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Research article

Sterility maintenance of reused disposable paper/plastic sterilization pouches in actual clinical practice

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ABSTRACT

Paper/plastic sterilization pouches are commonly used packaging material for steam sterilization. Reuse of these pouches is a general practice in Thailand despite a single-use recommendation. This study aimed to determine microbial contamination after reusing paper/plastic sterilization pouches in a dental clinic and storage in a closed environment for 6 months. Three hundred and twenty pouches underwent 3 times of clinical use in terms of packaging, autoclave sterilization, handling, and unpacking. A mouth mirror was packed in each pouch to be used in a clinic. After each use, a pouch would be carefully inspected for reusability and undergone packaging, sterilization, handling again. In all steps, sterilization monitoring was rigorously applied. After 3 times of use, a piece of filter paper was placed inside each pouch (instead of a mouth mirror), the pouch was autoclaved and stored in a closed environment for 6 months. Then the filter paper was retrieved for microbial cultivation. A negative control group comprised new pouches containing filter paper without storage and a positive control group comprised pouches with impaired integrity. All samples in both the reuse and the negative control groups had no microbial contamination. All samples in the positive control group showed contamination. These results suggested that reusing paper/plastic sterilization pouches could be a safe practice provided careful monitoring and inspection were employed.

1. Introduction

In a general dental practice, adequate supply of sterile dental instruments is a vital part of every dental procedure with packaging and sterilization as important processes to prevent infection [1, 2]. Steam sterilization using an autoclave is the most commonly used method of sterilization due to its simplicity, effectiveness, and practicality [3, 4]. Packaging helps preserve the sterility of sterilized instruments in storage before use by preventing microbial contamination from the external environment after the sterilization process. A number of studies on infection control cover various aspects including personal hygiene and personal protective equipment such as wearing gloves, disinfection and sterilization methods, sterilization monitoring, as well as knowledge of infection control [5, 6, 7, 8, 9]. Few studies focus on packaging, though. Previous studies found closed environments such as closed cabinets or drawers to be preferable to open environments such as open shelves or corridors and that safe storage time of sterile packages was at least up to 30 months [10, 11].

The most commonly used packaging material for steam sterilization is paper/plastic sterilization pouch due to its convenience of use, content visibility, and efficacy [12]. These paper/plastic sterilization pouches are disposable and recommended to be used only a single time [13]. However, a survey on autoclave dental packaging in Thailand found that such pouches were routinely reused in more than 80% of the private clinics and in about 30% of the hospital clinics surveyed [14]. Moreover, the pouches were used most frequently 3 times but could be up to 5 times. This is the only survey on the reuse or resterilization of the packaging. Moreover, there is no study on the impact of reuse/resterilization of packaging in actual practice. Several studies on the shelf-life of sterilized packs in clinics found contamination in the range of 0–1.6% in a period of up to 2 years [10, 15, 16]. These studies agreed that contamination was inadvertent and was not time-related; however, all these observations were from single-use pouches. There is no study on the incidence of contamination in reused pouches in actual clinical settings. The objective of this study aimed to assess sterility maintenance of reused pouches in practice. This study could provide information regarding the factors

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involved in the safety of reused paper/plastic sterilization pouches in a clinical setting.

2. Materials and methods

2.1. Study protocol and paper/plastic sterilization pouch preparation

This study consisted of an experimental group, a negative control group, and a positive control group.

2.1.1. Experimental group

A total of 320 paper/plastic sterilization pouches was prepared in exactly a similar pattern as shown in Figure 1. Each pouch was prepared from sterilization tubular rolls (SteriCLIN VP Medical Packaging, VP Marketing GmbH, Germany) at 30 cm long and contained a mouth mirror and an internal chemical indicator (3M Comply SteriGage Chemical Integrator, 3M, USA) inside. The paper/plastic sterilization pouch was heat sealed at both ends (Euroseal, Euronda S.p.A., Italy). The width of a sealed area was 1.2 cm. The external chemical indicator (autoclave tape) was placed outside. Then each pouch was autoclaved (Autoclave 1) (M11 UltraClave, Midmark Corporation, USA), checked for sterility, and stored for use in the actual clinical setting. The dental assistants were instructed to peel open the pouch on the indicated side (a trapezoid side) and only as wide as necessary, drop the mouth mirror, and keep the used opened pouch in the bag provided. This pouch was then inspected for reusability from intact seal along the unopened margin and intact integrity of the paper and plastic sides. The pouches that passed the quality check would be reused (Reuse 1). The process repeated with a mouth mirror and an internal chemical indicator placed inside the reused pouch. The pouch was resealed at a different position, autoclaved (Autoclave 2), checked for sterility, and stored in a clinic to be used again. The process repeated until each pouch was used in a clinic 3 times (Autoclave 3/Reuse 2). For the last time, a piece of 0.5×2 cm^2 filter paper (Whatman paper, Patterson Scientific, England) was placed inside (instead of a mouth mirror). Both internal and external chemical indicators were also employed as in previous cycles of sterilization. The pouch was then autoclaved (Autoclave 4/Reuse 3) and stored in a closed container. After 6 months of storage, the filter paper was checked for microbial contamination.

2.1.2. Negative control

This was a group with a paper/plastic sterilization pouch containing filter paper and an internal chemical indicator that passed one sterilization cycle. The pouch was prepared on the day of microbial cultivation (no storage).

2.1.3. Positive control

The paper/plastic sterilization pouch was prepared similar to that of the negative control group but with damaged integrity. A spatula was used to separate the paper side and the plastic side of the pouch for about 5 mm wide at 3 positions each side (Figure 2). The pouches passed one sterilization cycle and stored in a closed container. After 6 months, the filter paper was checked for microbial contamination.

A flowchart of the experimental protocol is summarized in Figure 3.

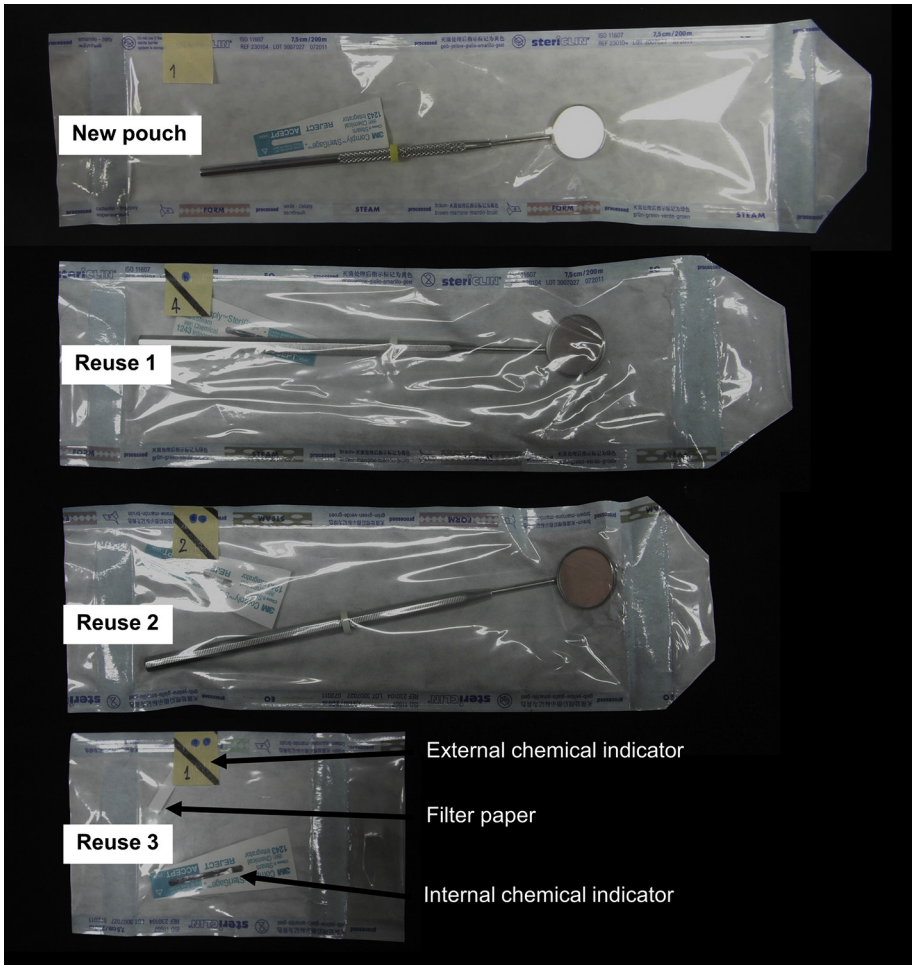

Figure 1. Representatives of pouches from each round of use.

Figure 2. Pouches from a positive control group showing a spatula separating the seal on the side of the pouch. The circled area shows the separated seal.

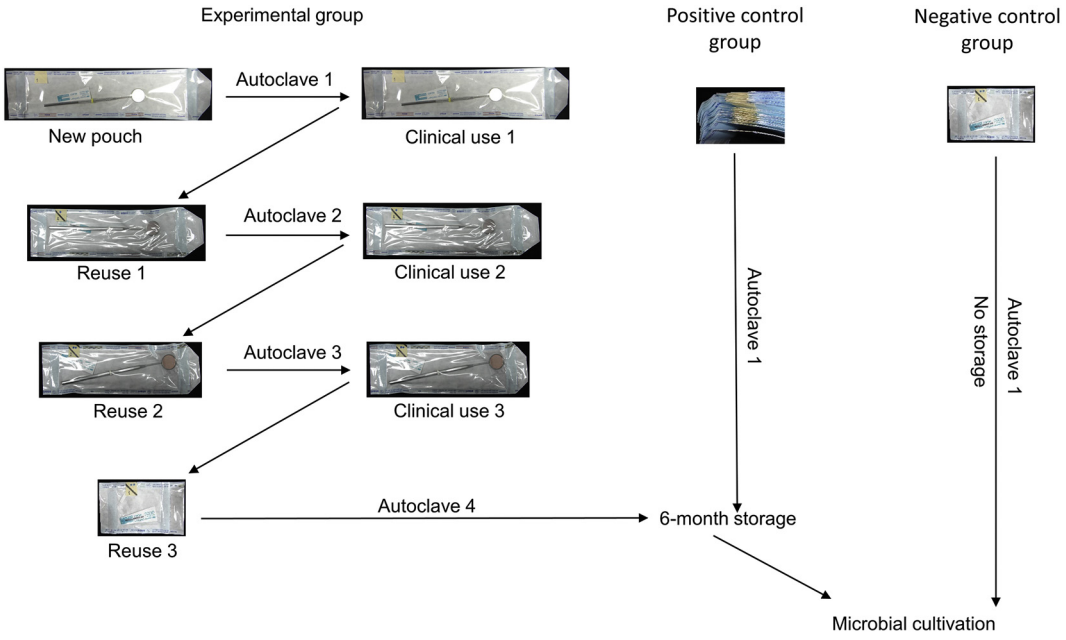

Figure 3. A protocol of the experimental design.

2.2. Sterilization process

All paper/plastic pouches were autoclaved at 121 °C and 15 psi for 30 min. 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 in all pouches from 3 experimental and 2 control groups using an internal chemical indicator and autoclave tape as an external chemical indicator. Both types of chemical indicators would change the color following the sterilization process. However, biological monitoring is the method most reliable since it directly detects the killing of a microorganism. The spore test tubes (3M Attest, 3M, USA) containing *Geobacillus stearothermophilus,* a highly resistant microorganism, were placed at the center and opposite corners of the autoclave tray. After the sterilization cycle, the spore test tubes were incubated at 56 °C for 48 h to evaluate for microbial growth. A positive culture indicates failure of an autoclave process. Biological monitoring is recommended on a weekly basis [1]; however, in this experiment, it was performed in every cycle of sterilization.

At the last step of sterilization before 6-month storage (Autoclave 4/ Reuse 3), the pouches from the experimental group and the positive control group were put in a tray and autoclaved together in the same cycle. Each cycle contained 70–72 pouches.

2.3. Microbial cultivation

Brain Heart Infusion (BHI) broth (Becton Dickinson, Maryland, USA) was used as the medium to culture for possible contamination of the filter paper [17]. Briefly, the broth was prepared and dispensed in a glass bottle one day in advance. After the specified storage period, the pouch was inspected for barrier integrity prior to opening. One experimenter would peel-open the pouch until the filter paper could be retrieved aseptically by a second experimenter who put the filter paper into the broth containing bottle. The incubation was at 37 °C for up to 2 weeks. In each round of incubation, there was filter paper from an experimental, a positive, and a negative control group as well as the control group of broth not containing filter paper. A negative culture was considered to be a clear broth. Even the slightest turbidity of the media indicated microbial contamination as the filter paper would not be clearly observed.

3. Results

Each of the 320 paper/plastic sterilization pouches were employed to undergo 3 uses in a real clinical setting (sterilization was performed prior to each use) with the last step using a piece of filter paper placed inside the pouch (Autoclave 4, as shown in Figure 3) and a storage of 6 months to finally check for microbial contamination. Among these, 291 pouches passed all 4 sterilization cycles. Twenty-nine pouches were excluded

after reusability inspection; 16 (5%) and 13 (4%) were excluded after the first and the second clinical uses, respectively. All pouches from the 3rd clinical use passed the reusability inspection and none was excluded. The most common cause of exclusion was peel opening on the wrong side (Table 1) making the pouch not resealable from too wide an opening.

In every cycle of sterilization, physical monitoring during the sterilization process showed that the conditions required were met, both external and internal chemical indicators showed the color change indicating the proper functioning of the autoclave machine. The spore tests showed negative results indicating the sterile status inside the autoclave.

All filter paper from 291 pouches passing Autoclave 4 (Figure 3), and were stored in closed containers for 6 months, showed no microbial contamination. Also, all filter paper from the pouches in the negative control group (n = 291) showed no contamination after 2 weeks of culture. On the other hand, all the filter paper retrieved from the pouches in the positive control group (pouches with damaged integrity) showed positive microbial growth within 2 weeks with 56% showing positive growth within 10 days in cultures (Figure 4).

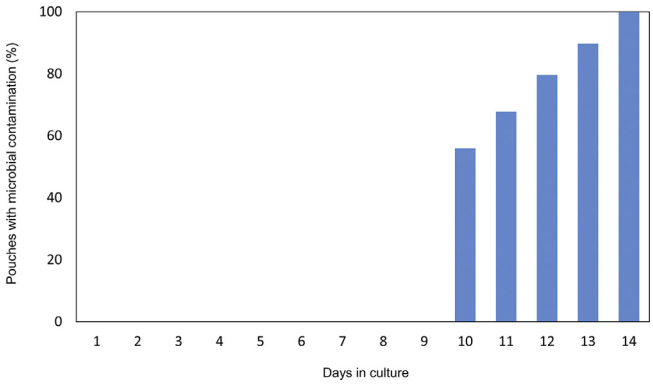

Figure 4. Percentage of the filter paper from the positive control group that showed microbial growth (n = 59).

4. Discussion

Our previous study found the intact barrier integrity of paper/plastic sterilization pouches after repeated sterilization in an ideal condition where the sterilization processes were repeated immediately (up to 6 times) with minimal handling and without real use in a clinic [17]. However, sterility maintenance of a pouch with 'resterilization' does not also mean sterility maintenance with 'reuse.' In the current experiment, we found intact sterility of paper/plastic pouches that were used 3 times clinically and were stored in closed containers for 6 months. This was the most common time of reuse from a survey in Thai dental clinics [14]. The last step of 6-month storage was to confirm the integrity of the pouch after repeated use, resterilization, and long-term storage. The design of this study did not compromise patients' care because the monitoring was rigorously performed and all pouches were inspected for intact integrity and positive color changes of the chemical indicators before opening of the pouches. This is the first study to prove that reusing paper/plastic sterilization pouches does not impair sterility maintenance of the pouches provided certain measures are taken into account to make the reuse safe in practice.

There are many factors that could affect sterility maintenance of the reused pouches such as intention to reuse the pouch, inspection before reuse, handling and unpacking, and education on infection control. In our experiment, we clearly advised the dental assistants of the intention to reuse the pouches and instructed them to carefully tear the pouches with a minimal opening just enough to release the mouth mirror to prevent a large tear. Otherwise, the pouch would not be reusable due to not enough space for resealing. In real practice, the space for packaging will be smaller after each use, thus the pouch will subsequently fit a smaller instrument each time. In our pilot study using 30 pouches with 10 dental assistants, 63% of the used pouches did not pass the reusability inspection due to an opening on the wrong side or puncturing of the pouch by a mouth mirror without peel opening. After the detailed instruction and an emphasis on careful opening, fewer pouches failed the reusability inspection (Table 1). The intention to reuse the pouch could be an important factor leading to a more careful opening of the pouch so as to not tear open it too much to prohibit resealing. From the results, the pouches that failed reusability inspection were mostly from the first two clinical uses. All pouches from the 3rd clinical use passed the reusability inspection, suggesting that after being acquainted with the procedure, the dental assistants were more able to tear the pouches successfully for reuse.

Table 1. The pouches excluded from the experiment.

Reasons for pouch exclusion	n
Wrong opening side	14
Broken side seal	11
Wrong opening side and broken side seal	2
Torn pouches	2
Total	29

Another crucial step is the inspection of the pouches in order to determine reusability before re-packaging and resterilization. In our experiment, we were very careful with the inspection of every used pouch. If the inspection was not done properly, it might lead to contamination of the instrument from using the damaged pouch as can be seen in the positive control group. We found positive microbial growth in a positive control group at about 2 weeks' time in culture. This is different from the previous study that found positive growth within 8 days of culture with most samples showing positive results within 24 h [17]. This could be because in the current study the damage to the pouch was less than that in a previous study where there were many holes in a single pouch. Our results prove that even with tiny openings in a pouch and with careful handling and storage in closed environments, the contamination is imminent provided the integrity of the pouch is lost. Thus, in circumstances when the operator is uncertain whether the pouch is contaminated or not, or if in doubt of any tear of the pouch, that pouch should not be reused. Reuse should only be done with the pouch that the operator is certain of its intact integrity and cleanliness.

This study used BHI broth to culture for potential contamination. This broth is suitable for culturing a wide variety of organisms including fastidious aerobic and anaerobic bacteria, yeasts, and fungi [18]. Other related studies employed Tryptic Soy Broth which is suitable mainly for culturing aerobic bacteria [10, 19, 20]. BHI broth appeared to be appropriate to check for contamination in this case as all samples in the positive control group consistently showed positive microbial growth (Figure 4). However, since no single broth or medium can be used to culture every organism, it might be thorough to use two types of broth to double-check for contamination.

The latest review on the quality of cross-infection control in dental laboratories worldwide revealed substantial flaws in infection control processes and disinfection practices even though patients' greatest concern is compliance with sterilization [21]. Many issues are involved in potential contamination including knowledge of infection control, personal hygiene, personal protective equipment, disinfection, and sterilization. It was found that sterilization monitoring was deficient not just in developing but also in developed countries [22]. A survey in Thailand similarly found that most dental clinics, both private and public, did not perform adequate sterilization monitoring [14]. Only 10% of the clinics surveyed performed all types of monitoring (physical, chemical, and biological as explained in Methods). Education on infection control is also an important factor as observed in our pilot study on puncture opening of the pouch with a dental instrument. Puncturing is not a

recommended practice due to potential contamination from outside of the pouch. This suggests that some dental assistants may not have adequate education on sterilization. Indeed, a survey in 2013 on Thai clinics found that only 44% of responders had formal education on infection control [14].

There were two previous studies assessing the sterility of resterilized paper/plastic pouches [23, 24]. Both found contamination in resterilized groups but the results were inconclusive. In one study, the contamination was found in one time resterilized pouches but not in 2–5 times resterilized pouches [24]. While in another study, the contamination was found in the new pouches as well as in the resterilized pouches [23]. Most studies on incidental contamination of the sterilized packages similarly found intact sterility provided proper storage and handling conditions suggesting that microbial contamination was event-related from inadvertent contamination during transfer, handling, and unpacking [10, 11, 15, 19]. These observations suggest that many factors are associated with potential contamination in a clinic and could be important when considering reusing the pouches especially the important contribution of human errors to the sterility maintenance of the packages. Thus, compliance with the guidelines on infection control is crucial [1, 25, 26]. For example, opening of the pouches should be performed based on aseptic technique with uncontaminated hands in areas not easily contaminated with human saliva/blood/tissue; when sterilizing sharp instruments, the sharp ends should be protected to prevent rupturing or puncturing the pouch. Such measures as recommended by the guidelines are important general infection control procedures to follow whether reusing is an issue or not.

Currently, there is no study on the reuse practice on how this process is actually done in various clinical settings or what the incidence of contamination is. Our study demonstrated that reusing paper/plastic sterilization pouches can be safe provided certain factors are carefully applied and monitored. The limitations that need to be considered are the tearing of the pouch and careful inspection before re-packaging. These issues require the operator to clearly understand the procedure and realize the importance of each step in advance. For convenience and safety, single-use of a paper/plastic sterilization pouch is still a recommended practice.

Declarations

Author contribution statement

J. Klumdeth: Performed the experiments.
N. Jantaratnotai: Analyzed and interpreted the data; Wrote the paper.
S. Thaweboon: Contributed reagents, materials, analysis tools or data.
P. Pachimsawat: Conceived and designed the experiments; Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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