In Vitro Study of the Microstructural Effects of Photodynamic Therapy in Medical Supplies When Used for Disinfection

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Abstract—**Cleaning and disinfecting surfaces and materials in health services are primary elements in infection control measures. For thermosensitive materials, the chemical agents used have disadvantages such as the odor of the products, which can cause allergic reactions to patients and the nursing staff. Photodynamic therapy (PDT) has been shown to be an effective technique in the treatment of infections caused by different microorganisms; however, nothing is known about the effects of this technique on the microstructure of hospital supplies. This *in vitro* study aimed to evaluate the effects of 0.2% peracetic acid, 1% sodium hypochlorite and PDT with 0.01% methylene blue on the composition and color changes of hospital masks and extensions. For this purpose, 100 mask samples and 100 extension samples were randomly distributed in 20 experimental groups (n = 10, 10 groups for each material), in which the applied substance was varied (sodium hypochlorite, peracetic acid and PDT) and the number of applications (without application, 1, 2 or 3 applications). The compositional analysis was performed by Fourier transform infrared spectroscopy, while the color changes were evaluated using image analysis by CIElab method evaluating the parameters L*, a*, b* and ΔE. The statistical analysis was performed at 5% significance level. It was observed that all agents altered the composition of the materials in a similar way. Although all agents promoted changes in different parameters evaluated, peracetic acid and methylene blue alone altered the final color perceived only in extensions. It was concluded that 0.2% peracetic acid, 1% sodium hypochlorite and PDT alter the chemical composition of both masks and extensions, and that such changes have a positive relationship with the number of treatments performed. These compositional changes may be related to the color changes promoted in both materials by all agents tested.**

Keywords— **Disinfection, laser, medical supply, photodynamic therapy.**

I. Introduction

Currently, the environment in health services has been the focus of special attention to minimize the spread of microorganisms that can cause serious infections, such as from multi-resistant microorganisms. The use of medications by inhalation has led to a greater use of nebulizers and there is a concern that these devices may contribute as a source of bacterial infection [1].

The cleaning and disinfection of surfaces and materials in health services are primary and effective elements in the control measures to break the epidemiological chain of infections. Nebulizers are medical hospital devices that are generally used in the treatment of respiratory tract disorders to relieve inflammatory, congestive and obstructive processes. They are devices that come into contact with colonized intact mucous membranes and minimally require an intermediate level of disinfection always after rigorous cleaning.

Currently, it is recommended that 0.2% peracetic acid (high-level disinfectant) and 1% sodium hypochlorite (intermediate-level disinfectant) be used as disinfectants for nebulizers. These agents are effective in eliminating bacteria, viruses, fungi and spores [1, 2, 3, 4]. However, disinfection by immersion in chemical solutions is operationally complex, presents occupational risks and the possibility of toxic residues remaining. Thus, the search for agents that guarantee the disinfection of medical supplies and that do not present such adverse effects, that are effective and of low cost, is necessary.

Photodynamic therapy (PDT) has been reported as an effective technique for the decontamination of biological tissues, such as periodontal pockets and peri-implant sites in dentistry, as well as skin and mucous membranes. The efficiency of this technique has been proven to eliminate bacteria, fungi and parasites [5]. There are no studies that report the use of PDT for decontamination of hospital devices.

Observing the absence of studies on this topic, we question whether the current disinfection techniques or PDT can physically and chemically modify the structure of medical devices that consist of polyvinyl chloride. The study of such changes may contribute to the proposal of an alternative decontamination technique for these inputs. Therefore, this study aims to evaluate the effects of 0.2% peracetic acid, 1% sodium hypochlorite and photodynamic therapy with methylene blue (PDT) on the composition and color of masks and extensions used in hospital nebulizers.

II. Material and method

For this study, 100 specimens of extension and 100 specimens of nebulizer masks were obtained. The material was cleaned with enzymatic detergent (Proaction -AS110-4E, Grow Química, Brazil) and sectioned in dimensions of 5 mm^2 using a sterile circular cutter. Samples were randomly distributed into experimental groups according to Table 1.

Table 1 Experimental groups of the present study

Experimental group	Material	Treatment	Repetitions
1	mask	Distilled water	1
2		Peracetic acid	1
3			2
4			3
5		Sodium hipo-clorite	1
6			2
7			3
8		PDT	1
9			2
10			3
11	extension	Distilled water	1
12		Peracetic acid	1
13			2
14			3
15		Sodium hipo-clorite	1
16			2
17			3
18		PDT	1
19			2
20			3

For groups 1 and 11, the samples were stored in sterile well plates with 1000µL of distilled water for 10 min. In groups 2, 3, 4, 12, 13 and 14, 0.2% peracetic acid (Proaction Peracetic 0.2%, Grow Química, Brazil) was used. For the treatments, the samples were individually immersed in 1000µL of peracetic acid for 10 min; then, they were rinsed with distilled water and placed in sterile well plates. In groups with repetition, treatments were repeated in the same way, respecting 10-minute intervals between them.

In groups 5, 6, 7, 15, 16 and 17, 1% sodium hypochlorite (Proaction 1%, Grow Química, Brazil) was used. For the treatments, the samples were individually submerged in 1000µL of sodium hypochlorite for 10 min, rinsed with distilled water and placed in sterile well plates. In groups with repetition, treatments were repeated in the same way, respecting 10-minute intervals between them.

In groups 8, 9, 10, 18, 19 and 20, the samples were individually submerged in 1000µL of methylene blue 0.01% at 10 mM (Sigma Aldrich, USA) for 10 minutes; afterwards, laser irradiation was performed for 8 minutes. Then, they were rinsed with distilled water. In groups with repetition, treatments were repeated in the same way, respecting 10-minute intervals between them. For irradiations, it was used a laser (λ = 660 nm, DMC Equipamentos, Brazil), with a fiber diameter of 600 µm, 50 mW, 8 J of total energy delivered, mode continuous with a distance of 1 cm from the sites to be irradiated in order to irradiate the entire well of a 96-well culture plate. Considering the well area (0.31 cm^2), the irradiance of 161.3 mW/cm^2 was obtained.

The compositional analysis was performed by Fourier transform infrared spectroscopy (FTIR). The spectra in the infrared region (4000 to 650 cm^{-1}) were obtained with a Frontier spectrometer (Perkin Elmer, USA), using the ATR accessory (attenuated total reflectance) with a diamond crystal. From each sample, spectra were collected in a central area of 1.5 mm^2. Each spectrum had a background spectrum subtracted during the acquisition and was obtained with 64 scans, with a resolution of 4 cm^{-1}. A descriptive comparative analysis was performed for all obtained spectra using the Origin 8.0 software. The comparison of the intensities of the absorption bands was performed only considering spectra of the same material, and was performed after normalization by the peak of highest intensity found for each material.

The color analyzes were performed adapting the methodology of CAL *et al*. (2006) [6]. For that, digital photos were obtained using a scientific CCD camera mvBlueFOX120a (Matrix Vision, Germany) and an objective lens model #53-301 (Edmund Optics, USA). The illumination was performed by a lighting system composed of three white LEDs arranged in a concentric way and with a standardized distance, avoiding shadow areas. The camera, the lighting system and the sample holder are positioned within a fully closed black box. Before the beginning of the image acquisition and after every 10 images taken, the optical power emitted by the LEDs was measured using a power meter (FieldMaxII, Coherent, USA). To enable the comparative study between the samples of the present study, the same reference sample was used for all images (a reference sample for masks and a reference sample for extensions), which was kept dry and in the same position. Thus, for all images, the samples were positioned two by two (test sample and reference sample), in a standardized position side by side, in a mark made on the sample support plate, to ensure that the energy received by each of them had the smallest possible variation in all acquisitions. To acquire the images, the wxPropView capture software was used for the Labview® environment. The camera gain and exposure time were adjusted to 10 dB and 106µs, respectively. The images were saved in bitmap format. For the analysis of the images, a routine in MATLAB® environment was elaborated to calculate the values of a*, b*, L* and ΔE, which indicates if there were changes in the color of the samples, as follows: $\Delta E^*ab = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$.

III. RESULTS AND DISCUSSION

Figure 1 shows the average FTIR spectra of the extension samples after treatment with peracetic acid. The application of peracetic acid reduced the intensity of the bands with absorption in 2957-2917 cm^{-1} and in 2851 cm^{-1}, which correspond to the group alkanes (CH stretching and bond, respectively). This reduction occurred in a similar way for all treatment repetitions, that is, the application of 1, 2 or 3 times did not cause significant differences in the reduction of the intensity of these bands. The application of peracetic acid also reduced the intensity of the absorption bands located at 1462 cm^{-1} (CH_2). The 873 cm^{-1} band had its complete disappearance after all treatments. There were no new bands, which indicates that there was possibly no adsorption of the agent in the material.

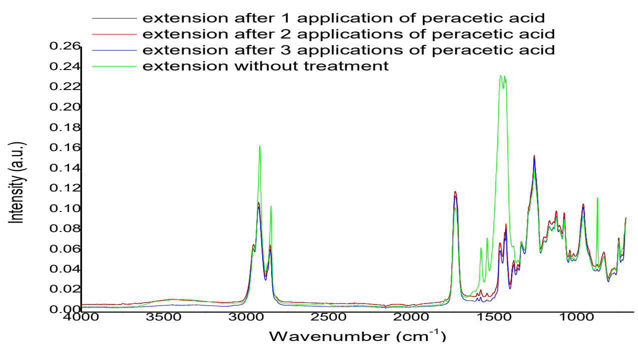

Fig. 1 Average infrared absorption spectrum of extensions before and after treatment with peracetic acid, in the region between 3250 - 2500 cm^{-1}

In the mask samples (Fig. 2), it is observed that this agent promoted a reduction in the intensity of the bands located in 2957-2851 cm^{-1} (CH group), as they have very few absorption bands in the infrared spectrum but, unlike what happened in extension samples, peracetic acid did not change the intensity of the 2957 cm^{-1} band. The 873 cm^{-1} band also completely disappeared after all treatments with peracetic acid. Due to this analysis, we can suggest that there was no total material degradation but that the changes produced by peracetic acid were similar in the two materials tested. Peracetic acid is a toxic and corrosive product, with the molecular formula $C_2H_4O_3$, which acts by oxidizing cellular constituents, releasing active oxygen that interacts with sulfur bonds of proteins, enzymes and other microbial metabolites [3]. This released oxygen can be a degrading agent for certain types of materials. Although the literature shows that this agent does not interfere with the compositional and mechanical properties of certain hospital materials [3], in the present study we show that peracetic acid interferes with the composition of masks and extensions used in hospital nebulizers; however, further studies are needed to clarify whether this chemical modification interferes with the durability of the material.

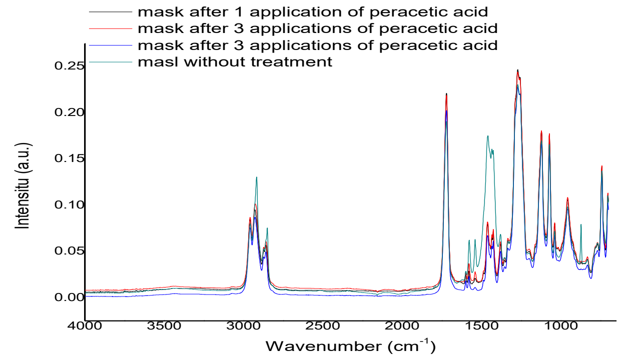

Fig. 2 Average infrared absorption spectrum of masks before and after treatment with peracetic acid, in the region between 4000 - 650 cm^{-1}

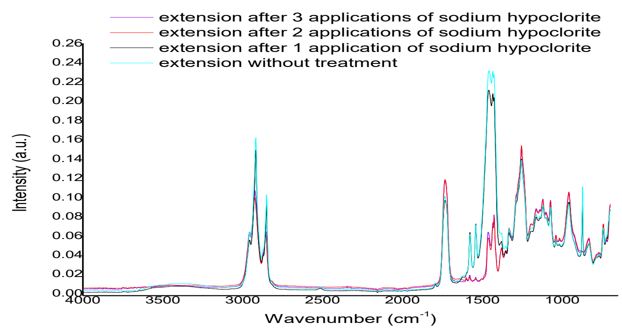

Fig. 3 Average infrared absorption spectrum of extensions before and after treatment with sodium hypochlorite, in the region between 4000 - 650 cm^{-1}

The application of sodium hypochlorite also promotes compositional changes in the extensions, being evidenced by the reduction in the intensity of the absorption bands in 2917 and 2851 cm^{-1} (Fig. 3), 1462-1436-1425 cm^{-1}, which correspond to the CH connection, and complete elimination of the 873 cm^{-1}. In masks, sodium hypochlorite apparently promoted the same chemical changes as peracetic acid (Fig. 4).

Sodium hypochlorite (NaClO) is an agent that dissolves and degrades fatty acids and organic material; also, lead to amino acid degradation and hydrolysis and reduces the surface tension of the remaining solution [4]. This agent is quite toxic in biological tissues, whose action goes beyond the concentration used and, for this reason, a concentration of 1% is recommended for disinfecting surfaces and hospital supplies [1, 2]. In this study, it was found that the action on masks and extensions was very similar to that promoted by peracetic acid, with little chemical modification but that does not imply the complete degradation of the evaluated materials.

In Figure 5, it was noticed that PDT significantly decreases the intensity of the absorption bands of 2917 and 2851 cm^{-1}, in the same way as observed for peracetic acid and sodium hypochlorite. Still, it was observed that the PDT after two applications promoted a displacement of the band from 1735 to 1721 cm^{-1}, as well as a significant increase in the intensity of the bands from 1723 and 744 cm^{-1}.

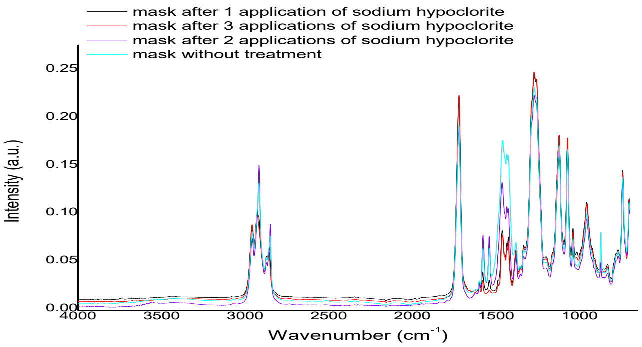

Fig. 4 Average infrared absorption spectrum of masks before and after treatment with sodium hypochlorite, between 3250 - 2500 cm^{-1}

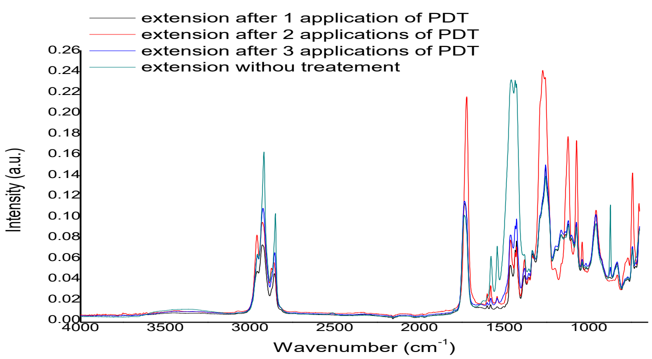

Fig. 5 Average infrared absorption spectrum of extensions before and after treatment with PDT, in the region between 4000 - 650 cm^{-1}

For masks, the effects of PDT were also similar to the effects promoted by the other agents (Fig. 6). It is noted a reduction in the intensity of the 2917, 2851, 1577, 1541, 1461, 1436, 1425, 1258, and 873 cm^{-1} bands (with total elimination of the latter) and an increase in the intensity of the 1720 cm^{-1} band in a similar way to that promoted by the others agents. However, unlike sodium hypochlorite, PDT does not change the 1016 cm^{-1} band.

It is known that during PDT, different reactive oxygen species are formed, such as singlet oxygen and superoxide anion, in addition to different free radicals [5]. According to the results observed in this study, it is suggested that these products are responsible for promoting changes in hospital masks and extensions, probably breaking chemical bonds in functional groups. Thus, even acting through different mechanisms, PDT has effects similar to the effects promoted by sodium hypochlorite and peracetic acid, which can compromise the structure of the materials tested in the long term; however, from the data presented, it is not possible to say whether such changes interfere with the useful life of the materials. If this interference occurs, it is likely to be of the same magnitude as that promoted by agents currently employed. However, it is noteworthy that PDT has advantages over these agents, such as the absence of side effects to biological tissues and the absence of odor, which makes it an attractive technique for use by the hospital team.

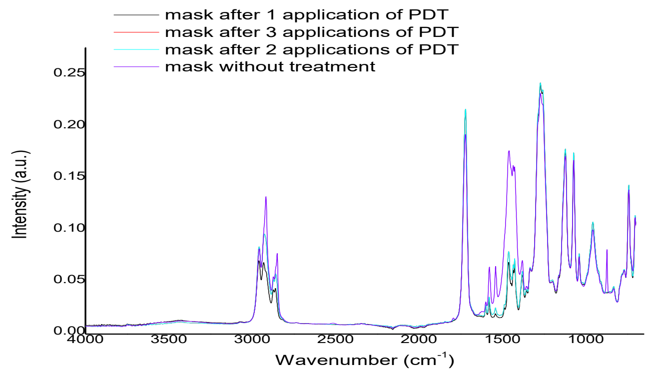

Fig. 6 Average infrared absorption spectrum of masks before and after treatment with PDT, in the region between 4000 - 650 cm^{-1}

Bearing in mind that all the agents tested promoted chemical changes in the masks and extensions, it was evaluated whether such changes can be visually perceptible, through a color change. For these analyzes, the effects of treatments on the parameters ΔL (luminosity, from white to black), Δa (from red (+a*) to green (-a*)), Δb (from yellow (+b*) to blue (-b*)) and ΔE (total color change, perceptible to the human eye when greater than 1) were compared to samples without treatment and treated with different repetitions.

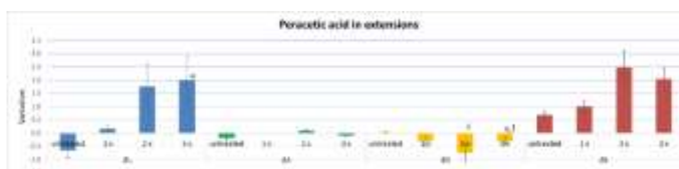

Fig. 7 Variation of ΔL, Δa, Δb and ΔE in the extensions before and after treatment with peracetic acid in different repetitions. The bars indicate standard error. Different letters show statistically different averages at the level of 5% according to the Student-Newmann-Keuls test

It was observed (Fig. 7) that peracetic acid did not promote statistically significant changes in the luminosity (ΔL) of the extensions; however, it changed the parameters Δa positively (became more reddish) and decreased the parameter Δb (became more bluish). In this way, this agent changed the perceived total color (ΔE> 1 after 2 and 3 repetitions).

In masks (Fig. 8), the application of peracetic acid under different repetitions promoted statistically significant changes in the parameters ΔL, Δa and Δb; however, it did not change the perceived total color. This finding is expected because the extensions have a transparent color and, for this reason, changes in the shade of this material are more noticeable than changes in masks, which have a green color.

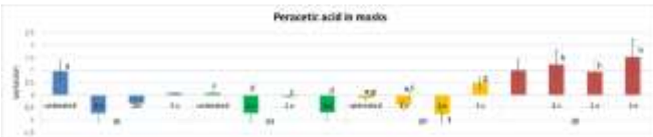

Fig. 8 Variation of ΔL, Δa, Δb and ΔE in the extensions before and after treatment with peracetic acid in different repetitions. The bars indicate standard error. Different letters show statistically different averages at the level of 5% according to the Student-Newmann-Keuls test

The treatment of extensions with sodium hypochlorite statistically increased ΔL and Δa, which means that it increased the luminosity and made the extensions more greenish, thus decreasing Δb (it became more bluish); however, it did not promote significant changes in the final perceived color of the extensions, as can be seen in Figure 9.

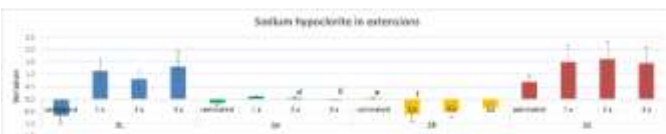

Fig. 9 Variation of ΔL, Δa, Δb and ΔE in extensions before and after treatment with sodium hypochlorite in different repetitions. The bars indicate standard error. Different letters show statistically different averages at the level of 5% according to the Student-Newmann-Keuls test

In the masks, the treatment with sodium hypochlorite only significantly decreased Δa (made it more greenish), but did not promote changes in the other parameters, that is, sodium hypochlorite also does not change the color of hospital masks, even after 3 repetitions of treatments (Fig. 10). These results suggest that the masks are composed of material more resistant to the action of sodium hypochlorite. Although the masks and extensions are composed of the same material (polyvinyl chloride), they have some differences related mainly to the presence of plasticizer [7]. The plasticizers widely used in health products are Di-isononyl phthalate and Trioctyltrimelite, which have an aromatic ring and ester group, and confer different mechanical and chemical characteristics for the materials. In this way, these materials can be broken down in different ways by the different disinfecting agents used in this work.

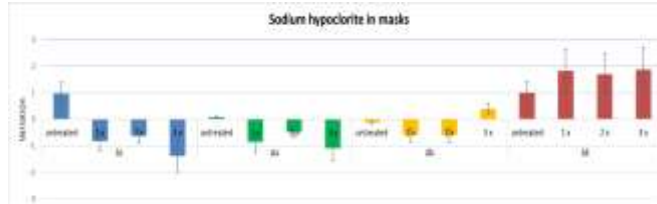

Fig. 10 Variation of ΔL, Δa, Δb and ΔE in masks before and after treatment with sodium hypochlorite in different repetitions. The bars indicate standard error. Different letters show statistically different averages at the level of 5% according to the Student-Newmann-Keuls test

The effect of methylene blue alone on the color of both extensions and hospital masks was also evaluated (Fig. 11 and Fig. 12). It was noticed that methylene blue alone promotes an increase in the parameter ΔL (increases the luminosity) and a significant decrease in the parameters Δa and Δb (makes the extensions greener and more bluish), which causes a significant change in the perceived color (ΔE > 1).

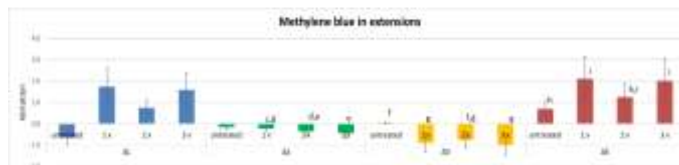

Fig. 11 Variation of ΔL, Δa, Δb and ΔE in extensions before and after treatment with methylene blue in different repetitions. The bars indicate standard error. Different letters show statistically different averages at the level of 5% according to the Student-Newmann-Keuls test

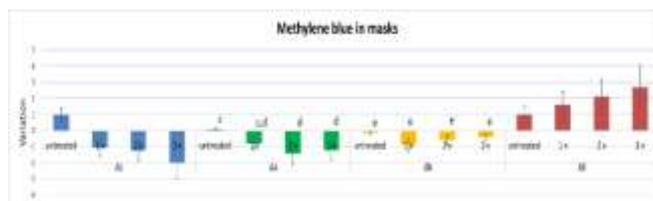

Fig. 12 Variation of ΔL, Δa, Δb and ΔE in the masks before and after treatment with methylene blue in different repetitions. The bars indicate standard error. Different letters show statistically different averages at the level of 5% according to the Student-Newmann-Keuls test

In masks, methylene blue alone significantly reduced luminosity, Δa and Δb (makes extensions greener and more bluish); however, although there was a tendency to change the total color of the samples, this trend was not statistically significant ($p < 0.05$). Methylene blue is a blue colorant with a $C_{16}H_{18}ClN_3S$ structure, which is responsible for such coloration. From the results of this work, even the few repetitions of treatment with this dye already reflect changes in the color of PVC materials, in a more significant way than those promoted by peracetic acid and sodium hypochlorite.

Finally, the effects of PDT with this same dye on the optical changes of PVC materials were tested. By analyzing Figure 13, it is possible to observe that, just like methylene blue alone, PDT also increases the luminosity and makes the extensions more bluish (significantly increases ΔL and significantly decreases Δb); however, it did not result in a significant change in the perceived color. In masks (Fig. 14), PDT only promoted a statistically significant decrease in the parameter Δa (made it more greenish), but did not significantly change any other color parameter. Thus, it is observed that PDT has a more pronounced effect on extensions (as expected, considering that the extensions are originally transparent in color), but do not change the color of the masks in up to 3 repetitions of treatments. Still, it was possible to verify that the color changes were promoted by the dye alone, and not by the photochemical reaction of the PDT. In other words, the release of singlet oxygen, superoxide anion or free radicals does not seem to be responsible for the color changes observed in hospital masks and extensions.

Fig. 13 Variation of ΔL, Δa, Δb and ΔE in the extensions before and after treatment with photodynamic therapy in different repetitions. The bars indicate standard error. Different letters show statistically different averages at the level of 5% according to the Student-Newmann-Keuls test

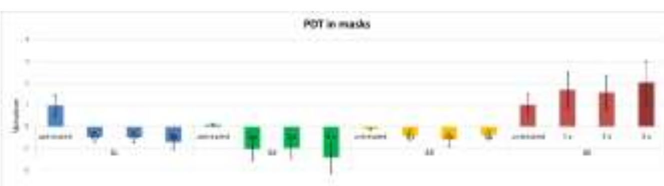

Fig. 14 Variation of ΔL, Δa, Δb and ΔE in the masks before and after treatment with photodynamic therapy in different repetitions. The bars indicate standard error. Different letters show statistically different averages at the level of 5% according to the Student-Newmann-Keuls test

Thus, considering the benefits of PDT in microbial reduction reported in the literature on the irradiation parameters studied in this study, as well as the compositional changes promoted in the tested materials, it is possible to infer that photodynamic therapy can be used as an alternative for decontamination of these materials. Yet, future microbiological as well as durability evaluations of the tested materials, are essential for this to be put into future clinical use.

IV. CONCLUSION

It can be concluded that 0.2% peracetic acid, 1% sodium hypochlorite and PDT alter the chemical composition both masks and hospital extensions, and that these changes have a positive relationship with the number of treatments performed. These compositional changes may be related to the color changes promoted in both materials by all agents tested.

Acknowledgment

To FAPESP (2017-21887-4), PROCAD-CAPES (88881.068505/2014-01), National Institute of Photonics (CNPq/INCT 465763/2014-6) and Multiuser Experimental Center of UFABC (CEM-UFABC). The authors also thank Prof. Emery C.C.C. Lins (UFPE) for access to the imaging equipment used in this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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