Research Article

# Comparison on the Efficacy of Chemical and Natural agents on Disinfection of Denture Bases – an *In Vitro* Study

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**ABSTRACT**

The aim of the study is tocompare the efficacy of chemical and natural agents on the disinfection of denture bases. The objective of the study is to evaluate the anti-fungal activity of natural oils such as Gingely oils and Sunflower oil against Chemical agents such as Glutaraldehyde and EDTA to determine the efficacy of various classes of denture disinfecting agents. A total of 60 heat polymerized acrylic denture strips are inoculated in the Candida albicans culture media for 48 hours and then incubated in the respective disinfectants for 8 hours. Swabs are taken from the strips and inoculated in SDA and incubated overnight at 37 degrees Celsius. After this the growth is checked. In the second method, the Candida is directly exposed to the disinfectant and incubated for a period of 6 hours. The growth is then checked and compared with the first method. 70% of growth was positive for Gingely oil, whereas 100% of growth was positive using sunflower oil. 60% of growth was positive for EDTA and Glutaraldehyde. The Friedmann test stated the mean ranks as chemical, 1.70 and oil, 1.30. So, the chemicals are better disinfectants than oils. The study revealed that Chemical agents like EDTA and Glutaraldehyde are better disinfectants for denture bases than Natural oils such as Sunflower and Gingely oils.

**Keywords:** Disinfectants, acrylic resin, Candida albicans, chemical agents, Natural oils.

## INTRODUCTION

A Complete denture is defined as a dental prosthesis, which replaces the entire dentition and associated structures of the maxilla and mandible. A complete denture restores the aesthetic, phonetic and masticatory functions of the individual.[1]A denture placed in the oral environment forms a bio film on the surfaces of the denture, which makes it susceptible for infections. Patients who wear dentures present with a variety of symptoms and abnormal intraoral findings. The advanced age of the average denture wearer and the nature of the denture bearing mucosa appear to influence the nature of the problems. Superimposed infection with candidial organisms and traumatic lesions are the most commonly encountered abnormalities.[2] Denture stomatitis had been reported in 11-67% of complete denture wearers[3]. Denture bio film is an important factor in in the pathogenesis of denture stomatitis. Candida species, notably Candida albicans, is the major fungal pathogen in humans. It is a dimorphic fungus capable of causing superficial mucosal infections, as well as systemic infections, in immune compromised individuals. The factors responsible for its pathogenesis are still not fully understood and increasing resistance to commonly used antifungal agents necessitates the search for new formulations and wearing denture is a long-term issue which warrants long term maintenance. Using a chemical disinfectant to maintain the denture and the oral hygiene will not be agreeable in long term denture wearers. Many plant extracts and essential oils have biological activity both in vitro and in vivo, which has justified research on traditional medicine focused on the characterization of their antimicrobial activity.[4]The antimicrobial activity shown by plant oils is mainly due to a number of phenolic and terpenoid compounds, which have antibacterial or antifungal activity.[5] In addition, it is expected that plant compounds with target sites other than those currently used by antimicrobials will be active against drug-resistant microbial pathogens. Yet, the information available regarding plants (particularly medicinal plants) that are active against this microorganism has, until recently, not resulted in effective formulations for human use. In this situation the plant products that are consumed in our day-to-day life can be explored to identify their antimicrobial property, which will be beneficial for the community

Candida albicans found in the bio film has been reported as an important agent for the installation and maintenance of denture stomatitis.[6] The prevalence of Candida albicans in the denture is significantly higher than that in mucosa.[7] 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 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.[8]

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 bio films on oral cavity tissues and denture surfaces as well as due to its resistance to anti-fungal agents. This

bio film grows extensively on acrylic resin denture material and its effective removal is a significant challenge by both chemical and mechanical methods.[9] Chlorhexidine is a broad-spectrum biocide effective against Gram-positive bacteria, Gram-negative bacteria and fungi. Chlorhexidine inactivates microorganisms with a broader spectrum than other antimicrobials (e.g. antibiotics). It has both bacteriostatic and bactericidal mechanisms of action, depending on its concentration and is the most effective antifungal agent in use.

Dentures can be cleaned mechanically, chemically or through a combination of both these methods. Mechanical methods are comprised of brushing, and ultrasonic treatment though the use of ultrasonic cleansers is limited due to the lack of information and discouraging cost. Brushing is simple inexpensive and effective method when used meticulously in removing denture bio film. However, abrasive action could result in the wear of the denture base and relining materials. Another disadvantage of the mechanical methods is among the physically challenged or geriatric denture wearers. So efficient chemical denture cleansers might be an important alternative to mechanical cleansing. Chemical methods include soaking in commercial (peroxides, acids, mouth washes and enzymes) or household (hypochlorides, sodium chloride vinegar). These solutions are simple to employ and can easily reach undercuts of the denture base, acrylic resins surface roughness remains unchanged and less susceptible to bio film accumulation.[10]

Oil pulling with sunflower and sesame oil has both historical and present significance in India for prevention of tooth decay, oral malodor, bleeding gums, etc.[11]It has been claimed that they activate enzymes and draw toxins out of blood.[12] It has been hypothesized that sesamin and fatty acid components of gingely oil are involved in its antifungal activity.[13]

Gingely oil is known to inhibit the growth of both the mycelial as well as yeast forms of Candida albicans. Sunflower oil also inhibits the growth but to a lesser extent than sesame oil.[14]

Gingely oil and sunflower oil have been proven to be safe and biocompatible materials.

Glutaraldehyde is an important dialdehyde that has found usage as a disinfectant and sterilant, in particular for low-temperature disinfection and sterilization of endoscopes and surgical equipment and as a fixative in electron microscopy. Glutaraldehyde has a broad spectrum of activity against bacteria and their spores, fungi, and viruses, and a considerable amount of information is now available about the ways whereby these different organisms are inactivated. [15]

Surface efficacy tests have shown that the application of sanitizers at an active glutaraldehyde concentration of 1000ppm (0.1%) kills almost all of the tested bacteria species within five minutes at T = 25°C. 1000ppm of Glutaraldehyde is efficacious against all of the tested bacteria under the representative conditions of this test. Glutaraldehyde based sanitizers are also effective against the viruses. [16]

Ethylenediaminetetraacetic acid (EDTA) is known as a chelating agent.It is a complex molecule with a claw-like structure, which binds and seizes divalent and trivalent metal ions such as calcium and aluminum to form a stable ring structure. EDTA has traditionally been useful as a metal chelator due to its high density of ligands and resulting affinity for metal ions with binding typically occurring through its two amines and four carboxylate groups. After intravenous administration, accessible metal ions are chelated forming stable soluble complexes, which are then excreted in the urine. EDTA is not metabolized, but elimination is decreased with renal dysfunction. Disodium EDTA inhibits the growth of Candida albicans in vitro. EDTA binds the calcium that is required for growth of this fungal species. [17]

## METHODOLOGY

The anti-microbial activity is tested by two methods in this study. The effect when found coated on the denture bases contaminated with candida suspension and evaluation of the action of the standardized concentration of the disinfectant when directly added to the broth containing the candida suspension.

### Sample fabrication

A total of 60 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 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.

### Preparation of suspension

The specimens for each group were immersed for 8hrs in the tested oils (sun flower oil, sesame oil) and chemicals (Glutaraldehyde and EDTA) after they had been infected with Candida albicans and incubated for 48hr. The bacteriological procedure was accomplished by using the standardized Candidal cell suspensions (600×106 CFU/ml), that is equal to Macfarland standard bacteriological solution tube no.2. The procedure involves preparing the Mac Farland Standard Bacteriological Solution (tube No.2) that composed of 0.2 ml. Barium Chloride of 1% and 9.8 ml. H2SO4 of 1%. The standard

strain Candida albicans was grown on SDA overnight and made as a suspension with sterile normal saline matching the McFarland Standard.

**Preparation of disinfectants**

Preparation of disinfectant: The chemicals and sterile distilled water were mixed in a sterile disposable container and transferred using 5mL syringe.

**Incubation**

From the suspension 100 microliter of the suspension is added to 25ml of artificial saliva and incubated overnight at 37 degree celcius aerobically. After incubation, the denture strips were removed and placed in sterile disposable containers containing the oils and chemicals separately. The denture strips were immersed and placed in the incubator for 6 hours. After 6 hours the strips were removed one by one using sterile forceps.

**Evaluating results**

The strips were rinsed in clean drinking water and placed in the containers containing the oils and the chemicals for 8 hours. Swabs are taken from the strips and inoculated in SDA and incubated overnight at 37 degrees Celsius and checked for the presence of growth. The plates that have candidial growth are taken as positive and no growth is taken as negative.

**Broth culture**

2% Glutaraldehyde, 2% EDTA and Sunflower and Gingely oils are taken in 5 cuvettes of 1ml each. The candidal suspension which was made with turbidity matching 0.5 McFarland standard is taken and 10 microliters of the suspension is added to disinfectants taken in the cuvette. It is allowed to react for 6 hours at room temperature. After the period of 6 hours, 10 microliters of this preparation was transferred to Sabouruads dextrose agar and incubated for 12 hours at 37 degree Celcius. The test was done along with a positive and negative control. The results were then verified.

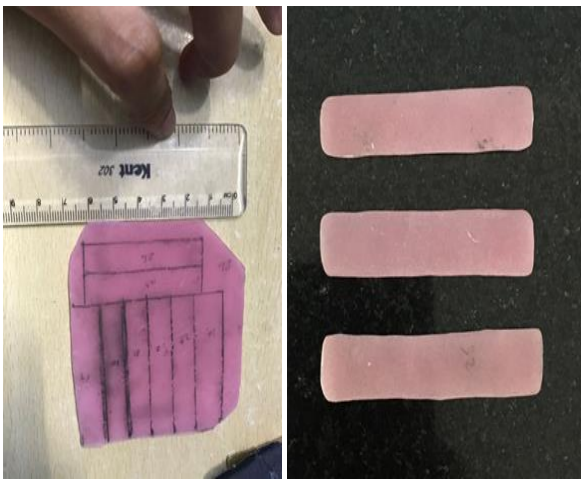


**Figure 1:** Measurement of Denture strips.

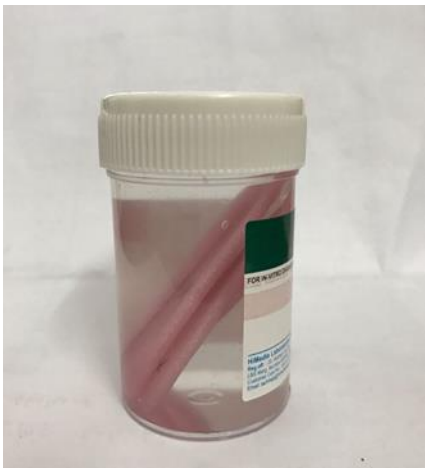


**Figure 2:** Denture strips placed in Candidal Solution

## RESULTS

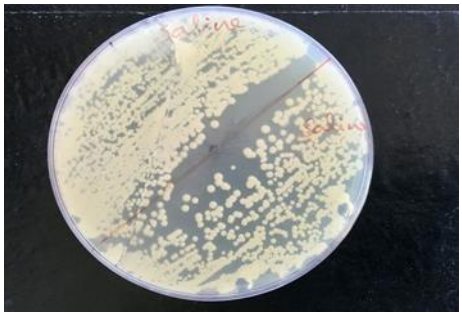


**Figure 3:** Saline – Positive control

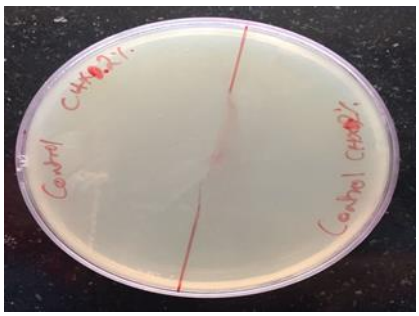


**Figure 4:** CHX 0.2% - Negative control

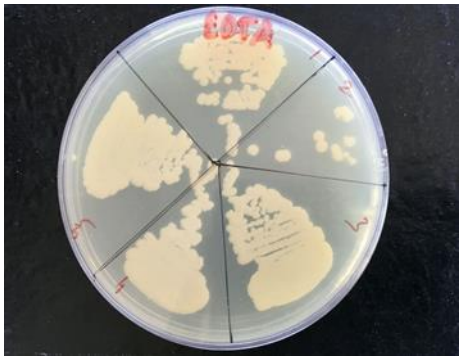


**Figure 5:** EDTA

**Figure 6:** Glutaraldehyde

**Figure 7:** Sunflower oil

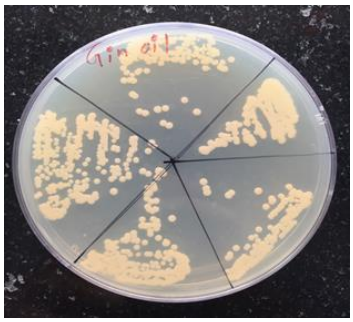


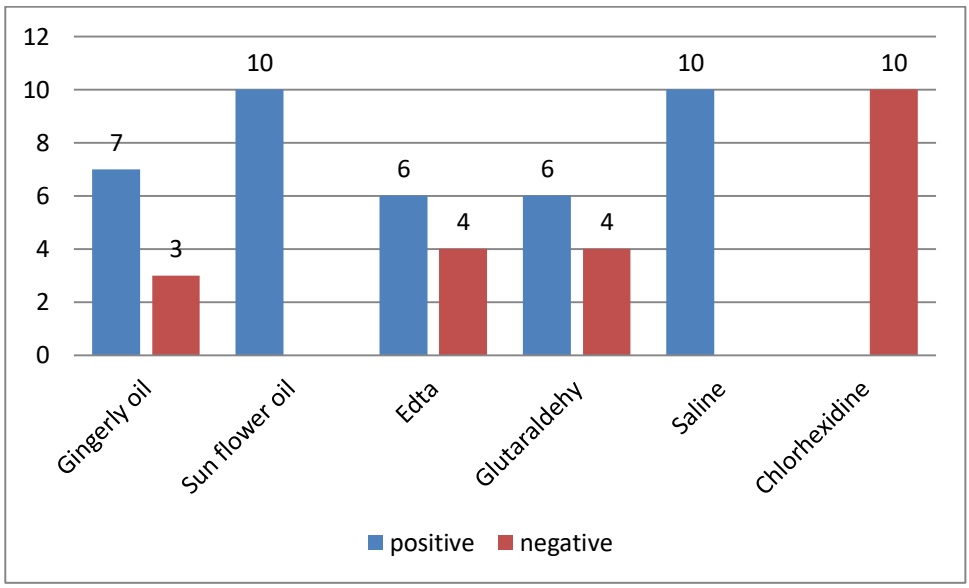
**Figure 8:** Gingely oil

**Method 1:** Contamination of denture bases with Candida suspension

**Analysis and Interpretation**

**TABLE 1:** Distribution of study population

| | POSITIVE | | NEGATIVE | | TOTAL | |
|---|---|---|---|---|---|---|
| | Frequency | Percentage | Frequency | Percentage | Frequency | Percentage |
| **Gingerly oil** | 7 | 70.0 | 3 | 30.0 | 10 | 100% |
| **Sunflower oil** | 10 | 100 | - | - | 10 | 100% |
| **EDTA** | 6 | 60.0 | 4 | 40.0 | 10 | 100% |
| **Glutaraldehyde** | 6 | 60.0 | 4 | 40.0 | 10 | 100% |
| **Saline** | 10 | 100 | - | - | 10 | 100% |
| **Chlorhexidine** | - | - | 10 | 100 | 10 | 100% |




Graph 1

From Table 1 that 70% of growth was positive and 30% of growth was negative using gingely oil, whereas 100% of growth was positive for sunflower oil. In the sense that the study was designed to evaluate the change in the proportion of positive and negative growth among patient's usage of natural oils and controls for mouth, it appears that Gingely oil is more effective than Sunflower oil. The chemicals, EDTA and Glutaraldehyde are more effective than the natural oils.

| Ranks | |
|---|---|
| | Mean Rank |
| oil | 1.30 |
| chemical | 1.70 |

The Friedman test compares the mean ranks between the oil and chemical, and how the groups differed. From the above mean rank table, chemical is 1.70 and oil is 1.30. So, the chemicals are better disinfectants than oils.

**Method 1:** Contamination of denture bases with Candida suspension

| Denature bases | Saline Positive control | Gingely oil | Sunflower oil | EDTA | Glutaraldehyde | CHX 0.2% |
|---|---|---|---|---|---|---|
| 1 | + | + | + | - | + | - |
| 2 | + | + | + | + | + | - |
| 3 | + | + | + | + | + | - |
| 4 | + | + | + | + | - | - |
| 5 | + | + | + | + | + | - |
| 6 | + | + | + | - | + | - |
| 7 | + | + | + | - | + | - |
| 8 | + | - | + | + | - | - |
| 9 | + | - | + | - | - | - |
| 10 | + | + | + | + | - | - |

**Method 2:** Direct exposure of Candida to Disinfectant

| Denature bases | Saline Positive control | Gingely oil | Sunflower oil | EDTA | Glutaraldehyde | CHX 0.2% (Negative control) |
|---|---|---|---|---|---|---|
| 1 | + | - | + | - | + | - |
| 2 | + | + | + | + | + | - |
| 3 | + | + | + | + | - | - |
| 4 | + | + | + | + | - | - |
| 5 | + | + | + | - | - | - |
| 6 | + | + | + | + | + | - |
| 7 | + | + | + | - | - | - |
| 8 | + | - | + | + | + | - |
| 9 | + | + | + | - | - | - |
| 10 | + | + | + | - | - | - |

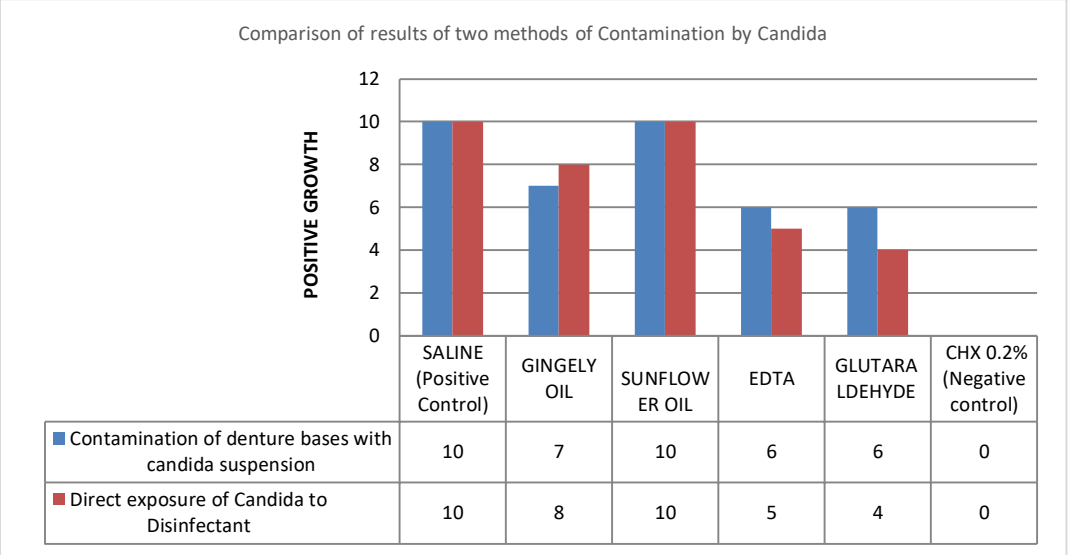
Hence, by both methods of contamination, the results show that EDTA and Glutaraldehyde are better disinfectants than Gingely oil and Sunflower oil.

## DISCUSSION

Some researchers have found that mechanical cleaning is better than chemical cleaning.[18] However chemical disinfectants have some advantages over mechanical cleaning such as effectivity and ease of use especially for elderly.[19] The adherence and colonization of microorganism may change by ongoing usage and cleaning cycle. Since continuous application of disinfectants may alter the surface characteristics [20], only one cycle of usage and cleaning was assessed in this study.

With the limitations, this study compared the efficacy of denture cleaners on contaminated specimens. Among all the agents evaluated against selected microorganisms, 2% Glutaraldehyde solution demonstrated the best cleaning effect on denture base material. Owing to the antimicrobial strength of Glutaraldehyde, very few colonizations were found in the specimens.[21]In another

study carried out, the results showed that Glutaraldehyde was the second active cleaner and sodium hypochlorite was the first.[22]2% glutaraldehyde penetrates into the resin surfaces, dissolutes the surface materials and inhibits the enzymatic activities of microorganisms [23]. This may explain the success over the other cleaners. The addition of inorganic cations has been proposed, in combination with the effect of alkaline pH, to increase bactericidal activity of Glutaraldehyde. The activity of glutaraldehyde is increased in the presence of magnesium ions (Mg), as more cellular alkaline phosphatase (PAse) is released. The presence of divalent cations at alkaline pH reinforces the bactericidal activity by concentration of the wall and/or plasmolysis of the bacterial cell. This potentiation of glutaraldehyde activity in the presence of Mg has also been reported in moulds and spores.[24]Glutaraldehyde acts on proteins by denaturation.




**Graph 2**

The antimicrobial effects of EDTA have been demonstrated for a range of clinical microorganisms that include Gram-negative and Gram-positive bacteria, yeast, amoeba and fungi.[25]Divalentcations such as $Mg^{2+}$ are essential for stabilizing the negative charges of the oligosaccharide chain of the LPS component. EDTA has been shown to remove $Mg^{2+}$ and $Ca^{2+}$ ions from the outer cell wall of Gram- negative bacteria, thereby liberating up to 50% of the LPS molecules and exposing phospholipids of the inner membrane, enhancing the efficacy of other antimicrobials. [26]

The antifungal activity of EDTA is said to be likely via the inhibitory effect 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. [27] At low concentrations EDTA has been shown to prevent bio films by inhibiting the adhesion of bacteria. [28] Furthermore, it has also been shown to reduce bio film colonization and proliferation.[29] Studies by Percival et al.[30] reported a significant reduction in bio films when treated with different EDTA compositions and solutions.

The use of natural products as disinfectants or denture cleansers is greatly advantageous over using systemic approach by antibiotics or local approach with synthetic products or some oral antibiotics. Al- Haroni[31] showed that orally directed therapies against bacteria is superior to the use of broad spectrum antibiotics. The advantages of using natural products as denture cleansers include: safety and biocompatibility, has no chance to develop bacterial resistance[32], effective as fungicidal and bactericidal agents, and has anti-tumor, anti-oxidant, anti-inflammatory, anti- bacterial activity, and stimulate the immune system[33,34], in addition to their low cost and availability in mostly every house.

Oil pulling with sunflower and sesame oil has been used extensively for many years in India to prevent teeth decay, oral malodor, bleeding gums, dryness of throat and cracked lips, and for strengthening the teeth, gums, and jaws. They are effective mouthwashes and especially effective on Streptococcus mutans in plaque and saliva of children when used for 10-15 min. The viscous oil turns thin and milky white. It is claimed that they activates enzymes and draws the toxins out of the blood. [35]

According to the results of this study, Gingely oil proved to be a better disinfectant than Sunflower oil. The mechanism of action of these oils may be due to its viscosity that act by oil pulling mechanism, saponification, or emulsification action [36, 37], this may be caused by their high content of poly unsaturated fatty acids. Gingely oil had strong antifungal action that may result from their content of the triply unsaturated omega-3 fatty acid, Sesamin, and sesamolin.[38]

## CONCLUSION

The study revealed that Chemical agents like EDTA and Glutaraldehyde are better disinfectants for denture bases than Natural oils such as Sunflower and Gingely oils. The oils may be mildly effective in cleansing of denture bases but Chemical agents have a greater efficacy. Also, the direct action of the disinfectants on Candida is almost similar to the action of the disinfectant on the contaminated denture strips.

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