Original Article

Low-environmental-impact Bamboo Toothbrushes Demonstrate Comparable Hygienic Condition to That of Plastic and Biomass Plastic Toothbrushes after Use and Storage

Yoshimasa Ishii,[1] Itaru Suzuki,[2] Haruka Sakazume-Suzuki,[2] Yukitoshi Kurakawa,[3] Toshikazu Uchiyama,[3] Yuichiro Okada,[4] Teruaki Nagashima,[5] Chieko Taguchi,[2] and Kazumune Arikawa[2]

[1]Department of Dental and Oral Surgery, Zama General Hospital, Zama, Kanagawa 252-0011, Japan
[2]Department of Community Oral Health, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271-8587, Japan
[3]Department of Operative Dentistry, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271-8587, Japan
[4]Department of Histology, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271-8587, Japan
[5]Nihon University Graduate School Dentistry at Matsudo, Community Oral Health, Matsudo, Chiba 271-8587, Japan

Article History

Received 26 October 2023
Accepted 15 November 2023

Abstract

Brushing with a toothbrush is effective for removing oral biofilms. However, toothbrushes can be a source of infectious diseases after use. Recently, the environmental impacts of plastics, such as marine pollution, have become an issue. This includes the disposal of plastic toothbrushes. Consequently, bamboo toothbrushes, with a low environmental impact, have become widely-used as alternatives. Although there are many reports on the hygiene of plastic toothbrushes after use, there are few reports on bamboo toothbrushes. Thus, we compared the hygiene of bamboo toothbrushes with those of plastic and biomass plastic toothbrushes after use and storage. Three types of toothbrushes were immersed in bacterial cultures and washed, and the number of bacteria on the toothbrushes after 12 and 24 h of storage was examined. The number of bacteria on the bamboo toothbrushes immediately after immersion was significantly higher than on the other two types, but this number was 95–99% lower after 12–24 h of storage. No differences were observed for different bacterial species (*Actinomyces oris* and *Streptococcus mutans*) or different storage conditions (wet and dry). However, pre-wetting before immersion significantly reduced the number of bacteria on the bamboo toothbrushes. These results indicate that the hygienic conditions of bamboo toothbrushes after 12–24 h of storage were comparable to those of plastic or biomass toothbrushes. Furthermore, pre-wetting the bamboo toothbrushes before use improved hygiene immediately after use. Bamboo toothbrushes are a better choice as they provide equivalent hygiene to that of plastic toothbrushes and also have a low environmental impact.

Keywords:
bamboo toothbrush, plastic, SDGs, *Actinomyces oris*, *Streptococcus mutans*

Introduction

The formation of oral biofilms is thought to be important in the pathogenesis of oral infections such as dental caries and periodontal disease. Oral biofilms are formed by the attachment of initial colonizers to the tooth surface, followed by the attachment and aggregation of a wide variety of oral bacteria to form mature biofilms (1). Although brushing with a toothbrush can remove oral biofilms (2, 3), various bacteria have been reported to adhere to toothbrushes following use. These include *Enterobacteriaceae* and *Micrococcaceae* (4), *Streptococcus*, *Actinomyces*, *Porphyromonas*, and *Fusobacterium* (5), which are

Correspondence to:
Itaru Suzuki
E-mail: suzuki.itaru@nihon-u.ac.jp
doi：10.5466/ijoms.22.23

Table 1. Characteristics of the test toothbrushes

material	bristles	length (mm)	Head length (mm)	Width (mm)	Thickness (mm)	Bristle length (mm)	total surface area of the bristles (mm^2)	Bristle hole	Bristle hardness
natural bamboo	nylon 610	180	25	10	5	10	1369	19	Regular
biomass plastic	PBT	158	25	11	5	11	1349	24	Regular
plastic	PBT	157	25	11	5	10	1401	24	Regular

PBT: polybutyleneterephthalate

associated with oral infections, *Staphylococcus aureus* (*S. aureus*), which is associated with nosocomial infections, *Candida albicans* (*C. albicans*), which is associated with oral candidiasis (6), *Klebsiella pneumoniae*, which is associated with the development of respiratory tract infections, and *Streptococcus pyogenes*, which is associated with the development of pharyngitis (7). Moreover, Ryan et al. reported that microflora from the environment may adhere to toothbrushes after storage (8). Interestingly, herpes simplex virus type 1 has also been detected (9).

In a study that investigated toothbrush storage methods after use, microorganisms grew easily on toothbrushes that retain moisture, forming a self-contained ecosystem (10). Therefore, toothbrushes may become a source of infection depending on how they are used and stored (11, 12). Currently, toothbrushes of various shapes and sizes are being marketed, influenced by various research reports and changes in consumer brushing objectives (3).

In 2015, the United Nations adopted "Transforming Our World: The 2030 Agenda for Sustainable Development," which includes the Sustainable Development Goals (SDGs) (13). One of the SDGs was "By 2025, prevent and significantly reduce all forms of marine pollution, including marine sedimentation and eutrophication, especially pollution from land-based activities." This goal describes the marine pollution caused by marine plastics and the concerns about the impact on ecosystems caused by the uptake and ingestion of marine plastics by marine organisms (14).

The contribution of plastic toothbrushes to marine pollution has been reported in many studies. Okano et al. reported five plastic toothbrushes per 100 m in a survey of persistent drifting debris ashore in Japan (15). According to a report by the United Nations Environment Programme (UNEP), the total amount of plastic waste per capita in Japan in 2014 was the second largest in the world (16). In addition to discarded trash, microplastics are also considered a problem (17), and microplastics are released by the daily use of plastic toothbrushes (18). In Japan, the "Law Concerning the Promotion of Resource Recycling of Plastics" was enacted in 2021, which stipulates that the emission of microplastics must be controlled by manufacturers of specific plastic-using products (19).

Given this, several alternatives to plastic toothbrushes are available, including bamboo toothbrushes. There is an abundance of bamboo in Japan, so it has been used for various functions, including and a fuel for biomass power generation (20). Bamboo toothbrushes have been shown to have a lower life cycle impact assessment than plastic or electric toothbrushes (21, 22). In other words, they are considered to have a lower environmental impact.

There have been numerous reports on toothbrush hygiene. However, to the best of our knowledge, there have been no reports on the hygiene of bamboo toothbrushes. Therefore, the purpose of this study was to investigate the hygienic conditions of bamboo toothbrushes after use and storage in an in vitro experimental system using bacteria that have been reported to be present in the oral cavity and are potential causes of oral infections and infective endocarditis.

Materials and Methods

Test toothbrushes

Three types of toothbrushes were used in this study: bamboo toothbrushes (Astep Inc.), biomass plastic toothbrushes (Company A), and plastic toothbrushes (Company A). The characteristics of the toothbrushes are presented in Table 1. All toothbrushes were commercially available, not the type used in medical institutions. New and unopened toothbrushes were used in the experiments.

Test strains

Actinomyces oris (*A. oris*) strain MG1 (23), an initial colonizer, and *Streptococcus mutans* (*S. mutans*) strain UA159 (24), a cariogenic bacterium, were used in this study.

Immersion of toothbrushes in bacterial culture

The method of immersing each toothbrush in the bacterial culture was based on several reports with some modifications (25, 26, 27). First, each strain was incubated in brain heart infusion (BHI) liquid medium at 37°C for 16-18 h under 5% CO_2. After centrifugation of the culture medium at 3000 × *g* for 10 min at 4°C, the pellet was washed with sterile phosphate buffer solution (PBS) and centrifuged again under the same conditions. After centrifugation, the supernatant was removed and the pellet was suspended in sterile PBS and used for subsequent experiments. The bacterial culture was then adjusted to OD_{600}=0.1 in sterile PBS and placed in a sterilized beaker.

Each unopened toothbrush was immersed in the adjusted bacterial solution for 3 min such that the head (25 mm) and neck (10 mm) were immersed. In an additional experiment, bamboo toothbrushes were immersed in sterile distilled water (DW) for 5 s before immersion in the bacterial culture to reproduce wet conditions before toothbrush use.

After immersion, each toothbrush was removed from the beaker and rinsed with sterile DW for 3 s to remove the excess bacterial culture. This method mimicked the washing of each toothbrush under running water and was based on the method described by Kim et al. (28). After washing, each toothbrush was placed in a dry or wet environment for 12 or 24 h. The storage environment in this study was established according to Katsuno et al. (29). Each toothbrush was placed upright in a disinfected box in a room (room temperature 27°C, humidity 40-50%) and covered with a lid to prevent falling bacteria from adhering to it. The box was not sealed to ensure constant ventilation. On the other hand, the wet environment was conducted in a sealed box with the humidity adjusted to 70-80%. At 0 h (immediately after immersion [baseline]), and after 12 and 24 h of storage, the toothbrushes were collected, disassembled into bristles and bodies directly or with sterile pliers, and immersed in 10 ml of sterile PBS. The bristles were then vortexed for 30 s to remove adhering bacteria and used as samples for quantification.

Evaluation of the number of adherent bacteria

Next, the number of bacteria adhering to the toothbrushes was quantitatively evaluated. Each sample containing adherent bacteria was serially diluted 10 to 1000 times using sterile PBS, and 100 μl of each sample containing adherent bacteria was seeded on BHI agar or Mitis Salivarius (MS) agar in triplicate. BHI agar medium was used to quantify *A. oris*, and MS agar was used to quantify *S. mutans*. The number of colonies formed on the agar was measured and the average number of adherent bacteria was calculated.

Statistical analysis

Three independent experiments were performed. One-way analysis of variance (ANOVA) was used for the statistical analysis of differences in the number of adherent bacteria among the three types of toothbrushes and at different storage times, followed by multiple comparisons using the Tukey or Games-Howell method. Student's t-test was used for statistical analysis of the differences in the number of adherent bacteria between the dry and wet bamboo toothbrushes. The significance level for each test was set at 0.05. Excel (Microsoft Excel 2019; Microsoft Co., Albuquerque, NM, USA) and SPSS (IBM SPSS Statistics 28; IBM Corporation, Armonk, NY, USA) were used for data analysis.

Results

Differences in the amount of bacteria on each toothbrush in a dry environment

First, we investigated differences in the number of oral bacteria on each toothbrush when stored in a dry environment. For *A. oris*, the highest number of adherent bacteria was observed on the bamboo toothbrush at baseline (0 h), followed by the biomass plastic and plastic toothbrushes; however, the differences were not statistically significant. The number of adherent bacteria after 12 h and 24 h of storage was lower than the number of adherent bacteria at 0 h for all toothbrushes; however, the differences were only statistically significant for the bamboo toothbrush. In addition, the number of adherent bacteria after 24 h of storage was lower than the number of adherent bacteria after 12 h of storage for all tooth-

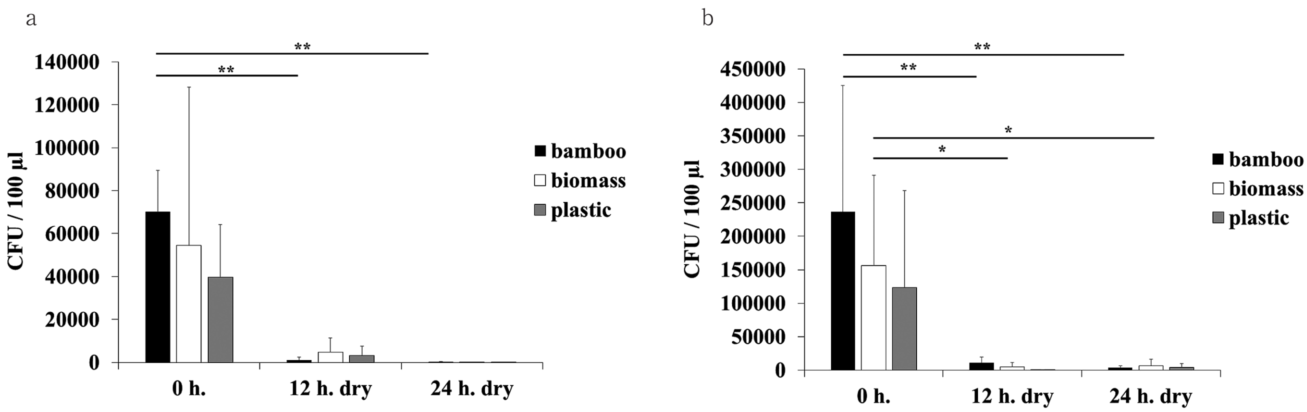

Fig. 1. (a) Number of *Actinomyces oris* on toothbrushes stored under dry conditions. (b) Number of *Streptococcus mutans* bacteria on toothbrushes stored under dry conditions. The vertical axis indicates the number of CFU per 100 μl and the horizontal axis indicates the storage time (0 h, 12 h, 24 h). Black bars indicate bamboo toothbrushes, white bars indicate biomass plastic toothbrushes, and grey bars indicate plastic toothbrushes. Data are expressed as mean ± standard deviation (SD) for the three independent experiments. Statistical analysis of the differences in the number of adherent bacteria between the three toothbrushes and storage time is performed using one-way ANOVA, followed by multiple comparisons using either the Tukey or the Games-Howell method. $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 2. Percentage reduction of bacteria on toothbrushes stored under dry conditions.

		12 h. dry	24 h. dry
A. oris	**Bamboo**	**98.49**	**99.82**
	Biomass	**91.41**	**100**
	Plastic	**92.11**	**100**
S. mutans	**Bamboo**	**95.51**	**98.46**
	Biomass	**96.63**	**95.59**
	Plastic	**99.56**	**96.69**

(%)

brushes; however, no statistically significant differences were observed (Fig. 1a).

We calculated the percentage decrease in the number of *A. oris* on each toothbrush from baseline (0 h) to 12 h of storage and from baseline (0 h) to 24 h of storage. The reduction rates of *A. oris* on the bamboo toothbrush were 98.49% and 99.82% after 12 h and 24 h of incubation, respectively. The reduction rate of *A. oris* on the biomass plastic toothbrush was 91.41% and 100% after 12 h and 24 h of storage, respectively. The percentage reduction of *A. oris* on the plastic toothbrush was 92.11% and 100% after 12 h and 24 h of storage, respectively (Table 2).

Next, the amount of *S. mutans* on each toothbrush was examined. At baseline (0 h), the bamboo toothbrush had the highest number of *S. mutans*, followed by the biomass plastic and plastic toothbrushes. The amount of *S. mutans* on each toothbrush was approximately 2-3 times higher than of the amount of *A. oris*, but the patterns were similar. The number of *S. mutans* decreased between 0 h and 12 h and between 0 h and 24 h on all toothbrushes with a statistically significant decrease for the bamboo and biomass plastic toothbrushes. No statistically significant reduction in bacteria was observed between 12 h and 24 h on any toothbrush (Fig. 1b). The percentage decrease in the amount of *S. mutans* on each toothbrush from baseline (0 h) to 12 h of storage and from baseline (0 h) to 24 h of storage was examined. The reduction rates of *S. mutans* on the bamboo toothbrushes were

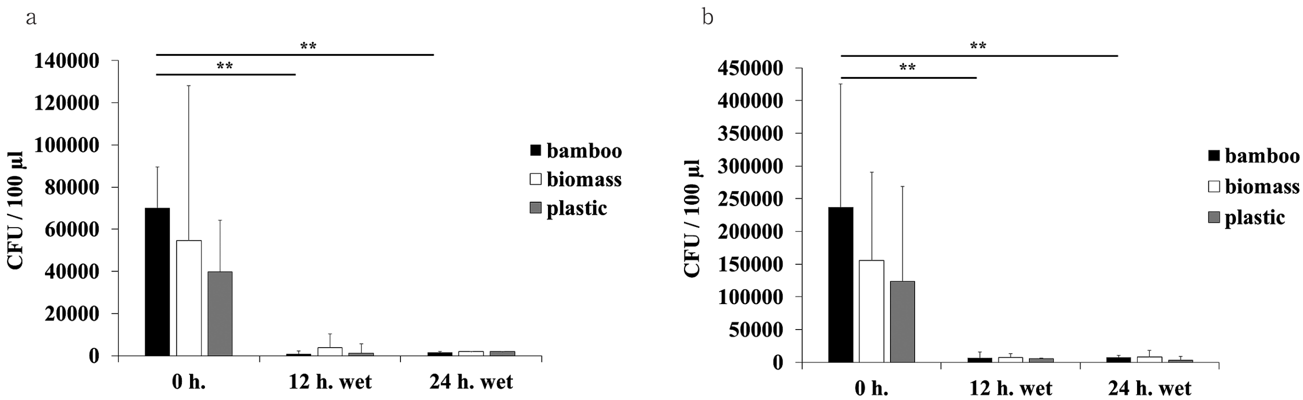

Fig. 2. (a) Number of *Actinomyces oris* on toothbrushes stored under wet conditions. (b) Number of *Streptococcus mutans* bacteria on toothbrushes stored under wet conditions. The vertical axis indicates the number of CFU per 100 μl, and the horizontal axis indicates the storage time (0 h, 12 h, 24 h). Black bars indicate bamboo toothbrushes, white bars indicate biomass plastic toothbrushes, and grey bars indicate plastic toothbrushes. Data are expressed as the mean ± standard deviation (SD) in the three independent experiments. Statistical analysis of the differences in the number of adherent bacteria between the three toothbrushes and storage time is performed using one-way ANOVA, followed by multiple comparisons using either the Tukey or the Games-Howell method. $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 3. Percentage reduction of bacteria on toothbrushes stored under wet conditions.

		12 h. dry	24 h. dry
A. oris	**Bamboo**	**98.74**	**97.64**
	Biomass	**93.16**	**96.53**
	Plastic	**97.07**	**95.02**
S. mutans	**Bamboo**	**97.18**	**96.83**
	Biomass	**95.45**	**94.60**
	Plastic	**95.59**	**97.25**

(%)

95.51 % after 12 h of storage and 98.46 % after 24 h of storage. The reduction rates for biomass plastic toothbrushes were 96.63% and 95.59% after 12 h and 24 h of storage, respectively. The reduction rate of *S. mutans* on plastic toothbrushes was 99.56% after 12 h of storage and 96.69% after 24 h of storage (Table 2).

Differences in the amount of bacteria adhering to each toothbrush in a wet environment

We investigated differences in the number of oral bacteria on each toothbrush when stored in a wet environment. Compared to baseline (0 h), the number of *A. oris* bacteria was reduced after 12 h and 24 h of immersion for all toothbrushes with statistically significant differences for the bamboo toothbrush. There was no statistically significant decrease in the number of bacteria after 24 h of storage compared to after 12 h of storage for all toothbrushes (Fig. 2a). The reduction rates of *A. oris* after 12 h and 24 h of storage were 98.74% and 97.64% , respectively for the bamboo toothbrush. The reduction rates for the biomass plastic toothbrushes were 93.16% and 96.53% after 12 h and 24 h of storage, respectively. The reduction rates of the plastic toothbrush were 97.07% and 95.02 % after 12 h and 24 h of storage, respectively (Table 3).

The number of *S. mutans* cells on each toothbrush was determined. Compared to baseline (0 h), all toothbrushes showed a decrease in the number of adherent bacteria after 12 h and 24 h of storage with a statistically significant decrease for the bamboo toothbrush only. As in the wet

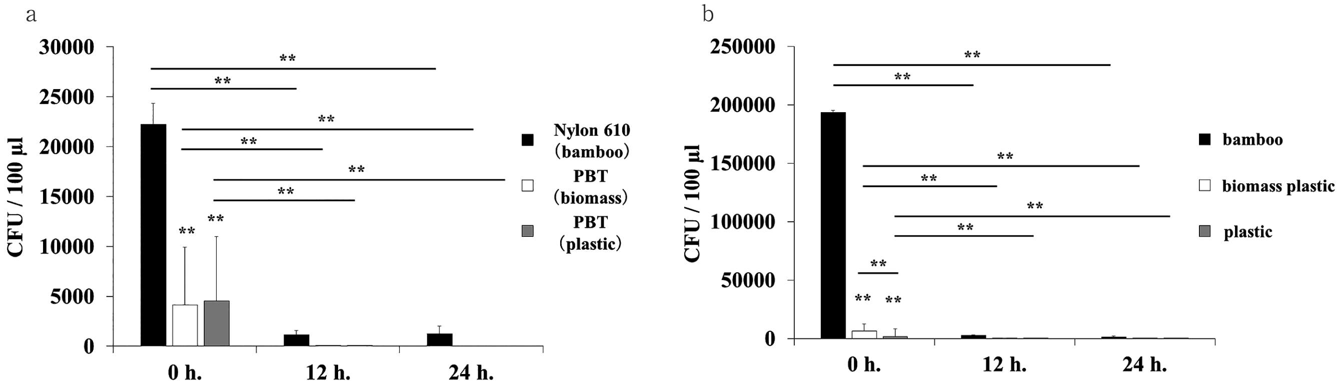

Fig. 3. (a) Number of *Actinomyces oris* on toothbrush bristles stored under dry conditions.
The vertical axis shows the number of CFU per 100 µl and the horizontal axis shows the storage time (0 h, 12 h, 24 h). Black bars indicate the nylon 610 (bamboo toothbrush), white bars indicate (biomass plastic toothbrush (PBT), and grey bars indicate the PBT (plastic toothbrush). Statistical analysis of the differences in the number of adherent bacteria between the three bristle materials and storage times is performed using one-way ANOVA, followed by multiple comparisons using either the Tukey or Games–Howell method. **P < 0.01.
(b) Number of *Actinomyces oris* on the head and neck of toothbrushes stored under dry conditions.
The vertical axis shows the number of CFU per 100 µl and the horizontal axis shows the storage time (0 h, 12 h, 24 h). The black, white, and grey bars indicate bamboo (bamboo toothbrushes), plastic (biomass plastic toothbrushes), and plastic (plastic toothbrushes), respectively. Data are presented as the mean ± standard deviation (SD) for three independent experiments. Statistical analysis of the differences in the number of adherent bacteria between the three toothbrush body materials and storage times is performed using a one-way ANOVA, followed by multiple comparisons using either the Tukey or the Games–Howell method. **P < 0.01.

Table 4a. Percent reduction of *A. oris* on toothbrush bristles after storage under dry conditions

	12 h. dry	24 h. dry
Nylon 610 (bamboo)	94.96	94.46
PBT (biomass)	99.92	100
PBT (plastic)	99.93	100

(%)

condition, there was no statistically significant decrease in the number of bacteria between 0 h and 12 h of storage and between 0 h and 24 h of storage for any of the toothbrushes (Fig. 2b). The decrease in the number of *S. mutans* on each bamboo toothbrush after 12 h and 24 h of storage was 97.18% and 96.83%, respectively. The reduction rates for the biomass plastic toothbrush were 95.45% and 94.60% after 12 h and 24 h of storage, respectively. The plastic toothbrush showed a 95.59% reduction after 12 h of storage and a 97.25% reduction after 24 h of storage (Table 3).

Differences in the amount of A. oris adhering to the bristles, head, and neck of each toothbrush in a dry environment

We examined the number of *A. oris* on the bristles, head, and neck of each toothbrush. The bamboo toothbrush bristles used in this study were made of nylon 610, and the biomass plastic and plastic toothbrush bristles were made of polybutyleneterephthalate (PBT). The number of bacteria on the nylon 610 bristles at baseline (0 h) was significantly higher than that on the PBT bristles. However, there was a significant decrease between baseline (0 h) and 12 h and between baseline (0 h) and 24 h of storage (Fig. 3a). The reduction in the number of adherent bacteria was 94.96% after 12 h of storage and 94.46% after 24 h of storage (Table 4a). Biomass plastic and plastic toothbrushes—both with bristles made of PBT—

Table 4b. Percent reduction of *A. oris* on toothbrush body after storage under dry conditions

	12 h. dry	**24 h. dry**
Bamboo	**98.69**	**99.27**
Biomass	**92.95**	**99.05**
plastic	**98.91**	**98.73**

(%)

showed similar trends. The number of bacterial cells on the bristles at baseline (0 h) did not differ significantly between the two types of toothbrush. However, after 12 h and 24 h of storage, the decreases were statistically significant (Fig. 3a). The reduction in the amount of adherent bacteria was 99.92–99.93% after 12 h of storage and 100% after 24 h of storage (Table 4a).

The amount of *A. oris* on the head and neck of the toothbrushes was also examined. For the bamboo toothbrushes, the amount of *A. oris* that adhered to the head and neck of the toothbrush at baseline (0 h) was significantly higher than that of the biomass plastic and the plastic toothbrushes. However, after 12 h and 24 h of storage, the number of *A. oris* decreased significantly (Fig. 3b). Interestingly, the number of *A. oris* on the bamboo toothbrush before decomposition was higher than that on the toothbrush after decomposition (Fig. 1a). The reduction rates for *A. oris* were 98.69% and 99.27% after 12 h and 24 h of storage, respectively (Table 4b). The number of bacteria on the biomass plastic toothbrush at baseline (0 h) was significantly higher than that on the plastic toothbrush. After 12 h and 24 h of storage, the decrease was statistically significant (Fig. 3a). The reduction rate of *A. oris* was 92.95% after 12 h of storage and 99.05% after 24 h of storage (Table 4b). The number of *A. oris* in the plastic was significantly reduced after 12 h and after 24 h of storage (Fig. 3a). The reduction rates for *A. oris* were 98.91% and 98.73% after 12 h and 24 h of storage, respectively (Table 4b).

Amount of A. oris on bamboo toothbrushes when pre-wetted with water

The results showed that at baseline (0 h), the number of attached bacteria on the dry bamboo toothbrushes was significantly higher than that on toothbrushes made of other materials (Figs. 1–3).

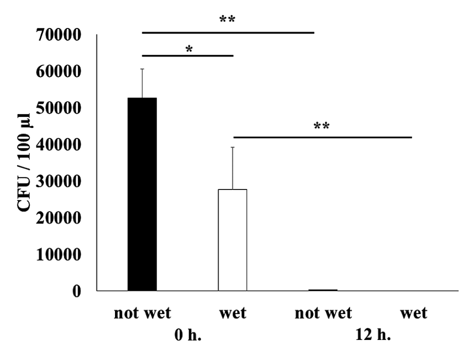

Fig. 4. Number of *Actinomyces oris* bacteria on bamboo toothbrushes pre-wetted with water.
The vertical axis shows the number of CFU per 100 µl, and the horizontal axis shows the conditions and storage time (0 h, 12 h). Data are presented as the mean ± standard deviation (SD) for the three independent experiments. Statistical analysis of the difference in the number of adherent bacteria between the two conditions is performed using the Student's t-test. $^{*}P < 0.05$, **, $P < 0.01$.

Table 5. Percent reduction of *A. oris* on wet or not wet bamboo toothbrushes

	12 h.
not wet	**99.37**
wet	**99.82**

(%)

Next, we conducted an experiment in which bamboo toothbrushes were immersed in sterile DW for 5 s immediately before immersion in the bacterial solution to reproduce the prewet conditions of the bamboo toothbrushes immediately before brushing. Immediately after immersion (0 h), the number of *A. oris* was significantly reduced in the pre-wetted condition compared with that in the non-wetted condition. In the pre-wetted condition, the number of *A. oris* on the bamboo toothbrushes after 12 h of storage was significantly reduced compared to that at baseline (Fig. 4). The number of adherent bacteria was 99.82% (Table 5).

Discussion

In recent years, toothbrushes made of various materials have been manufactured and marketed as alternatives to plastic toothbrushes. There have been several reports of bacteria and viruses have been detected on toothbrushes after use (5–12). However, few studies have focused on toothbrushes made of alternatives to plastics. Therefore, we investigated the differences in the number of bacteria adhering to conventional plastic toothbrushes, bamboo toothbrushes, and biomass plastic toothbrushes.

A. oris and *S. mutans*—the strains used in this study—can cause infective endocarditis (30, 31). *A. oris* and *S. mutans* are oral bacteria associated with oral infections. *A. oris* is an early adherent, adhering to tooth surfaces and serving as a scaffold for the formation of mature oral biofilms, contributing to dental caries and periodontal disease. On the other hand, *S. mutans* contribute to caries development by forming glucans. It has also been reported to be detected on toothbrushes used in the actual oral cavity (4, 5). Therefore, our experiments were conducted using *A. oris* and *S. mutans*. The amount of *A. oris* and *S. mutans* adhering to the bamboo toothbrushes was the highest when stored in a dry environment in this study (Fig. 1a, b). Bamboo has many holes in its cross-section owing to the sparseness of its fibers, which makes it a highly water absorbent material (32). On the other hand, biomass plastic is a plastic partly comprising biomass as the raw material, and its properties are not significantly different from those of conventional plastics (31, 33). In other words, the number of adhering bacteria was higher on bamboo toothbrushes compared to plastic and biomass plastic toothbrushes owing to water absorption by the bacterial solution and adhesion of the bacteria between the bamboo fibers. On the other hand, more adherent bacteria were observed when *S. mutans* was used compared to when *A. oris* was used. *S. mutans* utilizes glucosyltransferase (GTF) in the presence of sucrose to produce extracellular polysaccharides, contributing to the formation of a strong biofilm on the tooth surface (34). The experimental conditions of the present study involved adhesion to materials that are quite different to that of tooth surfaces, and the experiment was conducted in an environment in which no sugar source was present. A difference in the number of bacteria was observed immediately after toothbrushes were immersed in the bacterial solution. This suggests that differences between tooth surfaces and toothbrush materials may influence adhesion ability.

After immersion of the bamboo toothbrushes in the bacterial solution for 12 h and 24 h, the number of adhering bacteria was reduced by more than 98 % (Table 2). Furthermore, the number of adherent bacteria was comparable to that of the plastic and biomass plastic toothbrushes (Fig. 1a, b). Bamboo possesses antimicrobial properties against *S. mutans*, *S. aureus*, and *C. albicans* (35, 36). The main components are furans and phenolic compounds (36). This suggests that the antimicrobial activity of bamboo significantly reduces the number of attached bacteria. Spolidorio et al. reported that when *S. mutans* was deposited on plastic toothbrushes and allowed to stand, the amount of deposited bacteria increased up to 2 h after immersion, but was not detected after 8 h (34, 37). Furthermore, Wetzel et al. found that the number of adherent *S. mutans*, *Lactobacilli*, or *C. albicans* increased 2 h after children had used them; however, the authors reported a decrease in the number of attached bacteria after 8 h (38). Bunetel et al. also reported a significant decrease in the number of adherent *S. mutans* 24 h after immersion in a *S. mutans*-containing fluid (26). In other words, the results were consistent with those of the plastic and biomass plastic experiments. These results suggest that bamboo toothbrushes stored in a dry environment are as hygienic as plastic or biomass-based plastic toothbrushes after 12 h.

Interestingly, similar results were obtained when toothbrushes were stored in a wet environment (Fig. 2b). A study using interdental brushes reported that storage in humid environments affected bacterial growth (29). On the other hand, it has been reported that there is no correlation between the amount of microorganisms on toothbrushes after use and the storage method (39). Based on the results of this study, it was concluded that high or low humidity had no effect on the number of bacteria on toothbrushes. However, it is possible that the humidity in the boxes decreased during the experiment, which may have affected the results.

A typical toothbrush consists of the head, neck, and handle. The toothbrushes used in this study had a similar structure. The materials of the toothbrush body and bristles were different. Therefore, to clarify whether differences in the bristles affect the amount of bacteria adhered to the toothbrushes, toothbrushes were deconstructed into the bristle and toothbrush body, and the amount of bacteria adhered to each was quantitatively determined. The bristles of the bamboo toothbrushes used in this study were made of nylon 610, whereas those of the biomass plastic and plastic toothbrushes were made of PBT. On nylon 610, the amount of *A. oris* that adhered to the bristles immediately after immersion in the bacterial solution was significantly higher than that on PBT (Fig. 3a). In other words, the bristles of the bamboo toothbrush used in this study had a higher water absorption capacity than the bristles of the other two types of toothbrush, suggesting that it may be more susceptible to bacterial attachment. The reduction in the number of *A. oris* on the nylon 610 bristles was approximately 94% after 12–24 h of storage (Table 4a). In PBT, the bristles of the biomass and plastic toothbrushes, the amount of *A. oris* adhered to the bristles was significantly reduced after 12 and 24 h of storage (Fig. 3a). The reduction in the number of *A. oris* after 12–24 h of incubation was approximately 100% (Table 4a). Nylon 610 and PBT are excellent drying materials, and it has been reported that the drying rate is almost 100% after 100 minutes (40). This suggests that storing the bristles in a dry environment may have thoroughly dried them, making it difficult for the bacteria to exist as live cells. In contrast, quantification of the number of *A. oris* on the head and neck of the toothbrushes showed that the number of bacteria on the bamboo toothbrushes immediately after immersion was significantly higher than that on the biomass plastic and plastic toothbrushes (Fig. 3b). Interestingly, the number of *A. oris* on the bamboo toothbrush before decomposition was higher than that on the plastic toothbrush (Fig. 1a and 3b). This result suggests that the number of *A. oris* on the bamboo toothbrushes was higher than that on the pre-degradation toothbrushes, because the bacteria were detected in the holes where the bristles had been implanted. The reason for the statistically significant decrease in the number of *A. oris* on all toothbrushes after 12–24 h of storage was similar, possibly due to the antibacterial properties of bamboo and drying time (Fig. 3b).

These results indicated that the number of bacteria on the bamboo toothbrush after immersion in the bacterial solution decreased significantly after 12 h of storage and was comparable to that of plastic and biomass plastic toothbrushes. However, the number of bacteria on bamboo toothbrushes immediately after immersion in the bacterial solution tended to increase more than that on plastic and biomass plastic toothbrushes.

From these results, we concluded that immediately after use, bamboo toothbrushes were less hygienic than other toothbrushes. Therefore, we investigated a method to reduce the number of bacteria on bamboo toothbrushes immediately after immersion. The bamboo toothbrushes were pre-wetted with water prior to use. The number of *A. oris* on bamboo toothbrushes immediately after immersion was significantly reduced under pre-wet conditions compared with non-wet conditions. Under pre-wet conditions, the number of *A. oris* on the bamboo toothbrushes after 12 h of incubation was significantly reduced (Fig. 4). The reduction in adherent *A. oris* was 99.81% (Table 5). Because bamboo toothbrushes are water absorbent, it is possible that the concentration of bacteria on the toothbrushes was reduced by the water. In other words, pre-wetting bamboo toothbrushes with water immediately before use may lead to improved hygiene immediately after use. Sluijs et al. reported no significant difference in plaque scores between dry and pre-wet toothbrushes (41). In other words, there is a small possibility that pre-wetting the toothbrush just prior to use may have no effect on plaque removal. Therefore, studies on the effects of wet bamboo toothbrushes on plaque removal are required.

The use of a toothbrush is considered the most effective way to remove oral biofilms that are responsible for the development of dental caries and periodontal diseases (2). Several reports have been published on toothbrush replacement periods. Raiyani et al. reported that toothbrushes should be replaced after 3 to 4 weeks because toothbrushes used for 3 months were significantly more contaminated than toothbrushes used for 1 month (42). Suda et al. reported that the fibers of the bristles of a toothbrush that had been used 3 times a day for 3 min each time for 10 weeks became 2.5% thinner, which may lead to a decrease in plaque removal effectiveness (43). In other words, as the frequency of toothbrushing increases, the number of toothbrushes used is also expected to increase.

Currently, plastic toothbrushes are widely used; however, in view of the environmental impact of plastics, toothbrushes with a lower environmental impact should be selected(13–19). In this study, the hygienic conditions of bamboo toothbrushes with a lower environmental impact after 12–24 h of storage were comparable to those of plastic or biomass toothbrushes. Furthermore, pre-wetting the bamboo toothbrushes with water immediately before use improved hygiene immediately after use. In other words, the hygienic conditions of plastic, biomass plastic, and bamboo toothbrushes after storage were comparable; therefore, the use of bamboo toothbrushes with a lower environmental impact may be effective in achieving the SDGs from the perspective of the dental field.

This research had several limitations. First, the toothbrushes tested in this study were only assessed after one exposure to bacteria, simulating a single use only. Second, the number of bacteria present on the toothbrushes was not assessed between 0 h and 12 h. Third, the levels of bacteria were not assessed in actual human oral cavities. These three factors may limit the strength of the results. In future studies, we plan to examine the use of bamboo toothbrushes after multiple uses, 2–3 hours after immersion, and in the actual human oral cavity.

Acknowledgments

This research was supported by a research incentive donation from Astep Corporation. We would like to thank Editage.jp(www.edit-age.jp)for the English language editing.

Conflict of Interest

The authors declare that they have no conflicts of interest associated with this manuscript.

References

1. Kolenbrander PE, Palmer RJ Jr, Periasamy S, Jakubovics NS: Oral multispecies biofilm development and the key role of cell-cell distance. Nat Rev Microbiol, 8: 471–480, 2010.
2. Freire PL de L, Campos PH, Carvalho FG, Rodrigues JA, Diniz MB: Is pomegranate peels infusion effective for disinfection of toothbrushes? Revista De Odontologia Da UNESP, 45: 253–257, 2016.
3. Watanabe T, Kobayashi K: Recent toothbrush circumstances-Why is it wide flocking toothbrush now?-. JSP, 60: 87–94, 2018.
4. Zinn MK, Schages L, Bockmühl D: The toothbrush microbiome: impact of user age, period of use and bristle material on the microbial communities of toothbrushes. Microorganisms, 8: 1379, 2020. doi: 10.3390/microorganisms8091379.
5. Lee SY, Lee SY: Assessment of bacterial contamination of toothbrushes using Illumina MiSeq. Oral Biol Res, 43: 180–188, 2019.
6. Shang Q, Gao Y, Qin T, Wang S, Shi Y, Chen T: Interaction of oral and toothbrush microbiota affects oral cavity health. Front Cell Infect Microbiol, 10: 17, 2020. doi: 10.3389/fcimb.2020.00017.
7. Ferreira CA, Savi GD, Panatto AP, Generoso JD, Barichello T: Microbiological evaluation of bristles of frequently used toothbrushes. Dental Press J Orthod, 17: 72–76, 2012.
8. Blaustein RA, Michelitsch LM, Glawe AJ, Lee H, Huttelmaier S, Hellgeth N, Ben Maamar S, Hartmann EM: Toothbrush microbiomes feature a meeting ground for human oral and environmental microbiota. Microbiome, 9: 32, 2021. doi: 10.1186/s40168-020-00983-x
9. Frazelle MR, Munro CL. Toothbrush contamination: a review of the literature. Nurs Res Pract, 2012: 420630, 2012. doi: 10.1155/2012/420630
10. Saini R, Saini S: Microbial flora on toothbrush - at greater risk. Ann Niger Med, 4: 31, 2010. doi: 10.4103/0331-3131.73882
11. Neal PR, Rippin JW: The efficacy of a toothbrush disinfectant spray--an in vitro study. J Dent, 31: 153–157, 2003.
12. Asumang P, Inkabi SE, Inkabi S: Toothbrush bristles, a harbor of microbes and the risk of infection. Int J Oral Sci, 9: 25–27, 2019.
13. United Nations General Assembly: Transforming our world: the 2030 Agenda for Sustainable Development. UN Doc. A/RES/70/1(September 25, 2015).
14. Otsuka Y, Takada H, Nihei Y, Kameda Y, Nishikawa K: Current status and issues of microplastic pollution research. JSWE, 44: 35–42, 2021.
15. Okano T, Yasumoto M, Andou S: Properties and categories of non-biodegradable waste items found in beach litter. JSMCWM, 21: 94–105, 2010.
16. Valentina T, Ancuta M, Diana VZ, Marius MM, Dragos S: The influence of the plastic bags on the environment. Mater Plast, 55: 595–599, 2018.

17. Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, Rist S, Karlsson T, Brennholt N, Cole M, Herrling MP, Hess MC, Ivleva NP, Lusher AL, Wagner M: Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ Sci Technol, 53: 1039–1047, 2019.
18. Fang C, Gopalan S, Zhang X, Xu L, Niu J, Naidu R: Raman imaging to identify microplastics released from toothbrushes: algorithms and particle analysis. Environ Pollut, 337: 122510, 2023. doi: 10.1016/j.envpol.2023.122510
19. "Law for Recycling Plastic Materials" in Japan, e-gov portal, Ministry of the Environment. Available from: https://elaws.e-gov.go.jp/document?lawid=503AC0000000060, cited 14 Oct 2023.
20. Kodani J, Esaki K: Effect of the duration of abandonment on development of understory vegetation in bamboo forests. Jan J For Environ, 54: 19–28, 2012.
21. Lyne A, Ashley P, Saget S, Porto CM, Underwood B, Duane B: Combining evidence-based healthcare with environmental sustainability: using the toothbrush as a model. Br Dental J, 229, 303–309, 2020.
22. Guinée JB, Heijungs R, Huppes G, Zamagni A, Masoni P, Buonamici R, Ekvall T, Rydberg T: Life cycle assessment: past, present, and future. Environ Sci Technol, 45: 90–96, 2011.
23. Suzuki I, Shimizu T, Senpuku H: Role of SCFAs for fimbrillin-dependent biofilm formation of *Actinomyces oris*. Microorganisms, 6: 114, 2018. doi: 10.3390/microorganisms6040114
24. Senpuku H, Nakamura T, Iwabuchi Y, Hirayama S, Nakao R, Ohnishi M: Effects of complex DNA and MVs with GTF extracted from *Streptococcus mutans* on the oral biofilm. Molecules, 24: 3131, 2019. doi: 10.3390/molecules24173131
25. Schmidt JC, Bux M, Filipuzzi-Jenny E, Kulik EM, Waltimo T, Weiger R, Walter C: Influence of time, toothpaste and saliva in the retention of *Streptococcus mutans* and *Streptococcus sanguinis* on different toothbrushes. J Appl Oral Sci, 22: 152–158, 2014.
26. Bunetel L, Tricot-Doleux S, Agnani G, Bonnaure-Mallet M: In vitro evaluation of the retention of three species of pathogenic microorganisms by three different types of toothbrush. Oral Microbiol Immunol, 15: 313–316, 2000.
27. Shah S, Kunte S, Jagtap C, Jajoo S, Patel A, Shah P: Efficacy of two different mouth rinses in inhibition of the growth of *Streptococcus mutans* on toothbrush bristles. JDRR, 8: 261–266, 2021.
28. Bélanger-Giguère K, Giguère S, Bélanger M: Disinfection of toothbrushes contaminated with *Streptococcus mutans*. Am J Dent, 24: 155–158, 2011.
29. Katsuno Y, Sato T, Aiba C: The relationship between the bacterial contamination of interdental brushes and interdental brush cleaning and storage conditions. JNOHS, 9: 58–62, 2019.
30. Phichaphop C, Apiwattanakul N, Wanitkun S, Boonsathorn S: Bacterial endocarditis caused by *Actinomyces oris*: first reported case and literature review. J Investig Med High Impact Case Rep, 8: 2324709620910645, 2020. doi: 10.1177/2324709620910645
31. Nomura R, Matayoshi S, Otsugu M, Kitamura T, Teramoto N, Nakano K: Contribution of severe dental caries induced by *Streptococcus mutans* to the pathogenicity of infective endocarditis. Infect Immun, 88: e00897–19, 2020.
32. Sato K, Fujikawa T, Koga C: Improved effect of the high water content clay using the water absorptivity of bamboo. Geosynth Eng Pap, 29: 191–196, 2014.
33. Funabashi M, Kunioka M: Evaluation of biobased content of biobased polymer by measuring carbon-14 using accelerator mass spectrometry. Netsu Sokutei, 39: 143–150, 2012.
34. Ooshima T, Matsumura M, Hoshino T, Kawabata S, Sobue S, Fujiwara T: Contributions of three glucosyltransferases to sucrose-dependent adherence of *Streptococcus mutans*. J Dent Res, 80: 1672–1677, 2001.
35. Ramful R, Sunthar TPM, Kamei K, Pezzotti G: Investigating the antibacterial characteristics of Japanese bamboo. Antibiotics(Basel), 11: 569, 2022. doi: 10.3390/antibiotics11050569
36. Teraoka F, Takahashi J: Antibacterial activity of bamboo extract and its application in clinical dentistry part 1. Relationship between concentration of bamboo extract and antibacterial activity. JSDMD, 14: 219–224, 1995.
37. Spolidorio DM, Goto E, Negrini TD, Spolidorio LC: Viability of *Streptococcus mutans* on transparent and opaque toothbrushes. J Dent Hyg, 77: 114–117, 2003.

38. Wetzel WE, Schaumburg C, Ansari F, Kroeger T, Sziegoleit A: Microbial contamination of toothbrushes with different principles of filament anchoring. J Am Dent Assoc, 136: 758–806, 2005.
39. Oh JH, Lee MR, Seo JH, Chang YS: Micro-organism according to storage method of the tooth-brush. IJCPD, 9: 193–197, 2013.
40. Shinohara Y, Koshimune K: The experiment of moisture transfer on clothes: wetting and drying of fabrics: the development of elementary school home economics curriculum based on ESD. Bull Jpn Curric Res Dev, 37: 1–11, 2014.
41. van der Sluijs E, Slot DE, Hennequin-Hoenderdos NL, van der Weijden GA: Dry brushing: Does it improve plaque removal? A secondary analysis. Int J Dent Hyg, 16: 519–526, 2018.
42. Raiyani CM, Arora R, Bhayya DP, Dogra S, Katageri AA, Singh V: Assessment of microbial contamination on twice a day used toothbrush head after 1-month and 3 months: an in vitro study. J Nat Sci Biol Med, 6: S44–S48, 2015.
43. Suda Y, Suzuki S, Miyashita H, Hasegawa K: Dimensional changes of filament taken from the worn-out tooth brush. JSP, 22: 122–129, 1979.