

EFFICACY OF EDTA AND GLUTERALDEHYDE AS DENTURE DISINFECTANTS - In Vitro Study

Shabana Bowsiya¹, Dr. Naveen Kumar²

¹BDS 2nd year, ²Senior Lecturer
Saveetha Dental College,
Saveetha University, Chennai.

Abstract: Gluteraldehyde and Ethylenediaminetetraacetic acid (EDTA) are commonly used in dentistry as root canal disinfectants. They also have antifungal properties. Hence, they can be used as a denture disinfectant. In this study, the denture materials were contaminated with candidial suspension followed by exposure to the disinfectants. It was found that both the disinfectants were consistent with each other. However, EDTA has a better disinfecting property compared to gluteraldehyde. Since, they are fasible and easily available, they can commercially be used as denture disinfectants.

Keywords: Denture, disinfectant, EDTA, gluteraldehyde, Candidial infections

Introduction:

A complete denture is defined as a dental prosthesis, which replaces the entire dentition and associated structures of the maxilla and mandible (1). The many functions of complete denture include restoring the aesthetics, phonetics and mastication of the denture wearer. A denture becomes susceptible to infections due to the biofilm formed on its surface on placement in the oral environment. (2). A number of abnormalities and related intramural findings have been reported in denture wearers that can be attributed to the age and nature of mucosa (3, 4). One of the most common finding among these abnormalities are the Candidial infections superimposed with traumatic lesions. Almost 11-67% of the denture wearers have reported with Denture stomatitis (4). Candidas are species of yeast that occur in the oral environment and it is one of the chief causes for denture- induced stomatitis. This is due to the fact that Candida has various features that help in this role, namely it's adherence to denture surface and oral tissues, its ability to form biofilms and resistance to antifungal agents (7). Candida albicans is present in the denture surfaces in a significantly higher proportion than that in mucosa, it's prevalence rate in healthy individuals being 45-65% with an even greater ranges in children and young adults. Decreased flow of oxygen and saliva caused by the denture results in local acidic and anaerobic microenvironment of the underlying tissues that favours yeast growth. This ultimately increases the prevalence of Candida by 60-100% in denture wearers (6). Candida albicans that manifest in the biofilm, an important contributor to the pathogenesis of denture stomatitis, is essential for the installment and maintenance of denture stomatitis (5).

Effective removal of this biofilm is required through chemical or mechanical methods as this biofilm grows extensively on acrylic denture base material. Dentures can be cleaned mechanically, chemically or through a combination of both these methods. Mechanical method includes brushing and ultrasonic cleansers (8). But the ultrasonic method has limitations such as lack of information and discouraging cost. Mechanical methods on the other hand 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 for the physically challenged or geriatric denture wearers. An important alternative or adjunctive to mechanical cleansing is chemical denture cleansers. Chemical methods include soaking the dentures in commercial (peroxides, acids, mouth washes and enzymes) or household (hypochlorides, 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 other denture cleansing methods. One of the main disadvantages of mechanical denture cleansing methods is abrasion due to brushing is overcome by chemical methods as the acrylic resins surface roughness remains unchanged and the surfaces are less susceptible to biofilm accumulation (11).

There are many known denture disinfectants such a EDTA, sodium hypochlorite, sodium perborate, povidone iodine, hydrogen peroxide, etc.(15). In this study we study the effectiveness of citric acid and chlorhexidine as denture cleansers.

Methodology:

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 standardized concentration of disinfectant in a broth.

Sample fabrication:

A total of 40 heat-polymerized acrylic denture strips were obtained from a wax pattern with the dimension of 5x1cm. The wax pattern was invested with dental stone (type III gypsum) in a metallic flask. After the setting of dental stone, the wax was removed by dewaxing and heat-polymerized acrylic resin was mixed according to the manufacturers recommendation and packed into the mold at the dough stage. The metal flask was then closed and subjected to a short curing cycle. On completion of curing cycle, the flask was allowed to completely cool before opening and the denture sample was obtained. The denture sheets were cut into strips of 5x1cm dimension. The cameo surface of the strips were sandpapered and polished (wet and dry). On completion of processing, the strips were packed and autoclaved.

Contamination of suspension:

40 heat-cured denture acrylic strips were selected and sterilized by autoclaving at 15lbs for 30 minutes. These denture strips were immersed in sterilized uricol containers containing 50ml of sterilized artificial saliva. A *Candida albican* suspension was made to the turbidity matching 0.5 McFarland standard by immersing for 30 minutes. 100 micro liters of suspension was incubated for 3 days at 37Celsius after which it was taken out, and cleaned with mineral water and then immersed in 50ml 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 disinfectant, 2% gluteraldehyde and chelating agent i.e., EDTA were 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 positive control. After incubation for 48 hours, the denture samples were washed in drinking water and placed in sterile container containing disinfectant cleansing agent. 10 denture samples were placed in denture cleansing agent (gluteraldehyde and EDTA). 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 streak on the SDA plate. Repeat this for all the denture base samples. Incubate the SDA plates for 24 hours. After 24 hours, the growth pattern of *Candida albicans* was observed.

Broth culture:

The disinfectant material is taken in a standardised concentration in 5 currettes of 1 ml each, the *Candida* suspension which was made with turbidity matching 0.5 McFarland standard is taken and 10 micro litre 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 10 micro liter of this preparation was transferred to saborauds dextrose agar and incubated for 12 hours at 37 degree Celsius. The test was done along with a positive and negative control.

Experimental and control groups:

Four groups each containing contaminated specimen of 10 were assigned to various disinfectants.

Group 1: Chlorhexidine 0.2% (Positive control)

Group 2: Saline (Negative control)

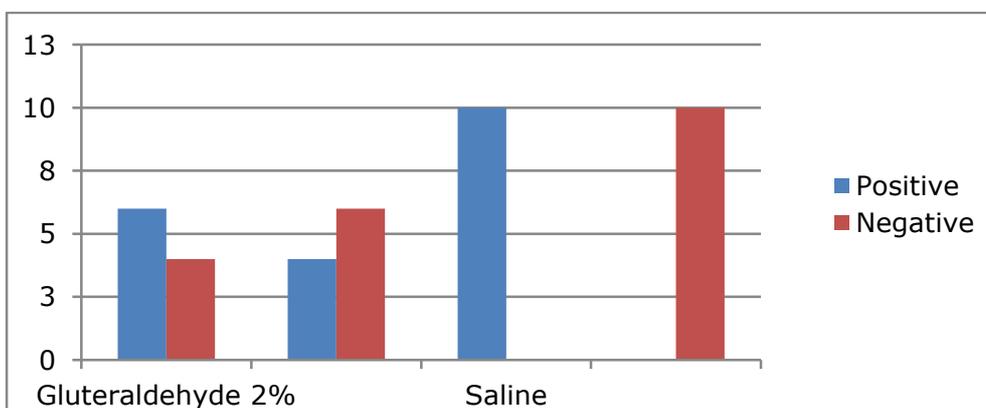
Group 3: gluteraldehyde 2%

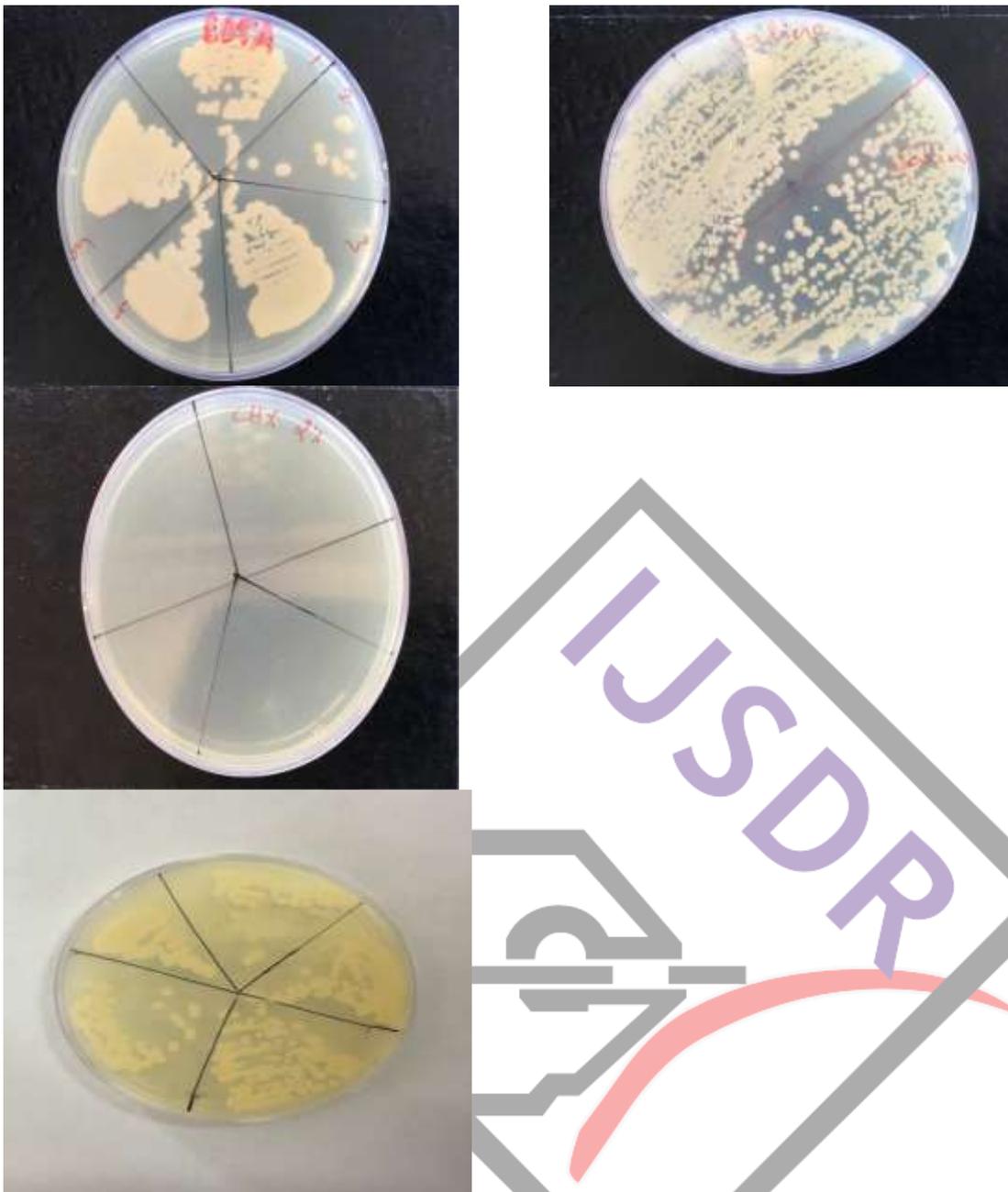
Group 4: EDTA 2%

Result:

The results obtained from both methods were consistent with each other. When tested against the two controls, it was found that EDTA had better disinfecting properties than gluteraldehyde.

Denture Cleansing agent	Positive	Negative
Gluteraldehyde 2%	6	4
EDTA 2%	4	6
Saline	10	0
Chlorhexidine 0.2%	0	10





Conclusion

Hence of the selected agents, six strips showed positive Candidal growth for glutaraldehyde and four strips showed positive Candidal growth for EDTA concluding that both the disinfectants are better denture cleansing agents.

Discussion

Glutaraldehyde has a broad spectrum of activity against bacterial spores, fungi, and viruses. It is highly active in alkaline pH than acidic (12). The primary target site is the cell wall of the fungi comprising of chitin. The mode of action of includes alkylation of hydroxyl, amino, carboxyl and sulfhydryl groups, cross-linking of proteins and macromolecules in the cell envelopes resulting in impaired DNA, RNA and protein synthesis (13).

Ethylendiaminetetraacetic Acid (EDTA) is a commonly used chelating agent used in dentistry. EDTA has antimicrobial and antibiofilm properties. It is found to be synergistic with other antimicrobial agents. The antifungal activity of EDTA is said to be likely via the inhibitory affect on growth causing fungal death by competing with siderophores for any of the trace iron and calcium ions that are essential for the maintenance of the life cycle of fungi (14).

Both disinfectants are equally effective against Candida growth as per the obtained results. Denture wearers will be keen on maintaining denture hygiene if the denture cleansing products are feasible and easily available. The commercially available denture cleansers may be unfeasible to the patient and this may them to neglect denture hygiene. The two disinfectants used in the study fulfill these criteria as they are cost effective 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 (16). The study was performed with a small sample size as a pilot study, and it leads the way to more large-scale studies with various other cost efficient disinfectants.

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