Effects of resterilization and storage time on sterility of paper/plastic pouches

Yada Puangsa-Ard[1], Sroisiri Thaweboon[2], Nattinee Jantaratnotai[3],
Praewpat Pachimsawat[1]

[1]Department of Advanced General Dentistry, Faculty of Dentistry, Mahidol University, Bangkok, Thailand,
[2]Department of Oral Microbiology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand,
[3]Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok, Thailand

Correspondence: Dr. Praewpat Pachimsawat
Email: praewpat.pac@mahidol.ac.th

ABSTRACT

Objectives: Paper/plastic pouches are widely used packaging materials for autoclaving medical and dental equipment. Although these pouches are recommended for single use, they are generally reused in Thailand. This study aimed to determine the ability of paper/plastic pouches to maintain sterility after multiple sterilization processes and stored in a closed environment for up to 6 months. **Materials and Methods:** A total of 6720 paper/plastic pouches were divided into four experimental groups: new pouches, 1 time, 3 times, and 5 times resterilized pouches. A piece of filter paper was placed inside each pouch, and the pouch was sealed, sterilized, and stored for up to 6 months. At the end of each storage period, the pouch was opened, and the filter paper was transferred to culture broth for microbial cultivation to determine sterility. Negative and positive controls were also used to validate the procedures. **Results:** All filter papers in the experimental groups, as well as the negative control group, remained sterile for up to 6 months of storage in a closed environment. On the contrary, all filter papers in the positive control group showed microbial contamination. **Conclusions:** In a closed storage condition, the paper/plastic pouches that passed multiple sterilization processes (up to 5 times resterilization) still maintained good barrier efficacy and remained sterile for up to 6 months.

Key words: Microbial contamination, paper/plastic pouches, resterilization, sterility, storage time

INTRODUCTION

Steam sterilization of medical/dental equipment is an important process to prevent infection in patients undergoing medical/dental procedures.[1] One critical aspect of sterilization is packaging, which plays an important role in preserving the sterility of medical/dental equipment and in preventing microbial contamination from the external environment after the sterilization process.[2] Paper/plastic peel pouches are widely used packaging materials for steam sterilization with an autoclave due to its convenience of use, content visibility, and efficacy.[3] The paper side is composed of complex cellulose fibers, and the other side is made of laminated transparent polyester/polypropylene.[3,4] Each side is fused together by heat sealing.[4] The pouches should be inspected to ensure complete closure of the package before and after sterilization.

Following the sterilization process, the equipment is generally not used immediately but stored for later use. Safe storage depends on the conditions of the storage

environment. Previous studies found that storing sterilized equipment in open environments, such as open shelves, resulted in faster microbial penetration than storing in closed cabinets with dustcovers,[5] and that using different packaging materials (reusable woven packs, disposable nonwoven packs, and polypropylene peel pouches) had no effect on safe storage time.[6] This indicates that the storage environment may be a more important factor than the type of packaging material in maintaining sterility.

A survey on autoclave dental packaging in Thailand found that paper/plastic pouch was the most commonly used packaging material for steam sterilization in both hospitals and private clinics.[7] Although these pouches are recommended for single use by the manufacturer,[8,9] almost all of these clinics reused the pouches. The most frequent times of resterilization were 3 times with 5 as the maximum times of reuse. However, the impact of pouch reuse and the integrity of reused pouches were not explored in that survey. Indeed, the work exploring the effects of resterilization on the packaging material is scarce. Most studies regarding resterilization focus on medical/dental instruments themselves, not on the packaging.[10,11] Even though packaging material is also an important factor in sterility maintenance.

Various factors can lead to loss of sterility of these pouches including the barrier efficacy of the paper/plastic pouch itself, packaging process, environmental factors (closed or open), and mishandling of the packages from human error. One critical factor of pouch reuse is the deterioration of barrier efficacy of these paper/plastic pouches after repeated sterilization processes. If resterilization process impaired the sterile integrity of the pouches and resulted in shorter storage time or risk of contamination, this information would be useful when considering reusing these pouches. The objective of this study was to determine the barrier efficacy of paper/plastic pouches on the sterility maintenance after resterilizing 1, 3, and 5 times and after storage in a closed environment for up to 6 months.

MATERIALS AND METHODS

Study protocol and preparation of paper/plastic pouches

There were four experimental groups and two control groups as shown in Figure 1.

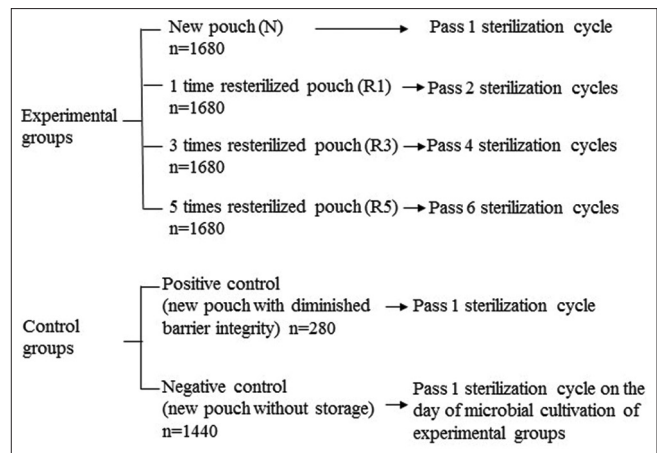The paper/plastic pouches were prepared from sterilization tubular rolls (Stericlin see-Through Reels,

Figure 1: Details of the experimental and control groups

VP Medical Packaging, Germany) at 7.62 cm × 12 cm apiece. Each pouch contained a piece of 0.5 cm × 2 cm filter paper (Whatman paper, Patterson Scientific, England) and a piece of internal chemical indicator (3M Comply SteriGage Chemical Integrator, 3M, USA) inside. Then, the pouches were sealed 1 cm from the bottom and 3 cm from the top with a heat sealer (Euroseal, Euronda S.p.A., Italy) as shown in Figure 2a. Each sealed pouch was inspected for bubbles, gaps, folds or creases, and holes and burn-through to ensure the integrity. Resterilized pouches were not sealed in the first cycles; they were sealed and put in the filter paper and internal chemical indicator only in the last cycle of sterilization [Figure 2b]. In the positive control group, the pouch was punched through both paper and plastic sides, and two cuts were also made at the sides of the pouch to deteriorate barrier integrity [Figure 2c]. Autoclave tape (3M ESPE Autoclave Steam Indicator Tape, 3M, USA) was placed on the plastic side of the pouch. The pouches were arranged in vertical position and the paper side was in contact with the plastic side of the next pouch without touching the chamber wall of an autoclave (M11 UltraClave, Midmark Corporation, USA). All samples were sterilized at 121°C and 15 psi for 30 min.

Sterilization monitoring

Three modes of monitoring were applied to every sterilization cycle: physical, chemical, and biological. Physical monitoring was direct observation of the gauges on the autoclave machine during the sterilization process. Chemical monitoring was done using an internal chemical indicator and autoclave tape as an external chemical indicator. Both types of chemical indicators would change the color following sterilization process. For biological monitoring, spore test tubes (3M Attest, 3M, USA) were placed at the

center and opposite corners of the autoclave tray. After the sterilization process, the spore test tubes were incubated at 56°C for 48 h to evaluate for microbial growth.

Storage

All sterilized samples were stored in closed plastic boxes for 1, 2, 3, 4, 5, or 6 months. Each box contained 125 samples (30 from each experimental group and 5 from the positive control group).

Microbial cultivation

After the specified storage period, the pouch was inspected for barrier damage before opening. The filter paper was aseptically removed and incubated in Brain Heart Infusion (BHI) broth (Becton Dickinson, Maryland, USA) at 37°C for up to 2 weeks. The turbidity of the media indicated microbial contamination.

RESULTS

Physical monitoring during the sterilization process showed that the conditions required were met, i.e., the temperature reached 121°C and the pressure was at 15 psi in every cycle of sterilization. Both internal and external chemical indicators were inspected on every package and all showed color change indicating the proper functioning of the autoclave machine. Biological monitoring with the spore test also showed negative growth in all samples indicating sterile status inside the autoclave.

All filter paper retrieved from new pouches, and 1 time, 3 times, and 5 times resterilized pouches demonstrated no microbial contamination after storage in a closed environment for up to 6 months [Table 1]. The negative control group was a new pouch sterilized on the same day that the microbial culture of the positive control group and experimental groups was carried out, without any storage. It represented the gold standard of pouch integrity. All pouches in the negative control group showed no microbial contamination. All samples in the positive control group (intentionally damaged pouches) showed microbial contamination at every storage period. The presence of positive microbial growth was observed mostly (90.3% of all positive control samples) within 24 h after incubation and all samples showed positive results within 8 days of culture [Figure 3].
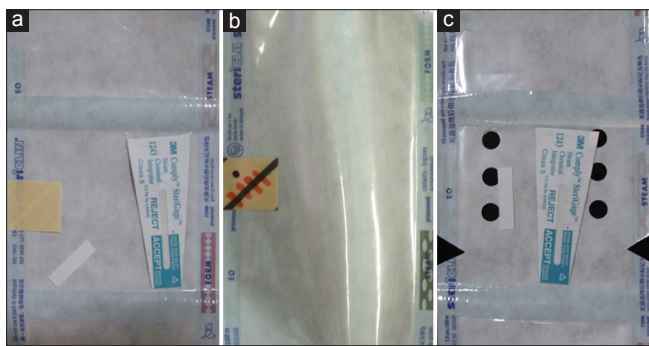

Figure 2: Paper/plastic pouch preparation. (a) Sealed pouch containing filter paper and internal chemical indicator. (b) Unsealed pouch sterilized in earlier cycles for R1, R3, and R5 groups. (c) Positive control pouch with holes and cuts to impair barrier integrity
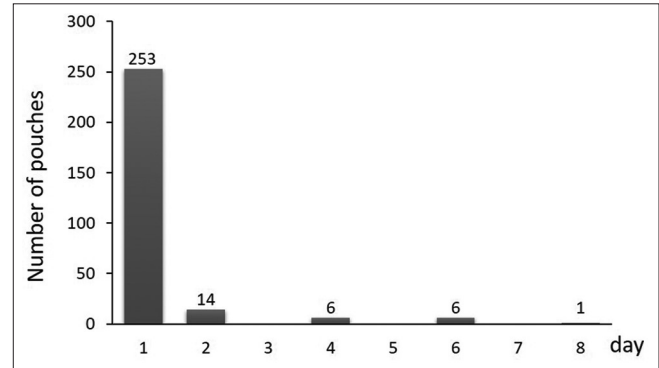

Figure 3: Time distribution for positive microbial culture of positive control group (*n* = 280)

Table 1: Results on microbial culture from new, resterilized, and control groups											
Storage time (month)	Experimental groups								Control groups			
	N		R1		R3		R5		Negative control		Positive control	
	+	-	+	-	+	-	+	-	+	-	+	-
0	0	240	0	240	0	240	0	240	-*	-*	40	0
1	0	240	0	240	0	240	0	240	0	240	40	0
2	0	240	0	240	0	240	0	240	0	240	40	0
3	0	240	0	240	0	240	0	240	0	240	40	0
4	0	240	0	240	0	240	0	240	0	240	40	0
5	0	240	0	240	0	240	0	240	0	240	40	0
6	0	240	0	240	0	240	0	240	0	240	40	0
Total	0	1680	0	1680	0	1680	0	1680	0	1440	280	0

*Same condition with new pouch (N) at this time point. +: Positive microbial culture, -: Negative microbial culture

DISCUSSION

Resterilized paper/plastic pouches could maintain sterility after at least up to 6 times of steam sterilization and up to 6 months of storage in a closed environment. This indicates that repeated sterilization is not a contributing factor in deteriorating the barrier efficacy of the paper/plastic pouches. To reuse a pouch, the used pouch has to be inspected thoroughly on both the paper and plastic sides, as well as along the sealing margins, for damages, tears, or holes. Areas of broken seal from peel opening or any damages are cut out, and resealing is done at a new undamaged position. In practice, only an undamaged pouch with adequate space for packaging and resealing at a new position can be resterilized; thus, the pouch will be smaller after each cycle of sterilization. However, this study was designed to investigate the barrier efficacy of the paper/plastic pouches in maintaining sterility after repeated sterilization in an ideal condition. Thus, filter paper was applied in place of medical/dental equipment to omit the possibility of sharp objects damaging the pouch integrity.

A previous study on paper/plastic pouch resterilization by Palananthana *et al*. used stainless steel wires packed inside the pouches and determined their sterility after being resterilized 1–5 times and stored in a closed cabinet for 4 months.[12] The results found 1.33% contamination in 1-time resterilized group at 2 months storage time, but there was no contamination in all other experimental groups (2, 3, 4, or 5 resterilization cycles and 3–4 months in storage), as well as the new pouch group. However, there was no negative control group to compare the findings. The results were ambiguous since the pouches that showed contamination were the ones with fewer cycles of sterilization (1 time resterilization) and a shorter storage time (2 months), while the pouches with more resterilization cycles (5 times resterilization) and longer storage time (4 months) could still maintain sterility. Our study found microbial contamination in all samples from the positive control group. All pouches in the positive control group were handled in a similar manner to pouches from the experimental groups and with gloves worn during transfer. This indicates that contamination may be from the ambient environment alone and that the barrier integrity is a very important factor in maintaining sterility of the package.

Studies on the shelf-life of new paper/plastic pouches have found contamination rates between 0% and 1.6%.[6,13-15] With low contamination rates, we calculated the sample size as 6720, which was significantly more than in past studies and with negative and positive controls to ensure enough chances for potential contamination. In all studies where contamination was detected, it was not time related but rather event related, as the negative control (without storage) also showed contamination not significantly different from the groups with longer storage times (up to 1 year).[13] There was the absence of contamination in the negative control group in our study. In studies without a negative control, microbial contamination was also not associated with storage time.[6] It was suggested that inadvertent contamination might have occurred during unpackaging and transfer of the medical/dental equipment.[14] Similarly, a prospective study which periodically checked for sterilized items shelved in hospital wards found no contamination for up to 2 years.[16] Indeed, with proper storage and handling conditions, the shelf life of sterilized equipment could be longer but no study has explored beyond a 2-year period. From these results, it can be concluded that microbial contamination is not dependent on storage time or the number of sterilization cycles.

This study used BHI broth for microbial culture to examine potential contamination. This medium has basically similar ingredients to Trypticase soy broth employed in other studies.[5,13-16] It can be used to culture both aerobic and facultative anaerobic bacteria.[17] We monitored the culture results for up to 2 weeks which was similar to the time period utilized in previous studies, as positive cultures might still be observed in the 2nd week.[13] Most positive microbial growth occurred within 24 h, similar to the time frame suggested by the manual,[17] and all samples were found positive within 8 days of culture. BHI appeared to be appropriate for culture of microbial contamination in this case, as the culture time was less than those using Trypticase soy broth, and all positive control samples consistently showed valid microbial growth.

Resterilization conditions of paper/plastic pouches in this study imitated an ideal condition where the sterilization process was repeated immediately with minimum handling. In real practice, reused pouches have a higher risk of event-related damage to the sterility of the pouches from unpackaging, re-packaging, handling, and transferring of pouches and equipment, as well as human error. This study was able to reveal only the intact barrier efficacy of

these pouches after repeated sterilization. A study on actual reuse practice with pouches containing medical/dental equipment is needed to validate these findings before the safety of reused pouches can be confirmed.

CONCLUSIONS

Resterilization of paper/plastic pouches for up to 5 times and storage in a closed environment for up to 6 months did not impair the barrier integrity of the pouches.

Financial support and sponsorship
This study was financially supported by Faculty of Dentistry, Mahidol University, Bangkok, Thailand.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Spry C. Understanding current steam sterilization recommendations and guidelines. AORN J 2008;88:537-50.
2. Kohn WG, Collins AS, Cleveland JL, Harte JA, Eklund KJ, Malvitz DM, *et al.* Guidelines for infection control in dental health-care settings--2003. MMWR Recomm Rep 2003;52:1-61.
3. Association of periOperative Registered Nurses. Recommended practices for selection and use of packaging systems for sterilization. AORN J 2007;85:801-2, 804-7, 809-12.
4. Mayworm D. Paper/plastic pouch heat seals. J Hosp Supply Process Distrib 1983;1:32-4.
5. Standard PG, Mackel DC, Mallison GF. Microbial penetration of muslin- and paper-wrapped sterile packs stored on open shelves and in closed cabinets. Appl Microbiol 1971;22:432-7.
6. Klapes NA, Greene VW, Langholz AC, Hunstiger C. Effect of long-term storage on sterile status of devices in surgical packs. Infect Control 1987;8:289-93.
7. Thanakitprapa P, Jaratrasamee A, Pachimsawat P. Autoclave monitoring and packaging in Bangkok dental offices, Thailand. M Dent J 2017;37:173-82.
8. Association for the Advancement of Medical Instrumentation. ANSI/AAMI ST79: 2010 & A1: 2010-Comprehensive Guide to Steam Sterilization and Sterility Assurance in Health Care Facilities. Arlington, VA: Association for the Advancement of Medical Instrumentation; 2010
9. Ostrom QT, Gittleman H, Stetson L, Virk SM, Barnholtz-Sloan JS. Epidemiology of gliomas. Cancer Treat Res 2015;163:1-4.
10. Maniglia-Ferreira C, de Almeida Gomes F, Ximenes T, Neto MA, Arruda TE, Ribamar GG, *et al.* Influence of reuse and cervical preflaring on the fracture strength of reciprocating instruments. Eur J Dent 2017;11:41-7.
11. Hogg NJ, Morrison AD. Resterilization of instruments used in a hospital-based oral and maxillofacial surgery clinic. J Can Dent Assoc 2005;71:179-82.
12. Palananthana M, Pachimsawat P, Thaweboon S. Microbial finding of reused laminated film pouches stored in closed cabinet for 4 months. M Dent J 2006;26:179-86.
13. Butt WE, Bradley DV Jr., Mayhew RB, Schwartz RS. Evaluation of the shelf life of sterile instrument packs. Oral Surg Oral Med Oral Pathol 1991;72:650-4.
14. Schwartz R, Davis R. Safe storage times for sterile dental packs. Oral Surg Oral Med Oral Pathol 1990;70:297-300.
15. Sattayasanskul W, Saetoh S, Thiengmontree A, Veerakeiatsoonthorn S. Effect of storage time on sterility of instrument in oral surgical packs. J Dent Assoc Thai 1990;40:54-8.
16. Webster J, Lloyd W, Ho P, Burridge C, George N. Rethinking sterilization practices: Evidence for event-related outdating. Infect Control Hosp Epidemiol 2003;24:622-4.
17. Power DA, Zimbro MJ. Difco & BBL Manual: Manual of Microbiological Culture Media. Sparks, MD: BD Diagnostic Systems; 2003.