Biofilm and the Dental Office

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The presence of adherent microbial biofilms in dental unit waterlines was first reported more than 30 years ago.[1] Recently, interest in these biofilms has reawakened. This can be attributed to increased awareness of potential occupational hazards in the dental office and concern about increasing numbers of dental patients considered to have diminished resistance to overt and opportunistic microbial pathogens (for example, elderly people, smokers, people with alcoholism, organ transplant and blood transfusion recipients, AIDS and cancer patients, people with diabetes, people with autoimmune diseases and people with chronic organic disorders).[2-10] Although no definable health effects have been associated with exposure to dental unit water, there have been documented reports of waterborne disease outbreaks in a broad range of other facilities, including hospitals, nursing homes, prisons, schools, restaurants, community waterworks and swimming pools. Responsible waterborne agents include significant bacterial human pathogens such as *Pseudomonas aeruginosa*,[11] *Escherichia coli*[12,13] and *Legionella* species,[14,15] as well as species of the highly resistant protozoan *Cryptosporidium*.[16,17]

This article provides a brief overview of the fundamental processes leading to biofilm formation and the significance these biofilms have in health care facilities, particularly hospital and dental settings. A statement adopted by the American Dental Association relating to dental unit waterlines appears as a sidebar. The ADA's statement encourages a consolidated effort to improve water quality in the dental office and sets out an aggressive, proactive research agenda for the control and prevention of biofilm formation in dental unit waterlines.

BIOFILMS

Upon immersion of any solid surface in an aquatic environment, macromolecules and

The author provides a brief overview of the basic processes involved in biofilm formation and explores the implications these biofilms have for health care facilities such as hospitals and dental offices. Included with this article are suggestions dentists may consider for improving water quality and a white paper on waterlines adopted by the ADA.

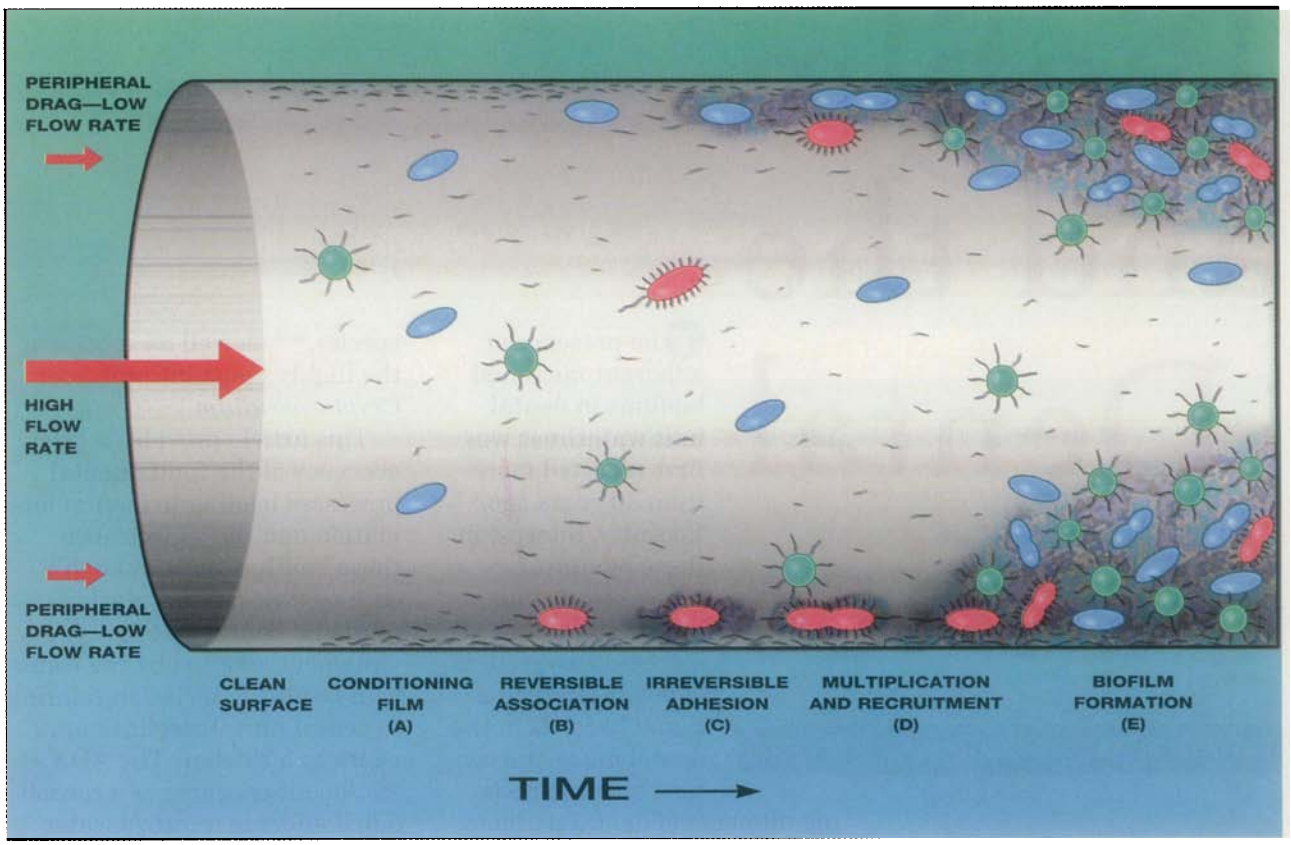

Figure 1. Biofilm formation in narrow-bore tubing. On adsorption of macromolecules from the aqueous phase and the formation of a conditioning film (A), bacteria may either associate reversibly with the surface (B) or adhere irreversibly (C). Subsequent division of adherent cells (D) and recruitment of planktonic cells from the bulk fluid phase results in biofilm formation (E).

other low-molecular-weight hydrophobic molecules in the water immediately begin to adsorb to the surface to form conditioning films (Figure 1).[18,19] These conditioning films alter the characteristics of the surface, which in turn may enhance the efficiency of bacterial adhesion.

The fundamental process leading to biofilm formation results from initial bacterial adhesion and may be either passive or active.[19] Some microorganisms may already possess the necessary attachment structures (for example, extracellular polymeric substances, fimbriae) to immediately form a firm passive attachment to a surface. Other bacteria require prolonged exposure to a surface to attach firmly. In this time-dependent process, termed active adhesion, biofilm formation begins through an initial reversible association between the microbe and the surface during which an as-yet-undefined physiological function (possibly EPS production) is induced (Figure 1). Irreversible adhesion and colonization is achieved through the secretion of EPS and subsequent microbial multiplication (Figure 1). The eventual production of a continuous fixed biofilm on the now-colonized surface is a function of cell division within the EPS matrix and the physical inclusion of other bacteria, fungi and parasitic agents from the free-floating microbial population in the surrounding water (Figure 1).[18,19]

Biofilms usually develop in response to adverse environmental conditions. Their development represents a universal strategy used by the microbial world to optimize the probability of survival. In comparison to planktonic (free-floating) microorganisms, sessile (attached) microbes have several survival advantages:

- retention (organisms serving as biofilm components are retained on surfaces in a cooperative ecosystem);
- nutrition (organisms serving as biofilm components have a nutritional advantage, as organic and inorganic nutrients are

bound by the biofilm matrix);
- resistance (biofilm formation confers microorganisms with a degree of resistance to antimicrobial substances, primarily as a result of the protection provided by the EPS matrix).

CONSEQUENCES OF BIOFILM IN THE HOSPITAL SETTING

The colonization and proliferation of microorganisms at surface-solution interfaces is a well-documented and routine occurrence in many facilities, including hospitals, nursing homes, prisons, schools, restaurants, community waterworks and industrial facilities. The consequences of such biofilm formation, which may be significant, include metal corrosion, energy losses, reduced surface efficiency and a variety of health effects.

Of particular interest and, perhaps, relevance to the dental office are the documented reports of waterborne infection and disease in hospital settings. Although numerous waterborne microorganisms may be potentially pathogenic, three genera in particular are excellent examples of causative agents of waterborne nosocomial (hospital-acquired) infection: *Pseudomonas* species, *Mycobacteria* species and *Legionella* species.

***Pseudomonas* species.** There have been reports of bacteria surviving in concentrations of chemical germicides that go significantly beyond the perceived limits of bacterial resistance. For example, microbial contamination of iodophor antiseptic solutions during their manufacture has been reported and has been associated with subsequent outbreaks of nosocomial infection.[20-22] Parrott and colleagues attributed peritoneal infections to the use of *P. aeruginosa*-contaminated poloxamer-iodine antiseptic.[22] An investigation of this outbreak revealed the polyvinylchloride water distribution pipes at the manufacturing facility to be heavily contaminated with biofilm, leading to the subsequent contamination of the antiseptic product.[23]

Contamination of chemical germicides during handling after their manufacture also has been associated with hospital-acquired infection. In one outbreak, *P. multivorans* was isolated from infected surgical wounds of nine patients.[24] The microorganism was later cultured from bottles of the topical antiseptic (0.005 percent chlorhexidine, 0.5 percent cetrimide) used to bathe the wounds. The source of contamination was identified as the piped water supply to the hospital, which had been used to dilute the antiseptic concentrate.

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***Mycobacteria* species.** Several species of nontuberculous mycobacteria (for example, *Mycobacterium avium* complex, *M. chelonei, M. fortuitum, M. gordonae, M. kansaii, M. terrae* and *M. xenopi*) have been isolated from hospital water systems, some of which have been associated with outbreaks of nosocomial infection.[25-28] In one such outbreak, *M. xenopi* isolated from hot water taps in a Veterans Administration hospital was implicated in 19 cases of pulmonary disease in hospitalized patients.[27] Disease transmission occurred through the formation of infectious aerosols (invisible airborne droplets 1 to 5 microns in size) of *M. xenopi* when patients used a shower, and consequent colonization of the patients through inhalation or ingestion of these infectious droplets.

***Legionella* species.** *Legionella* species are well-known as etiologic agents of both Legionnaires' disease and Pontiac fever. Forty-eight species and more than 51 serogroups of *Legionella* have been identified. Eighteen species have been associated with either fatal pneumonia (Legionnaires' disease) or a nonpneumonic, self-limiting flulike illness (Pontiac fever).[29]

All identified environmental sources of *Legionella* infection have been linked to contaminated water. Potential sources of legionellosis include contaminated water in cooling towers and air conditioners, hot tubs, shower head water and public fountains. Epidemiologic analyses of epidemic and sporadic cases have identified a variety of risk factors for the development of Legionnaires' disease. Prominent among these factors have been cigarette smoking,[30] advanced age,[31] chronic lung disease[31] and immunosuppression.[32,33]

One well-documented outbreak of Legionnaires' disease

occurred in a hospital in Stafford, England.[34] The source of infection was identified as the cooling water system of one of the air conditioning plants. Sixty-eight patients with confirmed cases of Legionnaires' disease were treated at the hospital, and 22 of these patients died. A further 35 patients were suspected of being infected. The epidemiological investigation revealed that all of these patients had recently visited the outpatient department of the hospital. An investigation of staff at the Stafford hospital revealed 30 percent of the 790 staff members to be seropositive for *Legionella* antibodies, while control subjects demonstrated a seropositivity rate of only 3 percent.[34]

Many other reports describe nosocomial outbreaks of Legionnaires' disease in which *L. pneumophila* was recovered from shower heads and the water supply.[35-40] A recent study documents that susceptible patients who used showers during hospitalization were at an increased risk of acquiring Legionnaires' disease in comparison with patients who sponge- or towel-bathed.[41]

In summary, the impact of contaminated water on nosocomial infection has been well-documented. Infection or disease results primarily through either the inhalation of infectious aerosols (for example, Legionnaires' disease, *M. avium* complex) or direct inoculation of traumatized tissue (for example, *Pseudomonas* species).

CONSEQUENCES OF BIOFILM IN THE DENTAL SETTING

The dental waterline provides an ideal environment for microbial colonization and proliferation, primarily due to the high surface:volume ratio in the tubing and the character of fluid dynamics in narrow, smooth-walled waterlines (Figure 1). Microorganisms in dental waterlines can come from a variety of sources. Most experts, however, suggest the public water supply to be a primary source.

It is important to note that microbial species colonizing dental units are mainly bacterial, fungal and free-living protozoan agents; viruses, such as the human immunodeficiency virus, cannot multiply in the dental unit waterline. Although it is possible that patient body fluids may be aspirated back into the waterlines during treatment, current infection control recommendations minimize the likelihood of this occurrence. Current recommendations include the installation and proper maintenance of anti-retraction valves and thorough flushing of the dental unit waterlines after treatment of each patient.[42,43]

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As a consequence of biofilm in dental waterlines, the water emitting from the high-speed handpiece, the air-water syringe and the ultrasonic scaler contains elevated concentrations of microorganisms. Microbial counts in the range of 1 million microorganisms per milliliter of water have been reported.[2-10]

Currently, there is no scientific documentation establishing that biofilm in dental unit waterlines represents a definable public health risk. This lack of evidence may reflect the absence of, or at least a very low rate of, disease transmission and is reassuring, as water is used during most dental procedures. The lack of definitive evidence, however, also may reflect the difficulty of establishing epidemiological links between infections with extended incubation times and antecedent dental procedures. Studies in the scientific literature are limited, but they do suggest that dental unit water may contain significant concentrations of *Pseudomonas* and *Legionella* species, both of which are potentially pathogenic to the susceptible host.

***Pseudomonas* species.** In 1987, two case reports were published in the British Dental Journal describing the placement of large amalgam restorations using matrix bands in two patients with cancer.[44] Three to five days after amalgam was placed, the patients returned to the dental office complaining of pain and swelling. On oral examination of both patients, the dentist observed that the swelling corresponded to the area where the matrix band had been used. Microbiologic culture of the infected sites recovered *P. aeruginosa*. The same pyocin type of *P. aeruginosa* was subsequently isolated from the dental unit waterlines in both case

involve some additional expense. They include the use of
- independent water reservoirs;
- chemical treatment regimens;
- daily draining and air purging regimens;
- point-of-use filters.

Preliminary data suggest that some combination of the above strategies will be necessary to control biofilm formation and to achieve the desired level of water quality. To date, however, there are insufficient data to establish the effectiveness of available methods. Industry and independent researchers should be strongly encouraged to explore as wide a range as possible of alternatives and adjuncts to the above listed options. Dental practitioners should always consult with the manufacturer of their dental units before initiating any waterline treatment protocol.

WATER QUALITY MONITORING

Simple and inexpensive methods to estimate the number of free-floating heterotrophic bacteria in dental unit water need to be developed to test the effectiveness of control measures. A well-designed water quality indicator should be self-contained and easy to use in-office; accurately detect a wide concentration range and type of aerobic mesophilic heterotrophic waterborne bacteria within a reasonable incubation time at room temperature; and be relatively inexpensive to use. The Council is aware that technology meeting these criteria is already available and could possibly be adapted for use in dentistry with minimal developmental cost.

TRAINING AND EDUCATION

The ADA should enhance its efforts to educate dental practitioners regarding microbial contamination and biofilm formation in dental unit waterlines, and the need for improvement in the quality of water delivered to patients. Additionally, manufacturers should maintain an active approach in training and educating the profession in the proper use and maintenance of their systems.

CRITICAL RESEARCH AND DEVELOPMENT NEEDS IDENTIFIED BY THE COUNCIL

- Research is needed to define the natural history of biofilms, specifically to more clearly determine the relationship of the numbers and types of microorganisms in the fixed population (sessile) to their free-floating (planktonic) counterparts.
- Improved, research-based methods need to be developed to effectively eliminate existing biofilm and prevent or control formation of new biofilm in dental unit waterlines.
- Alternative devices for monitoring the microbial quality of water used during dental care should be developed that are simple, reliable and cost-effective.

In summary, the Council recognizes that the scientific literature supports the need for improvement in dental unit water quality. The Council will continue to work with industry and the research community to address research and development needs that will allow the delivery of water of an optimal microbiological quality to the dental patient. The Council recommends dissemination of this information to dentists as part of the ADA's ongoing service to the profession and the public.

This statement was adopted by the ADA Board of Trustees, December 13, 1995, and the ADA Council on Scientific Affairs, September 28, 1995.

reports. The authors speculated that both infections were a result of direct inoculation of traumatized tissue with contaminated dental water. However, there remains the possibility that the specific microorganisms of concern in the waterlines originated from the respective patients.

***Legionella* species.** Several reports have demonstrated that *Legionella* species may colonize some dental units and have suggested that such contamination may result in occupational exposure to the microorganism through aerosolization of contaminated water.[3,9,10] Indeed, several studies have demonstrated higher seroprevalence rates for *Legionella* antibodies among dental personnel than among nonmedical control groups.[45-47] However, it should be noted that *Legionella* species are ubiquitous in the environment, and it is difficult to establish a definitive relationship between the presence of serum

antibody and the source of exposure without comprehensive epidemiological investigations.

Nevertheless, one such serological study analyzed samples from 107 dentists, dental assistants and dental technicians for antibodies to seven different *Legionella* species.[46] Thirty-four percent of the dental personnel showed a positive reaction to the polyvalent *L. pneumophila* antigen SG1-SG6 (Figure 2); *L. pneumophila* is the species considered most pathogenic to humans. Only 5 percent from a control group (nonmedical workers) tested positive. Dentists demonstrated the highest prevalence (50 percent) of *L. pneumophila* antibodies (Figure 2), followed by assistants (38 percent) and technicians (20 percent).

Higher seroprevalence rates for *Legionella* antibodies among dental personnel have not been directly correlated with higher rates of disease. Investigators have speculated that the higher prevalence of antibodies may reflect continuous exposure to small numbers of the organism, resulting in mild (Pontiac fever) or inapparent infections.[47]

As already mentioned, there are no definitive data linking dental exposure to contaminated water with specific disease incidents. However, as reviewed here, several retrospective studies among dental staff may suggest occupational exposure to potential pathogens. While exposure is not necessarily synonymous with infection or disease, reliance on the basic infection control principle of avoiding unwarranted exposure would seem a sound basis for professional decision making.

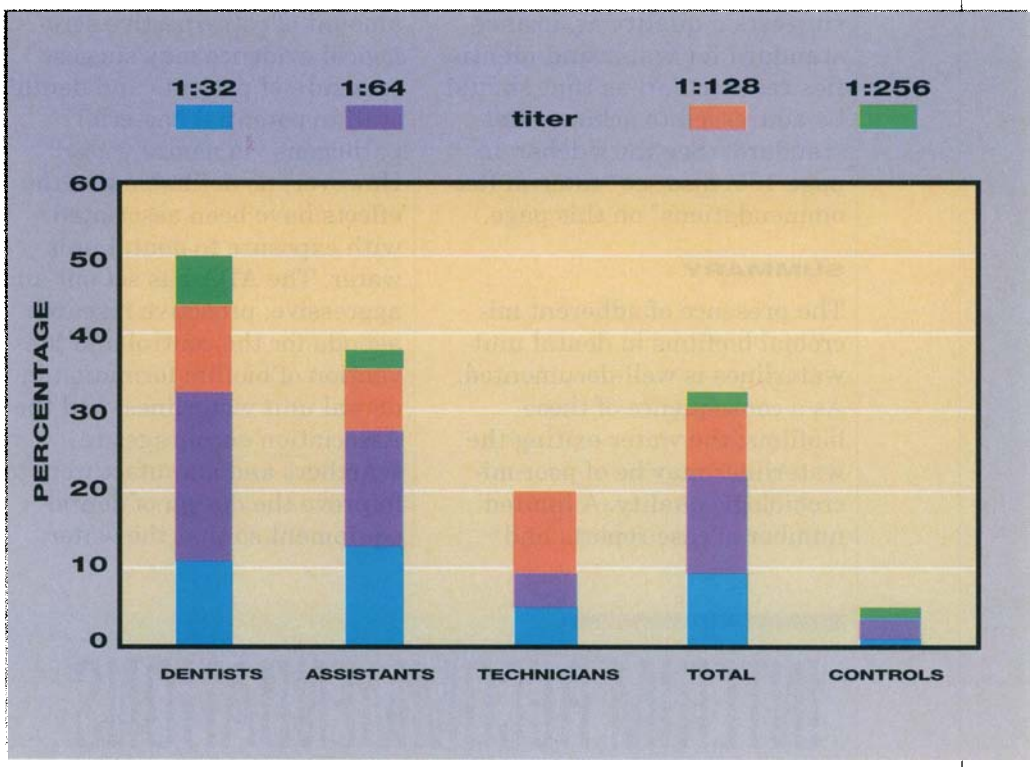

Figure 2. Prevalence of antibodies to *Legionella pneumophila* SG1-SG6. Twenty-three percent of the samples from people working in dental offices showed weak reactions (titers 1:32-1:64), and 11 percent showed higher antibody titers (1:128-1:256). The percentages for the control group were 4 percent and 1 percent, respectively ($P < .001$). (Reprinted with permission of Journal of Dental Research from Reinthaler FF, Mascher F, Stunzer D.[46])

STATEMENT ON DENTAL UNIT WATERLINES

The ADA, as early as 1978, suggested flushing dental unit waterlines with germicides as a means of controlling biofilm formation.[48] However, as it became clear that such a recommendation may not necessarily be appropriate for every dental unit in the marketplace, subsequent publications recommended that dentists follow dental unit manufacturers' recommendations for the proper maintenance of waterlines.[42,49]

Since that time, the ADA has hosted several workshops bringing together researchers and manufacturers in an effort to develop a consensus solution for improving the quality of dental unit water. To date, however, no scientific data evaluating the safety and effectiveness of potential solutions are available for the dental setting, and consequently no consensus solution has emerged. To continue to address the waterline issue proactively, the Association in August 1995 convened an expert panel of representatives of the dental profession, various governmental agencies, academia, research and industry. The panel developed a statement on dental unit waterlines that was adopted by the ADA Council on Scientific Affairs and subsequently by the Association. The statement outlines concerns relating to biofilm in the dental office,

suggests a quality assurance standard for water and identifies research areas that should be addressed to achieve that standard. (See the sidebar on page 185; also see "Interim Recommendations" on this page.)

SUMMARY

The presence of adherent microbial biofilms in dental unit waterlines is well-documented. As a consequence of these biofilms, the water exiting the waterlines may be of poor microbiologic quality. A limited number of case reports and amount of retrospective serological evidence may suggest exposure of patients and dental staff to potential bacterial pathogens via dental water. However, no definable health effects have been associated with exposure to dental unit water. The ADA has set out an aggressive, proactive research agenda for the control and prevention of biofilm formation in dental unit waterlines, and the Association encourages researchers and manufacturers to improve the design of dental equipment so that the water delivered to dental patients is of the best possible quality. ■

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INTERIM RECOMMENDATIONS FOR IMPROVING WATER QUALITY

In view of the collaborative research that will be required before any definitive recommendations can be made to improve water quality, the following interim recommendations are offered to the profession:

- Waterlines (without the handpiece attached) should be allowed to run and discharge water for several minutes at the beginning of each clinic day.[43] (This procedure is intended to reduce any overnight or weekend microbial accumulation.)
- High-speed handpieces should be run to discharge water and air for a minimum of 20 to 30 seconds after use on each patient. (This procedure is intended to aid in physically flushing out patient material that may have[42,43] entered the turbine and airlines or waterlines. Use of an enclosed container or high-velocity evacuation should be considered during discharge procedures to minimize the spread of spray, splatter and aerosols.)
- Dental personnel should routinely follow the instructions of the dental unit's manufacturer for the proper maintenance of waterlines.[42,43]
- Use of commercial options for improving water quality should be considered. (Dentists are cautioned that the research relating to the safety and effectiveness of some of the available options is limited. Consultation with the dental unit manufacturer may be advisable before instituting one, or any combination, of the currently available options.)
- Sterile saline or sterile water should be used as a coolant/irrigator when surgical procedures involving the cutting of bone are performed.[43]

The ADA, through its Council on Scientific Affairs, will continuously monitor research and development activities relating to the control and prevention of biofilm formation. One of these activities will be the Council's development of evaluation guidelines for equipment used in, or related to, the control and prevention of biofilm. The completion of such guidelines will allow manufacturers of appropriate products and equipment access to the Association's Acceptance Program. In this way, the profession may be made aware of dental products and equipment with the ability to meet suggested quality assurance standards for dental water.

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