A COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF VARIOUS DISINFECTANTS ON DIFFERENT DENTURE BASE MATERIALS

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Abstract

Aims: This in vitro study was conducted to evaluate and compare the antimicrobial efficacy of various disinfectants on different denture base materials.
Settings and Design: Invitro analytical study
Methods and Material: 64 specimens of PMMA, Polyamide, Cobalt chromium and PEEK were fabricated (n=16). Three denture cleansers were evaluated i.e sodium perborate, thyme oil and ozonated water. Distilled water served as control group. The contamination of specimens was done by Candida albicans. The contaminated specimens were disinfected by denture cleansers and cultured for candida albicans using Hi Chrome culture media. The colonies of candida albicans grown on Hi Chrome agar were counted. Data was tabulated and subjected to statistical analysis.
Statistical analysis used: Two-way analysis of variance (ANOVA) of mean values was performed followed by Tukey's HSD and 't' test for Post hoc analysis for pair wise comparison. The level of significance was set at $p \leq 0.05$.
Results: Among the denture base materials, PEEK exhibited least number of colonies of candida albicans, followed by cobalt chromium, polyamide and PMMA. Among the various denture cleansers, sodium perborate exhibited least number of colonies of candida albicans followed by ozonated water, thyme oil and distilled water. Sodium perborate and ozonated water found to be equally effective in reducing number of candida albicans colonies (statistically non-significant). Thyme oil reduced the number of colonies of candida albicans on cobalt chromium and PEEK while it was not effective on PMMA and polyamide.
Conclusions: Among the denture cleansers, sodium perborate and ozonated water found to be equally effective in reducing number of candida albicans colonies in all denture base materials. Thyme oil reduced the number of colonies on cobalt chromium and PEEK while it was less effective on PMMA and polyamide. Choice of denture cleansers must be based on type of materials, mode of action of denture cleansers and interaction of denture cleansers and denture base materials between them.

Keywords: PMMA, Polyamide, Cobalt chromium, PEEK, Denture cleansers, sodium perborate, thyme oil, ozonated water.

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1. Introduction

Removable dental prosthesis is one of the most common prosthetic restorations as it can be conveniently removed by the patients when not in use and replaced whenever necessary. Dentures are the most common removable dental prosthesis in dental practise and are, by far, the most affordable and common way that most patients choose to that most that most patients choose to replace their missing teeth. The reason behind this is that despite of all the other advancements in dental technology, removable prosthesis is the choice of treatment in patients with multiple local and systemic compromises.[1] Commonly used denture base materials are Polymethyl methacrylate (PMMA), Polyamide and Cobalt chromium. Polymethyl methacrylate is the material of choice for the removable prosthesis because it can meet the specifications required as a prosthetic base material.

It has important advantages such as having sufficient resistance to chewing forces, low-solubility characteristics in liquids, ease of application and repair an d it offers low cost and good physicochemical properties and acceptable aesthetics. However, low-impact stability and fatigue resistance including cause of allergic reactions in some patients are factors that lead to choosing an alternative base material. [2] Polyamide, also known as nylon, is a thermoplastic polymer. The flexibility, low density, impact resistance and the lack of cytotoxic effects are factors that increased its use as a denture base material. The absence of residual monomer s led to its preference for patients with allergies against acrylic resins.[2] Cobalt chromium is a metal denture base material. It is rigid even in a very thin section. It has various advantages like mandibular dentures are heavier, so the retention and stability are improved, improved thermal conductivity gives good sensory interpretation. However, it is more expensive, requires more time for fabrication, requires refractory cast material, difficult to fabricate and cannot be rebased.[2-3] In the past few years, a new dental polymer called polyetheretherketone (PEEK) which is a polycyclic, aromatic, thermoplastic material that is semi-crystalline and has a linear structure has made its place in denture materials. It has advantages like low elastic modulus, very light material with a low density, white, radiolucent, light weight with low density, rigid material with great thermal stability, non-allergic and has low plaque affinity.[4] The presence of a removable prosthesis in the oral cavity may result in accumulation of microbial plaque around and under the denture. This may induce certain pathological mucosal reactions like denture induced stomatitis, and angular cheilitis.[5] Various agents used for denture cleaning and disinfection include chemical agents, essential oils and Ozonized water. Sodium perborate is commonly used denture cleanser which is a peroxide type of denture cleanser. Essential oil like thyme oil is aromatic oily liquids obtained from plant materials such as flowers, leaves, herbs. The mechanism behind antimicrobial activity includes permeability and depolarization of the cytoplasmic membrane. Ozone, in the gaseous or aqueous phase, can also be used as denture cleansers. Ozone has a rapid and robust effect against bacteria through oxidizing cell walls and cytoplasmic membranes. The advantages of ozone in the aqueous phase are its potency, ease of handling, lack of mutagenicity, rapid microbicidal effects and suitability for use as a soaking solution.[6-9] There is inadequate research on comparative effects of these denture cleansers on different denture base materials. Therefore, the present study was taken up to evaluate and compare antimicrobial efficacy of various denture

cleansers on different denture base materials against Candida albicans.

2. Material & Methodology

Custom made metal discs (10mm in diameter and 3mm in thickness) were used to make the test specimens. 16 specimens of conventional heat cure acrylic resin (Group I) (Trevalon, Dentsply India Pvt. Ltd) were fabricated by compression moulding technique.

For fabricating specimens of (Group II) Polyamide denture base materials (Lucitone, Dentsply India Pvt. Ltd) injection molding system was used. 16 specimens of Polyamide denture base material were fabricated. Preparation of specimens for (Group III) Cobalt chromium (Wironit, Bego Germany) denture base materials was done by using conventional casting method. 16 specimens of PEEK (Group IV) (Arum PEEK Medium, Langeheide, Germany) were fabricated from pre-polymerized blocks of PEEK by computer aided milling.

All the specimens were sterilized in an autoclave at 120º C before implementation of the experiments. All specimens from each group were contaminated by Candida albicans. Freeze dried strains of Candida albicans (MTCC 227) were cultured on Hi Chrome agar at 37^0C for 48 hours in aerobic conditions. A stock solution adjusted to 0.5 McFarland scale was prepared from fresh Candida albicans colonies in tryptic soy broth. (Figure1A). 1ml stock suspension was added to each sterile glass tube containing 1ml of tryptic soy broth. The mixture was homogenized by vortexing. Then all specimens of each group were placed into separate glass tubes, ensuring they were completely immersed in Candida albicans suspension (Figure1B). The glass tubes containing specimens and Candida albicans suspensions were incubated at 37^0C for 48 hours. After incubation, broth media were discarded from the glass tubes and specimens were washed with sterile distilled water. Then, wash solutions were discarded from the tubes and were employed for disinfection. Specimens from the broth were disinfected by immersion procedure using different denture cleansers 3.8 % sodium perborate (Subgroup A), thyme oil (Subgroup B), ozonated water (Subgroup C) and Distilled water as control (Subgroup D). Denture disinfectant solutions were prepared and added into the new sterile glass tubes. 4 specimens each of Group I-IV were immersed in each denture cleansers for 10 min (Figure 2A-D). After 10 minutes, solutions were discarded and specimens were washed with sterile distilled water. After discarding wash solutions from all the tubes, the specimens were transferred into the new glass tubes containing 1 ml of 0.9% saline solutions. After vigorously vortexing, a saline solution from each tube was cultivated on Hi Chrome Agar and incubated at 37^0C for 48 hours. After incubation, the colonies grown on Hi Chrome Agar were counted as CFU/ml according to the dilution ratio (25µl). Colonies of Candida albicans formed in each group in different denture cleansers were counted (Figure 3A-D). Data was tabulated and subjected to statistical analysis for interpretation of results. Mean and standard deviation of number of colonies of Candida albicans of specimens in each group was calculated. Statistical analysis for the calculated data was done by two-way analysis of variance (ANOVA) followed by Post hoc Tukey's HSD and 't' test for pair wise comparison of Groups and Subgroups. Level of significance was set at the probability level of $p \leq 0.05$. Conclusion was drawn based on the statistical analysis.

3. Results

Table 1 shows summary of mean of colonies of candida albicans formed on different Denture base materials after disinfecting with different denture cleanser. On subjecting the values to two-way ANOVA for number of colonies of Candida albicans formed in all groups after

disinfecting with different denture cleansers, there was statistically significant difference between Groups (denture base materials), Subgroups (denture cleansers) and in interaction between Groups and Subgroups (denture base materials and denture cleansers). ($p<0.05$)

Table 1: Mean number of colonies of Candida albicans formed on test denture base materials after disinfecting with different denture cleansers

Groups	**Mean number of colonies of Candida albicans**				
	Sub Group A	**Sub Group B**	**Sub Group C**	**Sub Group D**	**Mean number of colonies in each denture base material**
I	**5.250 (±2.2175)^{a}**	**75025 (±49950)^{b}**	**68.00 (±34.098)^{c}**	**100000.00 (±0.000)^{b}**	**43774.56 (±12496.57)^{A}**
II	**17.750 (± 14.385)^{a}**	**25031.00 (±49950.00)^{a}**	**26.250(± 31.941)^{a}**	**100000.00 (±0.000)^{b}**	**31268.75 (±12499.08)^{A,B}**
III	**8.000 (±9.238)^{a}**	**37.750 (±11.786)^{a}**	**16.500 (±15.155)^{a}**	**100000.00 (±0.000)^{b}**	**25015.56 (±9.04)^{B}**
IV	**3.750 (±5.560)^{a}**	**42.25 (±48.197)^{a}**	**13.5(± 17.748)^{a}**	**100000.00 (±0.000)^{b}**	**25014.87 (±17.87)^{B}**
Mean number of colonies in each denture cleanser	**8.687 (±7.84)^{1}**	**25034.9 (±24989.9)^{2}**	**31.06 (±24.73)^{1}**	**100000.00 (±0.000)^{3}**	

Figure with different lowercase superscript indicate statistically significant difference between subgroup of all test denture base material.

Figure with different uppercase superscript indicate statistically significant difference between test denture base material. Figure with different numerical superscript indicate statistically significant difference between different denture cleansers

It was found that For Group I (PMMA) least number of colonies were seen with Subgroup A followed by Subgroup C, Subgroup B and Subgroup D respectively. There was non-significant difference in number of colonies of Candida albicans found between Subgroup B and D ($p>0.05$); between remaining all Subgroups significant difference were found ($p<0.05$). respectively. For Polyamide least number of colonies were seen with Subgroup A followed by Subgroup C, Subgroup B and Subgroup D respectively. There was significant difference found between Subgroup A and D, Subgroup B and D and Subgroup C and D ($p<0.05$) whereas remaining Subgroups non-significant difference were found ($p>0.05$).

For Cobalt chromium least number of colonies were seen with Subgroup A followed by Subgroup C, Subgroup B and Subgroup D respectively. There was non-

significant difference in number of colonies of candida albicans found between Subgroup A, C and D (P>0.05) whereas Subgroup D was significantly different from all other subgroups (p<0.05).

For PEEK least number of colonies were seen with Subgroup A followed by Subgroup C, Subgroup B and Subgroup D respectively. There was non-significant difference in number of colonies of candida albicans found between Subgroup A, C and D (P>0.05) whereas Subgroup D was significantly different from all other subgroups (p<0.05).

After comparison between denture base material immersed in various denture cleansers least number of candida albicans colonies exhibited in PEEK followed by Cobalt chromium, polyamide and PMMA respectively.

Among the denture cleansers least number of colonies found in sodium perborate, followed by ozonated water and thyme oil.

4. Discussion

Removable dental prosthesis is a prosthetic restoration, which compensate missing teeth, soft and hard tissue deficiencies. It is designed to replace the functional and aesthetic defects of patients with edentulism. Generally, dentures are fabricated for geriatric patients. Some young patients who are born with congenitally malformed teeth function or edentulous arches require complete dentures. Correct prosthetic use and daily hygiene are important factors for good oral health, greater longevity of the prosthesis, and health of the supporting tissue. No matter what kind of denture base material we are using, denture care is indispensable for oral health, otherwise denture become unsanitary and undesirable effects are expected like bad breath, unpleasant staining and biofilm, calculus accumulation on the denture which can lead to denture stomatitis, angular cheilitis and poor oral health. Denture stomatitis is a common infection characterized by inflammation of oral tissue and colonization of the intaglio surface of prosthesis by microorganisms. Contamination of a prosthesis can provide a source of cross contamination between patient and dental personnel[10]. Denture disinfection has been recommended as an essential procedure for maintaining adequate denture hygiene. Several agents are indicated for denture disinfection and maintaining the health of the denture, classified into mechanical and chemical agents. The use of a brush with hot or cold water is most commonly used method and the use of toothpaste might scratch and cause irregularities on the surface of the dentures, which further facilitates the adherence of the microflora.[11]

Alternatively, easy to use and efficient methods for cleaning dentures were immersion in chemical solutions. An ideal disinfectant should be readily available, cost effective, have good antibiotic action, should remove inorganic/organic deposits and stain and does not cause dimensional and surface changes in the denture base. 3.8% sodium perborate is the chemical agent used in this study. 3.8% sodium perborate is a white, odourless, economical and versatile water-soluble oxidizing agent which releases hydrogen peroxide, on reacting with water. It has bactericidal, anti-fungicidal effects and effectiveness in denture disinfection, eliminating biofilm and staining of the denture's surface, but it has some disadvantages for clinical use. In addition to its unpleasant odour and taste, the disadvantages of using it even in low concentrations for night time immersion, are possible colour changes, an increase in the roughness of the denture base material, and toxicity.

Due to its antimicrobial potential, plant products present an opportunity for the discovery of new drugs with antimicrobial activity. One such essential oil is thyme oil, it is an aromatic oily liquid obtained from plant materials such as flowers, leaves, herbs with antioxidant and antimicrobial properties against a wide range of pathogens, including Candida albicans and

dermatophytes. Liu et al (2017)[7] had stated that thyme essential oil is the best among plant extracts. Gutierrez et al.[7] had proved that thymol showed complete inhibition of microorganisms such as C. albicans, Aspergillus flavus and Goncalves had concluded that thyme oil was efficient against Streptococcus mutans.[12]

Ozonated water might be considered as a denture-cleaning agent due to its potent antimicrobial efficacy. Ozone is a natural gaseous molecule which is made up of three oxygen atoms. The advantages of ozone in the aqueous phase are its potency, lack of mutagenicity, ease of handling, fast microbicidal effects, and aptness for use as a soaking solution.

The results of the present study revealed that the number of colonies of candida albicans formed in PMMA acrylic resin were least in sodium perborate, followed by ozonated water, thyme oil and distilled water. Distilled water served as control media for the study. Sodium Perborate seems to be a highly effective denture cleanser for PMMA denture base materials. Ozonated water and sodium perborate was equally effective (non-significant difference) this result was in accordance with Yildirim bicer[13]. Budtz Jorgensen reported that the advantages of effervescent products are safe and do not damage acrylic resin even when constantly used. However, there are also contrasting reports in the literature suggesting that alkaline peroxide type denture cleansing agents may not have as much antimicrobial effect as others.

When all the denture base materials were compared for growth of colonies of Candida albicans after disinfecting it was found that least number of colonies seen in PEEK followed by Cobalt chromium, Polyamide and PMMA respectively. This result was in accordance to Aslanimehr[14] et al, they concluded that polyamide denture material has lesser biofilm development and candida count as compared to heat cure acrylic resin. However, this result was in disagreement by Yeliz hayran[15] et al, they found that Candida albicans exhibited higher cell viability in polyamide resin compared to PMMA resin.

Freitis fernandes et al reported that PMMA resin caused increased microbial attachment compared to polyamide resin. This result was disagreed with the study of Abuzer et al, according to them polyamide as a denture base material has a crystalline polymer structure with a low melting temperature and overheating, and the inappropriate rapid cooling process might disrupt the material structure on the other hand PMMA has an amorphous structure and deterioration of the material with heat or other causes is harder than polyamide. Also, the polarity of the resin was reported to affect antimicrobial efficacy, especially in PMMA resin against Candida albicans. Polarity or resin surface charge result from residual monomers in PMMA resin decreases the adhesion of microorganisms and prevent biofilm formation[16].

Among the denture cleansers least number of colonies found in sodium perborate, followed by ozonated water and thyme oil. This result was in agreement with the study Pradeep et al they found that ozonated water were less effective compare to sodium perborate. This result was in agreement with the study Varsha Ravichandran [16] et al found that thyme oil was less effective compare to sodium perborate.

Based on the result of the above study it was found that sodium perborate was the most effective denture cleansers for all material tested. Ozonated water was found to be equally effective to sodium perborate for all denture base materials. Thyme oil was effective for cobalt chromium and PEEK while it exhibited significant growth of colonies on polyamide and PMMA.

5. Conclusion

Within the limitation of the present study, conclusions drawn were that all specimens of all denture base resins disinfected with test denture cleansers exhibited a significant reduction in the number of

colonies of Candida albicans compared to distilled water (control), among the various denture base materials PEEK exhibited growth of least number of Candida albicans colonies followed by cobalt chromium, polyamide and PMMA, and among the denture cleansers sodium perborate exhibited growth of least a number of candida albicans colonies, followed by ozonated water, thyme oil and distilled water.

Results of the study showed that sodium perborate denture cleanser significantly reduced the number of candida albicans colonies on all denture base materials tested, ozonated water denture cleanser significantly reduced the number of candida albicans colonies on all denture base materials tested, similar to sodium perborate and thyme oil denture cleansers significantly reduced the number of candida albicans colonies on cobalt chromium and PEEK while it is not effective on PMMA and polyamide.

Denture cleaning is essential for maintaining the prosthesis and oral health. Daily use of denture cleansers is recommended to prevent microbial colonization on denture and promote good health. Choice of denture cleansers must be based on type of denture base materials, mode of action of denture cleansers and interaction of denture cleansers and denture base materials between them.

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List of legends

Figure 1: (A) Candida Albicans inoculated in tryptic soy broth (B) Transferring of specimens in Candida Albicans suspension

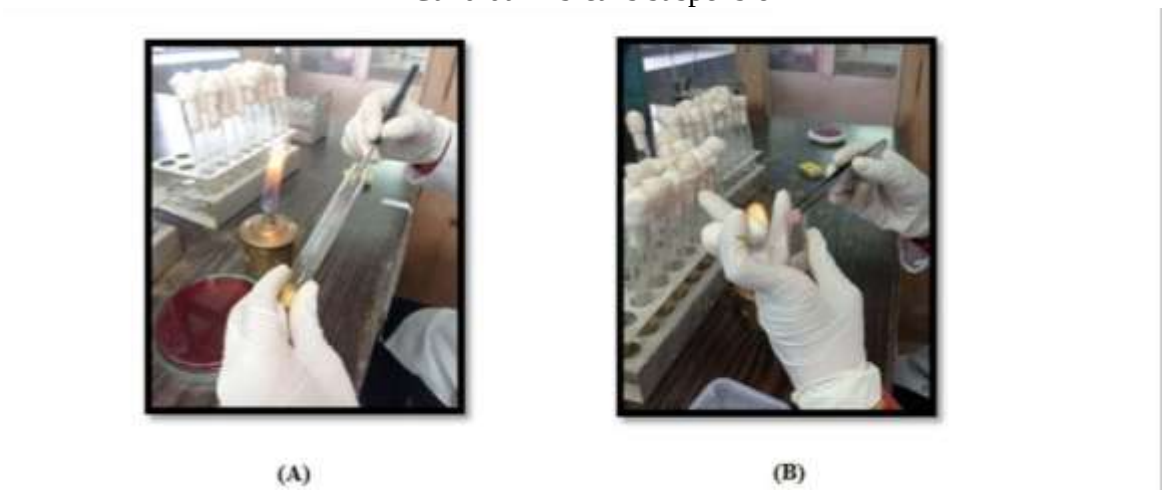

Figure 2: Specimens immersed in denture cleanser (A) Sodium Perborate (B) Thyme Oil (C) Ozonated Water (D) Distilled Water

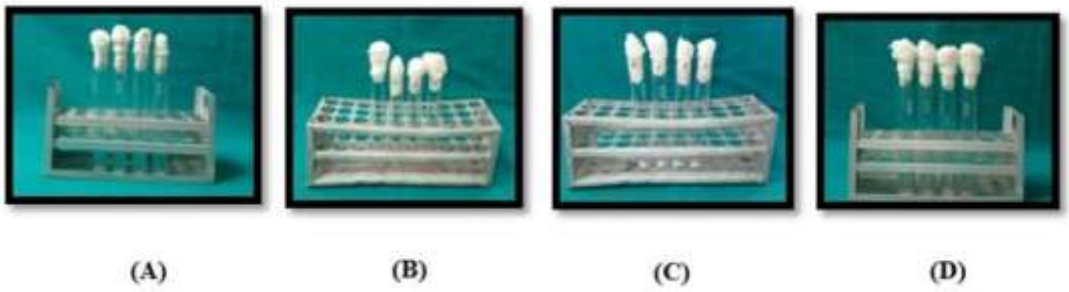

Figure 3: Number of colonies of Candida Albicans formed in different denture cleanser (A) Sodium Perborate (B) Thyme Oil (C) Ozonated Water (D) Distilled Water

(A) (B) (C) (D)