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Comparative Analysis of Biofilm Removal Efficiency and Tooth Wear between Mechanical Electric and Bioelectric Devices

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Abstract: Effective oral care is important to maintain a high quality of life. Therefore, plaque control can prevent the development and recurrence of periodontitis. Brushing with a toothbrush and toothpaste is a common way to remove plaque; however, excessive brushing or brushing with abrasive toothpaste can cause wear and tear on the dental crown. Hence, we aimed to quantitatively compare the plaque-removal efficiency and tooth wear of toothbrushes using bioelectric effect (BE) with those of mechanical electric toothbrushes. To generate the BE signal, an electronic circuit was developed and embedded in a toothbrush. Further, typodonts were coated with cultured artificial plaques and placed in a brushing simulator. A toothpaste slurry was applied, and the typodonts were eluted with tap water after brushing. The plaques of the typodonts were captured, and the images were quantified. For the tooth wear experiment, polymethyl methacrylate disk resin blocks were brushed twice a day, and the thickness of the samples was measured. Subsequently, statistical differences between the experimental toothbrushes and typical toothbrushes were analyzed. The BE toothbrush had a higher plaque-removal efficiency and could minimize tooth wear. This study suggests that the application of BE may be a new solution for oral care.

Keywords: bioelectric effect; oral health; plaque formation; plaque removal; tooth wear; gingival index

1. Introduction

Oral health is crucial for improving the quality of life [1]. However, according to the World Health Organization (WHO), 3.5 billion people worldwide suffer from oral diseases [2,3], with plaque formation being a primary cause [4,5]. Plaque is a biofilm that has been identified as a major cause of periodontitis or periodontal disease, which is directly linked to various health conditions such as stroke, diabetes, cardiovascular disease, and Alzheimer's disease [6–9]. Consequently, effective plaque removal is important in both public and private healthcare.

Biofilms are composed of polysaccharides and electrically polarized bacterial cells [10,11]. Biofilms are also extremely persistent, as they are 500–5,000 times more resistant to antibiotics than biofilms in their native state [12–14]. However, external electrical stimuli can disrupt the structural integrity of biofilms and affect the bacterial metabolic state [15,16]. The bioelectric effect (BE), which applies direct current (DC) and alternating current (AC), is effective in treating biofilms [17–19].

Recently, our group developed a toothbrush with BE, which was shown to reduce the gingival index in clinical trials [12,20]. We also demonstrated in an *in vitro* brushing simulator study that BE toothbrushes are significantly more efficient at removing plaque than conventional toothbrushes [21].

In the 1950s and 1960s, mechanical electric toothbrushes with high plaque-removal efficiencies were developed [22,23]. These toothbrushes have a higher plaque-removal rate than regular toothbrushes because of the added mechanical motion [24–26]; however, they cause tooth wear [27].

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Moreover, mechanical electric toothbrushes can accelerate tooth wear when used in conjunction with toothpaste [28].

Dental abrasion, a type of non-carious cervical lesion (NCCL), is defined as tooth wear caused by excessive brushing or brushing with an abrasive toothpaste [29] [Figure 1]. Tooth wear is slow, progressive, and irreversible; consequently, exposed cervical dentin can cause dentin hypersensitivity, a sharp pain that may require dental treatment [30]. The severity and prevalence of NCCL are likely to increase with age [31]. Therefore, preventive dental care is important, and new toothbrush devices that can prevent NCCL at a young age are required.

Figure 1. A 76-year-old female patient with multiple cervical abrasions evident due to her brushing habit.

In this study, we aimed to quantitatively compare the plaque-removal efficiency and tooth wear of toothbrushes using the bioelectric effect (BE) with those of mechanical electric toothbrushes, which have high plaque-removal efficiency but are disadvantaged in terms of tooth wear. Clinical trials are inconvenient for quantitative evaluation owing to inter-individual variability; however, *in vitro* simulators have been used to increase the reproducibility of the experiments [27,32–34]. We compared four types of toothbrushes (conventional toothbrushes, developmental toothbrushes with and without the BE, and mechanical electric toothbrushes) using an *in vitro* simulator. Based on the experimental results, we investigated the degree of plaque removal and tooth wear of the BE toothbrush. The BE toothbrush not only demonstrated effective plaque removal but also did not cause accelerated tooth wear.

2. Materials and Methods

2.1. Tested Toothbrushes and the Design of BE Toothbrush

We compared the plaque-removal efficiency and tooth wear of three toothbrushes with those of a BE toothbrush. A commonly used regular manual toothbrush, two developed toothbrushes (with and without BE), and a mechanical electric toothbrush were tested. The conditions of the proposed toothbrushes were specified to investigate the effect of bristles (typical toothbrush versus the proposed toothbrush) on plaque removal. The toothbrushes tested and their abbreviations are listed in Table 1 and Figure 2.

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Table 1. Description of condition for tested toothbrushes.

Toothbrushes	Abbreviations	Note
Oral-B Ultra-fine, Oral-B Laboratories, Boston, MA, USA	CB	Typical toothbrush
Non-bioelectric effect	BE-off	non-BE
0.7 V amplitude of 10 MHz with 0.7 V offset	BE-on	Applied BE
Oral-B iO3, Oral-B Laboratories, Boston, MA, USA	MB	Mechanical electric toothbrush

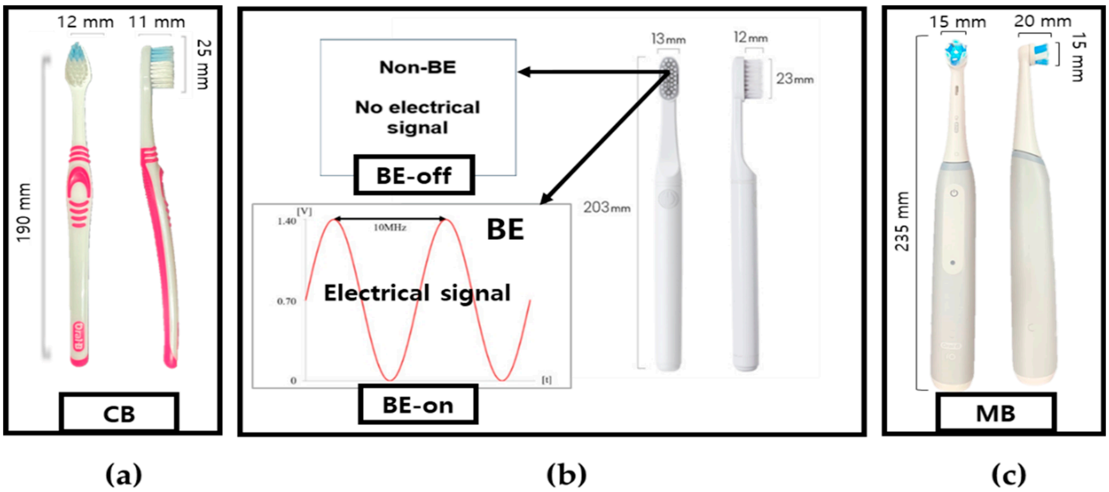

Figure 2. Actual figure of tested toothbrushes: (a) CB, (b) Proposed toothbrushes (applied non-BE: BE-off, applied BE: BE-on). (c) MB.

The BE applied to remove biofilms is a combination of electrical signals, specifically a 0.7 V sinusoidal signal at 10 MHz with a DC offset of 0.7 V, as shown in Table 2 and Figure 3 [12,20,21]. The BE signal frequency was selected based on a previous study [15]. The DC offset was set below an electrolysis threshold of 0.82 V. Our previous studies revealed that this signal does not induce electrolysis [20,21].

Table 2. Details of electric signal for BE.

Contents	Details	Comments
Intensity	0.7 V	Below-water electrolysis 0.82 V
Frequency	10 MHz	Effective frequency
Composition (AC:DC)	1:1	Effective biofilm treatment

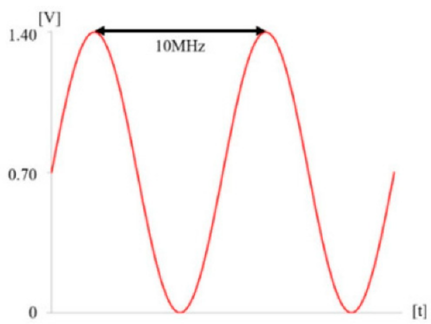

Figure 3. Schematic of electrical signal for BE.

An electronic circuit was developed to generate the BE signal, as shown in Figure 4 [12,20,21]. Subsequently, this circuitry was embedded in a toothbrush. A stainless-steel electrode was selected because of its corrosion resistance and conductivity, which are essential for supplying an electric field. The signal for the BE was output through the two electrodes.

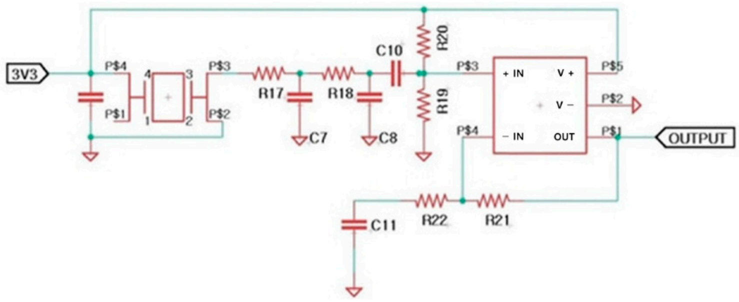

Figure 4. schematic of electronic system for generating electric signal for BE.

The current from the BE signal was determined to be safe [20,21] compared to the reported biocompatible current range [35].

2.2. The Brushing Simulator

The brushing simulator used in this study was developed in our previous study and is shown in Figure 5 [21]. The main components were a linear actuator, stepper motor, motor driver, piezoelectric pressure sensor, and an artificial tooth construct. An Arduino Uno (Arduino, Italy) was used for simulation control and data acquisition (DAQ).

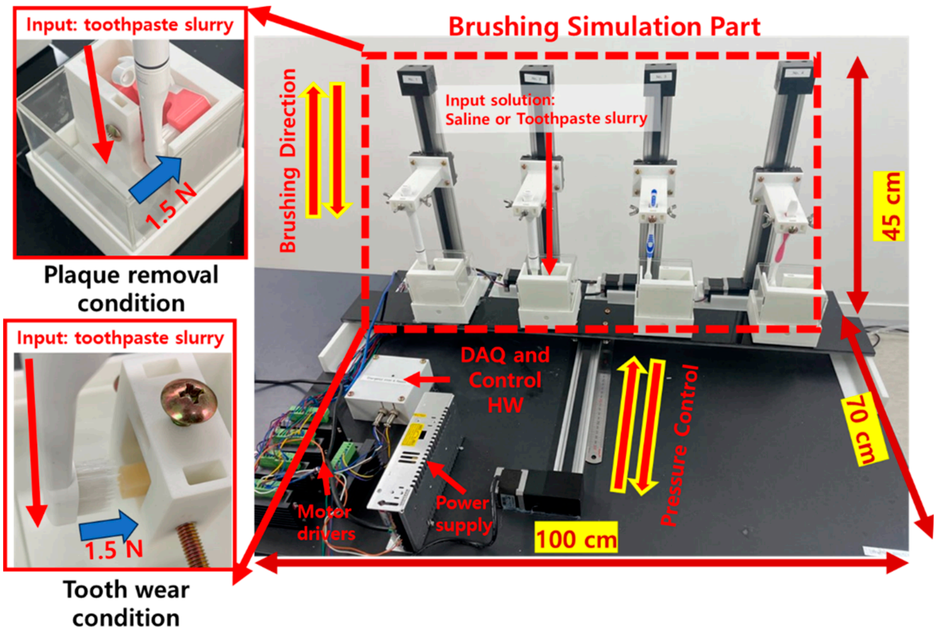

Figure 5. The development of the brushing simulator [21].

2.3. Plaque Culture

Streptococcus mutans (KCTC 3065, Korean Collection for Type Cultures (KCTC), Jeongeup, Republic of Korea) were cultured in a growth medium (LB Broth, Ambrothia Inc., Daejeon, Republic of Korea) at 37 °C for 48 h to provide sufficient time for maturation.

2.4. Experiment

In this study, experiments were conducted to investigate two aspects of toothbrushes: plaque removal and tooth wear, as shown in Figure 6.

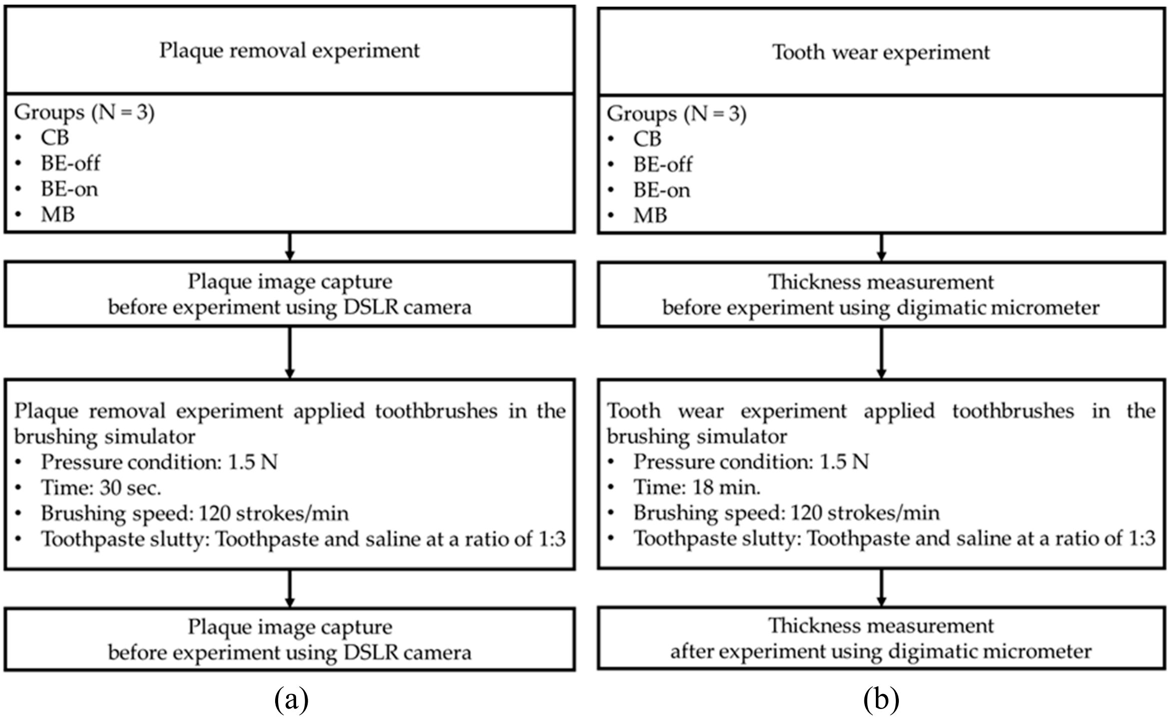

Figure 6. Graphic description of the plaque removal and tooth wear procedure: (a) plaque removal experiment. (b) Tooth wear experiment.

2.4.1. Plaque Removal Experiment

A plaque-removal experiment was designed to investigate the efficacy of plaque removal using each toothbrush. The typodonts were coated with cultured artificial plaques. Green marker spray (OccludeTM; Pascal, Bellevue, WA, USA) was used to visualize the plaque [34]. Subsequently, the coated typodonts were placed in a brushing simulator. ISO/TR 14569-1 specifies the load a toothbrush exerts on teeth as 0.5 to 2.5 N [36]. The force with which the toothbrush pressed on the teeth was set to 1.5 N, and the molars of the teeth were brushed [34]. Two minutes is generally recommended for toothbrushing [37]. The brushing speed was 120 strokes/min [36] for 30 s, and approximately one-fourth of the teeth were targeted. A toothpaste slurry (toothpaste: saline ratio of 1:3) was applied [21]. After brushing the teeth, typodonts were eluted with tap water for 30 s. This process removed isolated plaques for quantification. Our preliminary testing revealed that this process prevents unintended plaque elution [21]. The plaques of the typodonts were captured using a DSLR (Digital Single Lens Reflex, Nikon D3100, Japan) camera under controlled lighting conditions before and after the experiment. Images were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.4.2. Tooth Wear Experiment

A toothwear experiment was designed to investigate the tooth wear caused by each toothbrush. ISO/TR 14569-1 specifies the force applied to the tooth as 0.5 to 2.5 N [36]. The pressure applied to the tooth was set to 1.5 N. Additionally, the toothbrushing speed was set to 120 strokes/min [36]. Polymethyl methacrylate (PMMA) disk resin blocks (A3; Yamahachi, Gamagori, Japan) were used as test specimens. The specimens were kept in water at 37 °C for one week [36]. Typically, 28 teeth are present [38], and a brushing time of 2 min is recommended [37]. Each tooth was brushed for 4.3 s. For the convenience of calculation, assuming three sides of the tooth, this was set to 1.5 s. Teeth were brushed twice a day for 3 s per tooth. Therefore, the tooth wear test result was calculated as approximately 18 min/year. The thickness of the samples was measured using a digital micrometer before and after the experiment.

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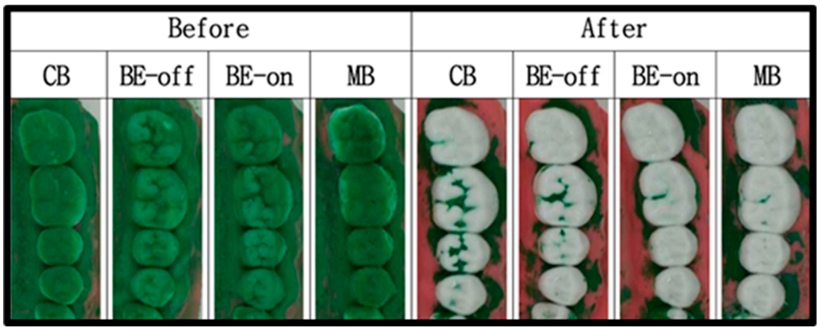
2.5. Statistical Analysis

Data are presented as means and standard deviations (SDs). One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was used to determine the significance of differences between the experimental toothbrushes and typical toothbrushes (CB). Paired t-tests were used to compare the differences between toothbrushes with BE (BE-on) and mechanical electric toothbrushes (MB). All statistical analyses were performed using R-studio version 4.3.1 (Posit, Boston, MA, USA). The p-values of less than 0.05 were considered significant.

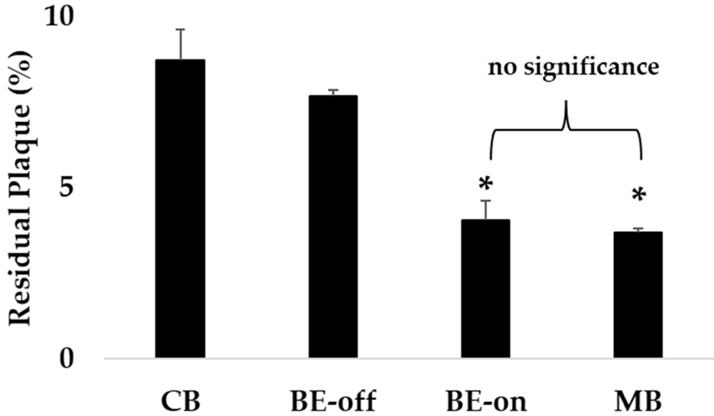
3. Results

3.1. Experiment of Plaque Removal

The experimental results are shown in Figure 7. No significant difference was observed in the residual plaque between the non-bioelectric effect (BE-off) and CB groups. A previous study reported that no significant difference was observed in the plaque-removal rate according to the shape of the bristle [39]. However, BE-on and MB treatments resulted in significantly reduced residual plaques. BE is effective in removing biofilms such as plaque, which is similar to our previous finding that the BE toothbrush significantly removed plaque [21]. Mechanical electric toothbrushes have a higher plaque removal rate than regular toothbrushes [24–26]. No significant differences were observed between the BE-on and MB groups. CB, BE-off, BE-on, and MB showed residual plaque percentages of 8.71 ± 0.89%, 7.67 ± 0.18%, 4.04 ± 0.58%, and 3.70 ± 0.12%, respectively.



(a)



(b)

Figure 7. Residual plaques in plaque removal experiment: (a) Representative image showing significant plaque reduction under BE-on and MB; (b) Results of residual plaque. Results are presented as means ± SDs. * $p < 0.05$ versus CB. There was no significant difference between BE-on and MB.

3.2. Experiment with Tooth Wear

Tooth wear was highest in the MB group, as presented in Table 3. No significant difference was observed between the other groups. Mechanical electric toothbrushes have been reported to induce greater wear than regular toothbrushes [27]. These results are similar to those reported previously. No significant difference was observed between the proposed toothbrushes (BE-off and BE-on) and the CB. Microcurrents do not affect enamel microhardness [40]. In addition, the BE signals used in this study are biocompatible [35].

Table 3. Results of thickness change due to tooth wear experiment in tooth wear experiment showing significant relative tooth wear under only MB. Results are presented as means ± SDs. * $p < 0.05$ versus CB. # $p < 0.05$ versus BE-on.

Toothbrush	Thickness changes due to tooth wear experiment (μm)
CB	7.33 ± 1.53
BE-off	6.67 ± 1.53
BE-on	8.67 ± 1.53
MB	19.67 ± 5.51*,#

The elastic modulus, hardness, and wear resistance of PMMA teeth are poor compared to those of natural teeth, leading to rapid wear of PMMA teeth [41]. Therefore, tooth wear was compared using relative rather than absolute values. MB showed 168.18 ± 75.10 % more wear compared to BE-on. However, no significant difference was observed in the wear between the BE-off and BE-on groups shown in Figure 8.

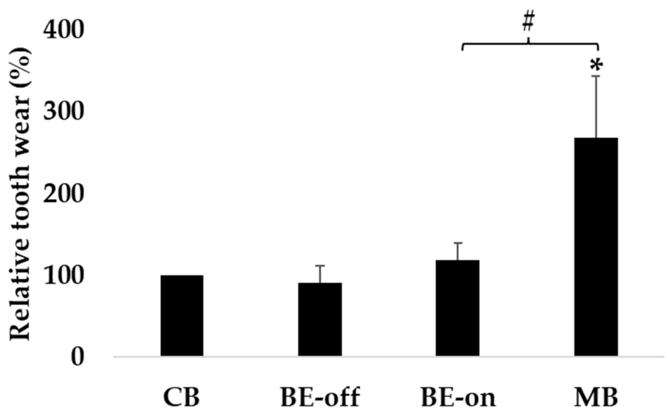


Figure 8. Results of relative tooth wear in tooth wear experiment showing significant relative tooth wear under only MB. Results are presented as means ± SDs. * $p < 0.05$ versus CB. # $p < 0.05$ versus BE-on.

4. Discussion

This study evaluated the plaque-removal efficiency and tooth abrasion of BE toothbrushes in an *ex vivo* setting. No significant difference in the number of residual plaques was observed between the CB and BE-off groups. BE-off does not involve the BE and should be considered a manual toothbrush. No significant difference in plaque-removal efficiency was observed owing to differences in bristles [39]. Similar results were observed in the present study.

A significant reduction in residual plaque was observed for MB and BE-on toothbrushes when compared with that of manual toothbrushes. MBs are more efficient for plaque removal than manual toothbrushes because of the mechanical rotation of their bristles [24–26]. We observed a significant reduction in the residual plaque when using BE-on compared with that of a manual toothbrush, a result similar to that of previous studies [21].

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The application of BE results in high biofilm treatment efficiency [17–19]. BE is caused by the propagation of electromagnetic currents, waves, and voltages [14–16]. Applying AC at a specific frequency can improve the porosity of the biofilm structure [17,18], whereas applying DC can induce changes in the electrolyte state [15,19]. The experimental conditions consisted of an electrical dielectric using a toothpaste slurry containing saline solution. Saline solutions were used as saliva in several studies [42,43]. Therefore, the BE-treated toothbrush proposed in this study is expected to have a higher removal efficiency than a manual toothbrush.

PMMA was used in the *in vitro* experiments to evaluate tooth wear during brushing. PMMA can be used for tooth wear testing despite the differences between real teeth and PMMA. PMMA is not only used as a tooth-filling material but is also used in various dental studies [40,44]. No significant difference was observed in the wear rate of PMMA for BE-off and BE-on compared with that of regular toothbrushes. However, MBs exhibited significant wear. BE-off is the state in which the BE is not applied; therefore, it is the same as a manual toothbrush. In the case of BE-on, no significant difference was observed in the wear rate.

Microcurrents had no effect on enamel [40]. BE uses a current of 40.7 ± 1.5 μA, so it is minimally harmful to the human body, as shown in our previous study [21]. Therefore, in this study, we expected that the BE energy applied to the toothbrush would not affect tooth wear. In contrast, the MB caused significant wear. MBs have been reported to cause tooth wear when used with toothpaste [27,28], and similar results were obtained in this study.

As the population ages and retains their natural teeth for longer periods, the prevention of tooth wear becomes increasingly important. The significantly smaller PMMA wear of BE-on compared with that of MB indicates that the application of the bioelectric effect does not accelerate the wear of permanent teeth.

Based on the above results, we expect that the application of BE will increase plaque-removal efficiency without accelerating the wear of permanent teeth. The BE toothbrush not only had a higher plaque-removal efficiency than a regular toothbrush but also had a plaque-removal rate equivalent to that of an MB. The tooth wear was observed to be similar to that of a manual toothbrush and significantly lower than that of an MB. These results suggest that BE toothbrushes have high plaque-removal efficiency and low wear rates, equivalent to those of MBs. In addition, clinical studies have seen toothbrushes significantly improve the gingival index [12,20]. Therefore, BE toothbrushes may provide a new solution for oral care.

Experiments using *in vitro* simulators not only have higher reproducibility than those of clinical trials but also enable quantitative experiments [21]. In this study, we quantitatively evaluated the plaque-removal efficiency and wear of BE toothbrushes. In our previous study, BE-treated toothbrushes were effective in improving the gingival index but not the plaque index [12,20]. This may be due to individual variation, which is a limitation of clinical trials. However, in this study, we observed a significant plaque-removal rate in the quantitative experiments.

Plaque can be a problem not only in teeth but also in other areas of the mouth, such as the tongue [45]. Moreover, they can occur in humans and pets [46]. Therefore, we plan to develop various types of oral products, including plaque-related tongue scrapers and oral care products for pets, using BE.

This study had some limitations. First, we used constant pressure and brushing speed; thus, changes in toothbrushing patterns in real life are not reflected [47]. Second, we evaluated plaque-removal efficiency in the same typodonts and area; thus, oral structures that vary from person to person are not reflected [48]. Third, tooth wear was evaluated using PMMA as the dental material; however, this may differ from actual teeth [41]. Therefore, additional research is needed to further apply the BE toothbrush for plaque removal and tooth wear in real environments.

5. Conclusions

The BE toothbrush not only showed an effective plaque-removal rate but also did not induce accelerated tooth wear. Proper toothbrush use should remove as much plaque as possible and minimize tooth wear. BE toothbrushes may be a new and more efficient alternative. Further

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investigations of various toothbrushing patterns, pressure conditions, intraoral structures, and materials are required. We plan to develop a range of oral care products containing BE in the future.

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Conflicts of Interest: The authors declare no conflict of interest.

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