‘COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF AQUEOUS OZONE, MICROWAVE IRRADIATION AND SODIUM HYPOCHLORITE SOLUTION ON HEAT POLYMERIZED POLYMETHYL METHACRYLATE DENTURE BASE MATERIAL AGAINST CANDIDA ALBICANS – AN *IN VITRO* STUDY.’

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IN

PROSTHODONTICS INCLUDING REMOVABLE, FIXED,

MAXILLOFACIAL AND IMPLANTOLOGY

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LIST OF ABBREVIATIONS USED

No.	Abbreviations	Full form
1	n	Number of samples in each group
3	p value	Probability of happening of an event
4	S.D.	Standard deviation
5	ANOVA	Analysis of variance
7	^{0}C	Degree Celsius
8	^{0}	Degree
9	mm	Millimetre
10	i.e.	that is
11	PMMA	Polymethyl methacrylate
12	µ	Micron
19	mins	Minutes
20	gms	Grams
21.	%	Percentage
22.	mg/l	Milligram per litre
23.	CFU/ml	Colony forming units per millilitre
24.	W	Watts
25.	g/mol	Grams per molecule

INTRODUCTION

The loss of teeth by an accident or disease has plagued mankind throughout ages and the means of replacing missing teeth structures by artificial materials continues to justify a large part of application of material sciences.[1]

Edentulism is the result of loss of all permanent teeth. Tooth loss is regarded as mutilating, terminal outcome of a multifactorial process involving a varied biologic process (caries, periodontal diseases, pulpal pathology, trauma, oral cancer) as well as non-biological factors related to dental procedures (access to care, patient's preferences, treatment options etc.).[2]

The treatment options for edentulous patients vary from a wide range from conventional complete dentures to fixed implant-supported restorations. In most of

the cases conventional complete dentures will remain the treatment of choice due to medical and financial reasons.[1]

The first dental prosthesis was believed to have been constructed in Egypt about 2500 BC. Dentures are believed to have surfaced as a mode of treatment for replacing missing teeth around 700 BC.[3] George Washington had a set of dentures which were fabricated from wood.[4] Although the art of firing porcelain was practiced in China in the 9th and 10th century, during the 18th century, gold and porcelain were the material of choice for denture base materials.[1]

The first porcelain denture with artificial teeth was fabricated by **Loomis (1854**). During the 19th century, Tortoise Shell (1850), Gutta Percha (1851), Vulcanite (1851), Cheoplastic (1856), Rose Pearl (1860), Aluminium (1867), Celluloid (1870) were used as denture base materials. Vulcanite remained the principal denture base material for the next 75 years. [1]

In the 20th century various materials like Bakelite (1909), Stainless steel (1921), Cobalt Chromium (1930), Acrylic Resin (1937), Self-cure Acrylic Resin, Epoxy Resin (1951), Polystyrene (1951), Nylon (1955), Polycarbonates (1967), High impact acrylic (1967), Visible L.C (1947), Pure Titanium (1998) were utilized for fabrication of dentures.[1]

During the latter part of the 19th century, polymers entered the field of denture base materials. The PMMA and its copolymers introduced by **Dr. Walter Wright (1937)** continues to be the most popular non-metallic material till date and is being used in 95% dentures.[5] PMMA has been the material of choice because of its biocompatibility, stability in the oral environment, superior esthetics, favorable

working characteristics, processing ease, accurate fit, and inexpensive equipment required for fabrication process.[6,7] Nonetheless, it has some problems such as polymerization shrinkage, weak flexural strength, low fatigue resistance, lower impact strength and poor antimicrobial property.[8]

The formation of plaque on surfaces of dentures is a common problem among denture wearers often leading to halitosis, poor esthetics, accumulation of plaque and consequent deleterious effects on the mucosa with its associated complications like denture stomatitis, inflammatory papillary hyperplasia, and chronic candidiasis.[9]

Surface irregularities or micro-porosities in the prosthesis, use of teeth with more natural contours tend to provide more recessed areas for accumulation of plaque, stains and debris thus, enhancing the growth of oral microbial flora including bacteria, viruses and fungi.[10] Approximately 65% of the complete denture wearers show denture induced stomatitis which is an inflammatory reaction of a denture bearing mucosa. It has a multifactorial etiology and Candida Albicans is reported as the primary etiological agent.[11] This may lead to denture stomatitis, a non-specific inflammatory reaction against microbial antigens, toxins, and enzymes produced by the colonizing microorganisms.

The role of *Candida* in the etiology of denture stomatitis is indicated by an increased number of yeasts on the dentures and the mucosa, increased levels of anti-*Candida* antibody titres in affected individuals and the clinical improvement of the mucosa caused by eradication of the yeast flora. The colonization of the fitting denture surface by Candida depends on numerous factors including adherence of

yeast cells, the redox potential of the site, interaction with oral commensal bacteria and surface properties of the acrylic resin.[12, 13]

The pathogenicity of denture plaque can be enhanced by factors stimulating yeast propagation, such as poor oral hygiene, high carbohydrate intake, reduced salivary flow, and continuous denture wearing. The additional important factors which can modulate the host-parasite relationship and increase the susceptibility to *Candida*-associated denture stomatitis may be aging, malnutrition, radiation therapy, diabetes mellitus, immunosuppression and possibly treatment with antibacterial antibiotics.[14]

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 and chemico-mechanical cleaning methods.[15] Traditional chemical based oral disinfectants including hypochlorites, peroxides, enzymes, acids, crude drugs and mouthwashes for dentures are efficient against pathogenic microbes however their volatile ingredients and byproducts are toxic and harmful to oral mucosa and supporting tissues.[16]

Mechanical methods have also recently been used like magnetic stirrers, agitators, sonic vibrators, and ultrasonic devices for denture cleaning. However, these methods have not been shown to clean dentures efficiently.[15]

The denture cleansing systems should be safe to both tissues and fabricated material, relatively inexpensive, involve minimum physical efforts and must be capable of removing plaque from the prosthesis.[17]

Disinfection of the PMMA by microwave irradiation is claimed to be simple, effective and an inexpensive method. Reduced microwave exposure times should be chosen in order to produce consistent disinfection without any detrimental effect on the acrylic resin material.[18]

Amongst the chemically disinfecting solution, sodium hypochlorite is the most commonly used and shows good bactericidal and fungicidal properties.[19] Its action is by directly acting on organic matrix of plaque causing dissolution of polymer structure because of the presence of undissociated hypochlorus acid which oxidases sulphydryl group of amino acids and proteins to the disulphide forms.[20, 21]

Recently, ozone; a natural gaseous molecule made up of three oxygen atoms has been found to be versatile bio-oxidative therapy in which it is administered via gas or dissolved in water or oil base to obtain therapeutic benefits. It can be used as an adjunct to conventional treatment strategy due to its powerful ability to inactivate microorganisms including C. albicans. The aqueous form of ozone, as a potential antiseptic agent shows less cytotoxicity than gaseous ozone.[22]

Therefore, the purpose of this study is to evaluate and compare the antimicrobial efficacy of aqueous ozone microwave irradiation and sodium hypochlorite solution on heat polymerized polymethyl methacrylate denture base material against candida albicans.

AIMS AND OBJECTIVES

AIM:

To evaluate and compare the antimicrobial efficacy of aqueous ozone, microwave irradiation and sodium hypochlorite solution on heat polymerized polymethyl methacrylate denture base material against C. albicans.

OTHER OBJECTIVES:

1. To evaluate the antimicrobial efficacy of 4mg/L aqueous ozone on heat polymerized polymethyl methacrylate denture base material against C. albicans.

2. To evaluate the antimicrobial efficacy of microwave irradiation at 650 watts for 3 mins on heat polymerized polymethyl methacrylate denture base material against C. albicans.

3. To evaluate the antimicrobial efficacy of 1% sodium hypochlorite solution for 10 mins on heat polymerized polymethyl methacrylate denture base material against C. albicans.

4. To evaluate if aqueous ozone can be used as a routine denture cleansing agent as compared to microwave irradiation and sodium hypochlorite solution.

REVIEW OF LITERATURE

Ozone

Leon J. Warshaw (1953)[23] investigated the effectiveness of high concentrations of ozone as a means of destroying organisms with which 3-D viewers have been deliberately contaminated. It was concluded that ozone concentration of 200 p.p.m. for 20 minutes completely eliminated a deliberate contamination by pure cultures of Escherichia coli, micrococcus pyogenes var. aureus (hemolytic strain) and Streptococcus pyogenes (beta strain). Exposure to this concentration of ozone for 30 minutes was effective in reducing deliberate contamination by candida albicans and a variety of fungi to a noninfectious level.

A Dyas, BJ Boughton, BC Das (1983)[24] conducted an experiment to determine the effectiveness of an inexpensive domestic ozone generator in maintaining an ultra-clean environment for immunosuppressed patients. Ozone concentrations varied between 0.3 and 0.9 ppm and were exposed for 4 hours. It was

seen that at this concentration ozone effectively inhibited the growth of all the bacterial species (E.coli, Proteus species, Pseudomonas aeruginosa, Serratia species, and three strains of Staphylococcus aureus) and Aspergillus fumigatus. Candida albicans was comparatively resistant however. In additional experiments using plates exposed to ozone 0.9 ppm for 4 h prior to bacterial inoculation, it was possible to show that the effect of ozone on the agar was not bactericidal. In the hospital room experiments at concentrations of ozone below 0.001 ppm, no bactericidal effect was seen with any of the organisms tested.

Hiroshi Murakami et al (1996)[25] evaluated the effectiveness of ozone as a denture cleaner against candida albicans. Two trial ozonizers (DC-1, DC-2) which generate ozone and one DC-0 which generates only air were used. The changes in the numbers of C. albicans in a concentration of 10 ppm after 30 min and 60 min and in air alone (DC-0) after 60min were examined. It was seen that in DC-1, the initial number of C. albicans at 4.85×10^3 CFU/ml had decreased after 30 min to 1.31×10^3 CFU/ml and after 60 min to 1.8×10^0 CFU/ml. In DC-2, the initial number of C. albicans was 4.10×10^3 CFU/ml; after 30 min the number had decreased to 8.45×10^2 CFU/ml and after 60 min to 1.5×10^0 CFU/ml. These figures were similar to those of DC-1. In air only, the initial number of C. albicans was 4.35×10^3 CFU/ml; after 60 min, the number had decreased to 3.80×10^3 CFU/ml, a decrease limited only to about 13% of the earlier reading. It was thus concluded that ozone was effective against C. albicans decreasing to about 1/10 after 30 min and to $1/10^3$ after 60 min.

Makoto Oizumi, Tetsuya Suzuki, Mitsuharu Uchida, Junichi Furuya, And Yugo Okamoto (1998)[26] evaluated and compared the microbicidal effect of gaseous

ozone with that of ozonated water in order to determine its usefulness as a method for disinfecting dentures. They tested the effect of ozone on 3 standard strains of oral microorganism namely Streptococcus mutans, staphylococcus aureus and candida albicans. It was seen that when gaseous ozone injection method was used, the numbers of cells of all three strains decreased to $1/10^5$ at 1 min and by 3 mins they were below the detection limit. In contrast, when ozonated water at 1 ppm and 3 ppm was used, C. albicans decreased to 1/10. Thus, it was concluded that direct exposure to gaseous ozone seems to be a more effective microbicide compared with ozonated water, and that gaseous ozone can be clinically useful for disinfection of dentures.

I. R. Komanapalli, B. H. S. Lau (1998)[27] studied the effects of ozone (O_3) on three types of microbes namely Bacteriophage λ, Escherichia coli and Candida albicans. Test suspensions were exposed to 600 ppm O_3 gas at room temperature. It was seen that Bacteriophage λ was completely inactivated at 10 min while Escherichia coli and Candida albicans were only inactivated by factors of 10^5 and 10^4 respectively at 40 min. Exposure of a mixed microbial suspension to O_3 for 5 min resulted in 100% killing of bacteriophages while the viability of E. coli remained unchanged. Various body fluids containing phages were exposed to O_3. Compared to buffered solution, the decrease in phage titers was significantly slower in whole blood, plasma, and albumin. Both E. coli and C. albicans had increased production of thiobarbituric-acid-reactive substances with increased O_3 exposure. Thus, it was concluded that microbes are inactivated by O_3 at different rates, possibly related to differential membrane permeability. The milieu in which microbes are present determines the effectiveness and outcome of O_3 treatment.

Miho Mizuguchi et al (2002)[28] examined the bactericidal effect of the ozone bubble method for Candida albicans. The survival rate was examined for three types of C. albicans, including the reference strain and clinical isolates, by changing the ozone concentration in three stages. As a result, the standard strain and the clinical isolate showed almost the same bactericidal effect, and the 99% inactivation time was about 26 minutes at an ozone concentration of 23 ppm, about 19 minutes at 41 ppm, and about 15 minutes at 49 ppm. Thus, it was concluded that the ozone bubble method is effective for killing C. albicans.

Hiroshi Murakami, Miho Mizuguchi, Masami Hattori, Yutaka Ito, Tatsushi Kawai, Jiro Hasegawa (2002)[29] examined the bactericidal and virucidal effectiveness of a denture cleaner that uses ozone (ozone concentration, 10ppm) against methicillin-resistant Staphylococcus aureus (MRSA) and T1 phage, respectively. It was seen that in the bactericidal activity test, with the ozone supply turned on, the number of bacteria was 3.1×10^3 CFU/mL at the beginning of the experiment, fell to 1.0×10^0 CFU/mL 10min later, and was 1.0×10^0 CFU/mL or less afterwards. In contrast, when the ozone supply was cut off (air bubble only), the number of bacteria was 3.4×10^3 CFU/mL at the beginning of the experiment, and had fallen to 3.0×10^3 CFU/mL 60min later (no statistically significant difference). In the virucidal activity test, the number of phages was 1.2×10^6 PFU/ mL before ozone treatment, fell to about 1/10 of that number 10 min later, and was 6.1×10^0 PFU/mL 40 min later. Thus, it was concluded that the use of ozone in this denture cleaner is effective against MRSA and viruses.

Nagayoshi M, Fukuizumi T, Kitamura C, Yano J, Terashita M, Nishihara T (2004)[30] examined the effect of ozonated water on oral microorganisms such as S. mutans, S. salivarius, S. sanguis, P. gingivalis, C. albicans and dental plaque. It was seen that almost no microorganisms were detected after being treated with ozonated water (4 mg/l) for 10 s. To estimate the ozonated water-treated Streptococcus mutans, bacterial cells were stained with Live/Dead BacLight™ Bacterial Viability Kit. Fluorescence microscopic analysis revealed that S. mutans cells were killed instantaneously in ozonated water. Some breakage of ozonated water-treated S. mutans was found by electron microscopy. When the experimental dental plaque was exposed to ozonated water, the number of viable S. mutans remarkably decreased. Ozonated water strongly inhibited the accumulation of experimental dental plaque in vitro. After the dental plaque samples from human subjects were exposed to ozonated water in vitro, almost no viable bacterial cells were detected. Thus, it was concluded that ozonated water was effective for killing gram-positive and gram-negative oral microorganisms and oral C. albicans in pure culture. Furthermore, ozonated water had strong bactericidal activity against the bacteria in plaque biofilm. In addition, ozonated water inhibited the accumulation of experimental dental plaque in vitro.

Kazuhito niinomi et al (2004)[31] compared the bactericidal potency between the freshly purified ozone gel containing 3 different ozone concentrations, the electrolyzed gel hard-type, and between the ozone gel immediately after purification and the ozone gel stored for 2 months after purification at room temperature. It was seen that the ozone gel containing 10 ppm ozone showed a bactericidal effect on E. coli, P. aeruginosa, S. mutans, B. subtilis, C. albicans all about 5 bacterial strains immediately after addition and, among them, only C. albicans was decreased to be

lower than the detection limit, and even after 6 h of reaction, only E. coli, K. pneumoniae, P. aeruginosa, S. mutans, S. aureus, C. albicans all about 6 strains became lower than the detection limit. This bactericidal potency was slightly stronger than that of electrolyzed gel hard-type. The gel containing 100 ppm ozone showed a greater bactericidal effect than the 10 ppm ozone gel, and the 1000 ppm ozone gel reduced E. coli, K. pneumoniae, P. aeruginosa, S. mutans, S. aureus, MRSA, S. epidermidis, C. albicans all about 8 bacterial strains to be lower than the detection limit immediately after addition, except for B. subtilis. The ozone gel immediately after purification and the ozone gel stored at room temperature for 2 months after purification showed similar bactericidal effects on E. coli and S. aureus regardless of reaction time. Thus, it was concluded that ozone gel can be used widely and clinically, because it has instantaneous and persistent bactericidal effects.

Kamila Leite Rodrigues et al (2004)[32] tested the ozonised sunflower oil, Bioperoxoil®, for its antimicrobial activity against some pathological strains in vitro together with its healing potential against Staphylococcus aureus in vivo. Bioperoxoil® was tested against S. aureus, Pseudomonas aeroginosa, Candida albicans, S. typhimurium and Escherichia coli suspensions using the agar diffusion method. Healing experiments were carried out with Wistar rats through topical application of 3.5 mg/ml of the ozonized oil up to the 7th day after inoculation with S. aureus. Bioperoxoil® showed anti-inflammatory effects against all strains tested, with minimal inhibitory concentration (MIC) values ranging from 2.0 to 3.5 mg/ml. Bioperoxoil® also demonstrated protective effects on skin connective tissue and to enhance wound healing during the treatment, as compared to a neomycin–clostebol association used as a positive control. The overall results indicated a significant

antimicrobial activity, anti-inflammatory and wound-healing properties for Bioperoxoil®, as compared to other antimicrobial agents commercially available.

Arita M, Nagayoshi M, Fukuizumi T, Okinaga T, Masumi S, Morikawa M, Kakinoki Y, Nishihara T (2005)[15] evaluated Microbicidal efficacy of ozonated water against Candida albicans adhering to acrylic denture plates. The heat-cured acrylic resins were cultured with C. albicans. After treatment of flowing ozonated water with and without ultrasonication, the number of attached C. albicans was counted. It was seen that after exposure to flowing ozonated water (2 or 4 mg/l) for 1 min, viable C. albicans cells were nearly nonexistent. The combination of ozonated water and ultrasonication had a strong effect on the viability of C. albicans adhering to the acrylic resin plates. There were no significant differences in antimicrobial activity against C. albicans between plates immersed in ozonated water with ultrasonication and those treated with commercially available denture cleaners. In addition, electron microscopic analysis revealed that small amounts of C. albicans remained on the plate after exposure to flowing ozonated water or immersion in ozonated water with ultrasonication. Thus, it was concluded that application of ozonated water may be useful in reducing the number of C. albicans on denture plates.

Carlos ESTRELA et al (2006)[33] evaluated the antimicrobial potential of ozone applied to 3 different solutions in an ultrasonic cleaning system against Staphylococcus aureus. A total of 120 mL of S. aureus were mixed in 6 litres of the experimental solutions (sterile distilled water, vinegar and sterile distilled water + Endozime AWpluz) used in an ultrasonic cleaning system (UCS). Ozone was produced by an electric discharge through a current of oxygen and bubbling with flow

rate at 7grams/hour ozone (1.2%) into the microbial suspensions. Bacterial growth was evaluated by turbidity of the culture medium. At the same time, 1 mL of bacterial samples was collected and inoculated in brain heart infusion agar (BHIA) plates. After incubation at 37°C for 48 h, the number of colony forming units (cfu) per mL on BHIA surface was counted. In dilution test in BHI tubes and in BHIA plates (cfu/mL), bacterial growth was not observed in any of the experimental solutions when ozone was added. Thus, it was concluded that the addition of ozone to an ultrasonic cleaning system containing different experimental solutions resulted in antibacterial activity against S. aureus.

Huth KC, Jakob FM, Saugel B, Cappello C, Paschos E, Hollweck R, Hickel R, Brand K. (2006)[34] investigated whether gaseous ozone ($4x10^6$ μg m^{-3}) and aqueous ozone (1.25-20 μg ml^{-1}) exert any cytotoxic effects on human oral epithelial (BHY) cells and gingival fibroblast (HGF-1) cells compared with established antiseptics [chlorhexidine digluconate (CHX) 2%, 0.2%; sodium hypochlorite (NaOCl) 5.25%, 2.25%; hydrogen peroxide (H_2O_2) 3%], over a time of 1 min, and compared with the antibiotic, metronidazole, over 24 h. Cell counts, metabolic activity, Sp-1 binding, actin levels, and apoptosis were evaluated. Ozone gas was found to have toxic effects on both cell types. Essentially no cytotoxic signs were observed for aqueous ozone. CHX (2%, 0.2%) was highly toxic to BHY cells, and slightly (2%) and non-toxic (0.2%) to HGF-1 cells. NaOCl and H_2O_2 resulted in markedly reduced cell viability (BHY, HGF-1), whereas metronidazole displayed mild toxicity only to BHY cells. Thus, it was concluded that the aqueous form of ozone, as a potential antiseptic agent, showed less cytotoxicity than gaseous ozone or

established antimicrobials under most conditions. Therefore, aqueous ozone fulfils optimal cell biological characteristics in terms of biocompatibility for oral application.

Marcelo Gonçalves Cardoso et al (2008)[35] evaluated the effectiveness of ozonated water in the elimination of Candida albicans, Enterococcus faecalis, and endotoxins from root canals. Twenty-four single-rooted human teeth were inoculated with C. albicans and E. faecalis, and 24 specimens were inoculated with Escherichia coli endotoxin. Ozonated water (experimental group) or physiologic solution (control group) was used as irrigant agent. Antimicrobial effectiveness was evaluated by the reduction of microbial counts. It was seen that ozonated water significantly reduced the number of C. albicans and E. faecalis at the immediate sampling, but increased values were detected after 7 days. Ozonated water did not neutralize endotoxin. Thus, it was concluded that ozonated water was effective against C. albicans and E. faecalis but showed no residual effect. Also, ozonated water was not able to neutralize endotoxins in root canals.

Dariusz Białoszewski et al (2010)[36] analyzed the basic bactericidal and fungicidal activity of ozonated water according to the European Standards EN 1040 "Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics" and EN 1275 "Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of basic fungicidal or basic yeasticidal activity of chemical disinfectants and antiseptics" against the collection of microorganisms recommended by the standards as well as a number of drug-resistant clinical isolates which included P. aeruginosa, S. aureus, E. coli, C. albicans and A. brasiliensis. A prototype device

for intra-operative ozone therapy was used. It was seen that the ozone concentration (1.3-1.5 μg/mL) in ozonated water was sufficient to kill almost all cells of the bacterial and yeast strains tested after 30 seconds. Effective action against A. brasiliensis spores required a longer time than those required in the case of bacterial cells or vegetative cells of yeast. Thus, it was concluded that ozonated water obtained using the prototype device and applied during intraoperative procedures is cost-efficient and may protect against infections.

Kiyotaka Yamada et al (2010)[37] studied the the antibacterial activity of water super-oxidised water containing high concentration of O^- (O^--water) against cultured planktonic cells of cariogenic bacteria, periodontopathic bacteria and C. albicans. O^--water was prepared using the AOE-750 (Oxy Japan Corporation, Japan) and its antibacterial activity against pure culture of Str. sobrinus, P. gingivalis, P. intermedia, A. actinomycetemcomitans, F. nucleatum and C. albicans was evaluated. Each oral microorganism (10^4 to 10^8 CFU/ml) was exposed to three concentrations of O^--water at room temperature or 37°C for 15 s to 24 h. it was seen that Exposure to O^--water resulted in a bactericidal effect against all cariogenic and periodontopathic bacteria tested. No significant fungicidal effect was observed on C. albicans. Thus, it was concluded that O^--water exerts an antibacterial effect on cariogenic and periodontopathic bacteria, suggesting its potential as a disinfectant in the prevention of bacterial contamination of dental equipment.

Dariusz Bialoszewski et al (2011)[38] investigated the bactericidal activity of ozonated water and that of a mixture of ozone and oxygen against eighteen clinical strains of Staphylococcus aureus and Pseudomonas aeruginosa exhibiting various

levels of antibiotic sensitivity. Bacteria were cultured in biofilm form on polystyrene titration plates for periods of 2 to 72 hours. The biofilms formed in this way were exposed to in statu nascendi ozonated water produced in a prototype device that had been tested in clinical conditions, or to a mixture of oxygen and ozone generated in the same device. Live cells in the biofilm were stained with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) bromide solution. It was seen that ozonated water was found to be an effective bactericidal agent against biofilms after as little as 30 seconds of exposure, while the bactericidal activity of the ozone-oxygen solution was much lower. Thus, it was concluded that unlike the ozone-oxygen mixture, ozonated water effectively destroys bacterial biofilms in vitro.

Julio César et al (2012)[39] evaluated the antimicrobial effects of ozonated water on the sanitization of dental instruments that were contaminated by Escherichia coli, Staphylococcus aureus, Candida albicans and the spores of Bacillus atrophaeus. A total of one hundred and twenty standardized samples of diamond dental burs were experimentally contaminated with all the 4 microorganisms for 30 min. After the contamination, the samples were exposed to ozonated water (10 mg/L O3) for 10 or 30 min. The control group was composed of samples that were exposed to distilled water for 30 min. After the exposure to the ozonated water, 0.1 mL aliquots were seeded onto BHI agar to count the colony-forming units per milliliter (CFU/mL) of E. coli, S. aureus, and B. atrophaeus. Sabouraud dextrose agar was used to count the CFU/mL of C. albicans. Results showed that for all of the microorganisms studied, the ozonated water reduced the number of CFU/mL after 10 and 30 min of sanitization, and this microbial reduction was dependent on the duration of the exposure to the ozonated water. Thus, it was concluded that the exposure to ozonated

water at a concentration of 10 mg/L for 10 or 30 min was sufficient to reduce the number of CFU/mL of E. coli, S. aureus, C. albicans and the spores of B. atrophaeus effectively on the surface of diamond burs, suggesting that ozonated water can be used for the sanitization of dental instruments.

Syed Sadatullah, Nor Himazian Mohamed, Fathilah Abdul Razak (2012)[40] analyzed the effect of 0.1 ppm ozonated water on 24-hour supragingival plaque (SP) samples in situ. SP was collected from the two most posterior teeth in the contra-lateral quadrants before and after a 30-second rinse with either distilled water (control group) or 0.1 ppm ozonated water (test group). The plaque was used to count the number of total bacteria, total anaerobic bacteria, Streptococcus mutans, and Candida albicans on selective agar media. The statistical analysis of the number of colony forming units (CFUs) obtained demonstrated a significant antimicrobial effect of ozonated water on the total bacteria ($p = 0.01$) and anaerobes ($p = 0.02$). A reduction in the post-rinse CFU count for Streptococcus mutans was also observed, but the effect was not statistically significant ($p = 0.07$). The Candida species was only grown from one sample. Thus, it was concluded that ozonated water at the 0.1 ppm concentration was effective in reducing the load of 24-hour plaque bacteria, but it did not eliminate them completely. Therefore, ozonated water rinsing may be an extremely useful addition to tooth brushing and flossing because it is bactericidal, easy to prepare and cost effective.

Tatsuya Fukui et al (2014)[41] examined Antimicrobial Effects of Ozone Gel Against Periodontal Bacteria namely E. coli, P. aeruginosa, S. aureus, C. albicans, Methicillin-resistant S. aureus (MRSA), S. epidermidis, K. pneumoniae, and Str.

Mutans and anaerobic strains such as P. gingivalis, P. intermedia, F. nucleatum, A. actinomycetemcomitans, and E. corrodens. These strains were treated with 10 ppm ozone gel with quenching after 3 h and with 100-ppm ozone gel with immediate quenching. Results showed that the number of CFUs was below the detection limit for P. gingivalis, P. intermedia, and F. nucleatum, E. coli, P. aeruginosa, C. albicans, K. pneumoniae, and S. mutans after treatment with 10 ppm ozone gel with quenching after 3 h. For Bacillus subtilis, an antimicrobial effect was observed after 3 h of treatment with 100 ppm of ozone gel. Thus, it was concluded that ozone gel is very useful for clinical application for oral surgery, implant treatment and peri-implant diseases because of their rapid antimicrobial effect on several bacterial strains.

Zargaran M, Fatahinia M, Zarei Mahmoudabadi A (2017)[42] evaluated the fungicidal effects of ozone on different forms of C. albicans. In addition, antifungal susceptibility profile of strains was assessed before and after exposure to ozone. Fifty strains of C. albicans were exposed to gaseous ozone at different times. Furthermore, biofilm formation and germ tube production were evaluated when yeast suspensions were exposed to ozone. In addition, antifungal susceptibility of ozone resistant colonies was investigated as compared to controls. It was seen that ozone was highly effective in killing C. albicans in yeast form and inhibition of germ tube formation during 210 and 180 seconds, respectively. Although with increasing exposure time biofilm production was considerably decreased, resistance to ozone was much higher among vaginal and nail isolates even after 60 min. All the strains were sensitive to fluconazole, caspofungin, and terbinafine pre- and post-ozone exposure. Resistance to amphotericin B was significantly enhanced after exposure to ozone. Thus, it was concluded that although ozone was highly effective on the yeast form of C. albicans

and it can inhibit the formation of germ tubes in C. albicans, the complete removal of biofilms did not happen even after 60 min. It seems that ozone therapy induces resistance to amphotericin B.

Laila E. Amin (2018)[43] biologically assessed ozone therapy on experimental oral candidiasis in immunosuppressed rats. Sixty male healthy rats were immunosuppressed with dexamethasone in their drinking water one week before candida infection. The animals were divided into four equal groups. Rats of group 1 were kept without any manipulation and those of group II were given oral inoculums of C. albicans on the dorsal surface of the tongue. Group III rats were handled as group II and instead the rats were treated by daily mycostatin drops local applicator as a routine treatment. Meanwhile, group IV rats were handled as group II and instead the rats were received daily intraperitoneal injection of 1 cm^3 of ozone oxygen gas mixture with concentration of ozone 70 $\mu g/cm^3$. After two weeks, all rats were euthanized and tongue specimens were prepared for histological staining with Haematoxylin & Eosin and CD3 immunohistochemical staining. Histological examination revealed that treatment with ozone therapy lead to gradual decrease in lingual papillary atrophy and invasion of candida yeast. Immunohistochemical study showed significant decrease in CD3 counting. Thus, it was concluded that ozone acts as an excellent fungicidal agent, also ozone is capable of alerting the immune system.

Amirtaher Mirmortazavi et al (2018)[44] evaluated the antifungal effects of home-generated ozonated water on the adhesion of the C. albicans attached to the surface of the denture base acrylic resins. Different concentrations of C. albicans were added to the tubes containing acrylic resin blocks, and then incubated for 2 h at 35°C.

The samples were assigned into three groups, each of which contained 42 samples, including normal saline (NS) solution as the negative control, nystatin (N) solution as the positive control, and ozonated water as the test group. The samples were washed and placed in an ultrasonic bath. Subsequently, the saline solution was cultured on Sabouraud dextrose agar. The concentrations of Candida were evaluated during the contact times. It was seen that the test group (i.e., ozonated water) with 114 colony-forming units (CFU) showed a significant reduction of Candida colonies, compared to the NS group with 2,172 CFU. The 120 mins and 1-minute incubation with ozonated water showed the highest and lowest effects on the viability of Candida adhered to the acrylic resin, respectively. Thus, it was concluded home-generated ozonated water can be applied to remove the Candida attached to the surface of the denture plates.

Erica Crastechini et al (2018)[45] evaluated the effect of ozonized olive oil (OZ) on the oral levels of Candida spp. in patients with denture stomatitis. Antifungal activity was screened against C. albicans and five non-albicans species (C. tropicalis, C. dubliniensis, C. krusei, C. guilliermondii, and C. parapsilosis). Also, the effects on C. albicans planktonic and biofilm were evaluated. 30 patients used OZ and 20 used sodium bicarbonate (SB) for 14 days. After 7 and 14 days, clinical evaluation, isolation and identification of yeasts were performed. Isolates were identified by phenotypic and genotypic tests. It was seen that ozonated oil showed in vitro antifungal activity against all species of Candida. Ozonated oil reduced the number of viable cells in C. albicans biofilms. Oral candidal levels were lower in relation to baseline both after after 14 days of treatment with SB and OZ. Remission of denture stomatitis was observed in all patients after 7 days of treatment in both groups. Thus,

it was concluded that ozonized olive oil can be a new alternative for the control of biofilm in patients with denture stomatitis.

Isha Khatri, Ganapathi Moger, Anil Kumar N (2019)[46] evaluated and compared the ability of ozonated water and topical clotrimazole in reducing the Candidal species colony‐forming unit (CFU) count in oral candidiasis. The study included 40 candidiasis patients of either sex, aged between 18 and 60 years. The patients were randomly assigned to either topical ozone therapy or topical clotrimazole groups. Group 1 patients were instructed to rinse with 5–10 mL of ozonated water for 1 min in the morning once daily for 5 days. Group 2 Patients were advised to apply the candid mouth paint to affected areas thrice daily for 2 weeks. Salivary Candidal CFU counts were assessed during and after the treatments. It was seen that there was gradual but significant reduction in Candidal CFU count in both groups. At the end of the treatment, Candidal CFU count reduction in ozone group was 60.5% whereas in clotrimazole group it was 32.3%. thus, it was concluded that ozone therapy was much more effective in reducing the patients with candidiasis to a state of carriers ozonated water might be useful to treat oral candidiasis.

Microwave Irradiation

Donna L. Dixon, Larry C. Breeding, and Tracy A. Faler (1999)[47] examined: (1) the efficacy of microwave irradiation against C albicans colonized on 3 soft denture liners and 1 heat-polymerized denture base resin, and (2) the effect of this irradiation on the hardness of the materials tested. In phase 1, an experimental protocol was developed. Sterilized specimens from 2 denture base soft liners and 1 heat-polymerized acrylic resin denture base material (n = 45 each) were inoculated with C albicans. Two thirds of the specimens were irradiated in a 60 Hz microwave oven for 5 minutes (dry). C albicans growth was then assessed with streaked blood agar plates and thioglycollate broth. One third of the specimens were not irradiated and served as controls. Pretest and posttest Shore A hardness values were obtained and compared. For phase 2, 15 specimens from each material group were subjected to irradiation (while immersed in water) for 5 minutes; and, 15 from each material were subjected to 10 and 15 minutes irradiation (dry), with subsequent sterility and change in hardness assessments completed as described in phase 1. In phase 3, 15 specimens from each material group were subjected to repeated 5 minutes irradiation cycles (while immersed in water), and changes in hardness were examined. Results showed that only the 5 minutes irradiated specimens immersed in water were effectively sterilized, as verified by the thioglycollate assay. The effect of repeated 5-minute irradiation cycles resulted in a significant change in hardness of the PermaSoft specimens. It was concluded that 5 minutes irradiation, while immersed in water, killed all C albicans present on the materials tested; and, repeated 5-minute irradiation significantly affected the hardness of only the PermaSoft material.

David W. Banting, Scott A. Hill (2001)[48] investigated thirty-four institutionalized subjects (mean age, 81 years) with a positive test for C. albicans pseudohyphae which were randomly assigned to test (microwave) and control (soak) groups. All subjects received the same course of topical antifungal medication (Nystatin 300,000 IU lozenges 3x daily for 14 days). Subjects in the microwave treatment group had their maxillary denture scrubbed and then microwaved for one minute at 850 Watts. This procedure was repeated three times. For subjects receiving the standard denture soak treatment, the nursing staff was instructed to disinfect the maxillary dentures in 0.2% chlorhexidine digluconate solution overnight for 14 days, changing the solution every two days. Infestation of the tissue surface of the maxillary denture with C. albicans progressively took place over the three-month period following treatment such that 8 (53%) of the microwaved dentures and 16 (84%) of the soaked dentures demonstrated pseudohyphae as determined by Gram stain. Infection of the soft tissues with the hyphal form of C. albicans also increased with time. After three months, one (8%) patient in the microwave group and 12 (63%) patients in the denture soak group demonstrated pseudohyphae on the cytological smears. Re-infestation of the denture surface and infection of the adjacent soft tissue were delayed dramatically in patients whose dentures were microwaved compared with those whose dentures were disinfected by being soaked in a chlorhexidine solution.

Ralf Buergers et al (2008)[49] evaluated and compared the efficacy of 10 denture disinfection methods in reducing Candida albicans (C. albicans) colonization on soft denture relining material. Circular specimens (diameter 8 mm) were made of soft denture relining material and thermally aged. Specimens were incubated with C.

albicans (strain 1386, DSMZ) followed by 1 of 10 disinfection procedures (6 soaks, 2 microwave irradiation regimes, 1 effervescent commercial cleansing product, and denture left dry overnight). Incubation with phosphate buffered saline (PBS) served as a control. Adhering fungi were quantified using a bioluminometric assay in combination with an automated plate reader for cell quantification. Scanning electron micrographs (SEMs) were made for validation. It was concluded that only soaking in sodium hypochlorite (1%; 10 min), microwave irradiation immersed in water (800 W; 6 min), and application of effervescent cleansing tabs (Blend-a-dent tabs; 10 min) proved to be effective against C. albicans colonization on soft denture relining material.

Ewerton G. de Oliveira Mima et al (2008)[50] evaluated the effectiveness of different exposure times of microwave irradiation on the disinfection of a hard chairside reline resin. Sterile specimens were individually inoculated with one of the tested microorganisms (Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, and Bacillus subtilis) and incubated for 24 hours at 37°C. For each microorganism, 10 specimens were not microwaved (control), and 50 specimens were microwaved. Control specimens were individually immersed in sterile saline, and replicate aliquots of serial dilutions were plated on selective media appropriate for each organism. Irradiated specimens were immersed in water and microwaved at 650 W for 1, 2, 3, 4, or 5 minutes before serial dilutions and platings. After 48 hours of incubation, colonies on plates were counted. Irradiated specimens were also incubated for 7 days. Some specimens were prepared for scanning electron microscopic (SEM) analysis. It was concluded that specimens irradiated for 3, 4, and 5 minutes showed

sterilization. After 2 minutes of irradiation, specimens inoculated with C. albicans were sterilized, whereas those inoculated with bacteria were disinfected. One minute of irradiation resulted in survival of all microorganisms. SEM examination indicated alteration in cell morphology of sterilized specimens. The effectiveness of microwave irradiation was improved as the exposure time increased.

K. H. Neppelenbroek et al (2008)[51] evaluated the effectiveness of microwave disinfection of maxillary complete dentures on the treatment of Candida-related denture stomatitis. Patients (n = 60) were randomly assigned to one of four treatment groups of 15 subjects each; Control group: patients performed the routine denture care; Mw group: patients had their upper denture microwaved (650 W per 6 min) three times per week for 30 days; group MwMz: patients received the treatment of Mw group in conjunction with topical application of miconazole three times per day for 30 days; group Mz: patients received the antifungal therapy of group MwMz. Cytological smears and mycological cultures were taken from the dentures and the palates of all patients before treatment at day 15 and 30 of treatment and at follow-up (days 60 and 90). The effectiveness of the treatments was evaluated by Kruskal–Wallis and Mann–Whitney tests. Microbial and clinical analysis of the control group demonstrated no significant decrease in the candida infection over the clinical trial. Smears and cultures of palates and dentures of the groups Mw and MwMz exhibited absence of Candida at day 15 and 30 of treatment. On day 60 and 90, few mycelial forms were observed on 11 denture smears (36.6%) from groups Mw and MwMz, but not on the palatal smears. It was concluded that miconazole (group Mz) neither caused significant reduction of palatal inflammation nor eradicated Candida from the dentures and palates. Microwaving dentures was effective for the treatment of denture

stomatitis. The recurrence of Candida on microwaved dentures at follow-up was dramatically reduced.

Paula Volpato Sanita et al (2009)[52] evaluated the effectiveness of microwave irradiation on the disinfection of complete dentures inoculated with American Type Culture Collection (ATCC) and HIV isolates of five species of Candida. Fifty dentures were made, sterilised and inoculated with the tested microorganisms (C. albicans, C. dubliniensis, C. krusei, C. glabrata and C. tropicalis). After incubation (37°C /48 h), dentures were microwaved (650 W/3 min). Non-irradiated dentures were used as positive controls. Replicate aliquots of suspensions were plated at dilutions 10^{-1} to 10^{-4} and incubated (37°C/48 h). Colony counts (cfu ml^{-1}) were quantified. Dentures were also incubated at 37°C for 7 days. Data were analysed with 2-way ANOVA and Tukey HSD tests ($\alpha = 0.05$). Dentures contaminated with all Candida species showed sterilisation after microwave irradiation. All control dentures showed microbial growth on the plates. The cfu ml^{-1} for C. glabrata was higher than those of C. albicans, C. dubliniensis and C. tropicalis whereas the cfu ml^{-1} for C. krusei was lower. The cfu ml^{-1} for clinical isolates was higher than those of ATCC yeast. Microwave irradiation for 3 min at 650 W resulted in sterilisation of all complete dentures.

Livia Nordi Dovigo et al (2009)[53] evaluated the effectiveness of microwave irradiation for disinfection of simulated complete dentures. Seventy dentures were fabricated in a standardized procedure, subjected to ethylene oxide sterilization, individually inoculated (10^7 cfu/mL) with Staphylococcus aureus (n = 20), Pseudomona aeruginosa (n = 20), and Bacillus subtilis (n = 30) and incubated for 24

hours at 37°C. After that, 40 dentures were selected for microwaving. For each microorganism, 10 dentures were submitted to microwave irradiation at 650 W for 3 minutes. In addition, 10 dentures contaminated with B. subtilis were irradiated for 5 minutes. Thirty non-microwaved dentures (n = 10 for each bacteria) were used as positive controls. Replicate aliquots (25 μL) of suspensions were plated at dilutions of 10^{-3} to 10^{-6} on plates of selective media appropriate for each organism. After incubation (37°C for 48 hours), colonies were counted (cfu/mL). TSB beakers with the microwaved dentures were incubated at 37°C for a further 7 days to verify long-term disinfection. The data were statistically analyzed by the Kruskal-Wallis test (α = 0.05). it was concluded that no evidence of growth was observed at 48 hours for S. aureus and P. aeruginosa on plates, and no turbidity was visible in the TSB beakers of these specimens after 7 days of incubation. Microwave irradiation for 3 minutes at 650 W produced sterilization of complete dentures contaminated with S. aureus and P. aeruginosa. Dentures contaminated with B. subtilis were disinfected by microwave irradiation after 3 and 5 minutes at 650 W.

Karen Tereza Altieri et al (2012)[54] evaluated the efficacy of two disinfectant solutions and microwave irradiation in disinfecting complete dentures contaminated with Methicillin-resistant Staphylococcus aureus (MRSA). The authors contaminated 36 simulated complete dentures with MRSA and divided them into four equal groups: a positive control group consisting of dentures that were not disinfected; a group that soaked in 1 percent sodium hypochlorite for 10 minutes; a group that soaked in 2 percent chlorhexidine gluconate for 10 minutes; and a group that underwent microwave irradiation at 650 watts for three minutes. The authors quantified colony counts and evaluated the long-term effectiveness of disinfection. It was concluded that

soaking in chlorhexidine gluconate solution and microwave irradiation resulted in complete disinfection of all dentures contaminated with MRSA in both the short and the long term. Soaking in sodium hypochlorite solution was effective only as a short-term disinfectant.

Mario Augusto Brondani, Firoozeh Samim and Hong Feng (2012)[55] conducted a critical review to synthesise and discuss the advantages and disadvantages of the use of a conventional microwave oven for cleaning and disinfecting complete dentures. One hundred and sixty-seven manuscripts published in English with full text were found, and 28 were accepted and discussed in the light of the advantages and disadvantages of the use of conventional microwave oven for cleaning and disinfecting complete dentures. It was concluded that there was no standardisation for microwave use for denture cleaning. Manual cleaning still seemed to be the optimal method for controlling fungal infection and denture stomatitis. However, such a daily routine appeared to be underused, particularly in long-term care facilities.

Plinio M. Senna, Wander J. da Silva and Altair A. Del Bel Cury (2012)[56] evaluated the influence of the area of Candida albicans biofilm on denture disinfection by microwave energy. Candida albicans biofilm was allowed to form for 72 h on resin discs, and three small coverage or seven large coverage discs were placed onto the palatal surface of sterile maxillary dentures. Each denture was immersed in 200 ml distilled water and individually irradiated at a power of 450, 630 or 900 W for different time intervals (1, 2 or 3 min) (n = 6). The effectiveness of disinfection was evaluated by counting the residual cells. The data were analysed by ANOVA and

Tukey's HSD test ($\alpha = 0.05$). Pearson's correlation test was performed to determine the correlation between effectiveness of sterilization and temperature.it was seen that dentures with a larger area of biofilm demanded a longer irradiation exposure to achieve disinfection ($p < 0.001$), irrespective of power setting, and in this time no yeast growth was detected. Dentures with small areas of biofilm were disinfected after 1 min at 900 W and 2 min at 450 or 630 W. A positive correlation was found between water temperature and effectiveness of disinfection ($r = 0.6170$; $p < 0.001$).

Mariana Montenegro Silva et al (2012)[57] compared the effectiveness of denture microwave disinfection and antifungal therapy on treatment of denture stomatitis. Sixty denture wearers with denture stomatitis (3 groups; n = 20 each), were treated with nystatin or denture microwave disinfection (1 or 3 times/wk) for 14 days. Mycologic samples from palates and dentures were quantified and identified with the use of Chromagar, and clinical photographs of palates were taken. Microbiologic and clinical data were analyzed with the use of a series of statistical tests ($\alpha = 0.05$). It was seen that both treatments similarly reduced clinical signs of denture stomatitis and growth on palates and dentures at days 14 and 30 ($p > 0.05$). At sequential appointments, the predominant species ($p < 0.01$) isolated was C. albicans (range 98%-53%), followed by C. glabrata (range 22%-12%) and C. tropicalis (range 25% - 7%). Thus, it was concluded that microwave disinfection, at once per week for 2 treatments, was as effective as topical antifungal therapy for treating denture stomatitis.

Silva M, Consani R, Sardi J, Mesquita M, Macedo A, Takahashi J. (2013)[58] evaluated the effect of microwave irradiation as an alternative method for

disinfection of different types of denture base acrylic resins. Twenty-four samples for each conventional, microwaved and characterized heat-cured acrylic resin were made and subjected to sterilization with ethylene oxide for the groups: 1) irradiated samples; 2) non-irradiated samples; and 3) samples without yeast. Each group was subdivided according to inoculation with C. albicans, C. dubliniensis and C. tropicalis. The samples were inoculated with 100 µL of inoculum of each species of Candida and later placed in an incubator at 37 °C for 1 hr to perform the first adhesion. After this time, each well was supplemented with sterile media and the plate was once again taken to a stove for incubation at 37 °C for 6 hr. The samples were immersed in 100 mL of sterile water and irradiated with microwave at 650 W for 3 min. Control samples were considered as the non-irradiated group. After incubation for 48 hr, irradiated and non-irradiated samples were subjected to a digital colony counter. It was seen that Control group (non-irradiated) showed microbial growth for resins and the means of ufc/mL were without statistically significant differences. Microwave irradiated samples (experimental group) promoted no viable colonies for all Candida species and types of acrylic resins. Thus, it was concluded that Microwave irradiation was an effective method for disinfection of the acrylic resins inoculated with C. albicans, C. dubliniensis and C. tropicalis.

Plinio M. Senna, Bruno S. Sotto-Maior, Wander J. da Silva and Altair A. Del Bel Cury (2013)[59] evaluated whether the addition of an enzymatic cleanser to microwave disinfection regimen would disinfect dentures with shorter irradiation time. Seven resin discs colonized with Candida albicans biofilm were placed on the palatal surface of sterile dentures to be randomly assigned to the following treatments: immersion in distilled water for 3 min with 0 (DW), 1 (DW + M1), 2 (DW + M2), or

3 min (DW + M3) of 450 W microwave irradiation; or immersion in denture cleanser for 3 min with 0 (DC), 1 (DC + M1), 2 (DC + M2) or 3 min (DC + M3) of 450 W irradiation. After the treatments, the viable cells were counted by a blinded examiner. The temperature was measured immediately after irradiation. The data were analyzed by ANOVA and Tukey post hoc tests ($\alpha = 0.05$). It was concluded that no viable cells were found after DC + M2, DC + M3, and DW + M3 treatments, of which DC + M2 achieved the lowest temperature. No significant difference was found between the effectiveness of DW, (DW + M1) and DC treatments ($p > 0.05$).

A. Z. Yildirim-Bicer, I. Peker, G. Akca, and I. Celik (2014)[60] evaluated alternative methods for the disinfection of denture-based materials. Two different denture-based materials were included in the study. Before microbial test, the surface roughness of the acrylic resins was evaluated. Then, the specimens were divided into 8 experimental groups ($n = 10$), according to microorganism considered and disinfection methods used. The specimens were contaminated in vitro by standardized suspensions of Candida albicans ATCC #90028 and Candida albicans oral isolate. The following test agents were tested: sodium hypochlorite (NaOCl 1%), microwave (MW) energy, ultraviolet (UV) light, mouthwash containing propolis (MCP), Corega Tabs, 50% and 100% white vinegar. After the disinfection procedure, the number of remaining microbial cells was evaluated in CFU/mL. it was seen that statistically significant difference ($P < 0.05$) was found between autopolymerised and heat-cured acrylic resins. The autopolymerised acrylic resin surfaces were rougher than surfaces of heat-cured acrylic resin. The most effective disinfection method was 100% white vinegar for tested microorganisms and both acrylic resins. This agent is cost-effective and easy to access and thus may be appropriate for household use.

Theodoros Klironomos, Aspasia Katsimpali, Gregory Polyzois (2015)[61] overviewed the current scientific knowledge concerning the effect of microwave disinfection on denture related material properties.they concluded that Microwave disinfection (650 W/3 min/3 cycles) is a safe alternative for the disinfection of denture bases and liners compared to the chemical one when the procedure is carried out in dry conditions, but could possibly cause dimensional changes of clinical significance when the irradiation takes place in wet environment. More than 3 cycles of microwave disinfection in these settings could adversely affect the physical-mechanical properties of denture base resins, liners or teeth. Microwave irradiation (650 W / 3 min) seems to have no detrimental effects of clinical importance on the flexural properties, impact strength and hardness of denture resins and the bond, flexural strength, porosity and hardness of denture liners.

Niloofar Mojarad, Zahra Khalili, Shima Aalaei (2017)[62] compared the efficacy of microwave radiation with that of chemical and mechanical techniques in disinfecting complete dentures contaminated with Staphylococcus aureus and Pseudomonas aeruginosa. Seventy‐two sterilized mandibular dentures were separately contaminated with S. aureus (n = 32) and P. aeruginosa (n = 32) and then incubated at 37°C for 48 h. The contaminated dentures were disinfected as follows: chemical disinfection with Corega tablets; chemical disinfection with 2% glutaraldehyde; mechanical disinfection by brushing the denture; and physical disinfection by 650‐W microwaves irradiation for 3 min with six samples in each subgroup. Six dentures served as negative control group, and six contaminated dentures with no disinfection served as the positive control group. To evaluate the

lasting time of disinfection, the containers with nutrient agar and dentures were stored for 7 days at 37°C to evaluate turbidity. Data were analyzed using Kruskal–Wallis and Mann–Whitney U‐test ($\alpha = 0.05$). It was seen that there was no evidence of bacterial growth in 48 h and turbidity after 7 days of incubation of dentures disinfected by microwaves, glutaraldehyde, and Corega tablets, which was statistically significant compared to the positive controls ($P < 0.001$). In mechanically disinfected dentures (brushing), bacterial growth was detected after 48 h which was statistically significant compared to the positive controls ($P < 0.001$) and turbidity was seen in all the nutrient agar plates.

Masoomeh Aslanimehr, Niloofar Mojarad, Safarali Ranjbar, Shima Aalaei (2018)[63] compared the effects of microwave irradiation, chemical techniques, and a mechanical method (i.e., brushing) on the disinfection of complete dentures contaminated with C. albicans. In this experimental study, sixty sterilized dentures were divided into six groups of 10 dentures each. The dentures (Groups 2–5) were contaminated with C. albicans and subjected to four disinfection procedures: Corega tablets, 2% glutaraldehyde, brushing, and microwave irradiation 650 W for 3 mins. It was seen that the dentures disinfected with microwave irradiation (650 W, 3 mins) and glutaraldehyde (2%, 10 min) exhibited no evidence of fungal growth after 48 hrs of incubation and also no turbidity in the TSB containers after 7 days of incubation. However, the dentures disinfected using the mechanical method and Corega tablets exhibited turbidity after 7 days and fungal growth after 48 h that was significantly more than that in the two other methods ($P = 0.000$) and less than that in the positive control group ($P = 0.000$).

Mario A Brondani and Adriana R Siqueira (2018)[64] critically reviewed the literature on protocols for complete denture disinfection using conventional microwave ovens. He concluded that there is still no established protocol; a firm protocol working as a gold standard might be difficult to attain given the variety of factors influencing the sseffectiveness of microwave use on denture disinfection and sterilization. Although underutilized in residential care, daily denture hygiene seems to still be the optimal method for controlling fungal infections and denture stomatitis.

Sodium Hypochlorite

J. A. Bell, S. L. Brockmann, P. Feil, D. A. Sackuvich (1989)[65] compared the biocidal effectiveness of chlorine dioxide and 5.25% sodium hypochlorite (diluted 1:10) on acrylic resin strips inoculated with Staphylococcus aureus, Candida albicans, or Escherichia coli in the presence of an organic load. Sterile acrylic resin strips were immersed in a solution containing 10% horse serum and 10^5 to 10^7 organisms/ml for each type of organism, then disinfected in chlorine dioxide, sodium hypochlorite, or 0.9 sterile saline for 30 seconds or 1,2, or 4 minutes. After disinfection, the strips were neutralized and incubated for 72 hours. The results showed a difference between the ability of chlorine dioxide and sodium hypochlorite to kill S. aureus and E. coli on acrylic resin strips when organic matter is present. However, the disinfectants exhibited similar effectiveness in killing C. albicans. It was concluded that chlorine dioxide achieved complete disinfection of all three organisms within 2 minutes, sodium hypochlorite achieved complete disinfection of all three organisms within 4 minutes, E. coli and S. aureus were the least resistant to chlorine dioxide and the most resistant to sodium hypochlorite and both disinfectants were effective within 2 minutes for C. albicans, but chlorine dioxide's biocidal activity was initially faster.

A. C. Pavarina, A. C. Pizzolitto, A. L. Machado, C. E. Vergani & E. T. Giampaolo (2003)[66] investigated and evaluated the effectiveness of an infection control protocol for cleansing and disinfecting removable dental prostheses. Sixty-four dentures were rubbed with sterile cotton swab immediately after they had been taken from patient's mouth. Samples were individually placed in the culture medium and immediately incubated at 37 ± 2^0C. The dentures were scrubbed for 1 min with 4% chlorhexidine, rinsed for 1 min in sterile water and placed for 10 min in one of the

following immersion solutions: 4% chlorhexidine gluconate, 1% sodium hypochlorite, Biocide (iodophors) and Amosan (alkaline peroxide). After the disinfection procedures, the dentures were immersed in sterile water for 3 min, reswabbed and the samples were incubated. He concluded that all samples obtained in the initial culture were contaminated with micro-organisms. All the lower dentures (no sonification) immersed in Biocide showed positive growth, and the upper dentures (sonification) were positive for growth in six of eight dentures. The 4% chlorhexidine gluconate, 1% sodium hypochlorite and Amosan solutions were proved to be effective in reducing the growth of the micro-organisms in the 10 min immersion period and the protocol evaluated in this study seems to be a viable method to prevent cross-contamination between dental personnel and patients.

W. Barnabe, T. De Mendonca Neto, F. C. Pimenta, L. F. Pegoraro & J. M. Scolaro (2004)[67] evaluated and compared the efficacy of sodium hypochlorite and coconut soap used as disinfecting agents in the reduction of denture stomatitis, Streptococcus mutans (SGM) and Candida albicans. The mucosal characteristics were evaluated according to Newton's classification at baseline, after cleansing the dentures with coconut soap for 15 days in group 1 (nine patients). In the other group (19 patients) the analysis was made before and after cleansing the dentures with coconut soap and with disinfection in a soak solution of 0.05% sodium hypochlorite for 10 min during 15 days. Microbiological tests were used to isolate C. albicans and SGM. Mann–Whitney and Wilcoxon tests were used to compare the mucosal characteristics and Fisher test and McNemar test to compare C. albicans and SGM levels. They concluded that statistical analysis at the 95% confidence level ($P < 0.05$) showed that: (i) the association of coconut soap and 0.05% sodium hypochlorite

significantly reduced clinical signs of denture stomatitis, (ii) C. albicans did not reduce in counts, (iii) SGM were reduced but not significantly and (iv) the association of coconut soap and 0.5% sodium hypochlorite was effective in controlling denture biofilm.

N. T. Sena, B. P. F. A. Gomes, M. E. Vianna, V. B. Berber, A. A. Zaia, C. C. R. Ferraz & F. J. Souza-Filho (2006)[68] investigated the antimicrobial activity of 2.5% and 5.25% sodium hypochlorite and 2.0% chlorhexidine gel and liquid as endodontic-irrigating substances against selected single-species biofilms. Single-species biofilms of Enterococcus faecalis, Staphylococcus aureus, Candida albicans, Prevotella intermedia, Porphyromonas gingivalis, Porphyromonas endodontalis and Fusobacterium nucleatum were generated on a cellulose nitrate membrane placed on agar medium. The biofilms were then immersed in the endodontic-irrigating substances for 30 s and also for 5, 10, 15, 30 and 60 min, with and without mechanical agitation. Sterile saline was used as control. They concluded that mechanical agitation promoted the effectiveness of the antimicrobial agents, resulting in less time to eliminate the same micro-organisms, except for S. aureus with 2.5% NaOCl. Antimicrobial agents in liquid presentation, especially 5.25% NaOCl and 2% chlorhexidine, killed the tested micro-organisms more rapidly. Saline did not inhibit the growth of any of the tested micro-organisms, with or without agitation, being statistically different ($P < 0.05$) from NaOCl and chlorhexidine. P. intermedia, P. gingivalis, P. endodontalis and F. nucleatum were eliminated in 30 s by all antimicrobial agents, with or without agitation, in contrast with the facultative and aerobe strains.

Francine Cristina da Silva et al (2008)[69] evaluated the effectiveness of disinfectant solutions (1% sodium hypochlorite, 2% chlorhexidine digluconate, 2% glutaraldehyde, 100% vinegar, tabs of sodium perborate-based denture cleanser, and 3.8% sodium perborate) in the disinfection of acrylic resin specimens (n = 10/group) contaminated in vitro by Candida albicans, Streptococcus mutans, S. aureus, Escherichia coli, or Bacillus subtilis as measured by residual colony-forming unit (CFU). In a separate experiment, acrylic resin was treated with disinfectants to monitor potential effects on surface roughness, Ra (μm), which might facilitate microbial adherence. Three hundred fifty acrylic resin specimens contaminated in vitro with 1×10^6 cells/ml suspensions of standard strains of the cited microorganisms were immersed in the disinfectants for 10 minutes; the control group was not submitted to any disinfection process. Final counts of microorganisms per ml were performed by plating method for the evaluation of microbial level reduction. In a parallel study aiming to evaluate the effect of the tested disinfectant on resin surface, 60 specimens were analyzed in a digital rugosimeter before and after ten cycles of 10-minutes immersion in the disinfectants. The results showed that 1% sodium hypochlorite, 2% glutaraldehyde, and 2% chlorhexidine digluconate were most effective against the analyzed microorganisms, followed by 100% vinegar, 3.8% sodium perborate, and tabs of sodium perborate-based denture cleanser. Superficial roughness of the specimens was higher after disinfection cycles with 3.8% sodium perborate ($p = 0.03$) and lower after the cycles with 2% chlorhexidine digluconate ($p = 0.04$).

Iara Augusta Orsi et al (2010)[70] evaluated the efficacy of disinfectants on the internal aspect of heat-polymerised acrylic resin contaminated with microbial strains.

Two hundred and fifty rectangular specimens were made of heat-polymerised acrylic resin, and then divided into five groups corresponding to the microbial strains (Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, S. mutans and Enterococcus faecalis). After contamination, the specimens were immersed in 1 and 2% sodium hypochlorite and 2% glutaraldehyde for periods of 5, 10 and 15 min. The specimens were placed into tubes containing different broths and incubated at 35°C and then visually analysed. It was concluded that disinfection for 10 min with 1% hypochlorite and 2% glutaraldehyde is effective in disinfecting the internal aspect of heat-polymerised acrylic resin.

Rutuja Madhukarrao Nirale, Ram Thombre, Girish Kubasad (2012)[71] compared the effect of sodium hypochlorite and microwave disinfection on the dimensional stability of denture bases without and with relining**.** A brass die was prepared by simulating an edentulous maxillary arch. It was used to fabricate 1.5 mm and 3 mm of thickness denture bases (n = 40). The 1.5 mm of thickness-specimens (n = 20) were relined with 1.5 mm of autopolymerizing relining resin. Five holes were prepared over crest of ridge of brass die with intimately fitting stainless steel pins which were transferred to the intaglio surface of specimens during fabrication of denture bases. For calculation of dimensional changes in denture bases, differences between the baseline area before and after disinfection of the specimens were used. The denture bases without and with relining were divided into 2 groups (each n = 20). It was concluded that the changes in dimensional stability of denture bases without relining and denture bases with relining were significant after microwave disinfection (650 W for 6 minutes) as compared to 0.525% sodium hypochlorite disinfection for 10 minutes). Denture bases without relining showed more dimensional changes

significantly than relined denture bases after microwave disinfection. Hence, chemical disinfection seems to be a safer method of disinfecting dentures in comparison with microwave irradiation, as disinfection by microwave irradiation causes alteration with regards to physical properties such as changes in dimensional stability.

Cristiane F. Carvalho et al (2012)[72] evaluated the hardness, roughness and mass loss of an acrylic denture base resin after in vitro exposure to four disinfectant solutions. Forty specimens were prepared and randomly assigned to 4 groups (n=10) according to the disinfectant solution: G1: control, stored in distilled water at 37°C; G2: 1% sodium hypochlorite; G3: 2% glutaraldehyde; G4: 4% chlorhexidine. G2 to G4 were immersed for 60 minutes in the disinfectant solution. Measurements were carried out both before and after immersion in the solution. The surface was analyzed with a surface roughness tester, a microdurometer FM-700 and a scanning electron microscope. Loss of mass was determined with a digital weighing scale. After disinfection procedures, values were analyzed statistically. It was concluded that immersion of specimens in 1% sodium hypochlorite solution produced a significant increase in roughness. No significant effect was observed on surface hardness of the tested specimens. G3 mass loss values were higher than values in G2 and G4. Qualitative evaluation by SEM showed varying degrees of surface change after immersion of the specimens in all the disinfectant solutions tested.

Delise Pellizzaro et al (2012)[73] investigated the effectiveness of combining brushing and cleansing agents in killing C. albicans biofilm. Disks of acrylic resin were made, sterilized, and inoculated with C. albicans (10^7 cfu/mL). After incubation (37°C/48 h), specimens were randomly assigned to 10 experimental groups (n=9): 5

subjected to brushing with distilled water or cleansing agents – dentifrice slurry, 2% chlorhexidine gluconate (CHX), 1% sodium hypochlorite (NaOCl), and Polident fresh cleanse® (combined method) - and 4 exposed to the cleansing agents without brushing (immersion). Non-cleansed specimens were used as positive controls. The viability of cells was evaluated by XTT reduction method. Results were analyzed by Mann-Whitney and Kruskal-Wallis tests (α=0.05). it was concluded that the combined method was significantly more effective ($p<0.0001$) in reducing biofilm viability than the immersion. Brushing with CHX and NaOCl resulted in 100% removal of the biofilm. Immersion in the agents reduced significantly ($p<0.0001$) the biofilm viability, with CHX being the most effective ($p<0.0001$). The use of the combined method of brushing with cleansing agents is an effective method to reduce C. albicans biofilm, being CHX and NaOCl the most effective solutions.

Ana Carolina Rodrigues Danzi Salvia et al (2013)[74] evaluated disinfection protocols, considering antimicrobial effectiveness and damage to the structures of prostheses. Solutions of 1% sodium hypochlorite, 2% chlorhexidine digluconate, 50% vinegar and sodium perborate were evaluated. Specimens were contaminated in vitro with standardized suspensions of Candida albicans, Streptococcus mutans, Escherichia coli, Staphylococcus aureus and Bacillus subtilis spores. Disinfection by immersion for 10 min was performed. Final counts of microorganisms were obtained using the plating method. Results were statistically compared by Kruskal-Wallis ANOVA and Dunn's test. The surface roughness of 40 specimens was analyzed before and after 10 disinfection cycles, and results were compared statistically using Student's t test. 50% vinegar was as effective as 1% sodium hypochlorite and 2% chlorhexidine digluconate against C. albicans, E. coli and S. mutans for the

disinfection of heat-polymerized acrylic resin. Sodium perborate based tabs exhibited antimicrobial activity only against S. mutans. Superficial roughness increased after cycles in 1% sodium hypochlorite.

Jovito Adiel Skupien, Fernanda Valentini, Noéli Boscato and Tatiana Pereira-Cenci (2013)[75] conducted a systematic review to determine the feasibility of a prevention protocol for Candida colonization in denture liners and an effective treatment after the fungi has colonized the material. It was concluded that the incorporation of nystatin (in general, 500 000 units) into tissue conditioners to prevent the onset of the disease and immersion in sodium hypochlorite for disinfection were most often used, and both methods were able to prevent or inhibit Candida colonization, depending on their concentrations. The 0.5% sodium hypochlorite concentration can disinfect tissue conditioners and denture liners. Microwave irradiation has also been described an alternative method of disinfection.

Mohannad H. Al-Saadi (2014)[76] evaluated the effectiveness of six disinfection methods and the influence of these methods on the adaptation of maxillary dentures. Acrylic resin specimens contaminated with fungi were exposed to the following disinfection treatments: 1. microwave oven (900 W) at full power for 5 min (with soaking the specimen in 250 ml water), 2. microwave oven at medium power for 5 min (with soaking the specimen in 250 ml water), 3. sodium hypochlorite 5.25 % for 5 min, 4. diluted sodium hypochlorite 1:420 for 5 h, 5. Chlorhexidine gluconate for 5 h, 6. effervescent tablets for 15 min, 7. soaking in 250 ml tap water for 15 min. Colony forming units (CFUs) of remaining cells were counted and compared with t test. Dimensional stability was evaluated using aluminum die simulating the

maxillary edentulous arch. Posterior palatal gaps were measured. Results showed that microwave irradiation (at full or medium power) and sodium hypochlorite 5.25 % for 5 min were able to reduce the CFUs of fungi by more than 4 $\log_{10}$ whereas diluted sodium hypochlorite, chlorhexidine gluconate, and effervescent tablets did not achieve a reduction of >2.8, 2.68 and 1.66, respectively. For dimensional stability test, t test revealed significant difference between control group and the microwave at full power group (p = 0.000). it was concluded that microwave oven at medium power and sodium hypochlorite (5.25 %) are effective and safe methods of disinfecting removable dentures.

Emilio Jose T. Rodriguez Acosta et al (2015)[77] evaluated the effect of 1% sodium hypochlorite (H1%) and 4% chlorhexidine gluconate (CG4%) on the adhesion of Candida albicans to denture base acrylic resins, as well as to verify the effect of the acquired salivary pellicle (ASP) formation on this process. A total of 300 acrylic specimens were immersed in distilled water (control) (n = 100), H1% (n = 100), or CG4% (n = 100) for 30 days. Twenty specimens were used in each experimental period (0, 1, 7, 15, 30 days). At the end of disinfection testing periods, 10 specimens of each group were exposed to human whole saliva to simulate ASP formation, and then all specimens were incubated with C. albicans ATTC 90028. Microorganism adhesion was analyzed by fluorescence microscopy, after staining with Acridine orange. Results showed that in the 30th disinfection cycle in relation to baseline, the H1% or CG4%, without ASP formation, reduced the C. albicans adhesion by approximately 80%; however, with ASP, this reduction after disinfection with H1% was higher (88%). The presence of ASP resulted in higher reduction of adhered fungal cells in comparison to resin without ASP, at the 1st H1% or CG4%

disinfection cycle, as well as at 30th H1% disinfection cycles. Thus, it was concluded that that the presence of saliva might influence the adhesion of C. albicans and improve the effectiveness of methods to reduce fungal adhesion.

Sheila Rodrigues de Sousa Porta, Silvia Carneiro de Lucena-Ferreira, Wander Jose da Silva and Altair Antoninha Del Bel Cury (2015)[78] evaluated the effect of sodium hypochlorite (NaOCl) on biofilms, colour stability and surface roughness (Ra) of complete dentures and patient acceptability. Fifteen participants were instructed to keep their dentures immersed daily in a 0.5% NaOCl solution for 3 min over 90 days. Swabs were taken from dentures and inoculated on CHROMagar and blood agar. The number of colony-forming units (cfu) was counted after a 48 hours incubation period. Colour stability was assessed using the CIE L*a*b* system. Ra was measured using a profilometer. Patient acceptability was checked based on their degree of satisfaction. It was concluded that 0.5% NaOCl solution was effective in reducing microorganisms without significant changes in colour or roughness of denture resin. The participants reported satisfaction with the cleaning results.

Amanda Peracini et al (2016)[79] evaluated the efficacy of cleanser solutions on denture biofilm removal by a crossover randomized clinical trial. Thirty two edentulous patients were instructed to brush their dentures (specific brush and liquid soap) three times a day (after breakfast, lunch and dinner) and to soak them (≥ 8 h) in: (C) Control - water; (AP) Alkaline peroxide; or (SH) 0.5% sodium hypochlorite. Each solution was used for 21 days (three cycles of 7 days). At the end of each cycle, the inner surfaces of maxillary dentures were disclosed (1% neutral red). Areas (total and stained biofilm) were measured (Image Tool software) and the percentage of biofilm

calculated as the ratio between the area of the biofilm multiplied by 100 and total surface area of the internal base of the denture. Data were compared by means of generalized estimating equation (α=5%) and multiple comparisons (Bonferroni; α=1.67%). Immersion in SH reduced biofilm to (%) (8.3 ± 13.3) compared to C (18.2 ± 14.9) and AP (18.2 ± 16.6). It was concluded that 0.5% sodium hypochlorite solution was most efficacious for biofilm removal. Alkaline peroxides may not lead to further biofilm removal in patients with adequate denture maintenance habits.

Duygu Karakis, Canan Akay, Burcin Oncu, Abbas Y. Rad, and Arife Dogan (2016)[80] compared the effects of some disinfectants, including ethanol extract of propolis (EEP) on the adhesion of Candida albicans to denture base resins. Seventy-two acrylic resin samples were prepared, half of which was polished and the other half was roughened. C. albicans strain ATCC 10231 was incubated on Sabouraud dextrose agar (SDA) at 37°C for 48 h. The adhesion period was completed by keeping the cells in this suspension for 90 min at 37°C. Specimens were then immersed in the following solutions: 1%, 2%, and 5% sodium hypochlorite; 4% chlorhexidine gluconate; and 10% EEP. Quantification of the antifungal activity of the chemical solutions was performed using the colorimetric MTT assay test. Results showed that all forms of sodium hypochlorite solutions yielded higher efficiency than 4% chlorhexidine gluconate and propolis solutions ($P < 0.05$), and 5% sodium hypochlorite was found to be the most efficient in both surface textures. However, it was only statistically more effective than 2% sodium hypochlorite in the roughened surface group ($P < 0.05$), while no significant differences between all forms of sodium hypochlorite solutions were observed in the polished surface group ($P > 0.05$).

Karuna Gajanan Pawashe, Shivsagar Tewary, Pronob Kumar Sanyal, Kumar Nilesh (2017)[81] evaluated and compared the effectiveness of four commercially available disinfectants on heat cure acrylic resin specimens contaminated with standard and clinical strains of two micro-organisms commonly inhabiting the oral microflora. Two hundred acrylic resin specimens (n=200), 10 in each group were contaminated in vitro with $1x10^6$ cells/ml suspensions of standard and clinical strains of micro-organisms (Candida albicans and Streptococcus mutans) and were immersed in four disinfectants (1% sodium hypochlorite, 2% chlorhexidine digluconate, 2% glutaraldehyde and 3.8% sodium perborate) for 10 minutes. The control group was not subjected to any disinfection process. Results showed that standard strains of Candida albicans (C) and Streptoccocus mutans (S) subjected to various disinfectants showed varied mean Colony Forming Units per ml (CFU/ml) from <10,000 to 25,000 and <10000 to <50,000 respectively. Clinical strains and of Candida albicans (C) and Streptococcus mutans (S) subjected to various disinfectants showed varied mean CFU/ml from <10,000 to 50,000 and from 10,000 to 50,000 respectively. Control groups showed maximum mean CFU/ml ($>10^5$). It was concluded that 1% sodium hypochlorite was found to be the most effective disinfectant for both Candida albicans and Streptococcus mutans. The least effective disinfectant being: 3.8% sodium perborate.

Assadollah Ahmadzadeh, Effat Abasi Montazeri, Sara Mogharabi, Maryam Jarahzadeh (2017)[82] evaluated the effect of antimicrobial solutions of 0.5% sodium hypochlorite, 100% vinegar, and 0.2% chlorhexidine on heat cure acrylic resin specimens contaminated with standard and clinical strains of S.mutans microorganism. A total of 60 acrylic sheets were made with dimensions of

17×6×1mm in one shape and one size and the same thickness. The specimens were divided into four subgroups of 15 and each group was immersed in sterile containers containing 0.5% sodium hypochlorite, 0.2% chlorhexidine, 100% vinegar, and water. A total of five samples were isolated from each group and were turbid in an interval time of 30min, 2hrs, and 4hrs, respectively. Results showed that all three disinfectant solutions resulted in complete elimination of S.mutan micro-organism provided that the immersion time lasts about thirty minutes. The long term effect of vinegar after 2hrs reduced to 80% and the effect of hypochlorite after 4hrs significantly reduced to 40%. It was concluded that among the three denture cleansers used in the present study, all were considered to be effective in cleaning and removing bacteria from denture at specific time interval.

Carine W. Pires, Sara Fraga, Aline C. O. Beck, Katia O. Braun, Paulo E. C. Peres (2017)[83] investigated the antimicrobial efficacy of different chemical agents used for denture cleaning. Biofilm samples collected from 10 removable dentures were subjected to 10 disinfection protocols: distilled water for 30 min (negative control); 1% sodium hypochlorite for 10 min (positive control); diluted sodium hypochlorite for 10 min; vinegar for 20 min; 0.2% peracetic acid for 5 min; alkaline peroxide solution for 5 min; alkaline peroxide solution for 30 min; 0.12% chlorhexidine digluconate for 10 min; 0.05% sodium salicylate solution for 10 min; and enzymatic detergent for 2 min. Each of the samples was plated on petri dishes with Mueller-Hinton agar. The numbers of microbial colonies after 48 h at 37ºC were compared using the Kruskal-Wallis and Dunn's tests ($\alpha = 0.05$). It was concluded that 0.2% peracetic acid and 0.05% sodium salicylate solutions were ineffective against bacterial growth, while enzymatic detergent and alkaline peroxide achieved an

intermediate effect. Diluted sodium hypochlorite, vinegar, and chlorhexidine digluconate can be considered adequate products for cleaning dentures due to their potential for inhibiting bacterial growth, similar to 1% sodium hypochlorite.

Aysegul Kurt, Gonca Erkose-Genc, Meltem Uzun, Tugrul Sarı, & Gulbahar Isik-Ozkol (2018)[84] evaluated the antimicrobial efficiency of three cleaning solutions and their effect on the physical properties of a denture base material. A heat-cured polymethyl-methacrylate (PMMA) denture base material (Meliodent) and three cleaning solutions (alkaline-peroxide, 30 minutes; 1% sodium-hypochlorite, 10 minutes; and 0.1% polymeric-guanidine solution, 5 minutes) were used. For antifungal activity test, 40 disc-shaped specimens were fabricated and allocated into a control group (distilled water) and 3 experimental groups (n = 10) according to the cleaning solutions. Antifungal activity against Candida albicans (ATCC 2091) was assessed with colony-forming units. An additional 40 rectangular plate specimens were fabricated for mechanical tests. Ten specimens were kept intact to be used as the control group for flexural strength test. The remaining 30 specimens were distributed into three groups according to the cleaning solutions. The surface roughness and Vickers hardness of the specimens were consecutively measured after 48 hours of water storage at 37 ± 2°C (t0), two disinfection cycles (t1), and 7 days of storage (t2) in one of the solutions. Results showed that the antifungal activities of polymeric guanidine and sodium hypochlorite were comparable to each other and significantly higher than alkaline peroxide ($p < 0.05$). The changes in the surface roughness of the specimens were statistically comparable among the cleaning solutions and time periods ($p > 0.05$); however, the decrease in the Vickers hardness of the specimens stored in sodium hypochlorite was significantly higher from t0 to t1

and t0 to t2 ($p < 0.05$) than other groups, resulting in comparable hardness changes. The flexural strengths of all groups were comparable with the control after t2 ($p > 0.05$).

Fernanda Valentini-Mioso, Tamires T. Maske, Maximiliano S. Cenci, Noeli Boscato, and Tatiana Pereira-Cenci (2019)[85] assessed the effectiveness of various chemical hygiene clinical protocols in reducing the microbial viability of biofilm formed on complete dentures. In this triple-blind (participants, dentist, and outcome evaluator) study, complete denture wearers without candidiasis were randomly divided into 4 groups (n=40) according to the chemical hygiene protocol: water (placebo), 0.5% sodium hypochlorite solution, 0.12% chlorhexidine gluconate solution, and 5% sodium bicarbonate solution. The biofilm formed on the palate intaglio and denture teeth was collected and assessed in each experimental phase for quantitative microbial viability at the seventh and 14th day after using the chemical protocol. Results showed that soaking dentures was not effective in decreasing Candida albicans, C. non-albicans, and lactobacillus counts. The use of sodium hypochlorite and chlorhexidine decreased total microorganisms and Streptococcus mutans counts for both palate and teeth compared with water and sodium bicarbonate. The intaglio of the dentures always presented higher microbial counts than did the denture teeth. It was concluded that the use of sodium hypochlorite and chlorhexidine and mechanical cleansing with a toothbrush decreased microbial viability in healthy complete denture wearers.

MATERIALS AND METHOD

Dental prostheses used by patients are exposed to normal oral microbial flora that includes fungi, bacteria, and viruses. Denture stomatitis is usual occurrence in denture wearers, resulting in an area of erythema underneath the denture. Its etiology is multifactorial, and may be associated with both local and systemic factors. As many as sixty seven percent of existing denture wearers are thought to have Candida-associated denture stomatitis. The role of Candida albicans in the pathogenesis of denture stomatitis has been well examined, and multiple strains of Candida have been found to colonize the denture base, as well as the oral tissues.[86] Dental professionals adjusting or repairing these prostheses may therefore be at risk of contacting infections from prostheses that have not been properly disinfected.[87]

Hence, this study was done to evaluate and compare the antimicrobial efficacy of aqueous ozone, microwave irradiation and sodium hypochlorite solution on heat polymerized polymethyl methacrylate denture base material against C. albicans.

Material and methods have been divided under the following headings:

I. Materials

II. Armamentarium and equipment

III. Methodology

1. Materials:

SR NO.	MATERIALS	MANUFACTURER	BATCH NUMBER
1.	Heat polymerized acrylic resin (Plate I, Fig 1)	DPI Heat Cure™, (Dental products ofIndia Ltd)	P-12152
2.	Die stone (Plate I, Fig 2)	Ultrarock; Kalabhai Karson Pvt Ltd, India	151227
3.	Sodium hypochlorite solution (Plate II, Fig 12)	M/s Anand Sales	520
4.	Cold mold seal(separating medium) (Plate III, Fig 17)	DPI Heat Cure™, (Dental products of India Ltd)	8117
5.	Distilled water (Plate III, Fig 22)	M/s Anand Sales	1120
6.	Sterile saline	eurolife healthcare Pvt. Ltd.	2E1240B

Materials used for contamination and inoculation of samples:

- Candida albicans (ATCC 25175) (ATCC – American type culture collection)
- Yeast and mold broth
- Sabouraud's agar plates

ARMAMENTARIUM AND EQUIPMENTS

1. Ozonator(Plate I, Fig 3)
2. Microwave (Plate I, Fig.4)
3. Glass Beaker (Plate I, Fig.5)
4. Sterile Syringe (Plate I, Fig.5)
5. Petroleum jelly (Plate I, Fig.5)
6. Glass bowl with metal plate (Plate I, Fig.5)
7. Petri dishes (Plate I, Fig.6)
8. Sterile swab sticks (Plate I, Fig.6)
9. Nickel wire inoculating loop (Plate I, Fig.6)
10. Automated densitometer (Plate II, Fig.7)
11. Ultrasonicator (Plate II, Fig.8)
12. Pipette with disposable sterile tips (Plate II, Fig.9)
13. Vortex Machine (Plate II, Fig.10)
14. Acrylizer with thermostat (Plate II, Fig.11)
15. Sodium Hypochlorite (Plate II, Fig.12)
16. Incubator (Plate II, Fig.13)
17. Rubber bowls, plaster spatula, lacron's carver (Plate III, Fig.14)
18. Mixing spatula (Plate III, Fig.14)
19. Varsity flasks and clamps (Plate III, Fig.14)
20. Sand paper (No. 120) (Plate III, Fig.15)
21. Camel hair brush (Plate III, Fig.15)
22. Vernier Caliper (Plate III, Fig.16)
23. Brass metal dies (Plate III, Fig.18)
24. UV Chamber (Plate III, Fig.19)
25. Parafilm (Plate III, Fig.20)
26. Hydraulic bench press (Plate III, Fig.21)

METHODOLOGY:

The basic methodology consisted of:

a. Metal Die preparation (Plate III, Fig.18)

b. Preparation of gypsum mold for fabrication of samples (Plate IV, Fig.23)

c. Preparation of heat polymerized acrylic resin denture base samples of 4 groups – Group C, Group O, Group M, Group S (Plate VI, Fig.24)

d. Sterilization and contamination of the samples

e. Disinfection of the samples in all the 4 groups– Group C, Group O, Group M, Group S

f. Procedure for serial dilution

g. Inoculation on Sabouraud's Dextrose Agar media (Plate V, Fig.27)

h. Counting of colonies after disinfection

A total of 100 samples were prepared with each group having 25 samples.

The samples were divided under the following groups: -

Control group	**Experimental Group**		
Group C (n=25)	**Group O (n=25)**	**Group M (n=25)**	**Group S (n=25)**
Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material without any disinfection.	Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material disinfected with 4mg/L aqueous ozone solution for 30 mins.	Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material disinfected with microwave irradiation at 650 Watts for 3 mins immersed in water.	Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material disinfected with 1% sodium hypochlorite solution for 10 mins.
TOTAL = 100			

a. Metal Die preparation (Plate III, Figure.18):

Preparation of molds were done by preparing metal dies for the fabrication of heat polymerized acrylic resin samples. 10 brass metal dies of dimension 15 mm in length, 15 mm in width, and 3 mm in height (15×15×3mm) were fabricated.[15] These fabricated metal dies had a threaded hole at the center and screws were used to engage these threaded holes to facilitate easy removal of dies from the stone mold.

b. Preparation of gypsum mold for the fabrication of samples (Plate IV, Fig.23):

The brass metal dies were used to prepare gypsum molds. Before investing them, the threaded holes on the dies were blocked with carding wax. A thin layer of petroleum jelly was applied on metal dies which were then invested in the lower half of the varsity flask. Die stone was used for base flasking and care was taken to embed half the thickness of the metal die in it.[88] Once the investment material had set, a thin layer of petroleum jelly was applied to the metal dies and investment material, following which counter flasking was done. The flasks were closed to ensure metal to metal contact between the base of the flask and its counterpart. After the investment material had set (1 hour) [89] the flasks were opened and the carding wax within the holes was removed. The dies were engaged with a screw and gently teased out. The molds were then immersed in hot water to remove any traces of petroleum jelly, wax and also to facilitate application of separating medium. These molds thus obtained were used for the fabrication of heat polymerized acrylic resin denture base material samples (PMMA).

c. Preparation of heat polymerized acrylic resin denture base samples: (Plate IV, Fig.24)

Using conventional heat polymerized denture base material (PMMA), 100 samples were prepared. Monomer and polymer were mixed in ratio of 1:2.5 by weight

as per manufacturer's recommendation.[90] Packing was done at dough stage and trial closure was performed. Final closure was carried out under a hydraulic bench press (Plate III, Fig 21) at a pressure of 3000 psi for 3 mins (according to the manufacturer's instructions). The flask was clamped and maintained under pressure for 1 hour.[91] It was then immersed in water in an acrylizer at room temperature. (Plate II, Fig. 11) The temperature was raised slowly up to 74°C and held for 2 hours. The temperature was again raised to 100°C and maintained for 1 hour.[90] After completion of this short curing cycle, the flasks were removed from the water bath and allowed to bench cool at room temperature prior to deflasking.[91] The polymerized samples were carefully removed. Samples with defects were discarded. Finishing of the samples was done using sandpaper (No. 120). Polishing was done with a felt buff cone and pumice, followed by polishing of the samples with rag wheel and polishing cake on a dental lathe.[91]

d. Sterilization and Contamination of the samples:

C. albicans (ATCC 25175) were cultured in yeast mold broth supplemented in glucose at 37° C for 24 hours. The broth containing C. albicans ($1.5x10^8$ colony-forming units/ml; CFU/ml) was immediately used as a fungus solution. The prepared resin samples were sonicated in water for 60 mins and later immersed in water for 1 day to remove the residual monomer, then dried in air and sterilised with 70% ethanol solution and UV treatment.

The resin samples were incubated with the fungus solution at 25° C for 3-5 days without agitation, and then washed 3 times with sterile saline to remove loosely attached micro-organisms. The cultured infected samples were finally stored in sterile petri dishes at room temperature for 20 mins and used for the experiment.[15]

e. Disinfection of the samples in all the 4 groups:

1. **Group C: -** Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material was not subjected to any disinfection method and left dry at room temperature. The colonies were counted later.

2. **Group O: -** Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material in 4mg/L aqueous ozone solution for 30 mins. The aqueous ozone was prepared by ozonation of purified water by insufflation of ozone gas at the rate of 4mg/L.

3. **Group M: -** Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material with microwave irradiation at 650 Watts for 3 mins immersed in water.

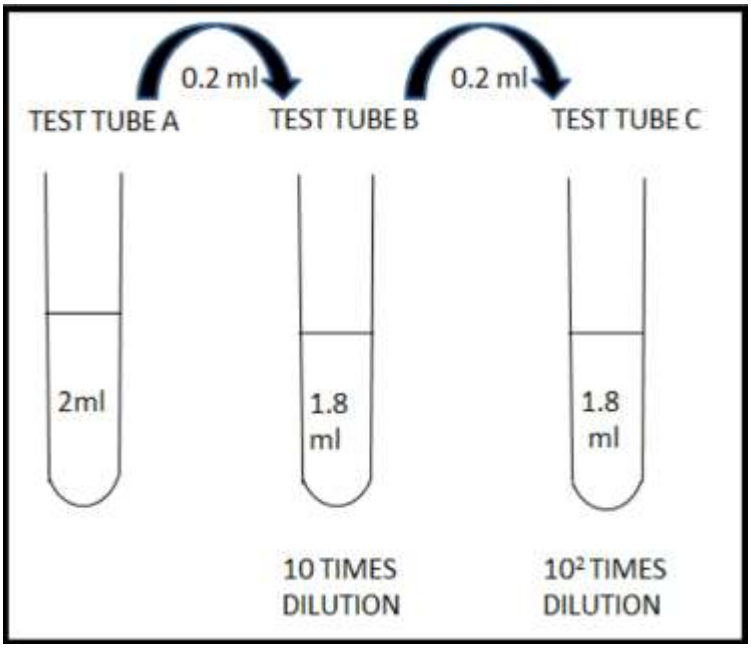
4. **Group S**: - Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material immersed in 1% sodium hypochlorite solution for 10 mins.

f. Procedure for serial dilution:

The plate count method was performed by diluting the original sample in serial dilution tubes, followed by the plating of the prepared serial dilutions onto the Sabouraud's Dextrose agar plates. The plate count method is based on viable cell counts. The typical counting ranges are 30 to 300 CFU per agar plate. Plates with more than 300 CFU are very difficult to count. Plates with less than 30 CFU are not statistically reliable.[92] Samples were added in test tubes and incubated at 37°C for 24 hours.[93] (Plate V, Fig.24)

In this procedure, single test tube was used for 1 sample. Thus, test tubes were divided into 4 groups (group C, group O, group M and group S) and each group consisted of 25 test tubes.

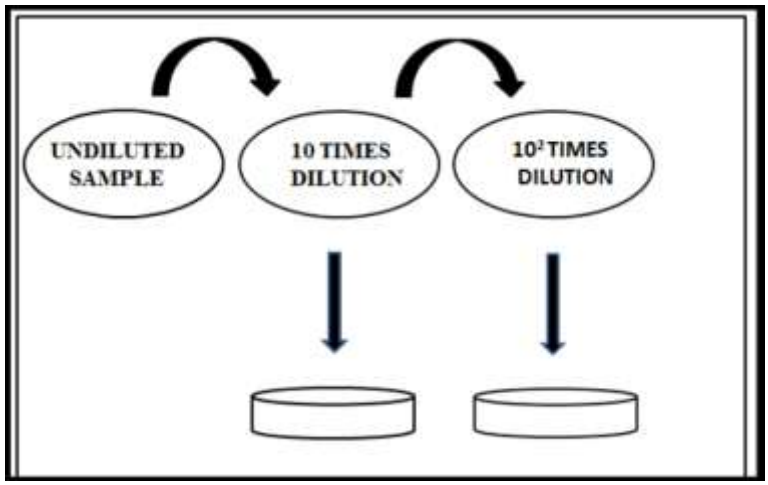
After 24 hours, samples with the colonies of Candida albicans were removed from the test tube and dispersed in a new test tube containing 2 ml of saline solution. This test tube was vortexed vigorously for 1 minute for even dispersion of colonies. Samples were removed from the test tubes and then allowed to stand for 9 minutes, followed by a short vortex to suspend any organisms present. Thus, all the colonies were evenly dispersed in a single test tube. Cell suspension was adjusted using an automated densitometer (DensiCHEK plus, Durham, North Carolina) (Plate II, Fig.7) to 0.5 McFarland standard, equivalent to an approximate concentration of $1.5x10^8$ colony forming units (CFU) per ml.[92,93]



TEST TUBE A: Containing 2 ml of saline solution with colonies Candida albicans evenly dispersed and density was adjusted to 0.5 McFarland Standard ($1.5x10^8$ CFU)

TEST TUBE B: Containing 1.8 ml of saline solution. Using a sterile 1 ml pipette (P‟ fact Prime variable volume pipette, Bioteknika healthcare Pvt. Ltd), 0.2 ml solution from test tube A was transferred to test tube B. This test tube now had a dilution of 10 times than original solution of test tube A.

TEST TUBE C: Containing 1.8 ml of saline solution. Using a new sterile 1 ml variable pipette, 0.2 ml solution from test tube B was transferred to test tube C. This test tube now had a dilution of 10^2 times than original solution of test tube A.



Total cell count for cultures from test tube A and B were > 300 CFU Total cell count for culture from test tube C was between 100 – 200 CFU

Therefore, serial dilution factor of 10^2 was considered for this study to get a viable cell count.[94]

e. Inoculation on Sabouraud's Dextrose Agar media:

0.2 ml of the saline was inoculated on Sabouraud's Dextrose agar plate and streaking was done with inoculating loop. The plates were then incubated in an incubator for 24 hours at 37°C.

g. Counting of the colonies after disinfection:-

After 24 hours, plates were examined for growth. The colonies were observed and then counted as the number of colony forming units (CFUs). The counting of CFUs assumes that every colony is separate and formed by a single viable microbial cell. The total colony counts obtained in CFU from the incubated agar plates and the respective dilution factor used can then be combined to calculate the original number of microorganisms in the sample as CFU per ml. Colony forming units per ml were calculated using the formula.[94]

$$\text{Colony formig units per ml (CFU / ml)} = \frac{\text{CFU x dilution factor}}{\text{plated Amount}}$$

PLATE I

Fig 1: Heat polymerized acrylic resin

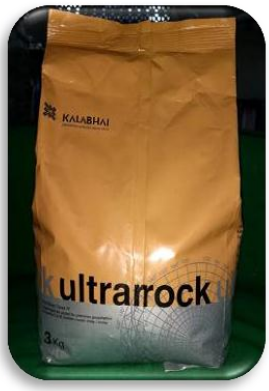


Fig 2: Die Stone

Fig 3: Ozonator

Fig 4: Microwave

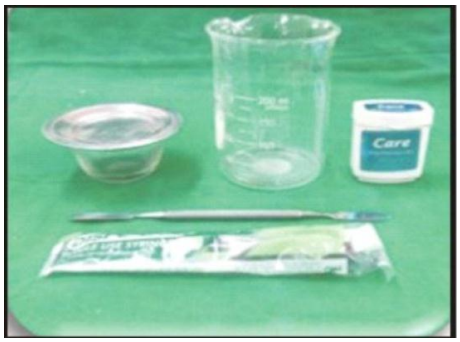


Fig 5: Glass beaker, Glass bowl with metal plates, Mixing spatula, sterile syringe, petroleum jelly

Fig 6: Petri dishes, Nickel wire inoculating loops, sterile swab sticks

PLATE II

ARMAMENTARIUM AND EQUIPMENTS

Fig 7: Automated Densitometer

Fig 8: Ultrasonicator

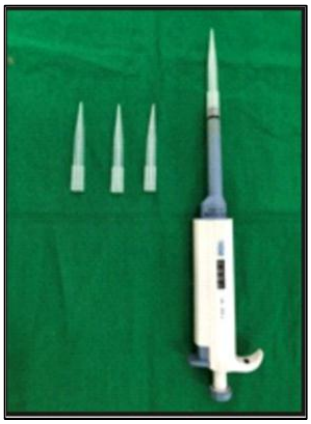


Fig 9: Pipette with disposable sterile tips

Fig 10: Vortex Machine

Fig 11: Acrylizer with thermostat

Fig 12: Sodium Hypochlorite

Fig 13: Incubator

PLATE III

ARMAMENTARIUM AND EQUIPMENTS

Fig 14: Rubber bowl, plaster spatula, lacron's carver and varsity flask & clamp

Fig 15: Porcelain jar, petroleum jelly, dapen dish, sandpaper (No. 120), camel hair brush, sterile syringe, mixing spatula

Fig 16: Vernier caliper

Fig 17: Separating medium

Fig 18: Brass metal dies

Fig 19: UV Chamber

Fig 20: Parafilm

Fig 21: Hydraulic bench press

Fig 22: Distilled water plant

PLATE IV

METHODOLOGY

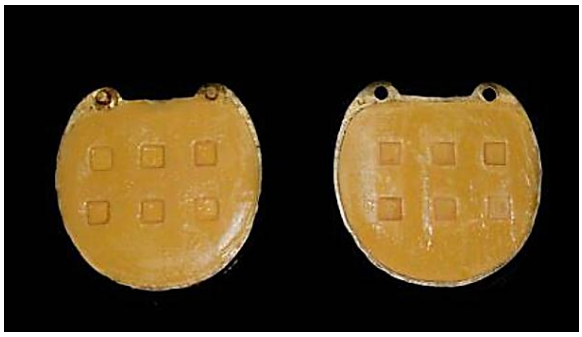


Fig 23: Preparation of gypsum mold to obtain samples

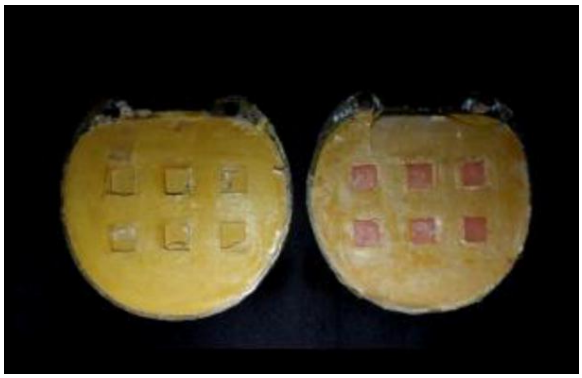


Fig 24: Preparation of heat polymerized acrylic resin denture base samples.

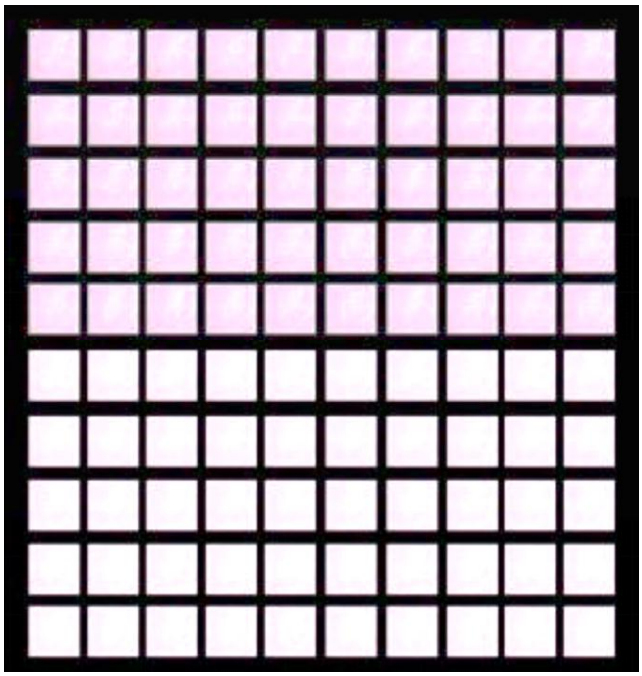


Fig 25: Total samples for the four groups : Group C, Group O, Group M, Group S

PLATE V

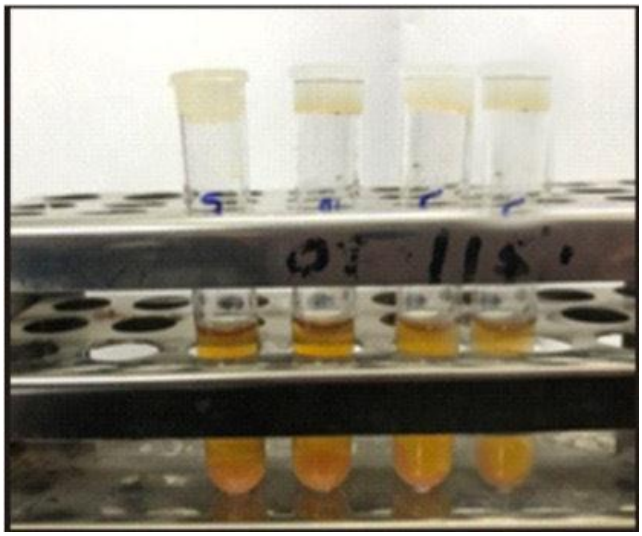
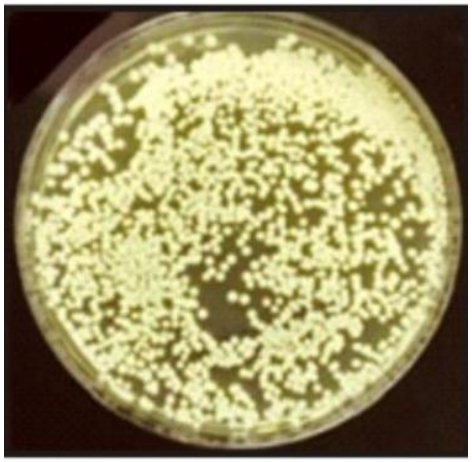
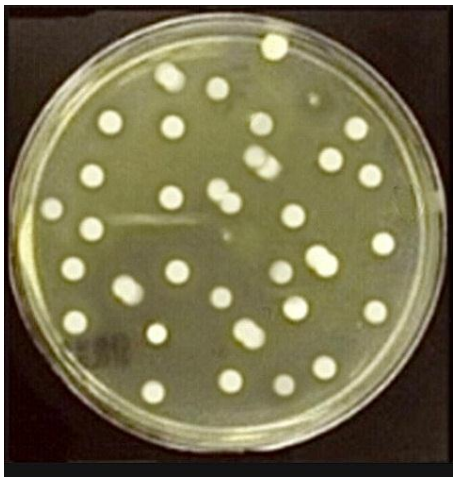


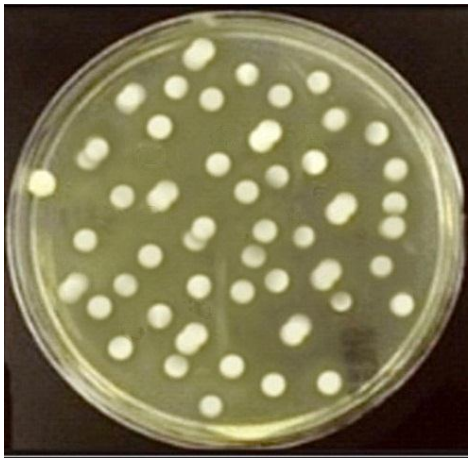
Fig 26 : Test tubes containing Yeast Mold Broth



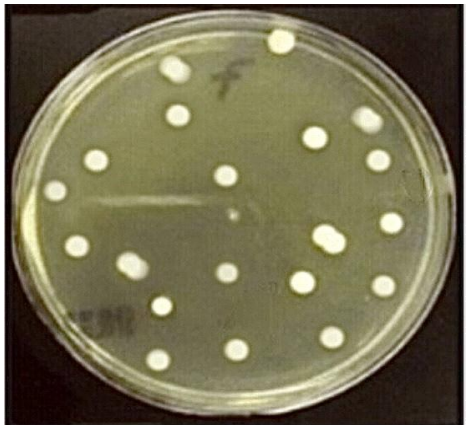
Group C



Group O



Group M



Group S

Fig 27: Sabouraud's Dextrose Agar Plates

Results

In this study comparative evaluation of the antimicrobial efficacy of aqueous ozone, microwave irradiation and sodium hypochlorite solution on heat polymerized polymethyl methacrylate denture base material against candida albicans was done.

A total of 100 heat polymerized acrylic resin samples were fabricated and divided into 4 groups.

Distribution of samples into groups

Sr no.	**Group**	**Code**	**n= no. of samples**
1.	Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material without any disinfection.	C	n=25

2.	Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material disinfected with 4mg/L aqueous ozone solution for 30 mins.	O	n=25
3.	Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material disinfected with microwave irradiation at 650 Watts for 3 mins immersed in water.	M	n=25
4.	Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material disinfected with 1% sodium hypochlorite solution for 10 mins.	S	n=25
Total number of samples			100

These acrylic resin samples were then cultured on Sabouraud's dextrose agar media and colony forming units per ml was calculated using the formula

Colony forming units per ml (CFU/ml) = $\frac{\text{CFU x dilution factor}}{\text{Plated Amount}}$

- The data were analyzed using the software SPSS ver. 20.0 and the statistical significance was tested at 5% level.
- The following statistical tests were employed for the analysis of the result:

A. The means were compared across groups for significance of difference using one-way analysis of variance (ANOVA).

B. Pair wise comparison of means were carried out and tested for statistical significance using TUKEY'S POST-HOC test.

Mean is the sum of all observations and divided by number of observations.

Median is the value of the variable that divides the distribution into two equal parts. i.e. 50 % observations will lie below and above it.

Standard Deviation is summarized as the amount of variation (change) in the observation from their average value (mean).

Standard Deviation: It is the most frequently used measure of deviation. It is defined as the root mean square deviation and is denoted by s or SD.

The formula used for calculating standard deviation:

$$SD = \sqrt{\frac{\Sigma(\overline{X} - X)^2}{n-1}}$$

Where:

$\overline{X}$= Mean
X = Values of the variables
Σ = Sum of the value
n = Number of observations
Min = Minimum Value
Max = Maximum Value

Results:

Table 1 provides the descriptive statistics of colony forming units x 10^2 per ml (CFU x 10^2/ml) across four study groups. In group C, the mean CFU x 10^2/ml was 308.40 ± 29.39, followed by group O, where the mean CFU x 10^2/ml was 0.63 ± 0.14.

For group M, the mean CFU x 10^2/ml was 1.11 ± 0.21 and for group S the mean CFU x 10^2/ml was 0.32 ± 0.12. The median of the group C, group O, group M and group S were 310.00, 0.60, 1.10 and 0.30 respectively. Other statistics like minimum and maximum values are also provided in the table.

Table 2 shows a comparison of mean colony forming units x 10^2 per ml (CFU x 10^2/ml) across four study groups using one-way analysis of variance (ANOVA). The resulting p-value obtained was 0.001, indicating a statistically significant difference in mean CFU x 10^2/ml across four study groups.

One-way ANOVA test resulted into a significant difference of means and hence, pairwise comparison of mean CFU x 10^2/ml was performed using Tukey post-hoc test with results shown in Table 3.

Table 3 shows significant difference in CFU x 10^2/ml among the group C with group O, group M and group S (p=0.001).

Difference in CFU x 10^2/ml among group O and group M was -0.48 which was non-significant (p= 0.999)

Difference in CFU x 10^2/ml among group O and group S was 0.31 which was non-significant (p= 1.000)

Difference in CFU x 10^2/ml among group M and group S was 0.0.79 which was non-significant (p=0.998)

DISCUSSION

Infectious diseases have become a major concern in the health care field. Therefore, the term infection control in dentistry is currently undergoing dramatic changes, and emphasizing on sterilization/disinfection procedures to prevent cross infection.[95] Increase in the incidence of communicable diseases particularly Hepatitis B and AIDS have led to significant changes in the attitude of both the dental surgeons as well as Para dental personnel. Potential source for transmission of infection from the patient to dental staff are via impressions, impression trays, gypsum and dental prosthesis.[96] Since, it is not possible to screen every patient for all the infections and diseases, transmission of disease should be blocked by a method that's practical. The center for disease control (CDC), ADA and FDI has advised to treat every patient as a potential source of infection.[97]

The objective of disinfection/sterilization of a denture is to obtain a clean decontaminated prosthesis by destruction of microorganisms. It is desirable that the

process should not involve any physical or chemical changes in the denture. Such changes may include alterations in linear dimensions, flexural strength, surface hardness and / or color etc.

Microbial plaque that accumulates on the tissue surface of dentures is composed of several oral microorganism species including Candida albicans, which is a major causative agent of denture-associated chronic atrophic candidiasis. A recent study demonstrated that denture plaque control is necessary for the prevention of denture stomatitis associated with candida albicans.[98] In addition, the tissue surfaces of dentures have been shown to be reservoirs of C. albicans, which is associated with stomatitis and disseminated fungal infectious diseases, and that elderly hospital patients have a high risk of aspiration pneumonia induced by various microorganisms on dentures.[99, 100]

Denture induced candidiasis is the most common disease in elderly denture wearers with a prevalence of 45-70 %.[101, 102] It is a multifactorial disease and Candida Albicans is the principal causative agent. Many therapeutic modalities are available ranging from denture disinfection to systemic antifungal therapy.[103] Despite this, the recurrence rate of denture induced candidiasis is high.[104, 105] It has been suggested that this is due to poor access of the antifungals onto the fitting surface, their poor penetration into the microbial biofilm on the porous denture material, as well as their rapid clearance by saliva and tongue movements.[106, 107]

In denture wearers, the occurrence of Candida is so high and the organism can be opportunistic, which is explained by the fact that dentures decrease the flow of oxygen and saliva to the underlying tissue producing a local acidic and anaerobic

microenvironment that favors yeast overgrowth. Additionally, Candida has affinity for the tissue surface of dentures and non-renewing surfaces such as teeth, dental fillings. Surface characteristics of denture base acrylic resins, such as hydrophobicity, have generally been acknowledged to be one of the factors contributing to the adhesion, which is a critical step in biofilm formation. Poor oral hygiene, practices such as failure to remove the denture while sleeping and poor denture cleansing allows the accumulation of biofilm, which is defined as structured microbial community that is attached to a surface, consisting of more than 10^{11} microorganisms per gram of dry weight and surrounded by a self-produced extracellular matrix.[108]

Candida biofilm formation on denture acrylic resin surfaces has been investigated and described previously. In vitro, attached budding yeast cells (2-4 hours) begin to filament after 4 hours and form pseudo and true hyphae until after 8 hours; neighboring cells and their filaments become entwined and form spatially organized woven structures. After 24 to 48 hours of undisturbed growth, the Candida biofilms increase in complexity, which consists of several different layers and all morphologic fungal forms. Candida hyphal formation also has been suggested as being important for the invasion of the host epithelium which allows dissemination of the organism and aids infection.[109]

Being the 'disease of the diseased', oral candidiasis predominantly affects immunosuppressed and medically compromised patients.[110] Oral candidiasis is often restricted to local mucosa reaction, however, immuno-compromised subjects may present with an aggravated systemic infection known as candidaemia, which can cause a 40% mortality rate or longer hospitalization time, leading to a higher cost to

the health system.[56] Oral candidiasis has become a significant challenge in patients with persisting risk factors and a recurrent need for antifungal treatment.[111] In particular, repeated courses of fluconazole have been shown to form a risk for persistent colonization with microbiologically and clinically resistant Candida. Oral candidiasis is a mixed biofilm infection which provides further challenges for its management.[112] Denture cleansing is essential to maintain the service ability of the denture, because of aesthetic concerns and for prevention of denture related stomatitis. Adequate denture hygiene is believed to be the most effective preventive and curative treatment for the pathogens.[113]

Several denture-cleaning procedures are used clinically for the reduction of denture plaque, stains and debris, and these are generally divided into physical, mechanical and chemical cleaning methods. However, it has been stated that mechanical cleaning methods are not sufficient for a complete reduction of microorganisms on denture plates. Mechanical methods have been used in combination with agitators, magnetic stirrers, sonic vibrators, and ultrasonic devices. However, these methods have not been shown to disinfect dentures efficiently. Thus, it is considered that chemical cleaning methods are more effective and crucial for daily denture care, 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.[15]

The significant reduction in the number of microorganisms observed by **Chetan et al**[9] suggests that the use of chemical cleansers is a suitable method for

cleaning dentures in geriatric patients. but their volatile ingredients and byproducts are toxic and harmful to oral mucosa and supporting tissues. This conclusion is supported by the finding of **Dills et al**[114] that brushing alone with a denture abrasive was less effective than chemical cleanser used for maintaining good denture hygiene.

Jagger et al[115] found in their survey that although brushing is the most widely used methods of denture hygiene, with increasing age of the patients the motor coordination is limited and patients may find it difficult to perform the daily hygiene routine effectively.

The efficacy of the hypochlorite is an important result considering the concentration employed, that presents antimicrobial action and can minimize adverse effects on materials.[116] Previous studies have shown the superiority of hypochlorite for removing biofilm when compared to peroxide, even in overnight immersions; however, the solutions were used at higher concentrations. Alkaline peroxide, as an oxidant product, may affect vital areas of the cell and generate in situ hydroxyl radicals, which exert a more specific action against anaerobes. In turn, hypochlorite ions have broader action, and therefore greater efficacy, thus explaining differences in removal of biofilm.[117] Sodium hypochlorite is an oxidizing agent and thereby destroys the cellular activity of proteins. Hypochlorite agents dissolve organic material, calculus and mucin, disinfect dentures and are good for stain removal. It acts directly on the organic matrix of the plaque, resulting in the dissolution of the polymer's structure, probably because of oxidation of the protein component or significantly reducing the adhesion of most Candida species to the oral epithelial cells. The antimicrobial activity of sodium hypochlorite is based on its higher pH >11.[118]

Schwartz [119] stated that a solution with a pH between 7 and 11 reduces the majority of microorganisms within an immersion time of 10 mins. The high pH of sodium hypochlorite compromises cytoplasmic membrane integrity with irreversible enzymatic inhibition, biosynthetic alterations in cell metabolism, and phospholipid destruction observed in lipidic peroxidation. Deviations in the pH of sodium hypochlorite may lead to changes in environmental conditions which, in turn, affect microbial metabolism as well as the surface properties of the microorganisms and solid surfaces. Thus, it is able to increase the electrostatic repulsion between the two units, thus disturbing the adhesion of microorganisms to the surfaces. The inhibitory effect of sodium hypochlorite maybe associated with non-specific interactions, such as surface hydrophobicity, involved in microbial adhesion to inert surface.[120] These characteristics allow the hypochlorite to reduce Candida sp. adhesive ability but it does not work as an anti-invasive barrier, as it is not able to prevent the production of proteinases by the Candida species. These findings show that sodium hypochlorite acts as an anti-fungi agent if used as a solution to submerge dentures in cases of denture stomatitis, even in short term immersions.[121]

A. C. Pavarina[66] investigated and evaluated the effectiveness of an infection control protocol for cleansing and disinfecting removable dental prostheses. It was concluded that, 1% sodium hypochlorite were proved to be effective in reducing the growth of the microorganisms in the 10 mins immersion period and the protocol evaluated in this study seems to be a viable method to prevent cross-contamination between dental practitioner and patients.

Francine Cristina da Silvaet al[69] performed a similar study in which they evaluated the effectiveness of disinfectant solutions (1% sodium hypochlorite, 2% chlorhexidine digluconate, 2% glutaraldehyde, 100% vinegar, tabs of sodium perborate-based denture cleanser, and 3.8% sodium perborate) in the disinfection of acrylic resin specimens (n = 10/group) contaminated in vitro by Candida albicans, Streptococcus mutans, S. aureus, Escherichia coli, or Bacillus subtilis as measured by residual colony-forming unit (CFU). The results showed that 1% sodium hypochlorite, 2% glutaraldehyde, and 2% chlorhexidine digluconate were most effective against the analyzed microorganisms, followed by 100% vinegar, 3.8% sodium perborate, and tabs of sodium perborate-based denture cleanser. Superficial roughness of the specimens was higher after disinfection cycles with 3.8% sodium perborate ($p = 0.03$) and lower after the cycles with 2% chlorhexidine digluconate ($p = 0.04$).

Iara Augusta Orsiet al[70] evaluated the efficacy of disinfectants on the internal aspect of heat-polymerised acrylic resin contaminated with different microbial strains. It was concluded that disinfection with 1% sodium hypochlorite for 10 mins is effective in disinfecting the internal aspect of heat-polymerized acrylic resin.

Hence, in this study immersion of acrylic resin samples in 1% sodium hypochlorite for 10 mins was used which showed similar results as seen in the above studies. It was seen that the mean cfu x 10^2/ml was 0.32 ± 0.12 **(Table 1)** which was the least as compared to the other groups. When compared to the control group the difference was highly significant ($p=0.001$)

Ozone

Ozone is a natural gaseous molecule made up of three oxygen atoms and molecular weight is 47.98g/mol. Ozone therapy is defined as a versatile bio-oxidative therapy in which oxygen is administered via gas or dissolved in water or oil base to obtain therapeutic benefits. Ozone, which is used for medical purposes, is a gas mixture comprised of 95 to 99.95% oxygen and 0.05 to 5% pure ozone. Many fields in dentistry could benefit from ozone therapy due to proven therapeutic advantages of ozone. Ozone has been used in dental treatment for over 3½ years by 4,000 dentists without any reports of side effects. In addition, in clinical studies in over 2,000 patients, no adverse effects have been reported.[122]

The word ozone originated from the Greek word ozein which means odor and was first used by German chemist Christian Friedrich Schonbein known as Father of ozone therapy (1799-1868) in 1840. In 1896, Nikola Tesla patented the first ozone generator in the U.S. During World War I Ozone gas was used for treating gaseous post-traumatic gangrene, infected wounds, burns and fistulas in German soldiers. Ozone therapy was accepted as an alternative medicine in the U.S.A. from 1880. German dental physician E.A. Fisch (1889-1966) was the first dentist who uses ozone in his dental practice.[122]

There are four different systems for generating ozone gas[122]:

I. **Ultraviolet system:** Produces low concentrations of ozone. It is used in esthetics, saunas, and for air purification.

II. **Corona Discharge system:** Produces high concentrations of ozone. It is the most common system used in the medical/dental field. Many room air purifiers frequently uses this method.

III. **Cold plasma system:** Used in air and water purification.

IV. **Electromagnetic:** This method used quartz glass tubes through which oxygen flows, with copper wire wound around the inner and outer tubes. A high frequency voltage is passed through the coils, producing a strong electromagnetic field.

Anti-microbial action[123]: Ozone is responsible for cell death which is non-specific but selective for microbial cells and does not cause damage to human body cells because of its major antioxidative property. It causes local damage of cytoplasmic membrane by ozonolysis of dual bonds and ozone-induced modification of intracellular contents which includes oxidation of proteins and loss of organelle functions due to secondary oxidant effects. Ozone is very effective against antibiotic restraint strains. Moreover, in viral infections, its action lies in intolerance of the infected cells to peroxides by reacting with the unsaturated fatty acids of lipid layer in cell membranes and change of activity of reverse transcriptase that takes part in synthesis of viral proteins.

Ozonated water is a powerful antimicrobial agent against bacteria, fungi, protozoa, and viruses. ozone, in the gaseous or aqueous phase, can kill bacteria, viruses and fungi. The efficacy of disinfectants is usually evaluated on the basis of a reduction in cultivable microorganisms. Ozonated water (0.5–4 mg/l) was highly effective in killing both gram positive and gram negative oral microorganisms.

Among them, the gram negative bacteria, such as the endodontopathic bacterium P. endodontalis and the periodontopathic bacterium P. gingivalis, were substantially more sensitive to ozonated water than the gram-positive oral streptococci and C. albicans in pure culture. The advantages of ozone in the aqueous phase are its potency, ease of handling, rapid microbicidal effects, lack of mutagenicity and suitability for use as a soaking solution for medical and dental instruments. Scanning electron microscopic analysis revealed absence of viable C. albicans cells remained on acrylic resin plates treated with flowing ozonated water or after immersion in ozonated water with ultra-sonication. C. albicans is detached from the resin plate through functional and structural disturbances in the cytoplasmic membranes. Neo Ozone Water-S apparatus is able to supply a large enough dose of ozonated water in a continual flow and should be useful for cleaning and disinfecting C. albicans adhering denture plates.[124]

Membrane permeability is a key element to cell viability, and the changes in permeability involve the loss of several vital processes linked to the cytoplasmic membrane. It is generally accepted that oxidation due to ozone induces the destruction of cell walls and cytoplasmic membranes of microorganisms and that differences in the sensitivity to ozonated water are probably due to differences in the structure of the cell walls of microorganisms. After the membrane is damaged by oxidation, the permeability of the membrane increases, and ozone molecules can readily enter the cells. A scanning electron microscopic analysis revealed some holes in the membrane when S. mutans cells were treated with ozonated water. Ozonated water had strong bactericidal activity against bacteria in plaque biofilm. In addition, ozonated water

inhibited the accumulation of experimental dental plaque in vitro. The use of ozone as denture cleaner is effective against methicillin resistant S. aureus and viruses.[124]

Huth et al[34] in their study declared that the aqueous form of ozone, as a potential antiseptic agent, showed less cytotoxicity than gaseous ozone or established antimicrobials (chlorhexidine digluconate-CHX 2% / 0.2%, sodium hypochlorite-NaOCl 5.25%, 2.25%; hydrogen peroxide-H2O2 3%) under most conditions. Therefore, aqueous ozone fulfills optimal cell biological characteristics in terms of biocompatibility for oral application.

Nagayoshi et al[30] tested the efficacy of ozonated water on survival and permeability of oral micro-organisms and dental plaque found that ozonated water had a rapid antimicrobial effect on oral microorganisms in pure culture, and that an ozone concentration of 2–4 mg/l was needed to kill the cells. The results of this study showed that there were no significant differences in microbicidal activity between ozonated water and povidone iodine in pure cultures of S. mutans and C. albicans. He also found that ozonated water with antiplaque activity might be effective as a disinfectant solution for dental instruments and removable dentures. Although rapid degradation is one of the major environmental advantages of ozonated water, this also produces a rapid reduction in microbicidal activity.

Arita et al[15] evaluated microbicidal efficacy of ozonated water against Candida albicans adhering to acrylic resin denture plates. The heat-cured acrylic resins were cultured with C. albicans. After treatment of flowing ozonated water with and without ultrasonication, the number of attached C. albicans was counted. It was seen that after exposure to flowing ozonated water (2 or 4 mg/l) for 30 mins, viable C.

albicans cells were nearly nonexistent. There were no significant differences in antimicrobial effectiveness against C. albicans between plates immersed in ozonated water and those treated with commercially available denture cleaners. In addition, electron microscopic analysis revealed that small amounts of C. albicans remained on the plate after exposure to flowing ozonated water or immersion in ozonated water with ultrasonication. Thus, it was concluded that application of ozonated water may be useful in reducing the number of C. albicans on denture plates.

Similar results were found in this study in accordance with the above studies with 4mg/l of aqueous ozone for 30mins. The results of this study showed that the mean CFU x 10^2/ml was 0.63 ± 0.14 (table 1) which was significant reduction in colonies as compared to the control group in which the mean CFU x 10^2/ml was 308.40 ± 29.39. Thus, the difference was highly significant (p=0.001). (Table 3)

Microwave Irradiation

Over an era of use of dentures, denture base materials may be colonized and deeply infected by microorganisms; such contaminated prostheses can provide a source of cross-contamination between patients and dental personnel. The microwave disinfection procedure was proposed to prohibit this cross contamination.

Rohrer and Bulard (1985)[125] were first to introduce microwave irradiation over the chemicals, as an effective, easy and inexpensive disinfection procedure. Microwaves are radio frequency waves, generated by a magnetron, that are close in frequency to television transmissions and aircraft radar. Most microwave ovens operate at 2,450 MHz with a power output range 400-1300 watts. Microwave fields have different properties when they contain certain type of materials. Metals are

microwaves reflective and do not heat. Some materials such as denture base acrylic resins are transparent to microwaves; they neither absorb nor reflect microwave fields nor do they heat. Materials such as water are microwave absorbent and heat within a microwave field.

Vela and Wu (1979)[126] studied the mechanism of microwaves by exposing various bacteria and fungi to microwaves of 2,450 MHz in the presence and in the absence of water. It was found that microorganisms were inactivated only in the presence of water and that dry or lyophilized microorganisms were not affected even by extended exposures.

Donna L. Dixon (1999)[47] performed a study where denture base resin contaminated with C. albicans were sterilized, when samples were immersed in water at the time of submission of microwave irradiation. However, when non-immersed samples were submitted to microwave irradiation (dry irradiation), sterilization was not achieved.

Rohrer and Bulard[125] showed that dentures contaminated with aerobic and anaerobic bacteria, including spore formers, were sterilized after microwave irradiation for 8 minutes at 720 W.

Webb et al[127] recommended microwave irradiation for 2 minutes at 650 W as a more effective sterilization method for dentures inoculated with *Streptococcus gordonii* than soaking them in sodium hypochlorite.

Nepelenbroek et al[93] demonstrated that acrylic resin specimens contaminated with individual suspensions of three bacteria (P. aeruginosa, S. aureus, B. subtilis)

were sterilized by microwave irradiation (6 minutes/650 W). These studies support that microwave irradiation may present a suitable alternative for disinfecting complete dentures and will possibly help overcome the limitations of current soak treatments; however, it has been observed that microwaving for 6 minutes at 650 W produced deleterious effects on some physical and mechanical properties of acrylic resin specimens.[128, 129] Thus, reduced microwave exposure times should be chosen to produce consistent disinfection without any adverse effect on acrylic resins.

Mima et al[50] observed that specimens of a hard chairside reline resin showed consistent sterilization of C. albicans, P. aeruginosa, S. aureus, and B. subtilis after exposure times shorter than 6 minutes at 650 W. Microwaving for 3 minutes promoted inactivation of all species evaluated. In addition, **Consani RLX et al[130]** showed that microwave disinfection for 3 minutes at 650 W significantly improved denture base adaptation when the traditional flask closure method was used.

Daniela Garcia Ribeiro[131] also confirmed microwave irradiation at 650 W for 3 minutes as a potential treatment to prevent cross-contamination. Thus, the samples were submitted to one microwave irradiation cycle at 650 W for 3 minutes.

Gregory L. Polyzois et al[132] Rafael Leonardo Xediek Consani et al[133] and studied the effect of microwave irradiation on the flexural strength, impact strength and Vickers hardness of denture base acrylic resin. They concluded that, the flexural strength, impact strength and hardness of denture material were not detrimentally affected by microwave disinfection. Thus, microwave irradiation can be an acceptable disinfection procedure for the prostheses, provided it shows consistent results every time it undergoes this procedure. Hence, if the prostheses undergo any modification or

even in routine denture hygiene procedure, they need to undergo disinfection procedure, repeatedly, to restrict cross-contamination.

Hence in this study microwave irradiation at 650 watts for 3mins in water was done. The results of this study were similar to the above-mentioned studies. The mean CFU x 10^2/ml was 1.11 ± 0.21 (table 1) after disinfection of acrylic samples with microwave irradiation, the difference being significant as compared to the control group (p=0.001). (Table 3)

The test samples utilized in this study were prepared of the dimension 15 mm in length, 15 mm in width, and 3 mm in height (15×15×3mm). The resin plates incubated with the fungus solution were stored in sterile petri dishes at room temperature and used for the experiment. Disinfection in all the 4 groups (Group C, Group O, Group M and Group S) was carried out. The plate count method was performed by diluting the original sample in serial dilution tubes, followed by the plating of the prepared serial dilutions onto the Sabouraud's Dextrose agar plates. Cell suspension was adjusted using an automated densitometer (Plate II, Fig.7) to 0.5 McFarland standard, equivalent to an approximate concentration of 1.5x10^8 colony forming units (CFU) per ml. [92, 93]

The results of this study shows that in group C, the mean CFU x 10^2/ml was 308.40 ± 29.39, followed by group O, where the mean CFU x 10^2/ml was 0.63 ± 0.14. For group M, the mean CFU x 10^2/ml was 1.11 ± 0.21 and for group S the mean CFU x 10^2/ml was 0.32 ± 0.12.

After comparison with each other, the results showed: (Depending on the number of formation of colonies)

Group C > Group M > Group O > Group S

Thus, it was concluded that all denture cleansing procedures i.e. disinfection of the samples in 4mg/L aqueous ozone for 30 mins, microwave irradiation at 650 Watts for 3 mins in water and 1% sodium hypochlorite solution for 10 mins showed significant reduction in Candida albicans biofilm as compared to the control group. This difference was statistically significant.

Clinical Significance:

When the entire spectrum of this study is analyzed, it becomes evident that the heat polymerized acrylic dentures disinfected with Sodium hypochlorite, Ozone and Microwave irradiation decreases the Candida albicans biofilm on the samples and thus, reduces the probability of related microbial diseases in the geriatric and handicapped patients to use plaque and candida free dentures regularly. Also, the concentrations of each disinfectant used in this study do not deteriorate the physical properties of the acrylic resin. It thus enhances the patient's perception and improves the adaptability of the patient to the denture. This in turn, aids in better comfort and contentment with the prosthesis in place.

Limitations of the Study:

The study was designed and carried out with utmost accuracy, however certain limitations encountered in the study are enlisted as follows:

1. Although all the samples were visually checked for any irregularities and porosities before testing, micro-porosities, and irregularities present in the samples may play an important role in reducing the efficiency of denture cleansing agents.

2. It is difficult to simulate the oral conditions appropriately as the study was carried out under laboratory conditions.

Scope for Further Studies:

1. Being an in-vitro study, the oral environment was not simulated completely. A clinical trial can be undertaken which would be more significant.

2. Also, the study was performed over a relatively short period of time compared to the average duration of denture use. Investigating the samples in vivo under controlled conditions would provide more appropriate data to support regular maintenance denture hygiene.

3. The antimicrobial effectiveness of denture cleansing agents can be evaluated against other microorganisms present in the oral cavity (Staphylococcus aureus, Streptococcus mutans E.Coli, Klebsiella, Fusobacterium etc.)

SUMMARY

Edentulism is considered a global public health problem that affects mainly elderly people. Adequate replacement of missing teeth is essential to maintain good general health and to improve the quality of life in old age. Conventional complete dentures are still the most common form of oral rehabilitation of edentulous patients, mainly due to their cost effectiveness, aesthetic appeal and easy maintenance. Complete dentures require special hygiene and maintenance care, since poor denture hygiene is associated with the proliferation of microorganisms and oral diseases, such as stomatitis and candidiasis. Denture stomatitis is an inflammatory reaction of oral tissues that are in contact with dentures and presence of intensely red, glistening, and slightly swollen palatal epithelium. Although bacteria or other yeast may be act as pathogens, Candida albicans in the microbial plaque on denture fitting surface is thought to be an important etiologic factor in the pathogenesis of denture-induced stomatitis. Epidemiological studies reported that the prevalence of denture stomatitis

among denture wearers to range from 15% to over 70%.Therefore, denture biofilm control may be a simple and effective method to maintain health. Biofilm control by traditional mechanical methods such as brushing may be influenced by the manual skills of the denture wearers and by their knowledge of how to perform denture hygiene procedures. Chemical cleaning methods may be an excellent complement to mechanical methods, since they reduce the quantity of microorganisms adhering to dentures, compensate possible limitations of brushing, have good acceptance by wearers, and are easy to use. Many products are available for the chemical cleaning of removable dentures, such as alkaline peroxides, alkaline hypochlorites, inorganic acids, disinfectants, and enzymes. The physical technique includes microwave irradiation which is one of the techniques used to disinfect the dentures of patients with candidiasis. Recently, Ozonated water has been proven powerful antimicrobial agent against bacteria, fungi, and viruses. It has also been reported that aqueous ozone can be mutagenic if used for a long period and in high concentrations. Thus, it is important to determine the effective concentrations for cleaning the denture to avoid hazardous side-effects of ozone.

Thus, the purpose of this study was to compare and evaluate the antimicrobial efficacy of aqueous ozone, microwave irradiation and sodium hypochlorite solution on heat polymerized polymethyl methacrylate denture base material against candida albicans.

For the purpose of the investigation, 100 heat polymerized acrylic resin samples of dimension 15 mm in length, 15 mm in width, and 3 mm in height (15×15×3mm) were fabricated. The resin plates were contaminated with the C.

albicans (ATCC 25175) solution at 25° C for 3-5 days without agitation, and then washed 3 times with sterile saline to remove loosely attached micro-organisms.

The samples were then subjected to different denture cleansing procedures. These were

1. **Group C: -** Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material without subjecting to any disinfection method and left dry at room temperature.

2. **Group O: -** Disinfection of heat polymerized polymethyl methacrylate denture base material in 4mg/L aqueous ozone solution for 30 mins.

3. **Group M: -** Disinfection of heat polymerized polymethyl methacrylate denture base material with microwave irradiation at 650 Watts for 3 mins immersed in water.

4. **Group S: -** Disinfection of heat polymerized polymethyl methacrylate denture base material in 1% sodium hypochlorite solution for 10 mins.

The plate count method was performed by diluting the original sample in serial dilution tubes, followed by the plating of the prepared serial dilutions onto the Sabouraud's Dextrose agar plates. Samples were added in test tubes and incubated at 37°C for 24 hours. After 24 hours, samples with the colonies of Candida albicans were removed from the test tube and dispersed in a new test tube containing 2 ml of saline solution. This tube was vortexed vigorously for even dispersion of colonies in a single test tube. The cell suspension was adjusted using an automated densitometer to

0.5 McFarland standard, equivalent to an approximate concentration of $1.5x10^8$ colony forming units (CFU) per ml.

The data was analyzed statistically using ANOVA followed by Tukey's post hoc test at a significance level of 5%.

Results showed that in group C, the mean CFU x 10^2/ml was 308.40 ± 29.39; in group O the mean CFU x 10^2/ml was 0.63 ± 0.14; in group M the mean CFU x 10^2/ml was 1.11 ± 0.21 and in group S the mean CFU x 10^2/ml was 0.32 ± 0.12.

There was a significant difference in CFU x 10^2/ml among the group C with group O, group M and group S (p=0.001). Difference in CFU x 10^2/ml among group O and group M was -0.48 which was non-significant (p= 0.999). Difference in CFU x 10^2/ml among group O and group S was 0.31 which was non-significant (p= 1.000). Difference in CFU x 10^2/ml among group M and group S was 0.0.79 which was non-significant (p=0.998).

Thus, the study results concluded that disinfection of acrylic resin with all denture cleansing procedures i.e. disinfection of the samples in 4mg/L aqueous ozone for 30 mins, microwave irradiation at 650 Watts for 3 mins in water and 1% sodium hypochlorite solution for 10 mins showed significant reduction in Candida albicans biofilm as compared to the control group. This difference was statistically significant.

CONCLUSION

Within the limitations of this in-vitro study following conclusions were drawn.

1. All Denture cleansing procedures i.e. disinfection of the samples in 4mg/L aqueous ozone for 30 mins, microwave irradiation at 650 Watts for 3 mins in water and 1% sodium hypochlorite solution for 10 mins showed significant reduction in Candida albicans biofilm as compared to the control group. This difference was statistically significant.

2. Amongst the three denture cleansing procedure, the most efficient denture cleansing procedure was immersion in 1% sodium hypochlorite solution for 10 mins followed by immersion 4mg/L aqueous ozone for 30 mins and microwave irradiation at 650 Watts for 3 mins. This difference was statistically non-significant.

3. Aqueous ozone can be used as a routine denture cleansing agent as compared to microwave irradiation and sodium hypochlorite solution.

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TABLES

Table 1: Descriptive statistics of mean colony forming units (Mean CFU × 10^2/ml) in four treatment groups

Groups	Mean	S.D.	Median	Minimum	Maximum
C	308.40	29.39	310.00	260.00	370.00
O	0.63	0.14	0.60	0.40	0.90
M	1.11	0.21	1.10	0.70	1.50
S	0.32	0.12	0.30	0.15	0.60

C: - Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material without any disinfection method and left dry at room temperature.

O: - Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material in 4mg/L aqueous ozone solution for 30 mins.

M: - Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material with microwave irradiation at 650 Watts for 3 mins immersed in water.

S: - Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material immersed in 1% sodium hypochlorite solution for 10 mins.

Table 2: Comparison of mean colony forming units (Mean CFU × 10^2/ml) among study groups

	Sum of Squares	df	Mean Square	F	p value
Between Groups	1775413.812	3	591804.604	2739.588	0.001*
Within Groups	20737.882	96	216.020		
Total	1796151.693	99			

One way ANOVA test; * indicates significant difference at $p \leq 0.05$

Table 3: Pair wise comparison of mean colony forming units (Mean CFU × 10^2/ml) among study groups

Comparison	Difference	p value
Group C vs. Group O	307.77	0.001*
Group C vs. Group M	307.29	0.001*
Group C vs. Group S	308.08	0.001*
Group O vs. Group M	-0.48	0.999 (NS)
Group O vs. Group S	0.31	1.000 (NS)
Group M vs. Group S	0.79	0.998 (NS)

Tukey's Post hoc test; * indicates significant difference at $p \leq 0.05$; NS: Non-significant

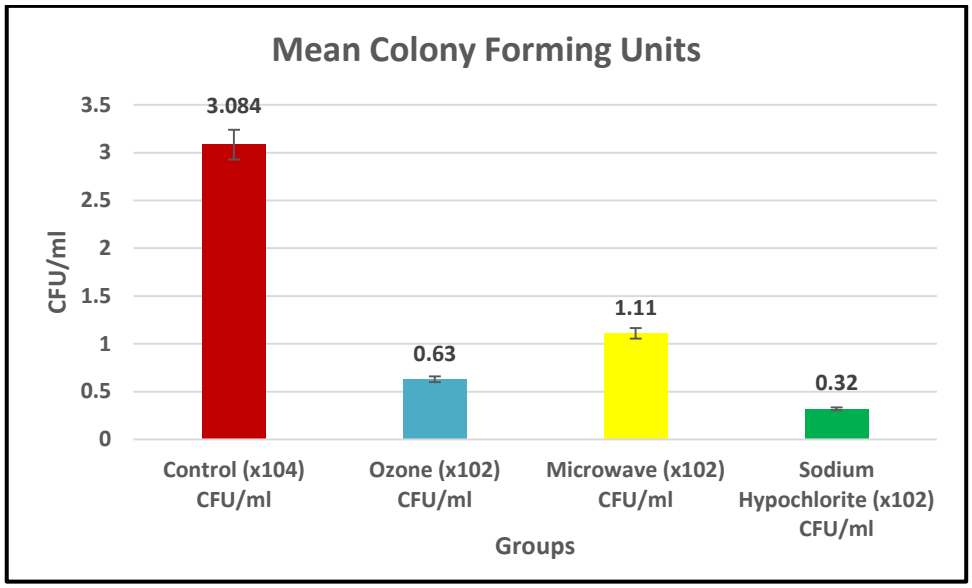
C: - Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material without any disinfection method and left dry at room temperature.

O: - Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material in 4mg/L aqueous ozone solution for 30 mins.

M: - Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material with microwave irradiation at 650 Watts for 3 mins immersed in water.

S: - Strains of C. albicans on heat polymerized polymethyl methacrylate denture base material immersed in 1% sodium hypochlorite solution for 10 mins.

GRAPH



Column chart with error bars showing mean colony forming units (Mean CFU × 10^2/ml) in four treatment groups

ANNEXURE

MASTER CHART

Colony forming units per ml (CFU/ml) after disinfection of heat cure acrylic resin denture base samples.

Sr. No	Group C (n=25) (Control): Heat polymerized Acrylic Resin (PMMA) without any disinfection	Group O (n=25) (Ozone): Heat polymerized Acrylic Resin (PMMA) material disinfected with 4mg/L aqueous ozone solution for 30 mins.	Group M (n=25) (Microwave): Heat polymerized Acrylic Resin (PMMA) material disinfected with microwave irradiation at 650 W for 3 mins immersed in water.	Group S (n=25) (Sodium Hypochlorite): Heat polymerized Acrylic Resin (PMMA) material disinfected with 1% sodium hypochlorite solution for 10 mins.
1.	$3.1X\ 10^4$ cfu/ml	$1.1X10^2$ cfu/ml	$0.8X10^2$ cfu/ml	$0.2X10^2$ cfu/ml
2.	$2.9X10^4$ cfu/ml	$1.4X10^2$ cfu/ml	$0.5X10^2$ cfu/ml	$0.4X10^2$ cfu/ml
3.	$3.5X10^4$ cfu/ml	$1.2X10^2$ cfu/ml	$0.5X10^2$ cfu/ml	$0.2X10^2$ cfu/ml
4.	$3.3X10^4$ cfu/ml	$1.1X10^2$ cfu/ml	$0.7X10^2$ cfu/ml	$0.3X10^2$ cfu/ml
5.	$2.8X10^4$ cfu/ml	$1.3X10^2$ cfu/ml	$0.5X10^2$ cfu/ml	$0.5X10^2$ cfu/ml
6.	$2.9X10^4$ cfu/ml	$1.0X10^2$ cfu/ml	$0.6X10^2$ cfu/ml	$0.3X10^2$ cfu/ml
7.	$3.2X10^4$ cfu/ml	$0.8X10^2$ cfu/ml	$0.7X10^2$ cfu/ml	$0.5X10^2$ cfu/ml
8.	$3.4X10^4$ cfu/ml	$1.1X10^2$ cfu/ml	$0.5X10^2$ cfu/ml	$0.4X10^2$ cfu/ml
9.	$2.8X10^4$ cfu/ml	$1.3X10^2$ cfu/ml	$0.8X10^2$ cfu/ml	$0.15X10^2$ cfu/ml
10.	$3.1X10^4$ cfu/ml	$0.9X10^2$ cfu/ml	$0.9X10^2$ cfu/ml	$0.27X10^2$ cfu/ml
11.	$2.6X10^4$ cfu/ml	$0.8X10^2$ cfu/ml	$0.5X10^2$ cfu/ml	$0.24X10^2$ cfu/ml
12.	$2.9X10^4$ cfu/ml	$1.1X10^2$ cfu/ml	$0.7X10^2$ cfu/ml	$0.6X10^2$ cfu/ml
13.	$3.2X10^4$ cfu/ml	$1.0X10^2$ cfu/ml	$0.5X10^2$ cfu/ml	$0.3X10^2$ cfu/ml
14.	$2.8X10^4$ cfu/ml	$1.4X10^2$ cfu/ml	$0.8X10^2$ cfu/ml	$0.2X10^2$ cfu/ml
15.	$2.7X10^4$ cfu/ml	$0.7X10^2$ cfu/ml	$0.6X10^2$ cfu/ml	$0.26X10^2$ cfu/ml

16.	$3.1X10^4$ cfu/ml	$1.2X10^2$ cfu/ml	$0.8X10^2$ cfu/ml	$0.35X10^2$ cfu/ml
17.	$3.4X10^4$ cfu/ml	$1.2X10^2$ cfu/ml	$0.7X10^2$ cfu/ml	$0.41X10^2$ cfu/ml
18.	$2.9X10^4$ cfu/ml	$1.5X10^2$ cfu/ml	$0.4X10^2$ cfu/ml	$0.2X10^2$ cfu/ml
19.	$3.2X10^4$ cfu/ml	$1.2X10^2$ cfu/ml	$0.6X10^2$ cfu/ml	$0.22X10^2$ cfu/ml
20.	$3.5X10^4$ cfu/ml	$1.3X10^2$ cfu/ml	$0.6X10^2$ cfu/ml	$0.4X10^2$ cfu/ml
21.	$3.2X10^4$ cfu/ml	$1.1X10^2$ cfu/ml	$0.7X10^2$ cfu/ml	$0.34X10^2$ cfu/ml
22.	$2.6X10^4$ cfu/ml	$1.2X10^2$ cfu/ml	$0.6X10^2$ cfu/ml	$0.5X10^2$ cfu/ml
23.	$3.3X10^4$ cfu/ml	$0.7X10^2$ cfu/ml	$0.8X10^2$ cfu/ml	$0.2X10^2$ cfu/ml
24.	$3.7X10^4$ cfu/ml	$1.1X10^2$ cfu/ml	$0.4X10^2$ cfu/ml	$0.3X10^2$ cfu/ml
25.	$3.0X10^4$ cfu/ml	$1.0X10^2$ cfu/ml	$0.5X10^2$ cfu/ml	$0.26X10^2$ cfu/ml