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REVIEW

Control & Prevention of Biofilm In Dental Office

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ABSTRACT:

The goal of infection control in any specialty practice is to minimize the risk from exposure to potential pathogens and create safe working environment to treat patient. In our routine dental practice one way to achieve this is by giving considerable importance to the quality of water used since patients and dental staff are regularly exposed to water and areolas generated from dental equipment. Biofilms are microbes that adhere to any moist surface including the inner surface of Dental unit water lines (DUWLs) and form a protective layer. A well developed or mature biofilm can contain bacteria, fungi, algae, protozoa and nematodes. Lumps of bacteria that are lost from the surface of the biofilm may be carried in to patient's mouth via spray or spatter from dental instruments and might place them to unnecessary risk. Recently, interest in these biofilms has been 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, autoimmune disease and chronic organic disorders). In developed countries recommendations were given by CDC (Center for Disease control) and ADA (American Dental Association) to control biofilms in DUWLs. In India where dental care is still in its infancy and awareness of patients is limited, it is duty of the dentists to control these biofilms in dental office.

Key words: ***Biofilm; Dental unit water lines (DUWLs), Infection control, CDC, ADA, Immunocompromised.***

INTRODUCTION:

For centuries, waterborne disease has been a persistent scourge of civilization. A dental unit is furnished with a system of thin, plastic tubes, called dental unit waterlines (DUW) delivering water to the different hand pieces. The water may circulate in a closed system, where it is taken from a container belonging to a unit. The quality of dental water is of considerable importance since patients and dental staff are regularly exposed to water and aerosols generated from dental equipment. The goal of infection control is to minimize the risk from exposure to potential pathogens and create safe working environment to treat patient.

With the increasing stress on infection control, because of contaminated water in dental units the dental profession is understandably interested in preventing any problems that might arise in this area.

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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 disease and people with chronic organic disorders).[1]

What is Biofilm?

A biofilm is the communal form in which bacteria prefer to live. The term 'biofilm' refers to the development of microbial communities on submerged surfaces in aqueous environments consisting primarily of complex, symbiotic microbial communities Human beings are themselves naturally colonized by biofilms (e.g., dental plaque).

Blake first reported presence of Biofilms in dental waterline in 1963. A biofilm can be present at any place where moisture and a solid surface exist. It consists of bacterial cells and other microbes that adhere to surface and form a protective layer. A well-developed or mature biofilm can contain bacteria fungi, algae, protozoa and nematodes. Virus such as human immunodeficiency virus (HIV) cannot multiply in dental unit waterlines.[2]

Definition of Biofilm:

Donlan and costerton defined biofilm as a microbial derived sessile community characterized by cells that are irreversibly attached to each other, are embedded in a matrix of extra cellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription.[3]

Nature of Biofilm:

Most biofilms are heterogeneous in species and morphology and are enveloped in a polysaccharide slime layer known as a glycocalyx.).The base of the biofilm is a bed of dense, opaque slime 5 to 10 mm thick. **(Fig.1)**. Bio films are 30 to 50 microns thick and strongly adhere to the tube wall. It is a sticky mix of polysaccharides, other polymeric substances and water, all produced by the bacteria. Bacteria and other microbes are distributed throughout the biofilm matrix, with like forms tending to associate in micro-colonies**.**

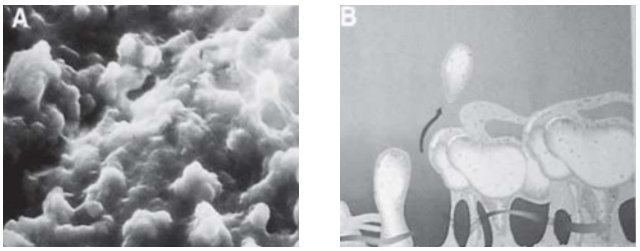

Figure 1. Biofilm architecture. A. Dental unit waterline magnified x 5,000, showing the polysaccharide slime layer and typical biofilm surface architecture. (Photo courtesy of the USAF Dental Investigation Service.) B. An artist's conception, revealing the presence of complex features such as channels for convective flow within the biofilm. (Illustration used by permission of the Center for Biofilm Engineering, Montana State University, Bozeman.)

(With due acknowledgments to Harold C, Slavkin taken from Biofilms Microbial Ecology and Antonivan Leeuwenhoek. JADA 1999, 130 (4): 492 -95)

Lumps of bacteria (microbial aggregates) are continually lost from the surface of this film into the tube lumen where they may be carried into the patient's mouth or into ambient air via spray or spatter from dental instruments. This film is not removed by flushing and microorganisms in biofilms are very resistant to chemical disinfections. Biofilms appear to be the source of the majority of bacteria found in dental unit water.[3]

Formation of Biofilm:

Bacterial biofilm, forms within a few days in a new dental unit and is universally present in all untreated dental unit waterlines. Biofilms usually develop in response to adverse environmental conditions. Their development represents universal strategy used by the microbial world to optimize the probability of survival. In comparison to planktonic (free-floating) microorganisms, sessile (attached) microbes have more survival rate that is due to **retention** (organisms serving as bio-film components are retained on surfaces in a co-operative ecosystem), **nutrition** (organisms serving as bio-film components have a nutrition advantage, as organic and in organic nutrients are bound by the bio-film matrix) and **resistance** (Biofilm formation confers microorganisms with a degree of resistance to anti-microbial substances primarily as a result of the protection provided by the EPS matrix.

The fundamental process leading to biofilm formation results from initial bacteria adhesion and may be either passive or active. Some microorganism may already posses the necessary attachment

structures of extra cellular polymeric substances (EPS), fibriae, 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 is induced. Irreversible adhesion and colonization is achieved though the secretion of EPS and subsequent microbial multiplication. [2, 3]

Biofilm and Dental unit:

DUWLs (Dental unit water lines) provide an environment conductive to rapid proliferation of biofilm. Due to convergence of 3 factors that is biology, physics and geometry that can be summarized in to 3 components:

Surface Colonization

Laminar Flow

Surface: Volume ratio

Surface Colonization:

Any material commonly used to deliver water to dental hand pieces and air / water syringes provide excellent substrates for the initial attachment of bacteria and the subsequent proliferation of biofilm. Most treated drinking water contains minerals-principally calcium carbonates that are deposited on water-bearing surfaces. Organic molecules subsequently concentrate on these surfaces and promote adhesion of bacteria suspended in water supplied by the municipal water systems. (Fig **2** demonstrates a typical sequence of biofilm formation on calcium carbonate deposited on polyurethane DUWL) Over time individual cells attached to the surface multiply to form micro colonies that ultimately coalesce to form a continuous sheet of bacteria protected by the glycocalyx.

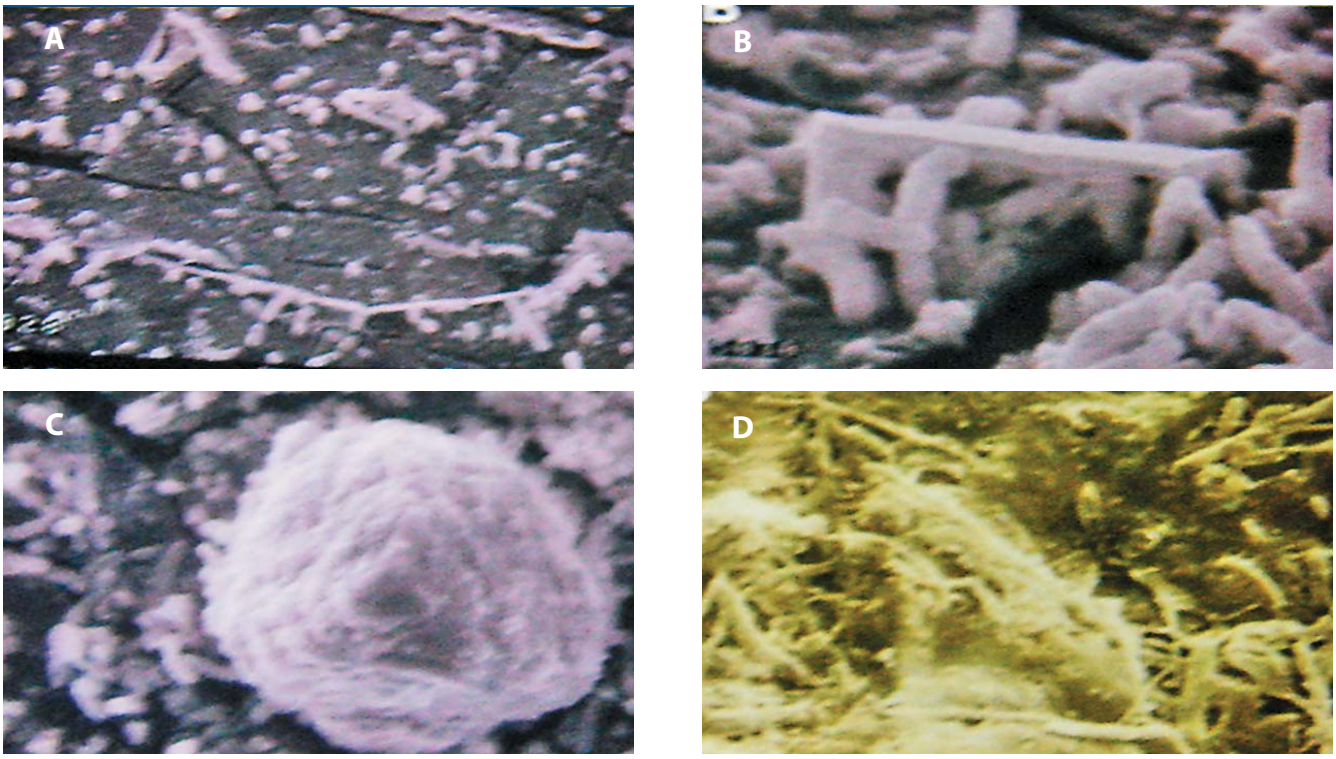

Fig. 2. Microscopic picture of Biofilm colonization sequence on dental unit waterline surface a) carbonate deposits, b) initial attachment, c) division of cells into microcolonies and d) biofilm formation

(With due acknowledgments to Brian G, Shearer taken from Biofilm and the dental office JADA 1996, 127 (2):181 -89)

Laminar flow:

Fluids moving through narrow- bare tubing characteristically assume a hydrodynamic pattern known as laminar flow. Closer to the tubing surface, frictional forces slow these movements of fluids until flow at the surface is stabilized; this crates an environment conducive to the formation of biofilm. In laminar flow systems, biofilm can flourish with minimal risk of being dislodged. This is one of the principal reasons that flushing of waterlines can eliminate suspended (planktonic) microorganisms, but usually is not effective in removing biofilms.

Surface: Volume ratio:

As the diameter of a water line-decrease, an increasingly larger surface area becomes available for colonization.[4]

Factors contributing to Dental Bio-Film formation:

A bio-film can form in any non- sterile fluid environment and dental unit waterlines provide an ideal environment for such films because of following factors

1) Dental water -line tubing has narrow bore, 2) high internal surface -area to volume ratio, 3) low water pressure and flow rates. This helps bacteria for prolonged exposure to the surface to attach firmly, 4) frequent period of stagnation- encourage accumulation of bacteria introduced from public water supply, 5) use of water heaters, heating water to near body temperature for patient comfort may enhance the number of micro- organisms pre-adapted for growth within a warm- blooded human host and 6) Pre-filter in dental units are used mainly to remove particulate matter from public water supply as it enters the dental unit. But it has no effect on particles that of bacteria and it also slows the flow of water, enhancing bio-film formation and may provide additional surface area for microbial colonization.[2]

Dental Waterlines and Health:

The question remains as to whether or not these microbial communities pose a risk, the answer has to be, potentially yes, particularly when one looks at the species of microbes As many of the gram-negative bacteria isolated from dental water sources are recognized as opportunistic pathogens in addition, aquatic mycobacteria, legionella species, fungi, protozoa and even nematode worms have been recovered.[3]

Microbial Flora

A range of microbiological flora have been identified in dental unit waterline samples by use of morphological and biochemical characteristics.

Pseudomonas aeruginosa	Achromobacter xylaxidans
Pseudomonas cepacia	Klebsiella pneumonial
Pseudomonas fluorescens	serratia marcessens
Pseudomonas resicularies	Nocardia spp.
Pseudomonas posimobilis	Streptococcus spp
Legionella spp	

Fungi Genera

Penicillium	Cladosporium
Alternaria	scopulariopsis.[5]

Pseudomonas aeruginnosa has been reported as being present in dental units. This gram-negative a rod is associated with a wide range of opportunistic infections and is a cause of pneumonia in hospitalized patients.

Mycobacterium species

Several species of non-tuberculous mycobacterium (e.g. *mycobacterium avium complex, M. chelonei, M.fortuitum, M.gordonae, M.kansaii, M. terrae and M. Xenopi) have been* isolated from dental unit water. These bacteria have been associated with outbreak of nosocomial infection.

Legionella species

These species are responsible for both Legionnaires disease and Pontia fever. Inhaling aerosols or aspirating water contaminated with bacteria transmits legionnaire's disease. Legionnaires' disease account for as many as 10,000 to 15.000 cases of pneumonia each year in the United States, with an estimated mortality rate of 5% to 15%. Risk factors that increase susceptibility to this disease include smoking, pre-existing respiratory disease and age.

Risk Individuals:

Conditions of at risk patients which make them more **susceptible** to infection from contaminateddentalwater: AIDS, tuberculosis(Tb), drug therapy, immunosuppressive medication, chemo the rapyradiationtherapy, cysticfibrosis, asthma, cancer, cardiacpatients, diabetes, Lupusery the matouspatients children, elderly, pregnancy, organ transplants, individuals with life threatening disease, leukemia, smoking and alcoholism.[5,6]

Biofilm as a public Health Problem:

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.

Consequences of Biofilm in Dental Setting

There are at least 4 ways in which waterborne microorganisms might cause infection in a patient undergoing dental work and that includes hematogenous spread during surgical procedures (Theoretical but possible),local mucosal contact (Oral/ conjunctiva), infection, inhalation

As a consequence of biofilm in dental waterlines, the water emitting from the high- speed hand piece the Air -water syringe and the ultrasonic scalar contains elevated concentration of microorganisms.[7]

Several studies have been reported in the literature stressing that these microbes emerging from dental unit water lines (DUWLs) pose a potential threat to both dentists and patients. Some of them include**:**

In 1974, Clark recovered gram-negative bacteria (pseudomonas species) from dental units and the nasal flora of 14 of 30 dentists while no clinical symptoms were reported, seeding of the respiratory tract with gram -negative bacteria has been identified as an antecedent event in the development of gram-negative pneumonia in hospitalized patients.

Clinical case reports

Martin published a case report describing *P. aeruginasa* wound infections in two immuno-compromised patients. The organism-isolated from the infected sites were matched by pyocin typing to bacteria recovered from the dental unit.

Atlas and Williams mentioned the case of a 65-year-old dentist who died after developing legionnaires' pneumonia although several species of Legionella bacteria were isolated in high number from a dental water source in his office well as in low levels from sources in his home the authors were unable to establish a conclusive dental association.[8]

In 1987, two case reports were published in the British Dental describing the placement of large amalgam restorations using matrix bands in two patients with cancer.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 cases. The authors speculated that both infections were a result of direct inoculation of traumatized tissue with contaminated dental water.

Several studies conducted in the United Kingdom and Germany has found higher titers of Legionella antibodies among dental personnel than among nonmedical control population. These increased titers are most likely due to chronic exposure to Legionella contaminated aerosols of dental water. Despite the higher titres no cases of Legionella Pneumonia among the exposed workers have been documented.

Lack of documented disease among these dental care personnel may reflect exposure to number of Legionella organisms that are insufficient to cause pneumonia; adequate host defense mechanisms in response to the Legionella challenge and continuous exposure to small amounts of Legionella which leads to light or sub-clinical infections detectable only by anti body measurement.

Legal case: A 1990 civil suit against a dental unit manufacturer was reported anecdotally by Dr. Robert Runnels. The plaintiff claimed that bacterial endocarditis and the need for subsequent prosthetic heart value surgery resulted from dental treatment with contaminated water. The same strain of gram-negative water bacteria (Moraxella) was isolated from the patient and the dental unit. The plaintiff intended to argue that the organism entered the unit as a result of retraction of oral flora that occurred because the dental unit was not equipped with an antiretraction value.[9]

Recommendations to control biofilm in Dental unit water lines (DUWLs)

Center for Disease control (CDC) (1993)

To install and maintain antiretraction valves to prevent fluids from being withdrawn in DUWLs

- To flush waterlines daily for several minutes and for 20-30 seconds between patients
- Use sterile solutions

ADA (1996)

- Manufacturing company to develop methods to control biofilms in DUWLs
- Goal for dental water to contain no more than 200 CFU/mL of heterotrophic bacteria in unfiltered output.[6]

Methods to control Biofilm:

Water Flushing:

- To flush waterlines daily for several minutes and for 20-30 seconds between patients.
- The efficacy of mechanical flushing alone to control microbial contamination in dental unit water is not well supported by the scientific literature.
- Flushing can temporarily reduce the number of organisms suspended in DUWSLs but there is no predictable effect on adherent biofilm.
- Flushing for several seconds between patients, however, may remove material that entered the water system during treatment.
- Flushing must be carried out in such a fashion as to avoid misting, which can contaminate the ambient air.
- The British Dental Association recommended that simple flushing of water lines is an expedient interim measure for use by all dentists until more effective methods are introduced.

Drying:

- Drying DUWLS overnight and on weekends by compressed air
- When used with flushing and 70% ethanol, produce no CFU/ML
- Drying alone provide no benefit, because of the protective effects of polysaccharide against desiccation.

Filtration:

- This involves use of micropore membrane filters usually placed on each water -bearing line near the hand piece or air water syringe to trap free floating (planktonic) micro- organisms.
- Filters can produce water that meets or exceeds the ADA goal thus they are adjunct in controlling quality of water delivered to patient

The potential **advantages** of filters include the reduction or elimination of Reliance on chemicals, the potential for damage to dental units and possible staff exposure to chemical residues.

Disadvantages include:

Need for frequent changes, lack of any effect on biofilms distal to the filter,filter has to be placed as close as possible to the water line, only trap Planktonic bacteria and not stop formation of biofilm,ultramicrobacteria - extremely small but viable bacteria may develop and may pass through filters.

Independent Reservoirs:

Self-contained water system: Also known as independent water supplies or reservoirs.

These systems isolate dental unit form public water supply and provide water or a specific treatment

solution from reservoirs filled and maintained by dental assistants.

Advantages: Chemical agent can be introduced through self- contained water systems. Water of known microbiologic quality is used.

Disadvantages: This system cannot reliably improve the microbial quality of dental unit water if chemical agents, which remove or inactivate biofilm inside unit, are not used.[7]

Chemical Treatment:

In this chemicals are used to inactivate or remove biofilms, can be used in three ways;

(a) Intermittent or shock treatment-here chemical agent is used periodically in dental waterline

(b) Continuous chemical treatment

(c) Combination of intermittent and continues treatment

Requirements of chemicals used include:

(a) Chemical germicides and cleaners used should be bio-compatible

(b) They should leave only safe levels of residues.

(c) Should be compatible with metal and synthetic materials used in dental unit construction.

(d) There should not be disinfectant by products (DBP) and if present, their effects on oral tissue should be minimum

Intermittent treatment regimens

- Use potentially biocidal concentration of germicide to remove biofilm.
- Experts refer to this approach as "shock treatment"
- The method includes delivering the agent for a specified contact time and frequency using an independent water reservoir that isolates the unit form the municipal water supply.
- A major advantage of intermittent chemical use is that the active agent is purged from the system before patient treatment.
- Disadvantages include the potential for surviving biofilm organisms to rebound between treatments, Potential staff exposure to chemicals, and the potential for adverse impact on metal, rubber and synthetic dental unit components.
- When biofilms present in dental unit water lines, are treated with chemical agents, chances exist for the generating of disinfectant by products DBP. In particular chlorine compounds including dissolved chlorine gas, chlorine dioxide, NaClO and monochloramine can react with the biofilm and other dissolved organic compound to produce a class of chemicals known as trihalomethanes or THM.
- THM are chloroform and related substance such as chlorinated acetates. Many of which are listed suspected human carcinogens.
- The U.S Environment protection Agency or EPA has established a limit on the level of THM permissible in drinking water at 100 parts per billion or ppb.

Continuous chemical treatment:

- In this either lower concentrations of potentially biocidal agents or less toxic (biostatic) substances in the water used for patient treatment.
- Although continuous treatment offers less potential for recolonization of water lines, it still may damage equipment.
- Since the agent is always present and may be aerosolized, the effects or chronic exposure on the health care worker must be considered.
- Enamel and dentin bond strength of dental adhesive material also may be affected.

1. Chlorine compounds -

a. 5.25% sodium hypoclorite (500 ppm) diluted 1:10.

b. Chloramines

c. Chlorine dioxide

2. ***Chlorhexidine gluconate -***

   E.g. *Bio 2000,* a glycerin based bur lubricant with Chlorhexidine 0.12 % + 12 % ethanol used continuously

3. ***Hydrogen peroxide***

   E.g. *Sterilex ultra and sanosil*-used intermittently

4. ***Citric acid 0.224 %*** – *(Bio-clear)* used continuously

5. ***Iodine - (Dentacide)*** used as a periodic waterline cleaner

6. Others- glutaraldehyde T4, Listerine, sodium fluoride

Sterile water delivery system

- They bypass or replace the DUWLs to provide sterile irrigants.
- Contain very low-level minerals & organic compounds.
- The sterile irrigants are provided through autoclavable tubing

Commercially available devices include:

Sterile Water pump (**Biotrol**) uses an electric peristaltic pump and a specially modified International Standards Organization, or ISO, connector to deliver water from a standard intravenous bag and IV tubing to air -driven hand pieces

AXCS Sterile irrigation (dentalEZ) uses bagged solution and a pressurized cuff to drive the solutions.

Disadvantages include

Expensive to purchase and operate, less convenient to use than conventional delivery systems.

and suck-back effect thereby leading to contamination.

Other Methods

- Ozone purified water
- Continuous ultraviolet germicidal irradiation (Uvgi) of tap water.
- Distilleries and deionizers
- Distilled-oxygenated water[10]

Role of Dentists in Biofilm prevention:

According to OSAP ((organization for safety and Asepsis procedures) Surgeons can take a number of steps toward improving the quality of dental unit water.

1. Water lines (without hand piece attached) should be allowed to run and discharge water for several minutes each morning at the beginning of clinic. This producer helps to reduce any overnight or weekend accumulation.
2. High-speed hand pieces should be run to discharge water and air for a minimum of 20 to 30 seconds age use on each patient. This helps in physically flushing out patient material that may have entered the turbines and airlines or waterlines.
3. Dental surgeons should routinely follow the recommendation by the dental unit manufacturer the proper maintenance of waterlines such as installation and maintenance of anti-retraction value to prevent oral fluids from being drawn into water lines (Retraction also referred as suck back). The entry of oral fluids and micro- organisms into waterlines as result of negative water pressure or other hydrodynamic process is known as retraction. Retraction of patient material by dental water systems offers potential for patient transmission either directly or by permitting colonization of water line biofilms by organisms from oral cavity.
4. Don'ts heat dental unit water. Although it is comfortable for patient but warming the water may amplify biofilm formation and select organisms pre adapted to growth in a human host.
5. Sterile saline or sterile water should be used as a coolant/irrigator when surgical procedures involving the cutting of bone are performed.
6. Use of commercial options for improving water supply should be considered. Such as using a separate water reservoir system to eliminate the inflow of municipal water into the dental unit.
7. Educate and train dental office staff on effective treatment measures to ensure compliance and minimize risks to equipment and personnel.

8. One should be aware of scientific and development in this area to know about improved technical approaches as they become available Contact the equipment manufacturer or dealer to obtain current recommendations for improving and maintaining water quality. When purchasing new equipment, select products that can reliably and economically maintain good water quality.
9. Water for dental unit waterlines should not stored too long.[11]

Successful control of biofilms in dental unit waterlines depends on technique factors; effective personnel training and n established standard operating procedures.

Conclusion:

Biofilms, once formed, serve as a reservoir significantly amplifying the numbers of free-floating microorganisms in the water exiting the waterlines. These biofilms might place immunocompromised patients at unnecessary risk. Hence reducing the number of microorganism presenting in DUWL is consistent with other empiric measures that form the basis of infection control. Although various methods are used for improving quality of water used in dental treatment, further search for effective, convenient and economic methods for day-to-day basis is required. In India where dental care is still in its infancy and awareness of patients is limited, it is duty of the dentists to control these biofilms in our dental unit water lines. This is also important so curb down the infectious diseases, which are prevalent in our society. IDA and DCI along with government should chart out guidelines so as to control another potential dilemma before it strikes.

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