Ozonated water an adjunct to tooth brushing and flossing. Myth or reality?

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The average concentration of dental plaque microorganisms in an adult with average condition of oral hygiene is about 2.5 X 10^{11}cells/g. The marginal gingiva alone harbours more than 325 cultivable bacterial species [1]. Up till a decade ago, an estimated 50% of the bacterial cells in plaque were yet to be cultured [2]. Most forms of oral diseases occur because of excessive accumulation of plaque. This leads to disturbance in the colonization resistance mechanism, which discourages attachment of exogenous microorganisms to the tooth surface [3]. Tooth brushing is the most common self-performed plaque control method. Its efficiency depends on the user's manual dexterity, age and motivation towards oral hygiene. Studies have shown that tooth brushing alone cannot adequately remove plaque from all the surfaces of teeth, especially the interdental area [4].

Ozone is a powerful non-antibiotic biocide. It is effective as a gas and dissolved in water. Its antimicrobial spectrum includes a wide variety of bacteriae, viruses and fungi. It acts by inducing oxidation of the cell wall and cytoplasmic membranes that leads to the lysis of the microorganism.Ozonated water is also very effective in inhibiting bacterial growth in biofilms. Treatment of experimental plaque with ozonated water has shown a significant reduction in the number of viable *Streptococcus mutans* [5]. Mere thirty seconds rinse of ozonated water can reduce the dental plaque bacterial load to almost half [6]. Its biocompatibility with oral epithelial cells has also been established [7]. The ease of preparation, and cost effectiveness can make it a very useful adjunct to tooth brushing and flossing. With more knowledge regarding its biocompatible concentration, ozonated water-wending machines in offices, schools, public places and homes can most certainly be a great idea towards maintaining oral health.

Keywords: Ozonated water; antimicrobial; Tooth brushing

1. The oral ecosystem

The oral cavity of human beings has a unique ecosystem, which includes a variety of microorganisms and saliva. These microorganisms live in a state of dynamic equilibrium within the hosts' oral cavity. The interaction between the normal oral flora and the host is considered the oral ecosystem. The different ecological niches in the oral environment influence the composition of the microflora at different sites. The resident microflora of the oral cavity is remarkably diverse and contains species unique to the mouth. It consists of a wide range of species of bacteria, viruses, mycoplasma, yeasts and occasionally protozoans like *Entamoeba gingivalis* [8]. Numerous microbiological studies have reported the diversity of the resident oral microflora, both at the genus and species level, at different ecological niches in the oral cavity [9].

Oral habitats that provide distinctly different ecological conditions include the mucosal surfaces like the tongue, lips, cheek and the non-shedding surfaces of the teeth. The tongue being a highly papillated surface acts as a reservoir for anaerobes. The mucosa of the lips, cheeks and palate is a habitat, which undergoes desquamation and keeps the microbial load on these surfaces low. Teeth are the hard non-shedding sites that provide surface for microbes to form biofilms. Different surfaces of the teeth support a distinct microflora because of the specific biological properties created in each of the different niches such as the fissures, proximal surfaces, smooth surfaces and dentures [10].

Other than the prevailing physical and biological properties of each site, the composition of microbiota at different sites is also sensitive to dietary fluctuation of the host, shedding of teeth, sloughing of epithelial cells, changes in physical parameters such as insertion of dentures and therapeutic treatment by antibiotics and radiotherapy. Other factors that influence the selectivity of microorganism in the oral ecosystem include pH, temperature, anaerobiosis, adherence and aggregation. The recognition of the influence of these physical, physiological and environmental factors on plaque composition has led to the concepts on oral disease recognition and prevention.

2. Dental plaque

Antoni Van Leeuwenhoek first observed the growth of a number of 'animalcules' having different morphology on the tooth surface [11]. Many years later with the development of the compound microscope, these 'animalcules' were called dental plaque. During the past two decades newer scientific methods such as confocal scanning laser microscopy have changed the view of dental plaque and it is now being studied as biofilm. A biofilm is a community of microorganisms that normally becomes attached to a surface under fluid conditions. Over 95% of nature's bacteria are estimated to exist in a biofilm [12]. Dental plaque is a biofilm that comprises of a matrix and microbes, which together form the tenacious substance that adheres to the surface of teeth and restorations.

The matrix contains polymers derived from the bacterial products and host saliva. The microbes in a biofilm have their own unique characteristics and not just the sum of the properties of the resident population. They do not exist as independent entities in the biofilm but function as a community [13]. They exhibit different properties from the same group of bacteria in the planktonic form that grow in a liquid culture. Thus, there is a high level of interest in the characteristics of bacteria existing as biofilms. The most significant difference between bacteria biofilm and bacteria growing in the planktonic form is that the former are more resistant to antibacterial agents and have more protection against host defences. Therefore, antibacterial agents that are effective on culture plates may not be so effective in a biofilm *in situ*.

2.1. Pathogenicity of dental plaque

Dental plaque is the precursor for initiation and progression of most oral diseases. It is invariably involved in the etiology of the most common oral diseases like dental caries and periodontal disease, as well as the less commonly occurring ones such as perimplantitis [14]. Over the past 120 years, the mechanism proving the role of plaque in the initiation of periodontal disease has gone through several changes. The views shifted from specific plaque hypothesis to non-specific hypothesis and back again to a theory of specific periodontal pathogen in plaque. In the early 1900's researchers assumed that plaque contained microorganism that caused dental caries and periodontal disease. This thinking was a result of the classical studies by Miller and others in the late 1800's. Using wet mounts, the technique available at that time, four different groups of suspected aetiological agents were observed which include spirochaetes, fusiforms, streptococci and amoeba. Thus, the specific plaque hypothesis came into existence [15]. Later in 1930's this theory was replaced with non-specific plaque hypothesis, which held the view that the entire bacterial flora in plaque, was responsible for periodontal disease [16]. According to this theory, identification of specific organism was not important and total plaque elimination was essential to control periodontal problems.

According to the specific plaque hypothesis other than the role played by specific pathogens in causing oral diseases such as caries and periodontal diseases, additional importance is also given to the host-bacterial interaction. It was proven in animal models that dental decay was a transmissible bacterial infection [17]. An electron microscopic study confirmed that spirochetes were present in connective tissue and the epithelium of patients with acute necrotizing ulcerative gingivitis [18]. This showed that specific bacteria are responsible for the etiology of dental caries and periodontal problems [19]. Non-disease associated plaque is plaque, which is not colonized by known pathogens. Therefore, the aim of treatment under specific plaque hypothesis is to convert odontopathic plaque to non-disease associated plaque instead of eliminating its accumulation [20]. This theory emphasises the role of specific pathogens in dental plaque biofilms, but at the same time, attention is directed towards responses of the host towards pathogenic microorganisms.

3. Plaque Control

Plaque control is the prevention of accumulation of plaque on the teeth and adjacent gingival surface. It is different from plaque elimination, which is total removal of the biofilm with its microbial as well as its organic and inorganic constituents. Plaque elimination is not desirable as it disturbs the colonization resistance mechanism, which discourages colonization of exogenous microorganisms to the tooth surface. Poor oral hygiene leads to increased accumulation of dental plaque. Most forms of gingivitis occur because of excessive accumulation of plaque. Excessive plaque at the gingival crevice area results in an inflammatory response and increase in flow of gingival crevicular fluid, which perturbs the microbial homeostasis at the crevicular area leading to gingivitis. A study by Lang et *al.*, [21] has shown that the rate of plaque accumulation increased prior to the appearance of clinical signs of gingivitis. The rate levelled off as gingivitis developed.

Two major reasons for plaque control are prevention of gingival inflammation and removal of bacterial species that have potential to cause dental caries and periodontal disease. Meticulous plaque control is an effective way of preventing and treating gingivitis. It is also a very important part of all the procedures involving the treatment and prevention of periodontal diseases [22].

3.1. Mechanical supragingival plaque control

Mechanical plaque control is the most common of all the conventional plaque control methods. Supragingival plaque removal on a regular basis is crucial in maintaining proper oral hygiene and preventing caries and periodontal disease. Loe et *al.*, [23] studied the accumulation of plaque in periodontally healthy individuals for a three-week period. After abstaining from any kind of oral hygiene methods for three weeks there was an increase in gingival inflammation which then regressed with the reinstitution of plaque control oral hygiene methods [23]. Further studies indicate that professional supragingival plaque control can result in alteration of the subgingival microflora which is associated with normal healthy periodontium [24].

3.1.1. Personal methods

The self-performed methods of plaque control include tooth brushing and interdental cleaning. Tooth brushing with a dentifrice has been practiced since ancient times. The more common perception of tooth brushing is cosmetic and aiding in fresh breath. With formulations containing anti-plaque agents, they have proved to reduce plaque and improve oral health [25]. It is the most common of all practiced methods but its efficiency depends on the users' manual dexterity and motivation towards oral hygiene. A powered toothbrush is a valuable alternate to manual toothbrush and was first introduced and studied in the early 1960's [26]. The earlier powered toothbrushes had a back and front motion. Subsequently brushes with circular motion were developed and more recently, they have been upgraded with higher frequency of vibrations. Studies have shown that powered brushes are very effective in reducing plaque [27]. They reduce plaque by 7% and gingival bleeding by 17% when compared with manual brushes [28].

Most commonly used interdental aids for plaque control are toothpicks and dental floss. Toothpicks are used where there are open interdental spaces due to recession of interdental papilla. Toothpicks can be round, rectangular or triangular in shape. The cleaning potential of the toothpick was more dependant on the shape than on the material [29]. Triangular toothpicks that confirm to the interdental space are superior in terms of plaque removal when compared to round and rectangular tooth picks. Dental flosses are used where the interdental papilla completely fills the interdental space. They can be waxed or unwaxed and studies have shown that the latter can be slightly more efficacious in interdental cleaning than the former. However, dental floss is significantly more efficient in removing interdental plaque than toothpicks, especially on lingual axial surfaces [30]. A single tufted brush is often effective in cleaning distal surface of the most posterior teeth and areas where interdental spaces are wider.

3.1.2. Professional methods

Professional plaque control methods include supragingival and subgingival scaling. Scaling is the process by which plaque and calculus are removed from supragingival and subgingival tooth surface. The primary aim of scaling is to remove plaque and calculus and restore gingival health. Sickles, curettes and ultrasonic scalers are commonly used instruments for scaling. Sickles are used to chip off calculus and remove deposits from the tooth surface. Curettes are more delicate and are used to debride supragingival and subgingival surfaces. Following hand instrumentation the clinical crowns are polished with a fine grain polishing paste and rubber cup to finish the procedure. Ultrasonic scalers are a very good alternate to hand scalers for scaling. The scaler tips are made of a metal core, which can be interchanged depending upon the tooth surface being cleaned. The metal core vibrates with an operating frequency between 25,000 to 40,000 cycles per second. This high-speed vibration produces heat and to minimize this heat the scalers are equipped with water irrigation. The vibrating tip when brought close to the tooth surface, loosens calculus and plaque thereby cleaning the surface.

3.2. Chemical supragingival plaque control

Chemicals can influence plaque both qualitatively and quantitatively. They act by stopping or lowering the proliferation rate of bacteria in plaque. Some act by preventing attachment of bacteria to the pellicle. The main use of antiplaque agents is to prevent plaque growth, control gingivitis and fight halitosis. They are also used to replace mechanical tooth brushing after surgical procedures to the oral cavity, acute and painful oral lesions where tooth brushing may result in elevation of pain, following scaling and root planing procedures and as an adjunct to normal mechanical methods [31]. However they are not significantly effective in cleaning interdental and subgingival plaque [32].

3.2.1. Anti-plaque agents

Phenolic compounds have been used for more than 100 years now. They are known to be germicidal and one product 'Listerene®' has been put to long-term studies, which showed reduction of plaque and decrease in severity of gingivitis [33]. Acidified sodium fluoride was first used as a mouthwash in the early 1940's. By the 60's fluoride mouthwashes showed promising clinical results in terms of plaque inhibition and caries prevention [34].

Herbal extracts like sanguinarine have been reported to possess anti-plaque properties and are used as mouthwashes and dentifrices [35]. Chlorehexidine gluconate, a bisguanide, has a wide spectrum antimicrobial activity. The currently accepted concentration of 0.12% chlorhexidine is supposedly the most effective chemical agent for plaque control. Clinical trials have shown a reduction of 55 to 60% of plaque and 45% of gingivitis [35].

Oxygenating agents like hydrogen peroxide are disinfectants, which are also effective in reducing inflammatory signs from the periodontium. Triclosan is an antimicrobial agent with activity against gram positive and gram-negative bacteria. It does not stain or alter the taste like chlorhexidine but has very poor substantivity [36]. Subgingival plaque requires irrigation or local delivery methods for transporting the anti-plaque agent to the periodontal pocket.

4. Ozone

Ozone is a pale blue gas with a strong odour. It is deep blue when in liquid form and is strongly magnetic. Its chemical formula is O_3 and has three atoms in each molecule. Being a powerful oxidant, it reacts with almost all biological substances. Ozone is capable of drawing electrons from a source, and in the process decrease the oxidation state of at least one of its oxygen atoms.

4.1. Mechanism of action

Ozone disinfects by rapidly rupturing the cell membrane of bacteria. It acts by inducing oxidation of the cell wall and cytoplasmic membranes that leads to the lysis of the microorganism. It is reported that bacteria suspended in phosphate buffered saline are completely inactivated after a 15 seconds exposure to ozone gas. Likewise, viruses suspended in a fluid low in organic material are also rapidly inactivated by ozone gas [37]. An ultrastructure study, using the scanning electron microscope, have shown that the cells of *Streptococcus mutans* treated with ozonated water showed the presence of holes in the membrane. The results suggested that the bactericidal activity of ozonated water might be through the disruption of its cytoplasmic membrane. Ozonated water is also very effective in inhibiting bacterial growth in biofilms. Treatment of experimental plaque with ozonated water has shown a significant reduction in the number of viable *Streptococcus mutans* [5]. The effectiveness of ozonated water on human plaque biofilm is comparable to other commonly used antimicrobial agents such as povidone iodine and benzylthonium chloride [5]. Suspensions of *Actinomyces naeslundii*, *Lactobacilli casei* and *Streptococcus mutans* in salt buffer, when exposed to ozone gas delivered by Healozone© were completely killed in 60 seconds [38].

4.2. Applications of Ozone in oral cavity

Dr. E.A.Fisch, a Swiss dentist, was the first to use ozone to treat dental pulpitis [39]. Ozone gas has been used to inactivate the causative microorganisms of primary root caries. It has also been used to treat sensitive cervical caries without altering the physical properties of the tooth enamel [40]. When used in endodontics as an irrigant it was not sufficient to disinfect the human root canals [41]. However, it is effective in inactivating *Streptococcus mutans*, methicillin-resistant *Staphylococcus aureus*, *Candida albicans*, and *Enterococcus. faecali*s from dentures [42]

4.3. Biocompatibility of Ozone

The first ozone generator was constructed in Germany. Nikola Tesla, a physicist, patented an ozone generator in late 19th century (Patents of Nikola Tesla 'Apparatus of producing Ozone' Patent Number 568,177 September 22, 1886). In nature, ozone gas is produced by the combination of sun's ultra-violet rays and oxygen in air. The corona discharge after a thunderstorm also results in ozone in air. A corona discharge ozone generator produces an electric discharge, which converts oxygen to ozone. Ultra-violet ozone generators utilize ultra-violet rays in producing ozone [43]. Electro-conductive diamond electrodes are also used to produce ozonated water [44]. Ozone gas readily dissolves in water to form ozonated water. Ozone gas is more powerful than ozonated water. Huth KC *et al* [45] studied the effect of ozone on oral cells and found high level of biocompatibility. No adverse effects were reported after treatment of human oral epithelial cells, gingival fibroblast cells and periodontal cells with aqueous ozone. There is sufficient evidence to establish the biocompatibility of ozone in *in-vitro* conditions [7].

5. Ozone as an adjunct to Tooth brushing and flossing

The major drawback with powered or manual toothbrushes is its reduced effectiveness in cleaning interdental area when compared to other surfaces. Whatever the technique employed tooth brushing results in incomplete removal of interdental plaque [46]. This is the reason why gingivitis is more severe in the interdental region than on the facial and lingual surfaces [47]. Tooth brushing along with flossing results in less plaque accumulation and gingivitis than tooth brushing alone [48]. Regular mechanical plaque removal leaves the tooth surface clean but not 'sterile' thus resulting in a healthy non-disease associated plaque. Factors like dexterity of the individual, motivation and age determine the extent of quantitative cleaning and removal of plaque. Rugg-Gunn [49] showed that school children tend to clean the labial surface of the anterior lower teeth more and spend less time on the lingual compared to the buccal surfaces. Right handed toothbrushers were shown to have more buccal plaque on right teeth [4].

Ozonated water is colour less liquid, which retains its concentration for a short while. At 0.1 ppm concentration ozonated water gargling did not produce any intra-oral or systemic side effects. Gargling at 0.1ppm was effective in reducing the *in-situ* microbial load of plaque to almost half [6]. Reduction of microbial plaque population is more desirable than its complete elimination. Freshly prepared ozonated water can be a potential alternate to chemical plaque control methods. However, the challenge of insufficient interdental cleansing needs evaluation. It can be manufactured in small scale with the same efficiency as in large scale. The ozonizers and water are common resources that are available to any urban population. Any ozonizer, which can provide fresh ozonated water at specific concentration, can be used as an ozonated water-wending machine. The ease of preparation, and cost effectiveness can make ozonated water gargling a very useful adjunct to tooth brushing and flossing. With more knowledge regarding its biocompatible concentration, ozonated water-wending machines in offices, schools, public places and homes can most certainly be a great idea towards maintaining oral health. Further research in this direction can be a huge step towards revolutionizing oral health.

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