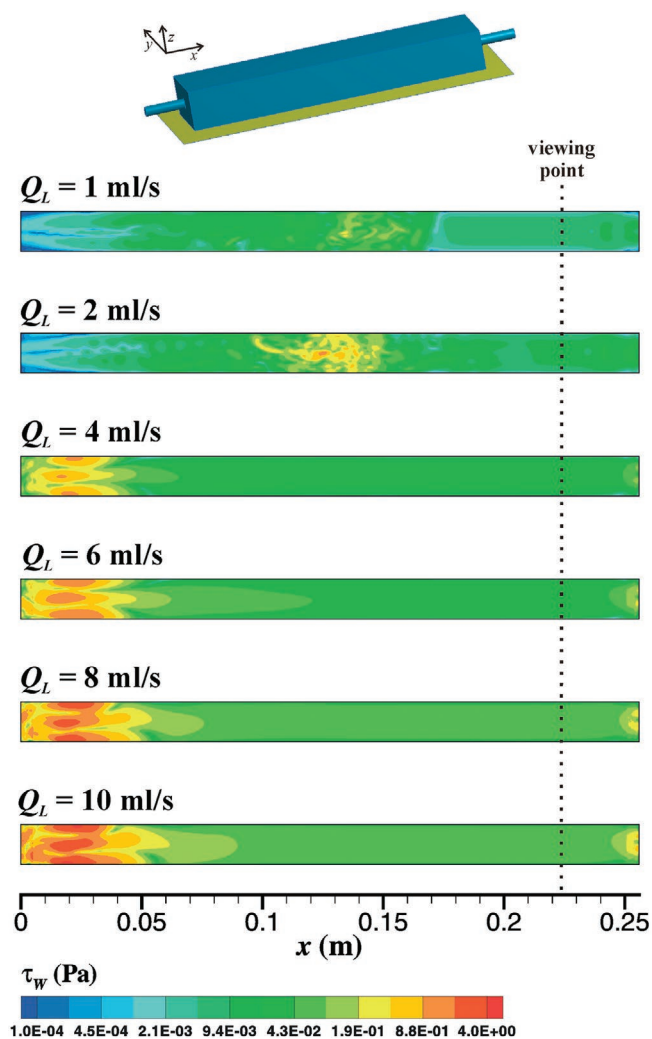
operation, the MRD has an entry section long enough to allow complete flow development before the area where the coupons are located. This is an important aspect as, otherwise, the shear stress field is not constant, and therefore large coupon-to-coupon variation will arise, or even large variations on a single coupon surface. Validation of these flow cells for a predetermined flow rate can be done using particle image velocity (PIV) techniques<sup>[169]</sup> and simulation of the flow by computational fluid dynamics.<sup>[166,168]</sup> MRDs contain a relatively high number of coupons (usually between 8 and 20) that enable destructive analysis of the biofilm in a long time-course assay<sup>[170]</sup> or the simultaneous testing of different surfaces. However, they are not designed to allow direct observation of the biofilm development and therefore coupons must be removed for examination, which can introduce artefacts due to sample handling.<sup>[171]</sup>

**Flow Cells:** There are several commercially available models of flow cells that can be mounted on a microscope stage and used with video capture systems enabling real-time observation of microbial adhesion and biofilm formation.<sup>[164]</sup> Some models are autoclavable, reusable, and compatible with confocal and epifluorescence microscopes<sup>[172]</sup> and the most common geometries are the flat plate and capillary flow cells.

Flat plate flow cells can contain one or two glass viewing ports (**Figure 7**). Models with two channels enable direct comparison of biofilms formed under two different conditions (a control and a specific treatment) in parallel. Some of the flat plate models include recesses to fit coupons that can be constructed from different materials to simulate cell adhesion to different surfaces although the low amount of biofilm that is produced may preclude further chemical/biochemical analysis. Flat plate flow cells are usually restricted to lower flow rates and to laminar flow applications, whereas capillary flow cells can be used at



**Figure 7.** Drawings of flat plate flow cells designed to enable in situ microscopic analysis of biofilm formation onto sample coupons (top image) or (coated) glass slides (bottom image) that can be used with transmission, fluorescence, or confocal microscopy.



**Figure 8.** Shear stress at the bottom wall of a parallel plate flow cell, illustrating the turbulence and altered flow conditions that may occur, for example, at the inlet of the flow cell. Reproduced with permission.<sup>[173]</sup> Copyright 2014, Elsevier.

higher flow rates but they are not very convenient for surface testing. For turbulent flow regimes, one must make sure that the flow cell is long enough to allow for full development of the flow in the viewing region (Figure 8).<sup>[173]</sup> Also sudden expansions or contractions between the connective tubing and the flow cell should be avoided to minimize entry effects.<sup>[169,174]</sup>

**Operating Conditions:** Flow cells and MRDs have been used to study bacterial cell adhesion and biofilm formation under hydrodynamic conditions that mimic the urinary tract.<sup>[158,170,173]</sup> In both types of systems, the flow rate should be adjusted to an average shear rate of around  $15 \text{ s}^{-1}$  as an estimate of the intraluminal urine flow, considering a daily production of urine around  $1.4 \text{ L}$ ,<sup>[151]</sup> an inner catheter diameter of  $2 \text{ mm}$ , and assuming operation in free drainage mode (see Section 5.3 and Box 2).<sup>[175]</sup> In general, in order to mimic the shear conditions of a urinary catheter in a parallel plate flow cell, the dimensions of the flow cell or the flow rate should be adjusted<sup>[166,173,176]</sup> to approach the required shear rate (Box 2).

#### 5.4. Evaluation

Independent of the microbial test selected, the last step is always the evaluation after bacterial/fungal exposure. Since several reviews on this topic have been published,<sup>[7,177]</sup> we here only include a brief overview (Figure 6). Culture methods on agar-plates are the classic, gold standard microbiological approach resulting in enumeration of viable bacteria, quantified by counting CFUs in a defined volume or area. However, the disadvantages are that this is time consuming and only suitable for culturable microorganisms.<sup>[7,164]</sup> Methods that do not require cultivation are based on the evaluation of metabolic activity of microorganisms using assays such as the neutral red uptake, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) or XTT (2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide) assays. However, these methods do not show whether the microorganisms are dead or only growth-inhibited, and they are not considered as validated and standardized as CFU counts are. Another possibility is the use of microscopy techniques allowing for the in situ analysis of organism morphology, bacterial activity,<sup>[178]</sup> as well as identification of microorganisms, for instance with fluorescence in situ hybridization (FISH, Figure 2).<sup>[179]</sup> Biofilms can also be visualized by staining of the biofilm extracellular matrix using specific probes<sup>[180]</sup> or using electron microscopy. However, these approaches generally do not provide information about numbers of viable bacteria.

A recent review of standardized testing methods has identified a shortage of suitable standard methods for testing antimicrobial materials or systems, especially methods allowing for testing under flow conditions.<sup>[7]</sup> In the absence of standardized methods, a multitude of experimental methods have been used around the world with the unfortunate consequence that studies of antimicrobial coatings or materials in the scientific literature are more or less impossible to compare. For example, large variability will often be obtained through the choice of test organism, medium composition, and flow conditions. Recommendations on tests to be used may best be derived from patient scenarios and the type of devices used. Table 2 summarizes the extremes expected during conditions encountered for catheters and ureteral stents. Extraluminally, flow as well as the bacterial numbers challenging the catheter surface will always be low. Intraluminally, flow will be low in case of free-flow catheters, but high during voiding in case of valve-controlled catheters. The “challenge dose” of bacteria will particularly depend on the colonization status of the bladder. In case of advanced stage UTI, the numbers of bacteria in the urine from the bladder will be (very) high. Therefore, the worst-case scenario for challenge of the catheter would be the situation of a patient with advanced stage UTI bladder colonization in combination with a free-flow catheter. In this case, the intraluminal surface of the catheter is likely to be at the highest risk for colonization and biofilm formation. The most robust testing of antimicrobial activity therefore should be performed with high inoculum under low or no flow. The test to assess the numbers of adherent bacteria can be any test validated with the gold standard of CFU assessment for bacteria in case of culturable bacteria. In case numbers of non-culturable bacterial species need to be assessed, this can most robustly be performed quantitatively by qPCR. For the analysis

**Table 2.** Combinations of flow conditions and bacterial challenge in different patient scenarios.

Patient condition	Catheter free-flow or with valve	Bacteria intra/extraluminally	Bacterial challenge concentration	Flow low/high
Non-colonized patient	Either	Extraluminally	Low	Low
Patient with bladder colonization/UTI	Free-flow	Extraluminally	Low	Low
		Intraluminally	High	Low
Patient with bladder colonization/UTI	Valve	Extraluminally	Low	Low
		Intraluminally	High	High
	Stent	Bacteria intra/extraluminally	Bacterial challenge concentration	Flow low/high
Non-colonized patient		Extraluminally	Low	Low
		Intraluminally	Low	Low
Patient with bladder colonization/UTI		Extraluminally	High	Low
		Intraluminally	High	Low

of spatial arrangement of different bacterial species within multispecies biofilms, FISH would be recommended.

### 5.5. In Vivo Urinary Tract Infection Models

The translation of novel antimicrobial material and device technologies for the urinary tract to the clinic has proven to be challenging. Preclinical in vivo testing is an important phase to design, produce, and reliably test the efficacy of any new antimicrobial-functionalized device. Preclinical in vivo studies mimic the pathogenesis of UTI in much greater complexity than is possible in vitro. Next to the valuable scientific insights such studies provide, they are often required by regulatory bodies for safety and efficacy evaluation of devices prior to their clinical testing and use in patients.<sup>[181]</sup> A wide range of preclinical in vivo UTI models are available with different levels of complexity, ranging from small rodent models to larger animal models. Thus, models are available for initial testing/screening of novel material and/or antimicrobial strategies in vivo. This includes subcutaneous mouse models,<sup>[30,182]</sup> non-functional implant models, such as murine<sup>[183]</sup> or rat UTI<sup>[184]</sup> models, to

fully functional device models, like the rabbit ureteral stent<sup>[52]</sup> and female pig catheter models.<sup>[185]</sup> Two comprehensive reviews on in vitro and in vivo model systems to study biofilm formation include a detailed overview of the UTI animal models currently available.<sup>[186,187]</sup> However, most emphasis in these models is on testing monospecies bacterial infections. There have been only a limited number of in vivo studies reported on fungal<sup>[188,189]</sup> and multispecies experimental UTI.<sup>[190]</sup> This reflects the relatively low level of interest in fungal and multispecies testing in vivo despite indications of synergies such as those previously described.<sup>[190]</sup>

The majority of in vivo models for device-related infection in the urinary tract are performed using clinical microbial isolates from hospitalized patients. This is clearly illustrated by the review by Lebeaux et al. in 2013 in which all models were using clinical isolates or pathogens that had been shown to cause UTI in the respective animals.<sup>[187]</sup> Their review lists two mouse models, three rat models, and two rabbit models (Table 3) that were using one or several strains of *E. coli*, *P. aeruginosa*, *P. mirabilis* and *E. faecalis* to study device-associated UTI. The presence of a device was mimicked by pieces of catheter or stent in the bladder, glass beads in the bladder, or in one of

**Table 3.** Diversity of strains used for in vivo models of device-associated UTI.

Model	Model type	Species	Strains	Reference
Mouse	Precolonized catheter segment (surgical) in bladder	<i>E. coli</i>	Uropathogenic K12 EMG2	[191]
Mouse	Catheter segment in bladder (both precolonized and with inoculation inside bladder)	<i>E. coli</i>	Cystitis isolate of UTI89	[192]
		<i>P. aeruginosa</i>	ATCC 19660	
		<i>P. mirabilis</i>	ATCC 51286	
		<i>E. faecalis</i>	OG1RF wild type and mutant strains	
Rat	Precolonized glass beads in bladder	<i>E. coli</i>	UTI isolate HM32 strain	[193]
Rat	Ureteral stent segment (surgical) in bladder, inoculated inside the bladder	<i>P. aeruginosa</i>	clinical isolate AN207, a slime producer	[194]
Rat	Tube segment in bladder, inoculated in the bladder	<i>P. aeruginosa</i>	UTI isolate 910735	[195]
Rabbit	Ureteral stent/catheter in bladder (both precolonized and inoculated in the bladder)	<i>P. aeruginosa</i>	Not specified	[52,196]
		<i>P. mirabilis</i>	296 (clinical isolate)	
Rabbit	Urethral catheter, inoculated after catheterization	<i>E. coli</i>	WE 6933, causing UTI	[197]
Mouse	Catheter segment (surgical), inoculated after catheterization	<i>C. albicans</i>	ATCC SC5314	[189]
Rat	Catheter segment, inoculated after catheterization	<i>C. albicans</i>	K1, DAY185	[188]



the rabbit models through placement of a urethral catheter. In some cases, the materials had a bacterial biofilm pregrown on their surface before placement into the bladder of the animals.

It is difficult to base any recommendation of strain selection on this literature review. It seems most studies have selected their unique strains and no comparisons between how these strains colonize or infect the material/device or the surrounding tissue are available. For the future, it could be of great value to identify a selection of clinical isolates that are well characterized, stable, and that could be used for both in vitro and in vivo testing of antimicrobial and antifouling materials.

## 6. Conclusions

The market for clinical care products is conservative, specifically in case of urinary catheters, which are considered as low-price products, often as a result of tenders driven to cut medical costs. The urinary catheter market is growing globally and will reach an annual volume of \$2.19 billion by 2022. In contrast to the size of the catheter market, national cost estimate from 2015 suggests that the annual total additional costs associated with CAUTI in the United Kingdom is £1–2.5 billion and in United states is \$36 billion, factors higher than the global total costs associated with catheter purchasing. The high risk of infection and its associated costs clearly underlines the need to provide patients with devices with the lowest possible risk of infection, and emphasizes the need for innovative products that reduce the incidence rate of device-associated UTI. Although standards are available for guiding the development of new devices with respect to biocompatibility (ISO 10993) and material characterization (Table 1), no such guidance exists for development of antimicrobial devices. Therefore, this review provides recommendations for design of standard test methods in addition to those provided in ISO 10993 for material and device safety. The considerations and recommendations presented and summarized in **Box 3** cover relevant aspects of antimicrobial material testing, including surface characterization, in vitro and in vivo tests, strain selection, and hydrodynamic conditions, complying to the complex pathology of device-associated UTI. Such

### Box 3 Recommendations for experimental design of in vitro antimicrobial urinary device testing.

- Biological testing should be performed in combination with thorough material characterization.
- Protocols should include test medium consisting of either large batches of pooled human urine or artificial urine. This medium is suggested for testing of the majority of surfaces of urinary tract devices, with the exception of extraluminal surfaces in the urethra, where addition of small amounts of serum is suggested to mimic the extraluminal interface with epithelial cells.
- Preconditioning of materials/devices with culture medium should be investigated using biologically relevant fluids above.

- Sterilization procedures for materials and devices should be developed and their effects on the materials/devices investigated.
- The microbial loading should ideally be  $10^7$ – $10^9$  CFU  $\text{mL}^{-1}$  for bacteria and  $10^5$  CFU  $\text{mL}^{-1}$  for fungi.
- The type of microorganisms (species and strains)
  - Short-term devices: we suggest performing tests with the bacteria *E. coli* and *E. faecalis* as well as with the fungus *C. albicans*.
  - Long-term stents and catheters with the complication of encrustation development: we suggest to include tests using *P. mirabilis* and *P. aeruginosa*. The experiments can be shortened by using an artificial urine that is already alkaline.
  - Despite clinical biofilms often being composed of several species, we suggest using monospecies systems until stable multispecies consortia have been developed and tested in the scientific literature. More research is needed trying to understand the ecology of multispecies microbial colonization and cohabitation in the urinary tract.
  - The chosen strains should be clinical isolates recovered from urinary tract devices, which are good biofilm producers under the conditions used during testing. Suitable strains should be characterized and made available to the scientific community through deposition in culture collections.
  - Strains should be chosen that are also suitable for in vivo evaluations.
- For testing of extraluminal surfaces in the urethra, static conditions or near-static conditions should be used. Typical tests which apply in situations encountered in the narrow space between the catheter and the urethral epithelial layer are the standard JIS-Z 2801 and “all in one” plating systems. These surfaces may be challenged with lower bacterial inoculum.
- For the intraluminal surfaces in the urethra, bladder, or devices in the ureter a setup allowing for flow is required to mimic what happens in vivo. Flow cells are suggested as experimental setups, since they may be easily designed to accurately mimic flow conditions in the device.

standard assays would enable comparisons of results obtained in different research labs both in industry and academia, as well as provide industry and academia with tools to assess the antimicrobial and/or antifouling properties for urinary tract devices in a reliable and relevant way.

## Acknowledgements

This perspective review has been written as a part of the EU-funded Trans Domain COST Action, TD 1305 iPROMEDA1 (improved protection of medical devices against infection), in the focus group for urinary tract devices. The aim of the work performed in this group has been to bridge different areas of expertise, discuss and give suggestions for ways in



which testing of medical devices aimed at preventing infection should be performed to follow best practices, as well as to enhance the predictability of methods for clinical outcome of a device with respect to infection. The authors would like to acknowledge COST (European Cooperation in Science and Technology) for funding of the network, as well as network participants that have given input in discussions forming the base for this paper, including Dr. Cesar Rodrigues-Emmenegger, Prof. Erik Reimhult, Prof. Peter Tenke, Dr. Botond Lakatos, Dr. Naseem Theilgaard, Dr. Reto Luginbuehl, Prof. Yannis Missirlis, Dr. Matthias Buhmann, Dr. Vesna Miskovic-Stankovic, Dr. Veronique Fontaine, Dr. Jose-Ramon Sarasua. Project PTDC/BTM-SAL/29335/2017, Portuguese Government, Fundação para a Ciência e Tecnologia (FCT), and designer Nuno Monge is acknowledged for assistance with the artworks of Figures 1, 3, 6, and 7.

## Conflict of Interest

The authors report no conflict of interest for this review.

## Keywords

antimicrobial, device-associated urinary tract infections, in vitro, in vivo, material testing

Received: October 11, 2018

Revised: January 18, 2019

Published online: March 18, 2019

- [1] World Health Organization (WHO), *Report on the Burden of Endemic Health Care-Associated Infection Worldwide*, WHO, Geneva, Switzerland **2011**. [https://www.who.int/gpsc/country\\_work/burden\\_hcai/en/](https://www.who.int/gpsc/country_work/burden_hcai/en/) (accessed: March 2019).
- [2] European Centre for Disease Prevention and Control (ECDC), *Annual Epidemiological Report 2015: Healthcare-Associated Infections Acquired in Intensive Care Units*, ECDC, Stockholm, Sweden **2015**. <https://ecdc.europa.eu/en/publications-data/healthcare-associated-infections-acquired-intensive-care-units-annual> (accessed: March 2019).
- [3] L. M. Weiner, A. K. Webb, B. Limbago, M. A. Dudeck, J. Patel, A. J. Kallen, J. R. Edwards, D. M. Sievert, *Infect. Control Hosp. Epidemiol.* **2016**, *37*, 1288.
- [4] A. P. Magiorakos, A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S. Harbarth, J. F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D. L. Paterson, L. B. Rice, J. Stelling, M. J. Struelens, A. Vatopoulos, J. T. Weber, D. L. Monnet, *Clin. Microbiol. Infect.* **2012**, *18*, 268.
- [5] World Health Organization (WHO), *Priority Pathogens*. WHO, Geneva, Switzerland **2017**. [https://www.who.int/medicines/publications/WHO-PPL-Short-Summary\\_25Feb-ET\\_NM\\_WHO.pdf](https://www.who.int/medicines/publications/WHO-PPL-Short-Summary_25Feb-ET_NM_WHO.pdf) (accessed: March 2019).
- [6] H. J. Busscher, H. C. van der Mei, G. Subbiahdoss, P. C. Jutte, J. J. van den Dungen, S. A. Zaat, M. J. Schultz, D. W. Grainger, *Sci. Transl. Med.* **2012**, *4*, 153rv10.
- [7] J. Sjollem, S. A. J. Zaat, V. Fontaine, M. Ramstedt, R. Luginbuehl, K. Thevissen, J. Li, H. C. van der Mei, H. J. Busscher, *Acta Biomater.* **2018**, *70*, 12.
- [8] M. Krishnamoorthy, S. Hakobyan, M. Ramstedt, J. E. Gautrot, *Chem. Rev.* **2014**, *114*, 10976.
- [9] ipromedai, [www.ipromedai.net](http://www.ipromedai.net) (accessed: March 2019).
- [10] a) A. M. Acosta-Miranda, J. Milner, T. M. Turk, *J. Endourol.* **2009**, *23*, 409; b) M. Monga, E. Klein, W. R. Castañeda-Zúñiga, R. Thomas, *J. Urol.* **1995**, *153*, 1817; c) K. Nikkhou, H. Z. Kaimakiotis, D. Singh, *J. Endourol.* **2011**, *25*, 1829.
- [11] T. M. Hooton, S. F. Bradley, D. D. Cardenas, R. Colgan, S. E. Geerlings, J. C. Rice, S. Saint, A. J. Schaeffer, P. A. Tambayh, P. Tenke, L. E. Nicolle, I. D. S. o. America, *Clin. Infect. Dis.* **2010**, *50*, 625.
- [12] a) A. A. Barros, C. Oliveira, A. J. Ribeiro, R. Autorino, R. L. Reis, A. R. C. Duarte, E. Lima, *World J. Urol.* **2018**, *36*, 277; b) P. Tenke, C. R. Riedl, G. L. Jones, G. J. Williams, D. Stickler, E. Nagy, *Int. J. Antimicrob. Agents.* **2004**, *23*, S67; c) N. Laube, L. Kleinen, J. Bradenahl, A. Meissner, *J. Urol.* **2007**, *177*, 1923; d) G. Zelichenko, D. Steinberg, G. Lorber, M. Friedman, B. Zaks, E. Lavy, G. Hidas, E. H. Landau, O. N. Gofrit, D. Pode, M. Duvdevani, *J. Endourol.* **2013**, *27*, 333; e) J. Y. Lock, M. Draganov, A. Whall, S. Dhillon, S. Upadhyayula, V. I. Vullev, H. Liu, *Conf. Proc. IEEE Eng. Med. Biol. Soc.* **2012**, *2012*, 1378.
- [13] N. D. Stone, M. S. Ashraf, J. Calder, C. J. Crnich, K. Crossley, P. J. Drinka, C. V. Gould, M. Juthani-Mehta, E. Lautenbach, M. Loeb, T. MacCannell, P. N. Malani, L. Mody, J. M. Mylotte, L. E. Nicolle, M.-C. Roghmann, S. J. Schweon, A. E. Simor, P. W. Smith, K. B. Stevenson, S. F. Bradley, *Infect. Control Hosp. Epidemiol.* **2012**, *33*, 965.
- [14] P. G. Pappas, C. A. Kauffman, D. R. Andes, C. J. Clancy, K. A. Marr, L. Ostrosky-Zeichner, A. C. Reboli, M. G. Schuster, J. A. Vazquez, T. J. Walsh, T. E. Zaoutis, J. D. Sobel, *Clin. Infect. Dis.* **2016**, *62*, e1. <https://doi.org/10.1093/cid/civ933>
- [15] W. A. Alfouzan, R. Dhar, *J. de Mycologie Médicale.* **2017**, *27*, 293.
- [16] R. C. L. Feneley, I. B. Hopley, P. N. T. Wells, *J. Med. Eng. Technol.* **2015**, *39*, 459.
- [17] J. M. T. Barford, A. R. M. Coates, *J. Infect. Prev.* **2009**, *10*, 50.
- [18] D. J. Stickler, *Nat. Clin. Pract. Urol.* **2008**, *5*, 598.
- [19] a) J. W. Costerton, Z. Lewandowski, D. E. Caldwell, D. R. Korber, H. M. Lappin-Scott, *Annu. Rev. Microbiol.* **1995**, *49*, 711; b) J. W. Costerton, J. C. Post, G. D. Ehrlich, F. Z. Hu, R. Kreft, L. Nistico, S. Kathju, P. Stoodley, L. Hall-Stoodley, G. Maale, G. James, N. Sotereanos, P. DeMeo, *FEMS Immunol. Med. Microbiol.* **2011**, *61*, 133.
- [20] C. Dariane, J. N. Cornu, E. Esteve, H. Cordel, C. Egrot, O. Traxer, F. Haab, *Progrès en Urologie.* **2015**, *25*, 306.
- [21] P. S. Stewart, J. W. Costerton, *Lancet* **2001**, *358*, 135.
- [22] I. Haraga, S. Abe, S. Jimi, F. Kiyomi, K. Yamaura, *J. Microbiol. Methods* **2017**, *132*, 63.
- [23] P. A. Tambyah, K. T. Halvorson, D. G. Maki, *Mayo Clin. Proc.* **1999**, *74*, 131.
- [24] L. E. Nicolle, *Drugs Aging* **2014**, *31*, 1.
- [25] D. Lange, B. H. Chew, in *Biomaterials and Tissue Engineering in Urology* (Eds: J. Denstedt, A. Atala), Woodhead Publishing, Cambridge, UK **2009**, Ch. 4.
- [26] D. J. Stickler, *J. Intern. Med.* **2014**, *276*, 120.
- [27] E. O. Kehinde, V. O. Rotimi, A. Al-Hunayan, H. Abdul-Halim, F. Boland, K. A. Al-Awadi, *J. Endourol.* **2004**, *18*, 891.
- [28] L. J. Cummings, S. L. Waters, J. A. D. Wattis, S. J. Graham, *J. Math. Biol.* **2004**, *49*, 56.
- [29] a) A. A. Barros, C. Oliveira, R. L. Reis, E. Lima, A. R. C. Duarte, *J. Pharm. Sci.* **2017**, *106*, 1466; b) K. Stærk, S. Khandige, H. J. Kolmos, J. Møller-Jensen, T. E. Andersen, *J. Infect. Dis.* **2016**, *213*, 386; c) J. J. Boelens, J. Dankert, J. L. Murk, J. J. Weening, T. van der Poll, K. P. Dingemans, L. Koole, J. D. Laman, S. A. J. Zaat, *J. Infect. Dis.* **2000**, *181*, 1337.
- [30] M. Riool, L. de Boer, V. Jaspers, C. M. van der Loos, W. J. B. van Wamel, G. Wu, P. H. S. Kwakman, S. A. J. Zaat, *Acta Biomater.* **2014**, *10*, 5202.
- [31] S. Holt, M. Grant, K. A. Thompson-Brazill, *Crit. Care Nurse.* **2017**, *37*, 78.

- [32] L. W. Sørbye, H. Finne-Soveri, G. Ljunggren, E. Topinková, R. Bernabei, *Age Ageing* **2005**, *34*, 377.
- [33] C. A. M. McNulty, *J. Infect. Prev.* **2009**, *10*, 70.
- [34] European Centre for Disease Prevention and Control (ECDC), *Point Prevalence Survey of Healthcare-Associated Infections and Antimicrobial Use in European Long-Term Care Facilities*. ECDC, Stockholm, Sweden **2013**. <https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/healthcare-associated-infections-point-prevalence-survey-long-term-care-facilities-2013.pdf> (accessed: March 2019).
- [35] R. A. Weinstein, R. O. Darouiche, *Clin. Infect. Dis.* **2001**, *33*, 1567.
- [36] R. Djeribi, W. Bouchloukh, T. Jouenne, B. Menaa, *Am. J. Infect. Control.* **2012**, *40*, 854.
- [37] M. Duvdevani, B. H. Chew, J. D. Denstedt, *Curr. Opin. Urol.* **2006**, *16*, 77.
- [38] L. E. Nicolle, A. C. G. Committee, *Can. J. Infect. Dis. Med. Microbiol.* **2005**, *16*, 349.
- [39] S. E. Staubli, L. Mordasini, D. S. Engeler, R. Sauter, H. P. Schmid, D. Abt, *Urol. Int.* **2016**, *97*, 91.
- [40] a) M. A. Halm, N. O'Connor, *Am. J. Crit. Care* **2014**, *23*, 505; b) M. J. Lobão, P. Sousa, *Acta Médica Portuguesa*. **2017**, *30*, 608.
- [41] L. E. Nicolle, *Antimicrob. Resist. Infect. Control* **2014**, *3*, 23.
- [42] P. Tenke, B. Köves, K. Nagy, S. J. Hultgren, W. Mendling, B. Wullt, M. Grabe, F. M. Wagenlehner, M. Cek, R. Pickard, H. Botto, K. G. Naber, T. E. Bjerklund Johansen, *World J. Urol.* **2012**, *30*, 51.
- [43] a) K. Schumm, T. B. Lam, *Cochrane Database Syst. Rev.* **2008**, CD004013, <https://doi:10.1002/14651858.CD004013.pub3> (accessed: March 2019); b) P. Jahn, K. Beutner, G. Langer, *Cochrane Database Syst. Rev.* **2012**, *10*, CD004997. <https://doi:10.1002/14651858.CD004997.pub3> (accessed: March 2019).
- [44] P. Singha, J. Locklin, H. Handa, *Acta Biomater.* **2017**, *50*, 20.
- [45] M. Riool, A. de Breij, J. W. Drijfhout, P. H. Nibbering, S. A. J. Zaai, *Front. Chem.* **2017**, *5*, 63.
- [46] K. Muramatsu, Y. Fujino, T. Kubo, M. Otani, K. Fushimi, S. Matsuda, *J. Epidemiol.* **2018**, *28*, 54.
- [47] R. Pickard, T. Lam, G. MacLennan, K. Starr, M. Kilonzo, G. McPherson, K. Gillies, A. McDonald, K. Walton, B. Buckley, C. Glazener, C. Boachie, J. Burr, J. Norrie, L. Vale, A. Grant, J. N'Dow, *Lancet.* **2012**, *380*, 1927.
- [48] P. Verleyen, D. De Ridder, H. Van Poppel, L. Baert, *Eur. Urol.* **1999**, *36*, 240.
- [49] N. S. Morris, D. J. Stickler, C. Winters, *BJU Int.* **1997**, *80*, 58.
- [50] a) B. H. Chew, R. F. Paterson, K. W. Clinkscales, B. S. Levine, S. W. Shalaby, D. Lange, *J. Urol.* **2013**, *189*, 719; b) A. A. Barros, A. Rita, C. Duarte, R. A. Pires, B. Sampaio-Marques, P. Ludovico, E. Lima, J. F. Mano, R. L. Reis, *J. Biomed. Mater. Res., Part B* **2015**, *103*, 608.
- [51] B. H. Chew, P. A. Cadieux, G. Reid, J. D. Denstedt, *J. Endourol.* **2006**, *20*, 949.
- [52] P. A. Cadieux, B. H. Chew, B. E. Knudsen, K. Dejong, E. Rowe, G. Reid, J. D. Denstedt, *J. Urol.* **2006**, *175*, 2331.
- [53] P. A. Cadieux, B. H. Chew, L. Nott, S. Seney, C. N. Elwood, G. R. Wignall, L. W. Gonneau, J. D. Denstedt, *J. Endourol.* **2009**, *23*, 1187.
- [54] T. John, A. Rajpurkar, G. Smith, M. Fairfax, J. Triest, *J. Endourol.* **2007**, *21*, 1211.
- [55] M. Multanen, M. Talja, S. Hallanvuori, A. Siitonen, T. Välimaa, T. L. Tammela, J. Seppälä, P. Törmälä, *Urol. Res.* **2000**, *28*, 327.
- [56] M. Multanen, T. L. Tammela, M. Laurila, J. Seppälä, T. Välimaa, P. Törmälä, M. Talja, *Urol. Res.* **2002**, *30*, 227.
- [57] D. Minardi, O. Cirioni, R. Ghiselli, C. Silvestri, F. Mucchegiani, E. Gabrielli, G. d'Anzeo, A. Conti, F. Orlando, M. Rimini, L. Brescini, M. Guerrieri, A. Giacometti, G. Muzzonigro, *J. Surg. Res.* **2012**, *176*, 1.
- [58] M. A. El-Feky, M. S. El-Rehewy, M. A. Hassan, H. A. Abolella, R. M. Abd El-Baky, G. F. Gad, *Pol. J. Microbiol.* **2009**, *58*, 261.
- [59] a) D. Lange, B. H. Chew, *Ther. Adv. Urol.* **2009**, *1*, 143; b) J. D. Denstedt, in *CP900, Renal Stone Disease, 1<sup>st</sup> Annual Int. Urolithiasis Research Symp.* (Eds: A. P. Evan, J. E. Lingeman, J. C. Williams), American Institute of Physics, College Park, MD **2007**, p. 272.
- [60] H. Wu, C. Moser, H. Z. Wang, N. Høiby, Z. J. Song, *Int. J. Oral Sci.* **2015**, *7*, 1.
- [61] F. Cieplik, D. Deng, W. Crielaard, W. Buchalla, E. Hellwig, A. Al-Ahmad, T. Maisch, *Crit. Rev. Microbiol.* **2018**, *1*. <https://doi:10.1080/1040841X.2018.1467876>
- [62] Frost and Sullivan, European market for urological devices, <http://www.frost.com/sublib/display-report.do?searchQuery=european+market+for+urological+devices%2Ceuropean+market+for+urological+devices&ctxixpLink=FcmCtx1&ctxixpLabel=FcmCtx2&id=M5FF-01-00-00-00&bdata=aHR0cHM6Ly93d3cuZnJvc3QuY29tL3NyY2gvY2F0YWxvZy1zZWYyY2guZG8%2FcXVlcnlUZXB0PWF1cm9wZWVhcnR0c3R1dXJvY29naWNoCtkZXXZyY2VzQH5AU2VhcnNoFjI3VsdHNAfkAxNTI5NTA1NTM3NDI4> (accessed: March 2019).
- [63] MarketsandMarkets, Urinary catheters market by product (intermittent, foley, condom), type (coated, uncoated), indication (urinary incontinence, benign prostate hyperplasia, bladder dysfunction, spinal cord injury), gender, end user—global forecast to 2022, <https://www.marketsandmarkets.com/Market-Reports/urinary-catheter-market-132934629.html> (accessed: March 2019).
- [64] a) TechNavio, Global urinary catheters market 2016–2020, <https://www.technavio.com/report/global-urology-devices-global-urinary-catheters-market-2016-2020> (accessed: March 2019); b) Grandview Research, *Urinary catheters market analysis by product, (intermittent catheters, foley/indwelling catheters, external catheters), by application, (benign prostate hyperplasia, urinary incontinence, others), and segment forecasts, 2013–2024*, <https://www.grandviewresearch.com/industry-analysis/urinary-catheters-market> (accessed: March 2019); c) Zion Market Research, *Catheter market by product (cardiovascular, urology, intravenous, neurovascular and specialty catheters)—global industry perspective, comprehensive analysis, and forecast, 2015–2021*, <https://www.zionmarketresearch.com/report/catheter-market> (accessed: March 2019)
- [65] a) T. Wassmann, S. Kreis, M. Behr, R. Buegers, *Int. J. Implant Dent.* **2017**, *3*, 32; b) T. Vladkova, *Int. J. Polym. Sci.* **2010**, 296094.
- [66] F. Poncin-Epaillard, T. Vrlinic, D. Debarnot, M. Mozetic, A. Coudreuse, G. Legeay, B. El Moulaj, W. Zorzi, *J. Funct. Biomater.* **2012**, *3*, 528.
- [67] a) G. Speranza, G. Gottardi, C. Pederzoli, L. Lunelli, R. Canteri, L. Pasquardini, E. Carli, A. Lui, D. Maniglio, M. Brugnara, M. Anderle, *Biomaterials* **2004**, *25*, 2029; b) L. Ploux, A. Ponche, K. Anselme, *J. Adhes. Sci. Technol.* **2010**, *24*, 2165.
- [68] F. Song, H. Koo, D. Ren, *J. Dent. Res.* **2015**, *94*, 1027.
- [69] J. M. Courtney, T. Gilchrist, *Med. Biol. Eng. Comput.* **1980**, *18*, 538.
- [70] A. A. Barros, C. Oliveira, E. Lima, A. R. C. Duarte, K. Healy, R. L. Reis, in *Comprehensive Biomaterials II*, Vol. 7 (Eds: P. Ducheyne, K. Healy, D. W. Huttmacher, D. W. Grainger, C. J. Kirkpatrick), Elsevier **2017**, Ch. 41.
- [71] S. Abramson, H. Alexandre, S. Best, J. C. Bokros, J. B. Brunski, A. Colas, S. L. Cooper, J. Curtis, A. Haubold, L. L. Hench, et al., in *Biomaterials Science* (Eds: B. D. Ratner, A. S. Hoffman, F. J. Schoen, J. E. Lemons), Elsevier Academic Press, New York **2004**, p. 67.
- [72] a) A. Al-Aown, I. Kyriazis, P. Kallidonis, P. Kraniotis, C. Rigopoulos, D. Karnabatidis, T. Petsas, E. Liatsikos, *Ther. Adv. Urol.* **2010**, *2*, 85; b) D. Lange, C. N. Elwood, B. H. Chew, *Biomaterials* **2011**, *24*, 459; c) B. H. Chew, D. Lange, *Nat. Rev. Urol.* **2009**, *6*, 440.
- [73] International Organization for Standardization (ISO), *ISO 10993-18:2005, Biological evaluation of medical devices—Part 18: Chemical characterization of materials*. ISO, Geneva, Switzerland **2005**. <https://www.iso.org/standard/41106.html> (accessed: March 2019).



- [74] ASTM International, *ASTM F2038-00(2011), Standard Guide for Silicone Elastomers, Gels, and Foams Used in Medical Applications—Part I: Formulations and Uncured Materials*. ASTM International, West Conshohocken, PA **2011**. <https://www.astm.org/Standards/F2038.htm> (accessed: March 2019).
- [75] ASTM International, *ASTM F2042-00(2011), Standard Guide for Silicone Elastomers, Gels, and Foams Used in Medical Applications Part II: Crosslinking and Fabrication*. ASTM International, West Conshohocken, PA **2011**. <https://www.astm.org/Standards/F2042.htm> (accessed: March 2019).
- [76] R. Yoda, *J. Biomater. Sci., Polym. Ed.* **1998**, *9*, 561.
- [77] P. Tengvall, in *Comprehensive Biomaterials* (Eds: P. Ducheyne, K. Healy, D. W. Huttmacher, D. W. Grainger, C. J. Kirkpatrick), Elsevier, Oxford, UK **2011**, p. 63.
- [78] A. Ponche, L. Ploux, K. Anselme, *J. Adhes. Sci. Technol.* **2010**, *24*, 2141.
- [79] H. Busscher, H. van der Mei, G. Subbiahdoss, P. Jutte, J. van den Dungen, S. Zaat, M. Schultz, D. Grainger, *Sci. Trans. Med.* **2012**, *4*, 153rv10.
- [80] a) J. M. R. Moreira, L. C. Gomes, K. A. Whitehead, S. Lynch, L. A. Tetlow, F. J. Mergulhão, *Food Bioprod. Process.* **2017**, *104*, 1; b) C. Rodriguez-Emmenegger, A. Decker, F. Surman, C. M. Preuss, Z. Sedlakova, N. Zydziak, C. Barner-Kowollik, T. Schwartz, L. Barner, *RSC Adv.* **2014**, *4*, 64781.
- [81] R. Murga, J. M. Miller, R. M. Donlan, *J. Clin. Microbiol.* **2001**, *39*, 2294.
- [82] M. Loza-Correa, M. Kalab, Q. L. Yi, L. J. Eltringham-Smith, W. P. Sheffield, S. Ramirez-Arcos, *Vox Sang.* **2017**, *112*, 401.
- [83] K. Anselme, L. Ploux, A. Ponche, *J. Adhes. Sci. Technol.* **2010**, *24*, 831.
- [84] a) M. J. Genet, C. C. Dupont-Gillain, P. G. Rouxhet, *XPS Analysis of Biosystems and Biomaterials*, Springer Science+Business Media, New York **2008**; b) J. F. Watts, J. Wolstenholme, *An Introduction to Surface Analysis by XPS and AES*, John Wiley & Sons, Chichester, UK **2003**; c) J. O'Connor, B. Sexton, R. S. C. Smart, *Surface Analysis Methods in Materials Science*, Springer, Berlin, Germany **2003**; d) A. Shchukarev, M. Ramstedt, *Surf. Interface Anal.* **2017**, *49*, 349.
- [85] a) K. B. Alici, I. F. Gallardo, *Sci. Rep.* **2013**, *3*, 2956; b) I. H. Parvez, D. Chapman, *Biopolymers* **1995**, *37*, 251; c) B. Stuart, *Infrared Spectroscopy: Fundamentals and Applications*, John Wiley & Sons, Chichester, UK **2004**.
- [86] T. Moriarty, A. Poulsson, R. ETJ, R. RG, in *Comprehensive Biomaterials*, Vol. 4 (Ed: P. Ducheyne), Elsevier Science, Amsterdam, Netherlands **2011**, p. 75.
- [87] M. V. Graham, N. C. Cady, *Coatings* **2014**, *4*, 37.
- [88] R. S. Friedlander, H. Vlamakis, P. Kim, M. Khan, R. Kolter, J. Aizenberg, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 5624.
- [89] K. Merrett, R. M. Cornelius, W. G. McClung, L. D. Unsworth, H. Sheardown, *J. Biomater. Sci., Polym. Ed.* **2002**, *13*, 593.
- [90] a) D. R. Clarke, *J. Mater. Sci.* **1973**, *8*, 279; b) K. D. Vernon-Parry, *III-Vs Review* **2000**, *13*, 40; c) L. F. Kourkoutis, J. M. Plitzko, W. Baumeister, *Annu. Rev. Mater. Res.* **2012**, *42*, 33; d) K. D. Jandt, *Surf. Sci.* **2001**, *491*, 303.
- [91] W. Wilson, M. Wade, S. Holman, F. Champlin, *J. Microbiol. Methods* **2001**, *43*, 153.
- [92] C. Ayala-Torres, N. Hernández, A. Galeano, L. Novoa-Aponte, C.-Y. Soto, *Ann. Microbiol.* **2014**, *64*, 1189.
- [93] A. M. James, in *Microbial Cell Surface Analysis* (Eds: N. Mozes, P. S. Handley, H. J. Bisscher, P. G. Rouxhet), VCH Publishers, New York **1991**, p. 221.
- [94] R. Xu, *Particuology* **2015**, *18*, 11.
- [95] a) E. Vogler, *Adv. Colloid Interface Sci.* **1998**, *74*, 69; b) R. J. Good, *J. Adhes. Sci. Technol.* **1992**, *6*, 1269; c) G. Bruinsma, H. van der Mei, H. Busscher, *Biomaterials* **2001**, *22*, 3217; d) N. Boks, W. Norde, H. van der Mei, H. Busscher, *Microbiology* **2008**, *154*, 3122.
- [96] N. Gusnaniar, H. C. van der Mei, W. Qu, T. Nuryastuti, J. M. M. Hooymans, J. Sjollem, H. J. Busscher, *Adv. Colloid Interface Sci.* **2017**, *250*, 15.
- [97] a) A. Marmur, *Soft Matter*. **2013**, *9*, 7900; b) A. Marmur, *Annu. Rev. Mater. Res.* **2009**, *39*, 473.
- [98] a) C. J. van Oss, *Colloids Surf., B* **2007**, *54*, 2; b) C. J. Van Oss, M. K. Chaudhury, R. J. Good, *Chem. Rev.* **1988**, *88*, 927; c) H. Radelczuk, L. Holysz, E. Chibowski, *J. Adhes. Sci. Technol.* **2002**, *16*, 1547.
- [99] C. M. Hansen, *Hansen Solubility Parameters: A User's Handbook*, CRC Press, London, UK **2007**.
- [100] H. van der Mei, R. Bos, H. Busscher, *Colloids Surf., B* **1998**, *11*, 213.
- [101] D. Akuzov, T. Vladkova, G. Zamfirova, V. Gaydarov, M. V. Nascimento, N. Szeglat, I. Grunwald, *Prog. Org. Coat.* **2017**, *103*, 126.
- [102] a) C. A. Schuh, *Mater. Today*. **2006**, *9*, 32; b) D. Lin, E. Dimitriadis, F. Horkay, *eXPRESS Polym. Lett.* **2007**, *1*, 576; c) J. Vlassak, W. Nix, *J. Mech. Phys. Solids* **1994**, *42*, 1223.
- [103] a) M. L. B. Palacio, B. Bhushan, *Mater. Charact.* **2013**, *78*, 1; b) H. Nili, K. Kalantar-zadeh, M. Bhaskaran, S. Sriram, *Prog. Mater. Sci.* **2013**, *58*, 1.
- [104] International Organization for Standardization (ISO), *ISO/DTS 19278, Plastics: Instrumented Micro-indentation Test for Hardness Measurement of Plastics Materials*. ISO, Geneva, Switzerland **2018**. <https://www.iso.org/standard/64253.html> (accessed: March 2019).
- [105] a) ASTM, *ASTM D395: 16e1, Standard Test Methods for Rubber Property—Compression Set*. ASTM International, West Conshohocken, PA **2016**. <https://www.astm.org/Standards/D395.htm> (accessed: March 2019); b) International Organization for Standardization (ISO), *ISO 37:2017, Rubber, Vulcanized or Thermoplastic: Determination of Tensile Stress–Strain Properties*. ISO, Geneva, Switzerland **2017**. <https://www.iso.org/standard/68116.html> (accessed: March 2019); c) International Organization for Standardization (ISO), *ISO 7619-1:2010, Rubber, Vulcanized or Thermoplastic—Determination of Indentation Hardness—Part 1: Durometer Method (Shore Hardness)*. ISO, Geneva, Switzerland **2010**. <https://www.iso.org/standard/50756.html> (accessed: March 2019); d) International Organization for Standardization (ISO), *ISO 527-1:2012, Plastics—Determination of Tensile properties—Part 1: General Principles*. ISO, Geneva, Switzerland **2012**. <https://www.iso.org/standard/56045.html> (accessed: March 2019); e) International Organization for Standardization (ISO), *ISO 527-2:2012, Plastics—Determination of Tensile Properties—Part 2: Test Conditions for Moulding and Extrusion Plastics*. ISO, Geneva, Switzerland **2012**. <https://www.iso.org/standard/56046.html> (accessed: March 2019); f) International Organization for Standardization (ISO), *ISO 868:2003, Plastics and Ebonite—Determination of Indentation Hardness by Means of a Durometer (Shore Hardness)*. ISO, Geneva, Switzerland **2003**. <https://www.iso.org/standard/34804.html> (accessed: March 2019); g) International Organization for Standardization (ISO), *ISO 604:2002, Plastics—Determination of Compressive Properties*. ISO, Geneva, Switzerland **2002**. <https://www.iso.org/standard/31261.html> (accessed: March 2019).
- [106] a) International Organization for Standardization (ISO), *ISO 14630, Non-active Surgical Implants: General Requirements*. ISO, Geneva, Switzerland **2012**. <https://www.en-standard.eu/iso-14630-non-active-surgical-implants-general-requirements/> (accessed: March 2019); b) International Organization for Standardization (ISO), *ISO 20696, Sterile Urethral Catheters for Single Use*. ISO, Geneva, Switzerland **2018**. <https://www.en-standard.eu/iso-20696-sterile-urethral-catheters-for-single-use/> (accessed: March 2019); c) Dansk Standard, *DS/EN ISO 20696:2018 Sterile urinrørskatetre til engangsbrug*. Fonden Dansk Standard, Nordhavn, Denmark **2018**. <https://webshop.ds.dk/Default.aspx?ID=120&q=ISO+20696> (accessed: March 2019); d) European Standards, *EN 1616 Sterile*

- Urethral Catheters for Single Use*. European Standards, Pilsen, Czech Republic **1997**. <https://www.en-standard.eu/csn-en-iso-20696-sterile-urethral-catheters-for-single-use/> (accessed: March 2019); e) ASTM, ASTM F1828-97, *Standard Specification for Ureteral Stents*. ASTM International, West Conshohocken, PA **1997**. <https://www.astm.org/Standards/F1828.htm> (accessed: March 2019); f) ASTM, ASTM F623-99(2013), *Standard Performance Specification for Foley Catheter*. ASTM International, West Conshohocken, PA **2013**. <https://www.astm.org/Standards/F623.htm> (accessed: March 2019); g) ASTM, ASTM F1828-17, *Standard Specification for Ureteral Stents*. ASTM International, West Conshohocken, PA **2017**. <https://www.astm.org/Standards/F1828.htm> (accessed: March 2019).
- [107] International Organization for Standardization (ISO), *ISO 10993-16:2017, Biological Evaluation of Medical Devices—Part 16: Toxicokinetic Study Design for Degradation Products and Leachables*. ISO, Geneva, Switzerland **2017**. <https://www.iso.org/standard/64582.html> (accessed: March 2019).
- [108] J. G. Shenderovich, B. Zaks, D. Kirmayer, E. Lavy, D. Steinberg, M. Friedman, *Eur. J. Pharm. Sci.* **2018**, 112, 1.
- [109] Food and Drug Administration (FDA), *Analytical Procedures and Methods Validation for Drugs and Biologics: Guidance for Industry* [U.S. Department of Health and Human Services], <https://www.fda.gov/downloads/drugs/guidances/ucm386366.pdf> (accessed: March 2019).
- [110] Particle Sciences, *Combination Devices: Particle Sciences—Technical Brief*, <http://www.particlesciences.com/news/technical-briefs/2010/combination-devices.html> (accessed: March 2019).
- [111] a) *Strategies to Modify the Drug Release from Pharmaceutical Systems* (Ed: M. L. Bruschi), Woodhead Publishing, Cambridge, UK **2015**, p. 63; b) A. M. Lowman, N. A. Peppas, in *Encyclopedia of Controlled Drug Delivery* (Ed: E. Mathiowitz), John Wiley & Sons **1999**, p. 397.
- [112] R. K. Malcolm, S. D. McCullagh, A. D. Woolfson, S. P. Gorman, D. S. Jones, J. Cuddy, *J. Controlled Release* **2004**, 97, 313.
- [113] N. Fong, L. A. Poole, Warren, A. Simmons, *J. Biomed. Mater. Res., Part B* **2013**, 101B, 310.
- [114] N. J. Irwin, C. P. McCoy, D. S. Jones, S. P. Gorman, *Pharm. Res.* **2013**, 30, 857.
- [115] International Organization for Standardization (ISO), *ISO 10993-1:2009. Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing Within a Risk Management Process*. ISO, Geneva, Switzerland **2009**. <https://www.iso.org/standard/44908.html> (accessed: March 2019).
- [116] International Organization for Standardization (ISO), *ISO 10993-3:2014. Biological Evaluation of Medical Devices—Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity*. ISO, Geneva, Switzerland **2014**. <https://www.iso.org/standard/55614.html> (accessed: March 2019).
- [117] International Organization for Standardization (ISO), *ISO 10993-5:2009. Biological Evaluation of Medical Devices—Part 5: Tests for In Vitro Cytotoxicity*. ISO, Geneva, Switzerland **2009**. <https://www.iso.org/standard/36406.html> (accessed: March 2019).
- [118] International Organization for Standardization (ISO), *ISO 10993-10:2013. Biological Evaluation of Medical Devices—Part 10: Tests for Irritation And Skin Sensitization*. ISO, Geneva, Switzerland **2013**. <https://www.iso.org/standard/40884.html> (accessed: March 2019).
- [119] International Organization for Standardization (ISO), *ISO 10993-12:2012. Biological Evaluation of Medical Devices—Part 12: Sample Preparation and Reference Materials*. ISO, Geneva, Switzerland **2012**. <https://www.iso.org/standard/53468.html> (accessed: March 2019).
- [120] a) M. Ramstedt, B. Ekstrand-Hammarstrom, A. V. Shchukarev, A. Bucht, L. Osterlund, M. Welch, W. T. S. Huck, *Biomaterials* **2009**, 30, 1524; b) E. Hidalgo, C. Domínguez, *Toxicol. Lett.* **1998**, 98, 169.
- [121] J. Sjollem, S. A. J. Zaat, V. Fontaine, M. Ramstedt, R. Luginbuehl, K. Thevissen, J. Li, H. C. van der Mei, H. J. Busscher, *Acta Biomater.* **2018**, 70, 12.
- [122] L. Vroman, *Colloids Surf., B* **2008**, 62, 1.
- [123] W. C. de Groat, *Br. J. Pharmacol.* **2006**, 147, S25.
- [124] a) P. I. Altman, in *Blood and Other Body Fluids* (Ed: D. L. Dittmer), Federation of American Societies for Experimental Biology, Washington, DC **1961**; b) Guyton, A. C., *Textbook of Medical Physiology*, WB Saunders, Philadelphia, PA **1981**.
- [125] D. S. Ipe, E. Horton, G. C. Ulett, *Front. Cell. Infect. Microbiol.* **2016**, 6, 14.
- [126] T. Brooks, C. W. Keevil, *Lett. Appl. Microbiol.* **1997**, 24, 203.
- [127] a) C. J. Alteri, H. L. Mobley, *Infect. Immun.* **2007**, 75, 2679; b) F. Toval, C. D. Köhler, U. Vogel, F. Wagenlehner, A. Mellmann, A. Fruth, M. A. Schmidt, H. Karch, M. Bielaszewska, U. Dobrindt, *J. Clin. Microbiol.* **2014**, 52, 407.
- [128] European Standard, *DIN EN 1616, Sterile Urethral Catheters for Single Use (Includes Amendment A1:1999)*. European Standard, Pilsen, Czech Republic **1999**.
- [129] a) J. Benton, J. Chawla, S. Parry, D. Stickler, *J. Hosp. Infect.* **1992**, 22, 117; b) L. Moulton, M. Lachiewicz, X. Liu, O. Goje, *J. Matern. Fetal Neonatal. Med.* **2018**, 31, 395.
- [130] a) R. Ruhai, H. Antti, O. Rzhapishevskaya, N. Boulanger, D. R. Barbero, S. N. Wai, B. E. Uhlin, M. Ramstedt, *Colloids Surf., B* **2015**, 127, 182; b) A. Roosjen, H. J. Busscher, W. Nordel, H. C. van der Mei, *Microbiology* **2006**, 152, 2673; c) O. Rzhapishevskaya, N. Limanska, M. Galkin, A. Lacoma, M. Lundquist, D. Sokol, S. Hakobyan, A. Sjöstedt, C. Prat, M. Ramstedt, *Acta Biomater.* **2018**, 76, 99.
- [131] O. Rzhapishevskaya, S. Hakobyan, R. Ruhai, J. Gautrot, D. Barbero, M. Ramstedt, *Biomater. Sci.* **2013**, 1, 589.
- [132] P. Behzadi, E. Behzadi, H. Yazdanbod, R. Aghapour, M. A. Cheshmeh, D. S. Omran, *Maedica* **2010**, 5, 277.
- [133] M. Mishra, S. Agrawal, S. Raut, A. M. Kurhade, R. M. Powar, *J Clin Diagn Res.* **2014**, 8, 44.
- [134] National Nosocomial Infections Surveillance, *Am. J. Infect. Control* **1996**, 24, 380.
- [135] M. S. Kabbani, S. R. Ismail, A. Fatima, R. Shafi, J. A. Idris, A. Mehmood, R. K. Singh, M. Elbaraby, O. Hijazi, M. A. Hussein, *J. Infect. Public Health* **2016**, 9, 600.
- [136] a) D. J. Stickler, S. M. Jones, G. O. Adusei, M. G. Waters, *J. Clin. Microbiol.* **2006**, 44, 1540; b) S. Milo, N. T. Thet, D. Liu, J. Nzakizwanayo, B. V. Jones, A. T. A. Jenkins, *Biosens. Bioelectron.* **2016**, 81, 166.
- [137] C. L. Clayton, J. C. Chawla, D. J. Stickler, *J. Hosp. Infect.* **1982**, 3, 39.
- [138] A. Alastruey-Izquierdo, M. S. C. Melhem, L. X. Bonfietti, J. L. Rodriguez-Tudela, *Rev. Inst. Med. Trop. Sao Paulo* **2015**, 57, 57.
- [139] a) T. Monsen, E. Lövgren, M. Widerström, L. Wallinder, *J. Clin. Microbiol.* **2009**, 47, 2496; b) E. Joyce, A. Al-Hashimi, T. J. Mason, *J. Appl. Microbiol.* **2011**, 110, 862.
- [140] a) D. J. Stickler, *Nat. Clin. Pract. Urol.* **2008**, 5, 598; b) V. Hola, F. Ruzicka, M. Horka, *FEMS Immunol. Med. Microbiol.* **2010**, 59, 525.
- [141] a) M. Burmølle, D. Ren, T. Bjarnsholt, S. J. Sørensen, *Trends Microbiol.* **2014**, 22, 84; b) J. S. Madsen, S. J. Sørensen, M. Burmølle, *Curr. Opin. Microbiol.* **2018**, 42, 104.
- [142] a) I. Pastar, A. G. Nusbaum, J. Gil, S. B. Patel, J. Chen, J. Valdes, O. Stojadinovic, L. R. Plano, M. Tomic-Canic, S. C. Davis, *PLoS One* **2013**, 8, e56846; b) K. W. Lee, S. Periasamy, M. Mukherjee, C. Xie, S. Kjelleberg, S. A. Rice, *ISME J.* **2014**, 8, 894.
- [143] E. M. Galván, C. Mateyca, L. Ielpi, *Biofouling* **2016**, 32, 1067.
- [144] J. Wang, B. Foxman, L. Mody, E. S. Snitkin, *Proc. Natl. Acad. Sci. USA* **2017**, 114, 10467.





- [145] B. M. Peters, M. A. Jabra-Rizk, M. A. Scheper, J. G. Leid, J. W. Costerton, M. E. Shirtliff, *FEMS Immunol. Med. Microbiol.* **2010**, *59*, 493.
- [146] a) P. Wu, D. W. Grainger, *Biomaterials* **2006**, *27*, 2450; b) R. G. Richards, T. F. Moriarty, T. Miclau, R. T. McClellan, D. W. Grainger, *J. Orthop. Trauma* **2012**, *26*, 703.
- [147] G. Viridi, D. Hendry, *Curr. Urol.* **2015**, *9*, 28.
- [148] N. A. Sabbuba, D. J. Stickler, M. J. Long, Z. Dong, T. D. Short, R. J. Feneley, *J. Urol.* **2005**, *173*, 262.
- [149] a) C. Wilson, S. S. Sandhu, A. V. Kaisary, *BJU Int.* **1997**, *80*, 915; b) K. German, P. Rowley, D. Stone, U. Kumar, H. N. Blackford, *BJU Int.* **1997**, *79*, 96.
- [150] P. Kristiansen, R. Pompeius, L. B. Wadström, *NeuroUrol. Urodyn.* **1983**, *2*, 135.
- [151] C. Rose, A. Parker, B. Jefferson, E. Cartmell, *Crit. Rev. Environ. Sci. Technol.* **2015**, *45*, 1827.
- [152] M. van de Lagemaat, A. Grotenhuis, B. van de Belt-Gritter, S. Roest, T. J. A. Loontjens, H. J. Busscher, H. C. van der Mei, Y. Ren, *Acta Biomater.* **2017**, *59*, 139.
- [153] a) B. Purevdorj, J. W. Costerton, P. Stoodley, *Appl. Environ. Microbiol.* **2002**, *68*, 4457; b) M. J. Vieira, L. F. Melo, M. M. Pinheiro, *Biofouling* **1993**, *7*, 67; c) L. Hall-Stoodley, P. Stoodley, *Curr. Opin. Biotechnol.* **2002**, *13*, 228; d) P. Stoodley, I. Dodds, J. D. Boyle, H. M. Lappin-Scott, *J. Appl. Microbiol.* **1998**, *85*, 19S; e) Y. P. Tsai, *Biofouling* **2005**, *21*, 267.
- [154] M. O. Pereira, M. Kuehn, S. Wuertz, T. Neu, L. F. Melo, *Biotechnol. Bioeng.* **2002**, *78*, 164.
- [155] M. Ferreira, O. Rzhepishevskaya, L. Grenho, D. Malheiros, L. Goncalves, A. J. Almeida, L. Jordao, I. A. Ribeiro, M. Ramstedt, P. Gomes, A. Bettencourt, *Int. J. Pharm.* **2017**, *532*, 241.
- [156] a) J. M. R. Moreira, J. S. Teodósio, F. C. Silva, M. Simões, L. F. Melo, F. J. Mergulhão, *Bioprocess Biosyst. Eng.* **2013**, *36*, 1787; b) J. M. R. Moreira, M. Simões, L. F. Melo, F. J. Mergulhão, *Colloid Polym. Sci.* **2015**, *293*, 177.
- [157] I. B. Gomes, A. Meireles, A. L. Gonçalves, D. M. Goeres, J. Sjollem, L. C. Simões, M. Simões, *Crit. Rev. Biotechnol.* **2018**, *38*, 657.
- [158] J. M. R. Moreira, J. Ponmozhi, J. B. L. M. Campos, J. M. Miranda, F. J. Mergulhão, *Chem. Eng. Sci.* **2015**, *126*, 440.
- [159] C. K. Hope, M. Wilson, *J. Microbiol. Methods* **2006**, *66*, 390.
- [160] A. C. Pavarina, L. N. Dovigo, P. V. Sanità, A. L. Machado, E. T. Giampaolo, C. E. Vergani, in *Biofilms: Formation, Development and Properties* (Ed: W. C. Bailey), Nova Science Publishers, New York **2011**, p. 125.
- [161] L. E. Fisher, A. L. Hook, W. Ashraf, A. Yousef, D. A. Barrett, D. J. Scurr, X. Chen, E. F. Smith, M. Fay, C. D. J. Parmenter, R. Parkinson, R. Bayston, *J. Controlled Release* **2015**, *202*, 57.
- [162] R. Y. R. Chua, K. Lim, S. S. J. Leong, P. A. Tambyah, B. Ho, *J. Hosp. Infect.* **2017**, *97*, 66.
- [163] D. J. Stickler, N. S. Howe, C. Winters, *Cells Mater.* **1994**, *4*, 387.
- [164] J. Azeredo, N. F. Azevedo, R. Briandet, N. Cerca, T. Coenye, A. R. Costa, M. Desvaux, G. Di Bonaventura, M. Hébraud, Z. Jaglic, M. Kačaniová, S. Knöchel, A. Lourenço, F. Mergulhão, R. L. Meyer, G. Nychas, M. Simões, O. Tresse, C. Sternberg, *Crit. Rev. Microbiol.* **2017**, *43*, 313.
- [165] W. F. McCoy, J. D. Bryers, J. Robbins, J. W. Costerton, *Can. J. Microbiol.* **1981**, *27*, 910.
- [166] J. S. Teodósio, F. C. Silva, J. M. Moreira, M. Simões, L. F. Melo, M. A. Alves, F. J. Mergulhão, *Biofouling* **2013**, *29*, 953.
- [167] M. O. Pereira, M. J. Vieira, L. F. Melo, *Water Environ. Res.* **2002**, *74*, 7.
- [168] J. S. Teodósio, M. Simões, L. F. Melo, F. J. Mergulhão, *Biofouling* **2011**, *27*, 1.
- [169] D. P. Bakker, A. van der Plaats, G. J. Verkerke, H. J. Busscher, H. C. van der Mei, *Appl. Environ. Microbiol.* **2003**, *69*, 6280.
- [170] A. S. Azevedo, C. Almeida, L. C. Gomes, C. Ferreira, F. J. Mergulhão, L. F. Melo, N. F. Azevedo, *Biochem. Eng. J.* **2017**, *118*, 64.
- [171] D. M. Goeres, L. R. Loetterle, M. A. Hamilton, R. Murga, D. W. Kirby, R. M. Donlan, *Microbiology* **2005**, *151*, 757.
- [172] A. C. Pavarina, L. N. Dovigo, P. V. Sanità, A. L. Machado, E. T. Giampaolo, C. E. Vergani, in *Biofilms: Formation, Development and Properties* (Ed: W. C. Bailey), Nova Science Publishers, New York **2011**, p. 125.
- [173] J. M. Moreira, J. D. Araújo, J. M. Miranda, M. Simões, L. F. Melo, F. J. Mergulhão, *Colloids Surf., B* **2014**, *123*, 1.
- [174] Stoodley, P., B. K. Warwood, in *Biofilms in Medicine, Industry and Environmental Biotechnology: Characteristics, Analysis and Control* (Eds: P. Lens, V. O'Flaherty, A. P. Moran, P. Stoodley, T. Mahony), IWA Publishing, Cornwall, UK **2003**, p. 197.
- [175] a) M. M. Velraeds, B. van de Belt-Gritter, H. C. van der Mei, G. Reid, H. J. Busscher, *J. Med. Microbiol.* **1998**, *47*, 1081; b) L. C. Gomes, J. M. Moreira, J. S. Teodósio, J. D. Araújo, J. M. Miranda, M. Simões, L. F. Melo, F. J. Mergulhão, *Biofouling* **2014**, *30*, 535.
- [176] H. Busscher, H. van der Mei, *Clin. Microbiol. Rev.* **2006**, *19*, 127.
- [177] M. Magana, C. Sereti, A. Ioannidis, C. A. Mitchell, A. R. Ball, E. Magiorkinis, S. Chatzipanagiotou, M. R. Hamblin, M. Hadjifrangiskou, G. P. Tegos, *Clin. Microbiol. Rev.* **2018**, *31*, e00084-16.
- [178] a) M. Wagner, H. Horn, *Biotechnol. Bioeng.* **2017**, *114*, 1386; b) K. W. Kim, *J. Microbiol.* **2016**, *54*, 703.
- [179] a) H. Frickmann, A. E. Zautner, A. Moter, J. Kikhney, R. M. Hagen, H. Stender, S. Poppert, *Crit. Rev. Microbiol.* **2017**, *43*, 263; b) K. Lagree, J. V. Desai, J. S. Finkel, F. Lanni, *Curr. Opin. Microbiol.* **2018**, *43*, 100; c) A. Moter, U. B. Göbel, *J. Microbiol. Methods* **2000**, *41*, 85.
- [180] S. Schlafer, R. L. Meyer, *J. Microbiol. Methods* **2017**, *138*, 50.
- [181] International Organization for Standardization (ISO), *ISO 10993-2:2006, Biological Evaluation of Medical Devices—Part 2: Animal Welfare Requirements*. ISO, Geneva, Switzerland **2010**. <https://www.iso.org/standard/36405.html> (accessed: March 2019).
- [182] M. Riool, A. J. Dirks, V. Jaspers, L. de Boer, T. J. Loontjens, C. M. van der Loos, S. Florquin, I. Apachitei, L. N. Rijk, H. A. Keul, S. A. Zaai, *Eur. Cells Mater.* **2017**, *33*, 143.
- [183] A. Waldhuber, M. Puthia, A. Wieser, C. Cirl, S. Dürr, S. Neumann-Pfeifer, S. Albrecht, F. Römmeler, T. Müller, Y. Zheng, S. Schubert, O. Groß, C. Svanborg, T. Miethke, *J. Clin. Invest.* **2016**, *126*, 2425.
- [184] C. Brown, *Lab Anim.* **2011**, *40*, 111.
- [185] N. A. Petersen, T. S. Kastberg, C. A. Asferg, H. J. Kolmos, T. E. Andersen, L. Lund, presented at *Danish Urological Society Autumn Meeting*, Kolding, Denmark, November 2017.
- [186] T. Coenye, H. J. Nelis, *J. Microbiol. Methods* **2010**, *83*, 89.
- [187] D. Lebeaux, A. Chauhan, O. Rendueles, C. Beloin, *Pathogens* **2013**, *2*, 288.
- [188] J. E. Nett, E. G. Brooks, J. Cabezas-Olcoz, H. Sanchez, R. Zarnowski, K. Marchillo, D. R. Andes, *Infect. Immun.* **2014**, *82*, 4931.
- [189] X. Wang, B. C. Fries, *J. Med. Microbiol.* **2011**, *60*, 1523.
- [190] a) C. E. Armbruster, S. N. Smith, A. Yep, H. L. T. Mobley, *J. Infect. Dis.* **2014**, *209*, 1524; b) W. H. Tay, K. K. L. Chong, K. A. Kline, *J. Mol. Biol.* **2016**, *428*, 3355.
- [191] K. R. Allison, M. P. Brynildsen, J. J. Collins, *Nature* **2011**, *473*, 216.
- [192] a) P. S. Guiton, C. S. Hung, L. E. Hancock, M. G. Caparon, S. J. Hultgren, *Infect. Immun.* **2010**, *78*, 4166; b) J. L. Kadurugamuwa,



- K. Modi, J. Yu, K. P. Francis, T. Purchio, P. R. Contag, *Infect. Immun.* **2005**, *73*, 3878.
- [193] M. Haraoka, T. Matsumoto, K. Takahashi, S. Kubo, M. Tanaka, J. Kumazawa, *Urol. Res.* **1995**, *22*, 383.
- [194] O. Cirioni, R. Ghiselli, C. Silvestri, D. Minardi, E. Gabrielli, F. Orlando, M. Rimini, L. Brescini, G. Muzzonigro, M. Guerrieri, A. Giacometti, *J. Antimicrob. Chemother.* **2011**, *66*, 1318.
- [195] Y. Kurosaka, Y. Ishida, E. Yamamura, H. Takase, T. Otani, H. Kumon, *Microbiol. Immunol.* **2001**, *45*, 9.
- [196] L. C. Fung, M. W. Mittelman, P. S. Thorner, A. E. Khoury, *Can J Urol.* **2003**, *10*, 2007.
- [197] a) M. E. Olson, J. C. Nickel, A. E. Khoury, D. W. Morck, R. Cleeland, J. W. Costerton, *J. Infect. Dis.* **1989**, *159*, 1065; b) R. Hachem, R. Reitzel, A. Borne, Y. Jiang, P. Tinkey, R. Uthamanthil, J. Chandra, M. Ghannoum, I. Raad, *Antimicrob. Agents Chemother.* **2009**, *53*, 5145; c) Z. Hazan, J. Zumeris, H. Jacob, H. Raskin, G. Kratysh, M. Vishnia, N. Dror, T. Barliya, M. Mandel, G. Lavie, *Antimicrob. Agents Chemother.* **2006**, *50*, 4144.