THE JOURNAL OF PROSTHETIC DENTISTRY

RESEARCH AND EDUCATION

Ultraviolet C as a method of disinfecting medical silicone used in facial prostheses: An in vitro study

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ABSTRACT

Statement of problem. Hygiene and disinfection are important factors for preserving facial prostheses and supporting tissue health. However, a method that does not accelerate degradation or color change is necessary.

Purpose. The purpose of this in vitro study was to evaluate the effectiveness of irradiation with ultraviolet C light-emitting diode (UV-C LED) light in the disinfection and initial color stability of the silicone (A-588-1; Factor II) used in facial prostheses.

Material and methods. One hundred and twenty specimens were made, contaminated by multispecies biofilm, and divided into 5 groups (n = 24) with different treatments: control, distilled water, 0.12% chlorhexidine, UV-C LED light, and dimethyl sulfoxide (DMSO) as the negative control. Cell viability was measured by the methyl tetrazolium salt method. Statistical analysis was performed by generalized linear models. Additional descriptive analysis was performed for color analysis by using 16 silicone specimens made with light and dark intrinsic coloring in 4 groups (controls and treatments n=4) submitted to UV-C LED light. The ΔE of the specimens was obtained by CIEDE200.

Results. The results of cell viability demonstrated a statistically significant difference among the groups ($P<.001$), with a microbial reduction after UVC-LED exposure compared with the control group. Regarding the color, the groups presented an average ΔE (light 0.205 and dark 0.308) compatible with visually imperceptible changes (light < 0.7 and dark < 1.2).

Conclusions. Irradiation with UV-C LED light decreased the in vitro microbial cell viability of the medical silicone used in facial prostheses, demonstrating initial color stability. (J Prosthet Dent 2021;■:■-■)

Maxillofacial prostheses are provided for head and neck reconstruction[1-15] with the objective of returning the patient to social and family life, promoting activity and confidence.[4,9,13] These prostheses are typically made from silicone, which is selected for its esthetic properties to make the prosthesis more natural.[14]

Even though it is considered the criterion standard in the manufacture of facial prostheses, silicone has disadvantages, including its degradation and color instability,[1,2,5-8,10-13,16-21] which can reduce the lifetime of the prosthesis to between 3 months and 2 years,[1,2,5,6,12-14,16,21,22] with replacements increasing costs and overloading rehabilitation services.[2,4,10-12]

Factors that lead to the degradation and color alteration of maxillofacial prostheses include environmental pollution, sun exposure, high temperature and humidity, the use of adhesives, the deposition of residues and microorganisms,[1,5-10,12,13,19-21,23-26] and skin secretions.[1,5,7,12,27,28] To extend their lifetime, prostheses should be cleaned carefully and appropriately because the use and incorrect handling of materials accelerate degradation and color change, still the main factor for early prosthesis replacement.[29]

Supported by grant no. 88887.488989/2020-00 from the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES)."
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Clinical Implications

According to this study, the UV-C LED is an accessible alternative for reducing the microbial contamination of silicone facial prostheses and improving long-term success.

Hygiene and disinfection are keys to maintaining maxillofacial prostheses and the supporting tissues.[1,8,9,12,13,19] Lack of ventilation, humidity, skin involvement, and the accumulation of biofilm on the surface of the prostheses can trigger skin irritations and infections,[1,2,8,11-13,22,25,30] which can result in a recontamination cycle. The difficulty in cleaning soft prosthetic materials has been documented, as they are permeable and irregular and consequently susceptible to microbial colonization.[1,12,31,32]

Several techniques are available for daily hygiene,[1,2,4-6,8-10,12,13,19,20,25,29,31,33] mainly using mechanical and chemical methods such as brushing or a solution of broad-spectrum disinfectant. However, no technique is completely effective in slowing down degradation, color change, or skin irritation.

Most studies on the hygiene of facial prostheses have focused on biofilms and cleaning the skin around the implants rather than assessing their effectiveness in cleaning the prosthesis itself and have not taken recontamination between the skin and prosthesis into consideration. The present study sought an alternative that is both straightforward to handle and accessible and that promotes microbial reduction in the silicone used for making facial prostheses, knowing that recontamination of the skin and prosthesis is important in the success of long-term rehabilitation.[11]

Ultraviolet C (UV-C) light has been used to reduce microbial contamination in different environments[34-41] and on surfaces through direct interaction with the microorganisms' genetic material, inactivating them and interrupting their contagion cycle. It is a viable and low-cost method, but the authors are unaware of testing on the silicone used for making facial prostheses. Therefore, the present study assessed whether medical silicone specimens (A-588-1; Factor II) used for making facial prostheses can be disinfected by using UV-C light-emitting diode (UV-C LED) irradiation and added a descriptive test to the initial color stability of this method. The hypothesis was that irradiation with UV-C LED light would cause a microbial reduction in silicone specimens and would not promote an initial color change.

MATERIAL AND METHODS

After the experimental treatments, viable microorganisms were quantified by measuring the optical density with the methyl tetrazolium (MTT) salt method,[42,43] providing a continuous quantitative variable. A color reading was made with a spectrophotometer (Delta Vista d8; Delta color), and the color change (ΔE) was calculated by using the CIEDE2000 formula,[44] providing a continuous quantitative variable.

The factors under study were 5 levels of treatment (no treatment control, distilled water, 0.12% chlorhexidine, UV-C LED light, and dimethyl sulfoxide [DMSO]) in a 24-hour biofilm. A sample size calculation was performed from an average Cohen effect size of 0.25, with a power of 0.8 and α=.05, indicating a total of 105 specimens. To account for possible losses, a total of 120 specimens were divided into the 5 groups (n=24).

For the viable microorganism analysis, 120 Ø6×3-mm specimens of medical silicone (A-588-1; Factor II) were made. The manipulated silicone was poured according to the manufacturer's instructions into a 3-mm-high aluminum pan sanitized with 70% alcohol. After the silicone had polymerized, the specimens were cut with a 6-mm sterile punch (Disposable Sterile Dermatological Punch 6 mm; Kolplast LTDA). All specimens were sterilized with ethylene oxide (Sterileno).

Two Gram-positive bacterial strains (*Streptococcus mutans* ATCC25175 and *Staphylococcus aureus* ATCC29213), a Gram-negative bacterial strain (*Escherichia coli* ATCC25922), and a yeast strain (*Candida albicans* ATCC10231) were used to carry out planktonic cultures to obtain multispecies biofilm to simulate a clinical contamination.[1,2,11,45] Each microorganism was cultivated individually in an appropriate culture environment (Müeller-Hinton agar medium for *S. aureus* and *E. coli*, brain-heart infusion medium for *S. mutans*, and Sabouraud dextrose medium for yeast).

From each culture, microbial suspensions made in the respective broth were prepared by using the serial dilution methodology in a 0.9% sodium chloride solution. After determining the microbial suspension concentration, a pool was prepared with the 4 microorganisms, each diluted to a concentration of 1.5×10^8 colony forming units/mL (CFU/mL) in their appropriate broth environment supplemented with 5% sucrose to provide biofilm growth.[42,46] The microorganism pool was used to obtain the multispecies biofilm to evaluate the antimicrobial potential of the proposed treatments.

The silicone specimens were divided into 5 groups (n=24) as follows: Gcontrol (control, without treatment); GH20 (distilled water); GCRX (0.12% chlorhexidine); GUVC LED (UV-C LED light); and DMSO (GDMSO). After being divided into groups, the silicone specimens were individually placed in a 24-well plate (Costar 3524)

inoculated with 1 mL of the microorganism pool suspension, except the DMSO (GDMSO), which was the negative group. The plates were incubated at 37 °C for 24 hours.[1,43]

After contamination, the silicone specimens were placed in a new 24-well plate and subsequently immersed in 2 mL of 0.12% chlorhexidine solution (Therapeutic Art Manipulation Pharmacy) or in distilled water for 10 minutes. The specimens in the UV-C LED treatment group were treated according to the guidelines established by the manufacturer (CleanBag; O2 Led), also for 10 minutes.

After the treatments, the silicone specimens were washed with sterile phosphate buffered saline (PBS) solution (0.15 M NaCl and 10 mM phosphate potassium, pH 7.4) under light agitation for 5 minutes. Nonadherent microorganisms were removed, and the specimens were placed in a new sterile 24-well plate containing 2 mL of the MTT [3- (4,5-dimethylthiazol-2yl) -2,5-diphenyltetrazolium bromide, SIGMA] diluted to a 0.5-mg/mL solution in PBS phosphate buffer.[43] The plates were light protected and kept in an incubator for 4 hours.

The silicone specimens were then transferred to new 24-well plates containing 1 mL of DMSO and agitated in a microplate shaker for 15 minutes to dilute the formazan salts formed after redox reaction in bacteria mitochondria to indicate the viability of the remaining bacteria after treatment.[43] Subsequently, 200 μL of each well were transferred to corresponding wells of a 96-well microplate so that the optical activity could be read on a microplate reader (Epoch ELx800; Biotek), whose filter wavelength was set to 570 nm.[43] The optical densities obtained were statistically evaluated, and the higher the optical density, the greater the cell viability. The group with only DMSO (GDMSO) was included to analyze the possible influences of this solvent on the solution's color, serving as a negative control group that also had its optical density measured and compared.

In addition, a color stability analysis was performed to determine any gross change in color stability that would initially invalidate the method. Sixteen 20×25-mm specimens were made by using the same technique as for the contamination specimens to be evaluated for color change after irradiation with the UV-C LED light. The specimen size was determined according to the specification of the spectrophotometer used. In half of these specimens, an intrinsic pigment (FI SK Pigments; Factor II) was introduced to simulate a light-colored skin, and, in the other half, an intrinsic pigment (FI SK Pigments; Factor II) simulating a dark-colored skin was introduced, resulting in 4 groups (n=4): G control clear; G clear; G dark control; and G dark. The control groups were not exposed to the UV-C LED light, and the light and dark groups were exposed to the UV-C LED light for 10 minutes in the CleanBag.

A spectrophotometer (Delta Vista d8; Delta color) with an optical configuration of D65 illumination, a 10-degree observer, and a computer software program (i7 Gold; Delta color) was used for color measurement. The CIEDE2000 formulation, the most current and accurate formula for detecting small color changes,[44] was used to determine color change (ΔE). The CIEDE2000 perceptibility and acceptability thresholds adopted for color change in light- and dark-skinned maxillofacial elastomers were 0.7 for perceptibility on light skin, 2.1 for perceptibility on dark skin, 1.2 for acceptability on light skin, and 3.1 for acceptability on dark skin.[47]

The data from the quantitative analysis of viable microorganisms did not show the assumptions required by the parametric test (normality of residues and homoscedasticity). For the comparison among the groups, generalized linear models were adjusted considering the gamma distribution fit in the log link function. A statistical software program (IBM SPSS Statistics, v25; IBM Corp) was used for the analysis (α=.05). Color stability was analyzed descriptively (n=4).

RESULTS

The results of the quantity of viable microorganisms (Table 1) demonstrated a statistically significant difference among the groups ($P<.001$). The higher the optical density, the greater the number of viable microorganisms. The color stability test demonstrated that the light material group had an average ΔE of below 1.2, and the dark material group had an average ΔE of below 0.7, which is considered visually imperceptible.[47] These results indicated that the UV-C LED light did not change the initial color of the material, regardless of the amount of pigment used (Table 2).

DISCUSSION

The study evaluated whether irradiation with UV-C LED light disinfected the medical silicone (A-588-1; Factor II) used to make facial prostheses. Additionally, color stability was analyzed in a descriptive manner. The null hypothesis was rejected, as a statistically significant difference was found among the groups in promoting microbial reduction and maintaining the initial color.

Cleaning and disinfecting maxillofacial prostheses is essential for maintenance and tissue health. However, cleaning is a difficult task, especially for older patients with limited manual dexterity or with the visual problems common in facial prostheses users.[1] In addition, materials and techniques that can be used without degrading or changing the color of the prosthesis or causing skin irritation are sparse.

The accumulation of biofilm and use of cleaning agents have been reported to accelerate prosthesis degradation, mainly by changing the color,[1,5-10,12,13,19-21,23-26,29] leading

Table 1. Analysis of optical density mean ±standard deviation as reason of time and treatment with wavelength of 570 nm filter

Experimental Groups	Coefficient	Wald X^2	Exp (β)	Optical DensityTime 24 h	Wald CI (95%)	*P*	Reduction Percentage of Optical Density from Control
Gcontrol (without treatment)	0.973	428.795	2.646	0.2204 ±0.055 A	0.2065-0.2352	<.001	Reference
GH20 (distilled water)	0.531	127.915	1.701	0.1417 ±0.021 B	0.1328-0.1512	<.001	35.71%
GCRX (chlorhexidine 0, 12%)	0.027	0.322	1.027	0.0855 ±0.002 C	0.0801-0.0913	.571	61.21%
GUVC (UV-C LED light)	0.410	76.247	1.507	0.1255 ±0.032 B	0.1176-0.1340	<.001	43.06%
GDMSO (DMSO)	ref	-	1	0.0833 ±0.003 C	0.0780-0.0889	-	62.21%

P<.001. Different letters indicate significant statistical differences with Bonferroni pairwise multiple comparison.

to early replacement and overloading the maxillofacial rehabilitation system. Therefore, the application of irradiation with UV-C LED light for prosthesis maintenance was of interest. The evaluation used a multispecies biofilm that simulated the microbiota composition of a prosthesis in use, and the microorganisms were selected according to previous studies.[1,2,11]

The MTT method was chosen to evaluate the use of LED UV-C light on silicone. It uses optical density measurement to determine the cellular viability of microorganisms after treatments, demonstrating whether there is microbial reduction. The groups that presented the lowest optical density values were the DMSO group (white reading) and the 0.12% chlorhexidine group. These groups were statistically similar and showed statistical differences from the other groups.

These results demonstrate the effectiveness of the 0.12% chlorhexidine group as a positive control (criterion standard) and are consistent with those of previous studies.[1,12] Thus, the 0.12% chlorhexidine group was effective in controlling microbial growth and was statistically superior to the other groups ($P<.05$). This chlorhexidine concentration was used because it has been defined in protocols for this function and it is easily obtainable. Nevertheless, chlorhexidine in different concentrations has been reported to produce color changes in silicone.[20,29]

The group that used the UV-C LED light showed a lower optical density of viable microorganism activity than the control group (no treatment control), being statistically different from each other ($P<.001$). Exposure to the UV-C LED light led to a microorganism reduction of 43.06% when compared with the group with no treatment (Gcontrol). The treatment with the UV-C LED light irradiation was not better than 61.21% chlorhexidine, possibly because of a shadow formed by the non-disorganization of biofilm. UV-C LIGHT is only fully effective when the entire surface is exposed to the irradiation.[34] In addition, the acrylic resin well plates can serve as a barrier to UV-C light.[34]

The device used in this study for irradiating UV-C LED light (Clean Bag) has 2 central LED lights with lower optical densities located in the central specimens of the plate, close to the irradiation source. Therefore, efficacy may have been improved if the specimens had been centralized on the irradiation of the UVC light. Nevertheless, irradiation with the UV-C LED was found to be a suitable alternative for disinfecting facial prostheses, being straightforward and quick to use and requiring minimal manual dexterity or visual acuity.

Table 2. Analysis of ΔE mean ±standard deviation of experimental groups in light- and dark-colored material

Material Color	Groups	ΔE
Light material	Control	0.155 ±0.081
	UV-C LED	0.205 ±0.163
Dark material	Control	0.463 ±0.372
	UV-C LED	0.308 ±0.209

The group that used distilled water (GH2O) obtained similar results to those of the group that used the LED UV-C light (GUVC), differing similarly from the group that was not treated (Gcontrol). This demonstrated that isolated washing is better than nothing, although, in practice, patients would use tap water rather than distilled water. The efficiency of microbial reduction in this group can be explained by washing away the microorganisms.

DMSO is the solvent used in the MTT method and was added as a white reading to prove that this solvent did not influence the color of the analyzed solutions. The DMSO group assists in safer results.

Chlorhexidine, which is considered the criterion standard in the disinfection of facial prostheses, has the disadvantage of altering the color.[20,29] Chamaria et al[29] evaluated the effect of chemical disinfection on the color stability of pigmented and unpigmented maxillofacial silicone elastomers. They used distilled water (control), antimicrobial soap, and 2% chlorhexidine. The color change with chlorhexidine proved to be clinically acceptable (mean ΔE=2.63 unpigmented and 2.42 pigmented), although it was visually perceptible and often led to early prosthesis replacement because of user dissatisfaction. Even distilled water showed a visually noticeable color change (ΔE average=1.82 unpigmented and 1.76 pigmented).

Paravina et al[47] determined the CIELab and CIEDE 2000 perceptibility and acceptability thresholds for color change in light- and dark-colored maxillofacial

elastomers. For light skin, the CIELab perceptibility was 1.1, and the CIEDE 2000 perceptibility was 0.7; the CIELab acceptability was 3.0, and the CIEDE 2000 acceptability was 2.1. The values corresponding to dark skin were 1.6 for CIELab perceptibility and 1.2 for CIEDE 2000; the CIELab acceptability was 4.4, and the CIEDE 2000 acceptability was 3.1.

In the present study, when color stability was further evaluated after irradiation with UV-C LED light, the light-colored material group had an average ΔE (0.205) below 0.7, and the dark-colored material group had an average ΔE (0.308) less than 1.2. This is visually imperceptible, and the results therefore indicate that UV-C LED light does not change the initial color of the medical silicone, regardless of the amount of pigment used. Only a single irradiation of UV-C LED light was used to determine whether a significant change occurred that would make the method infeasible.

Limitations of the present study included the small size of the specimens for contamination, the difficulty of reducing the shadow effect during UV-C LED irradiation caused by no disorganization of the biofilm, the layout of the LEDs on the available equipment, and the absence of long-term monitoring of the effect of UV-C LED light on color change.

Further longer term research should be carried out on actual prostheses to analyze the degradation of the prosthesis and color change and to create an efficient, safe, and economical way to promote the maintenance and longevity of the prosthesis and the health of the supporting tissues.

CONCLUSIONS

Based on the findings of this in vitro study, the following conclusions were drawn:

1. Irradiation with UV-C LED light decreased the microbial cell viability of the medical silicone used in facial prostheses.
2. The method did not adversely affect the color of the silicone and may be suitable for cleaning facial prostheses.

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https://doi.org/10.1016/j.prosdent.2021.06.036