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SCIENCE

BIOFILM IN DEVICE LUMENS AND PIPEWORK

FIGURE 1. Biofilm on a surface
Source: Pacific Northwest National Laboratory

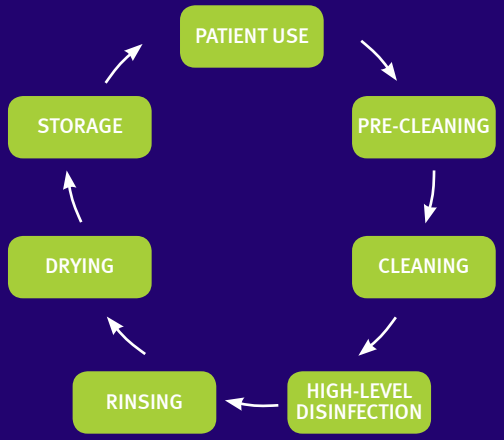

FIGURE 2. Reprocessing cycle of a medical device such as an endoscope

ABSTRACT

Biofilm constitutes an aggregation of microbial species such as bacteria and fungi which can accumulate on surfaces and cause infections. Biofilms are highly resistant to external factors such as disinfection and are a growing concern in infection control. The presence of biofilms in the lumens of medical devices and in water pipelines can put patient lives at risk. Chlorine dioxide has been shown to be effective against biofilm and could potentially be used to manage and prevent biofilm formation in device lumens and pipelines.

INTRODUCTION

Biofilm is a layer of microorganisms attached to a surface enclosed in a matrix of polysaccharide material known as extracellular polymeric substances (EPS). This matrix, produced by the microorganisms themselves, is mainly composed of polysaccharides, proteins, nucleic acids and lipids[1], and offers stability and protection to the organism. Biofilm is formed by bacterial and fungal species[2], however, the microorganisms themselves account for a small proportion of the entire biofilm (less than 10%), with the matrix accounting for over 90% of the structure.[1] Figure 1 shows biofilm on a surface.

The matrix forms a protective barrier which shields the microorganisms contained within it from desiccation and biocidal action[1], and many other external environmental factors which could damage or destroy the microorganisms in their planktonic state (meaning freely existing in solution).[3] There is evidence that in some cases certain biocides, such as alcohol, may actually fixate proteins to surfaces. It has been demonstrated that exposing contaminated surfaces to alcohol increased bacterial protein adherence and increased cleaning difficulty.[4]

Biofilms may form on a wide variety of surfaces, including indwelling medical devices or industrial or potable water system piping.[5] The water system biofilm is highly complex, composed of filamentous bacteria and containing corrosion products, clay material and fresh water diatoms. The biofilm on the medical device appears to be composed of a single, coccoid organism and the associated extracellular polymeric substance (EPS) matrix.[5]

Biofilm formation in the lumens of medical devices such as endoscopes and in pipelines of washer disinfectors and other water systems is a major concern for the healthcare industry. It poses a threat to both patients and medical staff.

BIOFILM IN MEDICAL DEVICES' LUMENS

Many studies have investigated the occurrence of biofilm in the lumens of endoscopes. Endoscopes are particularly difficult to decontaminate as they are heat-sensitive and therefore cannot be sterilised by heat. A common method of decontaminating lumened endoscopes is high-level disinfection using an automated endoscope reprocessor (AER).

High-level disinfection is only one part of the reprocessing process. Figure 2 portrays the entire reprocessing cycle a medical device, such as an endoscope, undergoes. Cleaning, rinsing, drying and storage are also important in this process. If these steps are inadequately performed, the high-level disinfection step can be compromised and can lead to the preservation of microorganisms and subsequent biofilm formation. According to Pajkos et al. (2004) the presence of biofilm may contribute to the failure of decontamination prior to reuse by protecting the microorganisms from the action of the disinfectant. Pajkos et al. (2004) showed that out of 13 endoscopes that had been sent to an endoscope-servicing centre, five (38.5%) were found to have biofilm in the suction and biopsy channels.[6] Alfa et al. (2017) found that flexible endoscopes can develop biofilm and accumulate fixed material called build-up biofilm (BBF) within lumens after repeated rounds of patient use and reprocessing.[7] The detected presence of biofilm suggests that current endoscope reprocessing methods are inadequate and do not effectively remove contamination. Sciortino et al.[8] (2004) evaluated endoscope reprocessing methods using an adenosine triphosphate (ATP) detection system. They concluded that the internal channels of older endoscopes were not being adequately cleaned to remove biofilms. Damage and defects of the device surface were associated with accumulation of microorganisms and residual soil.

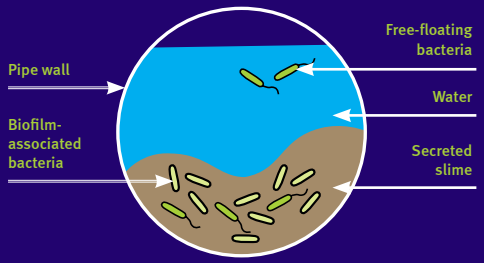

Figure 3. Biofilm forming inside a water pipe
Source: Centers for Disease Control and Prevention

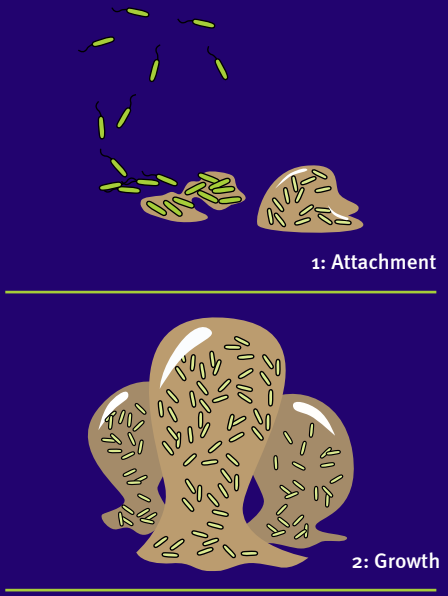

Figure 4. Biofilm life cycle
Source: Montana State University

Inadequate rinsing and drying of the device following disinfection are contributing factors of biofilm formation within endoscope lumens. Moisture which remains in endoscope channels after inadequate rinsing and drying can be contributing factors in the development of biofilm.[9] This moisture likely originates from water flowing through the AERs containing waterborne microorganisms such as *Pseudomonas aeruginosa* and *Mycobacterium* spp.[9] Moist environment provides optimal conditions for the microorganisms to grow and form biofilm. These biofilms hold microorganisms together which proliferate thus expanding this structure. The contaminated water which is pumped through the AER and used to rinse endoscopes after disinfection can result in re-contamination of the devices. Ofstead et al. (2018) assessed three hospitals for their endoscope reprocessing, drying and storage practices.[10] Fluid was detected in 22 of 45 (49%) endoscopes. Retained fluid was associated with significantly higher adenosine triphosphate (ATP) levels ($P < .01$), an indicator of living organisms. The majority of endoscopes (71%) harboured microbial growth, including mould and waterborne pathogens. ATP levels above background (=40 relative light unit (RLU)) were found on 69% of patient-ready endoscopes, and very high ATP levels (=200 RLU) were found on 22% of endoscopes.

Bacteria that remain in the moist environment of the AER at the end of the operating cycle may proliferate and form biofilms. Bacteria which get released from these biofilms further contaminate the AER leading to re-contamination of the final rinse water and may subsequently re-contaminate the disinfected endoscopes.[11] It is therefore crucial for the rinse water used to rinse devices post disinfection to be free of microbial contamination so that the disinfection step is not compromised. Rinsing with microbial-free water is as significant in the reprocessing cycle as adequate high-level disinfection.

BIOFILM IN AERs AND PIPEWORK

Biofilm is prevalent in pipelines, both of industrial and potable water systems. Biofilms found within water distribution systems can lead to corrosion of pipes and possible health risks.

Biofilm can also form in the pipework of AERs which results in contaminated rinse water for rinsing endoscopes after disinfection. In addition, contaminated water used for cleaning and disinfection cycles may compromise efficacy of the cleaning and disinfecting solutions. This can lead to recontamination or inadequate decontamination of the device which, if inadequately dried and stored, can provide favourable growth conditions for microorganisms and lead to biofilm formation and expansion. According to a survey performed by the National Institute for Public Health and the Environment (RIVM) in the Netherlands in 2008, less than 5% of the hospitals routinely monitored the quality of the water used for rinsing endoscopes in AERs.[11] This suggests that little importance is given to the quality of water. AER themselves are difficult to disinfect due to their complex design and certain surfaces can remain moist and can lead to bacterial proliferation and biofilm formation inside their channels. Endoscopes disinfected in contaminated AERs can become re-contaminated during rinsing with bacteria released from biofilms found within the machine piping.[12]

In order to prevent contamination of the water flowing through the AER pipelines and subsequent re-contamination of devices, it is paramount to maintain the AER microbe-free. AERs should undergo regular disinfection to prevent formation and build-up of biofilm.

Mahapatra et al. (2015) obtained 187 bacterial isolates from 45 water samples from drinking water pipelines. Single bacteria were isolated from seven samples, multiple bacterial combinations were found in the rest (38 samples). The isolates identified were *Acinetobacter* spp. (44), *Pseudomonas* spp. (41), *Klebsiella* spp. (36), *E. coli* (22), *Staphylococcus aureus* (14), *Aeromonas* spp. (2) and *Enterococcus* spp. (28). Biofilm was detected in 19.78% (37) of the isolates.[13] They found that most of the biofilm producers were *Pseudomonas* and *Acinetobacter* spp.

These bacterial cells can attach to the surfaces of the pipes, form biofilm and then be released into the flow of water.[13] **Figure 4** portrays the attachment and growth of biofilm and the subsequent release back into the environment. Aquatic microbes are well-adapted to the low nutrient level and cool water temperature of the distribution system and can easily survive when released into water. The organisms found within biofilms tend to become more resistant to antibiotics and certain disinfectants[13] and therefore serve as a pool of pathogenic organisms which, when released from biofilm, can lead to the spread of infections. Drinking water distribution systems (DWDS) support a diverse microbial community attached to the pipewalls where biofilms form.[14] Immunocompromised patients may be especially at risk of infection from contaminated water.

Chlorine dioxide dosed into the water at low levels is one of the few ways used to remove and prevent biofilm from water systems.[13]

CONCLUSION

Biofilm is a growing concern within the medical area because it can form and spread rapidly in the lumens of medical devices, especially endoscopes, and pipework of AERs.

Occurrences of biofilm in these lumens are largely due to improper reprocessing methods and physical difficulty in disinfection, respectively. Particular attention must be paid when reprocessing endoscopes as the lumens of such devices harbour microorganisms and if improperly disinfected or stored these can proliferate and form biofilms. If the pipes are contaminated with biofilm, bacteria released into the water flow can re-contaminate the device which is being rinsed after disinfection and can contaminate potable water which can be a source of infection. A rigorous decontamination method must be devised in order to control biofilm formation.

The study performed with chlorine dioxide presented in this paper shows this chemistry could offer a potential solution to this growing issue. Although further studies should be performed, these initial results are promising and provide a good indication of the potential of chlorine dioxide in the destruction and prevention of biofilm.

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CHLORINE DIOXIDE AND BIOFILM

Chlorine dioxide (ClO_2) has been shown to be effective against *Pseudomonas aeruginosa* biofilm grown in tubing simulating the internal channels found within endoscopes. It has also been found effective in reducing the amounts of protein and polysaccharides which constitute the biofilm structure.

In the study performed by Biotech-Germande laboratory[15] following EN ISO 15883[16], pieces of tubing were used to simulate the internal channels of endoscopes. The study aimed to assess whether chlorine dioxide could be used as a curative and/or preventative measure in the fight against biofilm. The study assessed efficacy of chlorine dioxide over a period of four weeks (20 working days). Efficacy of two treatments (preventative and curative) was assessed by analysing the number of viable bacteria, residual proteins and polysaccharides per surface unit of initially contaminated tubes and initially sterile tubes in order to assess biofilm removal and biofilm control, respectively. Each test line consisted of two tubes initially contaminated with Pseudomonas aeruginosa biofilm and one initially sterile tube.

The first test line was treated continuously with 10 litres of five parts per million (ppm) ClO_2 10 times a day as a preventative measure. The continuous flow of ClO_2 chemistry through the tube induced a quick a reduction in the number of viable bacteria fixed to the surface. The number of viable bacteria fixed to the tested surface decreased from 2.7×10^9 CFU/cm² to less than 1.0×10^2 CFU/cm² after just over two days (56 hours) (i.e. after 30 x 10 litres of 5ppm ClO_2 solution). This preventative treatment also resulted in reduction of the amount of proteins and polysaccharides. Residual amount of proteins and polysaccharides decreased from 44.5µg/cm² and 13.8µg/cm² to 2.2µg/cm² and 0.5µg/cm² after 392 hours and remained stable until the end of the test period with a mean value of ~1.4µg/cm² for proteins and ~1.5µg/cm² for polysaccharides. This repetitive treatment with 5ppm ClO_2 solution resulted in the reduction of the number of viable *P. aeruginosa* bacteria and the reduction of proteins and polysaccharides enabling the control of the biofilm present in the tubing.

The second test line was treated twice a day with a 50 ppm ClO_2 solution for five minutes as a curative measure. The ClO_2 chemistry in this curative assessment was added to the tap water and flushed through the tubing. Treatment twice a day with 50 ppm ClO_2 resulted in elimination of biofilm constituents. The number of viable bacteria decreased from 5.7×10^9 CFU/cm² to less than 5 CFU/cm² in less than 120 hours. With longer contact times, no viable bacteria were recovered from the initially contaminated surfaces of the tubing. This curative treatment also reduced the number of proteins and polysaccharides. Treatment for five minutes twice a day with a 50ppm ClO_2 solution induces a total and irreversible elimination of biofilm constituents (fixed bacteria, proteins and polysaccharides). This treatment showed that chlorine dioxide can be used to control the formation of biofilm.

The third test line was not treated with chlorine dioxide, it was supplied with 10 litres of tap water 10 times a day and is fitted with a 0.2µm filter which creates a bacteria-free water. This test line was used as a reference system for the production of bacteria free water. The filter allowed to control the microbial quality of the water produced however, despite its use, the water samples collected were not free from microbial contamination.

The fourth test line was not treated with chlorine dioxide, it was supplied with 10 litres of filtered tap water 10 times per day and acted as a control. Test performed with the control line showed a partial removal of biofilm which was a result of a wash-off effect of the water circulating through the tube and remaining bacteria could colonise nearby surfaces or contaminate the circulating water.

The two scenarios with chlorine dioxide (test line one and test line two) demonstrate that biofilm can be effectively removed and eliminated from tubing through flushing with chlorine dioxide solution.

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