PMF NEWSLETTER

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Letter from the Editor-in-Chief

This issue of the newsletter is important for two reasons. First of all, it contains an important review of water system biofilms by Mark Pasmore and Deborah Czarnecki. Many of the readers will remember Mark from his excellent presentation at the last PMF Biofilm conference. In this article Mark describes difficulties in sampling and testing for biofilm (rather than planktonic microorganisms), issues in controlling biofilm in water systems, and why this topic is one of concern to the pharmaceutical industry.

PMF Conference on Environmental Monitoring and Contamination Control - May 16-17 in Las Vegas, NV

The second item of note in this issue is that it is the first of our new *PMF Newsletter* editor, Robert Westney. Bob has graciously agreed to prepare this newsletter and will be working to improve the reliability of the newsletter's publication schedule.

Robert has over nearly 25 years of experience in the pharmaceutical industry, with experience in Quality Control Microbiology, Quality Assurance and Regulatory Affairs. He currently consults and is President of a specialty Contract Manufacturing Organization specializing in cryopreservation of in-house microbial isolates (http://www.cryologics.com).

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If you have an idea for an article or topic that would be of interest to the professional microbiologist, Bob wants to hear from you. He can be contacted at rwestney@cryologics.com.

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Controlling Biofilms in Pharmaceutical Water Systems: Lessons Learned from Clinical Water Systems

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Introduction

The term biofilm is becoming an increasingly recognized part of the scientific vernacular. It is used in many presentations and publications involving topics ranging from oil remediation to medical infections, and the breadth and scope of the biofilms topic seem to grow almost as fast as the use of the word. What is it about these microbial communities that creates such interest and what do we need to know to understand them?

In the medical field, the interest is largely associated with the ever increasing list of infections involving biofilms and the challenges related to controlling these highly tolerant and ubiquitous modes of life. These challenges create problems in not only clinical settings, but also in all of the industries that support clinics and use water in the development of their products (1). This is especially true for the pharmaceutical manufacturers that make injectable products, which require not just sterility, but also strict control of bacterial endotoxin and other pyrogens.

The risks related to infection and immune response to pyrogens have caused the pharmaceutical industry to hold themselves to rigorous standards of control of their water systems (2). However, as a community, pharmaceutical manufacturer's adherence to intellectual property requirements and proprietary operations often don't allow them to share information on microbial control strategies. Additionally, the data from contamination events are seldom published in publically available literature, making understanding the extent of the problem and controlling it all the more challenging. Biofilms are, however, a fact of life, and an improved understanding of these pervasive and persistent forms of microbial life can lead to improved control.

As a means to improve discussion of this topic, this article will compare pharmaceutical water system with those of clinical hemodialysis water systems, where published literature on biofilm and biofilm control exists (3). Hemodialysis water systems make a good comparison with industrial systems in that they both must deliver large quantities of pure water for the preparation of pharmaceutical/medical treatments (4). This comparison will also provide a means to relate the discussion of control biofilm in manufacturing systems to potential patient issues and outcomes.

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Background

Biofilm Basics

The increased use of the term 'biofilm' throughout the medical community has reduced the need to define the term; however, since different topics use slightly different definitions, this discussion will use the working definition: microbial communities adhered to a surface and held together by a polymeric matrix of their own synthesis.

The term community is a very important part of the definition. Microorganisms in biofilms are capable of responding as a collective to both stresses and opportunities, making them distinctly different from their planktonic counterparts. In these communities, members differentiate themselves to take various roles depending on nutrient availability, chemical signaling, and location in the biofilm. This differentiation of the microorganisms, in combination with the communal response and the large numbers of organisms typical of biofilms, creates various means for the microorganism to survive stresses to the biofilm (5). An example to illustrate the relevance of biofilms would be the tolerance of biofilms to antibiotics. In order for an antibiotic to be effective against a biofilm it must:

1) be effective against all the differentiated modes of growth and species present in the biofilm [some antibiotics are specifically effective against specific type of organism and/or specific targets such as rapidly dividing cells, and biofilms are usually composed of a wide variety of growth rates and species],

2) penetrate the biofilm [some compounds have difficulties penetrating the biofilm because they are attracted or repelled by the biofilm, or because they are consumed in reactions with the outer portions of the biofilm],

3) be effective within the local environment [biofilms alter their local environment often affecting such things as local pH and Oxygen concentration, both of which can change the efficacy of antibiotics],

4) reach the cells at concentrations sufficient to achieve kill [because of the altered state of some biofilm cells, lack of penetration, and/or changes in local environment; the concentration needed to treat biofilm cells is often significantly greater than that need to treat planktonic cells],

5) be sufficiently rapid so as not to allow adaptation or selection [in some cases, the communal response of biofilm allows the biofilm to change behavior or local environment to protect themselves from treatment; for example the elevated production of catalase by *Pseudomonas* in bio-

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films neutralizes low concentration peroxide treatment, protecting not only the Pseudomonas but also its neighbors.

These communities can establish themselves and develop from a single microorganism, leading to what are usually large microbial populations that tend to be heterogeneously distributed in population, structure, surface coverage and physiology (6). Like many other ecosystems, as the number of different species of organisms increases so does both the heterogeneity as well as the robustness/tolerance of the biofilm. These properties of high population, heterogeneity, tolerance and communal response to stress all create challenges for dealing with biofilms as is discussed in the following sections.

Comparing Manufacturing Water Systems to Clinical Water Systems

The natural reaction to hearing about the challenges that biofilms present is to ask "Is this an issue in my system?" Unfortunately the answer is inevitably yes. Every water system is exposed to microorganisms, and given time those microorganisms will form biofilms. This is illustrated in clinical hemodialysis systems, which are highly controlled water systems similar to aseptic processing systems used to fill many pharmaceuticals.

Hemodialysis systems operate by purifying municipal water to a very strict set of criteria and then mixing that water with components (usually sterile acid and base) to create dialysate. The dialysate is then used to treat renal patients' blood via transfer of waste across a specialized membrane termed a "dialyzer". These systems bear striking similarity to aseptic processing systems, in which water is brought in and purified to create water for injection (WFI) that is mixed with high purity raw materials to create the pharmaceutical formulations, which are then sterilized by filtration prior to aseptic filling.

Biofilms have been detected throughout hemodialysis systems, including the inlet lines, purification operations (deionization systems, purification membranes, etc.), the distribution lines, the dialysis machines, and the lines connecting the machines to the dialyzer. The only place where there are still questions as to clinical biofilm formation is on the dialyzer membranes, which are single use and/or receive the most rigorous disinfection. As discussed above, the water systems used in industry are similar to dialysis systems; thus the potential for biofilm formation in industrial systems must be considered as a risk, and a control strategy should be prepared. Therefore the question to ask is not "are biofilms an issue in my system?", but "do I have the proper controls and procedures in place to detect and control biofilms?"

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Detecting Biofilms in Water Systems

Biofilms are virtually ubiquitous throughout water systems. As mentioned above given microorganisms, water, and time biofilms will develop. However, knowing that biofilms are likely to develop, and being able to detect, identify, and quantify biofilm microorganisms are very different things. There are multiple problems with determining the presence of biofilm.

The first problem with detection of biofilms is sampling; as discussed above, biofilms have been observed throughout hemodialysis systems. To accomplish this detection, much of the sampling required destructive testing, that the soft and hard polymer materials used in the construction of these systems be cut open and the inner surfaces analyzed for biofilm using imaging techniques (7). Where destructive sampling was not feasible, the surfaces were scraped or swabbed and analyzed by imaging and/or enumeration methods. It is, however, unrealistic to sample an industrial water system in the same manner, because destructive sampling is impractical due to expense and material properties (i.e. stainless steel is the most common material used in manufacturing systems and the heat and mechanical abrasion associated with cutting of stainless steel usually destroy any biofilm present) and because there is not access to scrape or swab many locations in the systems. So most manufacturing facilities don't even try to sample for biofilms. Instead they sample for the planktonic microorganisms present in the water, with samples taken periodically from multiple locations within the system.

Planktonic sampling has additional issues such as the choice of the right location to sample for the planktonic microorganisms. Biofilms can form anywhere in the system, and they are not readily detected upstream of the colonization. All pharmaceutical manufacturers sample presterilized product for bioburden; however, the use of planktonic detection of the microorganisms presumes that the microbes
are evenly distributed throughout the water system; however, microbial release from biofilms, like biofilm colonization, is very heterogeneous. It usually happens as a point event, both in time and location. If that point of release is either after mixing or at the end of a filling it is likely that only a limited number of the product will be affected. The heterogeneous aspects of microbial release mean that non-continuous sampling, such as most bioburden testing (standard in the industry), is likely to miss the events depending on the scale of the release. Therefore, sampling will miss intermittent sloughing (large clumps of biofilm detaching) and seeding (microbially activated/controlled release of cells from the biofilm) events.

Once the planktonic sample is collected there are still challenges facing scientists looking for biofilms. After more than a century of use, the conventional measurement for microbial presence/enumeration is

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still the agar plate, a culture-based method. Although it is inexpensive and well understood, there are a number of limitations with this technique. The first is the limited variety of organisms that can be cultured on any given type of agar. Of a conservatively estimated 1.5 million species of microorganisms (8), <1% (9, 10, 11) can currently be cultured. Of the culturable microorganisms, even the media that can culture the widest variety of organisms still only grows a subset of the culturable organisms. The inability to detect and measure non-culturable organisms in the manufacturing environment, whether real or theoretical, presents a risk of underestimating the water-related microbial population when using agar plates. The risk of using agar plates is further complicated in that even detectable organisms can be underestimated when biofilms are involved. This heightened risk is due to biofilms sloughing events, where clumps of bacteria are released. These clumps have the potential to contain hundreds of thousands of microorganisms, but, when analyzed by the agar plate, are read as a single colony and therefore equated to a single microorganism. These combined factors mean that there is a risk that the agar plate method may dramatically underestimate the numbers of microorganisms in a system.

The industry has realized that there are issues with relying solely on agar methods of detection and enumeration, and has supplemented culture-based methods with endotoxin analyses. In addition to the issues mentioned above, culture-based methods can only detect viable microorganisms. Dead microorganisms, while they present no infection risk, are still capable of inducing an immune response and even anaphylaxis. The measurement of bacterial endotoxin allows manufacturers to achieve detection of non-culturable and even dead cells. However, even this system has limits because there is still the heterogeneous aspect of biofilms, and endotoxins are only expressed by Gram negative bacteria.

The medical and pharmaceutical industries are working to develop sensitive and comprehensive microbial detection techniques; however, in the interim, manufacturers must rely on the currently accepted methods. Since these methods are less than fully effective at biofilm detection it is critical that they be complemented with trending of the data. There is no substitute for vigilance and diligence. Biofilms are notorious for intermittent, unpredictable release of microorganisms and endotoxins. When biofilms are involved it is very common for a system to suddenly spike and then return to control for a number of days and then spike again. When Gram negatives are involved, as is usually the case with water systems, the microbial spikes are usually associated with spikes in endotoxin. Since these cases appear random, it is essential that long term data be collected both to set a baseline microbial level (i.e. when the system is in control) and to be able to evaluate if these intermittent increases represent a trend. If a trend is detected, stringent sanitization and cleaning must be performed to remove the biofilm, as will be discussed in more detail below.

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Biofilm Control

Challenges Associated with Biofilm Control

The three main challenges in controlling biofilms in pharmaceutical systems that have already been touched on are detection, tolerance to treatment, and the threat even inactivated biofilms represent due to endotoxins and other pyrogens. Additionally, biofilms are dynamic systems, continually changing and adapting. This means that they are unlikely to stay in one place. Once established, they are likely to grow and spread with the potential of taking over a system if not controlled. This dynamic nature also represents an increased threat of tolerance development. If the biofilm is not completely killed, the remaining bacteria have the potential to adapt, either through selection of the most tolerant organisms or by mutation. Even if the biofilm is killed but not removed, there are endotoxin/pyrogen threats, and just as importantly, the dead biofilm provides a potential home for recolonization with new bacteria. It has been shown that recolonization of a dead biofilm by contaminating bacteria takes a fraction of the time needed for bacteria to colonize a clean surface, creating the potential for a reoccurring problem (12).

Control Methods

If, at this point, you are a little nervous about biofilms in your system, that's great! A healthy respect for biofilms and microbial control is a good thing in a pharmaceutical system. The good news is that microorganisms and biofilms are controllable. Here are some suggestions to assist you as you consider your control procedures.

Have a Plan

Nothing is going to ruin your day and maybe your month more than to realize that your system has developed a biofilm, and you have no idea what to do to treat it. Therefore, it is important to have a plan in place ahead of time. The plan should have some metric for what test results will initiate a biofilm investigation. This might include repeated spikes in bioburden and/or endotoxin levels above the alert limit and should be considered whenever an investigation is initiated in response to exceeding an action limit. This should include trending, and the trending records should be maintained for the life of the system. As discussed above, biofilms are heterogeneous by nature and create seemingly random periods of control with periodic microbial spikes. In many cases this gets progressively worse as the biofilm ages and spreads.

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The plan should include predetermined decisions if alert limits are exceeded for dispositioning the product and for initiating further testing to differentiate biofilm from other microbial contamination. This may include additional sampling such as swabbing of tanks and pipes. The most probable sites for biofilms should be checked. These usually include dead legs (which should be removed from the system at all costs), fittings, connections, and locations of low shear (such as tanks, areas behind baffles, valving, etc.). Some industries with known biofilm problems such as the paper industry and the brewing industry (wine and beer) have in some cases gone to inline sensors that usually can detect biofilms earlier than is evident by planktonic counts. These inline sensors can also be used to determine if cleaning procedures are effective. Their drawback is that they only sample a single location and, like planktonic counts, can easily miss the location of biofilm development. Finally, if detected, the plan should have a sufficiently rigorous treatment procedure, that is different from the regular cleaning used on the system, to have known effectiveness against biofilms.

Biofilm Prevention/Control

The best thing that can be done to control biofilms is prevent them from developing. This is one of the cases where the old adage "an ounce of prevention is worth a pound of cure" really applies, because it is significantly more costly and time consuming to treat biofilms than to prevent them. Prevention involves setting barriers and procedures to first prevent microorganism from entering and then destroy any contaminating microorganisms before they can colonize and develop into biofilms. Regular, effective antimicrobial treatment and cleaning must be performed. The timeframe between these treatments needs to be sufficiently short, so that biofilm formation has not progressed beyond microcolonies, preferably not past initial attachment.

The evidence of the importance of biofilm control has been clearly demonstrated in multiple Hemodialysis Clinics across the nation (13). Cleaning procedures vary widely between clinics as do onsite water treatment facilities, distribution loops, dialyzer equipment and municipal water quality (14). As such, the control of bacteria and therefore biofilms ranges widely. There are multiple cases where Hemodialysis clinics that have not planned for and controlled biofilms have been shut down due to inability to meet microbial requirements, or worse, in some cases due to epidemic infections of the dialysis systems (15, 16).

Much of the practices necessary to prevent biofilms are already applied in the pharmaceutical industry. These include prevention measure such as closed systems, bioburden limits on raw materials, hot water recirculation loops for WFI, procedures to minimize contamination (full aseptic procedures for aseptic systems), and short/controlled times be-

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tween cleaning and regular sanitization/disinfection. The key to prevention of biofilms is that the sanitization and cleaning completely removes any microbial challenge. The validation of the cleaning and sanitization should take into account the worst case location (an example, if heat is the sanitizer then the heating curve of the slowest to heat location should be used for testing).

If a biofilm does form, it is highly recommended that a manufacturer have a plan for treatment ahead of time. A few manufacturers have gone as far as to validate a biofilm cleaning/sanitization procedure for just such a circumstance. In these biofilm treatment validations, the sanitizer is tested against biofilms formed from microbial isolates obtained from the manufacturing site, requiring stringent kill criteria.

Here are a few things to consider when choosing a cleaning/sanitization procedure. First aside from complete replacement of fouled components, mechanical removal (i.e. scrubbing/scouring) is almost always best for reducing biofilms. This is because this removes the microorganisms, their matrix (the glue or slime holding the biofilm together), pyrogens and often times the nutrients they use. It is not uncommon in the industry to have the tanks and other components scrubbed as part of a periodic cleaning procedure, and a power wash (adding air bubble or large light weight abrasives under high flow rates) can also be useful for enhancing biofilm removal. This type of cleaning should be complimented with sanitization, but it often removes the majority of the microorganism and endotoxin that create the risk to patients, and it further removes sufficient biofilm to greatly improve the sanitization efficacy.

Heat, although often ineffective against spores, can have efficacy at both preventing and treating biofilms (17). Biofilm microorganisms are usually only slightly more tolerant to heat than their planktonic counterparts, and the increased tolerance is usually due to increased mass and reduced thermal transfer (18). In other words biofilm take a little longer to heat up. So, if the heat treatment time is set sufficiently to treat biofilms this can be an effective sanitizer. However, the drawback to heat is lack of removal, so endotoxins and the potential for recolonization remain. For these reasons, heat treatment should always be complimented with effective cleaning procedures.

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When it comes to chemical treatment, there are many different disinfectants/sanitizers with varying efficacies against the varieties of microorganisms that could be involved (19). In general, it is the oxidative sanitizers that are the most effective for dealing with biofilms. These chemistries include bleach, peroxide, chlorine, ozone and similar products. The main reason for the effectiveness is that these chemicals are effective against an extremely wide variety of microorganism because they react widely with organic materials to degrade them to an oxidized state. Given sufficient time, these chemistries not only kill organisms, but reactively remove the organic materials associated with microorganisms and biofilms. Please note that the efficacies of the chemistries can vary widely when used outside of their optimal conditions. The manufacturer recommendations should be used to set optimal pH, temperature, and concentration, all of which must be maintained for the extent of the recommend treatment time. A common mistake in validating a sanitizer is not confirming that the concentration remains sufficient at the end of treatment to confer efficacy over the entire treatment.

Once treatment of the biofilm has been performed, the testing should be repeated to show removal. The manufacturing facility must return to data trending to confirm the system returns to baseline and maintains control, without the intermittent microbial spikes indicative of biofilms.

End Benefits of Biofilm Control

The effort required to control biofilms and maintain that control over time is significant. It must be performed in a diligent, consistent manner. In the end, however, it is both the manufacturer and more importantly, the patient using treatment that benefit. Preventing and quickly remediating biofilms reduce both manufacturing down time and loss of product. For the patient the risk associated with microorganisms or endotoxins evading detection and entering their body is further reduced. Controlling biofilms is a critical part of both patient and financial safety.

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About the Authors

Dr. Pasmore received his undergraduate degree in Chemical Engineering for University of Illinois, Champaign-Urbana, and graduate degrees in Chemical Engineering from University of Colorado, Boulder. He has held the position of Assistant Research Professor at Montana State University, Department of Chemical Engineering, where he performed research out of the Center for Biofilm Engineering. In his role as Medical Projects Supervisor at the Center for Biofilm Engineering his research involved the study and treatment of biofilms in infection. He has since held positions at STERIS Corporation and Baxter Healthcare Corporation where his research has involved the evaluation and development of sterilization and decontamination technologies. He has multiple peer-reviewed publications in the field, most recently a book chapter in the book The Role of Biofilms in Device-Related Infection. He is also involved in multiple professional organizations related to microbiology, disinfection/sterilization, and biochemical engineering.

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References

1. Riedewald, F. (1997) Biofilms in Pharmaceutical Waters. Pharm Eng. Nov-Dec, 8-19
2. Darouiche R. (2001) Device-Associated Infections: A Macroproblem that Starts with Microadherence, Healthcare Epidemiology, 33, 1567-1572
3. Jofre R, Rodrigez-Benitez P, Lopez-Gomez JM et al (2006) Inflammatory syndrome in patients on hemodialysis. *J Am Soc Nephrol* 17: S274 – S280
4. Pasmore M, Marion K (2009) "Biofilms in Hemodialysis." Role of Biofilms in Device Related Infections. Shirtliff M., et.al. Ed, Springer Publishing, Verlag Berlin Heidelberg, 167-192.
5. Hoenich NA, Ronco C, Levin R (2006) The importance of water quality and hemodialysis fluid composition. *Blood Purif* 24 (1): 11 – 8
6. Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial Biofilms: From the Natural Environment to Infectious Diseases. Nature Reviews, 2, 95-108
7. Stewart P, Franklin M (2008) Physiological heterogeneity in biofilms. Nature Reviews, 2, 199-210
8. Morin P (2000) Identification of the bacteriological contamination of a water treatment line use for hemodialysis and its disinfection. *J Hosp Infect* 45: 218 – 224
9. Anderson, T-H. (2003) Microbial eco-physiological indicators to asses soil quality. *Agricultural Ecosystems and Environment* , 98, 285-293.
10. Torsvik, V. G., Golsor, J., Daae, F, (1990) High diversity in DNA of soil bacteria. *Applied and Environmental Microbiology* , 56, 782-787.
11. Amann, R.I, Ludwig, W., Schleifer,K.H. (1995) Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation, *Microbiol Rev.*, 59, 143–169.
12. Atlas, R.M., Bartha, R. (1998) *Microbial ecology: fundamentals and applications.* Redwood, CA, USA: Benjamin/Cummings.
13. Little B, Ray R (2002) A Perspective on Corrosion Inhibition by Biofilms. Corrosion, 58(5), 424-428
14. Lonneman G (2004) When good water goes bad: how it happens, clinical consequences and possible solutions. *Blood Purif* 22: 124 – 129
15. Block C, Backenroth R, Gershon E, Israeli R, Simhon A, Popovtzer M, Shapiro MR (1999) Outbreak of bloodstream infections associated with dialysis machine waste ports in a hemodialysis facility. Eur J Clin Microbiol Infect Dis 18 (10): 723 – 725
16. Alter MJ, Tokars JI, Arduino MJ, Favero MS (2004) Control of infections associated with hemodialysis. In Mayhall CJ (ed) Hospital epidemiology and infection control, 3 rd edn. Lippincott, Williams & Wilkins, Philadelphia, 1139 – 1160
17. Roth VR, Jarvis WR (2000) Outbreaks of infection and/or pyrogenic reactions in dialysis patients. *Semin Dial* 13: 92 – 96
18. Holmes CJ, Degremont A, Kubey W et al (2004) Effectiveness of various chemical disinfectants versus cleaning combined with heat disinfection on *Pseudomonas* biofilm in hemodialysis machines. *Blood Purif* 22: 461 – 468
19. Stewart P (2003) Diffusion in Biofilms. Journal of Bacteriology, March, 1485-1491
20. Phillips G, Hudson S, Stewart WK (1994) Persistence of microflora in biofilm within fluid pathways of contemporary hemodialysis monitors (Gambro AK-10). *J Hosp Infect* 27 (2): 117 - 125

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USP Corner

Any questions concerning USP documents should be sent to Radhakrishna (Radha) Tirumalai, Ph.D. You can reach Dr. Tirumalai at: (706) 353-4514, via mail at United States Pharmacopeia, 126 Twinbrook Parkway, Rockville, MD 20852 or via e-mail at RST@USP.org. You can write representing your company, or as an individual scientist.

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