The Nature of Microbial Biofilms

Proper decontamination, cleaning and disinfection will make steam sterilization safer.

By Dr Mikael Zimmerman DDS, PhD, associate professor

Introduction

Biofilm is a microbial community characterized by cells that are attached to a surface or to each other and that are embedded in a matrix they have produced.

When the bacterial cell is free-floating and alone, it must carry out all necessary activities by itself. The biofilm functions as a large community or an organ where different tasks are carried out by different groups of cells, which all help each other survive and multiply.

The biofilm constitutes a highly effective defense barrier. Bacterial cells in the biofilm are protected from disinfectants, temperature changes, pH variations, drying, antibiotics and a host defence in the form of the human immune system.

Micro-organisms free-floating in liquid (water) are referred to as planctonic micro-organisms. Most research and testing of the effects of different disinfection methods are carried out on planctonic micro-organisms.

For chemical disinfectants to have a lethal effect on micro-organisms embedded in a well-organized biofilm, the concentration must be increased several thousand times compared to killing the very same micro-organisms in a free-floating, planctonic phase. This is not only the case with chemical disinfectants, the same circumstances are important to consider for several other antimicrobial activities, for example antibiotic therapies. All antimicrobial activities will have the best effect on micro-organisms in a planctonic phase before the biofilm will be established. Once the biofilm is well organized, any antimicrobial activity or agent must be increased in strength and/or concentration. In order to interfere with microbial life, or in other words to kill bacteria, the best action will be to destroy the biofilm. The individual micro-organisms will then lose their protection and once again be in a vulnerable planctonic phase.

This article will cover some of the basic functions of bacterial biofilms in order to highlight why cleaning and decontamination are so extremely important.

If items are not ultra-clean – sterilization will not be effective

For the above-mentioned reasons, bacteria always strive to create a well-organized biofilm with optimal functions. This rapid process in a humid environment will be established within seconds, will get organized in minutes and will be well-established with all necessary functions within a few hours. This is one of the reasons why instruments and articles must be cleaned and decontaminated as soon as possible after use.

Bacterial biofilm should not be confused with the bioburden of medical devices, even though biofilm is a kind of bioburden. The bioburden is more often than not defined as the microbiological load, for instance the number of contaminating organisms in the product/item prior to cleaning, disinfection

and sterilization. However, bioburden can also be biological materials such as blood, mucous fluids, saliva, etc.

Medical devices that require sterilization or disinfection must be thoroughly cleaned to reduce organic material and to destroy and remove bacterial biofilm. If items are not ultra-clean, sterilization will not be effective.

It is of utmost importance to fully understand that the mechanical removal of bacterial biofilms will actually be more important than sterilization itself! Even if the sterilization procedure should result in a total elimination of all living forms of micro-organisms in biofilms on instruments, many other harmful/toxic substances in the biofilm can be pernicious to patients, personnel, instruments and the environment.

Survival and procreation

Nature is calibrated on survival and procreation. This means that the most basic functions or needs of all forms of life will be to get a sufficient amount of nutrients and to be enough protected to be able to produce subsequent generations. In a human being, the genome (DNA) is securely encapsulated in the nucleus of the cells, and each cell includes its own copy of this enlocked information, i.e. an identical copy of the total encoded information for all functions, for instance activities and heritage for every single cell in an individual. Dealing with micro-organisms becomes so much easier when we recognize that human beings and micro-organisms have the two basic principles of survival and procreation in common. Like human beings, every single micro-organism is unique with its own genetic bank of information: the DNA.

Why bacterial cells will die and human cells will live

There is a fundamental difference between human cells and bacterial cells. In a human cell, called the *eucaryotic* cell, the DNA is securely encapsulated in a safe vault, the nucleus. In the bacterial *prokaryotic* cell, the DNA is floating around freely in the plasma (interior).

When the genetic material, which is also the focal point of total control, is locked into the nucleus, it is more difficult to read necessary information. The information in the DNA strain has to be read by minor “messenger molecules”, i.e. enzymes that can later transport the message outwards through the membrane of the nucleus to different functions within the cell. The advantage is that this process will make the human cell more stable and secure.

Having the DNA floating freely within the cell will facilitate immediate ordering and action—the prokaryotic DNA does not need to send the message by messenger enzymes. The great advantage is superior possibilities for rapid adaptation to different surroundings, but the price to be paid is vulnerability.

Another major and most decisive difference is that within a human cell there are “micro-cells” or so-called organelles which have very specific functions: power-station organelles, fat-storage organelles, protein-producing organelles, etc. The bacterial cell is carrying out the same survival actions as the human cell, but since it has no organelles (with a few exceptions), all important cell functions are being carried out within the cell membrane (the “eggshell”) around it.

The cell membrane is the contact area with the surroundings. In the human cell it is just an eggshell, but in the bacterial cell it contains all life-supporting activities. It is very easy to interfere with the

bacteria by disturbing or destroying the cell membrane, which is exactly what is done by the use of antibiotics, chemical disinfectants, etc. The human cells will not be affected by this kind of treatment, because the life-supporting functions are protected within the organelles, but the bacterial cell will die. In order to be much more adaptable to survive under many different and sometimes difficult conditions, the bacterial prokaryotic cells have to pay a high price in vulnerability.

Interfering with basic bacterial functions

Understanding all of this will make it very easy to prevent bacterial presence– it all comes down to destroying the possibilities for survival and procreation. Just by keeping an area clean, most of the "foodstuff" will be removed, thus interfering with bacterial nutrition.

The use of a strong enough chemical will destroy the cell membrane and thereby also nutrition intake and other important cell functions. These actions, however, have to be fast and effective enough to prevent the bacteria from the possibility to adapt to harsher living conditions.

Antibiotics have several different modes of action. The vast majority of actions influence the bacterial cell membrane by destroying functions for either nutrition or procreation or for both functions, thereby killing the bacteria slowly over time. One of the major problems with this slow killing is that this will give bacteria a possibility to counter-attack by adapting to altered living conditions and develop antibiotic resistance.

Genotype or phenotype

In all forms of procreation, there is an important difference in *genotype* and *phenotype*. The genotype is the genetic information (DNA) transferred from one generation to the next. The *phenotype* is the result of how the original *genotype* can become altered and influenced by a large variety of factors during life time. *Staphylococcus aureus* represents a *genotype* of a specific type of bacteria, and all strains of *Staphylococcus aureus* will have a lot of features in common, but there will be strains of this bacteria being more or less aggressive, more or less disease-causing, more or less sensitive to disinfection processes and more or less antibiotic-resistant. All of this depends on the surroundings which the species have adopted to: the *phenotype*. Human beings will receive the *genotype* once and from either one of two individuals: when the sperm reaches the egg cell at the moment of conception. Bacteria, on the other hand, multiply by dividing whereby the next generation gets its *genotype*, at first, from one individual. But bacteria can add on new *genotypes* during a life span! It has been shown that 15-20% of bacterial DNA is present in biofilms outside the bacteria itself (plasmids), which makes it possible for any bacterium to pick up useful *genotypes* and thereby alter its own possibilities. A well-known resistance gene is ESBL (Extended Spectrum Beta Lactamas), which often and wrongly is described as ESBL-bacteria. ESBL is an enzyme, or a piece of DNA, that can be found in bacterial surroundings, such as biofilms, and can be picked up by many bacteria thereby altering their *genotype* and becoming antibiotic-resistant. ESBL is just one example of many such factors.

Bacterial cell phones and calibrated action

Bacteria communicate with each other by using enzymes or hormones called auto-inducers. Recently, auto-inducers have been found to start a phenomenon called *Quorum sensing*. To make a certain thing happen, every single bacterium of the same strain will produce a small amount of a specific auto-inducer informing others what to do. The interesting and somewhat scaring fact is that by sensing the concentration of auto-inducers, this group of bacteria will know when they have

reached exactly the required minimal number of bacteria, and then they will all start the very same action simultaneously. They do not only communicate with each other, but they can also send signals to our human cells. For example, the bacterium *pseudomonas aeruginosa* has been found to produce auto-inducers that can turn the human immune system on and off. Once the bacterial biofilm has been established, it will function as a well-organized society where bacteria communicate by auto-inducers, will share genetic useful information through plasmids and will gain an enormous powerful action potential by Quorum sensing.

Selection of species

Dental plaque is a very interesting biofilm. It is a well-known fact to the dental profession that high sugar consumption in individuals colonized with *Streptococcus mutans* will promote dental caries. But at the same time, a high concentration of sugar is a well-known method for food preservation that hinders bacterial growth and rotting. Logically, a high sugar concentration in the dental plaque ought to work the same way and kill the bacteria in that biofilm. This is actually the case except for *Streptococcus mutans,* which by producing acid to lower the pH in the plaque will be protected against the killing capabilities of a high sugar concentration. This acid production action is controlled by auto-inducers and *Quorum sensing*. Similar situations can be created by using antimicrobials in a wrong way. In a certain bacterial population, which is being subject to lethal processes such as for example disinfection, the most vulnerable bacteria will die first, and the most resistant bacteria will survive until the bitter end. It does not have to be different species of bacteria, but could simply be different strains of the same species. The stronger surviving ones will start multiplying and establishing a subsequent and more resistant population. This is another type of selection which one must be very careful with from an infection control perspective.

Bacterial toxins – potent and dangerous

Bacteria per se do not cause diseases. To survive and have the possibility to procreate, the bacteria simply need food. To get nutrients, the bacteria excrete enzymes and exotoxins into the surrounding environment. The exotoxins will break down tissue and make it possible for the bacteria to absorb and digest fat, proteins and carbohydrates. Exotoxins usually have a local effect, but some toxins are extremely powerful and can be spread to the whole host body system.

Exotoxins are poisons excreted by living bacteria. Some bacteria, especially the gram-negatives, will include toxins in their cell membranes, the so-called endotoxins. When the bacteria are engulfed by our immune cells the endotoxin will be released inside the immune cells and actually kill them. Endotoxins can also be remnants of micro-organisms or parts of the cell walls with poisonous substances released when the bacteria disintegrate. If an object coated with a large mass of endotoxin-containing bacteria is sterilized, the sterilization processes will actually result in the release of endotoxins!

Endotoxins and exotoxins do not multiply and are normally harmless, but if they are allowed to enter the body system (such as the blood stream), they will produce toxic effects. If the endotoxic level from gram-negative bacteria is high, it could kill the patient due to the widening of blood vessels and drop in blood pressure. Toxins can also reduce the body's defence against infections, as both bacterial enzymes and toxins are important for the spreading of disease-causing bacteria in the tissue.

The importance of proper cleaning and disinfection prior to sterilization cannot be emphasized enough. Standard methods of sterilization, such as autoclaving, have none or little effect on bacterial toxin levels.

Sterility does not mean clean and safe

Sterility is defined as the total absence of any reproducing micro-organisms. There is confusion in the use of the term sterile; in the area of hygiene and infection control, it often refers to a medical device being clean, safe and free of disease-causing microbes. It is of great importance to fully understand that the term sterile means nothing less than not being able to reproduce.

In the internationally accepted definition, sterile items should have a sterility assurance level (SAL) of $SAL=10^{-6}$.This is often misinterpreted as only one living microorganism in one million sterilized items. The correct interpretation requires some clarification.

The normal measurable contamination level of a used instrument is around 1 million micro-organisms (10^6) per square centimeter. Let us suppose that the sterilization process starts with an instrument contaminated with 10^6 micro-organisms. If we presume that the sterilizer is filled with a number of instruments having the same contamination level initially, and some instruments are picked out at regular intervals during the sterilization cycle to measure the remaining number of living micro-organisms, then with an increased sterilization cycle time, the number of living micro-organisms becomes lower. This can be measured until there are no living microorganisms left. The number of living micro-organisms will now be zero. If it takes X minutes to reach the point when no living (viable) micro-organisms can be detected during which X minutes one can actually measure the reduction in the number of remaining living microorganisms, then by doubling that time to 2X minutes in a mathematical calculation, another 10^6 microorganisms would be killed in theory, but now continuing from a presumed level of zero microorganisms ending up with 10^{-6}. The extra time, to achieve the killing of microorganisms beyond zero, is a margin of safety that forms the basis for calculating sterilizer cycle times.

Having the same length of sterilization time, but starting with a cleaner instrument with only 10^1 (ten) microorganisms, the sterilization process will result in a much larger safety margin. To ensure proper and complete elimination of all biological materials prior to sterilization, it is crucial to control and handle the whole process of decontamination – cleaning and disinfection – correctly.

Removal of fat and proteins

Instruments and articles used for medical purposes will be soiled with proteins (blood, serum albumin, saliva etc), lipids (body fats) and carbohydrates. Gram-negative bacteria are much fattier (contain more lipids in the cell membrane) than gram-positive bacteria, where the cell membrane mostly consists of protein and carbohydrates. As a consequence, the gram-negative bacterial biofilm will be fattier, and the gram-positive bacterial biofilm will be more protein-rich. In the oral cavity, the dental plaque (biofilm) above the gingival sulcus (on the supra-gingival tooth surface will be more gram-positive, and the plaque (biofilm) in the gingival sulcus (on the sub-gingival tooth surface) more gram-negative (fat). So, dental instruments will be soiled with both fat and protein biofilms.

Proteins require low temperatures in the cleaning process, since they will coagulate at high temperatures. Coagulated proteins can become a protective shield for other contaminants. As a contrast, lipid components (fat) require high temperatures to be dissolved.

The sterilization process adds the wrapping

If correct handling, proper validation, process control and maintenance of the washer-disinfectors are maintained, the washer-disinfection process will actually result in sterile instruments – since the washer disinfector should be able to remove all biological material. No remaining biology means nothing left that could possibly reproduce. Correct handling also includes careful loading of the articles and instruments that should be processed in the washer disinfector.

The major difference between washer-disinfectors and sterilizers is that washer-disinfectors remove bacterial exotoxins and endotoxins and breakdown products on items as well as chemical residuals.

The major disadvantage of washer-disinfectors is that even if the process results in sterile products, the problem is how to get them out of the washer-disinfector in a dry and sterile condition and also how to maintain sterility. Logistics, transport and storage make it necessary to add a safe wrapping to washer-disinfected items. What the steam sterilizer really adds is the possibility of packaging the goods. The requirements for sterile instruments are that they must be sterile the very moment they are to be used, and they must therefore be packaged in special, close-fitting packages that do not allow penetration. All packaged/wrapped goods require sterilizing in steam-sterilizer processes with pre-vacuum and post-vacuum cycles. The wrapping must be of such a quality that nothing will grow and multiply and possibly contaminate the instruments inside during transport and storing.

Be careful with chemicals

Chemical disinfectants can be useful in carefully selected situations. For instruments and similar articles, chemical disinfectants must never be an alternative to disinfection with moist heat in any situation when it is possible to use moist heat. In most cases, chemical disinfectants can only kill micro-organisms, not remove a biofilm. There is also a problem in the removal of chemical residuals on the articles without recontamination.

Automated processes—the most reliable option

Physical cleaning is the most important step in a disinfection and sterilization process. Physical cleaning means the use of mechanical (kinetic) energy, the best being water with a rapid velocity and under high pressure. The aim is to remove residual bioburden and biofilm from all surfaces of the instruments/articles. This must be done without harming and destroying the surface of the items.

Cleaning involves the removal of organic substances and other residues from a surface or item, but cleaning as such has no microbial killing action and should therefore not be confused with decontamination and disinfection. However, certain cleaning procedures, e.g. in washer-disinfectors, also include decontamination and disinfection.

The physical cleaning per se does not remove the bio-burden. The most important function of the physical cleaning is that it damages the biofilm, tearing away parts of it and removing superficial layers of the biofilm. This facilitates penetration of the bioburden by the cleaning liquids of which the most important is water, because water molecules finally will remove the bioburden from the surface.

The process of manual cleaning must involve thorough scrubbing of all surfaces of the item and rinsing of the item in clean water, preferably running water. It has been proven that hand-washing of items in still water reduces the microbial bioburden. However, although the hand-washing procedure

is effective in reducing the microbial levels deposited on the surgical instruments, the risk of recontamination from micro-organisms in the water increases rapidly unless the water is changed frequently.

Automated processors offer the safest, most reliable option, provided they are suitably monitored and maintained.

Conclusions

On the basis of the knowledge about microbial biofilms and their development in general, certain preconditions have to be observed in relation to the work in dental clinics, and certain steps have to be taken to ensure complete sterile instruments for dental use:

- Sterilization only will **not**:
    - remove biofilm or bioburden
    - destroy exotoxins and endotoxins
    - result in an instrument safe for use if the instrument is not thoroughly cleaned, decontaminated and disinfected
- Sterilization only has little effect on plasmids that might involve factors such as resistance, virulence and pathogens.
- If instruments are not ultra-clean, sterilization will not be effective. Toxins as well as genetic plasmid material can continue to cause adverse and undesired effects. The sterilizing agent, saturated steam, will have difficulties penetrating any bioburden and/or bacterial biofilm, which will encapsulate microorganisms. The process will result in a non-sterile instrument.
- Decontamination, cleaning, disinfection and sterilization must be carried out in the right sequence, and each step meticulously.
- The wrapping of an instrument before sterilization is there in order to guarantee that instruments can be transported and stored under safe conditions to be sterile at the time and point of use.

With the knowledge at hand, and following the correct process delineated above as well as the points to be observed in particular, dentists do not have to see microbial biofilms as an insurmountable problem in their clinics.

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