Original Article

Effect of Different Disinfecting Solutions on Some Properties of Maxillofacial Elastomers Over Time

Qaiss B. Al-Jumaili[1], Salim A. Salim[2]*

Abstract

Objective: The use of disinfectant solutions for a long time can cause distortion in the maxillofacial prosthesis. The purpose of this study is to check the efficiency of three disinfectant solutions on bacteria and Candida and to evaluate the changes in the color and the hardness of the silicon with the increasing of the time of disinfection.

Methods: Twenty-eight samples of maxillo-facial silicon with dimensions of 4cm*1cm*3mm for each sample were used and divided into four groups. Group I: Normal saline 0.9 %, Group II: Sodium hypochlorite 3%, Group III: Thymol 2%, and Group IV: Neutral soap. The samples contaminated with isolated *Staphylococcus epidermidis* bacteria and *Candida Albicans*; each group immersed for 10 minutes and then 30 hours in testing solutions evaluating the growth of colonies. Hardness test was done for all testing groups by the use of Shore-A hardness tester after 30 hours of immersion for each group. Color stability was also done by the use of a digital Spectrophotometer for the same samples after the same period of immersion.

Results: Sodium hypochlorite and thymol solutions showed a highly significant difference on bacterial and Candida growth in both time intervals with no effect on hardness and color, Neutral soap showed a poor disinfection effect on microorganisms with a low significant change in the hardness and no effect on color stability.

Conclusions: Sodium hypochlorite 3% and Thymol 2% are very effective disinfection solutions on both bacteria and candida with very little effects on the hardness and color stability.

Keywords: *Maxillofacial, Disinfectant, Silicon hardness, Color stability.*

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1. *Khanzad Training Center, Erbil, Iraq.*
2. *Department of Prosthodontics, College of Dentistry, Hawler Medical University, Erbil, Iraq.*

** Corresponding author: drqais@yahoo.com*

Introduction

Facial expressions and appearance are important in a human's social and personal life. Facial deformities due to trauma, cancer, or congenital anomaly that lowers the patient's self-esteem and willingness to be a part of normal life[1]. Maxillofacial prosthetic rehabilitation aims to restore aesthetics and in some instances, function when vigorous tissue defects are present. Therefore, the aim of facial prostheses is to restore aesthetics and function[2]. There is no single material to date that fulfills all the ideal properties or requirements of maxillofacial prostheses[3]. Current materials used for maxillofacial prostheses are far from ideal, and there is a need for novel improved materials that mimic the patient's natural skin color and texture[4]. Silicone elastomer has achieved wide clinical acceptance, due to its many advantageous properties that consecrate it as the most appropriate material for facial prostheses such as biocompatibility, low chemical reactivity, ease of manipulation, and optical transparency.Furthermore, it can be pigmented to simulate skin tone; therefore, it enhances the aesthetic outcome of the prosthetic device[5,6].

These facial prostheses have a limited lifetime of 1.5-2 years on average[7]. However, this relatively short lifetime of facial prostheses is mainly caused by discoloration, deterioration of the prosthesis material by microbial ingrowth, material rupture, and aging[7-9]. Many studies have reported these changes after exposure to accelerated aging[10,11]. Moreover, significant changes also occurred due to skin secretions[12] and different disinfection treatments[11,13,14]. Color and surface changes, such as hardness, are often two main reasons for replacing a prosthesis since these are the alterations that patients usually perceive and are eye detectable[15-17].

Maintaining hygiene of the prosthesis is important for the health of the soft tissue underneath the prosthesis and for preserving the prosthesis itself in a good condition. Factors such as poor ventilation of the skin, accumulation of moisture, and compromised skin hygiene are presumed to be the most important factors causing skin irritations and infections[18,19]. The surface of the silicone prostheses can act as a reservoir for microorganism and yeast. Surface irregularities increase the possibility of harboring microorganisms, making the surface more difficult to be cleaned[20,21]. It is well known that the *Candida Albicans* (*C. Albicans*) tend to develop biofilms, which consist of a dense network of yeasts, hyphae, and pseudohyphae. This biofilm is highly resistant to the traditional antifungal agents and also to the host immune factors[22]. This makes biofilm-associated *C. Albicans* infections more difficult to treat and has led to the search for a new and effective treatment against biofilm-associated organisms[23].

Nowadays, there are different commercially available solutions such as sodium hypochlorite, chlorhexidine, and neutral soap, which can be used as a disinfectant. In addition to these solutions, it has been observed that plant-derived products (especially essential oils) can influence microbial biofilm production[23,24]. Thymol, one of the major components of thyme oil, has a phenolic structure and is credited with a series of pharmacological properties, including antimicrobial and antifungal effects[25,26], its activity against the sessile cells of *C. Albicans* biofilm has not yet been investigated and, as *C. Albicans* biofilms are known to be important factors underlying its virulence and pathogenicity. Accordingly, this study was designed to investigate the effect of sodium hypochlorite 3%, Thymol 2%, and neutral soap as disinfectant solutions on the growth of different microorganisms on the elastomeric silicon of maxillofacial prosthesis, and to evaluate its effects on the color stability and the hardness of the material with varying intervals of time as an aging process.

Materials and methods

Twenty-eight samples with dimensions of 4cm*1cm*3mm of M511-Addition (Platinum) Silicon rubber material from Technovent Co.TM had been used, which is designed and manufactured especially for the facial prosthesis. Customized metal mold consisted of two parts was designed and locally made larger than the ordinary dental flasks and molds to compensate for the size of a silicon sheet that divided into the mentioned samples. The two parts filled with plaster (type II gypsum) with a spacer of 3 mm thickness of wax sheets and pressed together, after setting of the plaster, parts opened and wax relieved, according to a manufacture instructions, elastomeric silicon of facial prosthesis (Liquid Silicon rubber) had been mixed with a 10:1 base and catalyst ratio by weight.

Intrinsic pigmentation (tan color) added to silicon to get a simulated skin color, a silicon poured in the mold and well distributed in the space, two parts of flask closed and pressed together and inserted in a dry air oven with a 90-degree centigrade for one hour, after cooling, the mold opened and the facial silicon sheet removed and washed out with water for cleaning, then the sheet of silicon cut into 28 pieces with equal dimensions for each to be used as a samples in the study (Figure 1).

The samples divided into four groups, seven samples for each. group, I treated with a normal saline 0.9% as a control group, group II treated with a sodium hypochlorite (NaOCl) 3% from Sigma Aldrich, group III treated with a thymol which is plant extract essential oil (also known as 2-isopropyl-5-methyl phenol, IPMP) which is a natural monoterpenoid phenol derivative of cymene (C10H14O) used as a crystal from Oxford lab chem and it solved in warm water to get 2% solution, group IV treated with a neutral soap as a liquid from natural psychos products.

Figure 1: Silicon material in a customized metal mold.

The following tests had been done for each group:

Bacteriological tests

All samples were contaminated with a *Staphylococcus Epidermidis* which is a Gram-positive facultative anaerobic bacterium isolated from the human skin[27], a swab was taken from each sample and implanted in a blood agar culture media. After 24 hours of incubation in a body temperature degree, bacterial colonies were calculated by colony-forming unit (CFU) for each sample. Samples immersed in their corresponding solutions for 10 minutes, Bacterial colonies calculated for each sample, then the samples immersed for 30 hrs in the same corresponding solutions, and bacterial colonies calculated again. The same procedures were repeated for the previously mentioned groups by using *C. Albicans*, which is opportunistic pathogenic yeast instead of Staphylococcus Epidermidis bacteria by the use of Sebaroud Dextrose Agar (SDA) culture media, *C. Albicans* colonies were also calculated each time by colony-forming unit (CFU) for each sample.

Hardness tests

Samples were tested before and after the disinfection procedures using a digital Shore-A Hardness tester, the method based on placing the sample on the solid plane of the device 12 mm away from the needle, a light pressure was applied on the samples to a full extent, after that the obtained values were calculated within 1 second, and repeat the procedure on different points on the sample with a 6 mm distance between them for 5 times to calculate the average value for each sample. This procedure was done for all samples before immersing them in the solutions, then the 4 groups immersed in their corresponding disinfectant solution and the control for 30 hrs which approximately simulate one year of service considering a daily immersion of the prosthesis in the disinfectant solutions by the patient for 5 minutes[29].

The hardness test was performed for the samples of each group after immersion for 30 hrs, the average reading for each sample was calculated and statistical analysis applied to check the effect of each solution on the hardness of the material.

Color stability test

Color changes were evaluated using a GENESYS 10S UV-Vis digital Spectrophotometer (Thermo Fisher Scientific, USA), which is working by dual-beam geometry with an internal reference detector and scan speed up to 3600 nm/min and wavelength range 190-1100 nm. Through entering the corresponding standard range of wavelength and measuring the absorption value (AV) for each sample before immersing the samples in the solutions. Later on, the four groups of samples immersed in their corresponding solution for 30 hours to simulate one year service assuming a 5 minutes daily treatment[8,30] and the procedure repeated for each sample. The average value was taken and the differences represent the amount of the color change, data collection and statistical analysis applied to check the effect of each solution on color stability.

Statistical analysis

ANOVA test was used to determine the effect of each disinfectant on microbial growth, hardness test and color stability test. Statistical significance was defined as $p \leq 0.05$ and all calculations were conducted using the SPSS software package (version 21; SPSS Inc., Chicago, IL, USA).

Results

Bacterial and fungal culture results

This study used turbidity standard McFarland, the data of Table 1 reveal that there is a significant difference in bacterial growth on silicone after contaminating it with staph epidermidis. This means that the number of colonies before decontamination were >10^5 colony forming units/ml. The bacterial colonies of silicone samples that soaked in normal saline 0.9% (control group) for 10 minutes is about > 10^5 cfu/ml, this indicated that the colonies of all samples were still survive. NaCLO showed no growth of bacteria on any of the 7 samples after 10 minutes of decontamination followed by thymol group in which only two samples were still infected in about >10^3 cfu/ml, while all neutral soap samples remained contaminated but with decreased bacterial colonies.

Table 2 shows a significant difference in bacterial growth on the samples after contaminating it with *staphylococcus epidermidis*. That indicates the number of colonies before decontamination were >10^5 colony forming units/ml.

The bacterial colonies of silicone samples that soaked in normal saline 0.9% (control group) for 30 hours is about > 10^5 cfu/ml, it remained without change in bacterial colonies number on the 7 samples. NaCLO 3% showed no growth of bacteria on any of the 7 samples after 30 hours. Thymol 2% group also showed no growth of bacterial colonies, while all neutral soap samples remained contaminated but with a high reduction in bacterial colonies growth. ANOVA test was used to compare between the groups and p-value was 0.001.

Regarding the fungal growth, Table 3 shows that there is a significant difference in fungal growth on silicone after contaminating it with *Candida Albicans*. The number of colonies before decontamination were >10^5 colonies forming units/ml. The colonies of silicone samples that soaked in normal saline 0.9% (control group) for 10 minutes was about > 10^4cfu/ml, the samples still had candida growth with slight reduction in number. While NaCLO 3% and Thymol 2% groups showed no growth of bacteria on any of the 7 samples of each group after 10 minutes of immersion and decontamination. Followed by neutral soap group in which only two samples were still infected in about >10^3 cfu/ml. ANOVA test was run to compare between the groups and P-value was 0.001.

Table 1: Bacterial decontamination (after 10 minutes).

Groups	No growth	> 10^3 cfu/ml	> 10^4 cfu/ml	> 10^5 cfu/ml	Total	p-value
Normal Saline	0	0	0	7	7	0.001
NaCLO 3%	7	0	0	0	7	
Thymol 2%	5	2	0	0	7	
Neutral soap	0	3	4	0	7	

Table 2: Bacterial decontamination (after 3 minutes).

Groups	No growth	> 10^3 cfu/ml	> 10^4 cfu/ml	> 10^5 cfu/ml	Total	p-value
Normal saline	0	0	0	7	7	0.001
NaCLO	7	0	0	0	7	
Thymol	7	0	0	0	7	
Neutral soap	0	5	2	0	7	

Table 3: Fungal decontamination (after 10 minutes).

Groups	No growth	> 10^3 cfu/ml	> 10^4 cfu/ml	> 10^5 cfu/ml	Total	P-value
Normal saline	0	0	7	0	7	0.001
NaCLO	7	0	0	0	7	
Thymol	7	0	0	0	7	
Neutral soap	5	2	0	0	7	

The data of Table 4 reveal that there is no growth of *C. Albicans*, and the number of colonies was 0 after 30 hours of immersion of each group of Silicon samples in NaCLO 3%, thymol 2%, and neutral soap. While the control group shows minimum growth of fungal colonies on the silicon samples even after 30 hrs of immersion. ANOVA test shown significant differences between the groups, and p-value was 0.001.

Hardness test results

According to data from Table 5, there are no significant changes in the hardness value of silicon material before and even after immersion for 30 hours in the control, NaCLO, and thymol. The only change had happened when the samples immersed in neutral soap, and the obtained value was slightly increased by approximately two hardness units.

Color stability test results

Table 6 shows that there is a clear color shift after treating silicone with different materials, these changes are statistically significant, and p-values are less than 0.05. Regardless of the study group and the material that has been used, the silicone changed in color in all groups, and the absorption unit (AU) decreased on average by one unit.

Table 4: Fungal contamination (After 30 hours).

Study groups	Mean	n	Standard deviation	p-value
NaCLO group / before	28.00	7	0.57	0.68
NaCLO group / after	28.28	7	1.60	
Neutral soap group / before	28.00	7	0.57	0.009
Neutral soap / after	30.28	7	1.38	
Thymol group / before	29.00	7	0.64	0.15
Thymol after / after	29.57	7	0.53	
Control group / before	29.00	7	0.57	0.10
Control after / after	30.28	7	1.43	

Table 5: Change in silicone hardness in different study groups (before and after). Fungal decontamination (after 30 minutes).

	No growth	$> 10^3$ cfu/ml	$> 10^4$ cfu/ml	$> 10^5$ cfu/ml	Total	p-value
Normal saline	0	2	5	0	7	0.001
Na CLO	7	0	0	0	7	
Thymol	7	0	0	0	7	
Neutral soap	7	0	0	0	7	

Table 6: Color of silicone before and after treatment with different study materials.

Study groups	Mean	n	Standard deviation	p-value
NaCLO group / before	28.00	7	0.57	0.68
NaCLO group / after	28.28	7	1.60	
Neutral soap group / before	28.00	7	0.57	0.009
Neutral soap / after	30.28	7	1.38	
Thymol group / before	29.00	7	0.64	0.15
Thymol after / after	29.57	7	0.53	
Control group / before	29.00	7	0.57	0.10
Control after / after	30.28	7	1.43	

Discussion

With the limitation of this study, three disinfectant solutions (sodium hypochlorite 3%, thymol 2%, and neutral soap) used in addition to the use of Normal Saline 0.9% as a control solution.

The results showed that sodium hypochlorite 3% had a high efficacy as a disinfectant solution, it showed zero colonies of *Staphylococcus Epidermidis* bacteria and Zero colonies of *C. Albicans* in both 10 minutes and 30 hours of immersion; this is matched the results of Hashizume et al.[31]; also it did not affect the hardness of the elastomeric silicone after 30 hours of immersion, but on the other hand, there was minimal change in color stability of the material for the same duration of use. Similarly, thymol solution showed the same findings to sodium hypochlorite, with the exception of the antibacterial efficacy after 10 minutes of immersion in thymol was slightly less. Regarding the neutral soap, it showed poor disinfectant results against bacteria and fungi after 10 minutes of immersion and better effect after 30 hrs of immersion, and it showed about two units change of Shore-A hardness test, and one absorption unit change for color stability test.

The null hypothesis was rejected in this study since the hardness and color changes presented differences among the disinfectant materials. The hardness values of the maxillofacial elastomers should remain between the appropriate range is wide (approximately 10-45 Shore-A) and depending on the replacing facial region since the facial area varies in hardness and stiffness[32]. Considering this range of changes in the hardness values of the examined samples could be characterized as clinically acceptable.

Mancuso et al.[33] reported that extrinsic factors, such as the absorption and adsorption of substances, causes discoloration. Goiato et al.[11,13] reported that the chemical cleansing agents could cause damage to the material's physical properties, such as an increase in absorption and solubility especially when the samples are immersed in hypochlorite solution during the disinfection procedure, a porous structure may have formed, and the additives to the silicone materials may promote water absorption and lead to reduce hardness. Finally, they also stated that the choice of a chemical agent for a prosthesis cleansing should be based not only on its antimicrobial properties but also its compatibility, in order to preserve as much as possible, the physical properties of the surface of the materials.

Conclusions

Within the limitation of this study, Thymol 2% is the most suitable disinfection solution that has a high antimicrobial efficacy; moreover, it does not affect the hardness and less effect on color stability of the maxillofacial silicone material. Sodium hypochlorite has high antimicrobial efficacy, but it slightly affects the color of the silicon, while the neutral soap had a poor antimicrobial effect.

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