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Original research

The effect of ozone water disinfection on color stability of nanoparticles reinforced maxillofacial silicones

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Purpose
The aim of this study is to evaluate and compare the color stability of nanoparticles reinforced maxillofacial silicone after disinfection with neutral soap, 4% chlorhexidine, and ozone water.

Materials and Methods
According to ISO 4823, a metal die was fabricated, and 96 samples were created using Room Temperature Vulcanizing silicone (RTV), Heat Temperature Vulcanizing silicone (HTV), and 3% silicone dioxide nanoparticle-reinforced RTV and HTV silicones. The samples were disinfected using neutral soap, 4% chlorhexidine, and ozone water for 10 minutes, three times a day, for 60 days. The samples were divided into four groups: Group 1 (RTV), Group 2 (3% SiO_2 nanoparticle-reinforced RTV), Group 3 (HTV), and Group 4 (3% SiO2 nanoparticle-reinforced HTV). The color stability of the maxillofacial silicones was evaluated before and after disinfection using a UV spectrophotometer. The obtained color stability values were statistically analyzed using two-way ANOVA and Tukey's HSD test. Values were considered significant when $p<0.05$.

Results
The 3% SiO_2 nanoparticle-reinforced HTV silicone showed better color stability compared to HTV and RTV silicones, with the least difference observed in the 3% SiO_2 nanoparticle-reinforced RTV.

Conclusion
Ozone water caused the least change in the color of maxillofacial silicone compared to other disinfectant solutions.

Keywords: *Color stability, disinfection, maxillofacial silicone, nanoparticles, ozone*

Introduction

The aim of each maxillofacial prosthodontist is to restore the patient's esthetics and phonetics, which will improve their self-esteem and help them lead a near-normal life (1). The field of maxillofacial prosthetics primarily deals with the replacement of maxillofacial structures by artificial substitutes, which may be fixed or removable (2).

Color is the prime factor recognized by many patients who wear facial prostheses (3-8). The main reasons for the discoloration of maxillofacial silicone material are microbial in-growth, rupture, and aging. The prosthesis is exposed to various environmental factors such as personal hygiene products, environmental pollution, UV rays, temperature, humidity, skin secretions, and disinfection solutions (9-15). Disinfection is essential for the protection of the prosthesis as well as the surrounding tissues (16).

Hygiene is important for the health of the soft tissues underneath the prostheses. Disinfection is a process used to eliminate microorganisms from the maxillofacial silicone prosthesis without affecting its color and

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mechanical properties. The chemical solution used for disinfection should not elicit any reaction in human tissues and should not affect the properties of the silicone. The choice of disinfectant is based on antimicrobial effect, biocompatibility, and preservation of the material's properties (17). Disinfectants such as effervescent tablets, neutral soap, plant extract, sodium hypochlorite solution, and 4% chlorhexidine are available to disinfect maxillofacial silicone materials (18).

Nanomaterials play a significant role in basic scientific innovation and clinical dentistry by changing the properties of materials (19). Nanomaterials such as titanium dioxide, barium sulfate, ceramic powder makeup, and zinc oxide coloring agents have been used as reinforcement materials in maxillofacial silicones. Materials with small particle sizes have large surface areas and strong interactions between the organic polymer and inorganic nanoparticles (20, 21). Silicon dioxide (SiO_2) nanoparticles (NPs) have more biomedical applications due to their biocompatibility (22-24). SiO_2 NPs are small in size; hence they have strong interactions with the organic polymer (25). Therefore, this study was conducted to evaluate and compare the color stability of maxillofacial silicone reinforced with SiO_2 nanoparticles after disinfection with 4% chlorhexidine, neutral soap, and ozone water. A hypothesis was formulated that there would be no differences in color stability among the maxillofacial silicone materials after disinfection.

Figure 1. *The representative photograph of the master die.*

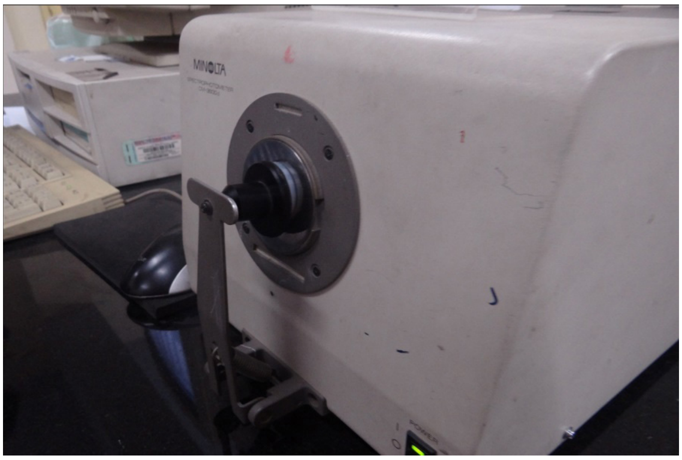

Figure 2. *The spectrophotometer device used in this study.*

Materials and Methods

Experimental design

According to ISO-4823, a master die in the shape of a ring, measuring 3 mm in height and 30 mm in diameter, was created (26) (Figure 1). A total of 96 disc-shaped samples were fabricated using room temperature vulcanizing silicone (RTV silicone, M511, Technovent P&O International, Delhi, India) and heat temperature vulcanizing silicone (HTV silicone, Copsil T-30, TN Resin, P&O International, Delhi, India). Then, 3% SiO_2 nanoparticles (30 to 50 nm) were incorporated into both the RTV silicone and HTV silicone, resulting in 6 samples for each group. The study commenced following the approval of the institutional review board (SRMU/M&HS/SRMDC/2017/F/003).

Sample distribution

The samples made from room temperature vulcanizing (RTV) silicone comprised Group 1. Room temperature vulcanizing (RTV) silicone reinforced with 3% SiO_2 nanoparticles comprised Group 2. Heat temperature vulcanizing (HTV) silicone comprised Group 3. Heat temperature vulcanizing (HTV) silicone reinforced with 3% SiO_2 nanoparticles (NPs) comprised Group 4. Each group was further subdivided based on the disinfection treatment. Samples that were not disinfected were considered as the control (Group A). The samples subjected to disinfection with neutral soap were considered as Group B. Samples disinfected with 4% chlorhexidine were considered as Group C. Samples disinfected with ozone water were considered as Group D.

Sample fabrication

Room temperature vulcanizing (RTV) maxillofacial silicone's base (10 gm) is mixed with 1 gm catalyst (10:1 ratio, totaling 11 gm) on a glass plate for 30 minutes using a stainless-steel spatula to achieve a homogeneous mixture. This mixture is then placed in a vacuum chamber for 20 minutes to remove air bubbles. The mixture is poured into a stainless-steel split mold, which is coated with a special separating medium and allowed to dry for 30 minutes. The samples are left to cure at room temperature (23 ± 2°C) for 24 hours. After curing, the samples are retrieved, and any excess material (flash) is trimmed off with a sharp scalpel. Similarly, heat temperature vulcanizing (HTV) silicone is mixed in a 1:1 ratio and poured into the stainless-steel split mold. It is then allowed to polymerize at 90°C for 1 hour in a hot air oven (Servo Enterprises, Chennai, India).

Experimental sample fabrication

RTV maxillofacial silicone (Technovent M511 RTV silicone, PO Internationals, Haryana, India) base (10 gm) and catalyst (1 gm) were weighed using a digital analytical balance. Initially, SiO_2 nanoparticles (30 to 50 nm) (Aerosil, Pharm, Mumbai, India) were added to the pre-weighed catalyst of the maxillofacial silicone and mixed for 10 minutes. Then, the pre-weighed base was added and mixed for 30 minutes on a clean glass plate using a stainless-steel spatula to achieve a homogeneous mix. This mixture was placed in a vacuum chamber for 20 minutes to obtain an air bubble-free sample. Next, the mixture was poured into a stainless-steel split mold coated with a separating medium and allowed to dry for 30 minutes, followed by polymerization at room temperature (23 ± 2°C) for 24 hours. After curing, the excess material was trimmed off with a scalpel.

The HTV silicone (Copsil T-30 TN resin, PO Internationals, Haryana, India) catalyst and base were taken in a 1:1 ratio. Initially, the SiO_2 nanoparticles were added to the pre-weighed HTV silicone elastomer base and mixed for 30 minutes. Then, the catalyst was added and mixed for 20 minutes on a clean glass plate using a stainless-steel spatula to obtain a homogeneous mixture. This mixture was placed in a vacuum chamber for 30 minutes to acquire an air bubble-free sample. The mixture was then poured into a stainless-steel split mold and allowed to polymerize at 90°C for 1 hour in a hot air oven. Finally, the excess material was trimmed off with a scalpel.

Disinfection procedure

The samples were disinfected using neutral soap (Johnson, Chennai, India), 4% chlorhexidine (Microshield, Chennai, India), and ozone water for 10 minutes. After disinfection, the samples were rinsed with water for 10 seconds. Ozone water was produced by passing a high-voltage electrical discharge at a constant flow rate into the apparatus and injecting it into the diffuser through the output tube at a concentration of 10 ppm. The ozone water was generated in the apparatus for 20 minutes. Ozone is an unstable compound that deteriorates very quickly, with a half-life of 40 minutes at 20°C. The disinfection procedure was repeated three times a day for 60 days. After each disinfection procedure, all samples were stored in a light-proof black box at a controlled temperature of 23 ± 2°C and a relative humidity of 50 ± 10%.

Evaluation of color stability

The color stability of the silicone was evaluated using a UV light reflection spectrophotometer (MINOLTA spectrophotometer CM-3600d, Chennai, India). The color changes were calculated using the CIE Lab* system. In this system, the "L" axis represents brightness, ranging from 0 (black) to 100 (perfect white). The coordinate "a" indicates the amount of red (positive values) and green (negative values), while the coordinate "b" represents the amount of yellow (positive values) and blue (negative values). The system calculates the value of ΔE (color change) between two readings using the following formula (25) : $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$

Statistical analysis

The values were statistically analyzed using SPSS version 17 (IBM, Armonk, NY, United States). The sample size was established using power analysis at a 95% confidence interval ($\alpha = 0.05$). The results were analyzed using two-way ANOVA and Tukey's HSD post hoc test. A significance value of $p<0.05$ was considered statistically significant.

Results

The mean and standard deviation of color change in the maxillofacial silicones after disinfection are listed in Table 1. The within-group comparisons were performed using two-way ANOVA and are presented in Table 2. Multiple group comparisons among the disinfection solutions were conducted using Tukey's HSD test. A significance value of $p<0.05$ indicated significant changes in color among the disinfection solutions for all the maxillofacial silicone materials (Table 3). The samples disinfected with ozone water exhibited the least color change compared to those disinfected with neutral soap and 4% chlorhexidine. Multiple group comparisons among the silicone materials were also conducted using Tukey's HSD test (Table 4). The results showed a significant difference in color change among RTV, HTV, and 3% SiO_2-reinforced RTV and HTV silicones.

Discussion

Maxillofacial silicone is a popular material used for the fabrication of maxillofacial prostheses due to its physical and mechanical properties. Typically, silicone pigmentation is achieved by adding opacifiers and nanoparticles to the base (15, 27). These additions block ultraviolet rays, thereby reducing color instability (18, 28).

Soap consists of water, alkali, and cassia. Evidence of the use of materials like soap dates to 2800 BC. Previous studies have shown that neutral soap has antibacterial and antimicrobial effects (29). Four percent chlorhexidine has a rapid antimicrobial action, making it useful as a topical antibiotic

Table 1. *Mean and standard deviation of color stability.*

Materials	Disinfection Solutions	Mean	Std. Deviation
RTV	Neutral soap	6.6533	1.19709
	4%chlrohexidine	7.9900	1.05451
	Ozone water	4.4000	1.41944
	Total	6.3478	1.91440
3%RTV	Neutral soap	4.0833	.58674
	4%chlrohexidine	6.5700	1.45737
	Ozone water	2.9900	.53826
	Total	4.5478	1.78524
HTV	Neutral soap	7.3533	.97885
	4%chlrohexidine	8.1033	1.04086
	Ozone water	4.1067	.69044
	Total	6.5211	1.98145
3%HTV	Neutral soap	4.2400	.94986
	4%chlrohexidine	5.8417	1.28672
	Ozone water	2.8617	.81969
	Total	4.3144	1.58745
Total	Neutral soap	5.5825	1.72176
	4%chlrohexidine	7.1263	1.50126
	Ozone water	3.5896	1.10624
	Total	5.4328	2.05237

Table 2. Two-way ANOVA results for color stability (R Squared = .782, Adjusted R Squared = .742).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	233.740a	11	21.249	19.516	.000
Intercept	2125.085	1	2125.085	1951.743	.000
Materials	73.001	3	24.334	22.349	.000
Disinfection solutions	150.903	2	75.452	69.297	.000
Materials * Disinfection solutions	9.836	6	1.639	1.506	.192
Error	65.329	60	1.089		
Total	2424.154	72			
Corrected Total	299.069	71			

Table 3. Tukey HSD test for pairwise group comparisons for disinfection solution.

(I) Disinfection Solutions	(J) Disinfection Solutions	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Neutral soap	4%chlrohexidine	-1.5437*	.30122	.000	-2.2677	-.8198
	Ozone water	1.9929*	.30122	.000	1.2690	2.7168
4%chlrohexidine	Neutral soap	1.5437*	.30122	.000	.8198	2.2677
	Ozone water	3.5367*	.30122	.000	2.8128	4.2606
Ozone water	Neutral soap	-1.9929*	.30122	.000	-2.7168	-1.2690
	4%chlrohexidine	-3.5367*	.30122	.000	-4.2606	-2.8128

Table 4. Tukey HSD test for multiple group comparison for maxillofacial silicone The error term is Mean Square(Error) = 1.089.

(I) Materials	(J) Materials	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
RTV	3%RTV	1.8000*	.34782	.000	.8809	2.7191
	HTV	-.1733	.34782	.959	-1.0925	.7458
	3%HTV	2.0333*	.34782	.000	1.1142	2.9525
3%RTV	RTV	-1.8000*	.34782	.000	-2.7191	-.8809
	HTV	-1.9733*	.34782	.000	-2.8925	-1.0542
	3%HTV	.2333	.34782	.908	-.6858	1.1525
HTV	RTV	.1733	.34782	.959	-.7458	1.0925
	3%RTV	1.9733*	.34782	.000	1.0542	2.8925
	3%HTV	2.2067*	.34782	.000	1.2875	3.1258
3%HTV	RTV	-2.0333*	.34782	.000	-2.9525	-1.1142
	3%RTV	-.2333	.34782	.908	-1.1525	.6858
	HTV	-2.2067*	.34782	.000	-3.1258	-1.2875

solution for the skin and as a wound cleanser. It is effective against all kinds of microbial agents and yeast (30, 31). Ozone water has antimicrobial, disinfectant, biocompatibility, and healing properties, which is why it has been proposed for various treatments in dentistry. Thus, neutral soap, 4% chlorhexidine, and ozone water were selected for the disinfection of maxillofacial silicone materials (32).

Polyzois et al. (15) mentioned in their study that a Delta E value greater than 2 units indicates a perceptible color change. All the ΔE values obtained in this research were higher than the threshold value for human eye perception. Therefore, the results indicate that significant color change occurred during the polymerization of the silicone after disinfection with neutral soap, 4% chlorhexidine, and ozone water (33). Nanoparticles act as UV shields; their electrons vibrate when exposed to UV radiation, scattering light among themselves. Thus, the smaller the nanoparticles, the better the protection against solar radiation (34). Nano-sized SiO_2, TiO_2, and ZnO have strong interfacial reactions with the organic polymer. This improves the physical and optical properties of the organic polymer and increases its resistance to environmental aging. Additionally, nanoparticles block UV rays, which enhances the color stability of silicone elastomer (35).

In this research, the samples were disinfected with neutral soap, 4% chlorhexidine, and ozone water for 10 minutes, three times a day, for 60 days, and significant differences in the color of RTV and HTV with 3% SiO_2 NP reinforced RTV and HTV silicones were observed. Group 4, containing 3% SiO_2 NPs reinforced HTV silicone, showed the least change in color after disinfection compared to Groups 1, 2, and 3 maxillofacial silicone materials. Ozone water also had the least effect on color compared to neutral soap and 4% chlorhexidine, thereby rejecting the null hypothesis.

Previous study results showed that titanium dioxide, barium sulfate, ceramic powder makeup, and zinc oxide nanoparticles reinforced maxillofacial silicone exhibited greater color stability (36). The results of the present study confirmed these findings, as SiO_2 NPs have strong interfacial reactions with the organic polymer. Aimee Maria Guiotti et al. (37) found the mean color stability of maxillofacial silicone for neutral soap to be 6.92 (1.56) and for 4% chlorhexidine to be 7.65 (1.35). In the present study, the color stability obtained for room temperature vulcanizing (RTV) silicone was 6.65 (1.19) for neutral soap, 7.99 (1.05) for 4% chlorhexidine, and 4.40 (1.42) for ozone water, which validated the results of the previous study. The results showed statistically significant changes in the color stability of maxillofacial silicone disinfected with neutral soap, 4% chlorhexidine, and ozone water ($P<0.05$). Ozone water showed the least significant difference in color stability compared to neutral soap and chlorhexidine disinfection because ozone water cannot penetrate as deeply as other disinfection solutions (38).

The ΔE value of room temperature vulcanizing (RTV) silicone, with 3% SiO_2 NPs in RTV, exhibited a lower ΔE value than heat temperature vulcanizing (HTV) silicone and 3% SiO_2 NPs in HTV silicone. Therefore, HTV silicone and 3% SiO_2 NP-reinforced HTV silicones can be recommended for the fabrication of maxillofacial prostheses due to their better color stability. A limitation of the study is that the manipulation of maxillofacial silicone material with NPs was done manually, and uniform mixing cannot be ensured. Ozone water caused the least change, but it might still be unacceptable regarding color change. Therefore, clinical correlation of the parameters is required to validate the study's results. Ozone water showed the least change in the color stability of maxillofacial silicone, so it can be used as a disinfection solution for maxillofacial silicones.

Conclusion

The 3% SiO_2 NPs-reinforced HTV silicone showed better color stability compared to HTV and RTV silicones, but the least difference was observed with 3% SiO_2 NP-reinforced RTV. Neutral soap, 4% chlorhexidine, and ozone water showed statistically significant changes in the color stability of maxillofacial silicone. Ozone water caused the least change in the color of maxillofacial silicone compared to other disinfectant solutions; hence, it can be recommended for the disinfection of maxillofacial silicones.

Türkçe öz: *Ozon suyu ile dezenfeksiyonun nanopartiküllerle güçlendirilmiş maksillofasiyal silikonların renk stabilitesi üzerindeki etkisi. Amaç: Bu çalışmanın amacı, nötr sabun, %4 klorheksidin ve ozon suyu ile dezenfeksiyon sonrasında nanopartiküllerle güçlendirilmiş maksillofasiyal silikonun renk stabilitesini değerlendirmek ve karşılaştırmaktır. Gereç ve Yöntem: ISO 4823'e göre bir metal kalıp üretildi ve oda sıcaklığında vulkanize edilen silikon (RTV), yüksek sıcaklıkta vulkanize edilen silikon (HTV) ve %3 silikon dioksit nanopartikül ile güçlendirilmiş RTV ve HTV silikonları kullanılarak 96 numune oluşturuldu. Numuneler, 60 gün boyunca günde üç kez, 10 dakika süreyle nötr sabun, %4 klorheksidin ve ozon suyu kullanılarak dezenfekte edildi. Numuneler şu dört gruba ayrıldı: Grup 1 (RTV), Grup 2 (%3 SiO_2 nanopartikül ile güçlendirilmiş RTV), Grup 3 (HTV) ve Grup 4 (%3 SiO_2 nanopartikül ile güçlendirilmiş HTV). Maksillofasiyal silikonların renk stabilitesi, dezenfeksiyon öncesi ve sonrası UV spektrofotometre kullanılarak değerlendirildi. Elde edilen renk stabilitesi değerleri, iki yönlü ANOVA ve Tukey'nin HSD testi kullanılarak istatistiksel olarak analiz edildi. Değerler, $p < 0.05$ olduğunda anlamlı kabul edildi. Bulgular: %3 SiO_2 nanopartikül ile güçlendirilmiş HTV silikon, HTV ve RTV silikonlarına kıyasla daha iyi renk stabilitesi gösterdi. En az fark, %3 SiO_2 nanopartikül ile güçlendirilmiş RTV'de gözlemlendi. Sonuç: Maksillofasiyal silikonun renginde en az değişikliğe neden olan dezenfektan çözeltisi ozon suyu oldu. Anahtar Kelimeler: renk stabilitesi; dezenfeksiyon; maksillofasiyal silikon; nanopartiküller; ozon*

Ethics Committee Approval: This study has been reviewed and approved by the local ethics committee (SRMU/M&HS/SRMD-C/2017/F/003).

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: ASC, MB participated in designing the study. SD, ASC, MB participated in generating the data for the study. SD, ASC, MB participated in gathering the data for the study. SD, ASC participated in the analysis of the data. SD, ASC participated in writing the paper. SD has had access to all raw data of the study. ASC has reviewed the pertinent raw data on which the results and conclusions of this study are based. SD, ASC, MB have approved the final version of this paper. ASC guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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