Original Article

The Efficacy of Surface Disinfectant Wipes After Exposure to Air by Un-capping the Container

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Abstract

The objective of this study is to determine whether air exposure of different disinfectant wipes by un-capping the container alter the bactericidal efficacy. Three commercially available disinfectant wipes with different active ingredients consisting of CaviWipes™ (QAC & Isopropyl alcohol), SporeClear™ (QAC & Biguanides) and Optim 33TB (Ionized H_2O_2) were used in the experiment. Forty milliliters of stimulated saliva were collected from 15 healthy volunteers in the morning before performing daily oral hygiene care. The saliva was spread on sterilized leather surfaces and air dry. The surfaces were then used to test the effectiveness of disinfectant wipes those had been air exposed for 1, 4 and 18 hours compared with a group that tightly cap the container. The remaining CFU/mL of bacteria on the surfaces were calculated to compare log reduction. Data were statistically analyzed by Kruskal-wallis test and Mann-Whitney U test. A value of $p<0.05$ was considered significant. This study revealed that air exposure seems to marginally affect the antibacterial capability of disinfectant wipes with different active ingredients as determined by log reduction. Despite non-alcohol containing formula of SporeClear™, air exposure seems to worsen its activity, though no statistically significant difference was observed. Bactericidal activity of CaviWipes™ and Optim 33TB were quite stable regardless of prolonged air exposure or the order of sheet pulled out from the container. The outermost sheet of SporeClear™ was more affected by prolonged air exposure. To sum up, bactericidal efficacy of disinfectant wipes was minimally affected by duration of air exposure. Activity of SporeClear™, a non-alcohol containing formula, was greater affected by time and order of sheet dependent according to its texture.

Keywords: Alcohol, Biguanides, Disinfectant wipes, Infection Control, Ionized Hydrogen peroxide

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Introduction

Preventing the spreading of infection during dental practice is a key process for providing patients safety. During dental treatment, it is inevitable that there will be contamination of fluids such as saliva, blood, oral fluids, etc. These fluids are enriched with microorganisms that may cause the spreading of diseases. Cleaning the clinical contact surfaces is one of a crucial component of standard precautions. Clinical contact surfaces must be wiped to clean and disinfect with chemical disinfectants at least of intermediate-level or hospital disinfectant with tuberculocidal claimed. The registration of chemical disinfectants in the United States is done by three agencies. Centers for Disease Control and Prevention (CDC) classify the chemical disinfectants into three different levels base on their efficacy: low-, intermediate-, and. high-level disinfectant.[1,2] Additionally, the US Environmental Protection Agency (EPA) registers the effectiveness of chemical disinfectants categorized into two groups based on their ability to kill Mycobacterium tuberculosis; the Hospital disinfectant with or without tuberculocidal claim.[1,2] The US Food and Drug Administration (FDA) register chemical disinfectants with sporicidal activity or referred as chemical sterilant. In Thailand, all of the chemical disinfectants use in medical and dental practices are registered by Thailand Food and Drug Administration.

Chemical disinfectants use for surface disinfection nowadays available on the market has been developed into easy-use format as pre-immerse cleaning sheets. The products in this format are very practical to disinfect the contaminated surfaces within a limited of time and therefore become very popular. The disinfectant wipe available nowadays on the market contains several active ingredients for examples alcohol, biguanides, quaternary ammonium compound, H_2O_2 etc. Generally, two forms of alcohol, Isopropyl and ethyl alcohol is widely used as antiseptics. However, the high rate of evaporation limits its utility as disinfectant.[1] Biguanides, well-known as Chlorhexidine, can disrupt cell membrane's permeability and causes cytolysis. However, biguanides demonstrated less sensitivity against Mycobacterium tuberculosis. To Spore-forming bacteria and TB, Biguanides can be only bacteriostatic.[3] Recently, several studies demonstrated that Hydrogen peroxide is more satisfied in bactericidal activity than other product.[4-6] All of the disinfectants mentioned previously are presented as active ingredient in the ready to use disinfecting towelette available in the market nowadays. When utilized this kind of products, it is required that the cap of the container must keep tightly close to avoid the evaporation of active ingredients, especially the product contain alcohol.[1] The relative evaporation rate of isopropyl alcohol is 21 times faster comparing with ether and 1.7 times comparing with n-Butyl acetate.[6,7] From observation, it was found that clinical practitioners often forget to close the container's lid, which may affect the product's effectiveness. However, there is no clear evidence stating the correlations between the time period if the container is left open and the reduction of the efficiency of disinfectant wipe. This information will be useful for products selection and usage suggestion. This research aimed to compare the efficacy of the disinfectant wipe with different active ingredients with the time the products' containers were left open. The efficacy of the first sheet and the following two sheets were also examined when the containers' lid was left open. The data from this research will be useful for the development of guidelines for infection control in the dental clinic.

Materials and Methods

We utilized disinfectant wipes with different active ingredients available commercially by mainly focus on two particular types of products; alcohol containing formula and the non-alcohol containing formula. The selected disinfectant wipes and its active

ingredients include CaviWipes™ (isopropyl alcohol and QAC) (Metrex, Romulus, Michigan, USA.), Optim 33TB (Ionized H2O2) (SciCan, Toronto, Canada.) and SporeClear™ (Biguanides and QAC) (Hu-Friedy Mfg. Chicago, Illinois, USA.)

The disinfectant wipes were divided into 4 groups; immediately after open, left the cap open for 1, 4 and 18 hours, respectively. The 1st, 2nd and 3rd sheet in the container were used to test for bactericidal activity against cultivable oral bacteria.

Saliva Collection

Saliva samples from 15 volunteers have been collected using stimulated saliva collection methods. The volunteers were asked to chew a piece of paraffin to stimulate the salivary flow in the morning before performing routine oral hygiene. The saliva was collected approximately 40 milliliters. With this method, dental plaque from different areas of the mouth including the tooth surface will be removed by mechanical cleansing. The samples were kept at 4°C till being used.[8,9] The saliva from each volunteer was used in the experiment conducted independently. The saliva from each individual was spreaded onto 37 leather samples including 36 test groups (3 products, 4 durations and 3 sheets) and one for control group (sterile distilled water). The experiments were performed repeatedly using saliva from all 15 subjects.

Surface preparation, disinfection and sample collection

Pieces of leather, 20 x 20 cm in size, and glass slab frame with a square hole of size15 x 15 cm were custom made to mimic surface contaminated with saliva. All of the materials were sterilized by autoclave prior to use. The assembly of these tools for the experiments was shown in Figure 1.

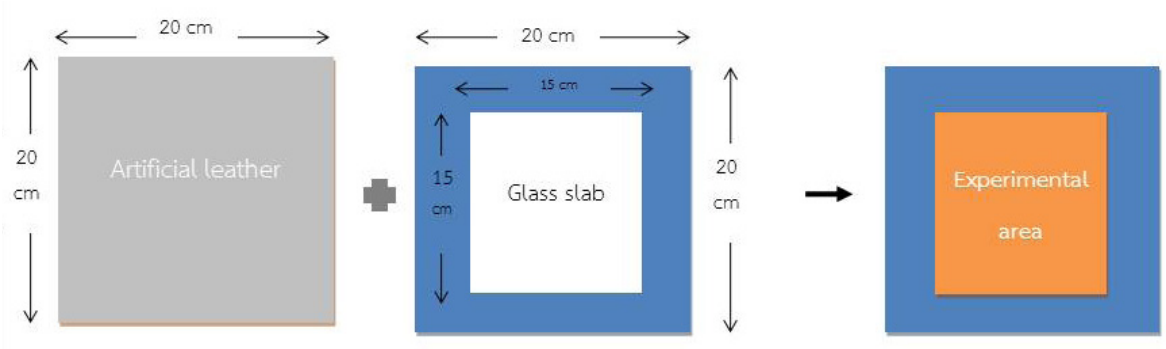

Figure 1 *Diagram of saliva contaminated leather surfaces preparation*

A saliva sample of 0.1 mL was dropped and spread by sterile cotton swab onto the surfaces and leave to dry. The experiment area mimicking saliva contaminated surface was marked with a pencil before taken the glass slab off. The disinfectant wipes of different composition, air exposure time and order of the sheet were used to wipe the surface and leave for appropriate contact time those recommended by the manufacturer.

In order to test the effect of air exposure against the efficacy of 3 different disinfectant wipes available commercially, the disinfectants' containers were left open to expose the wiping materials to air for 18, 4, 1 hours and not exposure. Then the 1st, 2nd and 3rd sheet of disinfectant wipes in each time point were used to disinfected saliva contaminated surface. We controlled the wiping manner to be in the same pattern by wiping in left to right direction continuously from top to bottom of the area. Only one side of a sheet was used per experiment area.

The bacterial samples remained on leather surfaces were then collected with sterile cotton swab and PBS (Phosphate Buffered Solution) by swabbing

motion all over the surfaces. Sterile scissors were used to cut the tip of cotton swab into eppendorf tube containing 1 mL of sterile PBS. The tubes were then shaken with vortex mixer for 3 minutes and sonicated (Sonics VCX750 Newtown, Connecticut, USA) for 30 seconds at 20 % amplitude to break clump of bacteria. After that, a serial ten-fold dilution of the samples were performed and 100 µL of appropriate dilution were plated onto blood agar plate. The plates were incubated at 37°C for 24 hours. The recoverable colony on blood agar were then enumerated and calculated to Log reduction by comparing with initial number of bacteria on the surfaces. Bacteria recovered from surfaces wiped with sterile distilled water were kept as negative control. We repeated the experiment by utilizing saliva samples collected from different 15 subjects.

The texture examination of wiping materials under stereomicroscope.

We examined closer to the texture of wiping materials under a stereomicroscope (Olympus SZH10 Shibuya-ku, Tokyo, Japan) to compare texture of wiping materials of three different disinfectant wipes.

Statistical analysis

Data were statistically analyzed by Kruskal-wallis test and Mann-Whitney U test. A value of $p<0.05$ was considered significant.

Result

When appropriately kept in tightly closed container, the disinfectant wipes could reduce bacteria on the surface up to log 4.90, log 5.33 and log 5.16 reduction, for CaviWipes™, SporeClear™ and Optim 33TB respectively (Fig. 2). Whereas bacteria on the surfaces was found to reduce by log 1.66, if the contaminated surfaces were wipe with distilled water alone.

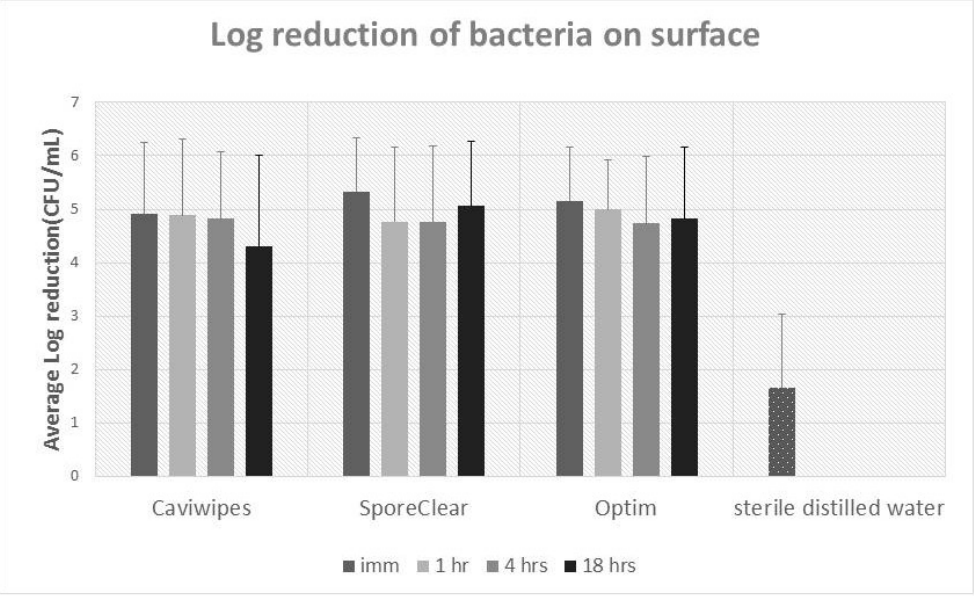

Figure 2 *Average log reduction of bacteria on surfaces after disinfected by 1st sheet of various disinfectant wipes with different air exposure times*

If the container's cap was left open, disinfection efficacy of the first sheet of every product reduced in different pattern as demonstrated in fig. 2. CaviWipes™, which is alcohol containing product, was relatively stable after exposure to air for 1 and 4 hours. Its efficacy starts to reduce after being exposed to air for 18 hours. Unexpectedly, the efficacy of an alcohol-free product, SporeClear™, reduced at as early as 1 hr and thereafter. Efficacy of Optim 33TB, ionized H_2O_2, is quite stable regardless of how long it had been exposed to air. However statistically significant results were not observed in any tested groups.

If the container's cap was closed tightly, a comparable bactericidal efficacy was observed among 1st, 2nd and 3rd order of sheet in the container of all tested products (Fig. 3a). It should be noted that the efficacy of the 2nd and 3rd sheet was slightly higher than the 1st sheet when the container's lids were left open, especially in SporeClear™ group, though no statistical significant was observed (Fig. 3b-3d).

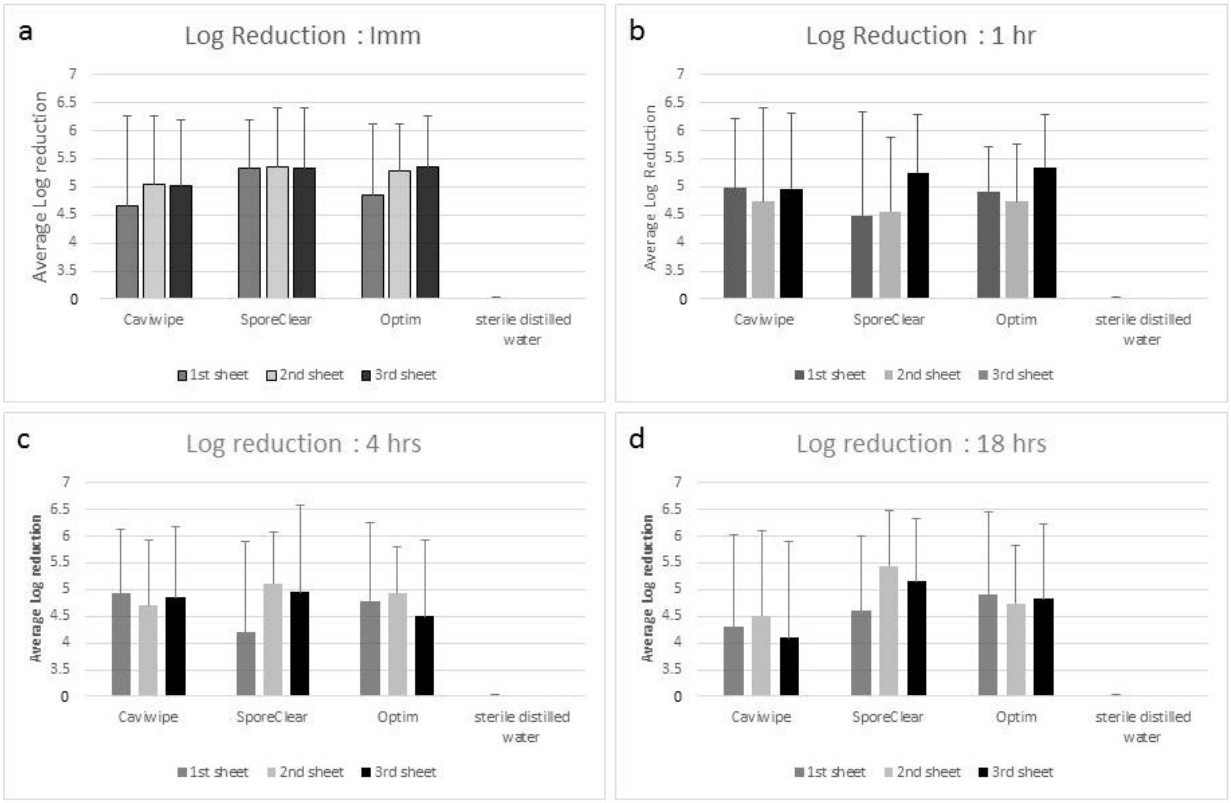

Figure 3 *Average log reduction of bacteria on surfaces after disinfected by various disinfectant wipes. a) Tightly capped the container's lid (Immediately used). b) Uncapped the container's lid for 1 hr. c) Uncapped the container's lid for 4 hrs. d) Uncapped the container's lid for 18 hrs*

We examined closer to the texture of wiping materials under stereomicroscope (Fig. 4). It was clearly demonstrated that CaviWipes™ and Optim 33TB utilized similar pattern of texture of materials consisting of a thick and dense fibrous sheet with small circular pattern made from thinner material. SporeClear™ had a different pattern of texture with obviously looser fibrous without circular pattern.

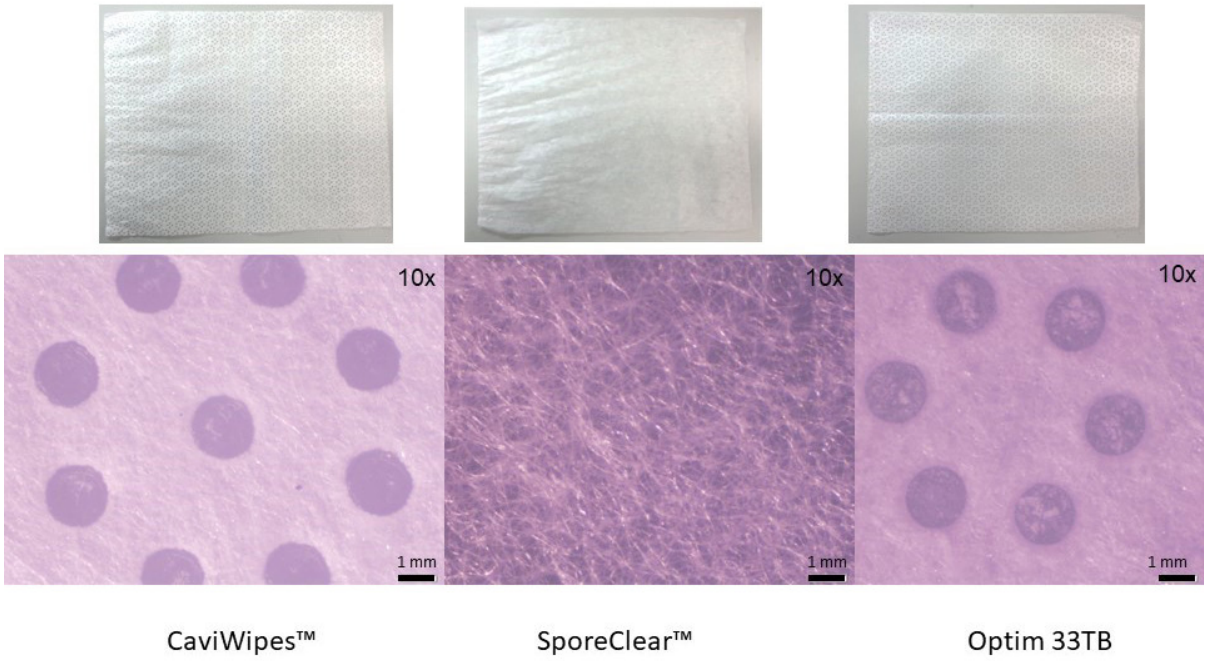

Figure 4 *Surface texture of disinfectant wipes under stereomicroscope*

Discussion

Our study was designed to test the bactericidal efficacy of different disinfectant wipes against cultivable oral bacteria and the effect of air exposure. The results indicated that within the tested period, the efficacy of all disinfectant wipes were not significantly reduced by air exposure time. It is quite surprising to us that a non-alcohol containing product, SporeClear™, demonstrated slightly reduction in its efficacy at as early as 1 hour of air exposure and thereafter. Whereas the alcohol-containing product, CaviWipes™, starting to reduce its efficacy after 18 hours of air exposure. This result was not consistent with previous concern regarding the evaporation rate of alcohol.[9] The texture examination of wiping material clearly shows the difference in texture of material between SporeClear™ and the other two brands. Thicker and denser fibrous sheet with small circular pattern made from thinner material of CaviWipes™ and Optim 33TB might help retained the disinfectant on the wiping material. The standardized methods for evaluating the effectiveness of chemical disinfectants, the decrease required in the initial inoculum is a minimum of logarithms.[10] In our study, all of the three products with different active ingredients demonstrated effectiveness close to log 5 reduction when use immediately. The effectiveness was slightly reduced, though not significant, with prolonged air exposure.

Besides texture of wiping material, cap tightness may also result in different efficacy. We have noticed that each product's container has different cap tightness which may affect the seal of the container. Meanwhile leaving the cap open may dry out disinfectant from the outer most of the sheet that exposes to air, it is noticeable that the portion of wet, unexposed sheet inside the container still retained its efficacy to reduce the bacterial count comparable to those of tightly capped container. However, our experiment was conducted within a short period of time which might not fully resemble those situations occur in clinic which multiple prolonged air-

exposure might occur repeatedly. Therefore, our suggestion is to tightly close the cap of container of disinfectant wipe every time after use. Turning the bottle up-side down to distribute the disinfectant equally when not use is also recommend. In conclusion, bactericidal efficacy of disinfectant wipes was not affected by duration of air exposure up to 18 hours tested.

Acknowledgements

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