ABSTRACT

Aim: This article reviews the issue of dental unit waterline (DUWL) contamination which affects all the clinical and hospital settings. The contaminating microorganisms commonly isolated from these settings and the most pathogenic among them have serious consequences. Over the years several measures are inculcated for decontamination of water, their advantages and shortcomings have been addressed. Options using nanotechnology which are available in the market are described briefly.

Materials and Methods: A manual and electronic search was conducted. Google and PubMed were searched for relevant material from studies up to 2013. Medical Subject Headings words looked for were “Nanotechnology,” “Water purification,” and “Biofilms.” Reviewed findings were summarized by topic, using the Preferred Reporting Items for Systematic Reviews and Meta-analyses statement for reporting. Seventy articles were shortlisted for articles pertaining to our topic of discussion. A systematic approach was followed by two independent reviewers and included eligibility criteria for study inclusion, data extraction, data synthesis, and drawing of conclusion.

Results: Dental waterline contamination is widespread in any type of dental setting having serious implications on clinicians and patients alike, especially elderly and immune-compromised. Hence, international bodies like center for disease and control and American Dental Association have come up with stringent measures for maintenance of water quality. A gamut of procedures has been tried to overcome this problem ranging from chlorinated products, water filters to the usage of distilled water. The use of nanoemulsions, nanofilters, nanomembranes, etc., and their applicability for routine usage is discussed.

Conclusions: Biofilm formation in DUWLs is inevitable with the subsequent release of part of microbiota into the otherwise sterile dental settings. These consequences can be quite serious on clinicians and dental patients. Though conventional measures in water decontamination have been partly successful, the quest for more foolproof methods has led to the use of latest technology, i.e., nanotechnology. The most practical option has to be chosen based on the ease of their usage.

Key words: Bacteria, biofilms, dental unit waterlines, medically compromised patients, nanotechnology, water purification

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complex architecture, making most elimination techniques impenetrable. The various measures include the point of use filters, use of sterile water system, chemical treatment of water, etc. Each of them is fraught with their own set of advantages and disadvantages. The progress of material science has ascended from macro to micro and even nano levels.

**MATERIALS AND METHODS**

A detailed MEDLINE search was conducted, and the articles pertaining to our topic were included. Only articles printed in the English language were considered. Articles pertaining to nanotechnology and its usages in water purification, methods and its applications in the treatment of dental unit waterlines (DUWLs) were included. The MEDLINE database was searched from 1987 till 2013 using the following Medical Subject Headings: “Nanotechnology,” “Water purification,” “Biofilms,” and “DUWLs.” A total of 94 articles were chosen addressing nanotechnology, nanoparticles, and water purification. Based on the study titles and abstracts, 70 literature pieces were finally selected and shortlisted, such that they fulfilled the inclusion criteria. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) was used to describe and summarize the results of our review, and the 27 PRISMA were used.

**Inclusion criteria**

- Studies that were published in authentic international and national journals
- Studies which were either of a randomized controlled clinical trial type or of a peer-reviewed category articles
- Studies which clearly stated objectives of their research and/or hypothesis to be tested.

**Exclusion criteria**

Studies which did not meet the required inclusion criteria or did not clearly relay the information were excluded.

**Data collection and analysis**

Initially, titles and abstracts of the selected studies identified by the previously described search strategies were screened independently by two reviewers so as to determine whether they should be included in the review. Selected studies were independently reviewed by the reviewers using the criteria defined above.

The literature review resulted in the following data, which has been put together and presented systematically.

**DENTAL UNIT WATERLINE MICROSTRUCTURES**

**Biofilm**

The microorganisms may originate in the waterline from any source, namely suck-back from patients mouth or those inherently present in the water form a biofilm adherent to the inner part of tubing that supplies the operatory. There are basically five steps in the biofilm development:

- Stage 1: Initial attachment
- Stage 2: Irreversible attachment
- Stage 3: Maturation I
- Stage 4: Maturation II
- Stage 5: Dispersion.

**Clinical significance**

Till recently it was inconceivable that DUWL contamination can lead to actual fatality although two such reports have been quoted in older literature without adequate substantiation. The complacency upon this matter is warranted no more, due to the alarming case of death of an 82-year-old Italian lady. This woman died due to complications of Legionnaire’s disease - A fatal type of pneumonia caused by *Legionella* bacteria which in the present case was specifically acquired during dental treatment. While dealing with medically compromised patients, utmost care needs to be taken to prevent secondary infections. The commonly isolated species found in DUWLs are Gram-negative bacteria. These lead to the production of endotoxins, which have been shown to trigger or exacerbate asthma in susceptible individuals. This is evidenced by the study done on dental students who developed asthma on commencement of their dental training. They also suggested a temporal association between occupational exposure to contaminated DUWL with aerobic counts of >200 cfu/mL at 37°C.

**Organisms identified**

An array of microbes has been isolated from the biofilms in the DUWL. Among all microorganisms isolated from DUWL, the ones having a pathogenic potential are *Legionella pneumophila*, *Mycobacterium* spp., *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus* spp., and *Stenotrophomonas maltophilia*. The prevalence of
legionellae species in DUWL has been reported to vary between 0% and 68%. Most of the isolated organisms are Gram-negative mesoheterotrophic water bacteria.

A detailed, quantitative, and qualitative analysis of water and biofilm from DUWL indicates that yeast-like fungi such as the Candida albicans, Candida curvata are found in the samples. Other species isolated from dental water units undergoing a continuous waterline treatment were Aspergillus flavus, Penicillium expansum, Exophiala mesophila. Some of these fungi are also causative organisms of respiratory infections in immunocompromised patients [Table 1].

The reports of the emergence of new multiple-drug resistant organisms (MDRO) found in nonclinical environments and the spread of these pathogens in the clinical setting have all accentuated the need to monitor these organisms. Most of the MDROS are Gram-negative microorganisms having serious consequences due to their strong antimicrobial resistance. Among them, P. aeruginosa is one such organism causing serious respiratory infections in patients. P. aeruginosa has been reported to survive for months on dry surfaces, and its ability to persist and grow even in antimicrobial solutions like triclosan, makes it a disquieting concern for medical staff.

S. maltophilia is a globally emerging Gram-negative MDRO that is commonly recovered from water treatment and distribution systems, wastewater plants, sinkholes, lakes, rivers, faucets, tap water, bottled water, contaminated chlorhexidine-cetrimide topical antiseptic, hand-washing soap, contact lens solutions, ice machines, and sink drains. The point of significance with respect to S. maltophilia is its ability to adhere to plastics and materials used in intravenous cannulae, prosthetic devices, DUWLs, and nebulizers.

Conventional measures against dental unit waterline contamination

Some of the water treatment methods in usage are biocidal agents such as chlorine and its derivatives, use of ultraviolet light, boiling of water, ultrasonic irradiation at a low frequency, water distillation, reverse osmosis, filtration with fiber and ceramic filters, activated carbon, solid block, pitcher and faucet-mount filters, bottled water, ion exchange, water softener, ozone systems, and activated alumina “Altered” Water. Among these, the commonly used treatments are analyzed below.

Filtration

The purpose of using filters on the dental waterline is to eliminate bacteria from the water entering the hand-piece. Filters should be inserted just distal to the point of entry of water into the hand-piece for maximum efficiency. A pore size of 0.2 μ is recommended. Though filters eliminate bacteria, their effect on the biofilm in the prefiltration segments of waterlines is nil unless concomitant treatment to remove them is included.

Flushing

American Dental Association recommends that waterlines should be flushed thoroughly for several minutes at the start of each clinical day. Flushing for 20 min, which would be impractical prior to most dental surgeries, would reduce the bacterial count to zero. However, the persistent nature of contamination is demonstrated when 30 min later, shedding of bacteria from the biofilm returns the total colony counts to within preflush range. Flushing for several seconds between patients, however, may remove materials that may have entered the water system during patient treatment.

Biocides and chemical disinfectants

Biocides have been used in an attempt to remove the biofilm and eliminate planktonic bacteria. These include chlorhexidine gluconate, povidone iodine, ethanol, hypochlorite, peroxide, and glutaraldehyde. The intrinsic resistance of the biofilm ecosystem has hampered their value. For usage of disinfectants, there is a dilemma between effective disinfection and formation of harmful disinfection by-products (DBP).

Nanotechnology

Nanotechnology, the purposeful machining of matter to a particle size in the range of <100 nm, holds a definite scope for creating newer materials and devices which utilize the unique phenomena realized at those length scales. This is because of their high reactivity due to the large surface to volume ratio.

Materials which can be denoted as nanomaterials are smaller than 100 nm, in at least one dimension. When diminished
to this level, these materials exhibit unique size-dependent properties which vary from their larger counterparts, many of which have been explored for applications in water and wastewater treatment. The smoothly scalable size-dependent properties which have been exploited are the properties of nanomaterials which relate to the high specific surface area, such as fast dissolution, high reactivity, and strong sorption. The discontinuous properties, such as superparamagnetism, localized surface plasmon resonance, and quantum confinement effect.\textsuperscript{[31]}

**Synthesis of nanoparticles**

Fabrication of nanomaterials is done using different techniques. Nanoparticles can be produced from larger structures (top down) by the use of ultrafine grinders, lasers, and vaporization followed by cooling. As the particles become more complex, there is a preference to use bottom-up approach by arranging molecules to form complex structures with new and useful properties.

- **Self-assembly:** Manipulation of physical and chemical conditions such as pH, temperature, and solute concentrations can induce self-assembly of molecules to form fibrous nanostructures\textsuperscript{[22]}
- **Layer by layer deposition:** By layering of sodium silicate and poly (allylamine hydrochloride) on gold, followed by calcination in a furnace, platforms of bilayer membranes used for protein analysis can be fabricated\textsuperscript{[23]}
- **Preparation of functional nanoparticles by thermal plasmas:** Induction of thermal plasmas help in the formulation of functional nanoparticles of silicide and boride. Injected powders are evaporated and reacted with boron in thermal plasma\textsuperscript{[24]}
- **Gas phase synthesis and sol-gel processing:** Gas phase synthesis and sol-gel processing are the major sections into which most attempts for the synthesis of nanoparticles fall under. Nanoparticles with diameters ranging from 1 to 10 nm with consistent crystal structure, surface derivatization, and high degree of monodispersity have been processed by both gas-phase and sol-gel techniques\textsuperscript{[25,26]}
- **Crystallization:** Utilizing solutions with differing amounts of amino acids, hydroxyapatite-aspartic acid crystals were created\textsuperscript{[27]}
- **Biogenic strategy:** The self-assembled organic materials which form templates or scaffolding for inorganic components are examples of sub-levels in structural hierarchy with its complex biologic set up\textsuperscript{[28]}
- **Microbial synthesis:** Even fungus *Aspergillus fumigatus* can be used for the production of silver nanoparticles which is carried out extracellularly\textsuperscript{[29]}
- **Biomass reactions:** Gold nanorods and nanoparticles with other shapes were produced by incubation of dead oat stalks with an acidic aqueous solution of gold ions\textsuperscript{[30]}
- **Alternative preparation methods:** To produce wider range of grain sizes, precipitation with controlled mixing and monitoring of nucleation could be used. Other methods are filter dye applications in photographic films and spectral sensitizing dyes for use in silver halide grains\textsuperscript{[31]}
- **Other strategies:** Additional nanoparticle synthesis techniques include sonochemical processing and high energy ball milling\textsuperscript{[32]}

**APPLICATIONS OF NANOTECHNOLOGY IN DENTISTRY**

Nanotechnology has vastly been used in the field of dentistry over the past couple decades, and the applications are only increasing. Nanodental techniques may evolve through several stages of technological development, first using genetic engineering, tissue engineering, and tissue regeneration, and later growing whole new teeth *in vitro* and installing them. Ultimately, the nanorobotic manufacture and installation of a biologically autologous whole replacement tooth including both mineral and cellular components find the usage of nanotechnology.

The applications are enumerated:\textsuperscript{[33-35]}

- **Tooth denaturation and caries prevention** (remineralization)
- **Enhancing dental durability and cosmetics** (nanoporous silica filled composite)
- **Diagnosis and treatment of oral cancer** (nanoelectromechanical systems, optical nanobiosensor are used for diagnosis, while systems like brachytherapy with nanomaterials, drug delivery across the blood-brain barrier, and nanotechnology in photodynamic therapy are utilized for treatment of oral cancers)
- **Orthodontic nanorobots**
- **Nano adhesive** (Polyhedral Oligomeric Silsesquiox - They can be used as either additives or replacements for traditional plastics)\textsuperscript{[36]}
- **Nano cream**
- **Nano impression** (Nanofillers in the vinylpolysiloxanes, produce a unique addition siloxane impression material. The material gives an advantage of better flow, improved hydrophilic properties, fewer voids and in turn, enhanced detail precision)
- **Hypersensitivity Cure** (nanosized particles in the desensitizing toothpaste; reconstructive dental nanorobots selectively and precisely occlude selected tubules in a small time frame, using native biological materials)
- **Nano Anesthesia** (colloidal suspension containing millions of active analgesic dental nanorobot “particles” may be released on the patient’s gingivae. These travel through the gingival sulcus and the dentinal tubules to reach the pulp. Once installed in the pulp, the analgesic dental nanorobots may be commanded by the dentist to shut down all sensitivity in any tooth that requires treatment)
- **Nanofiber scaffolds**
Nanofibers, <100 nm in diameter, including nanorods, nanoplatelets, nanotubes, nanofibrils, and quantum wires are other major nanomaterials being widely explored for various applications and with more fervor in the field of periodontology.

Periodontal regeneration can be achieved by best mimicking the structure of the extracellular matrix and allowing adhesion and proliferation of the cells at the defect site. Development of electrospun fibers and arrangement of the same in the form of scaffolds are moving a step closer toward the ideal properties of the guided tissue regeneration membranes. The nano-sized bone graft materials and drug delivery systems are proving to achieve higher regeneration than the systems available at present.

- Nano-Bio Chip Technology (used as a detector for the various markers in the saliva and gingival crevicular fluid)
- Nanofiltration (for water purification).

Nanotechnology for water decontamination

The ever increasing dearth of potable water has almost reached a crisis which could be abated utilizing advancements in nanoscience and engineering. The tools are nanoabsorbents, nanocatalysts, bioactive nanoparticles, nanostructured catalytic membranes, submicron nanopowder, nanotubes, magnetic nanoparticles, granules, flake, and high area metal particle supramolecular assemblies with characteristic length scales of 9–10 nm including clusters, micromolecules, nanoparticles, and colloids having a significant impact on water quality in the natural environment.[37] Carbon nanotubes (CNTs) have attracted a lot of attention as very powerful adsorbents, for a wide variety of organic compounds from water. Examples include dioxin,[38] polynuclear aromatic hydrocarbons,[39–41] dichlorodiphenyltrichloroethane and its metabolites,[42] polybrominated diphenyl ethers,[43] chlorobenzenes and chlorophenols,[44,45] trihalomethanes,[46,47] bisphenol A, nonylphenol,[48] phthalate esters,[49] dyes,[50] etc.

Among the plethora of materials, four categories could be evaluated for water purification: (1) Carbonaceous nanomaterials, (2) metal-containing nanoparticles, (3) zeolites, and (4) dendrimers. Their versatile physicochemical properties make them particularly attractive as separation and reactive media for water purification.[24]

Carbon-based nano-adsorbents

CNTs eliminate organic chemicals more efficiency than activated carbon on adsorption.[51] Oxidized CNTs have high adsorption capacity for metal ions with fast kinetics. Electrostatic attraction and chemical bonding on the surface of functional groups (e.g., carboxyl, hydroxyl, and phenol) of CNTs assist in adsorption of metal ions.[52]

Metal-based nano-adsorbents

Cost effectiveness is a major factor for metal oxides such as iron oxide, titanium dioxide, and alumina for removal of metals and radionuclides.[53] Metal-based nanomaterials have been explored to remove a variety of heavy metals such as arsenic, lead, mercury, copper, cadmium, chromium, nickel, and have shown great potential to outcompete activated carbon.[54]

Polymeric nano-adsorbents

Dendrimers are tailored adsorbents that are capable removing both organics and heavy metals. Their interior shells can be hydrophobic for sorption of organic compounds, while the exterior branches can be tailored for adsorption of heavy metals [Figure 2].[55]

INDIVIDUAL TECHNIQUES

Pollution detection and sensing

Sensors for the detection of different compounds can be fabricated from nanostructured materials.[56] An example is silver nanoparticle array membranes that can be used as flow-through Raman scattering sensors for water quality monitoring.[57] The particular properties of CNTs make them very attractive for fabrication of nanoscale chemical sensors, especially for electrochemical sensors.[58–61]

Nanofiltration

Water treatment for drinking water production using nanofiltration membranes is one of the easier techniques.[62] These are pressure-driven membranes with properties intermediate between those of reverse osmosis and ultrafiltration membranes and possess pore sizes between 0.2 and 4 nm. Nanofilter (NF) membranes have been shown to remove turbidity, microorganisms, and inorganic ions such as Ca²⁺ and Na⁺. CNTs have been arranged to form hollow monolithic cylindrical membranes,[63] which were found to be efficient for removal of bacteria or hydrocarbons. Silver nanoparticles have been assessed for drinking-water treatment due to their strong and broad spectrum of antimicrobial activities.[64–66] Silver nanofilters have varieties, among which the highest bacteria removal efficiency was by the Ag/cation resin filter. These filters completely removed all targeted bacteria, namely Escherichia coli, Salmonella typhimurium, Shigella dysenteriae, and Vibrio cholera.[67]

Below is a schematic of a composite nanomaterial packed bed reactor for purification of water contaminated by mixtures of (i) metal ions, (ii) organic solutes, and (iii) bacteria [Figure 3].[68]

Adsorbents

Slurry reactors or adsorbers have included nano-adsorbents into their existing treatment procedures. These are most efficient in powder form since all surfaces of the adsorbents are utilized, and the mixing greatly facilitates the mass transfer. Nano-adsorbents can also be used in fixed or fluidized adsorbers in the form of pellets/beads or porous granules loaded with nano-adsorbents.[69]
Contemporary innovations in nanotechnology for water decontamination

Nanoemulsions

Nanoemulsions are unique disinfectants with a uniform population of droplets of high energy ranging in diameter from 100 nm to 300 nm. These nanoemulsions have expanded biocidal activity even against enveloped viruses, spores, and fungi, along with bacteria by disruption of their outer membranes. A study to investigate the efficacy of cetylpyridinium chloride containing nanoemulsion disinfectants on bacterial loads at different time intervals was undertaken. The modus operandi is nonspecific disruption of bacterial cell membranes, which does not necessarily result in the development of resistant strains. Nanoemulsions can be diluted and stored at a broad range of temperatures for up to 2 years. The present study the majority of microbes found were Gram-negative water bacteria common in dental waterlines. The significant reduction in live bacteria observed in nanoemulsion-treated DUWL tubing showed that nanoemulsion effectively reduced DUWL communities.

Mechanism of action

The kinetics of the nanoemulsion is attributed to their production, wherein nanoemulsions are under produced under high shear forces in a microfluidizer. The shear energy is stored in oil droplets, giving them the high energy. This energy is passed on to bacteria upon fusion of the droplet with the bacteria, disrupting the bacterial membrane. Adjuncts used in a nanoemulsion are cetylpyridinium chloride and quaternary ammonium salt. The positive charge is placed on the nanodroplet by cetylpyridinium chloride, by its incorporation as a co-surfactant to the nanodroplet. There are multiple modes of action for antimicrobial activity of cetylpyridinium, namely disruption of intermolecular interactions, cellular membrane, cellular permeability controls, and inducing leakage of cellular contents. It could be concluded that nanoemulsion containing cetylpyridinium chloride could be quite effective in DUWL decontamination.

NANOTECHNOLOGY FOR DECONTAMINATION: THE HIGHPOINTS, PITFALLS

In spite of all the accomplishments of nanotechnology, there is still a wide debate about the safety of nanoparticles and their potential impact on environment and biota. Advantages of using nanotechnology for water purification

- Water decontamination is effective even at low concentrations
- The overall bulk form of nanomaterials needed for treatment are less, hence waste generated posttreatment is minimal
- Nanoparticles have higher surface energy thereby novel reactions can be accomplished at a nanoscale, which is impossible with the analogous bulky material
- Innumerable factors have been attributed to the chemical reactivity enhancement of nanoparticles: Small particle size leading to increase in specific surface area, increase in the surface energies for easier delocalization of electrons, and perfection in atomic organization in the lattice.
DUWL decontamination using nanotechnology

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The specific sections of nanotechnology water treatment which could be incorporated fully are

- Nanomaterials do not have a strong antioxidant property like conventional disinfectants, hence they do not produce DBPs.\(^n\)

Disadvantages of using nanotechnology for water treatment

Ecotoxicity in water has risen due to engineered nanoparticles production. This can be rectified by the use of model wastewater treatment, by clearing sludge for removal of oxide nanoparticles. TiO\(_2\) nanoparticles aggregate severely when added to water. Aqueous fullerene particles can get coagulated in the presence of salt, so on and so forth.\(^n\)

Nanotechnology-based water treatment technologies would be able to compete with conventional treatments only if the cost and systems utilizing nanomaterials become comparable to conventional methods. One of the myths people carry about future nanotechnology is that it is the panacea for all ailments.

CONCLUSION

Nanotechnology though relatively recent has gradually captured every area of medical and dental science. As in any of the technological advancements, the economy, human and environmental issues are barricade for the widespread application in the immediate future. The traditional methods of water decontamination do not actually cater to every type of dental setup. The usage of chlorine-containing disinfectants is more feasible in everyday practice, although the pollution to the clinicians and the dental assistants is alarming when quarantined. Even though ultrafilters are used, there are only a scattering if studies to support their efficacy. The custom made distilled water tanks are quite cumbersome for the wide-spread usage.

The compelling reasons for their utility are their advanced stage in research and development, availability and feasibility, and lastly compatibility with inherent systems. Aerosol contamination from the inherent water supply will definitely point toward the inadequacies in the decontamination techniques, thereby accentuating the need for incorporation of innovative technological advancements in the form of nanotechnology in the area of screening and assessment of water quality, and counteracting the same at nano levels in the form of nanoemulsions and filters.

More and more mutant and virulent species of microorganisms evade even the multiple levels of infection control are evolving. Since, water usage is ubiquitous and water being the primary vehicle of transport of these organisms, provision of pristine water in all the clinical areas is indispensable.

Currently, though nanotechnology-based water decontamination is in its embryo stages, the days are not far, when it could become the routine tenets of disinfection due to its comprehensive benefits.

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Conflicts of interest

There are no conflicts of interest.

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