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Use of an electrolyzed superoxidation solution to disinfect non-invasive mechanical ventilation masks

Uso de una solución electrolizada de superoxidación para desinfectar mascarillas de ventilación mecánica no invasiva

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ABSTRACT. Introduction: non-invasive mechanical ventilation masks are non-reusable supplies in high demand in respiratory therapy services. Determining whether they can be successfully disinfected could help to optimize resources. Neutral pH electrolyzed superoxidation solutions are effective and harmless high-level disinfectants used in the hospital environment. **Objective:** to evaluate the effectiveness of an electrolyzed neutral superoxidation solution to eliminate the bacterial load of noninvasive mechanical ventilation masks and its effects on the mask material. **Material and methods:** 49 masks used in patients with non-infectious diseases or pneumonia of the Respiratory Therapy Service of the Ismael Cosío Villegas National Institute of Respiratory Diseases were randomly distributed into the experimental group of electrolyzed solutions of neutral pH superoxidation (n = 22) and the orthophthalaldehyde control group (n = 27). Bacteriological sampling was performed before and after disinfection and the bacterial load was characterized in five of each group. Disinfection was by immersion in electrolyzed solutions of neutral pH 0.004% superoxidation, for five minutes or for 45 minutes in orthophthalalaldehyde. New masks subjected to three disinfection cycles were analyzed by scanning electron microscopy. **Results:** disinfection with neutral pH electrolyzed superoxidation solutions eliminated 100% of the bacterial load. The neutral pH electrolyzed superoxidation solution was effective against the nosocomial opportunistic bacteria *Staphylococcus aureus, Corynebacterium striatum, Enterobacter cloacae, Enterobacter aerogenes* and *Enterococcus faecalis* from the masks. Scanning electron microscopy revealed that three disinfection cycles did not generate structural damage to the material. **Conclusion:** the disinfection method with neutral pH

RESUMEN. Introducción: las mascarillas de ventilación mecánica no invasiva son insumos no reutilizables de alta demanda en los servicios de terapia respiratoria. Determinar si pueden ser desinfectadas exitosamente podría ayudar a optimizar recursos. Las soluciones electrolizadas de superoxidación de pH neutro son desinfectantes de alto nivel efectivas e inocuas utilizadas en el ámbito hospitalario. **Objetivo:** evaluar la efectividad de una solución electrolizada de superoxidación neutra para eliminar la carga bacteriana de mascarillas de ventilación mecánica no invasiva y sus efectos sobre el material de éstas. **Material y métodos:** 49 mascarillas utilizadas en pacientes con enfermedades no infectocontagiosas o neumonía del Servicio de Terapia Respiratoria del Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas se distribuyeron, de manera aleatoria, en el grupo experimental de soluciones electrolizadas de superoxidación de pH neutro (n = 22) y el grupo control ortoftalaldehído (n = 27). Se realizó un muestreo bacteriológico antes y después de la desinfección y en cinco de cada grupo se caracterizó la carga bacteriana. La desinfección fue por inmersión en soluciones electrolizadas de superoxidación de pH neutro al 0.004%, por cinco minutos o por 45 minutos en ortoftalaldehído. Las mascarillas nuevas sometidas a tres ciclos de desinfección, se analizaron mediante microscopia electrónica de barrido. **Resultados:** la desinfección con soluciones electrolizadas de superoxidación de pH neutro eliminó 100% de la carga bacteriana. La solución electrolizada de superoxidación de pH neutro fue efectiva contra las bacterias oportunistas nosocomiales *Staphylococcus aureus*, *Corynebacterium striatum*, *Enterobacter cloacae*, *Enterobacter aerogenes* y *Enterococcus faecalis* de las mascarillas. La microscopia electrónica de barrido reveló que tres ciclos de desinfección

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electrolyzed superoxidation solutions was effective in eliminating the bacterial load without causing damage to the mask material.

Keywords: electrolyzed superoxidation solution, noninvasive mechanical ventilation mask, high-level disinfection, reuse of medical devices.

no generan daños estructurales en el material. **Conclusión:** el método de desinfección con soluciones electrolizadas de superoxidación de pH neutro fue efectivo para eliminar la carga bacteriana sin generar daños en el material de las mascarillas.

Palabras clave: solución electrolizada de superoxidación, mascarilla de ventilación mecánica no invasiva, desinfección de alto nivel, reúso de dispositivos médicos.

INTRODUCTION

The use of single-use masks for the administration of nebulized drugs, oxygen therapy and non-invasive mechanical ventilation (NIMV) results in a high consumption of material and economic resources for respiratory therapy services in the country's public hospitals. Applying effective disinfection techniques that allow reuse of these supplies could be a strategy to optimize this area. Since this type of equipment is semi-critical, it should be subjected to standardized high-level disinfection protocols to ensure its effectiveness and guarantee the integrity of the material.[1-3]

Currently, at the Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas (INER), Mexico City, a disinfection protocol is applied for reusable semi-critical equipment consisting of an enzymatic washing cycle and a 40-minute orthophthalaldehyde (OPA) immersion cycle, followed by rinsing with sterile water and drying.[4,5] However, the use of OPA has disadvantages such as skin, clothing and surface staining.[6,7] In addition, there have been reports of health effects on technical personnel and anaphylaxis in patients treated with materials disinfected with this substance.[8,9]

In contrast, neutral pH superoxide electrolyzed solutions (SES) are non-toxic, non-corrosive and environmentally friendly high-level disinfectants.[10-13] They are produced through the controlled electrolysis of an aqueous solution of sodium chloride that generates active oxygen and chlorine species, such as hydrogen peroxide and hypochlorous acid.[14] These act by scavenging electrons and breaking chemical bonds of the outer envelopes in microorganisms.[15] Consequently, it generates protein denaturation in viruses and osmotic lysis in single-celled organisms.[16] The effectiveness of neutral SES has been documented on fungi, spores, viruses and bacteria, including multidrug-resistant strains and biofilms.[17-19] Because of these properties, they are used in the disinfection of hospital environments such as intensive care units, in healthcare settings and in rooms with specialized equipment, such as radiology and tomography rooms.[10-13]

This study evaluated the effectiveness of a SES with neutral pH at 0.004% of active species of chlorine and oxygen to eliminate the bacterial load of single-use NIMV masks used by patients with non-infectious diseases of different types of pneumonia, in parallel with a disinfection control protocol for semicritical equipment routinely applied in the INER using OPA. The effects of three disinfection cycles with SES on the mask material were also analyzed by scanning electron microscopy (SEM).

MATERIAL AND METHODS

Collection of non-invasive mechanical ventilation (NIMV) masks. Experimental study. The sampling method used to collect the NIMV masks (AcuCare™ F1-0 NV LGE, ResMed) was by convenience. They belonged to consecutive cases that met the selection criteria. Those from patients admitted to the clinical area of the Respiratory Therapy Service of the INER with non-infectious diseases were included. Specifically, with chronic obstructive pulmonary disease (COPD), obesity-hypoventilation syndrome, asthmatic crisis, pleural effusion, pulmonary tumor, type II respiratory failure, pulmonary cancer, venous thrombosis, pulmonary multinodular disease and pulmonary hypertension. Masks used by patients with pneumonia, nosocomial pneumonia and multiloculated empyema were also included. Masks used by patients diagnosed with tuberculosis, human immunodeficiency virus (HIV), hepatitis B and C were not included.

Five to six masks were collected per week according to the guidelines of the Manual for Cleaning and Disinfection of Reusable Equipment in the Respiratory Therapy Service.[4] Each mask was assigned with an identification number and randomly assigned to two groups identified as: the experimental group SES and the control group OPA. At the end of the study, 22 were in the SES group and 27 in the OPA group. Likewise, each week, one to two masks, from those assigned to each group, were randomly selected for bacteriological contamination typing until there were a total of five per type of disinfectant.

Determination of bacteriological load. Microbiological control of the internal part of the masks was performed by swabbing (sterile rayon-headed plastic swabs, 3M™ RediSwab). The swabs were placed inside tubes containing 10 mL of Letheen broth (3M™ RediSwab Letheen Broth RS96010LET). 100 μL of broth and a 1:10 dilution was seeded in duplicate on trypticasein soy agar (TSA) plates and incubated for 48 hours at 37 °C. The bacterial load was determined by counting colony forming units (CFU) and adjusting for the total volume of Letheen broth. The

identification of microorganisms was carried out with biochemical tests and the VITEK® 2 automated method, based on the protocols established in the Institute's Clinical Microbiology Laboratory.

Disinfection process for non-invasive mechanical ventilation masks. The masks of the experimental group SES were disinfected following the steps: 1) immersion of the device for three minutes in a 4 mL/L water dilution of the enzymatic detergent (Endozime® AW Plus); 2) rinsing with tap water; 3) immersion for five minutes in SES at 0.004% active chlorine and oxygen species and REDOX potential 750-950 mV (Estericide® QX, Sanitary Reg. No.: 0363C2006 SSA); 4) drying; and 5) wiping with sterile gauze.

Those in the OPA control group received the routine process of washing and disinfection of the institution's semi-critical material consisting of: 1) immersion for three minutes in the enzymatic detergent dilution (Endozime® AW Plus); 2) rinsing with flowing water; 3) immersion for 40 minutes in 0.55% OPA solution (CIDEX® OPA); 4) thorough rinsing with sterile water; 5) drying; and 6) wiping with sterile gauze.

At the end of the protocols, a microbiological control was performed as described above. Both disinfectant solutions were used according to the manufacturer's instructions, and were changed when the appearance of suspended particles was detected. Adequate OPA activity was verified using the test strips indicated by the supplier, while reuse of the SES was carried out according to the manufacturer's indications. The technical personnel in charge of performing the disinfection processes were informed about the characteristics of SES and its safety. However, they were asked to apply the same biosafety measures as with the use of OPA: use of gloves, mask and gown. They were also required to report any discomfort during the process. Personnel were not blinded to the treatments that each mask must receive. This work was performed from November 2017 to August 2018. The masks disinfected in this protocol were not reused on patients.

Analysis of structural damage in the material of non-invasive mechanical ventilation masks by scanning electron microscopy. Six new single-use NIMV single-use BiPAP masks (AcuCare™ F1-0 NV LGE, ResMed) were used. They were randomly assigned to the experimental group SES (n = 3) or the control group OPA (n = 3). All masks were subjected to three consecutive disinfection cycles as described above. Photographs were taken of the masks under the same light and perspective conditions. The analysis of structural changes in the material was performed with a JEOL JCM-600Plus scanning electron microscope (SEM). The samples were covered with graphite and gold. Images were acquired using a high vacuum SED detector with a spot size of 4.5, a working distance of 8.1 and at 15 kV. Micrographs were obtained at magnifications of 5,000× and 10,000×. The studies were carried out at the Department of Metallurgical Engineering, Faculty of Chemistry, National Autonomous University of Mexico, Mexico City.

RESULTS

The SES disinfection protocol was effective in decontaminating NIMV masks used by patients. Immersion of NIMV masks in SES for five minutes eliminated 100% of the bacterial load, regardless of the initial amount, both in masks used in patients with non-infectious diseases and in masks used in patients with pneumonia of different etiology *(Table 1)*. The same result was obtained when OPA was used as a disinfection control. It was evident that the two disinfection processes completely eliminate bacterial contamination; therefore, no statistical analysis was applied since there are no differences between them that can be compared. That is, SES had the same results as the OPA used as a control.

Regarding bacterial typing, in the five masks randomly selected from the SES group, prior to disinfection, two were found to be colonized by Enterococcus faecalis, one by Enterobacter cloacae, another by Enterobacter aerogenes and the last by Staphylococcus aureus and Corynebacterium striatum *(Table 2)*. Immersion in SES for five minutes completely eliminated these typically nosocomial opportunistic bacteria from the masks. In the case of the OPA group, of the five masks chosen at random, two were contaminated with Staphylococcus aureus, one with Enterobacter cloacae, one with Enterococcus faecalis and one with Pseudomonas aeruginosa *(Table 2)*. Similarly, immersion for 45 minutes in OPA eliminated these microorganisms from the masks.

In terms of the duration of each disinfection process, the use of SES required an average of 20 minutes per mask. Fifteen minutes of handling were required, comprising the stages of washing with enzymatic detergent to eliminate organic matter, rinsing with running water, drying after immersion in SES and cleaning with sterile gauze. In contrast, disinfection with OPA requires, in addition to the 40 minutes of immersion, 20 minutes of pre- and post-handling. In particular, thorough washing with sterile water is necessary to remove OPA residues. It is then that the use of SES as a disinfectant save about 40 minutes per mask. Finally, the technical staff did not report any physical discomfort associated with handling SES.

The SES disinfection protocol did not induce structural damage to the NIMV mask material. At the macroscopic level, photographs of the masks at the end of the disinfection protocols, acquired under the same light conditions, showed that those immersed with SES had a translucent appearance after three disinfection cycles *(Figure 1)*. In contrast, those immersed three times in OPA presented a yellowish opacification *(Figure 1)*.

Table 1: Comparison of disinfection methods with neutral pH electrolyzed superoxidation solution and orthophthalaldehyde.

Disinfection method							
Neutral pH superoxide electrolyzed solutions (SES)				Orthophthalaldehyde (OPA)			
Mask ID	Patient diagnosis	CFU (10^3)/mL	Bacterial death (%)	Mask ID	Patient diagnosis	CFU (10^3)/mL	Bacterial death (%)
1	Obesity-hypoventilation syndrome	4.6	100	2	Asthmatic crisis	80	100
5	Obesity-hypoventilation syndrome	110	100	3	Left pleural effusion	68	100
21	Interstitial pneumonia	40	100	4	Left pleural effusion	0	100
25	Lung mass	0	100	8	Left pulmonary tumor	0	100
27	Exacerbated COPD	26	100	12	Multiloculated empyema	4	100
31	Obesity-hypoventilation syndrome	110	100	13	COPD	6	100
34	Nosocomial pneumonia	18	100	17	Multiloculated empyema	290	100
36	Atypical pneumonia	4.2	100	18	Respiratory failure type II	1.3	100
39	Diffuse interstitial pneumonia	380	100	20	Lung cancer	0	100
40	Lung mass	2.8	100	23	Diffuse interstitial pneumonia	45	100
44	Mixed respiratory failure	280	100	26	Obesity-hypoventilation syndrome	98	100
46	Respiratory failure type II	0.4	100	29	Exacerbated COPD	0	100
47	Pneumonia	6.1	100	30	Exacerbated COPD, respiratory insufficiency type II	2,100	100
48	Pneumonia	13	100	33	COPD	1.7	100
59	Venous thrombosis	0	100	38	COPD/respiratory insufficiency type II	120	100
64	Interstitial pneumonia	300	100	45	COPD	230	100
68	COPD	420	100	50	Pneumonia	8.5	100
71	Pulmonary multinodular disease	0	100	51	Lung tumor	460	100
72	Pneumonia	600	100	52	Diffuse interstitial pneumonia	140	100
73	Pneumonia	680	100	53	Unspecified	11	100
75	Respiratory failure type II	1.1	100	54	Pulmonary hypertension	250	100
83	Pneumonia	22	100	57	Pneumonia	9.6	100
				58	Pneumonia	8.5	100
				61	Diffuse interstitial pneumonia	68	100
				62	Diffuse interstitial pneumonia	0	100
				67	Diffuse interstitial lung disease	0	100
				77	COPD	2	100

ID = identification. CFU = colony forming units. COPD = chronic obstructive pulmonary disease.

Table 2: Typing of microorganisms in five single-use non-invasive mechanical ventilation masks before the disinfection process.

Disinfection method					
Neutral pH superoxide electrolyzed solutions (SES)			Orthophthalaldehyde (OPA)		
Mask ID	Patient diagnosis	Identified MO	Mask ID	Patient diagnosis	Identified MO
31	Obesity-hypoventilation syndrome	*E. cloacae*	50	Pneumonia	*S. aureus*
40	Lung mass	*S. aureus* *C. striatum*	54	Pulmonary hypertension	*E. cloacae*
44	Mixed respiratory failure	*E. faecalis*	57	Pneumonia	*E. faecalis*
64	Interstitial pneumonia	*E. faecalis*	58	Pneumonia	*P. aeruginosa*
73	Pneumonia	*E. aerogenes*	77	COPD	*S. aureus*

ID = identification. MO = microorganism. COPD = chronic obstructive pulmonary disease.

However, analysis of the surface topography of the masks by scanning electron microscopy (SEM) revealed a similar appearance between the samples immersed in SES or OPA. No apparent structural damage was observed that could suggest aggressiveness of the disinfectants with the material of the masks *(Figure 1)*. It is worth mentioning that the SEM detected the presence of metallic particles deposited on the surface of the new masks, this means, before being subjected to the disinfection procedures (data not shown). This could be attributed to the manufacturing or packaging processes of these medical devices. It should be noted that after the disinfection protocols were performed, the load of these particles decreased.

DISCUSSION

In this work it was determined that a 0.004% SES of active chlorine and oxygen species can eliminate the bacterial load of NIV masks used by patients with non-infectious diseases and different types of pneumonias. It was shown to be effective against Enterococcus faecalis, Enterobacter cloacae, Enterobacter aerogenes, Staphylococcus aureus and Corynebacterium striatum, which are of particular medical importance, as they are multidrug-resistant nosocomial opportunists with the ability to form biofilms on medical devices.[20] After immersion for five minutes in the electrolyzed solution, the bioburden was completely eradicated without density dependence at the beginning of the disinfection process. This is consistent with a number of reports demonstrating that SES has bactericidal action against multidrug-resistant strains and biofilms.[17-19,21,22] This antimicrobial activity is due, broadly speaking, to the active species of chlorine and oxygen, which by oxidizing mechanisms generate osmotic lysis, denaturation of proteins and lipids and damage to genetic material.[15,16] It should be noted that currently and to the best of our knowledge, there is no evidence to indicate resistance of any pathogen to the germicidal action of SES. For this reason, they are used as high-level disinfectants and cold sterilants. Likewise, it has been shown that neutral SES is a non-corrosive disinfectant, so it is applied in the disinfection of specialized equipment such as those found in computed tomography and nuclear magnetic resonance rooms.[13] According to this evidence, SES did not induce changes in the masks that were appreciable at the macroscopic level. Consequently, the analysis of their surfaces by SEM showed that immersion in SES for three consecutive cycles does not induce structural damage in the material. In this regard, a recent study established that immersion of polyvinyl siloxane impressions for dental prostheses for 10 minutes in SES does not alter the reproduction of surface details or texture,[23] which corresponds to the results of this work.

To establish whether the disinfection of the masks with SES was efficient, OPA was used as a disinfection control in this study. This is a disinfectant routinely used in the decontamination of semi-critical material, which means that its effectiveness has been validated by health organizations around the world.[1] The results clearly showed that SES eliminates the entire bacterial load as well as immersion in OPA. It was also determined that both have activity against the same bacteria colonizing the masks: Staphylococcus aureus, Enterobacter cloacae and Enterococcus faecalis. The difference between them was, in the scope of this work, in the duration times of the disinfection processes. While disinfection with SES required 20 minutes, decontamination with OPA required 60 minutes. Another difference detected was a yellowish opacification of the masks that was evident at the macroscopic level in those subjected to three cycles of immersion with OPA. However, these macroscopic changes induced by OPA require further evaluation to determine if it affects their functionality.

It is important to point out that there are two aspects that are crucial for the reuse of medical materials. One, to establish that biofilms are not formed on them; and two, to determine that there are no disinfectant residues

that could compromise the patient's health. In this regard, there are reports that associate the presence of traces of OPA in endoscopes with anaphylactic reactions and cytotoxic effects.[8,24] Therefore, the use of this substance requires thorough rinsing of the material exposed to it. However, deficient or careless rinsing is a risk factor for the development of biofilms.[25] Likewise, tolerance of gram-positive and biofilm strains to OPA has been reported.[26,27] On these points, the use of SES has the advantages of not requiring rinsing and of being a non-toxic substance.[28] However, for the use proposed in this work, specific safety studies should be carried out.

SES have proven to be innocuous in animal models, in human cells and in hospital settings.[13,28] In fact, one of their most important applications is in wound disinfection.[29] In relation to this aspect, in this work the technical personnel did not report any discomfort when handling SES. However, it should be noted that biosecurity measures were used and that no objective measure of this point, such as a questionnaire, was made, so this is an anecdotal observation.

In summary, the results suggest that it is feasible to use a SES to decontaminate this type of masks, since in short times it was effective in eliminating the bacterial load, without depending on the amount of biomass. In addition, it did not induce structural damage in the mask material when subjected to three consecutive immersion processes. This could indicate that their useful life would be extended by up to three disinfection cycles.

Although the results obtained suggest that SES can be used as a disinfectant for this type of mask, we recognize the limitations of the present work. Firstly, the sample size, which should be increased to be statistically representative. Furthermore, bacterial load typing should be carried out on all the masks to clearly establish against which species the biocidal action of SES is effective or if there are any resistant bacteria. Additionally, the study must be extended to other pathogens such as fungi and viruses before it can be validated for use in patients. In this sense, if it is planned to reuse the masks, it is also necessary to make sure that there are no biofilm growths on them. In addition, the assessment of the quality of the material should be carried out on masks coming from patients and then subjected to disinfection. Likewise, it is imperative to include physical analyses that establish the correct functionality of this material after disinfection, considering that it is designed to be used only once.

CONCLUSIONS

Five-minute immersion in SES at 0.004% active chlorine and oxygen species was effective in decontaminating

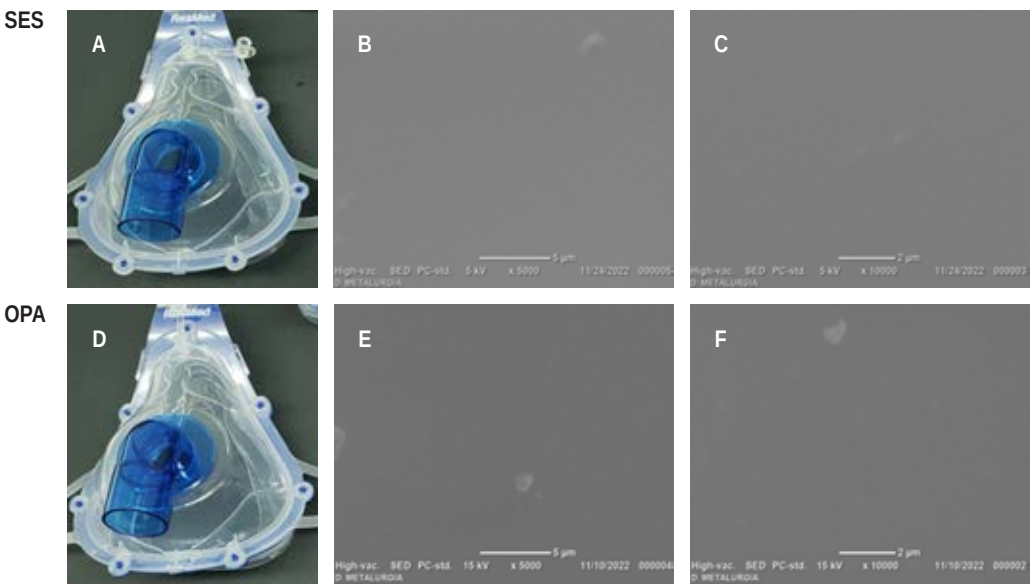

Figure 1: Material analysis of noninvasive mechanical ventilation (NIMV) masks subjected to three cycles of disinfection with superoxide electrolyte solution (SES). After three consecutive cycles of disinfection with SES or orthophthalalaldehyde (OPA) the masks were processed for examination by scanning electron microscopy. **A)** Photograph of the physical appearance of the SES-treated masks is shown. **B)** Scanning electron microscopy micrograph at 5,000× magnification is shown. No fractures or any damage to the material are visible. **C)** 10,000× magnification is shown. No fractures or damage to the material are visible. **D)** Corresponds to the physical appearance of a mask subjected to three disinfection cycles with orthophthalalaldehyde. A yellowing of the mask can be seen. **E and F)** Scanning electron microscopy micrographs at 5,000× and 10,000× magnification, respectively. No fractures or other changes in the topology of the material were observed at any magnification.

bacterially loaded single-use NIMV facemasks. This broad-spectrum disinfectant eliminated nosocomial opportunistic species Enterococcus faecalis, Enterobacter cloacae, Enterobacter aerogenes, Staphylococcus aureus, and Corynebacterium striatum from the masks. In addition, after three consecutive immersion cycles, the disinfectant did not cause damage to the mask material. All of the above suggests that it is feasible to use SES to disinfect single-use NIMV masks and extend their useful life. However, this is a first approach that must be complemented with other studies and validated before it can be used in patients. Specifically, it must be determined whether it eliminates other pathogens, such as viruses and fungi, as well as multidrug-resistant strains and biofilms. Functionality and safety studies must also be performed.

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