Review Article

In vitro dynamic bladder models for studying urinary tract infections: a narrative review

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Abstract: Experimental models of the bladder are key to studying the pathogenic mechanism of catheter-related bacterial biofilm infection. Although numerous studies have reported multiple models, these model designs were heterogeneous. This study aimed to review the status quo and explore the problems associated with *in vitro* dynamic bladder models for studying urinary tract infections (UTIs). The PubMed and SinoMed databases were searched from their inception to February 2020. Studies regarding *in vitro* bladder models related to UTIs were reviewed based on a bibliometric evaluation of their basic characteristics and model analysis. A total of 74 papers and 44 bladder models were included in this study. The results were as follows: (I) urine transmission devices: 10 studies applied the gravity effect of culture media, while the others used peristaltic pumps, and 11 of them combined stirring or rotating forces. The flow rates in all studies ranged from 15 μL/min to 50 mL/min. (II) Bladder model: two studies reported on simulating the bladder using plastic bags, while the others reported on glass cylinders or fermenters with a capacity of 200 to 700 mL. *E. coli* and *P. mirabilis* were the main bacterial strains. (III) Infection carrier: six studies reported planktonic bacteria as their infection carrier, while 45 studies reported silica gel, rubber, polyurethane, silicone, polytetrafluoroethylene, or perfusion bag. (IV) Infection medium: 25 studies reported the culture medium. Thirty-two studies reported artificial urine, while 17 studies reported human urine. (V) Research analysis: 45 studies investigated the bacterial biofilm formation in the bladder model. Thirty-six studies compared the effects of various drug coatings, diverse material surfaces, or different materials. Only five studies compared distinct bladder models. The included studies' main defects were the single simulation of bladder urodynamics, divers parameter settings, and non-standard experimental modeling. Our analysis showed for the first time that *in vitro* dynamic bladder models could provide new ideas for exploring the mechanism and prevention of bacterial biofilm infection in urinary implanted biomaterials. Due to the limitations of the included studies, more high-quality studies are needed to verify the conclusions above further.

Keywords: Bacterial biofilm infection; *in vitro* techniques; models; biological; systematic review; urinary bladder

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Introduction

Urinary tract infection (UTI) is the most common hospital-related infection, and 70–80% of these infections are associated with the use of a urethral catheter. The crucial part of UTIs' pathogenesis is the formation of bacterial biofilm on the surface of biomaterials, which can lead to refractory biofilm resistance and immune damage (1-4). In other words, the bacterial biofilm is considered the key to biomaterial-related infections, although its mechanism of formation is still unclear (5). The discovery of mechanisms for the prevention and control of biofilm infection has become the primary research hotspot. In recent years, the study of biofilm infection from the perspective of fluid mechanical biology provided a new research direction. Numerous studies have been conducted on static planktonic bacteria. However, the materials for urinary tract implantation relating to the biofilm bacterial infection are exposed to a complex dynamic urinary flow environment. From the perspective of mechanical biology, organisms are affected by the mechanical environment since mechanical factors act on the entire body and organs' biological processes, including positive functional adaptation reconstruction, negative structural damage, and disease occurrence. At present, a variety of successful bacterial biofilm models have been reported. As far as *in vitro* research is concerned, the design of experimental models that closely reflect actual human bladder urodynamic conditions is key to studying the pathogenic mechanism of catheter-related bacterial biofilm infection, and a new anti-biofilm infection technology is required. However, previous studies' bladder models differed in their ability to simulate human urinary flow effectively. In particular, the hydrodynamics status and standardization were inconsistent.

This review systematically evaluated the current research status and investigated the problems associated with *in vitro* dynamic bladder models for studying UTIs. We strongly believe that this research can provide a valuable reference for constructing *in vitro* models of UTIs. This study may also offer some insight into the mechanism and prevention of catheter-related complicated UTIs. We present the following article following the Narrative review checklist (available at http://dx.doi.org/10.21037/apm-20-2061).

Methods

Research selection

Inclusion and exclusion criteria

All previous studies that were based on an *in vitro* bladder model related to human UTI were included in this review. The selected research articles varied in the study's model and design, and language preference was given to studies published in Chinese and English only. Studies with incomplete data, repeated publications, *in vivo* animal experiments and human studies, and those without a bladder model as the research topic were excluded from this study.

Literature retrieval

The PubMed and SinoMed databases were electronically searched from the date of inception of the database to February 2020. The references of relevant studies were also searched. Free words were used for retrieval; for example, *in vitro*, *ex vivo*, bladder, and model.

Literature evaluation and analysis

Literature selection was conducted by reading the articles' titles and abstracts, and unrelated articles were excluded. Furthermore, the full texts of the articles were thoroughly checked to determine the relevance of the research. Inconsistencies were resolved by negotiation. At present, there is no authoritative quality evaluation tool for *in vitro* research (6-9), although some scholars suggest the OHAT risk-of-bias tool and Toxicological data reliability assessment tool (ToxRTool) (10-12). However, these methods are mainly used to evaluate the quality of animal research, and therefore, were unfit for the present research purpose. For this reason, a quality assessment of the included studies was not performed. The selected articles were classified and analyzed according to bibliometrics analysis, bladder model, model bacteria, infection medium, and carrier.

Discussion

Basic characteristics of included studies

We obtained 1,850 articles (1,776 in English and 174 in Chinese) and 22 references. Two reviewers (Guo-Bing Xiong and Ai-Bo Liu) independently read the titles

and abstracts of the studies. In total, 120 articles [112 in English (including 22 references), two in Japanese, and six in Chinese] were included in the preliminary screening. Finally, 74 articles published in English, including 14 articles obtained by searching the reference lists of relevant articles were included in this study. The basic characteristics of the 74 articles are summarized in https://cdn.amegroups.cn/static/public/apm-20-2061-1.pdf. The selected 74 studies were published between 1966 and 2020, and the decade distribution of studies as follow: 23 studies reported 2011–2020 (13-35), 10 studies reported 2001–2010 (36-45), 17 studies reported 1991–2000 (46-62), 10 studies reported 1981–1990 (63-72), 13 studies reported 1971–1980 (73-85), only one study reported <1971 (86).

Model construction and analysis

According to the human urinary bladder and urine flow, i.e., continuous urine secretion, one-way flow from the kidney, ureter, and bladder to the urethra, cyclical bladder urine storage, and intermittent urination, we summarized the key components of the models into four categories. These included the devices for bacterial culture medium storage and collection of waste liquid, the power devices for urinary flow transmission (gravity, pump, combined agitation, or rotation to the latter), bladder models (combined with infection carrier), and the input and output pathways of the culture medium. All of the equipment was assembled to simulate the human urinary bladder system.

Some of the selected literature established *in vitro* bladder models based on the same models or with some modifications [two bladder models (13,15) were modified from the model reported by Abbott *et al.* (21); eight bladder models (16,17,19,30,32,33,35,38) were modified from the model reported by Stickler *et al.* (49); two bladder models (32,39) were modified from the model reported by Gaonkar *et al.* (43); one bladder model (28) was modified from the model reported by Fu *et al.* (36); two bladder models (31,34) was modified from the model reported by Andersen *et al.* (37); one bladder model (45) was modified from the model reported by Stickler *et al.* (55); one bladder model (48) was modified from the model reported by Getliffe *et al.* (59); six bladder models (19,41,50-53) were modified from the model reported by Stickler *et al.* (58); one bladder model (62) was modified from the model reported by Stickler *et al.* (64); 11 bladder models (63,65-70,72,73,75,76) were modified from the model reported by Greenwood *et al.* (77); one bladder model (75) was modified from the model reported by Greenwood *et al.* (78); seven bladder models (79-85) were modified from the model reported by O'Grady *et al.* (86)]. Hence, among the 74 studies, a total of 44 *in vitro* bladder models were finally included.

Components of the model and its analysis

The power devices of urinary flow

Ten studies (14,22,39,42,43,50,51,53,56,74) used the culture medium's gravity, while the others applied peristaltic pumps to exert power for fluid transmission. Of these, 11 studies (13,21-23,44,68,69,72,78,85,86) were combined with stirring or rotating instruments and were set to a certain flow rate (15 μL/min to 50 mL/min). Meanwhile, 18 studies (13,15,16,21,23,24,30,53,57,60,61,68,69,70-73,75) established the parameters of residual urine and micturition to simulate the clinical physiology or pathological urination and urine retention, so that the urine flow modes of the models more closely reflected the unsteady flow stress conditions of human bladder urine environments.

We have summarized the power apparatuses of urinary flow in the selected bladder models on human urinary flow's anatomy and physiology, which were the key technologies used to construct the models. These involved the pump, fluid gravity, or incorporated stirring or rotating devices into the models to provide urine transmission and urination power.

Bladder model

Only two studies (14,42) simulated the bladder using plastic bags, while the remaining studies used glass bottles or cylinders with a heterogeneous capacity of 200 to 700 mL. From a biomechanical standpoint, bladder tissue's elemental mechanical properties include elasticity, viscoelasticity, and plastic deformations (87,88). As a soft biological shell, the human bladder is considered to be a viscoelastic material in biomechanics; the flow pattern of the urine in the bladder is neither a static model nor a simple laminar model, importantly the latter one which is often neglected in the past (89-92). Therefore, it has been suggested that the design of experimental simulated bladders models should be further improved based on existing technologies and crafts. In particular, under the premise of a lack of ideal materials for bladder stimulation, the hydrodynamic design of urinary flow should simulate the unsteady flow stress conditions (turbulent stress) as much as possible (93). Moreover, the bladder's capacity should be standardized; either equal to

the physiological capacity of the human bladder or reduced according to a specific proportion so that it can work with the enhanced mechanical-biological features of the dynamic bladder urine environment.

Infection carrier

E. coli and *P. mirabilis* were the main typical strains for infection research, with a suitable consistency and the clinical isolation strains of catheter-related to UTI. Such refractory infections are complicated by the unique ability of *P. mirabilis* to form crystalline biofilms based on their crystalline nature owing to ureolytic biomineralization, eventually leading to encrusted and blocked implanted biomedical devices. This is especially important for indwelling urethral catheters and ureteral stents during daily clinical practice (94,95).

Infection vectors

Sixty-seven studies reported the infection vectors, including infected urine (64), rubber (76), planktonic bacteria (21,77), polymer (42,46) with the former polyolefin copolymer, glass (23,31) with the former sponge simultaneously, polyurethane (19,20,44,45), latex (39,43,50,58,60), Foley catheter (22,47,48,53,56,59) with no statement of the specific materials, agar plates (13,15,57,62,65,68,70,71,73), and silicone (14,16-18,24-30,32,33-38,40,41,49,51,52,54,55). The other eight studies (20,23,31,43-45,58,60) also reported some materials, while the remaining 17 studies (61,63,65,67,69,72,74,75,78-86) did not explicitly report infection vectors.

At present, most of the urinary catheters and stents used in urological practice are made up of silica gel and polyurethane. Some differences existed in the included studies, and thus, it is suggested that future research should focus on these two kinds of medical biomaterials. It is particularly emphasized that the initial bacterial adhesion of bacterial biofilms is mediated by multiple factors, among which the properties of biomaterials participate in the entire biofilm formation process, especially the physicochemical mechanism. They are involved in early bacterial adhesion and in the late stages of what is called "surface-programmed" biofilm growth, which is another important research direction to pursue (96-98).

Infection media

The culture medium also played an important role. Twenty-five studies reported different culture mediums, such as tryptone liquid culture (36) and MHB culture medium (13-15,21,56,61,63,66-72,74,75,77-84). Meanwhile, 17 studies reported human urine (20,24,25,28,29,33,46,48,49,57,58,61,65,71-73,76), and the remaining 32 studies reported artificial urine. Undoubtedly, urine that could be used as a culture medium is the closest simulation of the human environment. Although studies have been carried out using an artificial urine formula, the standardized protocol for artificial urine or the urine of healthy volunteers, especially the latter's homogeneity problem, remains to be further explored (99).

Research design and content analysis

Forty-five studies were conducted to explore bacterial biofilm formation in bladder models, which corresponds with the current research focus. A total of 36 studies were performed with comparative experimental designs, mainly consisting of comparisons between various coatings of drugs or different materials, including between static and dynamic models in three studies (20,27,30), the comparative analysis of culture fluids in two studies (56,57), comparisons between multiple interventions in 21 studies (13,14,17-20,22,28-30,36,38,39,41,43,50,53-55,58,64), comparisons between infection vectors in eight studies (37,40,42,44,45,52,53,58), and comparisons between experimental temperature in one study (37).

There were two primary categories of interventions; antibiotics and drug coating. (I) Antibiotic interventions included the following: antibacterial phage (28,36), norfloxacin and nano silver ion (20), lavage antibacterial solution (14,22), fosfomycin (13), *E. coli* lysates-IAA (urease inhibitor)-allicin (17), biodegradable aqueous polyurethane polymer with ciprofloxacin and streptomycin (19), single agent or double or triple combinations of 1% polygalacturonic acid, 0.4% octanoic acid and 0.3% hydrogen peroxide (22), connecting a 9V direct current line containing silver (41), Jack Bean urease of acid bladder irrigation solution (20 mg urease plus 1 L urine), different bladder irrigators (Uro-Tainer, Bladder Syringe, Optiflow, 1% hydrochloric acid) (48), bladder irrigation solution (0.9% normal saline, sulpir G, 1% mandelic acid) (59), various concentrations of urease inhibitor, acetohydroxamic acid or fluoroimide (51), drainage systems mono flo, S4, P4, pp2000n, and sustained-release device embedded silver ion (55), 4 mA Iontophoresis-gentamicin (57), and chlorhexidine (64). (II) Drug coatings included the following: silver coating (50), polytetrafluoroethylene silver nanocomposite coating (18),

ciprofloxacin coating (58), chlorhexidine-sulfadiazine silver/chlorhexidine-silver sulfadiazine-triclosan coating/silver hydrogel-nitrofurazone coating (43), nitrofurazone/triclosan impregnation (39), silver hydrogel/antimicrobial peptide coating (24), minocycline-rifampicin coating (29), and acylase coating of QS destructing enzyme (30), and triclosan (0% *vs.* 0.5%, 1%, 4%) coating/pure solvent-hydrogel coating/hydrogel, and iodized or hydrogel plus polyhexamethylene biguanide (PHMB) coating (38).

From this, it is clear that there is obvious diversity in research. On the one hand, it implies that the problem of anti-infection, especially biofilm infection, is serious, and also suggests that the efficacy, safety, and cost of existing interventions should be summarized promptly to provide a reference for the future development of safe and effective coated medical implants with wide adaptability.

In total, only five studies reported a comparative experiment of *in vitro* bladder models (20,27,30,56,57), which are summarized in Table S1. Among these, Frant *et al.* (20) suggested that biofilm formation was more prevalent in the BioEncrustation dynamic bladder model and that more substances were observed in the urine scale. Meanwhile, Rasmussen *et al.* (56) suggested that bacterial infection increased, and retrograde infection was found to be more prevalent in the bladder model group without a urine meter. These two studies demonstrated the value of a dynamic bladder model for the mediation of bacterial infection. The small number of studies involving direct comparison experiments with different bladder models is a particular regret of the present study. However, we can summarize meaningful results from the different design techniques of urine transport pattern and force in existing bladder models.

Moreover, the principle of hydrodynamics is applied for a random movement of bladder urine flow. The intracavity indwelling catheter tip and water bag's characteristics are to create a unique stress environment of unsteady flow, i.e., turbulent shear stress, which can be formed in the body when the catheter is indwelled in the bladder. Therefore, the question arises about the formation of bacterial biofilm models simulating human bladder urine flow (100,101). There are complexities and difficulties in the construction of an *in vitro* bladder model. Despite the significant advancements that have been reported in numerous related articles regarding the 44 bladder models included in this study, the design and craft remain unclear and technical limitations still exist. Most published studies have only emphasized the simple movement of fluid by gravity or the unidirectional constant flow under the effect of an electric pump, etc. However, they have failed to consider the following situations comprehensively: the effect of the viscoelastic properties of the bladder, the consequence of the indwelling urinary catheter, the occurrence of urine retention phenomenon, as well as the volume of urine transformation, urination flow rate, residual urine, periodic gradual urine filling, and short-term micturition. In practice, the real state of human urinary flow is turbulent shear stress, as discussed above, yet this concept and its compound stress environment have not been fully simulated (89-93). The existing model design ideas are relatively simple, but the design technology and modules are not standardized (102).

Henceforth, we aim to systematically review the main components and key construction technologies of the *in vitro* bladder models in existing studies and examine their defects to overcome difficulties in constructing *in vitro* bladder models and improve their performance for studying urinary biomaterial infection. This will help provide a worthwhile reference for bladder model construction that closely reflects the actual bladder urine flow conditions. The direct comparison of the bladder models was only reported in five studies; hence, more verification of the bladder model performance is needed. We can ensure the subsequent studies' authenticity only when the scientific feasibility and repeatability of the experimental model can be fully confirmed. This review could provide new research directions and translate basic research achievements into clinical practice.

There are some limitations in this study that should be noted. Firstly, the bladder models of the included studies were not standardized, some research details were unclear, the technical case-based designed schemes lacked a set of standards, and the evaluation indexes were multifarious. This made it difficult to compare the results of various studies and multiple bladder models, and thus, it could only be summarized and analyzed according to the existing data through simple quantitative and qualitative analysis. Secondly, the simulation and further verification of the real stress conditions and even the compound stresses of the bladder urine flow have not yet been reported. Importantly, the *in vitro* models lack the characterizations of bladder tissue architecture and the host stress responses, and therefore cannot provide a sufficient reference. Thirdly, a quantitative meta-analysis was not available due to the lack of homogeneity between the research data. Furthermore, quality evaluation of the *in vitro* research could not be performed. Lastly, literature retrieval was limited to articles

published in English and Chinese from the PubMed and SinoMed databases, respectively; thus, selection bias was difficult to avoid.

Conclusions

To our knowledge, this is the first systematic review of *in vitro* dynamic bladder models for studying UTIs. In conclusion, the existing research suggests that the *in vitro* bladder model can provide new ideas for exploring the pathogenic mechanisms, prevention, and control of bacterial biofilm infections related to bio-implants. Nevertheless, based on the principles of fluid and viscoelastic biomechanics and mechanical biology, model designs should focus on simulating the complex stress environment of the actual bladder urine flow, combined with the construction of a bacterial biofilm generation carrier, culture medium, standardization of the bladder model, as well as verification of the scientific feasibility and repeatability of the model. At present, we are investigating the effect of *in vitro* urine turbulent shear stress of the bladder model on the formation of bacterial biofilm in the hope of obtaining some meaningful results (103).

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/apm-20-2061). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Supplementary

Table S1 Data of bladder model comparison

First author	Year	Model	Outcome
Frant M (20)	2018	Encrustator® Model	Only a small amount of crystal deposit (~0.057 mg/cm^2) formed on the surface of the polyurethane catheter, and the main components were magnesium ion and phosphate; only a small amount of calcium, potassium, sodium and oxalate ion were detected.
		BioEncrustation Model	A significantly higher concentration of sediment (~0.37 mg/cm2) formed, which was mainly composed of sodium and oxalate ions. In addition, a large number of divalent cationic magnesium and calcium were detected. The surface phosphate ion concentration is comparable.
Azevedo AS (27)	2017	Static model	The growth curve of the dynamic model is similar to that of the two bacterial biofilm formed on the artificial urine silicone test piece in the previous study. Fish combined with CLSM to evaluate the spatial distribution of biofilm. Compared with the dynamic condition, the single species biofilm in the static condition has a higher thickness value
		Dynamic laminar flow model	Single microbial biofilm showed that the number of culturable cells of botulinum toxin and scrub typhus increased significantly within 48 h ($P < 0.05$), in addition, the growth rate of scrub typhus was faster (0.4879 h-1) comparing with E. coli (0.2831 h-1), and the dynamic culture conditions had a negative impact on the cell concentration.
Ivanova K (30)	2015	Static model	Foley catheter coated with acylase in the dynamic system has the same trend of inhibiting biofilm formation as that in the static system
		Dynamic model	Crystal violet and fluorescence image analysis showed that the formation of acylase coated bacterial biofilm was inhibited by 80% when Pseudomonas aeruginosa ATCC10145 was infected in a dynamic environment.
Rasmussen A (56)	1996	Bladder model with no urine flow meter	The bacteria were positive after three days of culture.
		Bladder model with urine flow meter	There was no positive bacterial culture, and the retrograde bacterial infection was suppressed. Among them, Urometer 500 meter had no bacterial positive or retrograde infection after nine days of culture compared with the other two meters ($P = 0.04$)
Wong HY (57)	1995	Discharge valve opened per 4–6 h in the bladder model *vs.* continually	The results of bacterial growth did not change in either the intermittent filling, emptying of the drain valve set, or the continuous drainage simulating the Foley catheter drainage environment.

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