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## The Effectiveness of Using Different Solutions to Sanitize Contaminated Toothbrushes

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The purpose of this study was to estimate the amount of bacteria left on toothbrushes after brushing and the efficacy of their sterilization by spraying some antibacterial agents. For controlled conditions, only one test subject was instructed to brush his teeth once a day after dinner with a new toothbrush until the experiment ended. Then the toothbrush was sealed in a sterile pack and moved to the laboratory. The bristles of this toothbrush were cut into quarters to spray them with 4 different kinds of solution: 1 (control – distilled water), 2 (toothpaste solution 10%), 3 (vinegar 50%), and 4 (chemical spray - grain alcohol 70%). After spraying the bristles, bacteria, normally *S. mutan*, was collected and cultured in LB agar for 24 hours. There was a significant decrease in the number of bacteria when comparing the control to sprays 2 and 4, which overlapped in the error bar. Therefore, using either a 10% toothpaste solution or 70% grain alcohol solution is the most effective way to sanitize contaminated toothbrushes.

*toothbrush, hygiene, antibacterial, disinfect, sanitize*

### INTRODUCTION

Oral health is very important both socially and pathologically. People receive dental care, but we rely heavily on brushing our teeth for dental health. According to Cobb [1, pp. 263-264], bacterial contamination of toothbrushes can cause serious mouth infections. But most people use the same toothbrush for several weeks or even months. In a prior experiment we conducted for this paper, a toothbrush used for 30 days in a bathroom environment showed a significant degree of contamination.

Scientists have also studied what causes a toothbrush to become contaminated. They found that there are two sources for bacterial contamination of toothbrushes: bacteria not only originate from the oral cavity [2, p. 93] but also from the environment where the toothbrushes are stored [3, p. 99].

Methods for sanitizing toothbrush have already been suggested, such as UV rays [4, p. 24], immersion in disinfecting solutions [5, p. 381], spraying chemical decontaminating solutions on the bristles [3, p. 99],

using a microwave oven [6, p. 641], and using a dishwasher [7, p. 616].

The purpose of this paper is that to evaluate the efficacy of spraying chemical decontaminating solutions on the bristles, which is the most effective way of sterilizing a toothbrush at home. The solutions used for making the spray are distilled water, toothpaste solution, vinegar, and chemical sprays on the market that can be easily found and are not toxic. The vinegar solution was selected because one study [8, pp. 31-32] suggested that it had the best price to performance ratio in sterilizing toothbrushes.

Because toothbrushes are so easily contaminated by normal use and because most people use their toothbrushes for long periods of time before replacing them, it is important for health reasons to sterilize toothbrushes by some process.

### METHODS

#### Study Design

There was some possibility that we cannot get meaningful data if toothpaste was used by the test

subject when brushing his teeth because bacteria in the oral cavity are vulnerable to being destroyed by the toothpaste. To prevent this from happening, the experiment was conducted without toothpaste when the test subject brushed his teeth.

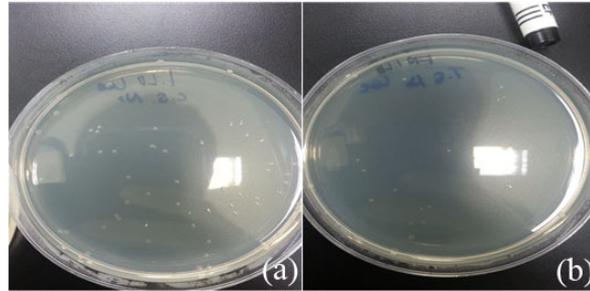
Only one test subject was used in this experiment to control for the bacterial differences in each person's oral cavity. The test subject was forbidden to use any antibacterial products in his oral cavity and had to brush his teeth once a day right after dinner until the experiment ended. The subject was directed to brush his teeth for 3 minutes in the evening immediately after dinner under our supervision. Above mentioned, totally 4 solutions were used in this paper. Table 1 shows the composition of solutions.

Type	Composition
Spray 1	Distilled Water 100%
Spray 2	Toothpaste solution 10 wt%
Spray 3	Vinegar 50%
Spray 4	Toothbrush sanitizer 100% (Grain alcohol 70%)

**Table 1.** Composition of tested solutions

Toothpaste, Vinegar, Toothbrush sanitizer, spray gun made up of each spray.

There were three prior experiments conducted in this research which were used to help refine these methods. They were conducted with toothpaste. The first was for a toothbrush used for 1 day, and the second was for a toothbrush used for 3 days. Although only distilled water was used to clean the toothbrushes, little or no bacteria was found on the agar. Therefore, the method of only brushing one time with no toothpaste was selected to obtain a proper amount of bacteria (see Figure 1).



**Figure 1.** The result of prior experiment.

(a) toothbrush used for 1 day (b) toothbrush used for 3 days Although they were cleaned only with distilled water, there was no bacteria found on either plate.

Bacteria can be transferred from a toothbrush to a plate by two methods: by scratching the toothbrush with a sterilized pin and rubbing it onto the plate or by washing the toothbrush in an Agar solution and pouring it onto the plate. With regard to the plate, both a Nutrient Agar plate and an LB Agar plate could be used to incubate bacteria. Through a third prior experiment, the washing method and the LB Agar plate were selected to more easily count the number of colonies.

### Experimental Method

The test subject brushed his teeth without toothpaste under the above-mentioned conditions. Immediately after brushing, the toothbrushes were sealed in a sterilized pack. The bristles of these toothbrushes were quartered with a sterilize knife. Each of quartered bristles was put on different plates, and each plate was sprayed with one of 4 differnt solutions. The method of spraying was to spray two times for each plate and from a distance of 5cm. Then the sprayed plates were washed with agar and cultured in the LB Agar plates. These agar plates were incubated at a temperature of 37°C for 24 hours. After the incubation period, the number of colony forming units was counted using a microscope and the CFU/mL was determined.

### RESULTS

Figure 2 and Table 2 summarize the data from all 12 bristle parts from 3 toothbrushes. Significant decrease rates comparing with control, spray 1 were found on spray 2 and 4, which are overlapped in error bar.

Although the average value of spray 2(0.84 CFU/mL  $\pm$  0.36) is slightly less than spray 4(0.92 CFU/mL  $\pm$  0.38), overlapped range is too large to say that definitely the data of spray 2 is less than spray 4.

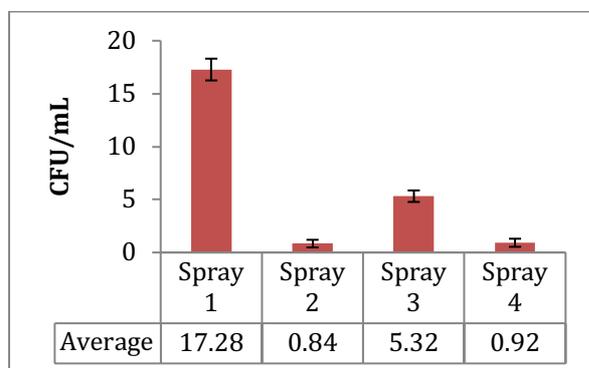


Figure 2. CFU/mL according to sprays.

	Spray1	Spray2	Spray3	Spray4
1 <sup>st</sup> (CFU/mL)	15.36	0.12	4.4	0.16
2 <sup>nd</sup> (CFU/mL)	17.6	1.20	5.28	1.24
3 <sup>rd</sup> (CFU/mL)	18.88	1.20	6.28	1.36
<b>Average</b>	17.28	0.84	5.32	0.92
<b>Std. Dev.</b>	1.78	0.62	0.94	0.66
<b>Std. Err.</b>	1.03	0.36	0.54	0.38
<b>Sterility</b>	-	95.1%	69.2%	94.7%

Table 2. Overall data of experiment.

## DISCUSSION

First and second prior-experiments showed that meaningful data cannot be obtained if toothpaste is used while brushing teeth. Above mentioned, the result of third prior-experiment showed toothbrush that cleaned with distilled water is contaminated seriously and LB Agar with Agar-washing method is most effective way to incubate bacteria. Because humidity and temperature cannot be controlled, to ignore these environmental facts, sterilized packs were used in this experiment. Based on these facts, this experiment was built.

There are two noticeable things in the result. Nelson-Filho *et. al.* [5, pp. 383-384] stated that chemical sprays are one of the most effective ways to sanitize a contaminated toothbrush; however, both toothpaste solution and chemical spray are quite effective in this

experiment. The difference between two experiments can be explained by whether toothpaste is used for brushing or not. Nelson-Filho *et. al.* [5] had test subjects use toothpaste while brushing, so there is the possibility that some bacteria could have been killed by just the toothpaste. As noted earlier, toothpaste was not used while brushing in this experiment, so there are still many bacteria that can be destroyed by simply using toothpaste on the toothbrushes.

The other noticeable thing is the effectiveness of vinegar solution. Komiyama *et. al.* [8, p. 33] stated that a vinegar solution is quite good at getting rid of bacteria, and, because vinegar is also cheap, it is the most cost effective way to sanitize toothbrushes. Through this experiment, however, using vinegar solution was not a good way to sanitize toothbrushes. There are two facts to explain this difference. One thing is the difference of vinegar solution. Moreover, apple vinegar was used in this experiment, so the apple sugar may have helped the bacteria to grow. Another thing is that toothpaste was not used while brushing. If toothpaste was used, it may help to sanitize. Considering these facts, the sterilization effectiveness of vinegar remains in doubt.

In the section of study design, prior experiments were introduced. They showed their own meaningful data. In 1<sup>st</sup> and 2<sup>nd</sup> prior experiment, just a few bacterial colonies were found. These results coincide with another study [9, pp. 84-85]. These results can be interpreted that if toothbrushes are used less than 3 days, most of bacteria can be removed by spraying all the solutions.

From all of the obtained results of experiments, if a spray with toothpaste solution or chemical spray is used once every 3 days, it can help prevent bacterial infections from oral cavity. However, there are some limitation in our paper because it ignored the environmental sources of bacteria found in a bathroom environment. Therefore, more data from the environment is needed to accurately find the correct number of bacteria and to decrease the contamination rate by some chemical solution.

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