UNIVERSIDADE PAULISTA
PROGRAMA DE PÓS-GRADUAÇÃO STRICTO SENSU EM ODONTOLOGIA

AVALIAÇÃO DA EFICIÊNCIA DA LUZ ULTRAVIOLETA C COMO MÉTODO DE DESINFECÇÃO DAS PRÓTESES OCULO PALPEBRAIS: ESTUDO IN VITRO.

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Paulista – UNIP, para obtenção do título de Mestre em Odontologia.

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RESUMO

O objetivo deste estudo foi avaliar a eficácia da irradiação com Luz UV-C de LED na desinfecção de protótipos reais de próteses oculos-palpebrais confeccionadas em silicone medicinal (A-588-1; Factor II). No modelo prótese oculo-palpebral, foram confeccionadas 24 amostras e contaminadas, por 24 horas, em um pool de microrganismos simulando uma situação clinica real. As amostras foram divididas em quatro grupos (n=6), sendo eles: sem tratamento; clorexidina 0,12%; luz UV-C de LED; e dimetilsulfóxido (controle branco) e submetidas aos tratamentos propostos. A viabilidade celular dos microrganismos foi mensurada pelo método do sal corante de tetrazólio e as densidades ópticas analisadas estatisticamente. A análise estatística foi realizada por modelos lineares generalizados. Os resultados de viabilidade celular demonstraram diferença estatisticamente significativa entre os grupos (p<0,0001), com redução microbiana após exposição à luz UV-C de LED, em comparação ao grupo sem tratamento. Conclui-se que o tratamento com a luz UV-C de LED diminuiu a viabilidade celular microbiana *in vitro* das próteses faciais confeccionadas em silicone medicinal.

Palavras-chave: reabilitação, luz ultravioleta, desinfecção, próteses faciais, contaminação

ABSTRACT

The goal of this study was to assess the efficacy of ultraviolet-C light emitted by light emitting diodes (UV-C LED) in disinfecting real prototypes of oculo-palpebral prostheses made of medical silicone (A-588-1; Factor II). Twenty-four samples were made using an oculo-palpebral prosthesis model. Subsequently, they were contaminated for twenty-four hours in a pool of microorganisms simulating a real clinical situation. The samples were divided into four groups, namely: no treatment; 0.12% chlorhexidine; UV-C LED; and dimethyl sulfoxide (blank control), and submitted to the proposed treatments. The cell viability of the microorganisms was measured using the tetrazolium salt assay.The optical densities were statistically analyzed. Generalized linear models were used to perform thestatistical analysis. Cell viability results indicated statistically significant differences between groups ($p<0.0001$), with microbial reduction after exposure to UV-C LED irradiation, in comparison to the control group. It was concluded that treatment with UV-C LED decreased the in vitro microbial cell viability of facial prostheses made of medical silicone.

Key-words*:* rehabilitation, ultraviolet light, disinfection, facial prostheses, contamination

SUMÁRIO

Sumário

1. INTRODUÇÃO

As próteses maxilo-faciais são amplamente utilizadas para reconstruções de cabeça e pescoço, com o objetivo de devolver ao paciente afetado pelas deficiências anatômicas, sejam elas por trauma ou por processos patológicos, retorno à vida social e familiar. Tenta-se promover a volta às suas atividades habituais, além de devolver confiança para a realização das tarefas diárias. [1,5]

Atualmente, as próteses faciais são confeccionadas em silicone, por este material possuir diversas características estéticas, apresentando aspecto mais natural. Mesmo sendo considerado o material que apresenta o padrão ouro para a confecção de próteses faciais, o silicone medicinal apresenta algumas desvantagens, sendo elas, rápida degradação e instabilidade da cor. Desse modo, o tempo de vida útil dessas próteses é de cerca de três meses a dois anos, necessitando assim de repetições mais freqüentes, tornando-se um problema devido ao alto custo do material.[1, 6,8,9,10,11,12,13,14]

Dos fatores que levam à degradação e alteração da cor das próteses extra orais podemos citar a poluição, a exposição solar, exposição a altas temperaturas e umidade, a utilização de adesivos que ajudam na sua fixação, a presença de microorganismos e as secreções da pele, presentes no leito receptor da prótese.[1, 6]

A higienização cuidadosa das próteses é de extrema necessidade. No entanto, a forma incorreta de higienização e a utilização de materiais de desinfecção aceleram sua degradação e alteração de cor. Sendo este um dos principais fatores que causam a necessidade de troca ou substituição precoce. A higiene e a desinfecção das próteses são as principais chaves de manutenção das mesmas e dos tecidos de suporte que as sustentam. A falta de ventilação e a umidade presentes na interface prótese e tecidos de sustentação ocasionam o acúmulo do biofilme, resultando na presença de irritações e infecções locais. Gera-se assim um ciclo de recontaminação. As dificuldades na utilização de materiais e substâncias de higiene das próteses maxilo-faciais tem sido documentadas.[1,6]

As técnicas utilizadas atualmente para a higienização e desinfecção protéticas são as mecânicas, como a escovação, e os métodos químicos, que consistem na utilização de soluções de amplo espectro desinfetantes. Contudo, quanto mais efetivas as técnicas de desinfecção, ou a soma delas, maior a degradação, alteração de cor e irritação do tecido de sustentação.[8,9,10,11,12,13,14]

Na busca de materiais alternativos para desinfecção das próteses maxilo-faciais, um estudo cita a luz ultravioleta C como método efetivo para essa função em amostras simples de silicone medicinal.[1] A luz ultravioleta C (UV-C) vem sendo usada para redução da contaminação microbiana em diferentes meios e superfícies.Ela age diretamente no material genético dos microrganismos, desorganizando esse material assim como inativando e interrompendo seu ciclo de contágio, sem mudar a estrutura dimensional dos objetos. É considerado um meio de desinfecção viável e de baixo custo. [15,16,18]

Em busca de técnicas que sejam efetivas na desinfecção das próteses maxilo-faciais, que diminuam a degradação e não cause alteração de cor do silicone, o presente estudo foi desenvolvido com o objetivo de avaliar a eficácia da luz ultravioleta C no desempenho dessa função em protótipos de próteses confeccionadas em silicone medicinal (A-588-1; Factor II).

2. ARTIGO

The Journal of Prosthetic Dentistry

Assessment of the Efficacy of Ultraviolet-C Light as a Method for Disinfection of Oculo-Palpebral Prostheses: *In Vitro* Study

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ABSTRACT

Problem encountered: Infection cycles of observed between the facial prostheses and the tissues that support them.

Purpose: The purpose of the present study was to assess the efficacy of ultraviolet C light as a method of disinfection for prototypes of facial prostheses made of medical silicone (A-588-1; Factor II).

Materials and Methods: Twenty-four prototypes of facial prostheses made of medical silicone were made following a model of oculo-palpebral prosthesis and subsequently contaminated with a pool of microorganisms. They were divided into four groups (n=6), namely: no treatment; 0.12%chlorhexidine; UV-C LED light for 20 minutes; and DMSO (white control and submitted to the proposed treatments). The MTT cell viability assay was used to measure the optical density of the microorganisms present after the proposed treatments.

Results: After measuring the data, a generalized linear model was adjusted to statistically assess the effects of treatments on the viability of microorganisms. All the groups exhibited statistical difference among the min comparison to the untreated group (p<0.05).

Conclusions: The group of prototypes subjected to treatment with UV-C LED light irradiation exhibited lower cell viability in its medical silicone structure. Therefore, it may be an appropriate method for disinfecting maxillofacial prostheses.

Clinical Implications

According to this study, Ultraviolet C Light is an alternative for disinfecting facial prostheses, as it reduces microbial contamination without altering the structure of the prostheses.

Keywords: rehabilitation, ultraviolet light, disinfection, facial prostheses, contamination

INTRODUCTION

Maxillofacial prostheses are widely used for head and neck reconstructions. The objective is to allow patients affected by anatomical deficiencies—whether due to trauma or pathological processes—to return to social and family life. The intention is to promote the performance of normal activities, in addition to restoring confidence in carrying out daily tasks.[1,5]Currently, facial prostheses are made of silicone. This material has several aesthetic characteristics and a more natural appearance. Even though it is considered the gold standard for manufacturing facial prostheses, medical silicone has some disadvantages, such as rapid degradation and color instability. This way, the useful life of these prostheses is about three months to two years, thus requiring more frequent replacements, becoming a problem due to the high cost of the material.[1,6,8,9,10,11,12,13,14]

The factors that lead to the degradation and alteration of the color of extra oral prostheses are pollution, exposure to high temperatures and humidity, sun exposure, the use of adhesives that help in their fixation, the presence of microorganisms, and skin secretions present in the recipient bed. Careful cleaning of prostheses is extremely necessary. However, the incorrect form of cleaning and the use of disinfection materials accelerate prostheses degradation and color change. This is one of the main factors that cause early exchange or replacement. Hygiene and disinfection of prostheses are the main keys for maintaining them and the supporting tissues. The lack of ventilation and the humidity present in the prosthesis-supporting tissues interface cause the accumulation of biofilm, resulting in the presence of local irritations and infections. This process generates a cycle of recontamination. It is worth mentioning that difficulties in using hygiene materials for maxillofacial prostheses have already been documented.[1,6]

The techniques currently used for cleaning and disinfecting prostheses are mechanical, such as brushing, or based on chemical methods, which consist of the use of broad-spectrum disinfectants. However, the more effective the disinfection techniques, or the sum of them, the greater the degradation, color change and irritation of the supporting tissues.[8,9,10,11,12,13,14]

In search of alternative materials for disinfecting maxillofacial prostheses, some studies cite ultraviolet C (UV-C) light as an effective method for this function in simple samples of medical silicone.[1]UV-C light has been used to reduce microbial contamination in different media and surfaces. It acts directly on the genetic material of microorganisms, disorganizing it as well as inactivating and interrupting its contagion cycle. It is considered a viable and low-cost means of disinfection.[15,16,18]

The cycles of infections occurring between facial prostheses and the tissues that support them made it necessary to search for techniques that can be effective in the disinfection of maxillofacial prostheses, without causing changes in the color of the silicone and reducing its degradation. Therefore, the present study was conducted with the objective of assessing the efficacy of UV-C light in disinfecting prototypes of prostheses made of medical silicone

(A-588-1; Factor II).

Material and Methods

The study design was entirely based on using the amount of viable microorganisms as a response variable, measured by optical density, using the MTT assay after treatments. This variable was classified as continuous quantitative. The factors under study were four different treatments—i.e., no treatment, 0.12%chlorhexidine, UV-C LED light, and dimethyl sulfoxide (DMSO) as blank control—and the time of contamination with the pool of microorganisms

(24hours). The sample calculation was performed using an average effect size of 0.25, as classified by Cohen, with a power of 0.8 and $\alpha = 0.05$, totaling 24 samples.

In order to assess the viability of microorganisms, we made a total of twenty-four prototypes of prostheses made of medical silicone (A-588-1; Factor II), following a model of oculo-palpebral prosthesis, based on the photo collection of Instituto Mais Identidade (Brazilian non-profit organization that promotes oral and maxillofacial rehabilitation), with the creation of a digital impression model. After obtaining the model, it was molded with dense condensation silicone and the mold was filled with a colorless mixture of medical silicone (A-588-1; Factor II), as established by the manufacturer's instructions. Before using the prototypes, they were submitted to sterilization with ethylene oxide, performed by Sterileno sterilization company (Araçoiaba da Serra, SP, Brazil).

Two Gram-positive bacterial strains composed of the microorganisms *Streptococcus Mutans* ATCC25175 and *Staphylococcus Aureus* ATCC29213, a Gram-negative bacterial strain composed of the microorganism *Escherichia coli* ATCC25922, and a yeast strain composed of the fungus *Candida albicans* ATCC10231 were used to perform planktonic cultures and obtain multispecies biofilm, with the purpose of simulating a real situation of contamination. Each strain was cultured individually in an appropriate culture medium (Müeller-Hinton agar medium for *S. aureus* and *E. coli*; brain-heart infusion broth for *S. mutans*; and Sabouraud dextrose agar for yeast) to be later used in the experiment.

Mother plates were obtained for each organism, from which replications were subsequently made. From these replicates, microbial suspensions were made in the respective broth media and their concentrations determined through the serial dilution methodology in 0.9% sodium chloride solution. After determining the concentration of the microbial suspensions, a pool was prepared with the four microorganisms, each one diluted at a concentration of 1.5 x 108 colony forming units per ml (CFU/ml), in their appropriate broth media, each being glycosylated with 5% sucrose. This pool was used to obtain the multispecies biofilm for assessing the antimicrobial potential of the proposed four treatments. (Figure 1) Subsequently, the prototypes were divided into four groups, namely: untreated group (control); chlorhexidine group (0.12% chlorhexidine); UV-C LED group (UV-Clight, 20 min); and DMSO group (blank control, which did not undergo any treatment and only had the optical density measured later in the methodology).

After being divided into groups, the prototypes were placed individually in a sterile 100- ml beaker with 40 ml of the suspension containing the pool of microorganisms, in their respective culture media. (Figure 2)The beakers were covered with plastic wrap and incubated at 37°C for 24 hours in an oven. Subsequently, they were submitted to the treatments proposed by the study.

After 24 hours of the contamination process, the prototypes were transferred to new 100-ml beakers containing 40 ml of sterile saline solution (0.9%NaCl) buffered with phosphate (phosphate buffered saline [PBS],0.15 M NaCl,10 mMpotassium phosphate, pH 7.4) to remove non-adhered microorganisms. The prototypes were subsequently washed under agitation for a period of one minute on a stirrer. (Figure 3)Then, they were immersed in 40 ml of 0.12% chlorhexidine solution supplied by Farmácia de Manipulação Arte Terapêutica (São Paulo, SP, Brazil), for the stipulated time of 10 minutes. (Figure 4)The prototypes of the disinfection group with UV-C LED light were treated for a period of 20 minutes, as established for the group by the manufacturer, from the CleanBag® ultraviolet light object purifying device made by O2 Led. This device was developed in the form of a suitcase, with its interior mirrored, carrying a translucent support for the disposition of the prostheses, thus making it possible that all the interfaces of the prostheses received the irradiation of the UV-C LED light. Four UV-C light beams were adapted and used for six suitcases for conducting the present study, so that there was no need to disinfect the suitcases. All of them were calibrated at 254nm, and all the prostheses were arranged in the same position in the device installed inside the suitcase. (Figures 5 and 6)

After the treatments, the prototypes were transferred to new sterile beakers containing

40 ml of a dye solution of the MTT cell viability assay [3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide,(SIGMA, USA)] diluted to 0.5% in PBS. (Figure 7) Subsequently, they were completely wrapped in aluminum foil and transferred to an oven for a period of four hours at

37 °C. After four hours, the beakers were removed from the oven and the prototypes were transferred to new sterile beakers, each containing 40 ml of DMSO, which were shaken for 15 minutes in a stirrer to remove and dilute the formed formazan salts, which indicate the viability of bacteria remaining from the treatment. (Figure 8) Then, we transferred 200 µL from each beaker that held each prototype in duplicate to 96-well plates, which were subsequently taken to read the optical activity in a micro plate reader(BioTek- Epoch ELx800; Sellex Inc., Washington, DC, USA), whose filter wavelength was set at 570 nm (48). (Figure 9) The optical densities obtained were statistically assessed, and the higher the optical density, the greater the cell viability.

A group with only DMSO (GDMSO) was added in this step, so that there was no influence of this solvent on the color of the solutions that would be analyzed. This group served as a blank one that also had its optical density measured and statistically compared. After descriptive and exploratory data analysis, a generalized linear model was fitted in order to assess the effects of the treatments—i.e., no treatment, 0.12%chlorhexidine,UV-C LED, and DMSO -blank control—on the viability of microorganisms. All analyzes were performed using the

R program (R Core Team [2022]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria), with a significance level of 5%.

Results

It can be observed in Table 1 and in Figure 10 that the DMSO group (blank control) exhibited lower viability of microorganisms than the other groups (viability = 0.04;p<0.05). On the other hand, the untreated group exhibited higher viability than the other groups (mean viability = 0.70; ranging from 0.40 to 1.34;p<0.05). The UV-C light group exhibited lower viability (mean viability = 0.25; ranging from 0.22 to 0.36) than the untreated group (p<0.05); however, it exhibitedhigher viability than the chlorhexidine group (mean viability = 0.09; ranging from 0.06 to 0.12) and the DMSO group (p<0.05).

Discussion

The goal of the present study was to assess whether UV-C light was effective in disinfecting facial prostheses made of medical silicone. The null hypothesis was rejected, since there was a statistically significant difference between the groups assessed when the question was the microbial reduction related to the treatments. The rehabilitation of patients with maxillofacial prostheses is a great challenge. Helping integrate them back into society means restoring their quality of life.[1,6]

The disinfectants currently used are responsible for the degradation of prostheses made of medical silicone. These prostheses are composed of a material that confers certain mobility, which makes them even more susceptible to degradation with the use of these agents. The alterations reported in previous studies are related to structure and color, making it necessary, often early, to replace these prostheses, overloading the rehabilitation services provided to these patients.

Ariani (2015) made a comparison between disinfectants, reporting their efficacy for cleaning maxillofacial prostheses, with chlorhexidine being the agent that exhibited the highest efficacy among the compared methods. Malateaux et al. (2021) compared the disinfectants already known and used in the cleaning of prostheses, and added the UV-C light irradiation method. These authors concluded that, in silicone specimens, the method presented promising results regarding the disinfection and maintenance of the specimens, since the chemical agents previously used had degraded the structure of the medical silicone and produced a change in its color.

The present study simulated a real situation of microorganisms present in the supporting tissues of the face and prostheses, a combination that has already been reported in previous studies.[1] In an attempt to obtain results that are more faithful to clinical reality, prototypes of real maxillofacial prostheses were made using an oculo-palpebral model, based on a database of patients who had undergone their rehabilitation at Instituto Mais Identidade.

The MTT assay was chosen to assess the cell viability present in the prototypes after being submitted to the proposed treatments. This method, through optical density, demonstrates whether or not there is microbial reduction, and the greater this density, the greater the viability of microorganisms. The groups with the lowest optical density value were the DMSO group (blank control) and the 0.12% chlorhexidine group.

The results of the present study indicate that the group treated with 0.12% chlorhexidine obtained the best results, which confirms what has already been observed in previous studies, i.e., that this treatment is the gold standard in the disinfection of prostheses, with its concentration in accordance with the described protocols. However, its limitation is observed in reports from these same studies, regarding the degradation that its use causes in prostheses, especially when dealing with the alteration of their color. Malateaux et al. (2021) assessed the color stability of medical silicone after subjecting the specimens to disinfection treatment with UV-C LED. These authors reported a significant difference in promoting microbial reduction and maintaining the initial color of medical silicone.

In the group in which the treatment was performed with UV-C LED, there was less optical activity—which means less activity of microorganisms—in comparison to the control group or the group without treatment, thus being statistically different ($p<0.05$). Exposure of prostheses to UV-C light promoted a reduction in the amount of microorganisms, with lower viability (mean viability = 0.25; ranging from 0.22 to 0.36) than the group without treatment.

The group treated with UV-C LED obtained less satisfactory results than the group treated with chlorhexidine, and the lack of disorganization of the biofilm may be cited as an explanation, since the UV-C light may have penetrated only its most superficial layer.

It is known that UV-C light is not effective in shadow areas. Malateaux et al. (2021) reported that the difficulty in reducing the shadow effect during UV-C LED irradiation caused by the non-disorganization of the biofilm was a limitation. A possible disorganization of the biofilm tends to decrease the shadow area found in this dense biofilm. The DMSO group was added as a blank control group, since this solvent is used in the MTT assay and its optical density could change the optical density of the analyzed solutions, thus making the results found more accurate. It is necessary to conduct further studies addressing the technique assessed in the present study, so that its improvement and its use in real prostheses can become viable.

Conclusions

According to the results obtained in the present study, it was possible to observe lower cell viability in the samples subjected to UV-C light, thus indicating the disinfection effect of this method, i.e., the irradiation of UV-C LED light was effective in promoting the reduction of the microorganisms. Therefore, we can consider that this methodis adequate for disinfecting maxillofacial prostheses. Its clinical use can be of great importance, given that the device has proven its efficiency in promoting the disinfection of the prostheses. This way, with the development of the appropriate protocol, the longevity of the useful life of the prostheses can be increased, since this method does not cause degradation of silicone, the material with which these prostheses are made.

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Figures and Table:

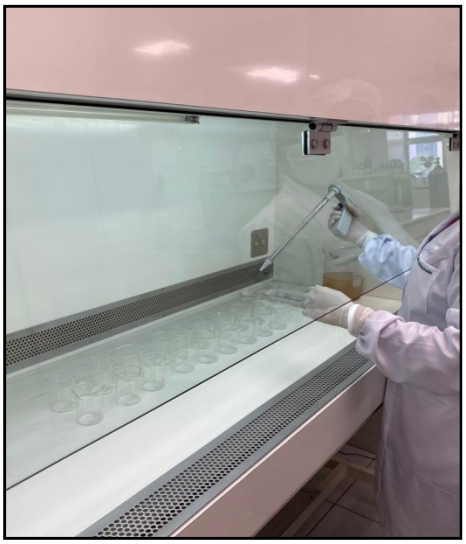

Fig. 1 – Manipulation of the pool of microorganisms.

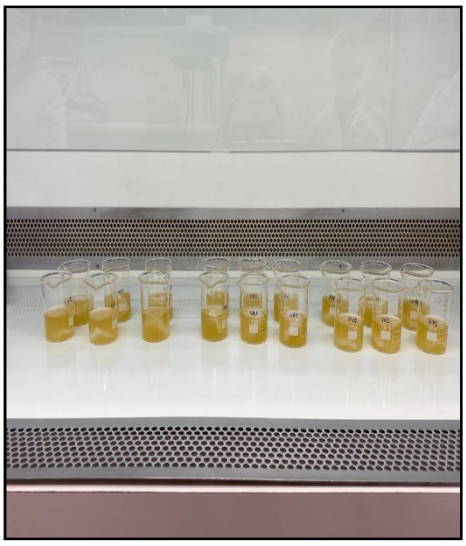

Fig. 2– Prostheses in the manipulation process.

Fig.3– Protheses being washed in a shaker .

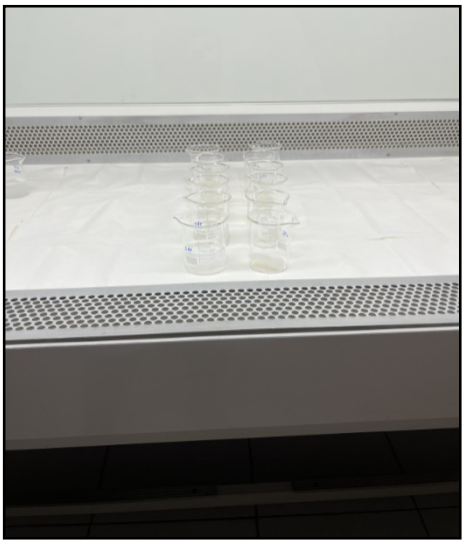

Fig 4–Chloerhexidine treatment group.

Fig. 5–Cleanbag Device.

Fig. 6– UVC Led treatment group.

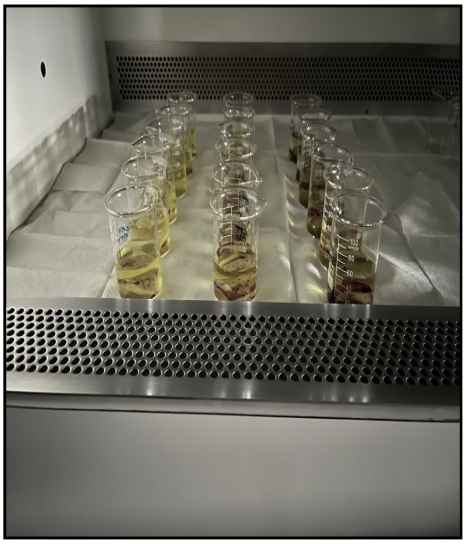

Fig .7 –Prostheses submerged in MTT coloring salt.

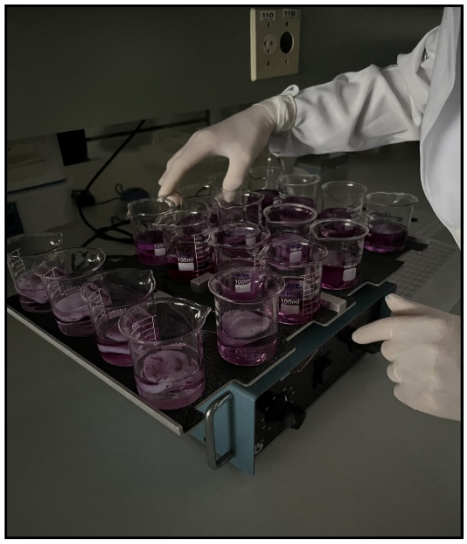

Fig. 8– Prostheses in DMSO solvent.

Fig. 9– 96-well plate.

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Table 1. Viability of microorganisms by the MTT assay in each treatment.

Group	Mean (standard deviation)	Median (minimum and maximum values)
No treatment	0.70 (0.34) a	0.61 (0.40-1.34)
Chlorhexidine	0.09 (0.02) c	0.08 (0.06-0.12)
UV-C light	0.25 (0.05) b	0.23 (0.22-0.36)
DMSO (blankcontrol)	0.04 (0.00) d	0.04 (0.04-0.04)

Note.p<0.0001; different letters indicate statistically significant differences ($p \leq 0.05$).

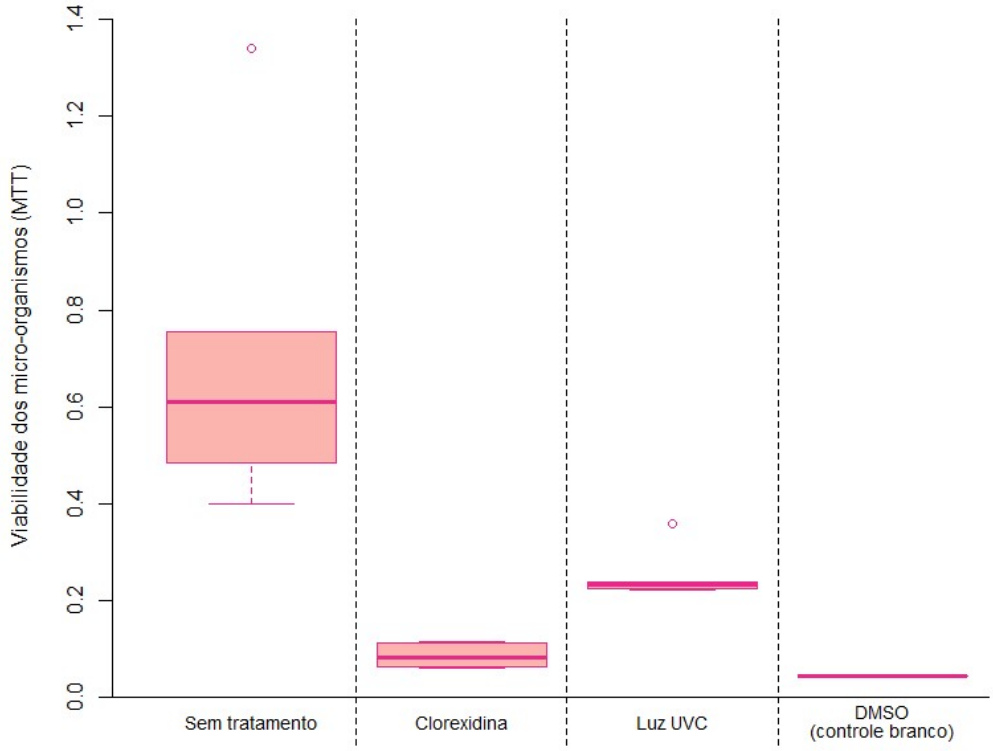

Figure 10. Box plot of the viability of microorganisms by the MTT assay in each treatment.

3. CONCLUSÃO GERAL

De acordo com os resultados apresentados no presente estudo, pode-se identificar menor viabilidade celular nas amostras submetidas à Luz UV-C, ficando demonstrado o efeito de desinfecção desse método, ou seja, a irradiação da luz UV-C de LED foi eficaz em promover a redução dos microrganismos. Dessa forma, podemos considerar um método adequado para desinfecção das próteses maxilo-faciais. Sua utilização clinica pode ser de grande importância, uma vez que o dispositivo comprovou sua eficiência em promover a desinfecção das próteses, dessa maneira, com o desenvolvimento do protocolo adequado, pode-se aumentar a longevidade da vida útil dessas próteses, por se tratar de um método que não causa degradação na cor desse silicone, material com o qual essas próteses são confeccionadas.

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