

## Comparison of Microbial Adherence to Silicone Elastomers for Maxillo-Facial Prostheses

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**Abstract:** This study aimed to compare the adhesion of three microorganisms on silicone elastomers materials surfaces used in maxillo-facial prostheses. *Candida albicans*, *Streptococcus mutans* and *Staphylococcus aureus* were incubated separately with silicone elastomers ( $n = 18$ ) for 30 days at 37°C. The counts of viable microorganisms in the accumulating biofilm layer were determined and converted to colony-forming units per cm<sup>2</sup> unit surface area. Scanning electron microscope (SEM) was used to evaluate the microbial adhesion. Statistical analysis was performed using Kruskal-Wallis. Significant differences in microbial adhesion were observed on silicone elastomers after the cells were incubated for 30 days ( $p < 0.001$ ). SEM showed evident of microbial adhesion on silicone elastomers surface.

**Key word:** Adhesion • Silicone Elastomers • Candida Albicans • Streptococcus Mutans • Staphylococcus Aureus

### INTRODUCTION

Silicone elastomers have been used for over 50 years to fabricate maxillo-facial prostheses for individuals with facial defects, however, silicone elastomers exhibits low surface-free energy, hydrophobicity and poor wettability [1, 2]. For in vivo applications; silicone elastomer prostheses are processed against dental stone. The resultant surface is a kind of replica of the surface topography of the dental stone and as such not particularly smooths [3]. Silicone elastomers and denture bases provide an ideal interface for microorganism's colonization and aggregation in the oral cavity [4-7]. On the other hand, are evermore permeable and more susceptible to microbial colonization; and similar to the denture resins, surface irregularities present on silicone elastomers could increase the likelihood of microorganism colonization on their surfaces [8-11]. However, it is equally important to evaluate the adhesion of these microorganisms in vitro due to most of external maxillofacial prosthesis fabrication by silicone elastomers and which combined with defects in the maxilla, tracheal stent, nasal, midfacial and large orbital defects. Hence,

from these combinations create the maxillofacial prostheses non ideal prostheses in size, morphology, extension and locations. This study evaluated and compared the adhesion of three microorganisms on silicone elastomers surfaces for maxillo-facial prostheses.

### MATERIALS AND METHODS

**Samples Preparation:** The silicone elastomer (Si) samples were fabricated using a half sheet of wax sheet pattern and all surfaces were flamed gently to provide a smooth surface texture. This wax sheet pattern was invested in die stone type IV using a water/powder ratio of 23ml/100 gm. The investing die stone was mixed manually and mechanically by using vacuum chamber machine and a split mould poured in dental flask. Separating agents were applied to the desired surfaces of the selected samples. After gypsum material had completely set, the flask was boiled for 10 minutes in eliminating wax unit to melt and eliminate the wax and the two section of the flask were flushed with detergent agent in boiling water followed by flushing with clean water by clean soft bristle brush. Silicone elastomer material was supplied in a disposable

double cartridge system and a mixing ratio of (1:1) (Multisil Epithetik transparent, Germany) and applied on dental gypsum moulds and packed into the mould with 100 bar pressure by using hydraulic press. The material was mixed according to manufacturer's instructions and processed in gypsum mould and curing for 30 mins at 60°C by using polymerization unit to complete polymerization process. The flask was left at the bench in order to cool completely for 2 hours and left in the flask overnight before removal. The samples were fabricated by one operator at 20–25°C room temperatures. Following of polymerisation process, silicone elastomer samples were gently removed from the gypsum moulds and the excess flashes were trimmed away with sterile scissors. The final of the silicone elastomer sheet was cut into rectangular shape samples (10x10x2) mm by using a scalpel and sharp surgical blade #10. An atomic force microscopy (AFM) was employed to measure the surface roughness ( $R_a \sim 0.8 \mu\text{m}$ ) of samples surface. However; following of surface roughness measurement, the samples were cleaned in isopropyl alcohol for 10 min and for 5 min in distilled water in an ultrasonic bath. Furthermore, the Si samples were kept in desiccators for 24 h and then sterilized prior to use in an autoclave at 121 °C for 15 min.

#### Microbial Culture Activation and Growth Conditions:

Three microbial strains of American Type Culture Collection Type (ATCC), namely, *S. mutans* (ATCC 35668), *S. aureus* (ATCC 25923) and *Candida albicans* (ATCC 90028), were obtained from the Department of Microbiology and Immunology, Dental and Medical School of USM. These strains were received as glycerol-preserved stocks stored at –73 °C. To expose the Si samples to a standard microbial suspension in the culture broth, Si material samples placed on the bottom of screw cap Falcon tubes 15 ml capacities by using sterile artery forceps. For *S. mutans*; the suspension was then incubated at 37 °C under anaerobic conditions with 5% CO<sub>2</sub> until an optical density (OD) of 1.0 at 540 nm nephelometric turbidity units was reached. Furthermore, the *S. aureus*; the suspension was incubated constantly at 37 °C under aerobic conditions until an OD of 0.5 at 660 nm nephelometric turbidity units was reached and for *C. albicans* suspension was incubated constantly at 37°C under aerobic conditions until the suspension reached an OD of 0.3 at 540 nm nephelometric turbidity units. A negative control sample of the Si was incubated without *microorganisms* cells inoculum. No microbial growth was observed in the culture medium and the lack

of contamination was verified by Gram staining and light microscopy. Sterile growth media was renewed at an interval of 3 days for 30 days.

**Quantitative Measurement of Microbial Biofilm:** An in vitro microbial adhesion assay was conducted; after the microorganisms were incubated for 30 days. The microorganisms' cells were collected from each sample by gently rinsing and washing twice in sterile phosphate buffer solution (PBS). These steps were carefully performed to clean the incubation broth medium and remove loosely attached *C. albicans* cells. The fixed, attached biofilm cells were scraped from each specimen surface by using a sterile sharp head blade and vortexes for 10 min to diffuse *C. albicans* cells aggregation [12].

**Colony-Forming Units (CFU) Counts:** Microbial biofilms cells and suspension were serially diluted to determine the number of CFU per mL<sup>2</sup>. This method was used to estimate the number of each microorganism on a surface of samples. Each sample surface containing microorganism biofilm was scraped off and suspended in 1.5 mL micro tubes containing 1000 µL of sterile phosphate buffer solution (BPS) and vortexes for 10 min. The final dilution 100 µL of the *S. mutans* dilution cells was spotted on brain heart infusion agar (BHI) plate and both were incubated for 48 h at 37 °C under anaerobic conditions. The *S. aureus* dilution cells were spotted on brain heart infusion agar (BHI) plate and *C. albicans* dilution cells were spotted on Sabouraud dextrose agar (SDA) plate and were incubated for 24 h at 37 °C under aerobic conditions. The number of CFU per agar plates was counted the following day by using an automated multifunctional plate reader (AcoLyte, Model No. 7500, ISYN; UK). The final number of the microorganisms was counted using the following formula: (number of colonies) × 10 × (reverse of dilution value) [13, 14] and the data were used to observe any significant differences of microbial numbers on PMMA materials.

#### SEM Analysis of Microorganism's Morphology:

SEM (Fei, Model Quanta FEG 450; Holland) was used to evaluate the microorganism's attachment to Si surface. The specimens were immersed in 70% ethanol for 15 min to remove the cells and medium debris. Afterward, these specimens were mounted on aluminum stubs by using a double-sided adhesive carbon tape to reduce the overcharging effect on the specimens when

imaging process was coated with pure gold by using a desktop sputtering coating machine (Leica, SCD 005; Austria).

**Statistical Analysis:** Microorganism's counts were converted to CFU/mL<sup>2</sup>, due to nonparametric data analysis, Median and Interquartile rang (IQR) were calculated. The results were statistically analyzed by Kruskal-Wallisad Mann-Whitney to detect significant changes in the microbial counts on Si material surface. SPSS version 20 (IBM) was used for statistical analysis.  $P < 0.05$  was considered statistically significant. SEM images were recorded to support captured data.

## RESULTS

Figure 1 represents the results of the median and IQR of microbial adhesion (CFU/mL<sup>2</sup>) per cm<sup>2</sup> for 30 days. The number of adherent *C. albicans*, *S. mutans* and *S. aureus* cells per mL<sup>2</sup> attached to the Silicone surface was significant difference (Kruskal-Wallis;  $p < 0.001$ ). Mann-Whitney post-hoc U-test further showed that significantly higher adherent of *S. mutans* cells were found on the Si surface than *C. albicans* and *S. aureus* ( $p < 0.001$ ). No significantly differences adherent between *C. albicans* and *S. aureus* cells were also observed ( $p < 0.335$ ).

SEM Figure 2 (a, b and c) shows the SEM results of *C. albicans*, *S. mutans* and *S. aureus* adhered on the Si surface after incubation period at 30 days.

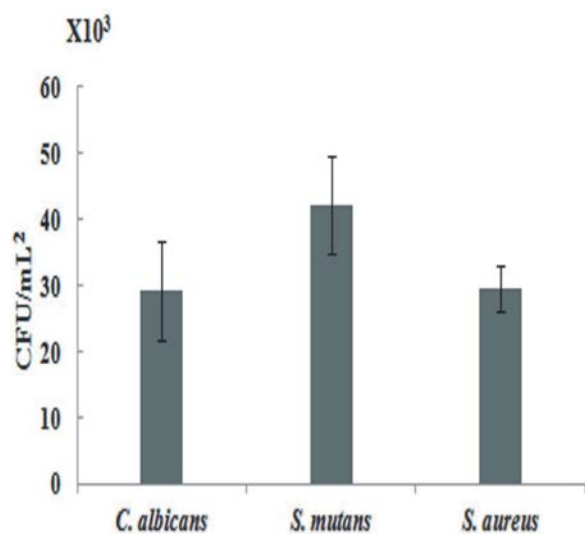


Fig 1: Qualitative biofilm amounts during the experiment. Error bars represent the Interquartile rang (IQR).

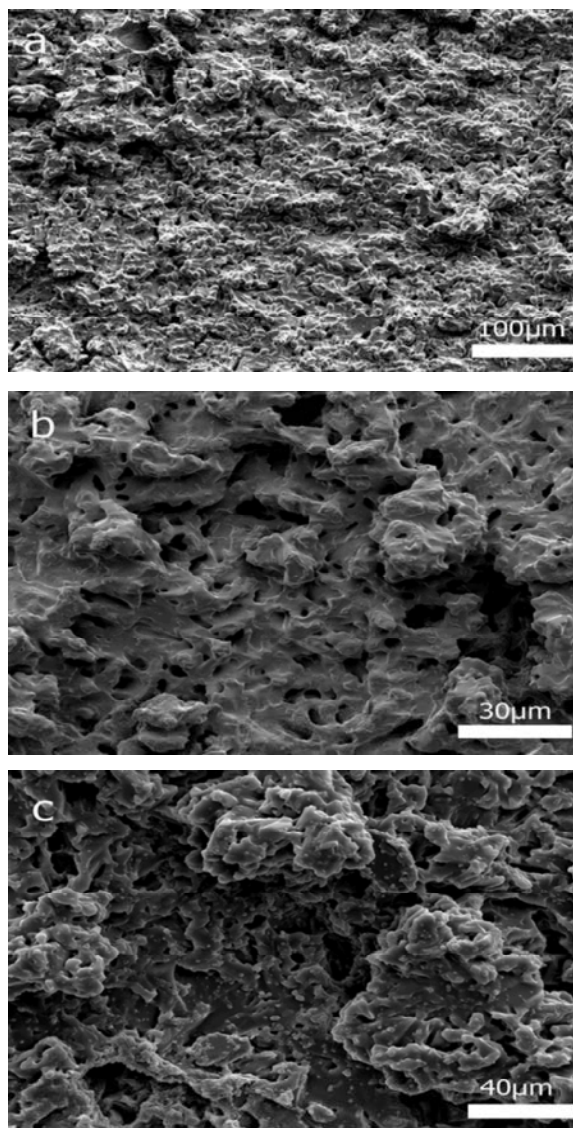


Fig 2: SEM images of different microorganisms on silicone elastomers surface (a) *C. albicans* (b) *S. mutans* (c) *S. aureus*.

## DISCUSSION

Silicone elastomer surface roughness is directly correlated with the roughness of gypsum mould used to fabricate prostheses and is a hydrophobic material and its roughness is directly correlated with the roughness of the handmade stone mould used to fabricate the prostheses [15, 16]. *Candida* sp. is the most commonly found microorganisms on dentures (65.5%), followed by *S. mutans* (53.3%) and *S. aureus* (34.4%) [17, 18]. Further studies suggested that the adhesion of *C. albicans* on

silicone elastomer materials may be different from that in other *Candida* sp. or other microorganisms, such as *S. mutans* or *S. aureus* [19-24]. In this study, the silicone elastomers provide greater adhesion of *C. albicans*, *S. mutans* and *S. aureus*, Figs. 2 (a), (b) and (c). This result is in agreement with other studies attributed to the increase in surface area caused by roughness and porosity favouring microorganism's adhesion [25-27]. The porosity of maxillofacial silicone prostheses and the related physical and biological features can be considered as a drawback for the use of these materials and are a major concern in clinical practice. Moreover, the porosity of prosthetic materials may provide a pathway for microbial invasion and contribute to surface irregularity. In this study, the SEM findings showed fair porosity, surface irregularity and absence of density on the silicone surface behind of microorganisms Figs. 2 (a), (b) and (c). According to these finding of results of surface of silicone in the decrease of surface roughness and the porosity of surface topography, we have to investigate our results by incubation with the prevalent common microorganisms in the oral cavity. Furthermore, the porosity supports microbial accumulation and colonization and this enhances the infection of the silicone elastomers surface. The results of study agree with that of Nevzatoğlu *et al* [28]. However, the results of in-vitro studies should be generalized to the clinical setting with caution and consideration of their limitations in simulating clinical conditions. On the other hand, silicone elastomers are increasingly used for fabrication of external maxillofacial prostheses and no biological analysis is performed prior to their application in clinical setting because there is a general consensus that their application has very limited or negligible health risks for patients.

## CONCLUSION

The constant presence of these microorganisms on external maxillofacial prostheses may contribute to the individual's re-infection, causing undesirable infections and propagation of microorganisms in dental offices, leading to cross-contamination.

**Conflict of interest:** The authors declare that they have no conflicts of conflict.

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