



## Sacred Lotus as an Impression Disinfectant and its Effect on the Dimensional Stability of an Elastomeric Impression Material

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### Abstract

**Purpose of study:** This study was achieved to evaluate the antimicrobial activity of Lotus essential oil as an additive to condensation silicon impression material during mixing and to evaluate its effect on dimensional stability of impression material.

### Materials and Methods

**Microbiological Test:** The strains used throughout this study to investigate the effect of augmenting condensation silicon impression material with lotus oil were *Pseudomonas aeruginosa* ATCC No. 9027, *Escherichia coli* ATCC No. 8739, *Staphylococcus aureus* ATCC No. 6538, *Streptococcus mutans* ATCC No. 700610 and *Candida albicans* ATCC No. 10231).

**Dimensional Change Test:** Twenty impressions of Standardized metal die as per ADA specification number #19 were made. Standardized photographs were taken at different time intervals (0, 1, 6, 12 and 24 hours) and the pictures were measured with image processing software.

### Results

**Microbiological Results:** The results demonstrated the effectiveness of lotus oil in reducing the count of examined strains in comparison with the control, a single exception was noted in case of *Staphylococcus aureus*.

**Dimensional stability results:** A significant dimensional change immediately after setting, after 6 and 12 hours with addition of Lotus oil to impression material. There was no significant dimensional change after one and 24 hours in comparison with control group.

**Conclusion:** Addition of Lotus essential oil as a disinfectant to condensation silicon impression material was highly efficient against various pathogens and the dimensional changes were complying the standards of the American Dental Association (ADA) specification 19, which states that acceptable dimensional change should be not more than 0.5%.

**Keywords:** Lotus Oil; Elastomeric Impression; Condensation Silicon; Disinfection

### Introduction

The history of complete denture impression procedures has been influenced largely by the development of impression materials. In the last decade, several investigators have recommended using newer elastomeric for final impressions to replace the older and more traditional materials. Four basic types of elastomer impression materials are currently in use in the dental profession: silicone rubbers which polymerize by a condensation reaction, polysulfide (mercaptan) rubbers, polyethers, and silicones which polymerize an addition reaction [1].

The most commonly used impression is condensation silicon impression material, a high viscosity material for border molding and a low viscosity material for secondary impression but the pro-

duction of ethanol as a sub-product of the polymerization and its evaporation likely affect the dimensional stability of the material so the impression should not be stored for more than 30 minutes following removal from the mouth [2].

Disinfection of the dental impressions is very important procedure to prevent transmitting of serious diseases from patients to all dental personnel.

Dental impressions can easily become contaminated with a patient's blood and saliva. Such fluids can contain overt pathogens, including the viral agents associated with hepatitis, herpes simplex, and HIV/AIDS, in addition to tubercle bacteria, *Streptococcus* and *Staphylococcus species*, *Bacillus species*, and *Enterobacter species*. Some of these microbes can exist for extended periods outside their human hosts [3].

The elastomeric impression materials are currently recommended to be disinfected by immersion [4] by using Glutaraldehyde, chlorine compounds, iodophors, phenolic and alcohol compounds is the most common. although there has been some concern over the effect of disinfection procedures on the dimensional stability [5].

The accuracy of lab-constructed appliances depends on the accuracy of the stone cast, and thus the accuracy of the clinical impression is important. impression materials undergo dimensional changes during setting and the use of disinfectants particularly immersion ones has also been reported to cause dimensional [6].

The first reference on the uses of EOs for therapeutic reasons was found in the Ebers papyrus. This document listed in detail more than 800 EOs remedies and treatments.

In ancient Egypt, lotus was used to cure many diseases, recently it is proved that Lotus has anti-oxidative, anti-inflammatory activity antiviral activity, and antibacterial activity [7]. Lotus essential oil is natural material so there is no any adverse reaction or side effect as with any chemical or manufactured materials.

However, it was observed that disinfection of the impression is not performed systematically in routine dental practice, studies were evaluate the addition of disinfectant to the impression material during mixing [8].

The purpose of current study was to evaluate anti-microbial activity of addition essential oil of Lotus as disinfectant to elastomeric impression material during mixing and evaluate its effect on dimensional stability of impression material.

## Material and Methods

### Microbiological Test

The count of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans* (CFU/g) was used to investigate the effect of augmenting condensation silicon impression material with lotus oil (was prepared in faculty of Pharmacy, Pharos University in Alexandria).

The study groups were divided into three main groups:

1. **A negative control group:** Impressions by using condensation silicon light body (Alphasil® perfect light, Müller-Omicron, Germany) without addition of lotus oil or microbial strains.
2. **A positive control group:** Impressions with addition of standardized inoculum of microbial strains
3. **A test group:** Impressions with addition of standardized inoculum of microbial strains and lotus oil.

Stainless model (Figure 1) with polished surface divided into sixteen ( $10 \times 10 \text{ mm}^2$ ) squares separated by 2 mm deep grooves and aluminum tray  $6 \times 6 \text{ cm}^2$  were fabricated.

Stainless steel model was immersed for 2 minutes in a previously standardized suspension of strains equivalent to  $10^8 \text{ cfu/ml}$  by adjusting the optical density at 600 nm.

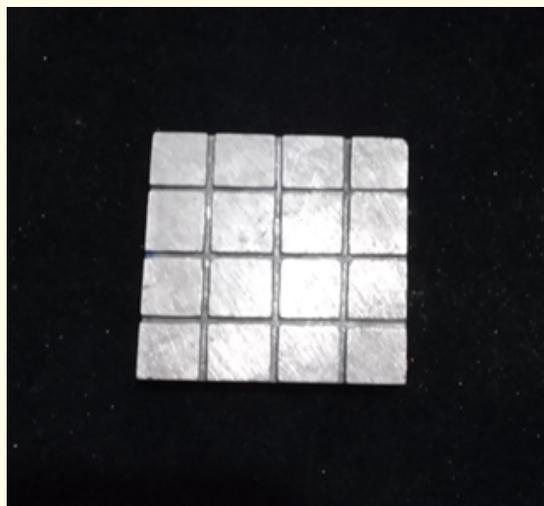


Figure 1: Stainless model.

Impression (2-paste putty system) of stainless steel model was taken after addition of one drop of lotus oil for every 3 cm of impression past during mixing, after complete setting, stainless steel model has been removed from the impression. Flat sixteen square impression sections were cut off from the impression and removed by means of sterile cutter blades. Each sample was weighed under aseptic conditions and transferred to a tube containing 10 ml of sterile physiological saline. Each sample was mixed thoroughly using a vortex mixer for 60 seconds and serially diluted up to  $10^{-4}$ . Each dilution of *Pseudomonas aeruginosa* ATCC No. 9027, *Escherichia coli* ATCC No. 8739, *Staphylococcus aureus* ATCC No. 6538, *Streptococcus mutans* ATCC No. 700610 was inoculated on tryptone soya agar plates and incubated aerobically at  $37^\circ\text{C}$  for 3 days. Dilutions of *Candida albicans* ATCC No. 10231 were cultured on Sabouraud dextrose agar and incubated aerobically at  $37^\circ\text{C}$  for 5 days. After incubation the number of colony forming units (CFU) was counted using a colony counter and multiplied by the dilution factor to calculate CFU/g of impression.

### Dimensional change test

Standardized metal die as per ADA specification number #19 was fabricated as shown in (Figure 2).



Figure 2: Standardized metal die as per ADA specification number #19.

Twenty impressions of the metal die were made, the impression material was placed on the metal die, covered by glass slab and 1 kg weight was applied to seat the plate firmly against the mold.

Impressions were divided into two groups control and test group.

1. **Control group:** impression was mixed as manufacturer (2-paste putty system).
2. **Test group:** one drop of lotus oil was added for every 3cm of impression material past during mixing of the impression (2-paste putty system).

Standardized photographs of impressions were taken at different time intervals (0, 1, 6, 12 and 24) hours, using digital camera with an ad-hoc device. The device enabled the position of the camera and the distance to the object to be standardized and included an add-on so that all impressions would be placed in the same position (Figure 3).



Figure 3: Ad-hoc device.

The pictures were analyzed with image processing software (Image J) by measuring the length of horizontal Line from the inner profile of the two cross vertical Lines of the impression of stainless steel die (Figure 4). Each measurement was repeated three times and the mean of the three measurements was calculated.

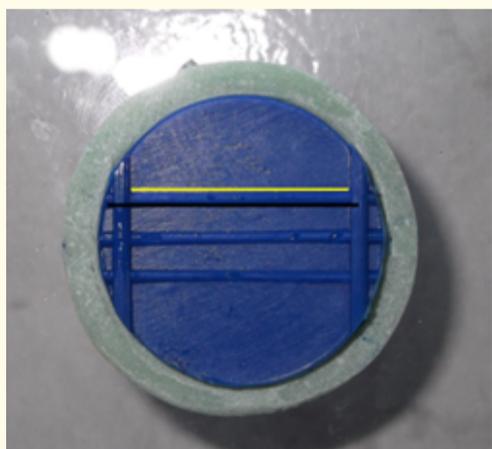


Figure 4: Measuring the distance from the inner profile of the two cross vertical lines of impressions.

The data were subject to analysis of variance (ANOVA) by using Microsoft Excel 2010 to estimate the p-value ( $\alpha < 0.05$ )

Results

Microbiological Results

The results presented showed that statistical analysis using one-way ANOVA for different groups of impression revealed highly significant differences ( $p < 0.05$ ) between the tested groups and the control group. On the other hand, no significant difference ( $p < 0.05$ ) has been shown between the test group of *Staphylococcus aureus* and the control group (Table 1 and Figure 5).

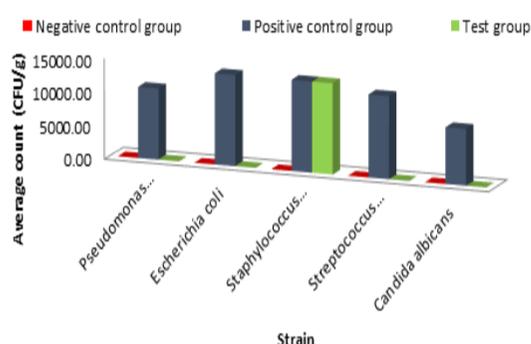


Figure 5: Percentage of reduction the count of examined strains in comparison with the control.

Strain	Count reduction (%)	One-way ANOVA p-value
<i>Pseudomonas aeruginosa</i>	99.57	$3.96 \times 10^{-37}$
<i>Escherichia coli</i>	99.73	$7.84 \times 10^{-40}$
<i>Staphylococcus aureus</i>	0.05	0.9177
<i>Streptococcus mutans</i>	100	$1.78 \times 10^{-33}$
<i>Candida albicans</i>	100	$7.32 \times 10^{-21}$

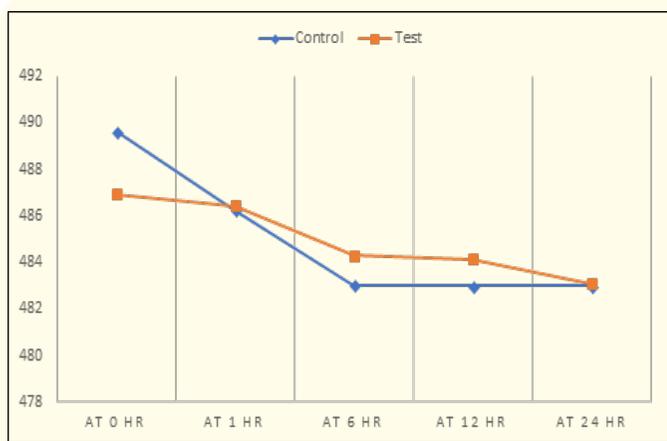
Table 1: Percentage of reduction the count of examined strains in comparison with the control.

The results demonstrated the effectiveness of lotus oil in reducing the count of examined strains in comparison with the control, a single exception was noted in case of *Staphylococcus aureus*.

Dimensional stability results

The results presented showed that statistical analysis using one-way ANOVA revealed significant dimensional change immediately after setting, after 6 and after 12 hours with addition of Lotus oil to impression material and was no significant dimensional change after one and 24 hours in comparison with control group.

There was significant shrinkage with control group after one hour, 6 hours, 12 hours and after 24 hours in comparing with the material immediately after setting. (Table 2 and Figure 6).



**Figure 6:** Comparison between test and control groups regarding dimensional changes at (0, 1, 6, 12 and 24 hours) in pixels.

Length Line	Control	Test	p
At 0 hr	489.64 ± 0.09	486.95 ± 1.13	0.003*
At 1 hr	486.27 <sup>#</sup> ± 0.06	486.43 ± 0.88	0.760
At 6 hr	483.04 <sup>#</sup> ± 0.04	484.29 <sup>#</sup> ± 0.15	< 0.001*
At 12 hr	483.0 <sup>#</sup> ± 0.0	484.14 <sup>#</sup> ± 0.23	< 0.001*
At 24 hr	483.0 <sup>#</sup> ± 0.01	483.09 <sup>#</sup> ± 0.37	0.686

<sup>#</sup>: significant with 0 hr. at p ≤ 0.05

**Table 2:** Comparison between test and control groups regarding dimensional changes at (0, 1, 6, 12 and 24 hours) in pixels.

Normally quantitative data was expressed in mean ± SD and was compared using student t-test.

There was almost no dimensional changes with test group at 1 hour in comparison with the dimension at 0 hour, but there was significant shrinkage at 6 hours, 12 hours and 24 hours in comparison with 0 hours (immediately after setting).

### Discussion

New researches have shown that 67% of materials sent to dental laboratories are infected by various microorganisms [9].

The most frequently identified microorganisms are *Streptococcus* species, *Staphylococcus* species, *Escherichia coli* species, *Actinomyces* species, *Anitratu* species, *Pseudomonas* species, *Enterobacter* species, *Klebsiella pneumonia* and *Candida* species [10].

American dental association (ADA) has issued guidelines to attain the top method of impression disinfection to limit cross contamination during dental clinical and laboratory procedures [11].

The most common techniques for disinfection are immersion and spraying the disinfecting agents on impressions. The method and the time of disinfection process depends on the type of impression material and the ability of the impression material to keep its dimensional stability without any changes after disinfection process [12].

The most common disinfectant materials Glutaraldehyde Iodophores and Phenol, but they have many health effects. Glutaraldehyde could cause throat and lung irritation, difficulty in breathing and asthma, nasal irritation, allergic dermatitis and conjunctivitis. Iodoform as disinfectant material is corrosive and staining. Phenol is toxic and skin irritant [13].

Flower extracts and their essential oils have many traditional uses, such as in the preparation of foods and herbal remedies with minimal known “side-effects” on human health. Being natural, they are accepted to be highly safe for consumption [14].

In current study lotus essential oil was used as disinfectant material for condensation silicon impression material. It was shown that Lotus essential oil was highly effective against *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus mutans*.

The current study showed that lotus essential oil was highly effective against *Candida albicans*. which in agreement with previous study, where Lotus rhizome extract were evaluated against five different strains of fungi and yeast, including *Candida albicans*, *Aspergillus niger*, *A. fumigatus* and *Trichophyton mentagrophytes*; the extract showed potential activity in all the strains tested [15].

Essential oils are secondary metabolites containing a mixture of compounds that are based on 5 carbon isoprene structure (terpenes) occurring as diterpenes, triterpenes, tetraterpenes (C20, C30, and C40), hemiterpenes (C5), and sesquiterpenes (C15). The antimicrobial activity of terpenes involves disruption of cell membrane by their lipophilic constituents [16].

Terpenes are termed terpenoids when they contain additional elements, typically oxygen. Most of the antimicrobial activity in EOs is found in the oxygenated terpenoids (e.g. alcohols and phenolic terpenes), while some hydrocarbons also exhibit antimicrobial effects [17].

The result of current study showed that Lotus oil did not exhibit an inhibition activity against *Staphylococcus aureus*. These results are in conformity with the results obtained by previous study [18].

The dimensional stability of an impression material reflects its ability to maintain the accuracy of the impression over time. These materials should remain stable without further changes for a long period of time [19].

Dimensional accuracy and stability of impression materials may be affected by disinfected materials and the methods of disinfection. Although most of the reviewed authors report occasional statistically significant dimensional changes related to the disinfection procedure, almost all agree that these are not likely to have a clinical significance [20,21].

In current study, Lotus essential oil was added to the impression material during mixing procedure, so there was no possibility for forgetting disinfection of impression before sending it to laboratory or to find that the disinfection process takes time, or it

is tedious as some of dentist consider it.

This study evaluated and compared the dimensional changes occurred in condensation-silicon impression material as a result of addition of lotus essential oil as disinfectant material during mixing, it showed that the impression material had significant shrinkage comparing with control group immediately after setting, this could be explained as, If oxygen is present in terpenoids (component in essential oils that is responsible for the antimicrobial and antiviral activity), its functional nature is generally as alcohol, aldehyde, ketone or carboxylic groups and its evaporation likely affect the dimensional stability of the material [22].

With control group, the impression material showed that maximum shrinkage occurred at first six hours and the material almost stable for next eighteen hours this in agreement with another studies, where dimensional changes have been observed in condensation silicone impressions at all times (ranges from immediately after mouth removal to 13 weeks). There is a consensus that condensation silicone impressions should not be stored for more than 30 minutes following removal from the mouth [2] The production of ethanol as a sub-product of the polymerization and its evaporation likely affect the dimensional stability of the material [3].

The tested group showed dimensional instability after 6 hours and 12 hours comparing with the material immediately after setting and there was no significant difference in dimensional change between tested and control group after twenty-four hours. The results showed that there was no significant dimensional change between tested group and control one hour after setting.

Dimensional changes with tested group comparing with control group, immediately after setting and one hour later were within the standards of ADA specification 19, which states that acceptable dimensional change should be not more than 0.5% [23] So the impression should not be stored for more than one hour. and it is recommended to pour the cast immediately after removing the impression from the mouth.

It was very difficult to compare the results of current study regarding the dimensional changes of condensation silicon impression material after self-disinfecting with Lotus essential oil because the material used for disinfection and method of application did not discuss before and this study is the first one deal with using natural remedy as disinfectant and adding it during impression mixing.

## Conclusion

Promising results of this study encourage self-disinfection of condensation silicon light body impression material by lotus essential oil as it showed:

A highly significant reduction in the count of *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus mutans* and *Candida albicans* immediately after setting of impressions material.

Mixing of lotus essential oil with impression material had no effect on dimensional stability especially after one and twenty-four hours comparing with control. But immediately after settings, impression had dimensional change within the standards of ADA specification 19, which states that acceptable dimensional change should be not more than 0.5%.

## Conflict of Interest

There was no conflict of interest.

This article was represented as poster in Greater New York Dental Meeting 92<sup>nd</sup>, New York 2016.

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