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Ultraviolet C as a method of disinfecting medical silicone used in facial prostheses: An in vitro study – Part 2

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ABSTRACT

Statement of problem. Disinfection is an important factor in preserving facial prostheses and maintaining tissue health. However, whether disinfection with ultraviolet C is an effective disinfection method is unclear.

Purpose. The purpose of this in vitro study was to evaluate the effectiveness of irradiation with different exposure durations of an ultraviolet-C light-emitting diode in the disinfection of the silicone (A-588–1; Factor II) used in facial prostheses.

Material and methods. A total of 216 specimens were prepared, contaminated by multispecies biofilm, and divided into 9 groups (n=24) for different treatments: chlorhexidine 0.12% (G CHG), ultraviolet-C light-emitting diode for 5 minutes (G UVC5), ultraviolet-C light-emitting diode for 10 minutes (G UVC10), ultraviolet-C light-emitting diode for 20 minutes (G UVC20), their respective untreated controls (Gcontrol CHG, Gcontrol UVC5, Gcontrol UVC10, Gcontrol UVC20), and dimethyl sulfoxide (G DMSO) as the negative control. Cell viability was measured by using the methyl tetrazolium salt (MTT) method. Two statistical analyses were performed. First, a 2×3 ANOVA was carried out to compare the control groups (Gcontrol UVC5, Gcontrol UVC10, and Gcontrol UVC20) and the experimental groups of UV-C LED light with different exposure durations (G UVC5, G UVC10, and G UVC20). The second analysis was performed using generalized linear models to compare the optical density of the groups (G UVC5, G UVC10, G UVC20, G CHG, and G DMSO).

Results. Cell viability results demonstrated a microbial reduction after exposure to the ultraviolet-C light-emitting diode for 20 minutes (G UVC20) compared with untreated controls ($P<.05$). The 5- and 10-minute exposures were statistically similar to their respective control groups ($P>.05$). The 20 minutes exposure had the lowest average optical density value, being statistically different from the 5-minute exposure ($P<.05$). A 20-minute exposure to the ultraviolet-C light-emitting diode (G UVC20) was similarly effective when compared with the standard disinfection treatment (G CHG) and dimethyl sulfoxide (G DMSO) ($P>.05$).

Conclusions. Irradiation with an ultraviolet-C light-emitting diode for 20 minutes decreased the in vitro microbial cell viability on the medical silicone used in facial prostheses. (J Prosthet Dent 2024;132:844.e1-e6)

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Clinical Implications

According to this study, exposure to an ultraviolet-C light-emitting diode for 20 minutes could become an alternative disinfection method for silicone facial prostheses, improving long-term success.

The human face translates emotions and allows us to express ourselves to the world. Patients with facial mutilations have serious problems that lead to a low quality of life, and a maxillofacial prosthesis may be indicated to restore function and esthetics, returning people to society.[1–9]

A symbiosis is present in the skin between the resident microbiota and the host. The use of facial prostheses can lead to an imbalance in this harmony, favoring the formation of a niche for bacteria and yeasts. A biofilm can form mainly because of the humidity and temperature between the skin and the prosthesis, generating a cycle of recontamination and requiring the patient to perform proper and rigorous hygiene.

Disinfection of facial prostheses is important and challenging,[1,3,4,8–10] as the disinfection agents and methods currently in use are associated with early prosthesis degradation if handled incorrectly, including color change and skin irritation.[11] Chlorhexidine is the standard for disinfecting facial prostheses[1,3,12]; however, an early color change has been reported at the different concentrations used,[11,13] making it necessary to replace the prosthesis within a short time.[1–6,14–16]

Studies on silicone disinfection by Cevik and Yildirim-Bicer[17] evaluated the effect of various disinfectants and accelerated aging on the change in color and hardness of medical silicone. They concluded that 0.2% chlorhexidine may be a safe method of disinfecting all types of silicone prostheses that require extended clinical use, but perceptible color changes occurred, limiting the duration of use. Perceptible and acceptable changes in the color of the medical silicone used in the manufacture of maxillofacial prostheses were determined by Paravina et al.[18]

Innovative methods for the decontamination of facial prostheses have been studied by Cevik et al[19] evaluated the antimicrobial effects of nanotitanium dioxide and disinfectants on maxillofacial silicones. They reported that the incorporation of nanotitanium dioxide into the silicone as an opacifier was effective and that chlorhexidine was the most effective cleaning agent, but they did not evaluate the change in color and properties of the silicone.

The effectiveness of ultraviolet-C light to disinfect the medical silicone used in the manufacture of facial prostheses and the initial color stability was reported in Part 1 of the present study,[12] determining that it was an effective disinfection method after a 10-minute exposure but was inferior to chlorhexidine. Limitations were also identified, including determining the optimal exposure time and the arrangement of light-emitting diodes (LEDs) for the equipment used.

Ultraviolet-C light has been used for the decontamination of different surfaces because it acts directly on the genetic material of microorganisms, inactivating them and interrupting their contagion cycle.[20–27] In addition ultraviolet-C light disinfection is inexpensive, rapid, does not cause skin irritation, and can be performed by individuals with poor visual acuity, important since most people with facial prostheses are visually impaired.[12]

The objective of this study was to analyze the different durations of application of the method of disinfection with ultraviolet-C LED light using refurbished equipment. The null hypothesis was that a longer exposure time to ultraviolet-C LED light would provide similar disinfection to the standard treatment with chlorhexidine.

MATERIAL AND METHODS

The specimens were made from a medical silicone (A-588–1; Factor II) and were contaminated with a multispecies biofilm and disinfected with chlorhexidine 0.12% (Therapeutic Art Manipulation Pharmacy) and Ultraviolet-C light (CleanBag II; O2 Led). After conducting the experimental treatments, viable microorganisms were quantified by measuring the optical density using the methyl tetrazolium salt (MTT) method,[28,29] resulting in a continuous quantitative variable.

The sample size of 216 specimens (n=24) was determined from a Cohen mean with an effect size of 0.25, with a power of 0.8 and α=.05, and with the sample calculation performed in a previous study as the reference.[12] The methodology used in this study was adapted from that Malateaux et al,[12] who investigated the efficacy of UV-C LED light on the medical-grade silicone used in facial prostheses.

The medical silicone (A-588–1; Factor II) was manipulated and poured according to the manufacturer's instructions into a 3-mm-high aluminum pan, previously cleaned with 70% alcohol. After the polymerization time, the specimens were cut with a 6-mm sterile punch (Disposable Sterile Dermatological Punch 6 mm; Kolplast LTDA) to make 216 Ø6×3-mm specimens which were sterilized with ethylene oxide (Sterileno). The specimens were handled with sterile gloves thereafter.

Two strains of Gram-positive bacteria (*Streptococcus mutans* ATCC25175 and *Staphylococcus aureus*

ATCC29213), a strain of a Gram-negative bacterium (*Escherichia coli* ATCC25922), and a strain of yeast (*Candida albicans* ATCC10231) were used to obtain planktonic cultures and multispecies biofilms to simulate clinical contamination.[1–3,30] Each microorganism was individually cultured 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).[12]

The microbial suspensions of each culture made in the respective broths were manipulated by using the methodology of serial dilution in 0.9% sodium chloride solution. After determining the concentration of the microbial suspension, a pool was prepared with the 4 microorganisms, each diluted to a concentration of 1.5×10^8 colony forming units (CFU)/mL in their appropriate broth environment supplemented with 5% sucrose to promote biofilm growth.[29–31] The pool of microorganisms resulted in a multispecies biofilm, simulating an actual infection and allowing the antimicrobial potential of the proposed treatments to be evaluated.[12]

The silicone specimens were divided into 9 groups (n=24): Gcontrol UVC5 (control – no treatment); G UVC5 (UV-C LED light 5 minutes); Gcontrol UVC10 (control – no treatment); G UVC10 (UV-C LED light 10 minutes); Gcontrol UVC20 (control – no treatment); G UVC20 (UV-C LED light 20 minutes); Gcontrol CHG (control- no treatment); G CHG (0.12% chlorhexidine); and G DMSO (dimethyl sulfoxide). After being divided into groups, the silicone specimens were placed individually in a 24-well plate (Costar 3524; Corning Inc) inoculated with 1 mL of the suspension of the pool of microorganisms, except dimethyl sulfoxide (G DMSO), which was the negative group. The plates were incubated at 37 °C for 24 hours.[1,12,28]

After contamination, the silicone specimens were placed on a new sterile 24-well plate. For the G CHG group, the specimens were immersed in 2 mL of 0.12% chlorhexidine solution (Therapeutic Art Manipulation Pharmacy) for 10 minutes. Specimens from the UV-C LED treatment groups (G UVC5, G UVC 10, and G UVC 20) were subjected to treatments according to the time specified for each group: 5 minutes (G UVC 5), 10 minutes (G UVC 10), and 20 minutes (G UVC 20) using the equipment (CleanBag II; O2 Led) (Fig. 1) with a mirrored interior and no exposure to external light, safe for handling, set and calibrated at a wavelength of 254 nm (USR-200 UV Portable Counter and UV Spectral Irradiance Meter, VERSION v2.00.108; Everfine), with 24 LEDs, centering each specimen arranged on the 24-well plate on an LED (Fig. 2) with direct exposure to light. The control groups (Gcontrol UVC5, Gcontrol UVC10, Gcontrol UVC20, Gcontrol CHG), and DMSO did not undergo any treatment.

After the experimental treatments, the silicone specimens were washed with sterile phosphate buffered saline solution (PBS) (0.15 M NaCl and 10 Mm potassium phosphate, pH 7.4) under mild agitation for 5 minutes to remove microorganisms that had not adhered. Each specimen was then placed on a new sterile 24-well plate containing 2 mL of diluted MTT [3-(4,5-dimethylthiazol-2yl)–2,5-diphenyltetrazolium bromide] (Sigma- Merck) in 0.5 mg/mL of PBS.[28] The specimens were kept immersed in methyl tetrazolium salt (MTT) and in an incubator at 37 °C for 4 hours.

Figure 1. Clean Bag II (O2 Led).

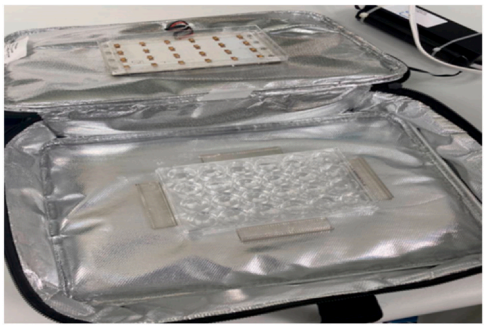

Figure 2. Interior of Clean Bag II (O2 Led) with 24-well plate arrangement.

After 4 hours, the silicone specimens were placed on new 24-well plates containing 1 mL of dimethyl sulfoxide (DMSO) and shaken on a microplate shaker for 15 minutes to dilute the formazan salts that form to indicate the viability of bacteria remaining after treatment.[31] Then, to read the optical density in a microplate reader (EpochELx800, Biotek; Agilent Technologies) with a filter wavelength adjusted to 570 nm,[28] 200 µL from each well were transferred to the corresponding wells of a 96-well plate. The optical densities obtained were statistically evaluated so that the higher the optical density, the greater the cell viability. As a negative control group, for which optical density was also measured and compared, the group with dimethyl sulfoxide (G DMSO) was included to analyze the possible influences of this solvent on the color of the solution.[12]

The increase in time exposure (5, 10, and 20 minutes) in groups treated with UV-C light (independent variables) could lead to an exponential decrease in the

optical density of viable microorganisms (outcome variable). The polynomial degree was analyzed to choose the appropriate approximation function for the response variable. The model fit was analyzed through the forward selection process (from the lowest to the highest degree) with the analysis of the model's significance. With this, the optical densities of viable microorganisms were found to show a linear behavior in the function of the treatment groups. Therefore, a linear model was adopted. A data exploratory analysis was performed to verify the assumptions of a parametric analysis (normality of residuals, homoscedasticity, independence of errors, and additivity of the model). The quantitative analysis of viable microorganisms did not present normal distribution because of the behavior of the G CHG and G DMSO. Therefore, 2 statistical analyses were performed: a 2×3 analysis of variance (ANOVA) for the comparation of the control groups – without treatments (Gcontrol UVC5, Gcontrol UVC10, and Gcontrol UVC20) and the experimental groups of UV-C LED light for different durations (G UVC5, G UVC10, and G UVC20). The second analysis was performed to compare the optical density of the groups (G UVC5, G UVC10, G UVC20, G CHG, and G DMSO) to verify the effectiveness of the experimental treatments. For this comparison, generalized linear models were adjusted by considering the quality of adjustment using the lowest Akaike information criterion (AIC).[32] A statistical software program (IBM SPSS Statistics, v25; IBM Corp) was used for the analysis (α=.05).

RESULTS

The descriptive analysis of the groups showed similar means and medians with a low standard deviation (Table 1). In the analysis of the polynomial degree, a significant linear relationship was observed (P=.033). Thus, the reduction of viable microorganisms occurs linearly during the observed time (Fig. 3). When analyzing the control groups (without treatment) at 5, 10, and 20 minutes, no significant change in optical density with a 570-nm wavelength filter between them was observed (P>.05) (Table 2). However, the results for the UV-C light groups showed that the 20-minute exposure had the lowest average optical density value, being statistically different from the 5-minute exposure (P<.05) (Table 2). The 10-minute exposure was statistically similar to both the 5- and 20-minute exposures (P>.05). In the comparison between the groups with UV-C light and their respective controls (without treatment) at the same analysis time, only the 20-minute UV-C light group was statistically different from the control group at the same time (Table 2). The 5- and 10-minute exposures were statistically similar to those of their respective control groups (P>.05) (Table 2). These findings demonstrate that UV-C light had a greater bactericidal potential at 20 minutes. The comparison between treatments with UV-C light at different exposure times (5, 10, and 20 minutes) and chlorhexidine with DMSO (the reference) showed that the UV-C light 20 and chlorhexidine groups were statistically similar to DMSO (P=.812, P=.849, respectively). However, the groups treated with UV-C light for 5 and 10 minutes were statistically different from DMSO (P<.001, P=.042, respectively). The results demonstrated that UV-C light had the greatest bactericidal potential after an exposure of 20 minutes (Table 3).

Table 1. Descriptive analysis of optical density as function of treatment and time with wavelength of 570 nm filter

Experimental Group	Mean ±Standard Deviation	Median (Minimum-Maximum Values)
Gcontrol CHG	0.1147 ±0.0101	0.1150 (0.10 –0.13)
G CHG	0.0965 ±0.0038	0.0960 (0.09 – 0.11)
Gcontrol UVC5	0.1287 ±0.0173	0.1220 (0.11 – 0.17)
G UV5	0.1368 ±0.0942	0.1175 (0.10 – 0.58)
Gcontrol UVC10	0.1300 ±0.0167	0.1255 (0.11 – 0.18)
G UVC10	0.1143 ±0.0166	0.1120 (0.10 – 0.16)
Gcontrol UVC20	0.1322 ±0.0236	0.1305 (0.10 –0.20)
G UVC20	0.0970 ±0.0049	0.0950 (0.09 – 0.11)
G DMSO	0.0947 ±0.0064	0.0930 (0.09 – 0.12)

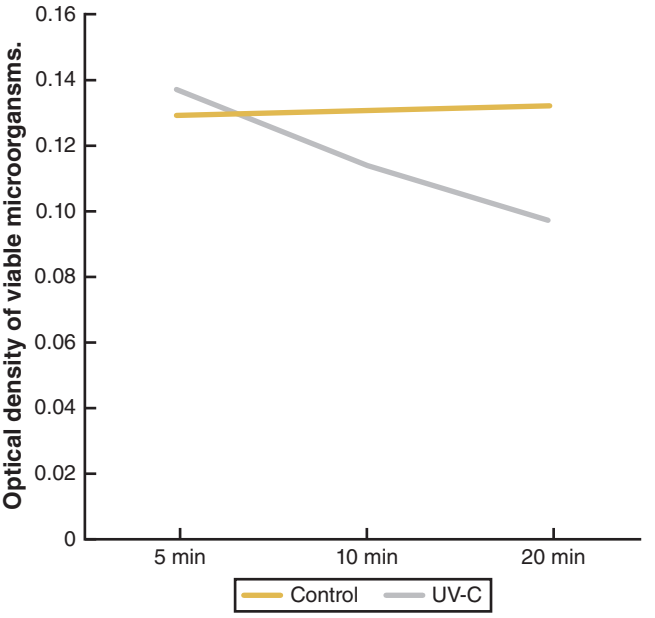

Figure 3. Optical density of viable microorganisms in treatment groups at different times using a 570-nm wavelength filter.

DISCUSSION

The null hypothesis that a longer exposure time to ultraviolet-C LED light would provide similar disinfection to the standard treatment with chlorhexidine was not rejected, as no statistically significant difference was

Table 2. Mean ±standard deviation of optical density as function of treatment and time with wavelength of 570 nm filter

Experimental Groups	Time		
	5 min	10 min	20 min
Control	0.1287 ±0.0173 Aa	0.1300 ±0.0167 Aa	0.1322±0.0236 Aa
UV-C LED	0.1368 ±0.0942 Aa	0.1143 ±0.0166 Aab	0.0970±0.0049 Bb

Different letters indicate statistically significant differences ($P<.05$). Uppercase letters for groups control with UV-C LED within same time (vertical comparison). Lowercase letters compare different time exposures within same treatment (horizontal comparison).

Table 3. Estimation of parameters of optical density as function of treatment with 570-nm wavelength filter

Groups	Regression Coefficients	Wald Chi-squared	*P*
CRX 10 min	0.002	0.036	.849
UVC 5 min	0.042	19.148	<.001*
UVC 10 min	0.020	4.130	.042*
UVC 20 min	0.002	0.057	.812
DMSO	Ref	-	-

found between the group with the longest exposure to UV-C light (G UVC20) and chlorhexidine.

In part 1 of this study,[12] the effectiveness of UV-C LED light was verified for the disinfection of the medical silicone used for making facial prostheses, with a single exposure time of 10 minutes, as recommended by the manufacturer for disinfecting other surfaces and objects. The same study[12] assessed whether the initial color of the medical silicone changed after the exposure time. Promising results were obtained, without any initial color change, but the action of the UV-C LED light was still inferior to that of the standard chlorhexidine, and the authors suggested additional studies to develop the method of disinfection of facial prostheses with UV-C LED light for different lengths of time and remodeling of the equipment used.

The relationship between cell viability based on optical density (the higher the optical density, the greater the cell viability) and the use of UV-C LED light over time was verified in this study part 2, with a linear decrease as the duration of exposure increased. Analysis of the UV-C LED control groups (without treatment) at 5, 10, and 20 minutes revealed no alteration in optical density with a filter with a wavelength of 570 nm. When applying UV-C LED light, a 20-minute exposure was observed to result in the lowest mean value of optical density, being statistically different from the 5-minute exposure group. The optical density of the 10-minute exposure group was statistically similar to both the 5- and 20-minute exposure groups ($P>.05$). Comparing the UV-C-exposed groups and their respective controls (no treatment) with the same durations of exposure, only the 20-minute UV-C LED light group was statistically different from its respective control group ($P<.05$), determining that UV-C LED light had the greatest bactericidal potential after a 20-minute exposure.

Exposure times to UV-C LED light were determined based on the exposure time recommended by the device manufacturer for surface treatment in general (10 minutes), this time being reduced by half in the 5-minute group and doubled in the 20-minute group to create a time-dependent exposure curve and determine the optimal exposure time for the medical silicone used for facial prostheses. However, the function showed a linear behavior.

When comparing all groups, including the 0.12% chlorhexidine and DMSO (negative control) groups, the G CHG, G DMSO, and G UVC20 groups were observed to have lower optical density values, being statistically similar to each other and statistically different from the other groups, demonstrating lower cell viability. DMSO was the solvent used for the methyl tetrazolium salt (MTT) method to dilute the formazan salts formed after redox redaction in bacteria mitochondria to indicate the viability of the remaining bacteria after treatment and was also added as a blank to ensure that it had not influenced the color of the analyzed solutions.

Chlorhexidine, considered the standard treatment for disinfecting facial prostheses,[1,3] has the disadvantage of altering prosthesis color.[11,13,17] The results obtained in this study enabled a comparison between the method of disinfection using chlorhexidine and the method of disinfection using UV-C LED light with an exposure time of 20 minutes, the latter having the possible advantage of not degrading the surface of the medical silicone used to make prostheses or changing their color,[12] both being disadvantages of the chlorhexidine method.[11,13,17] UV-C light, in turn, has been reported not to change the structure and color of other surfaces and has been shown not to change the initial color of medical silicone.[12]

Remodeling the arrangement of LEDs inside the device used also influenced the results. In a previous study,[12] the device had 2 central LED lamps and the more central specimens on the plate had the lowest optical densities, being closest to the irradiation source, suggesting that the efficiency of disinfection can be improved if the specimens are centered under the UV-C LED. In the present study, a device with 24 central LED lamps was made, centralizing the UV-C LED light irradiation on the specimens and obtaining uniform optical densities among specimens, regardless of their position on the plate. The color change that might occur when using this new device format requires further investigation.

The nondisorganization of the biofilm was a limitation in the previous study and may have interfered with the results.[12] However, this limitation was addressed in the present study, as only an increase in exposure to UV-C LED light was sufficient to make the method statistically similar to chlorhexidine, the standard disinfection treatment.

Limitations of the present study included the lack of statistical comparison of the results obtained with the results obtained in the previous study. More research should be carried out on actual prostheses to analyze prosthesis degradation and color change after exposure to UV-C LED light for 20 minutes so that a protocol for its use can be developed.

CONCLUSIONS

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

1. A 20-minute of exposure to UV-C LED light was as effective as the standard disinfection treatment with chlorhexidine
2. The cell viability in medical silicone used for facial prostheses decreased at different exposure times.

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