

The Role of Bacterial Biofilms in Infections and Effective Prevention Strategies for Biofilm Formation in Urinary Catheters

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Abstract

Biofilm formation is a complicated microbiological process and one of the distinctive features of pathogenic bacteria. Biofilms are a cluster of bacterial cells enclosed in extra polymeric substances and irreversibly attached onto a surface. Biofilms have a major impact on public health as biofilm associated bacteria are able to survive and populate in indwelling medical devices, causing severe nosocomial and recurrent infections. Biofilm-embedded bacteria have unique characteristics which are harder to destroy than the planktonic forms. Especially biofilm bacteria are highly resistant to antimicrobial agents. Treating patients undergoing long term urinary catheterization is becoming complicated by encrustation and blockage due to crystalline biofilm formation inside the lumen of catheters. Further, all types of indwelling catheters including silver or nitrofurazone-coated devices are

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vulnerable to biofilm formation.

Therefore, there is an urgent requirement to develop either urinary catheters with other alternative coatings or new urinary drainage systems which may lead to reduction of infections and morbidity. Other than that, controlling biofilm formation is a necessary requirement during both manufacturing and usage of catheters. Antifouling and biocidal coatings on the catheters are currently being investigated and it is proved that herbal products also express anti-biofilm activity.

This review is largely based on previous literature describing biofilm formation and their role in catheter associated urinary tract infection. Further, mechanism of biofilm formation in indwelling urinary catheters, antibiotic resistance patterns, and the detection methods of biofilm are briefly described. Understanding the mechanisms of biofilm formation and the antibiotic resistance patterns is of utmost importance which will result in the development of new remedies for biofilm infection.

Keywords: Antibiotic Resistance, Biofilm Formation, Urinary Catheters

Introduction

The Structure and Formation of Microbial Biofilms

Biofilm is defined as accumulation of the microbial organisms that is permanently attached to biotic and abiotic surface and generally enclosed in self-produced extracellular polysaccharide substances (EPS) (Sayal *et al.*, 2014; Wijesinghe *et al.*, 2019). Biofilm production is a complicated microbiological process which is a major pathogenic event in the infection. It involves a sequence of steps, including irreversible attachment, accumulation, and maturation (Alves *et al.*, 2014; Tiwari & Ghnawate, 2017). When the bacterial colonies attach to surfaces, the bacterial cells upregulate genes which are specific for adhesion. Further, bacteria itself secrete a sticky gum like material for anchorage called as extracellular substances (Sayal *et al.*, 2014; Tiwari &

Ghnawate, 2017; Wijesinghe *et al.*, 2019). Also, the bacteria populate by multiplying rapidly and spreading along the surface within the EPS of the biofilm. Some of the bacteria detach from the surface and again become into planktonic form and then attach to a new colonised surface (Tiwari & Ghnawate, 2017). This phenomenon helps the metastasis process of biofilm.

Initially the thickness of biofilm is approximately 200 μm and over time it reaches a thickness of $\sim 500 \mu\text{m}$ (Singha *et al.*, 2017). Types of EPS and the diversity of biofilm vary according to the bacterial species and strain (Osungunna & Onawunmi, 2018). Staphylococcal polysaccharide, *Pseudomonas aeruginosa* alginate, streptococcal and lactobacilli glucans and fructans are well known examples for biofilm-associated EPS. Biofilm is composed of small percentage of bacteria compare to EPS which occupies a large portion of biofilm (Tiwari & Ghnawate, 2017). EPS binds the bacteria together in a sticky web of twisted EPS fibers. The fibers interconnect the cells and anchor them to a surface, therefore EPS is considered as the backbone of biofilm (Murugan *et al.*, 2016). The formation of EPS permits the emerging biofilm community to develop a three-dimensional structure (Murugan *et al.*, 2016; Singha *et al.*, 2017). EPS and the accompanying web-like structure are known as extra cellular matrix or biofilm matrix. Bacterial colonies are found within the biofilm matrix (Tiwari & Ghnawate, 2017). Physical and the physiological properties of biofilm are determined by the EPS and the bacterial cells respectively (Murugan *et al.*, 2016).

Biofilms have water channels which transport the essential nutrients and oxygen to internal cells (Alves *et al.*, 2014; Tiwari & Ghnawate, 2017), and remove the waste products and toxins from the cells. Extracellular matrix facilitates communications among the microbial cells via biochemical signals (Tiwari & Ghnawate, 2017), such as acyl-homoserine lactone and oligopeptides facilitates communication in gram negative bacteria and gram positive bacteria respectively in an event called as quorum sensing (Maharjan *et al.*, 2018). Also, environmental factors have a major impact on formation of biofilm including EPS production

(Murugan *et al.*, 2016; Singha *et al.*, 2017). The mechanisms of how environmental factors influence biofilm formation yet to be justified (Murugan *et al.*, 2016).

Pathogenesis and Antibiotic Resistance Mechanisms of Biofilms

Biofilm develop on the surfaces of living tissues such as the urinary bladder and abiotic surfaces generally in indwelling devices (Bardoloi & Yogeasha Babu, 2017). Biofilms are considered as one of the serious medical threats to patient's health as the matrix prevent the pathogenic microorganism against the host defense mechanism (Sayal *et al.*, 2014 ; Maharjan *et al.*, 2018) and its unique characteristics enable intrinsic resistance to antibacterial drugs. Biofilm associated bacteria acquire 100- to 1000-fold increase in antibiotic tolerance compared to planktonic bacteria (Murugan *et al.*, 2016). Bacteria become more resistant after adhering to the surface and subsequently develop more resistance over the following days of biofilm production (Tiwari & Ghnawate, 2017).

Generally biofilm consists of multi drug-resistant bacterial species separated by interstitial fluid (Alves *et al.*, 2014; Bardoloi & Yogeasha Babu, 2017). Biofilm also consists of gram positive bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and Gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (Patel *et al.*, 2016 ; Bardoloi & Yogeasha Babu, 2017).

Biofilm bacteria exhibit different antibiotic resistant strategies than the normal pathogenic bacteria, and the resistance techniques depend on the slow penetration of the antibiotics, different rates of bacterial growth and metabolism as well as horizontal transmission of drug-resistant gene between the closely attached cells (Alves *et al.*, 2014; Tiwari & Ghnawate, 2017 ; Maharjan *et al.*, 2018). Also, biofilm bacteria have the ability to form unique subpopulation like spore formation. The subpopulations have different phenotypes and persist in a dormant state which are highly resistance to antibiotics and cause recurrent infections (Murugan *et al.*, 2016; Singha *et al.*,

2017). In addition to that, the biofilm matrix consists of concentrated extracellular enzymes including formaldehyde dehydrogenase, formaldehyde lyase and β -lactamases. The enzymes are capable of inactivating the hydrophilic positively charged antibacterial substances (Murugan *et al.*, 2016).

A knowledge of biofilm formation and antibiotic resistance profile of bacterial species are critical to find new techniques for therapeutic use. Since the biofilm bacteria is highly resistance to antibiotic, there are considerable drawbacks in the treatments of patients with this type of infection (Osungunna & Onawunmi, 2018; Sayal *et al.*, 2014). Especially, antibiotic resistivity against commonly used antibiotics for the urinary tract infection has been increasing worldwide. It was found that the resistance pattern may vary with the bacterial stain (Murugan *et al.*, 2011). According to the reports from different countries, drugs Colistin, Ofloxacin, Fosfomycin, Vancomycin, Meropenem, Imipenem, Piperacillin and Tazobactam remain as the antibiotic of choice in the treatment of biofilm infections (Dumaru *et al.*, 2019; Maharjan *et al.*, 2018; Osungunna & Onawunmi, 2018; Sabir *et al.*, 2017). On account of antibiotic resistance pattern, the highest resistance was observed with Ampicillin and Ceftazidime, followed by Amoxicillin Clavulanate and Tetracycline (Maharjan *et al.*, 2018; Sabir *et al.*, 2017).

Biofilm in Indwelling Urinary Catheters

Indwelling medical devices support to increase therapeutic results of patients, particularly those requiring life supporting devices and protect their lives, however these devices are more prone to get colonization of biofilm bacteria (Dror *et al.*, 2009 ; Alves *et al.*, 2014 ; Bardoloi & Yogeesh Babu, 2017). About 30% of biofilm producing bacteria have been identified from indwelling medical equipment including endotracheal tubes, central venous catheters and urinary catheters. Among the medical devices, indwelling urinary catheters are known to be the major common site of biofilm producing bacteria (Patel *et al.*, 2016 ; Bardoloi & Yogeesh Babu, 2017). The bacteria can originate in the inner or

outer part of the device (Sayal *et al.*, 2014). The time needed for the biofilm to be produced on the device is based on the bacterial group and the type of device material. But a day is enough to produce a thick biofilm on the complete surface of a polymeric device (Alves *et al.*, 2014; Maharjan *et al.*, 2018).

Urinary tract infections play a major part in hospitalized infections and nearly 97 % of the cases were related to urinary catheters. About 60 % of infections developed due to the presence of biofilm (Alves *et al.*, 2014). Generally during initial admissions at hospitals, around 12 to 16% of adult inpatients have an indwelling urinary catheter (Maharjan *et al.*, 2018). Almost 50 % of catheterized patients acquire infections within a period of less than one week. Patients catheterized for nearly 4 weeks have a 100 % possibility of getting infections (Alves *et al.*, 2014). A study carried out in India indicates that *E.coli* (29.74%) was the most commonly isolated followed by *K. pneumoniae* (21.84%), *P. mirabilis* (15.42%) from the urine samples of patients with indwelling urinary catheters (Sayal *et al.*, 2014).

If the urinary catheter is inserted in the same area for a longer period, it increases the likelihood of these microorganisms producing biofilms and results in chronic and recurrent urinary tract infections (Sayal *et al.*, 2014). If the infection is not treated, it may consequently lead to bacteraemia, bacterial vaginosis, chronic renal infection, acute pyelonephritis, bladder cancer and in certain cases death (Alves *et al.*, 2014).

Crystalline Biofilm Formation in Urinary Catheters

Bacteria use the catheter as a pathway to enter into the bladder (Tiwari & Ghnawate, 2017). Urease is an enzyme produced by bacteria which are colonized in the urine of catheterized patients (Stickler, 2014). *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus* species, some *Providencia* species, *Staphylococcus aureus* and coagulase-negative *Staphylococci* produce urease in urine and form a unique type of crystalline biofilm on catheters (Stickler, 2014; Tiwari & Ghnawate, 2017). Especially, *Proteus mirabilis* is the most common bacteria which forms crystalline biofilms in the urinary catheters (Stickler, 2014).

Formation of Crystalline biofilms involves in sequences of steps. Initially urease enzyme produced by bacterial species break down the urea in urine and release ammonia. Ammonia increases the pH level of urine and subsequently results in the formation of calcium and magnesium phosphate crystalline. Accumulation of the crystalline materials occurs in the urine that remains inside the catheter lumen and develop rapidly within the biofilm matrix (Broomfield *et al.*, 2009; Stickler, 2014; Tiwari & Ghnawate, 2017). Also the crystalline materials harden and obstruct the lumen of catheter and finally block the urine flow. Blockage of urine flow results in painful bladder distension and if the urine from the distended bladder refluxes into the kidney, it causes pyelonephritis (Stickler, 2014; Tiwari & Ghnawate, 2017). However, if the particular causative agent is not identified it can make the disease more severe and in some cases, it can be fatal (Alves *et al.*, 2014). All available catheter types are vulnerable to encrustation and blockage including silver or nitrofurazone-coated ones (Stickler, 2014). High morbidity and the mortality rates of catheter associated urinary tract infection increase the hospital stay and cost of the treatment (Maharjan *et al.*, 2018).

Detection Methods of Biofilm

Conventional methods are economical and reliable for the routine screening and diagnosis (Dumaru *et al.*, 2019). Biofilm can be easily demonstrated by phenotypic methods such as tube method, Congo Red Agar (CRA) method and tissue culture plate method. Besides that genotypic methods have also been carried out to screen biofilm production as indirect proof of the presence of biofilms (Bardoloi & Yogeasha Babu, 2017). Molecular methods considerably widened a spectrum of microbes detected in biofilms. The Modern Next-Generation Sequencing (NGS) techniques is beneficial in studying highly diverse bacterial communities (Kotaskova *et al.*, 2019). NGS targeting 16S rDNA is able to analyse the bacterial composition in catheter related infection and therefore it is suggested as a promising diagnostic tool for catheter related infections (Okuda *et al.*, 2018).

The new test methods are currently used for biofilm detection such as DNA based methods including quantitative real time PCR, multiplex PCR, Sanger sequencing, next generation sequencing and micro array analysis. Further, confocal scanning, laser microscopy with molecular probes, Fluorescence In Situ Hybridization (FISH) or labelled antibodies-electron microscopy are used to detect biofilms (Magana *et al.*, 2018; Petrachi *et al.*, 2017). Virulence genes responsible for biofilm formation can be amplified with the usage of gene specific primers, even in the uncultured pathogen present in the sample (Kırmusaoğlu, 2019). Researches who worked with biofilm structures have identified virulence genes responsible for biofilm formation such as *cup A* gene in *P. aeruginosa*, *ica* gene in *S. aureus* and *esp* gene in *E. faecalis* (Falaki & Mahdavi, 2017; Murugan *et al.*, 2016; Pérez-Osorio *et al.*, 2010). 16S rDNA sequencing is the most common housekeeping genetic marker to identify bacterial species. 16S rDNA molecule among various bacteria have highly conserved nucleotide sequence interspersed with regions of more variables that are specific to genes or species (Jenkins *et al.*, 2012; Kai *et al.*, 2018; Kotaskova *et al.*, 2019; Maciel *et al.*, 2009). Each bacterial species has its unique 16S rDNA sequences with differences (<0.5%) (Janda & Abbott, 2007). Phylogenetic analysis using 16S rDNA sequences is considered to be a highly reliable and important bioinformatics tool (Roy *et al.*, 2014; Yang *et al.*, 2016).

Phylogenetic is the study of the evolutionary relatedness among groups of organisms. Molecular phylogenetic uses sequence data to infer these relationships for both organisms and the genes they maintain (Ziemert & Jensen, 2012). Evolutionary relationship among the biofilm bacterial species are determined by phylogenetic analysis (Murugan *et al.*, 2016). Phylogenetic tree is a branching diagram where the simple tree representation of the evolution makes the phylogenetic analysis easier to comprehend and represent as well (Roy *et al.*, 2014).

Effective Strategies and Novel Approaches to Prevent Biofilm Formation

Nowadays mortality rates increase and there is an urgent

requirement to develop strategies to prevent and eradicate biofilm production on urinary catheters (Alves *et al.*, 2014). In this standpoint, early detection of biofilm producers is crucial (Maharjan *et al.*, 2018). Also analysing the biofilm development with regard to age, gender, hospital unit, bacterial species (Alves *et al.*, 2014) and their antibiotic susceptibility may provide a wide knowledge regarding treatment and prevention of such infections (Sayal *et al.*, 2014). Several studies indicate that microbial studies alone will not eradicate CAUTIs (Singha *et al.*, 2017). In order to meet all the challenges of catheter associated infection, development of an alternative indwelling catheter system is an important requirement which should have the same mechanism as natural physiology of urine elimination and emptying urine completely without damaging the bladder and appropriate for all users (Feneley *et al.*, 2015). Researchers need to understand the interaction between the pathogens and materials with regard to development of infection. The necessity for better catheter materials has significantly increased (Singha *et al.*, 2017).

A research study reports that Azithromycin-Ciprofloxacin-impregnated urinary catheters inhibit bacterial colonization, biofilm formation, and inflammation in a murine model of foreign-body-associated urinary tract infections caused by *Pseudomonas aeruginosa* (Saini *et al.*, 2017). Another research study indicates that silver-polytetrafluoroethylene (Ag-PTFE) coated catheters substantially inhibit encrustation compare to uncoated ones. Currently coated catheters which are effective for long term catheterization are commercially not available. Silver has antibacterial properties (Singha *et al.*, 2017). Silver coated catheters are available in the market, but antibacterial properties of them are inactivated due to the sticky biofilm formation on the extra luminal part of the catheters. PTFE has an excellent non-stick properties and hence it is suggested that the Ag-PTFE would be a promising antibacterial coating for urinary catheters (Wang *et al.*, 2019). Research on Antifouling and biocidal materials would be most useful in designing competent and simple catheters. Antifouling coatings have the ability to prevent bacterial attachment to the surfaces,

whereas biocidal materials kill the pathogens. Silver ions, Triclosan, Chlorhexidine, Chlorine, Tributyltin and Nitric oxide are the chemicals which show biocide activity (Singha *et al.*, 2017). It has been proven in a research study that Chlorhexidine sustained-release varnish-coated urinary catheters efficaciously reduce urinary catheter-associated biofilm formation (Segev *et al.*, 2013).

Besides that, discovering natural agents could provide novel strategies for catheter associated biofilm infections. Research reports from various countries indicate that significant amount of plant species have anti-bacterial and anti-biofilm activity. Garlic shows inhibitory effect in quorum sensing mechanism which plays a vital role in biofilm formation in bacteria. Moreover, plant *Cocculas trilobus* has the ability of blocking the adherence of bacteria onto surfaces. As well as Cranberry Polyphenols, *Herba parrinae* extract, and *Ginkgo biloba* extract have strong inhibitory effects on biofilm formation (Lu *et al.*, 2019). Furthermore it is proved that catheters impregnated with herbal plant powders including *Andrographis paniculata*, *Euphorbia hirta* and *Terminalia chebula* have antibiofilm activity. Seeking novel antimicrobials from ethnomedicinal plant may provide a new pathway for preventing biofilm infections (AnilKumar, 2015).

Conclusions

Almost all cases of CAUTI are biofilm-related infections (Bardoloi & Yogeesh Babu, 2017). Biofilms and their control procedures have been mentioned in several aforementioned studies and complications of biofilm are thought provoking and which raises serious concerns (Maharjan *et al.*, 2018). Understanding the nature of biofilm and their connection with the environmental factors may help to discover treatment and management methods for CAUTI (Murugan *et al.*, 2016). In Sri Lanka, even though few works have been reported on utilizing the beneficial effect of biofilm bacteria (eg: biofilm biofertilizers) in the field of agriculture (Herath *et al.*, 2013), there are lack of research done on biofilm infection in health-related field.

Diagnosis and the prevention methods of catheter associated urinary tract infection require expertise, facilities and surveillance activities. Due to inadequate surveillance and reporting system, magnitude of catheter associated UTI has been underestimated and developed into a common problem in the developing countries. Lack of knowledge with regard to development of microorganisms in biofilms lead to slow progression of detecting biofilms in medical devices (Awoke *et al.*, 2019). Therefore, research related to catheter associated biofilm is most important and it will raise the awareness of this condition to the general public of Sri Lanka.

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