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Down to the Bristles: The Best Storage Method for Your Toothbrush

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ABSTRACT

Based on new technology, often dental hygienists are faced with the question of, "What is the best storage method for toothbrushes?" It is important for dental patients to know how to properly store their toothbrushes to prevent exposure to other harmful environmental bacteria, such as *Escherichia Coli* (*E. coli*). The purpose of this experiment was to assess the most effective storage method by determining which one produced the least amount of environmental bacteria. This simple experiment utilized six methods of storage: American Dental Association's recommendation to keep stored in a dry, upright environment, dipped in antiseptic mouth rinse then placed in a holder in the bathroom, a Steri-Pod, a traditional travel holder, a UV sanitizer, and one simply placed in a toothbrush holder. Each toothbrush was unused and placed in the same bathroom within the designated storage method. One brush was cultured at the start of the experiment to determine a baseline for the amount of bacteria naturally occurring on packaged toothbrushes. After two weeks, a culture was taken from each of the brushes and allowed ample time to grow. The growth was visually measured, counted, and compared. We hypothesize that the toothbrush dipped in antiseptic mouthwash will produce the least amount of bacterial growth.

Editor's note: Tina Barnett, Stephanie Iascone, and Tricia Wilson won a 1st place scholarship award at the 2014 Scholars' Day for their presentation on this topic.

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Down to the Bristles: The Best Storage Method for Your Toothbrush

Our mouths are filled with many different types of bacteria. Most are harmless and simply find the mouth a welcoming place to reside. Two of the most common harmful types of bacteria in our mouth are *Streptococcus mutans* (*S. mutans*) and *Porphyromonas gingivalis* (*P. gingivalis*). *S. Mutans* is responsible for tooth decay and *P. gingivalis* is responsible for periodontal disease. There are also bacteria in the environment that have the possibility of landing on your toothbrush if not stored properly. Like the bacteria in your mouth, they can be harmful or have no effect on your body. People often want to know what the best method is for storing your toothbrush. Depending on what storage method you choose, there are differences in the amount of environmental bacterial growth on your toothbrush.

METHOD

While looking up previous research on the subject, we found that companies are not mandated to sterilize toothbrushes before packaging. We were interested to see how much environmental bacteria would be on a toothbrush straight from the package. A modified standard plate count was conducted on the toothbrush straight from the package.

A modified standard plate count is often used in microbiology so it is easier to count the number of colonies that grew. In our case, there were five different dilutions that were made for that one toothbrush. The dilutions were as follows: 1:10, 1:100, 1:1,000, 1:10,000, and 1:100,000. In order to do the dilutions, we needed to make a wash so we were able to spread it onto an agar. We added 10mL of sterile water to a 50mL conical tube. The toothbrush head was aseptically placed in the conical tube, wrapped with parafilm, and vortexed for one minute to create the wash. The agar that we chose to use for this part of our experiment was the Trypticase Soy Agar (TSA). From the 10mL wash that we created, 1mL was dispensed onto a TSA agar (1:10). From the wash, 1mL was dispensed into a 9mL blank (A), and 1mL was dispensed into a 99mL blank. From the wash, 0.1mL was dispensed onto a TSA agar (1:100). From blank A, 0.1mL was dispensed onto a TSA agar (1:1,000). From the 99mL blank, 0.1mL was dispensed onto a TSA agar

(1:10,000). From the 99mL blank, 1mL was dispensed into a 9mL blank (B). From blank B, 0.1mL was dispensed onto a TSA agar (1:100,000). Each of the dilutions was spread onto the plate using a glass hockey stick spreader. The plates were then incubated for three days at room temperature and two days at 35°C. The plates were assessed and the results were gathered from the 1:10 dilution plate. The toothbrush that came straight from the package ended up having 90 environmental bacterial colonies on it (see Figure 1).



Figure 1: Bacterial colonies on an unsterilized, new toothbrush

For our experiment to find the best storage method for your toothbrush, we used six different toothbrushes and six different storage methods. The six toothbrushes were sterilized before the experiment was started to make sure that each toothbrush started out with zero colonies of bacteria. The storage methods we used were as follows: to keep stored in a dry, upright environment (American Dental Association's (ADA) recommendation), dipped in antiseptic mouth rinse and then placed in a holder in the bathroom, a Steri-Pod, a traditional travel holder, a UV sanitizer, and one simply placed in a toothbrush holder. The toothbrushes were stored in their designated storage methods in the same bathroom for two weeks. Each day the toothbrushes were run under tap water to simulate daily brushing and routine environmental exposure.

The reason we picked the storage methods that we did was because they seemed like some of the most popular storage methods and we wanted to test how some of the new technology works. One of the more popular storage methods was the American Dental Association's recommendation to keep the toothbrush stored in a dry, upright environment. If more than one brush is stored in the same holder or area, it is important to keep the brushes separated to prevent cross-contamination. For the first storage method, the toothbrush was stood upright in a cabinet with the door shut (Council of Scientific Affairs, 2011).

The second storage method used Listerine, an antiseptic that comes from a formula of four essential oils that kill millions of bacteria on contact. The fixed combination of eucalyptol, menthol, methyl salicylate, and thymol does not compare to others. Because of the antiseptic properties in the mouth, we decided to try it on a toothbrush and see if it would have the same results. After the toothbrush was run under tap water, it was dipped in Listerine and placed in a holder in the bathroom (Listerine, 2014).

The Steri-pod is a relatively new technology that kills bacteria with vapors. Inside each Steri-pod toothbrush sanitizer is a laboratory formulated thymol compound. The compound is encapsulated in plastic with small holes that allow the thymol vapors to escape and surround the toothbrush bristles. All you have to do is clip the Steri-pod to the head of your brush and it will begin to work. The manufacturer recommends replacing the Steri-pod every three months. For our experiment, the third toothbrush was stored in a holder on the sink and the Steri-pod was clipped to the head (Bonfit America Inc., 2011).

The fourth storage method used a traditional travel holder with holes to allow moisture to escape. The toothbrush was placed inside and the holder was laid on the counter. For the fifth storage method, a UV sanitizer was used. UV sanitizers work by penetrating thin-walled bacteria. The light alters the genetic structure and the bacteria die. UV light is not visible to the naked eye, and because of its frequency, has germicidal properties. When the nucleus is penetrated, the DNA is irreparably damaged which results in bacterial death or the inability to reproduce itself. The technology of the UV sanitizer is the same technology used in hospitals. For the machine we used, the

toothbrush head was placed in the sanitizer and ran for its allotted time before turning off automatically (Guardian Technologies, 2008).

The last storage method was a toothbrush placed in a holder. There was nothing covering the toothbrush and it was about three feet away from the toilet just sitting in the holder.

A modified standard plate count was done on these six toothbrushes after two weeks of storage in their specified methods. The standard plate count was done the same way on these six toothbrushes as it was done on the toothbrush straight from the package. The only change was that instead of five dilutions, there were six. The dilutions were as follows: 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000. The wash was created the same way as done previously. The agars that we chose to use for this part of our experiment were the TSA and the Eosin-Methylene Blue Agar (EMB). The dilutions were made in the following ways. From the 10mL wash, 1mL was dispensed onto a TSA agar (1:10). From the wash, 1mL was dispensed into a 9mL blank (A) and 1mL was dispensed into a 99mL blank. From the wash, 0.1mL was dispensed onto a TSA and EMB agar (1:100). From blank A, 0.1mL was dispensed onto a TSA agar (1:1,000). From the 99mL blank, 0.1mL was dispensed onto a TSA and EMB agar (1:10,000). From the 99mL blank, 1mL was dispensed into a 9mL blank (B). From blank B, 0.1mL was dispensed onto a TSA agar (1:100,000). From blank B, 1mL was put into a 9mL blank which created blank C. From blank C, 0.1mL was dispensed onto a TSA agar (1:1,000,000). Each of the dilutions was spread onto the plate using a glass hockey stick spreader. The plates were then incubated for three days at room temperature and two days at 35°C.

RESULTS

The data were collected and analyzed. Based on the growth consistency on the TSA plates, the dilutions 1:10 and 1:100 were used to determine the best storage method. The toothbrush with no storage had 330 environmental bacterial colonies on the 1:10 dilution and 140 on the 1:100 dilution. The toothbrush that was dipped in antiseptic mouthwash had 30 environmental bacterial colonies on the 1:10 dilution and zero on the 1:100 dilution. The toothbrush that was stored in the Steri-pod had 1,200 environmental

bacterial colonies on the 1:10 dilution and 190 colonies on the 1:100 dilution. The toothbrush that was stored in the travel holder had 30 environmental bacterial colonies on the 1:10 dilution and 10 on the 1:100 dilution. The toothbrush stored in the UV sanitizer had too many environmental bacterial colonies to count on the 1:10 dilution and 50 on the 1:100 dilution. The toothbrush that was stored in a dry, upright environment (ADA recommendation) had 10 environmental bacterial colonies on the 1:10 dilution and 150 colonies on the 1:100 dilution. However, because there should be more colonies on the 1:10 dilution and not the 1:100 dilution, we believe the plates for the ADA recommendation were switched. We believe that there were actually 150 environmental bacterial colonies on the 1:10 dilution and 10 on the 1:100 dilution.

The EMB plates were used to test for fecal matter. The results show no growth on any of the EMB plates. Figures 2 through 7 show the environmental bacterial

growth on all six storage method plates for the 1:10 dilution. Figure 8 shows the results of the experiment. As mentioned above, the UV sanitizer 1:10 dilution plate had too many colonies to count, so for the visual purposes of the graph, we put 2,000 as the number of colonies on the plate.

Based on our results, the best way to store your toothbrush is to dip it in an antiseptic mouth rinse after brushing. The essential oils and alcohol kill the bacteria and therefore, that toothbrush had the least number of environmental bacterial growths. The dilution of 1:10 had 30 bacterial colonies on it and the 1:100 had zero colonies. The plastic travel case was a close second with 30 environmental bacterial colonies on the 1:10 dilution and 10 on the 1:100 dilution. Since both of the 1:10 dilutions had 30 colonies on them, we had to decide which storage method was better. We decided since the 1:100 dilution of the toothbrush dipped in antiseptic mouthwash had zero colonies, it is considered the best storage method.

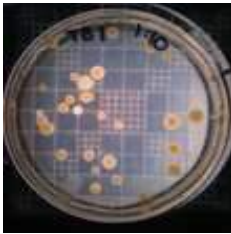


Fig. 2: No Storage

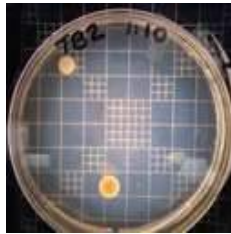


Fig. 3: Dipped in antiseptic

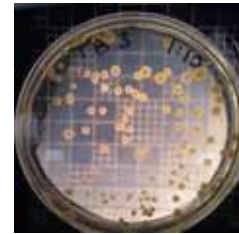


Fig. 4: Steri-pod

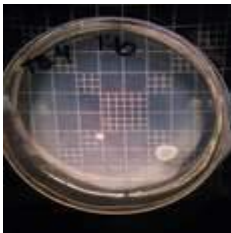


Fig. 5: Carry case

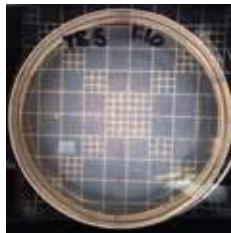


Fig. 6: ADA recommendation

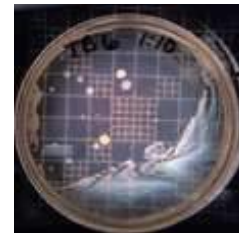


Fig. 7: UV sanitizer

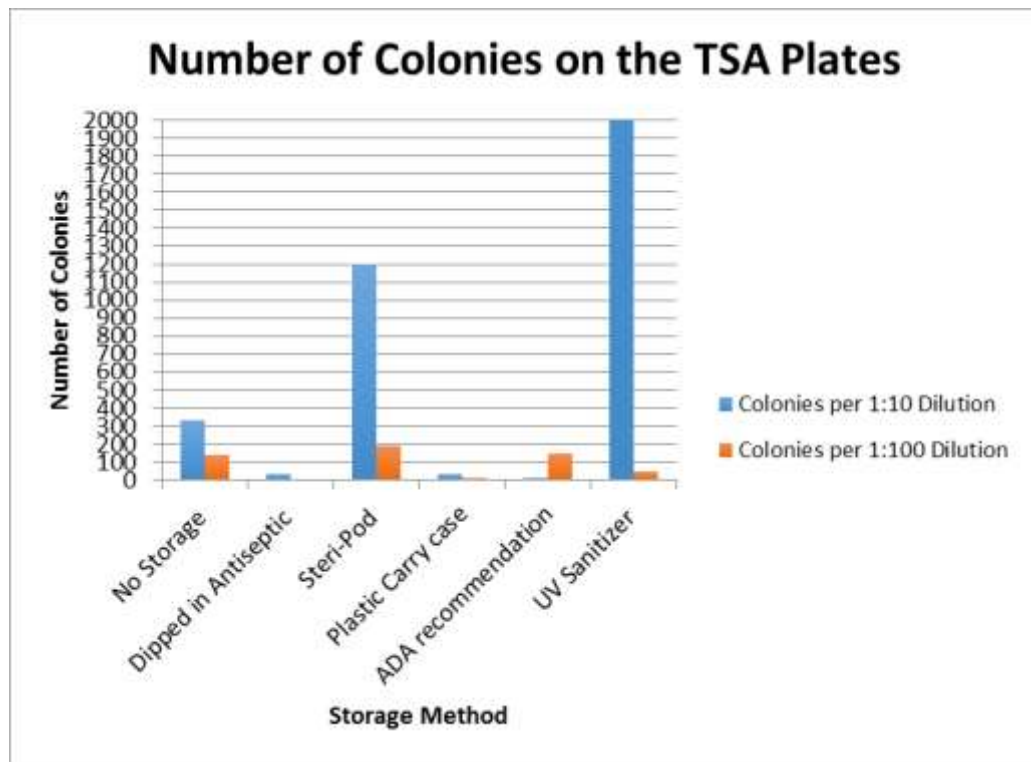


Figure 8: Colonies per 1:10 and 1:100 dilution by storage method

CONCLUSION

In the future, we would like to expand on this knowledge and include the actual use of each toothbrush within the oral cavity and then have the brushes placed in their designated storage method. We would then test if the oral cavity bacteria increases or decreases in each storage method. This would follow the same processes as our initial experiment. We would include a larger sample size in which people who have active periodontal disease and active decay will use the brushes. The brushes would then be tested for the specific oral bacteria, *P. gingivalis* and *S. mutans*, which are responsible for these diseases. Our goal is to determine the best storage solution so patients can avoid reintroducing disease causing bacteria into their oral cavity and for early prevention of these bacteria in the oral cavity in children.

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