Biofilms are often considered something new, but they are not. Biofilm is known by many other names, such as dental plaque or even shower slime. It was first scientifically described by Zobell and Anderson in 1936. During their study of seawater, they found that nutrients can become concentrated on a solid surface and allow the formation of what they described as micro-organic films. Today, we call these structures "biofilms," and we understand more about the complicated ways in which microorganisms interact and even cooperate within biofilms. Biofilms are therefore simply the natural growth of microorganisms on or at a surface. As biofilms naturally harbor (and are made up of) microorganisms, great attention has recently been given to understanding and determining how to eliminate this microbial threat.

Biofilm will form anywhere there is a surface and a liquid, whether in a water pipe, the wet lumen of an endoscope, or in the valves of the heart. Biofilm can be formed by many types of actively growing microorganisms, such as bacteria, fungi, algae and protozoa. It can be as thin as a few layers of cells or thick enough to completely seal up a channel that is several inches wide. Biofilm is highly varied and rarely made up of a single species of microorganisms. Typically, many types of microorganisms live and cooperate within a biofilm; it is, in fact, a billion-year-old mechanism used by microorganisms to survive in those environments where something is trying to wash them away. Microorganisms, like all forms of life, have a simple goal: to survive and reproduce. Forming a persistent, protective biofilm helps them do this. Obviously, this also has the effect of helping them survive efforts at cleaning, disinfection and even sterilization.

The one key component that helps biofilm remain on a surface is production of a sticky matrix in which the microorganisms live. This matrix, also known as an extracellular polymeric substance (EPS), is the glue that holds biofilm together and keeps it attached to a surface in a liquid environment. Once the microbes have attached to the surface and, while growing, have produced the matrix to help keep them there, other microorganisms join and the complexity of the biofilm increases. These first steps can take place in just a few hours. Then, over the next few days the biofilm continues to mature. The microbial cells start to interact with each other as the attached bacteria reproduce and work together for survival. The microbes can use chemical signals to communicate to each other within the biofilm, as well as allow parts of the biofilm to detach and start a new biofilm elsewhere. Once a biofilm reaches this mature point, it becomes very difficult to eradicate.

The four steps of biofilm formation can be described as attachment, aggregation, formation and detachment (Figure 1). During the attachment phase, microorganisms (in particular bacteria) attach to a surface where nutrients that allow them to grow have accumulated, such as a small scratch or crevice on a device surface or water pipe. After attachment, the bacteria begin to divide and other microorganisms can join the biofilm during the aggregation phase.

Learning Objectives
1. Define what biofilms are and their impact on reprocessing.
2. Describe steps in the management and prevention of biofilm development during reusable device processing.
3. Describe how to choose products that can reduce the risks associated with biofilms.
These microorganisms can also multiply and protect themselves in the biofilm matrix. Finally, after the biofilm has fully developed into a well-established matrix, the microbial cells will begin a programmed detachment that will release viable microorganisms to seek a new location to begin the entire process over.

Biofilms can pose problems in many different areas in healthcare facilities, including water supply systems, environmental surfaces, and dental lines, as well as on medical devices such as stents, catheters, implants, and reusable devices that contain lumens. Consider, for example, the inside of a lumened device. Following clinical use these areas can be damp, soiled, and contaminated with microorganisms from the patient procedure, offering an ideal opportunity for biofilm to develop. This is a particular concern if the device is left in this state for a long time (hours to days). If a mature biofilm is allowed to form within the lumen of such devices and they are then not correctly processed, there is a real possibility that microorganisms can persist there, and subsequently cross-contaminate another patient, posing a risk of infection. Other risks can include damage to devices (by producing chemicals that attack device materials) and the production of toxic substances (such as endotoxins) that can affect patients.

**How to prevent biofilm**

With the ability to form wherever there is a liquid and a surface, it is difficult to totally prevent microorganisms from forming biofilms. Bacteria and other microorganisms will always do whatever they can to survive. A medical device surface in combination with water or another liquid solution, especially a nutritive solution such as blood or mucus, provides a perfect environment for these microorganisms to adhere and multiply, forming a biofilm. Further, the biofilm’s protective matrix shields the microorganism and presents a challenge to cleaning and antimicrobial agents, making successful device processing much more difficult.

Due to the complexity of biofilm and its cycle of development, there is no one single step that can be used to reduce the risk of biofilm formation. Since biofilm can develop at any stage of the device reprocessing cycle, it is important to employ a holistic approach and use best practices at all stages of reprocessing to be the most effective (Figure 2).

One of the most important steps in controlling biofilm risks is to prevent its formation from the start. Since biofilms develop best on a moist nutrient-rich surface, any prevention, remedial or eradication regimen must include disruption of these conditions. Biofilms can be formed within a few hours. Device processing, particularly cleaning, should then be conducted as soon as possible after use. Allowing soil to remain on a device under moist conditions for extended periods of time before cleaning is a perfect situation to allow biofilm to develop. This risk can be reduced by removal of evident soil at the point of use and also by the use of safe transport chemicals that are designed to prevent the growth of bacteria/fungi and initiate the cleaning process.

When biofilms do form, they can be best controlled at younger stages of development than in maturity. Cleaning is an integral part of reducing the formation of biofilm. Marion et al. recommends the use of an effective and efficient cleaning agent prior to high level disinfection or sterilization as part of a preventive regimen used to reduce the risk of biofilm formation. Best practices should include a step that ensures that the devices are cleaned using a cleaning agent that not only penetrates the hard-to-clean crevices and joints where the microbes lurk, but also one that can penetrate and destroy the protective extracellular polymeric substances matrix formed by the biofilm.

High quality enzymatic detergents can be effective and efficient cleaning agents when specifically designed to penetrate, solubilize and remove biofilms from surfaces. When used according to manufacturer’s instructions, they will strip away the extracellular matrix and expose any remaining bacteria or other microorganisms to the lethal effects of high level disinfectants or sterilization processes. During this process it is important to select for use well-formulated cleaning agents specifically designed for medical device cleaning. An example of biofilm removal with such a cleaning process is shown in Figure 3. It is important to remember that cleaning is a multi-step process that generally includes pre-cleaning, cleaning, and thorough rinsing to ensure an effective process. The cleaning process should also include a mechanical step (such as brushing, use of ultrasonics, etc.) that will aid in the physical removal of soil and biofilm. Cleaning alone should not be assumed to completely eliminate the risk of biofilm, as the reprocessing cycle must include adequate disinfection and/or sterilization after cleaning.

Overall, it is important that the steps employed to control biofilm include disruption of the biofilm through effective cleaning followed by a high level disinfection or sterilization step to kill any remaining microorganisms on or in the device. If biofilm is not adequately removed the effectiveness of the terminal disinfection or sterilization process may be at risk. The presence of biofilm can even compromise sterilization processes.

Unlike critical devices that require sterilization, high-level disinfection can be employed on semi-critical devices in many instances where a sterilization process is not practical or suitable. In such instances, chemical high level disinfection can be used for heat labile devices. Chemical disinfectants may be aldehyde-based solutions, such as glutaraldehyde and OPA, or oxidative chemistry based solutions, such as those utilizing hydrogen peroxide or peracetic acid. Oxidizing agent based high level disinfectants have in some cases been shown to be very effective at removing biofilm through their oxidative chemical activity, in contrast to aldehyde-based disinfectants that may fix or cross-link the residual biofilm materials and thereby promote the development and persistence of the biofilm (Figure 4). FDA-cleared high level disinfectants and sterilants can do an excellent job at killing microbial pathogens, but it is critical that prior to the disinfection or sterilization step measures are taken to release microbes entrapped within biofilm by achieving thorough cleaning.
There is an additional risk with use of high level disinfectants, in that water of variable quality can be used to rinse devices following disinfection. The number of water rinses can range from one to six rinses, depending on the specific label claims of the disinfectant. Untreated water pipes can contain biofilm and therefore water passing through these pipes can become contaminated with microbe-bearing particles shed from the biofilm, and transferred to the processed medical device during rinsing. Rinse water quality should be therefore be carefully controlled to reduce the risk of cross-contamination. In addition, although the level of contamination may be low, if the device is not dried prior to storage dampness can allow for the development of biofilm over time in and on the device. Therefore, such devices — in particular flexible endoscopes — should be stored dry.

**Selecting products that are effective against biofilm**

Selecting products that are effective against biofilm can be difficult. The Environmental Protection Agency (EPA) regulates products used as disinfectants on environmental hard surfaces. The Food and Drug Administration (FDA) regulates products used as cleaners, disinfectants, or sterilization processes on medical devices. While both agencies are actively evaluating how to recommend best methods of cleaning, disinfection or sterilization as part of a rigorous device processing cycle. It is important to take care when interpreting any biofilm-related claims. Cleaning products such as enzymatic detergents can be evaluated for their ability to clean biofilm from surfaces to prevent mature biofilm formation, as shown above, but they are not evaluated for any anti-microbial capability. Cleaning products can range dramatically in their cleaning effectiveness, as well as their ability to remove biofilm-related substances from surfaces and in the presence of differing qualities of water. Product claims should therefore be closely inspected and supported with data. For disinfectant products, there is no standard methodology for evaluating a disinfectant’s ability to kill organisms trapped within a biofilm. Methods to test products against biofilm have been developed and continue to be validated through consensus method development teams in conjunction with the regulatory authorities in the USA. FDA-cleared high level disinfectants and sterilants are, of course, rigorously tested against multiple classes of microorganisms, including those that may cause biofilm, but it is still not fully understood what the impact of an improperly processed medical device can have on disinfection success if the conditions are right for the formation of a highly mature biofilm. Similarly, sterilization processes are rigorously tested and provide robust systems for microbial inactivation, but are not specifically tested for effectiveness in the presence of biofilm.

As is often the case when evaluating complex and diverse species of microorganisms, there is no one magic method for success in preventing or eradicating biofilm. No one method of cleaning, disinfection or sterilization would be expected to be effective. But each of these methods can be effective as part of a well-controlled device processing cycle. Just as important as product selection and proper use, one must always follow recommended guidelines on device reprocessing to achieve decontamination and disinfection/sterilization of medical devices. Like recommendations to brush and floss frequently to prevent the development of bacterial plaque in the mouth before it gets to the point that only painful scraping will remove it, proper care and handling of devices during reprocessing will prevent biofilm from eventually causing a problem for a patient.

**Conclusions**

Biofilms are simply naturally growing microorganisms. These complex system of microbial interaction simply uses extracellular polymeric substances (EPS) as a glue to help the diverse community of microorganisms survive in harsh climates. These same survival traits can make biofilm very difficult to remove and inactive once it becomes mature in or on a medical device. It is important to take the proper transport and cleaning steps to prevent mature biofilm from forming. Further, cleaning must effectively remove biofilm so that it can be followed by effective high level disinfection or sterilization as part of a rigorous process to ensure devices are rendered safe and effective for reuse with each and every reprocessing cycle.

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Biofilm and the reprocessing of medical devices

Circle the one correct answer:

1. What statement best describes biofilm?
   A. Single-celled planktonic species circulating in a liquid environment
   B. Aggregation of microorganisms easily eliminated by rinsing the devices
   C. A population or community of microorganisms encased in extracellular polymeric substance matrix
   D. None of the above

2. What is the function of the extracellular polymeric substances (EPS)?
   A. Is a key component for the aggregation of microorganisms in a biofilm
   B. It shields microbes and offers resistance to antimicrobial and cleaning agents
   C. Is the glue that holds biofilm together
   D. Keeps microbes rigorously attached to a hard surface in the liquid environment
   E. All of the above

3. What are the four phases of biofilm development?
   A. Attachment, aggregation, formation, detachment
   B. Attachment, replication, sentience, spread
   C. Synthesis, resistance, chemotaxis, modulation
   D. Growth, lag, stasis, death

4. The ___________ forms a strong adhesive and protective matrix that shields the microbes and offers resistance to antimicrobial and cleaning agents.
   A. Upper polysaccharide substances
   B. Extracellular polymeric substances matrix
   C. Bacterial cell wall
   D. Dead bacterial cells

5. Microbes in a biofilm use chemical signals to communicate to each other and guide various processes.
   A. True
   B. False

6. At what stage of the device reprocessing cycle are biofilms likely to develop?
   A. Transport stage
   B. Cleaning stage
   C. Packaging stage
   D. All of the above

7. Mature and complex biofilms on a solid-liquid interface are easier to clean or eradicate because they are approaching their death phase.
   A. True
   B. False

8. Standard methods have been approved by both the EPA and FDA to evaluate disinfectants’ ability to remove and inactivate biofilm.
   A. True
   B. False

9. The best practices to combat biofilm on medical devices should ensure that the devices are
   A. Cleaned with an effective and efficient cleaning agent
   B. Appropriately disinfected or sterilized
   C. Completely dried
   D. All of the above

10. Biofilm is a newly emerged “superbug.”
    A. True
    B. False

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