Biofilms: What Are They, and What Challenges Do They Present to Health Care Facilities?

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When in school, you probably took a microbiology course with an associated laboratory. Lab experiments often involved culturing bacteria for further study. Culture growth was maximized by using nutrient-rich, liquid media incubated at ideal temperatures. The goal was to isolate and characterize free-flowing, suspended microorganisms.

This approach works well in a laboratory where the separation of individual species and strains of many organisms can be necessary. In the "real world," however, microbial life exists in a very different form. Bacteria, for example, live and flourish in environments with myriad other microbial forms. These habitats are called biofilms, and it has been estimated that 99% of all bacteria exist and thrive in biofilms.[1]

The following brief article highlights a few basic biological details about biofilms. Using that historical background information, our discussion will then focus on their adaptability and challenge these microbial accumulations present in health care settings, where patients and personnel may be exposed to potentially infectious biofilms.

What Are Biofilms?

The simplest definition of a biofilm is that it is a thin, slimy film of microorganisms, primarily bacteria, that adheres onto a surface. Microorganisms that come together in biofilms are irreversibly connected with a surface and are enclosed in an extracellular, primarily polysaccharide, matrix.[1,2] The matrix both promotes growth in a sessile environment and can also protect its inhabitants from external antimicrobial chemicals, such as disinfectants, UV light, and antibiotics. The discovery of biofilms is credited to Anton Van Leeuwenhoek, who used his simple microscope to first observe microorganisms he called animalcules within plaque scraped from tooth surfaces around 1676.

Besides occurring in natural water sources, biofilms also form on a wide variety of other inanimate and tissue surfaces, including man-made industrial and potable water systems, medical devices, and dental water delivery systems. Representative sources are shown in **Figure 1**. Examples of devices and systems in health care facilities that have been linked to biofilms and waterborne infections are presented in **Figure 2**.

Figure 1. Examples of Biofilm Sources

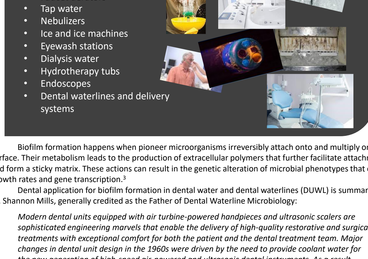
Industrial
- Oil pipelines
- Processing lines
- Cooling towers
- Hydrotherapy tubs

Dental Devices
- Water delivery systems

Medical
- Catheters
- Endoscopes
- Sinks/Faucets
- Shower heads
- CPAP units

Natural Surfaces
- Mammalian mucosa
- Heart valves
- Plaque

Figure 2. Health Care Facilities as Potential Sources of Waterborne Infections



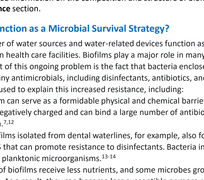
Biofilm formation happens when pioneer microorganisms irreversibly attach onto and multiply on a surface. Their metabolism leads to the production of extracellular polymers that further facilitate attachment and form a sticky matrix. These actions can result in the genetic alteration of microbial phenotypes that can alter growth rates and gene transcription.[3]

Dental application for biofilm formation in dental water and dental waterlines (DUWL) is summarized by Dr. Shannon Mills, generally credited as the Father of Dental Waterline Microbiology:

> *Modern dental units equipped with air turbine-powered handpieces and ultrasonic scalers are sophisticated engineering marvels that enable the delivery of high-quality restorative and surgical treatments with exceptional comfort for both the patient and the dental treatment team. Major changes in dental unit design in the 1960s were driven by the need to provide coolant water for the new generation of high-speed air-powered and ultrasonic dental instruments. As a result, dental units contain lengths of narrow-bore plastic tubing that deliver compressed air to power handpieces and water to cool and irrigate the operative site. Although usually unseen by both clinician and patient, this maze of small-bore plastic tubing offers an optimal environment for the proliferation of complex microbial communities known as biofilm.[4]*
>
> *Since the phenomenon of bacterial colonization of dental water delivery systems was first described in 1963,[5] numerous reports have confirmed that water produced by dental units and other dental equipment can contain very high levels of bacteria. While most dental unit water samples exhibit colony counts ranging from 1,000 to 10,000 colony forming units per milliliter (CFU/mL), counts greater than 1,000,000 CFU/mL have been documented. In contrast, the standard established by the Environmental Protection Agency (EPA), the American Public Health Association (APHA), and the American Waterworks Association (AWWA) for potable and recreational water is only 500 CFU/mL of non-coliform bacteria.[4]*

Biofilms can form anywhere there is water and an appropriate substrate for microbial attachment. While they may appear to be a random, amorphous accumulation when viewed under a light microscope, assays developed and viewed within recent decades using tools such as Scanning Electron Microscopy (SEM) have been able to reveal a complex, organized ultrastructure (**Figure 3**).[6]

Figure 3. SEM of Established Biofilm in DUWL

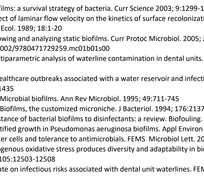


Structures include multiple canals that allow for nutrients to circulate and areas within the biofilm organization where microbial metabolism and growth can greatly vary. This is exemplified by observations showing that bacteria in the upper biofilm layers multiply faster due to their access to nutrients, while those near the attachment surface have more difficulty and thus metabolize and replicate slowly.[7]

A variety of other structures are also detectable and contribute to biofilm growth, such as extracellular polymeric substances (EPS), bacterial flagella, fimbriae (i.e., pili), and outer membrane proteins. Flagella on motile bacteria enhance the early stages of bacterial attachment onto surfaces. Studies have suggested that by facilitating microbial attachment, motile bacteria are able to attach and colonize more rapidly than nonmotile bacteria.[8] EPS is the major matrix component in biofilm and contains high concentrations of water. This is important because EPS hydration can prevent biofilm desiccation. The thickness of EPS is also variable within different biofilm regions, as the matrix can be concentrated more around bacterial cells and microcolonies and less densely distributed in spaces between these microcolonies.

Occasionally, portions of a biofilm can become fragile due to mechanical reasons or possible nutrient problems. The result is some bacteria are released from the biofilm into the water source and can be found as free-flowing, planktonic organisms (**Figure 4**).[9]

Figure 4. Portions of Mature Biofilm Breaking off in a DUWL

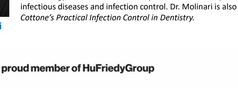


How Rapidly Can Biofilms Develop in DUWL?

The time course for attachment onto tissue and inanimate surface differs depending on the organisms involved and the empirical assay used to follow biofilm progression. In vitro static biofilm systems, for example, are particularly useful when studying early stages of biofilm formation, including initial adherence to the surface and microcolony formation. Many commonly studied bacteria can initially form a biofilm within 48 hours.[9]

The resultant biofilms containing multiple interacting bacteria grow rapidly if not removed, and an investigation of biofilm dynamics has indicated that bacterial counts can reach or exceed 200,000 CFUs of heterotrophic bacteria/mL (CFU/mL) within five days.[10] **Figure 5** shows sagittal sections from a DUWL to visually demonstrate the speed of microbial proliferation in tubing with narrow lumens.

Figure 5. Biofilm in a DUWL After Installation and 3 Weeks of Use



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For more detailed information on the composition and structure of biofilms, refer to the articles included in the **Reference** section.

How Do Biofilms Function as a Microbial Survival Strategy?

Unfortunately, a number of water sources and water-related devices function as reservoirs for a variety of waterborne infections in health care facilities. Biofilms play a major role in many of these documented outbreaks.[11] One aspect of this ongoing problem is the fact that bacteria enclosed in biofilm communities can become resistant to many antimicrobials, including disinfectants, antibiotics, and even antibodies. Several hypotheses have been used to explain this increased resistance, including:

1. A thick EPS in biofilm can serve as a formidable physical and chemical barrier, preventing their penetration. In addition, EPS is negatively charged and can bind a large number of antibiotic molecules before they reach susceptible bacteria.[7,12]
2. Investigation of biofilms isolated from dental waterlines, for example, also found that different bacteria can produce certain EPS that can promote resistance to disinfectants. Bacteria in a biofilm ecosystem can be far more resistant than planktonic microorganisms.[13-14]
3. The deepest layers of biofilms receive less nutrients, and some microbes grow slower, or even recede into a semi-dormant state. As a result, they can become less susceptible or more resistant to antimicrobials.[15-18]
4. Biofilms contain millions of heterogenous microorganisms in confined environments. Microbial multiplication under conditions where antimicrobial metabolites (i.e., hydrogen peroxide, hypochlorous acid) are also generated by other bacteria in close proximity to each other can increase the chances for genetic mutation during replication. Acquired resistance to selected antibiotics was found to be one of the resultant possibilities.[17-19]

We want to thank Dr. Molinari, and we invite you to evaluate our wide range of products designed to protect clinicians, patients, and the practice during dental procedures. For more information, visit palmerohealth.com, call 800-344-6424 or email customerservice@palmerohealth.com.

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