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THE ROAD TO SUSTAINABILITY IN DENTISTRY – IS THE REUSE OF STERILIZATION SLEEVES VIABLE?

Catarina Amaral Pereira Ribeiro Fernandes

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The Road to Sustainability in Dentistry – Is the Reuse of Sterilization Sleeves Viable?

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ABSTRACT

Introduction: Reducing plastic consumption from hospitals and medical/dental offices is a difficult task since medical areas have benefited most from its use. Currently recycling these plastics in the health sector carries risks of cross-infection and contamination, so the current methods of destructing them are those that provide the population with more security. However, some plastics do not come into contact with the patient, such as sterilization packaging. This is where hospitals and clinical practices could focus since there are more sustainable and eco-friendly solutions to minimize single-use plastic and mitigate the effect of the waste produced. However, one of the complications related to the reuse of these plastics is the fact that they are marked for single-use by their manufacturers.

Objective: This study aims to question the safety and efficacy of reusing sterilization packages, without compromising its sterilization conditions.

Materials and Methods: 36 samples of paper/plastics sterilization sleeves were tested in this investigation and they were divided into 3 groups (experimental group – reuse sleeves; negative control group – new sleeves; and a positive control group – samples air contaminated). The experimental group included sleeves that were opened and a gauze was introduced, then, they were closed again and sterilized, representing the re-use of the sleeves. After the sterilization cycle, samples were stored for 1 day (T_0), 7 days (T_1), 31 days (T_2), and 153 days (T_3). After the specified storage period, the sleeves were opened and the pieces of gauze were removed aseptically and incubated in *petri* plates with Nutrient Agar at 37°C for 3 days. After incubation, the *petri* plates were inspected and the microbial contamination was verified and classified as present or absent. This assay was performed in triplicate and on three different occasions, making a total of 108 samples analyzed.

Results: Observation of the *petri* plates regarding the experimental group showed no sign of contamination. The same happened to the negative control group. The remaining *petri* plates containing the positive controls presented a high number of colony-forming units.

Conclusion: This study shows that sterilization sleeves can be used a second time while maintaining sterility and integrity conditions even for long periods (153 days – 5 months of storage) and in an open environment.

RESUMO

Introdução: Reduzir o consumo de plástico dos hospitais e consultórios médicos/dentários é uma tarefa difícil, uma vez que as áreas médicas têm inúmeros benefícios com a sua utilização. Atualmente, a reciclagem destes plásticos no sector da saúde acarreta riscos de infeção e contaminação cruzadas, pelo que os métodos atuais de destruição dos mesmos são os que proporcionam mais segurança à população. No entanto, alguns plásticos não entram em contacto com o paciente, tais como as mangas de esterilização. É aqui que os hospitais e os clínicos se poderiam concentrar, uma vez que existem soluções mais sustentáveis e ecológicas para minimizar o plástico de uso único e mitigar o efeito dos resíduos produzidos. No entanto, uma das complicações relacionadas com a reutilização destes plásticos é o facto de serem assinalados pelos seus fabricantes como de uso único.

Objetivo: Este estudo pretende testar a segurança e eficácia da reutilização de mangas de esterilização, sem comprometer o seu ambiente asséptico.

Materiais e Métodos: 36 amostras de mangas de esterilização de papel/plástico foram testadas neste trabalho sendo divididas em 3 grupos (grupo experimental – mangas reutilizadas; grupo de controlo negativo – mangas novas; e um grupo de controlo positivo – amostras contaminadas com ar). O grupo experimental incluiu mangas que foram abertas e uma gaze foi introduzida, sendo novamente fechadas e esterilizadas, representando assim a reutilização das mangas. Após o ciclo de esterilização, as amostras foram armazenadas durante 1 dia (T_0), 7 dias (T_1), 31 dias (T_2) e 153 dias (T_3). Após este período de armazenamento, as mangas foram abertas e as gazes retiradas assepticamente e incubada em placas de *petri* com Agar Nutriente a 37°C durante 3 dias. Após o período de incubação, as placas de *petri* foram inspecionadas e a contaminação microbiana foi verificada e classificada como presente ou ausente. Este ensaio foi feito em triplicado em três momentos distintos, somando um total de 108 amostras analisadas.

Resultados: A observação das placas de *petri* do grupo experimental não mostrou sinais de contaminação. O mesmo aconteceu com o grupo de controlo negativo. As restantes placas de *petri* contendo os controlos positivos apresentaram um elevado número de unidades formadoras de colónias.

Conclusão: Este estudo mostra que as mangas de esterilização podem ser utilizadas uma segunda vez, mantendo as suas condições de esterilidade e integridade mesmo em longos períodos de tempo (153 dias – 5 meses de armazenamento) em ambiente aberto.

KEYWORDS

Reuse of paper/plastic sterilization sleeves; Sustainable Healthcare; Eco-friendly Dentistry; Waste Management; Environment.

INTRODUCTION

Plastics are everywhere. Raw materials such as cellulose, coal, natural gas, salt and petroleum form plastics. Under stable conditions, these materials are heated and broken down into smaller molecules called monomers that form covalent bonds with each other, in a process called polymerization and allow the creation of polymers. Various combinations of monomers provide plastic resins with various characteristics, such as strength or molding ability [1, 2].

In 1907 in Belgium, the first truly synthetic polymer, Bakelite, was developed. During World War II, mass manufacturing of plastics began and has since continued to grow [1]. Presently, it is estimated that more than 8.3 billion tonnes of plastic have been produced since the early 1950s, where 90% or more entered the wastewater stream [3, 5]. The United Nations Environment Program (UNEP) states that currently, more than 300 million tonnes of plastic are produced each year and half of that is for single use. The prediction is that by the end of 2050, around 12 billion tonnes of plastic will be wasted on the environment [3, 6]. About 99% of plastics, all of which are dirty, non-renewable materials, are made from chemicals obtained from oil, natural gas and coal. If current trends persist, the plastic industry could account for 20% of the overall consumption of oil worldwide by 2050 [7, 8].

When we talk about plastic in the medical sector, it was valued worldwide at 18.9 billion euros in 2019 [9, 2]. In Europe, it is expected that by 2024 this market will reach a value of 4 billion euros [10] due to its growing demand. The World Health Organization (WHO) explains that 85% of health sector waste is non-infectious. However, only a small percentage is recycled, being that most end up in landfills (79%) or incinerated (12%). This leads to the medical sector representing around 4% of global greenhouse gas emissions – if it was a country, it would be the fifth most polluting country in the world [11, 12].

According to the Eco Dentistry Association (EDA), some 680 million plastic and paper protections, as well as 1.7 billion sterilization instruments and packaging per year are sent to landfills or disposed of in the environment [13, 14, 15], which raises another

problem – overexposure to Bisphenol A (BPA) and Di(2-ethylhexyl)phthalate (DEHP) and microplastics (small particle between 100 nm to 5 mm). Recent studies show that large amounts of microplastics end up in the human diet and have been found for example in seafood, honey, bottled water and alcohol as a result of their deterioration in the environment [6, 16, 17, 18, 19]. In our body, due to the inability of our immune system to eliminate plastic, this situation can lead to chronic inflammation and cancer [20]. Also, microplastics in our bodies have repercussions when in contact with antibiotics. In fact, the correlation between antimicrobial resistance and increasing plastic pollution is being investigated. The conclusion is that when in contact with antibiotics, plastics can promote genetic mutations in bacteria so that they can acquire resistance to them, creating possible threats to human health [21, 22].

Reducing plastic consumption from hospitals and medical/dental offices is a difficult task since medical areas have benefited most from its use because plastic is economical, heat resistant, long-lasting, versatile, requires less energy to be produced, compared to metal or glass, is biocompatible and offers a sterile environment. These assets that make plastic the ideal material for single-use, are also the ones that make it impossible for nature to completely eliminate it. In addition, these are the plastics that currently represent 85% of all plastic produced in health [23]. Currently recycling these plastics in the health sector carries risks of cross-infection [24, 25] and contamination [26] so the current methods of destructing them are those that provide the population with more security.

However, some plastics do not come into contact with the patient, such as sterilization packaging – as shown in Figure 1 – which consists of a paper-plastic bag with one side made of medical grade paper and the other side made by a thin plastic film - Polyethylene Terephthalate (PET) or Polyethylene (PE). Paper and plastic are bonded in the corners by heat and the pouch is sealed [27]. These single-use packages that cover medical instruments are sterilized in the autoclave [28, 29, 30]. The sterilized instruments are then unpacked and the wrapper is thrown into the bin. This is where hospitals and practices could focus since there are more sustainable and eco-friendly solutions to minimize single-use plastic and mitigate the effect of the waste produced [31].

Figure 1. Paper/plastic sleeves prepared, from tubular sterilization rolls.
Source: https://stokmed.pl/gb/sterilization/111-5876-mediroll-paper-foil-sterilization-sleeve.html

This investigation arises to debate the importance and necessity of plastics. The problem addressed is not only the usage of plastic but also the way it is discarded. One of the complications related to the reuse of these plastics is the fact that they are marked for single-use by their manufacturers [32, 33]. In the impossibility of replacing plastic with other materials, because of its many assets, this study aims to question the safety and efficacy of reusing sterilization packages, without compromising its sterilization conditions [34, 35].

The question and hypothesis raised is the following – Can sterilization sleeves be used a second time, maintaining the conditions of sterility?

MATERIAL AND METHODS

Between November 19, 2020 and May 10, 2021, a cross-sectional epidemiological – observational analytical study – was performed in the Department of Orthodontics of the Faculty of Dental Medicine of the University of Porto in collaboration with the Center of Biological Engineering of the University of Minho where paper/plastics sleeves were tested/divided on an experimental group (EG) (n=12), a negative control group (NCG) (n=12) and a positive control group (PCG) (n=12) as shown in Figure 2, with the aim of knowing if sterilization sleeves could be used a second time, maintaining the conditions of sterility. The experimental group is the one with the sleeves that will be opened and re-sterilized. The negative control group is the one that will have new sleeves to assure the conditions of sterilization and to compare with the experimental group and, at last, the positive control group has the sleeves that have been opened and that will show the possible contamination after opening.

The sample calculation, i.e. the sample size – number of sterilization sleeves that were included in the study – was carried out taking into consideration that the objectives of the study were: (1) the evaluation of the presence of contaminated sleeves in each of two groups (EG and NCG) at 4 different moments – 1 day later (T_0); 7 days later (T_1); 31 days later (T_2) and 153 days later (T_3); (2) the comparison of the contamination levels between the experimental and negative groups at each moment; (3) the comparison of the contamination levels between the 4 moments in each group.

Thirty-six paper/plastic sleeves were prepared, from tubular sterilization rolls (MEDISTOCK®, France), with 7.5 cm by 15.0 cm each and divided into 3 groups of 12 samples each. After their preparation, sterilization sleeves were randomly selected to undergo a sterilization cycle in an autoclave (HS–22 K_{5+} WHITE, GENTINGE®, Sweden). After that cycle, these bags that were going to suffer new sterilization – EG – were inspected for openings, presence of water drops inside, bends and creases, or burns to ensure/maintain integrity – exclusion criteria – and opened if all those criteria were assured.

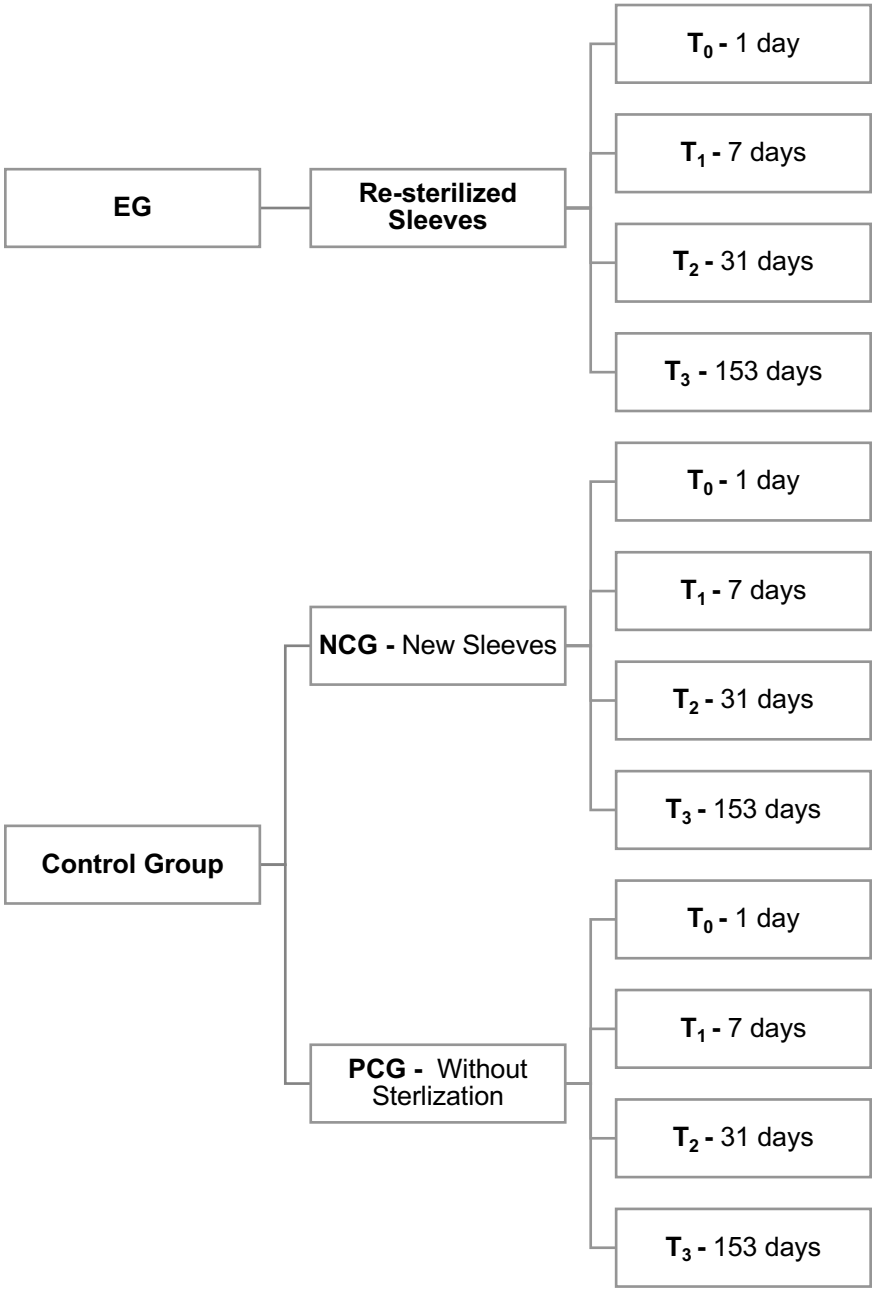

Figure 2. Study design – details of the experimental and control groups.

A piece of gauze, measuring 3 cm × 2 cm (Bastos Viegas®, S. A., Portugal) was placed in each of the new sleeves (NCG) and re-used sleeves (EG). Afterward, the sleeves from NCG were sealed 1 cm from the base and 3 cm from the top with a thermal sealer (EuroSeal® 2001, Euronda S.p.A., Italy) and the sleeves from the EG were resealed at 6 cm from the top with the same sealer. In this step, the exclusion

criteria were once again applied – to ensure/maintain integrity. Autoclave tape type 1 (Dental Autoclave Sterilization Indicator Tape for Steam, Toscana, Italy) which change the color following the sterilization process, indicating if the unit was correctly exposed to the sterilization process with visual confirmation [29], was used as external chemical control of the sterilization and was placed on the plastic side of the sleeves that were going to be re-sterilized.

All sleeves were arranged in a horizontal position and the paper side was in contact with the plastic side of the next sleeve without touching the chamber wall of the autoclave. Samples were sterilized at 121°C and 15 psi for 33 minutes. Samples from PCG were not sterilized to prove contamination on the gauze.

After the sterilization cycle, samples were stored in an opened plastic box for 1 day (T_0), 7 days (T_1), 31 days (T_2), and 153 days (T_3). The samples were left at room temperatures (≅ 20ºC) and humidity.

After the specified storage period, the sleeves were inspected for barrier damage before being opened. The pieces of gauze were removed aseptically (MSC-Advantage™ Class II Biological Safety Cabinets, Thermo Fisher Scientific™, USA) and incubated (General Incubator with Built-in Roller or Shaker – NB-205Q, N-BIOTEK, South Korea) in *petri* plates with Nutrient Agar 20g/L (Research Products International – RPI, USA) at 37°C for 3 days. After the incubation period, the *petri* plates were inspected and the microbial contamination was verified and classified as present or absent. This assay was performed in triplicate and on three different occasions, making a total of 108 samples analyzed.

RESULTS

All sleeves tested passed the reusability inspection, none of the samples was discarded. In Table 1 is possible to see the results obtained after the observation of the Nutrient Agar *petri* plates containing the gauzes resultant from the different groups assayed (EG, NCG and PCG). As it is possible to observe samples from EG showed no sign of contamination even after 5 months of storage, similarly to the NCG and in opposition to the PCG. PCG, intentionally contaminated by exposure to normal laboratory environment, showed extensive microbial growth in all samples after all the entire period of incubation tested. Throughout the experiment, 3 samples from each group – EG, NCG and PCG – belonging to T_3 had to be excluded due to external contamination of the culture medium plates (n=6).

Table 1. Presence or absence of contamination in each test group

Time Point	Negative Control Group	Experimental Group	Positive Control Group
T_0	Absence (n=9)	Absence (n=9)	Presence (n=9)
T_1	Absence (n=9)	Absence (n=9)	Presence (n=9)
T_2	Absence (n=9)	Absence (n=9)	Presence (n=9)
T_3	Absence (n=6)	Absence (n=6)	Presence (n=6)

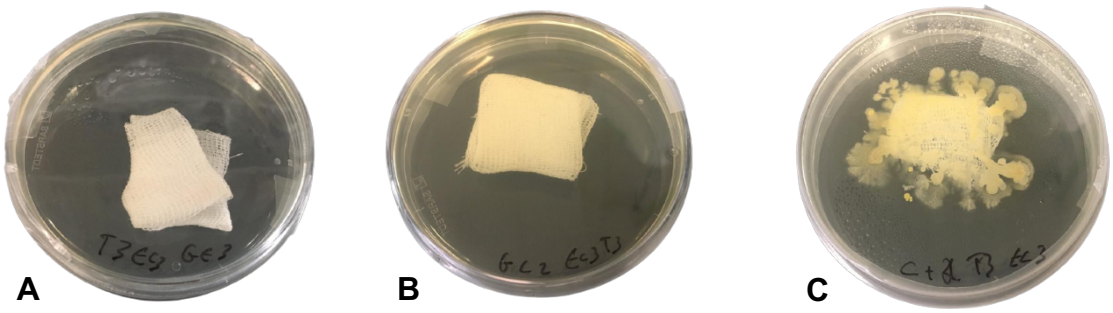

Figure 3. Microbiological result of EG, NCG and PCG, respectively.

DISCUSSION

The present investigation represents a preliminary exploratory study focused on the evaluation of the reuse of sterilization paper/plastic sleeves commonly used as packaging material in healthcare sector and was performed in triplicate and on three different occasions, making a total of 108 samples analysed respecting inclusion and exclusion criteria. The design of this study was intended to guarantee the independence of the results and prevent interferences between them as also to prove that these results were not just an eventuality.

Studies in the healthcare sector on the reuse of medical material [36, 37, 38, 17, 39, 40, 41] triggered the interest in this theme since it was little described in the literature. In fact, the results from this study show that sterilization sleeves can be used a second time while maintaining sterility and integrity conditions when compared to new sterilization sleeves, even for long time periods (153 days – 5 months of storage) and in an open environment.

To keep patients safe, avoiding cross-infection is a high priority in the healthcare sector [42, 43, 44 , 45] and, for sure, there should not be any financial or material barriers in preventing the risk of healthcare-acquired infection. However, nowadays healthcare waste is causing significant [1, 6, 18, 19, 20, 31] environmental contamination from single-use plastics, which is, in turn, harming human health anyways. Being aware that infection control is critical, this study intended on the one hand, to provide a solution that was safe in each procedure performed in healthcare establishments and, on the other hand, that would be the safest choice for the environment. This sustainability effort in healthcare could have an impact on people's health not only within hospitals and clinics but in general, reducing the microplastics and pollution as previously described. For example, if a dental clinic uses a sterilization roll of 200 meters per week to sterilize its materials, with this new approach the dental clinic would start spending one sterilization roll every two weeks, avoiding 200 meters of plastic to be thrown into the environment weekly, while bringing economic advantages to the dental clinic.

It is noteworthy that the results of this present work are in line with recent investigations presented by J. Klumdeth *et al.* [35] and Puangsa-Ard, *et al* [34] in 2020 and

2018 respectively. In their first analysis, no microbial contamination was found in either the reused or new sleeves. In the second analysis, all filter papers stored in both reused and new sleeves remained sterile for up to 6 months in a closed environment. These findings indicated that reusing paper/plastic sterilization sleeves could be a great start on this adjustment to a more sustainable practice. Also, in these studies their positive control group suffered decreased barrier integrity on the sterilization sleeves before being autoclaved. This had the purpose of demonstrating that contamination is event-related unlike the present study where the positive control group wanted to show the contamination that the gauze was exposed to when opened. Furthermore, this present study used Nutrient Agar as the culture medium to measure potential contamination in contrast to the previous studies that used BHI Broth. BHI Broth is a liquid non-selective medium used for the cultivation and maintenance of bacteria, yeasts, and fungi, taken from clinical samples. Nutrient Agar is a general-purpose solid medium recommended for the growing and maintenance of a wide variety of less fastidious microorganisms. However, since no single medium or broth can be used to culture all organisms, it may be thorough to use two types of medium and/or broth or specific/selective culture media for double-checking contamination in the future [46]. Lastly, their study used biological indicators to designate the quality of the autoclave and in the present study, it was choosing not to since the aim of this investigation was to evaluate the quality and safety of the paper/plastic sterilization sleeves and not the performance of the sterilization process. The use of these biological indicators is not common practice in most dental clinics according to studies performed by Vázquez-Rodríguez *et al.* [25] and Oosthuysen *et al.* [24]. Nevertheless, for future investigations regarding this subject, it could be recommended to use these indicators to study this additional factor.

As mentioned by some researchers an adequate storage is crucial to maintain sterility [34, 47, 28]. In addition, they reinforce that those conditions of the storage environment are a more relevant factor than the kind of packaging material. Considering this, in the present study the sterilization sleeves were stored in an open environment where samples were more susceptible to microbiological contamination since they were exposed to a microbe rich environment (open environment, microbiology laboratory), making them more propitious to event-related contamination.

It should be emphasized that the sleeves that can go through “re-sterilization” need to fit minimal criteria. The sleeves used in this study were subjected to little handling, which differs from actual clinical practice. This handling made them less propitious to event-related contamination which allowed all of them to be reused (e.g. no dental or sharp instruments were used inside these sleeves that could puncture and compromise the reusability of the sleeves). This is the only step that would have to change in the way that healthcare workers handle these sleeves – they would have to open the sleeves carefully, splitting only the necessary to remove the instruments inside or, instead, reuse sleeves that wrap non-cutting medical instruments, such as research trays. Being larger sterilization sleeves, they can be reused to wrap and sterilize smaller instruments, therefore the reuse could be done in this perspective as well.

Needless to say, that sleeves that are punctured, damaged in any way that not the usual tearing, or contaminated with blood, saliva, or other visual residues should not be re-sterilized, with the risk of crossed-infection. Additionally, the health practitioners have the major responsibility of ensuring the proper functioning and autoclave quality, not only for their daily conventional sterilizations but especially when considering this more sustainable method.

In further research, it would be important to repeat the experience by trying to recreate what can be done in an everyday practice, use a larger sample, medical instruments inside the sleeves and using different culture media to ensure that there really is no contamination of the paper/plastic sterilization sleeves by any type of microorganism. One subject that remains to be explored is to determine the breaking point of how many times these paper/plastic sterilization sleeves can be reused and for how long can they preserve sterilization.

CONCLUSION

The hypothesis of this study was confirmed and in fact paper/plastic sterilization sleeves can be used a second time while maintaining the conditions of sterility and integrity, even for long periods (153 days – 5 months of storage) and in an open environment which means that, even taking into account the methodological limitations of this research, the results obtained show that the reuse of sterilization sleeves in everyday clinical practice may be a viable practice.

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The Road to Sustainability in Dentistry – Is the Reuse of Sterilization Sleeves Viable?

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Keywords:	Reuse of paper/plastic sterilization sleeves, Sustainable Healthcare, Eco-friendly Dentistry, Waste Management, Environment
Categories:	Sterilization < Primary Research Field (Sub Field), Testing method < Primary Research Field (Sub Field), Instrument and technique < Primary Research Field (Main Field)

The Road to Sustainability in Dentistry – Is the Reuse of Sterilization Sleeves Viable?

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In general, medical and dentistry communities as well as manufacturers encourage the single use of materials aiming to prevent cross-contamination and to guarantee patients safety. However, often it involves the use of plastic and therefore it constitutes a major environmental issue. In order to encourage the reuse of this kind of materials in the health sector and namely paper/plastic disposable sleeves with multi-use applications during clinical use, it was investigated if sterilization sleeves can be used a second time, maintaining sterility. A set of 108 samples was used and they were divided into 3 groups: 1-time reused sleeves (experimental group), new sleeves (as a negative control group), and samples contaminated (positive control group). Samples were stored in an open environment and sterility was assessed over time up to 5 months. All samples in both the new and reused sleeves group had no microbial growth. On the other hand, positive control presented an extensive contamination. Even in an open environment, the 1-time reused paper/plastic sleeves maintained sterility and integrity and also, they can be safely reused for at least 5 months.

Keywords: Reuse of paper/plastic sterilization sleeves; Sustainable Healthcare; Eco-friendly Dentistry; Waste Management; Environment.

INTRODUCTION

Reducing plastic consumption from hospitals and medical/dental offices is a difficult task since medical areas have benefited most from its use because plastic is economical, heat resistant, long-lasting, versatile, requires less energy to be produced, compared to metal or glass, is biocompatible and offers a sterile environment. These assets that make plastic the ideal material for single-use, are

also the ones that make it almost impossible for nature to completely eliminate it. In addition these are the plastics that currently represent 85% of all plastics in health [1]. Currently recycling these plastics in the health sector carries risks of cross-infection [2, 3] and contamination [4] so the current methods of destruction are those that provide the population with more security [5, 6].

However, some plastics do not come into contact with the patient, such as sterilization packaging which consists of a paper-plastic bag with one side made of medical grade paper, and the other side made by a thin plastic film - Polyethylene Terephthalate (PET) or Polyethylene (PE). Paper and plastic are bonded in the corners by heat, and the pouch is sealed [7]. These single-use packages that cover medical instruments are sterilized in the autoclave [8, 9, 10]. The sterilized instruments are then unpacked and the wrapper is thrown into the bin. This is where hospitals and practices could focus since there are more sustainable and eco-friendly solutions to minimize single-use plastic and mitigate the effect of the waste produced [11, 12, 13, 14].

The problem we address is not only the usage of plastic but also the way it is discarded. One of the complications related to the reuse of these plastics is the fact that they are marked for single-use by their manufacturers [15, 16]. In the impossibility of replacing plastic with other materials, because of its many assets, this study aims to question the safety and efficacy of reusing sterilization packages, without compromising its sterilization conditions [17, 18, 19]. Therefore, the question and hypothesis raised is the following – Can sterilization sleeves be used a second time, maintaining the conditions of sterility?

MATERIAL AND METHODS

Thirsty-six paper/plastic sleeves were prepared, from tubular sterilization rolls (MEDISTOCK®, France), with 7.5 cm by 15.0 cm each, and divided in 3 groups of 12 samples each – 1-time reused sleeves (experimental group), new sleeves (as a negative control group), and samples contaminated (positive control group).

After their preparation, sterilization sleeves were randomly selected to undergo a sterilization cycle in an autoclave (HS–22 K_{5+} WHITE, GENTINGE®, Sweden). After that cycle, the bags that were

going to suffer new sterilization – Experimental Group (EG) – were inspected for openings, presence of water drops inside, bends and creases, or burns to ensure/maintain integrity – exclusion criteria – and opened if all those criteria were assured. A piece of gauze, measuring 3 cm × 2 cm (Bastos Viegas®, S. A., Portugal) was placed on each of the new sleeves – negative control group (NCG) – and re-used sleeves (EG). Afterward, the sleeves from NCG were sealed 1 cm from the base and 3 cm from the top with a thermal sealer (EuroSeal® 2001, Euronda S.p.A., Italy), and the sleeves from the EG were resealed at 6 cm from the top with the same sealer. In this step, the exclusion criteria were once again applied – to ensure/maintain integrity.

All sleeves were arranged in a horizontal position and the paper side was in contact with the plastic side of the next bag without touching the chamber wall of the autoclave. Samples were sterilized at 121°C and 15 psi for 33 minutes. Samples from positive control group (PCG) were not sterilized to prove contamination on the gauze.

After the sterilization cycle, samples were stored in an opened plastic box for 1 day (T_0), 7 days (T_1), 31 days (T_2), and 153 days (T_3) at room temperatures (≅ 20ºC) and humidity. After the specified storage period, the sleeves were inspected for barrier damage before being opened.

The pieces of gauze were removed aseptically (MSC-Advantage™ Class II Biological Safety Cabinets, Thermo Fisher Scientific™, USA) and incubated (General Incubator with Built-in Roller or Shaker – NB-205Q, N-BIOTEK, South Korea) in Nutrient Agar (Research Products International – RPI, USA) – at 37°C for 3 days.

After incubation the plates were inspected and the microbial contamination was verified and classified as present or absent. This assay was performed in triplicate and at three different occasions, making a total of 108 samples analyzed.

RESULTS

All sleeves tested passed the reusability inspection, none of the samples was discarded. In Table 1 is possible to see the results obtained after the observation of the nutrient agar plates containing the gauzes resultant from the different groups assayed (EG, NCG, and PCG). As it is possible to

observe in Figure 1, samples from EG showed no sign of contamination, even after 5 months of experiment, similarly to the NCG and in opposition to the PCG. PCG, intentionally contaminated by exposure to normal laboratory environment, showed extensive microbial growth in all samples after the entire period tested. Throughout the experiment, 3 samples from each group – EG, NCG, and PCG – belonging to T_3 had to be excluded due to external contamination of the culture medium plates (n=6).

DISCUSSION

To keep patients safe, avoiding cross-infection is a high priority in the healthcare sector [6) 20) 5) 21)], and for sure there should not be any financial or material barriers in preventing the risk of healthcare-acquired infection. However, nowadays healthcare waste is causing significant [13, 22) 23) 11, 12) 14)] environmental contamination from single-use plastics, which is, in turn, harming human health anyways. With the knowledge that infection control is critical, this study wanted to provide a solution that was safe and environmentally friendly. Thus, the present investigation represents a preliminary exploratory study focused on the evaluation of the reuse of sterilization paper/plastic sleeves commonly used as packaging material in healthcare sector.

In these conditions, new sleeves (NCG) and 1-time reused sleeves (EG) kept sterility and integrity conditions over time during 5 months showing that they can be reused ate least a second time. It is noteworthy that the results of the present work are in line with recent investigations presented by J. Klumdeth *et al.* [19)] and Puangsa-Ard, *et al* [18)]. In both studies, no microbial contamination was found in either the reused or the new sleeves and those remained sterile for up to 6 months in a closed environment. As mentioned by some researchers an adequate storage is crucial to maintain sterility [8, 17) 18)]. In addition, they reinforce that those conditions of the storage environment are a more relevant factor than the kind of packaging material. Considering this, in this study the sterilization sleeves were stored in an open environment where samples were more susceptible to microbiological contamination. Moreover, since there is the possibility of the presence of unnoticeable damage, and although the material used in this assay (gauze) are not as prone to compromise the reusability of the sleeves, unlike sharp and rough materials, the sleeves used in this study were subjected to some handling and exposed to a microbe rich environment (open environment, microbiology laboratory), making them more

propitious to event-related contamination. These findings indicated that reusing paper/plastic sterilization sleeves could be a great start on this adjustment to a more sustainable and environmental practice.

It should be emphasized that although none of the sleeves was discarded and no contamination was observed, the sleeves that can go through "re-sterilization" need to fit minimal criteria and there is the need of some special care. Thus, the reuse of paper/plastic sleeves implies some special handling/procedures and careful and thorough reusability inspection by healthcare workers in order to guarantee their sterility and reusability.

CONCLUSION

The hypothesis of this study was confirmed, and in fact, sleeves can be used a second time while maintaining the conditions of sterility and integrity, even for long periods (153 days – 5 months of storage) and in open environment. Further studies are needed to determine how many times these paper/plastic sleeves can be reused and for how long can they preserve sterilization.

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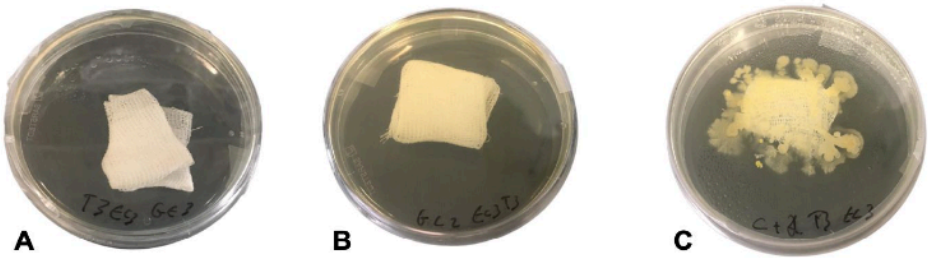

Figure 1. Microbiological result of EG, NCG and PCG respectively.

Table 1. Presence or absence of contamination in each test group

Time Point	Negative Control Group	Experimental Group	Positive Control Group
T_0	Absence (n=9)	Absence (n=9)	Presence (n=9)
T_1	Absence (n=9)	Absence (n=9)	Presence (n=9)
T_2	Absence (n=9)	Absence (n=9)	Presence (n=9)
T_3	Absence (n=6)	Absence (n=6)	Presence (n=6)