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A COMPARATIVE EVALUATION OF EFFECTIVENESS OF OZONATED WATER OVER COMMERCIALLY AVAILABLE DENTURE CLEANSING TABLETS ON CANDIDA SPECIES BIOFILM IN HEAT CURED PMMA RESIN PLATES.

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Abstract:

Background/aims: *Ozone (O_3) is a highly reactive molecule with antimicrobial properties against bacteria, fungi and viruses, which have been proven in different studies. We examined the effect of ozonated water on Candidaalbicans on acrylic denture plate and compared its efficacy with commercially available denture cleansing tablets*

Methods: *The heat-cured acrylic resins were cultured with C. albicans. After treatment of ozonated water and denture cleansing tablet dissolved solution the number of attached C. albicans was counted by digital colony counter in terms of Colony Forming Units CFU/ ml.*

Results: *After treatment with ozonated water (4 mg/l) for 60 min, the colony forming units were markedly depleted as compared with the control group but aseptic. The antimicrobial activity against C. albicans between plates immersed in ozonated water were slightly greater than those treated with commercially available denture cleaners.*

Conclusion: *Our results suggest that application of ozonated water may be useful in reducing the number of C. albicans on denture plates.*

Introduction

Ozone is known to be a potent oxidizer and has the ability to oxidize any known biological entity. A recent study demonstrated that denture plaque control is essential for the prevention of denture stomatitis associated with Candidaalbicans[1]. In addition, the fitted surfaces of dentures have been shown to be reservoirs of Candida albicans, which is associated with stomatitis and disseminated fungal infectious diseases, and it is well known that elderly hospitalised patients have a high risk of aspiration pneumonia induced by microorganisms on dentures[2,3]. Denture-cleansing methods are generally divided into mechanical and chemical cleaning methods. However, it has been reported that mechanical cleaning methods are insufficient for a complete reduction of microorganisms on denture plates[4]. Thus, it is considered that chemical cleaning methods are more effective, in which hypochlorites, peroxides, enzymes, and acids are generally employed as immersion-type chemical solutions for denture cleaning. However, some chemical agents used for denture cleaning are known to damage acrylic resin and metal

alloy materials, and are also relatively expensive compared to ozonated water used in the present study[5].

Ozonated water has been shown to be a powerful antimicrobial agent against bacteria, fungi, protozoa, and viruses[6]. It is recognized that dental plaque is a kind of bacterial biofilm[7,8] in which bacterial growth is the primary factor governing the relative abundance of different microorganisms including C. albicans. In a recent study by Nagayoshi et al he stated that ozonated water was useful in reducing the number of infections caused by oral microorganisms. This suggested that oral microorganisms might be inactivated by ozonated water at different doses[9]. It has been reported that ozonated water can be mutagenic if used for a long period and in high concentrations[10]. In the present study, we evaluated the antimicrobial efficacy of ozonated water against Candida albicans on resin denture plates in vitro by counting the colony forming units and compared it with the effectiveness of commercially available denture cleansing tablets with sodium mono phosphate perborate as its main content.

Material and Methods

Growth conditions for Candida albicans and preparation of test plates

The experimental denture plates were prepared using heat-cured acrylic resin (25 mm x 2 mm x 2mm) (Asian Acrylates Acrylin H Heat Cure, Dbm Pink, Mumbai) according to the manufacturer's instructions. The resin plates were ground with#180 emery paper, sonicated in water for 60 min, and immersed in water for 1 day to remove the residual monomer, then dried in air.

Sterile 1 X PBS (Phosphate Buffered Saline); pH 7.4

- NaCl
- KCl
- Na_2HPO_4
- KH_2PO_4

Potato Dextrose Broth

24g Nutrient broth was suspended in 1000ml of distilled water and was autoclaved at121°C; 15lbps for 15 minutes.

Potato Dextrose Agar plates(1 L)

The medium was prepared by dissolving 39 g of the commercially available Potato Dextrose Agar Medium (HiMedia-GM096) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure, 121°C. After autoclaving, the media (20ml) was allowed to cool to 60°C and was poured to pre sterilized Petriplates. The plates were allowed to solidify in a laminar air flow chamber.

Fungal culture:

Candida albicans (ATCC 10231) was used for the study.

Preparation of biofilm and sample addition

Candida albicans was prepared (growth adjusted to 0.5% McFarland's standard). PMMA Blocks were incubated in respective test tubes containing Candida albicans for 5 days at room temperature for the formation of biofilm.

Experimental and Control groups

Contaminated samples (n =10) were grouped using simple random allocations as follows:

GROUP A is incubated samples (n =10) that left untreated were considered as the control group

GROUP B is incubated samples (n =10) which were immersed in ozonated water.

GROUP C is incubated samples (n =10) which were treated with commercially available denture cleansing tablet dissolved solution.

Cleaning procedures

Un treated remained as control group and was considered as Group A.

Ozonated water was generated by portable ozone generator DENT OZONE, ADC Inc. Mumbai) (Fig 1). The concentration of ozonated water in the aqueous phase is adjusted to concentrations of 4 mg/l for 60mint and this group was considered as Group B

Commercially available denture cleansing tablet with sodium mono phosphate perborate as its main content dissolved in 150ml of distilled water for 24hrs (as per manufacturer's `instructions) and this group was considered as Group C

Enumeration of CFUs/mL

After treatment with Ozonated water and Denture cleansing tablets, the treated blocks were rinsed three times with sterile 1 X PBS (pH 7.4) to remove unattached Candida *sp* and debris. Then the blocks were added with 3 ml sterile 1 X PBS (pH 7.4) and vortexed for 10 minutes, the supernatant containing detached bio film was transferred to sterile eppendrof tubes, respectively. 10μL was plated uniformly on potato dextrose agar plates and incubated at 37^{0}C for 24 hrs.

The plates were checked for colony forming units (CFUs) after 24 hours of incubation. The CFUs were then enumerated using a Digital Colony Counter. The CFUs in Control and test was compared for determining the inhibition of cell adherence which was expressed in terms of CFUs/mL.

TABLE 1

Sample code		No. of Blocks	CFUs/mL
Heat cured PMMA Blocks in	Control	10	1344 x 10^2
	Ozonated water	10	200 x 10^2
	Denture cleansing tablets dissolved solution	10	408 x 10^2

Statistical analysis

Statistical analysis was performed using software STATISTICA (Version 7.0; StatSoft Inc.). Significant statistical differences between treatments were examined using Student–Newman–Keuls test. Differences were considered statistically significant at $P < 0.05$.

Results

We examined the effects of different cleaning methods on the adherence of Candida albicans to resin plates (Table 1). This showed that heat cured PMMA blocks treated with ozonated water of 4mg/l concentration for 60 minutes shows less number of colony forming units per millilitre of Candida albicans. They are most effective in reducing the growth of Candida albicans, but was not completely aseptic.

The control group had the highest colony count of Candida albicans.

Comparison of microbicidal efficacy between ozonated water and denture cleansers

We compared the microbicidal efficacy of ozonated water with commercially available

Denture cleanser tablet [sodium monophoshate per borate]. Our results showed that denture cleanser tablets are effective in removing Candida

Fig 1. Portable ozone generator (DENT OZONE)

albicans from PMMA, but its potency is less than that of ozonated water. They showed more CFU/ml than ozonated water and less than that of control group.

Scanning Electron Microscopic Observation of Candida albicans
(Magnification 100x)

Figure 1 shows scanning electron microscopic picture of control group (Mag 100x50μm), which was the untreated group showing high number of colony forming units per millilitre. (Group A)

Figure 2 shows scanning electron microscopic picture (Mag 100x50μm), of Group B, the group which was treated with ozonated water showing least number of CFUs/ ml of candida albicans .

Figure 3 shows scanning electron microscopic picture (Mag 100x50μm), of Group C, the group which was treated with commercially available denture cleansing tablet, showing less number of CFUs / ml of candida albicans than control group and less effective in removing candida than group treated with ozonated water.

Discussion

In this study, two different denture hygiene methods are presented, which differed in their ability to remove Candida albicans from denture samples, thus supporting a rejection of the null hypothesis for C albicans removal.

With the onset of the 21st century, infectious disease specialists are being asked to manage a greater number of patients with serious infections who are over 65 years old, and are gaining a new perspective on the emerging problem of geriatric infectious diseases.

Recently, because of growing interest, there have been a great number of clinical studies of a variety of infectious diseases in geriatric populations, and it has come to be generally accepted that the etiology, clinical manifestations, therapy, and prognosis of pneumonia in elderly patients are quite different than in younger adults[11].

In the dental field, it is necessary to find innovative methods and techniques that can prevent, reduce, or eliminate oral microorganism colonization in elderly patients who have denture plates[12]. Further, it has been reported that there is a strong association between poor denture hygiene and oral candida colonization[13]. Results of another recent study indicated that compromised elderly patients above 70 years of age had a higher number of C.albicans than patients in their 60s[14]. These findings suggest that plaque accumulation on the dentures of handicapped elderly patients could create an appropriate environment for the growth of C. albicans. Many researchers have reported that surface roughness is a key factor in the entrapment of microorganisms on denture surfaces. Verran

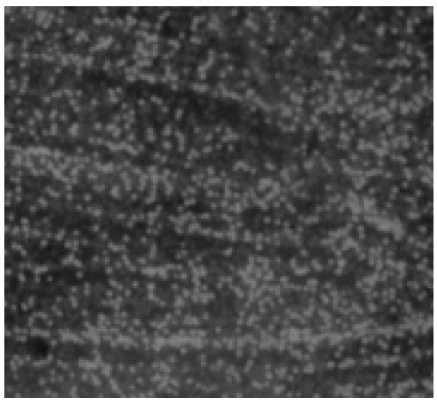

Figure 1

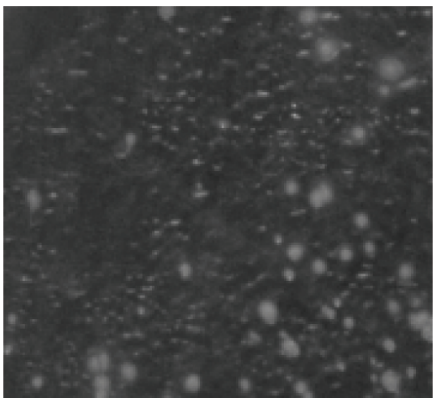

Figure 2

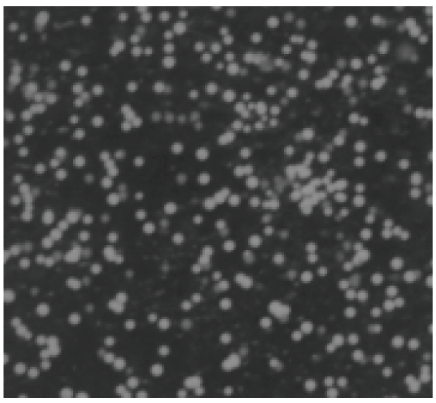

Figure 3

and Maryan[15] found that an increase in surface roughness facilitated C. albicans retention on acrylic resin surfaces. However, few microbiological studies have been conducted to develop a cheap and reliable apparatus that is easy to use. In the present study, we prepared the resin plates (25 mm x 2 mm x 2 mm) with a rough surface to create an appropriate environment for the growth of C. albicans, and examined the effect of ozonated water on the viability of C. albicans in order to understand its effect on dentures and compare its effectiveness with commercially available denture cleansing tablet with sodium mono phosphate per borate as its main content.

And the data we obtained from the study is that the cleansing efficiency is higher in ozonated water treated group than that of group treated with commercially available denture cleansing tablet.

Arita et al. observed that accumulation of microorganisms, especially C. Albicans, on acrylic resin denture surfaces was significantly reduced by rinsing with ozonated water accompanied by ultrasound. This effect depends on the concentration of ozone and the highest effect is with ozone concentration of 4 mg/ml.[16]

A denture cleanser releasing ozone bubbles with a concentration of 10 ppm has been developed and is effective in reducing the number of C. albicans following a 30–60 min exposure.[17]

Suzuki et al. showed that ozone is successfully used in dental casting alloys, such as a removable partial denture which unlike other detergent materials in the markets, it does not have any negative effect on the surface and weight properties of these alloys.[18]

Oizumi et al. showed that ozone gas is more effective in removing denture microorganisms than ozonated water.[19] A study by Ekren et al. showed the effect of ozone on bond strength of soft liners to acrylic resin materials. Ozone reduces the bond strength of soft liners such as mollosil and molloplast B to acrylic resin dentures and the higher the exposure to ozone is, less bond strength will be obtained.[20] It has been reported that ozone has astrong oxidizing power with a reliable microbicidal effect[21, 22, 10], and it is generally accepted that oxidation mediated by ozone destroys cell wall and cytoplasmic membranes of bacteria and fungi[23]. After a membrane is damaged by oxidation, its permeability increases, causing the microorganism to die.

There are several applications for ozone in the field of prosthodontics like patients suffering from periimplantitis were investigated by Karapetian et al.[24] They compared the effectiveness of conventional, surgical and ozone therapy methods to cure periimplantitis. They reported that the main challenge seems to be the decontamination of the implant surface, its surrounding tissue and the prevention of recolonization with periodontal pathogenic bacteria. And the most effective bacterial reduction was recorded in the ozone-treated patient group.

Conclusion

Although several studies have examined ozone in various fields of dentistry, this is still a novelty and further studies are needed. It seems that ozone can effectively remove bacterial plaques and create a suitable environment for the performance of different dental restorations and dentures.

In this study we propose that it is possible to apply ozonated water for denture cleaning, and this method effectively saves expense at home for the aged.

Ozone therapy has a wide range of applications in almost every field of dentistry. Its unique properties include immunostimulant, analgesic, antihypnotic, detoxicating, antimicrobial, bioenergetic and biosynthetic actions. It is atraumatic, painless, non-invasive nature and relative absence of discomfort increase patient's acceptability and compliance .

However it is necessary to pay attention to the side effects of ozone, that include epiphoria, rhinitis,

cough, headache, nausea and vomiting[25].

This study was conducted in Biogenix Research Center, Trivandrum. Candida albicans (ATCC® 10231TM) was used for the study.

Since this culture was prepared in vitro without any patient involvement, there is no scope for ethical issues.

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