Emerging Impact of Biofilms on the Environment of Care.

Mark Hodgson, Sarah Finn PhD, Medentech Ltd

Recently, several published studies have highlighted the role of biofilm on dry environmental surfaces in harboring and protecting multidrug-resistant bacteria in healthcare establishments. Vickery *et al*. demonstrated that biofilms on dry hospital surfaces are far more widespread than previously recognized (1). A following study by Hu *et al*. found bacteria dwelling in biofilm on over 90% of surfaces tested from an Intensive Care Unit (ICU), after two terminal cleanings (2). Furthermore, over 50% of these contained multidrug-resistant (MDR) bacteria (2). This work suggests that many commonly used disinfectants are not effective against bacteria within biofilms and that unless we consider the potential impact of these biofilms we are unlikely to attain the goal of reduced infection rates.

Mature biofilms are complex, multi-species structures that could contain bacteria, fungi, algae, yeasts and protozoa. Viruses can also become encased and protected by biofilms produced by other species. Biofilms are the most common form of growth for microorganisms and are a key survival factor. Biofilms provide protection against desiccation, Ultra Violet (UV) light, antimicrobials (including disinfectants and biocides) and provide an environment for microorganisms to access nutrients and share genetic material (3–6). The formation of biofilms in damp and aquatic environments has been well documented for some time. Briefly, there is firstly a loose attachment of the microorganism to a surface, followed by strong adhesion. This is followed by the excretion of extracellular polymeric substances (EPS), which eventually makes up 75 – 90% of the biofilm and acts as a protective barrier (7). As the population within the biofilm grow and multiply the complexity of the microbial population within the biofilm increases and the size (area and depth) of the biofilm increases.

Included in the identified activities within biofilm is the sharing of genetic material such as plasmids that may contain antibiotic resistance genes (8, 9). By sharing plasmids, it is possible for a previously antibiotic susceptible bacterial population within a biofilm to become resistant by acquiring these mobile genetic elements, thus increasing the spread of MDR bacteria.

A further defense mechanism for biofilms are specialized survivor cells called “persisters”, a small dormant sub-population of the overall population (10). When the biofilm is disturbed, such as through the action of disinfectants or mechanical cleaning, the persister cells can survive and subsequently rapidly repopulate the biofilm with new residents and could even increase the thickness of the biofilm to provide enhanced protection against future attacks

Most of this is not particularly new to biofilm researchers as the interaction of organisms and survival mechanisms has been studied for decades. What is new is that while thus far much of research work has been focused on wet or damp environments, the work by Vickery *et al.* revealed that there are extensive biofilms found on “dry” surfaces in the healthcare environment (1). This is changing how we should think about cleaning and disinfection. Firstly, these biofilms are typically not large, often only a few microns thick, fitting into microscopic crevices found on most surfaces. Secondly, many of the commonly used disinfectants are not effective against biofilms. Almatroudi *et al.* cultivated dry biofilms of *Staphylococcus aureus* in vitro and exposed them to bleach at concentrations up to 20,000 ppm (11). Live cells were still

detectable after exposure and reformed the biofilm in a number of days (11). Another study found that up to 11% of MRSA and 80% of Pseudomonas aeruginosa cells in biofilm survived after treatment containing either benzalkonium chloride, chlorohexidine or triclosan (12).

The biofilm provides a reservoir where bacteria can survive on dry surfaces for prolonged periods without dehydration. There is some debate as to how biofilms form on otherwise dry surfaces, as the majority of microorganisms have an optimal water activity of 0.95 – 0.98, and a minimum for most bacteria of 0.88 - 0.91 (13, 14). This measure is relative to pure distilled water which has a water activity of 1. Vickery *et al.* hypothesized that in the hospital setting, these dry surfaces biofilms may form when a surface is temporarily wet due to incidental spills, condensation or high humidity (1). After initial biofilm formation, the EPS would then protect the microorganisms from desiccation (1). Work published by Otter *et al.* demonstrated that bacteria were detectable on surfaces for longer than would typically be considered possible with planktonic bacteria (15). Many Gram negative bacteria such as *Klebsiella* and *Staphylococcus* were shown to survive for 6 weeks on dry surfaces, where classically significant declines in population within a few days would be expected (15).

Biofilms have been shown to be >1500 times less susceptible to biocides compared to planktonic cells (1, 12). Oxidizing chemistries have been found to be more effective than a range of other chemicals, including alcohols, phenols and quaternary ammonium compounds (16). This may be due to the oxidizing agents targeting multiple cell and biofilm targets (16–19). Commodity bleach even at 2 to 4 times typical doses is not fully effective (11). For products that use a two-step process of clean then disinfect, it is important that the disinfectant be applied in a relatively short time frame after the cleaning phase, as treatment with surfactant alone will not achieve total biofilm inactivation which could lead to rapid regrowth (20).

Users should also be aware of the implications for UV “robot” cleaners. Biofilm provides significant protection for bacteria from the impact of UV irradiation. One researcher indicates that the dose of UV required to provide a four log kill of bacteria protected by biofilm is four to five times greater than if there is no biofilm present (21). In effect to attain the same level of kill as demonstrated on test strips one would need to run the device for four to five times as long, biofilm is natures very own SPF 30. All surfaces should be cleaned and disinfected prior to the application of a UV system; it is important that no surfaces are missed and that any use of UV occurs within a short time frame of the manual clean and disinfection.

To protect patients and to ensure a safe working environment for our staff and visitors it is vital that environmental cleaning practices address the root cause of pathogen reservoirs in patient care areas. Unless we use products that are effective in eliminating bacteria living within biofilm we will not be in position to truly reduce risk.

References

1. **Vickery K**, **Deva A**, **Jacombs A**, **Allan J**, **Valente P**, **Gosbell IB**. 2012. Presence of biofilm containing viable multiresistant organisms despite terminal cleaning on clinical surfaces in an intensive care unit. J Hosp Infect **80**:52–55.

2. **Hu H**, **Johani K**, **Gosbell IB**, **Jacombs ASW**, **Almatroudi A**, **Whiteley GS**, **Deva AK**, **Jensen S**, **Vickery K**. 2015. Intensive care unit environmental surfaces are contaminated by multidrug-resistant bacteria in biofilms: Combined results of conventional culture, pyrosequencing, scanning electron microscopy, and confocal laser microscopy. J Hosp Infect **91**:35–44.

3. **Espeland EM**, **Wetzel RG**. 2001. Complexation, stabilization, and UV photolysis of extracellular and surface-bound glucosidase and alkaline phosphatase: Implications for biofilm microbiota. Microb Ecol **42**:572–585.

4. **Le Magrex-Debar E**, **Lemoine J**, **Gell MP**, **Jacquelin LF**, **Choisy C**. 2000. Evaluation of biohazards in dehydrated biofilms on foodstuff packaging. Int J Food Microbiol **55**:239–243.

5. **Stewart PS**, **Costerton JW**. 2001. Antibiotic resistance of bacteria in biofilms. Lancet ProQuest Sci Journals pg **358**:9276.

6. **Mah TFC**, **O'Toole GA**. 2001. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol.

7. **Hall-Stoodley L**, **Costerton J**, **Stoodley P**. 2004. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol **2**:95–108.

8. **Dionisio F**, **Matic I**, **Radman M**, **Rodrigues OR**, **Taddei F**. 2002. Plasmids spread very fast in heterogeneous bacterial communities. Genetics **162**:1525–1532.

9. **Molin S**, **Tolker-Nielsen T**. 2003. Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilisation of the biofilm structure. Curr Opin Biotechnol.

10. **Lewis K**. 2007. Persister cells, dormancy and infectious disease. Nat Rev Microbiol **5**:48–56.

11. **Almatroudi A**, **Gosbell IB**, **Hu H**, **Jensen SO**, **Espedido BA**, **Tahir S**, **Glasbey TO**, **Legge P**, **Whiteley G**, **Deva A**, **Vickery K**. 2016. Staphylococcus aureus dry-surface biofilms are not killed by sodium hypochlorite: implications for infection control. J Hosp Infect.

12. **Smith K**, **Hunter IS**. 2008. Efficacy of common hospital biocides with biofilms of multi-drug resistant clinical isolates. J Med Microbiol **57**:966–973.

13. **Lund BM**, **Baird-Parker TC**, **Gould GW**. 2000. The Microbiological Safety and Quality of Food. Aspen Publisher, Gaithersburg, MD.

14. **Farkas J**, **Doyle M**, **Beuchat L**. 2007. Physical methods of food preservation, p. 685–712. *In* Doyle, P, Beuchat, LR, Montville, TJ (eds.), Food Microbiology: Fundamentals and Frontiers. American Society for Microbiology Press, Washington, DC.

15. **Otter JA**, **French GL**. 2009. Survival of nosocomial bacteria and spores on surfaces and inactivation by hydrogen peroxide vapor. J Clin Microbiol **47**:205–207.

16. **Toté K**, **Horemans T**, **Vanden Berghe D**, **Maes L**, **Cos P**. 2010. Inhibitory effect of biocides on the viable masses and matrices of Staphylococcus aureus and Pseudomonas aeruginosa biofilms. Appl Environ Microbiol **76**:3135–3142.

17. **Cy L**, **Yc C**, **Lp S**, **Cj S**, **Infect H**, **Commission E**, **Committee S**, **Identified N**, **Risks H**, **Quae E**, **Is H**, **Microbiol M**. 2012. Biofilm Resistance 1–7.

18. **Lee D**, **Howlett J**, **Pratten J**, **Mordan N**, **McDonald A**, **Wilson M**, **Ready D**. 2009. Susceptibility of MRSA biofilms to denture-cleansing agents: Research Letter. FEMS Microbiol Lett **291**:241–246.

19. **Otter JA**, **Vickery K**, **Walker JT**, **deLancey Pulcini E**, **Stoodley P**, **Goldenberg SD**, **Salkeld JAG**, **Chewins J**, **Yezli S**, **Edgeworth JD**. 2015. Surface-attached cells, biofilms and biocide susceptibility: Implications for hospital cleaning anddisinfection. J Hosp Infect **89**:16–27.

20. **Simões M**, **Simões LC**, **Machado I**, **Pereira MO**, **Vieira MJ**. 2006. Control of Flow-Generated Biofilms with Surfactants. Food Bioprod Process **84**:338–345.

21. **Elasri MO, Miller R. 1999,** Study of the Response of a Biofilm Bacterial Community to UV Radiation. Applied and Environmental Microbiology, May p. 2025–2031