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EVALUATION OF THE ANTIMICROBIAL EFFICACY OF OZONATED WATER AND THE EFFECT ON COLOR STABILITY OF DENTURE BASE RESIN: AN IN VITRO STUDY

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ABSTRACT

Purpose: The purpose of this study was to evaluate antimicrobial efficacy of ozonated water on heat cure polymethyle methacrylate denture base acrylic resin and its color stability before and after use of ozonated water. **Materials and method:** Ninety Heat cure Polymethyle methacrylate denture base acrylic resin samples having 25x10x3 mm of dimension were fabricated. These samples were infected with the *Candida albicans* and treated with ozonated water with three concentrations (2mg/L, 4mg/L, 6mg/L) at three time intervals (5, 10, 30 minutes). After that Colony of *candida albicans* counted and color stability checked with spectrophotometer. **Results:** One-way ANOVA showed highly statistically significant difference in 2mg/L, 4mg/L and 6mg/l concentration of ozonated water on *Candida albicans* at 5, 10, and 30 minutes ($p<0.001$). Reduction of count of *Candida albicans* seen in all groups but 6mg/L at 30 minutes of time interval showed marked reduction of count of *Candida albicans.* While analysing color stability result showed that Tukey's post hoc test was statistically significant in all groups ($p=0.004$). **Conclusion:** Ozonated water was very effective for reducing *Candida albicans* count on heat cure polymethyle methacrylate denture base acrylic resin samples. Significant color change was seen on heat cure denture base acrylic resin samples after use of ozonated water.

INTRODUCTION

Heat cure Polymethyl methacrylate (PMMA) is the most commonly used denture base resin.[1,2] No matter what kind of denture base material we are using, denture care is indispensable for oral health.[1,3] Microbial plaque that accumulates on the fitted surfaces of dentures is composed of several oral microorganism species including Candida albicans, which is a major causative agent of denture-associated chronic atrophic candidiasis.[4,5]

Denture cleaning is an important measure that can prevent cross-contamination and contributes to patient's health, denture longevity, and overall quality of life.[1] Several denture-cleaning methods are used clinically for the reduction of denture plaque, debris, and stains, and these 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.[4,6]

Using brushes with hard bristles and too much force would cause scrapes and surface roughness in soft liners. Rough surface of denture base acrylic resin encourages plaque and pigment accumulation and jeopardizes aesthetics. Chemical cleaners are recommended as the method of choice, especially in elderly patients who have reduced motor-nerve capabilities to perform a mechanical cleansing technique.[7,8,9,10]

Ozonated water has been shown to be a powerful antimicrobial agent against bacteria, fungi, protozoa, and viruses.[4,11-19] However, very few studies have been done in the past evaluating the antimicrobial efficacy of ozonated water on heat cure denture base acrylic resin. Hence, this in vitro study was taken to evaluate antimicrobial activity of ozonated water and its effect on colour stability on heat cure denture base acrylic resin.

MATERIALS AND METHOD

A total of 90 heat cure polymethyl methacrylate denture base acrylic samples having 25x10x3 mm of dimension were fabricated which were divided into three groups i.e, Group A 5 minutes, Group B 10 minutes and Group C 30 minutes. These groups were further divided into three subgroups according to the different concentration of ozonated water i.e, Subgroup 1: 2 mg/L, Subgroup 2: 4 mg/L and Subgroup 3: 6mg/L concentration of ozonated water.

Agar plates were prepared by using Sabouraud dextrose agar to culture *Candida albicans*. All heat cure polymethyl methacrylate denture base acrylic samples were infected with the *Candida albicans*. These samples were immersed in ozonated water in three concentrations and at three-time intervals. Colony of *candida albicans* counted after treating with ozonated water. Color of all the samples measured with spectrophotometer before and after treatment with ozonated water.

RESULTS

Table 1 showed count of *Candida albicans* before and after ozonated water on adherence of *Candida albicans* on heat cure denture base acrylic resin samples with different concentration at 5 minutes time interval. The mean count of *Candida albicans* was found to be highest Group A1 (2mg/L) 41000 (±11005.04). One-way ANOVA test showed the F value was 121.872, which was highly significant (p<0.001).

Table 2 showed count of *Candida albicans* before and after ozonated water on adherence of *Candida albicans* on heat cure denture base acrylic resin samples with different concentration at 10 minutes time interval. The mean count of *Candida albicans* was found to be highest Group B1 (2mg/L) 34000(±11737.0). One-way ANOVA test showed the F value was 79.919, which was highly significant (p<0.001).

Table 3 showed count of *Candida albicans* before and after ozonated water on adherence of *Candida albicans* on heat cure denture base acrylic resin samples with different concentration at 30 minutes time interval. The mean count of *Candida albicans* was found to be highest Group C1 (2mg/L) 23000(±9486.0). One-way ANOVA test showed the F value was 56.216, which was highly significant (p<0.001).

Table 4 showed in comparison of effect of different concentration of ozonated water on color stability (ΔE) of denture base resin at 5 minutes time interval. The mean color stability of group A2 of denture base resin was found to be highest 0.0536 (±0.0278). Tukey's post hoc test showed F value was 6.758 which was significant (p=0.004).

Table 5 showed comparison of effect of different concentration of ozonated water on color stability (ΔE) of denture base resin at 10 minutes time interval. The mean color stability of group B3 of denture base resin was found to be highest 0.0705 (±0.1728).

Table 6 showed comparison of effect of different concentration of ozonated water on color stability (ΔE) of denture base resin at 30 minutes time interval. The mean color stability of group C3 of denture base resin was found to be highest 0.0861 (±0.0316).

Table 1: Comparison of different concentration of ozonated water on *Candida albicans* at 5 minutes time interval.

Group A (5mins)	Before treatment *C. Albicans* count Mean (SD)	After treatment *C. Albicans* count Mean (SD)	Change in *C. Albicans* count Mean (SD)
Group A1 (2mg/L)	500000 (0.0)	41000 (11005.04)	459000 (11005)
Group A2 (4mg/L)	500000 (0.0)	3570.0 (2297.84)	496430 (2297.0)
Group A3 (6mg/L)	500000 (0.0)	193.0 (136.14)	499807 (136.14)
One way Anova 'F' test	F = 0.0	F = 121.872	F = 121.872

p>0.05 – no significant difference (NS), **p<0.001 – highly significant

Table 2: Comparison of different concentration of ozonated water on *Candida albicans* at 10 minutes time interval

Group B (10 mins)	Before treatment *C. Albicans* count Mean (SD)	After treatment *C. Albicans* count Mean (SD)	Change in *C. Albicans* count Mean (SD)
Group B1 (2mg/L)	500000 (0.0)	34000 (11737.0)	466000 (11737.8)
Group B2 (4mg/L)	500000 (0.0)	1240.0 (950.0)	498760 (950.08)
Group B3 (6mg/L)	500000 (0.0)	153.0 (192.12)	499847 (192.12)
One way Anova 'F' test	F = 0.0	F = 79.919	F = 79.919

p>0.05 – no significant difference (NS), **p<0.001 – highly significant

Table 3: Comparison of different concentration of ozonated water on *Candida albicans* at 30 minutes time interval.

Group C (30 mins)	Before treatment *C. Albicans* count Mean (SD)	After treatment *C. Albicans* count Mean (SD)	Change in *C. Albicans* count Mean (SD)
Group C1 (2mg/L)	500000 (0.0)	23000 (9486.0)	477000 (9486.8)
Group C2 (4mg/L)	500000 (0.0)	800 (731.81)	499200 (731.81)
Group C3 (6mg/L)	500000 (0.0)	95 (77.78)	499905 (77.78)
One way Anova 'F' test	F = 0.0	F = 56.216	F = 56.216

p>0.05 – no significant difference (NS), **p<0.001 – highly significant

Table 4: Comparison of effect of different concentration of ozonated water on color stability (ΔE) of denture base resin at 5 minutes time interval.

Group A (5mins)	Mean	SD	One way Anova F test	p value, Significance
Group A1 (2mg/L)	0.0212	0.0163	F = 6.758	p = 0.004*
Group A2 (4mg/L)	0.0536	0.0278		
Group A3 (6mg/L)	0.0597	0.0293		

Table 5: Comparison of effect of different concentration of ozonated water on color stability (ΔE) of denture base resin at 10 minutes time interval.

Group B (10 mins)	Mean	SD	One way Anova F test	p value, Significance
Group B1 (2mg/L)	0.0385	0.0352	F = 4.265	p =0.025*
Group B2 (4mg/L)	0.06	0.0180		
Group B3 (6mg/L)	0.0705	0.0172		

Table 6: Comparison of effect of different concentration of ozonated water on color stability (ΔE) of denture base resin at 30 minutes time interval.

Group C (30 mins)	Mean	SD	One way Anova F test	p value, Significance
Group C1 (2mg/L)	0.042	0.0162	F = 8.884	p =0.001*
Group C2 (4mg/L)	0.0527	0.0227		
Group C3 (6mg/L)	0.0861	0.0316		

DISCUSSION

Denture disinfection is achieved mechanically by brushing and chemically by use of chemical agents like, hydrochlorides, peroxide, various acids and enzymes.[17] Chemical methods for disinfecting dentures include either soaking or immersion in solutions such as vinegar, sodium hypochlorite, glutaraldehyde, iodoform, chlorine dioxide, or alcohol solutions.[31] These chemicals have been reported to damage acrylic resin by altering the surface properties of acrylic resin therefore their use is not recommended.[17,32] The interphase region between the PMMA polymer bead and the polymer matrix was most affected by the ethanol.[33] Disinfection with ozonated water is new method, present study examined the antimicrobial activity of an ozonated water and its effect on color stability. In previous studies different concentrations of ozonated water at various time interval was used. Mostly used concentrations were in between 0.5 mg/L to 10 mg/L at various time interval to know the antimicrobial efficacy of ozonated water against the microorganisms like bacteria, fungi, protozoa and viruses.[4,13,15,16,18,19] In the present study 2 mg/L, 4 mg/L and 6 mg/L concentration of ozonated water were used at time interval of 5 min, 10 min and 30 minutes.

Candida albicans count in all the samples at 5 minutes time interval but concentration of 6 mg/L showed marked reduction of *Candida albicans* count. One-way ANOVA showed highly statistically significant difference in all the groups ($p<0.001$). Nagayoshi et al. in their study find that range of 0.5-4 mg/L was highly effective for killing *Candida albicans*.[12] Kucuk et al. used same concentration and time and got similar results.[19] Yoshida et al. used 4mg/L concentrated ozonated water for 5 minutes and find reduction in microorganism.[16] Habeeb et al. in their study find that ozone concentration of at least 4 mg/L was needed to kill the cells.[23]

6 ml/L concentration of ozonated water for 10 minutes remarkably reduced *Candida albicans* count. One-way ANOVA showed highly statistically significant difference in all the groups ($p<0.001$). Arita et al. find 4 mg/L ozonated water for 10 minutes was effective for sanitization of *Candida albicans*.[4] Yoshida et al. advocated that 4 mg/L of ozonated water for 10 min is sufficient for inactivation of virouses.[16]

Very few *Candida albicans* count were seen in 6 mg/L concentration of ozonated water was used for 30 minutes of time interval. On applying One-way ANOWA showed highly statistically significant difference ($p<0.001$). Cesar et al.[24] find that the concentration of 10 mg/L for 30 minutes was sufficient to reduce the number of colonies forming unit of *Candida albicans*. According to Cesar et al.[24] use of ozone for 5 minutes period had bacteriostatic effect and 30 min use of ozone had bactericidal effect. The present study showed that to increase period of immersion of samples in ozonated water increases the disinfection efficacy. The result of present study is in accordance with the results of past studies.[12,13,15,17,20,21,25,26,27, 28,29,30]

No study was found in literature about the color change of heat cure denture base acrylic resin after treating with ozonated water or in any form of ozone. Therefore, the result of present study cannot be compared with other studies. Previous studies shown that color change in denture base materials is caused by changes in the matrix of the material and solubility, water sorption, leakage, surface roughness, and chemical degradation may also be factors.[22] The colour change was found to be variable. It could be due to different absorption property of materials.

The null hypothesis was rejected as ozonated water change the color of heat cure denture base acrylic resin.

Clinical implication of the present study was that ozonated water is highly effective to disinfect the heat cure denture base acrylic resin. It substantially reduced *Candida albicans* pathogenic load from the heat cure denture base acrylic resin. On the other hand, even 2 mg/L concentration of ozonated water change the color of denture base acrylic resin. Therefore, ozonated water can be used in daily dental practice to disinfect the dentures made from heat cure denture base acrylic resin with caution.

There are certain limitations of the present study. Since it was an in vitro study, dynamic intra oral conditions cannot be simulated. In the present study only 3 concentrations of ozonated water were used. Further studies including less concentration of ozonated water, variation of time interval with larger sample size and with denture cleansing agents will be conducted. The effect of ozone on other physical and mechanical properties of the acrylic should be assessed in order to confirm the safe use of this disinfection method without any undesirable effects.

CONCLUSION

The following conclusions were drawn from this in vitro study:-

1. Ozonated water was very effective for reducing *Candida albicans* count on heat cure polymethyl methacrylate denture base acrylic resin samples.
2. As the time interval and concentration of ozonated water increases the colonies of *Candida albicans* were reduced.
3. As the time interval and concentration of ozonated water increases, it changes the color stability of heat cure polymethyl methacrylate denture base acrylic resin samples.
4. Even, ozonated water with 2mg/L concentration affected color change of heat cure polymethyl methacrylate denture base acrylic resin so it should be used with caution.

REFERENCES

1. Porwal A, Khandelwal M, Punia V, Sharma V. Effect of denture cleansers on color stability, surface roughness, and hardness of different denture base resins. J Indian Prosthodont Soc., 2017; 17(1): 61–7.
2. Hersek N, Canay S, Uzun G, Yildiz F. Color stability of denture base acrylic resins in three food colorants. J Prosthet Dent, 1999; 81: 375-9.
3. Salman M, Saleem S. Effect of different denture cleanser solutions on some mechanical and physical properties of nylon and acrylic denture base materials. J Baghdad Coll Dent, 2011; 23: 19-24.
4. Arita M, Nagayoshi M, Fukuizumi T, Okinaga T, Masumi S, Morikawa M, et al. Microbicidal efficacy of ozonated water against Candida albicans adhering to acrylic denture plates: Effect of ozonated water on Candida albicans. Oral Microbiol Immunol, 2005; 20(4): 206–10.
5. Barbeau J, Seguin J, Goulet JP, de Koninck L, Avon SL, Lalonde B, et al. Reassessing the presence of Candida albicans in denture-related stomatitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2003; 95: 51–9.
6. Dills SS, Olshan AM, Goldner S, Brogdon C. Comparison of the antimicrobial capability of an

abrasive paste and chemicalsoak denture cleaners. J Prosthet Dent, 1988; 60: 467–70.
7. Nakhaei M, Mirmortazavi A, Ghanbari M, Ahmadi Z. Effect of ozone and two common denture cleaners on tensile bond strength and surface hardness of a silicone soft liner. Front Dent, 2019; 16(5): 351–6.
8. Paranhos HF, Silva-Lovato CH, Souza RF, Cruz PC, Freitas KM, Peracini A. Effects of mechanical and chemical methods on denture biofilm accumulation. J Oral Rehabil, 2007; 34(8): 606-12.
9. Diwan R. Materials Used in the Management of Edentulous Patients. Prosthodontic Treatment for Edentulous Patients. St Louis CV Mosby Co., 2004; 190-207.
10. Cakan U, Kara O, Kara HB. Effects of various denture cleansers on surface roughness of hard permanent reline resins. Dent Mater J., 2015; 34(2): 246-51.
11. Kim JG, Yousef AE, Dave S. Application of ozone for enhancing the microbiological safety and quality of foods: a review. J Food Prot., 1999; 62: 1071–87.
12. Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. J Endod, 2004; 30(11): 778–81.
13. Huth KC, Quirling M, Maier S, Kamereck K, Alkhayer M, Paschos E, et al. Effectiveness of ozone against endodontopathogenic microorganisms in a root canal biofilm model. Int Endod J., 2009; 42(1): 3–13.
14. Kshitish D, Laxman VK. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: a clinical and microbiologic study. Indian J Dent Res., 2010; 21(3): 341–8.
15. Goztas Z, Onat H, Tosun G, Sener Y, Hadimli HH. Antimicrobial effect of ozonated water, sodium hypochlorite and chlorhexidine gluconate in primary molar root canals. Eur J Dent, 2014; 8(4): 469–74.
16. Yoshida G, Ando M, Sugita Y, Maeda H, Kato D, Suzuki R, et al. Effect on osteogenesis of cleaning titanium implants with ozonated water. J Hard Tissue Biol., 2016; 25(2): 149–56.
17. Garg J, Dhawan P, Madhukar P, Mahesh S, Tandan P. A comparative in-vitro study of two commercially available denture cleansers as antifungal agent against ozone water on heat cure resin. JPID, 2019; 2(3): 103-7.
18. Agostini F, Faccini M, Fitarelli F, Ortiz MAL, Salmeron S, Oliveira RCG, et al. In vitro comparison of antibacterial effect of ozonated water and ozonated gas. Ozone: Sci Eng., 2021; 43(4): 394–400.
19. Küçük F, Yıldırım S, Çetiner S. Cytotoxicity assessment of different doses of ozonated water on dental pulp cells. BMC Oral Health, 2021; 21(1): 32.
20. Abdallah RM, Aref NS. An in vitro assessment of physicomechanical properties of heat-cured denture base resin disinfected by ozonized water. World J Dent, 2020; 11(2): 146–50.
21. Cardoso MG, de Oliveira LD, Koga-Ito CY, Jorge AOC. Effectiveness of ozonated water on Candida albicans, Enterococcus faecalis, and endotoxins in root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2008; 105(3): e85-91.
22. Hong G, Murata H, Li Y, Sadamori S, Hamada T. Influence of denture cleansers on the color stability of three types of denture base acrylic resin. J Prosthet Dent, 2009; 101(3): 205–13.
23. Habeeb DHM, Al-Mizraqchi DAA-M, Ibraheem DAF. Antibacterial effect of ozonated water on adherent mutans streptococci (in vitro). Mustansiria Dental Journal, 2018; [2022 Oct 10]; 6(1): 20–7.
24. César J, Sumita TC, Junqueira JC, Jorge AOC, do Rego MA. Antimicrobial effects of ozonated water on the sanitization of dental instruments contaminated with E. coli, S. aureus, C. albicans, or the spores of B. atrophaeus. J Infect Public Health, 2012; 5(4): 269–74.
25. Bialoszewski D, Pietruczuk-Padzik A, Kalicinska A, Bocian E, Czajkowska M, Bukowska B, et al. Activity of ozonated water and ozone against Staphylococcus aureus and Pseudomonas aeruginosa biofilms. Med Sci Monit, 2011; 17(11): BR339-344.
26. Matar I, El-Sharkawy A, Mohamed NS, Mawsouf MN. Clinical and radiographic evaluation the effect of ozone therapy on tissue surrounding implant retained mandibular overdentures 2016 Revista Española de Ozonoterapia, 2016; 6(1): 51-62.
27. Pinheiro SL, Silva CC da, Silva LA da, Cicotti MP, Bueno CE da S, Fontana CE, et al. Antimicrobial efficacy of 2.5% sodium hypochlorite, 2% chlorhexidine, and ozonated water as irrigants in mesiobuccal root canals with severe curvature of mandibular molars. Eur J Dent, 2018; 12(01): 094–9.
28. Savabi G, Savabi O, Nejatidanesh F, Bagheri K, Karimi L. Prevention of cross-contamination risk by disinfection of irreversible hydrocolloid impression materials with ozonated water. Int J Prev Med., 2018; 9(1): 37.
29. Mirmortazavi A, Rajati Haghi H, Fata A, Zarrinfar H, Bagheri H, Mehranfard A. Kinetics of antifungal activity of home-generated ozonated water on Candida albicans. Curr Med Mycol, 2018; 4(2): 27–31.
30. Sghaireen MG, Alzarea BK, Alduraywish AA, Alam MK, Srivastava KC, Khader Y, et al. Effect of aqueous ozone solution irrigation on healing after treatment with dental implants: A cross-over randomized controlled clinical trial. J Hard Tissue Biol., 2020; 29(4): 263–6.
31. Ahila SC, Subramaniam E. Comparative evaluation of dimensional stability and surface quality of gypsum casts retrieved from disinfected addition silicone impressions at various time intervals: An in vitro study. J Dent Oral Hyg, 2012; 4: 34–43.
32. Amalan A, Ginjupalli K, Upadhya N. Evaluation of properties of irreversible hydrocolloid impression

materials mixed with disinfectant liquids. Dent Res J., 2013; 10: 65–73.
33. Jaiswal P, Pande N, Banerjee R, Radke U. Effect of Repeated Microwave Disinfection on the Surface Hardness of a Heat-Cured Denture Base Resin: An In vitro Study. Contemp Clin Dent, 2018; 9(3): 446-451.