Efficacy of Sodium hypochlorite vs Sodium perborate as Denture Disinfectants - In Vitro Study

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Running Title: Efficacy of Sodium hypochlorite vs Sodium perborate as Denture Disinfectants

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Abstract
Aim: To find the efficacy of sodium hypochlorite and sodium perborate as denture disinfecting agents

Materials and methods: Autoclaved heat cure acrylic denture strips were immersed 25ml of sterilised artificial saliva and 0.5 McFarland standard Candida albicans suspension. It was incubated for 3 days at 37° Celsius, after that it was cleaned with mineral water and then immersed in 50ml of disinfectant for 6 hours. 2% sodium hypochloriteand 4% sodium perboratewere used as denture cleansing agents, saline and 0.2% chlorhexidine were taken as the negative and positive control respectively.After 6 hours, a swab was taken from the rough surface of the denture base sample and streaked on the SDA plate. It was incubated for 24 hours. Growth pattern of Candida albicans was observed. A broth culture was made to confirm the results obtained in the above study

Background: The prevalence of *Candida albicans* in the denture is significantly higher than that in mucosa.Sodium hypochlorite is a reducing agent & Sodium perborate is an oxidizing agent and helps in removing stains and kills the microbes on the denture. Denture disinfectant is used to control growth of microorganisms like *Candida albicans* preventing denture related stomatitis

Results: It is concluded that 2% sodium hypochlorite is found to be better denture disinfecting agent compared to 4% sodium perborate.

Conclusion: 2% sodium hypochlorite is found to be better denture disinfecting agent compared to 4% sodium perborate

***Keywords*: Denture disinfectants, denture stomatitis, Candida albicans, Sodium hypochlorite, Sodium perborate**

Introduction

A complete denture is defined as a dental prosthesis, which replaces the entire dentition and associated structures of the maxilla and mandible.[1] A complete denture restores the aesthetic, phonetic and masticatory functions of the individual. A denture placed in the oral environment forms a biofilm on the surfaces of thedenture, which makes it susceptible for infections [2]. Patients who wear dentures present with a variety of symptoms and abnormal intra oral findings [3]. The advancing age of the denture wearer and the nature of the denture bearing mucosa appear to influence the nature of the problem. Superimposed infection with Candida organisms and traumatic lesions are the most commonly encountered abnormalities. Denture stomatitis had been reported in 11-67% of complete denture wearers [4]. Denture bio film is an important factor in the pathogenesis of denture stomatitis. Candida albicans found in the biofilm has been reported as an important agent for the installation and maintenance of denture stomatitis.[5] The prevalence of Candida albicans in the denture is significantly higher than that in mucosa. In healthy individuals it has a prevalence rate of 45-65% with a higher in children and young adults. In denture wearers the prevalence of candida increases to 60-100% due to the decreased flow of oxygen and saliva caused by the denture to the underlying tissue producing a local acidic and anaerobic micro-environment that favours yeast overgrowth. [6]

Candida species are yeasts and within the oral cavity. It is one of the main causative organisms of denture- induced stomatitis which is primarily due its ability to adhere and form biofilms on oral cavity tissues and denture surfaces as well as due to its resistance to anti-fungal agents [7] This biofilm grows extensively on acrylic resin denture material and its effective removal is a significant challenge by both chemical and mechanical methods. Dentures can be cleaned mechanically, chemically or through a combination of both these methods. Mechanical methods comprises of brushing and ultrasonic treatment through the use of ultrasonic cleansers. [8] The ultrasonic cleaning method is limited due to the lack of information and discouraging cost. Brushing is easier, inexpensive and an effective method when used methodically in removing denture biofilm. However, abrasive action could result in the wear of the denture base and relining materials [9]. Another disadvantage of the mechanical methods is among the physically challenged or geriatric denture wearers. Hence the efficient chemical denture cleansers could be an important alternative or adjunctive to mechanical cleansing. Chemical methods include soaking the dentures in commercial (peroxides, acids, mouth washes and enzymes) or household (hypochloride, sodium chloride vinegar) products [10]. These chemicals are easy to use and can easily reach undercuts of the denture base which are otherwise overlooked during denture cleaning. The acrylic resins surface roughness remains unchanged compared to the abrasion due to brushing and the surfaces are less susceptible tobio film accumulation [11].

Sodium perborate is a white, odour-less compound with the chemical formula $Na_2B_2O_4(OH)_4$[12]. It is an oxidizing agent with antimicrobial activity and their chemical effects can remove stains by releasing oxygen [13].Sodium perborate is widely used in dentistry as a bleaching agent for treating discolored teeth [14]. Sodium hypochlorite is a reducing agent with antibacterial and anti fungal activity [15]. Sodium hypochlorite does not affect the surface roughness but the chances of deteriorating the denture base material by corrosion and bleaching is possible [16].Sodium hypochlorite is commonly used as a root canal irrigant in dentistry [17]. Chlorhexidine acts against a wide range of microbes like Gram positive, Gram negative bacteria, dermatophytes, lipolytic viruses, fungi, yeasts. Based on the concentration it can be bacteriostatic or bactericidal [18].

There are many known denture disinfectants such as EDTA, Chlorhexidine, Sodium hypochlorite, Sodium perborate, Povidone iodine, Hydrogen peroxide, etc. In this study we have chosen to study the effectiveness of the very commonly available agents, namely Sodium hypochlorite and Sodium perborate [19, 20].

Materials and Methods

The effect of disinfectant was tested by two methods. One was by contamination of denture bases with candida suspension and the second method was by testing the effect of the standardised concentration of disinfectant in a broth.

Sample fabrication

A total of 40 heat-polymerized acrylic denture strips were obtained from a wax pattern with a standardised dimension of 5x1 cm. The wax pattern was invested with dental stone (type III gypsum) in a metallic flask. After the setting of dental stone, de-waxing is done by immersing the flask in a water bath at a temperature of 70-80°C for about 10 minutes [21]. Heat-polymerized acrylic resin was mixed according to the manufacturer's recommendation and packed into the mould at the dough stage. The metal flask was then closed and subjected to a short curing cycle at 740°C for 2 hours followed by a terminal boiling at 1000°C for 1 hour [22]. On completion of curing cycle, the flask was allowed to completely cool before opening and the denture sample was obtained. The denture strips of 5x1cm dimension were checked for any imperfections. The cameo surface of the strips was sandpapered and polished [22]. On completion of processing, the strips were packed and autoclaved.

Contamination of specimen

40 heat cured denture acrylic denture strips were selected and sterilized by autoclaving at 15lbs for 30 minutes. These denture strips were immersed in sterilizeduricol containers containing 25ml of sterilized artificial saliva. A Candida albicans suspension was made to the turbidity matching 0.5 Mcfarland standard by immersing for 30 minutes. 100µl of suspension is added to the artificial saliva and well shaken to ensure a good mix. The denture strips in the above suspension was incubated for 3 days at 37℃after which it was taken out, and cleaned with mineral water and then immersed in 50ml of disinfectant and kept for 6 hours. A subculture was made on Brain Heart Infusion agar and incubated for 24 hours.

Preparation of disinfectants
Commercially available oxidising agent 2% sodium hypochloriteand 4% sodium perboratewere used as denture cleansing agents in this study. Saline was taken as the negative control and 0.2% chlorhexidine containing commercially available mouthwash was taken as the positive control. After incubation for 48 hours, the denture samples were washed in drinking water and placed in a sterile container containing denture cleansing agent. 10 denture samples were placed in each denture cleansing agent (sodium hypochloriteand sodium perborate). The denture samples were left in the denture cleansing agent for 6 hours.

Culture preparation
After 6 hours, a swab was taken from the rough surface of the denture base sample and streaked on the SDA plate. This process was repeated for all the denture base samples. The SDA plates were incubated for 24 hours. After 24 hours, the growth pattern of Candida albicans was observed.

Broth culture
The disinfectant material is taken in a standardized concentration in 5 curettes of 1ml each, the candida suspension was made with turbidity matching 0.5 McFarland standardsand it is taken. 10µl of the suspension is added to disinfectants taken in cuvette. It was allowed to react for 6 hours at room temperature. After the 6 hour period 10µl of this preparation was transferred to saboraud's dextrose agar and incubated for 12 hours at 37℃. The test was done along with positive and negative control.

Experimental and control groups
Four groups each containing 10 contaminated specimenswere assigned to various disinfectants.

Group 1: Chlorhexidine 0.2% (Positive control)
Group 2: Saline (Negative control)
Group 3: Sodium hypochlorite2%
Group 4: Sodium perborate4%

Results
75% of the denture bases did not showany growth of Candida and and 25% of the denture bases developed Candida growth. Out of which, 90% negative results and 10% positive Candida growth was seen on use of 2% Sodium hypochlorite, whereas 40% of the positive results and 60% of negative results on using Sodium perborate. Finally, 100% of the denture bases showed no growth of Candida on using chlorhexidine. This study was designed to evaluate the change in the proportion of positive and negative growth among patient's usage of chemical agents. When tested against the two controls, it was found that Sodium hypochlorite is better compared to Sodium perborate.

Agent	Positive	Negative
2%Sodium hypochlorite	1	9
4%Sodium perborate	4	6
Saline	10	0
Chlorhexidine	0	10

Table 1: The table below denotes the results of effectiveness of disinfecting agents against denture strips contaminated with Candida suspension.

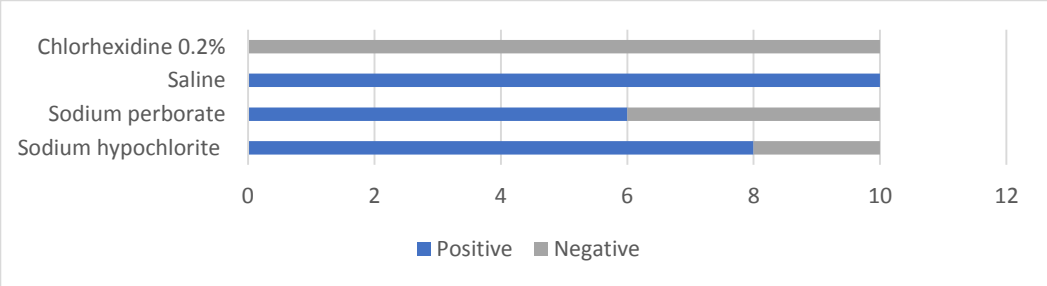


Figure 1: Graphical representation of the results

Figure 2 shows Chlorhexidine 0.2%

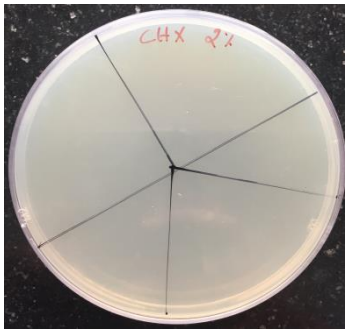
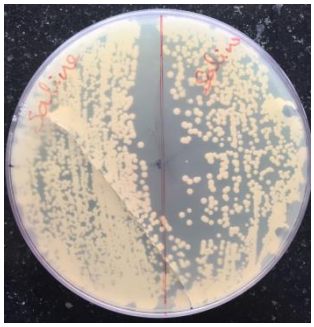


Figure 3 shows Saline



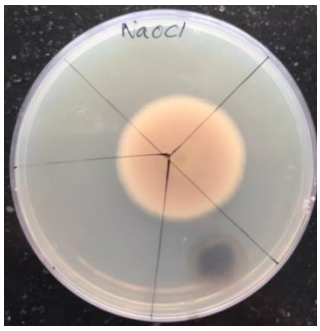


Figure 4 shows 2% Sodium hypochlorite

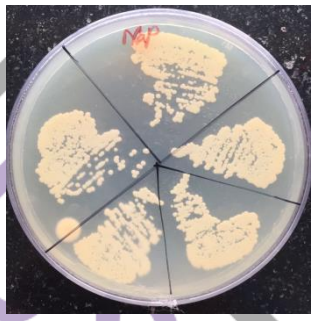


Figure 5 shows 4% Sodium perborate

Discussion

Sodium perborate is a white, odourless, economical and versatile water soluble oxidizing agent which releases hydrogen peroxide on reacting with water [23]. Hydrogen peroxide is a broad spectrum antimicrobial agent effective against dormant forms of known high resistance microbes such as bacterial spores, protozoal cysts and infectious proteins such as prions [24]. It degrades the microbes by chemical oxidation of the cellular contents [25].

Sodium hypochlorite is a reducing agent with antimicrobial activity [19]. It is an organic, fat solvent which degrades fatty acids and convert them into salts, alcohol and neutralises amino acids forming water and salt [26].The high pH of sodium hypochlorite interrupts the cytoplasmic membrane integrity with biosynthetic alterations in cellular metabolism, irreversible enzymatic inhibition, phospholipid degradation [27].It also reduces the adhesion of *Candida albicans* to the epithelial cells of oral cavity [19]. From the above results, 2% Sodium hypochlorite is found to be more effective compared to 4% Sodium perborate. These resultsare similar to a study done by Cem et al., where 2% Sodium hypochlorite was found to be better compared to other chemical disinfecting agents [19]. Nikawa and coworkers have concluded a similar result that chemical agents are easier to use and it is more efficient in reducingthe formation of biofilm [15]. Denture wearers maintain the denture hygiene if denture cleansing products are feasible and easily available. The commercially available denture cleansers may not be feasible to the patient and this may lead them to neglect denture hygiene. The two disinfectants used in the study fulfill these criteria as they are cost effective, easily available and they have the ability to effectively disinfect a denture. For best results, mechanical cleansing of the denture using toothbrush or nailbrush should be done prior to chemical disinfection [28].

Conclusion

From the above results it is concluded that 2% sodium hypochlorite is found to be better denture disinfecting agent compared to 4% sodium perborate.

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