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Original Article

The bactericidal and biofilm removal effect of super reducing water on *Streptococcus mutans* in three types of orthodontic brackets

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Abstract

Purpose: To investigate the bactericidal and biofilm removal effect of super reducing water (SRW) on *Streptococcus mutans* (*S. mutans*) adhered to orthodontic brackets, *in vitro*.
Methods: Three types of brackets were bonded to aluminum disks. After the formation of *S. mutans* biofilms on the surfaces, the brackets were divided into three groups (n = 44 each) based on their exposure to SRW: group 1, no treatment; group 2, treated for 5 min; and group 3, treated for 10 min. Total viable counts, adenosine triphosphate measurements, crystal violet assay, and scanning electron microscopy were used to evaluate the effect of SRW.
Results: The bacterial counts in groups 2 and 3 were significantly lower than those in group 1 ($P < 0.001$); however, no significant differences were observed between groups 2 and 3. Marked decreases in the number of bacterial colonies and extent of biofilm formation were observed in groups 2 and 3 compared to group 1. No significant differences in the number of bacterial colonies and amount of biofilm were observed among the three types of brackets in each group.
Conclusion: These findings indicate the bactericidal and biofilm removal effect of SRW treatment on *S. mutans* adhered to orthodontic brackets.

Keywords: alkaline electrolyzed water, orthodontic bracket, *Streptococcus mutans*, super reducing water

Introduction

Plaque control in orthodontic patients has often been inadequate due to the complexity of the appliances used [1]. The accumulation of dental plaque increases the levels of *Streptococcus mutans* (*S. mutans*) and *Lactobacilli* [2,3], eventually leading to the development of dental caries and white spot lesions in these patients [4,5].

Interactions between early bacterial colonies affect the formation, structure, and pattern of the biofilm and prevent the accumulation of dental plaque [6]. Therefore, the use of chemicals, in addition to daily mechanical cleansing methods, which can interfere with the properties of the biofilm, has been suggested in patients undergoing orthodontic treatment [7,8]. Most commercial chemicals used for cleaning have complicated formulas and contain antimicrobial agents [9-11]. Chemical cleaning methods that do not disturb the normal oral ecosystem and can significantly reduce both plaque and biofilm formation are preferred for daily use [12].

Functional electrolyzed water is produced by the electrolysis of ordinary tap water, which contains a small amount of salt, without the addition of any harmful chemicals [13]. It has been used as a disinfectant and cleaner in various fields, such as the food industry [14,15] and medicine [16]. Functional electrolyzed water can be classified into three major types based on the conditions used for electrolysis: acidic, neutral, and alkaline [17]. Some studies have shown the bactericidal and cleansing effects of acidic and neutral electrolyzed water on bacteria adhered to orthodontic brackets [18,19]. However, the effect of alkaline electrolyzed water on these microorganisms has not been evaluated in orthodontic patients.

The pH of the super reducing water (SRW; S-100, A.I. System Products Corp., Kasugai, Japan) used in the current study was 12.3. The cleaning, antimicrobial, anti-dust, rust-preventing, anti-septic, and burn-healing activities of SRW have been demonstrated previously [20-22]. The present study evaluated the effect of SRW on *S. mutans* in three types of orthodontic brackets in order to determine its bactericidal and biofilm removing capabilities. The null hypothesis stated that there were no significant differences in *S. mutans* adherence and biofilm formation among the different types of orthodontic brackets in the presence or absence of SRW.

Materials and Methods

An a priori power analysis was performed to calculate the sample size for one-way analysis of variance (ANOVA) using the G*power software (version 3.1.9.6; Franz Faul University, Kiel, Germany) at an effect size of 0.4 (Cohen's large-effect size), α error probability of 0.05, and power of 0.8. Based on the analysis, a total of 66 orthodontic brackets were required; thus, the number of orthodontic brackets in each group was set at 22.

SRW

SRW is a type of functional electrolyzed water comprising large amounts of electrons; it is produced by the electrolysis of natural water, followed by the application of an electric current and pressure using a special diaphragm system [20]. This water has an osmolarity of 100 mOsm, a specific gravity of 1.002, an oxidation-reduction potential of -344 mV, and a pH of 12.0-12.4 [13].

Brackets

Metal, ceramic, and plastic standard edgewise premolar brackets with 0.018 slots (Tomy International, Kanda, Japan) were used in this study.

Aluminum disk preparation

The three different types of brackets were bonded to an aluminum disk (diameter, 13 mm) using a 4-META/MMA-TBB adhesive resin (Super Bond; Sun Medical, Moriyama, Japan; Fig.1). A total of 132 aluminum disks were prepared to evaluate the effects of SRW on the adherence of *S. mutans* to the brackets. Each specimen was sterilized in 70% ethanol for one day and 0.1% sodium hypochlorite for 20 min, followed by two washes in sterile distilled water for 10 min immediately before use.

Bacteria preparation

S. mutans (ATCC27175) was anaerobically cultured in Brain Heart Infusion (BHI) broth overnight at 37°C and 10% CO_2, using the BD Bacto Brain Heart Infusion (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The cultured bacterial suspensions were then transferred to 1.5 mL tubes and centrifuged at 9,000 rpm for 5 min. The clear supernatant

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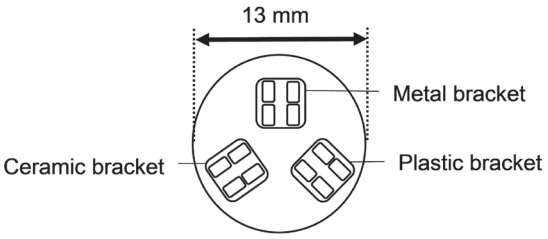

Fig. 1 The three types of brackets were bonded to an aluminum disk (diameter, 13 mm).

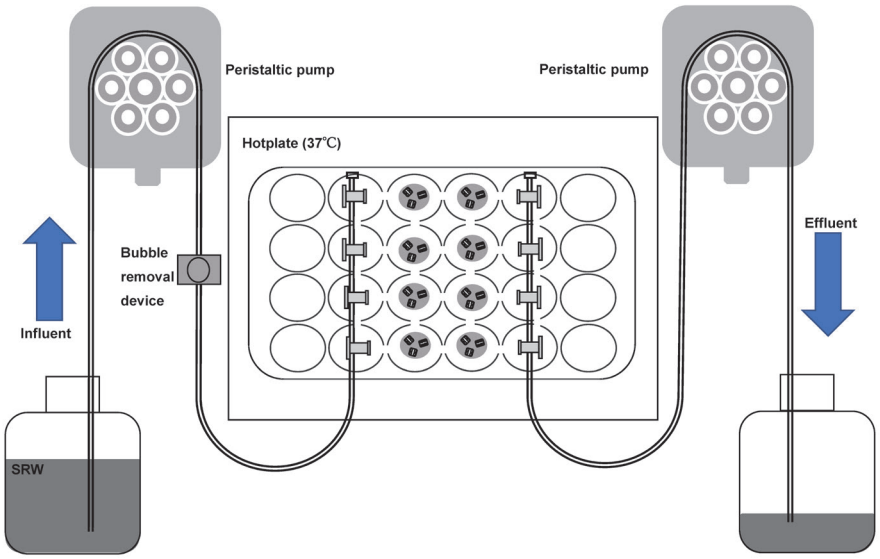

Fig. 2 Action of SRW using the flow system and a modified 24-well plate

was removed, and phosphate-buffered saline (PBS; pH 7.4) was added to the bacterial pellet inside the tube.

After vortexing the PBS and the bacterial pellet, the resultant bacterial suspension was centrifuged under the same conditions described earlier. These steps were repeated twice, following which the clear supernatant was removed, PBS was added, and the mix was vortexed again. The resultant bacterial suspensions in the 1.5 mL tubes were collected and transferred to a 50 mL tube. The volumes of the suspensions were adjusted using PBS to a final concentration of 0.30 at an optical density (OD) of 600 nm.

In vitro biofilm formation

The specimens were placed in a 24-well plate coated with artificial saliva (Salivent Aerosol; Tenjin Pharma, Kasumigaseki, Japan) at 37°C and 10% CO_2 for 1 h. Then, they were transferred to another well with 1 mL of bacterial suspension and incubated for 2 h to facilitate the initial bacterial adhesion. The bonding surface of the bracket was placed facing the bottom of the well.

Cell culture inserts were placed in 12-well plates, and each pre-incubated specimen was placed onto the insert. BHI containing 1% sucrose as culture solution was poured onto the cell culture inserts with the specimens to form bacterial biofilms on the surfaces of the brackets. The plate was incubated with gentle swaying for 48 h, and the culture solution was changed every 12 h.

Evaluation of the effect of SRW

The specimens were removed from the cell culture inserts and gently washed with PBS to remove the non-adhered bacteria. A total of 132 specimens were equally divided into three groups as follows (n = 44): group 1, no SRW treatment; group 2, treated with SRW for 5 min; and group 3, treated with SRW for 10 min.

The action of SRW was performed using the flow system and a modified 24-well plate (Fig. 2) [23]. Sixteen wells in the 24-well plate were connected by cutting the borders using a heated tweezer. The specimens in group 2 were placed in 8 of the 16 connected wells; the surfaces without the brackets faced the bottom of the wells. Likewise, specimens from group 3 were placed in the remaining 8 wells.

SRW was allowed to act on the specimens at 37°C for 5 min (group 2) and 10 min (group 3), following which the specimens were washed twice with PBS for 5 min. The 44 specimens in each group were randomly divided into two subgroups (n = 22, each) to measure the total viable counts and adenosine triphosphate (ATP) values; additionally, the crystal violet assay was performed to analyze the extent of biofilm formation. The random division was performed using the random numbers table to avoid potential differences in the amounts of bacteria based on the location of the biofilm and position of the specimen in the well.

Total viable count and ATP measurement

Each bracket was gently removed from the 22 specimens and transferred to new tubes containing 1 mL of PBS. The tubes were vibrated using an ultrasonic cleaning machine (US Cleaner; As One, Osaka, Japan) for 2 min and vortexed for another 2 min to remove the bacterial biofilms from the surfaces and form the bacterial suspension. Each bacterial suspension was homogenized and serially diluted to 10^5 of the initial concentration.

The diluted suspension (0.1 mL) was plated onto sheep blood agar medium and incubated at 37°C and 10% CO_2 for 48 h, following which the number of colonies on the medium was calculated (in colony-forming units/mL; CFU/mL). Another 0.5 mL of the bacterial suspension was used to measure the ATP bioluminescence intensity (relative light unit; RLU) using a luminometer (Lumitester PD-30; Kikkoman, Nishi-Shinbashi, Japan).

Crystal violet assay

Each bracket was gently removed from the specimens, and the base was placed facing the bottom of the well in a 48-well plate. Crystal violet solution (0.1%; 0.5 mL) was poured into the well containing the bracket. The surplus solution was gently removed from the well after 10 min, and each bracket was washed twice with distilled water at 10 min intervals.

The biomass, which comprised the biofilm bound to crystal violet, was extracted using 1 mL of 33% acetic acid for 10 min. The extracted solution (200 µL) was poured into the wells of a microplate, and the OD value was measured using a microplate reader (Imark Microplate Reader; Bio-Rad Laboratories, Hercules, CA, USA) at 570 nm to evaluate the amount of biofilm formation. The OD value was measured three times for each bracket, and the mean of the three measurements was calculated.

Scanning electron microscope observations

Additional specimens were prepared for scanning electron microscope (SEM) observation. Each bracket was gently removed from the aluminum plate and fixed in 2.5% glutaraldehyde solution at 4°C for one day. The specimens were dehydrated in 70%, 80%, and 90% ethanol for 2 h each, followed by dehydration in 100% ethanol three times at 30 min intervals. The brackets were degassed in a vacuum desiccator for one day, sputter-coated with platinum, and observed under an SEM (Tm4000plus Miniscope; Hitachi High-Technologies, Tokyo, Japan) using an emission electron voltage of 5-15 keV.

Statistical analyses

The Excel software BellCurve (version 3.21; Social Survey Research Information, Tomihisa, Japan) was used for statistical analyses. The means and standard deviations of the total viable counts and the ATP and OD values were calculated in each bracket from each of the three groups. One-way ANOVA and Tukey's test were used to analyze the effects of SRW on the total viable counts and ATP values in each bracket type. The Kruskal-Wallis and Steel-Dwass tests were used to determine significant differences in the action times of SRW on each bracket. Two-way ANOVA followed by the Tukey test was used to analyze the main effects of SRW on the different bracket types after the crystal violet assay. The parametric and nonparametric tests were performed after testing the normality of the distribution (Kolmogorov-Smirnov test) and the homogeneity of variance (Leven test). The level of significance was set at $P < 0.05$.

Results

Total viable count and ATP measurement

The total viable counts and ATP values in each type of bracket were significantly lower in groups 2 and 3 than in group 1 ($P < 0.001$); no significant differences were observed between groups 2 and 3 (Figs. 3, 4). The metal brackets in group 1 exhibited the lowest total viable counts and ATP values, followed by the ceramic and plastic brackets in ascending order, with significant differences ($P < 0.001$) and in groups 2 and 3, there were no significant differences between three bracket types.

Crystal violet assay

Two-way ANOVA indicated significant differences in the mean amounts of biofilm formed among the three groups ($P < 0.001$) and three bracket types ($P < 0.001$); no significant interactions were observed between these two factors. Tukey's test showed significantly lower amounts of biofilm formation in groups 2 and 3 than in group 1 ($P < 0.001$), with no significant difference between groups 2 and 3. Furthermore, the metal brackets exhibited a significantly lower amount of biofilm than the ceramic and plastic brackets ($P < 0.01$), with no significant difference between the ceramic and plastic brackets (Fig. 5). Typical images of metal brackets in each group stained with crystal violet are shown in Fig. 6.

SEM observations

Figure 7 shows the typical SEM findings of the bacterial colonies and biofilm on the surface of each type of bracket in each group. Abundant bacterial colonies and increased biofilm formation were observed on the surfaces of the three types of brackets in group 1 compared to those in groups 2 and 3; no differences in the two parameters were observed between groups 2 and 3. No differences in the number of bacterial colonies and the amounts of biofilm were observed among the three types of brackets in each group.

Discussion

This study revealed significant differences in the extent of bacterial adherence and biofilm formation among the different types of orthodontic

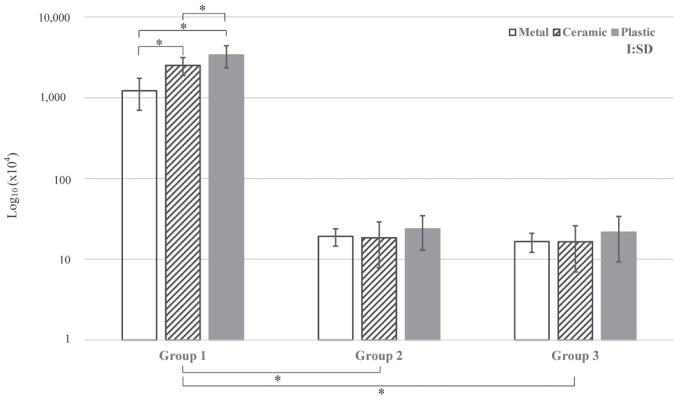

Fig. 3 Log reduction in the colony-forming unit (CFU)/mL measured after treatment with SRW. Comparisons between the experimental groups were performed using the Kruskal-Wallis test and Steel-Dwass test, and those among the three types of orthodontic brackets were compared using one-way ANOVA and Tukey's test. ($n = 22$; $*P < 0.01$). Group 1, no SRW treatment; Group 2, treated with SRW for 5 min; Group 3, treated with SRW for 10 min. SD: standard deviation.

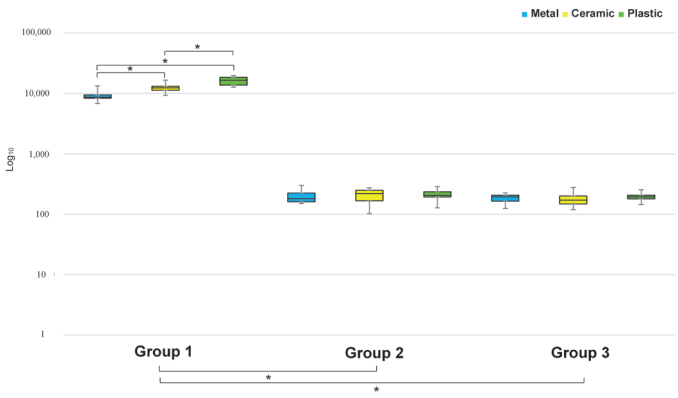

Fig. 4 Log reduction in ATP measured (RLU) immediately after SRW treatment. ($n = 22$; $*P < 0.01$). Group 1, no SRW treatment; Group 2, treated with SRW for 5 min; Group 3, treated with SRW for 10 min; SD: standard deviation.

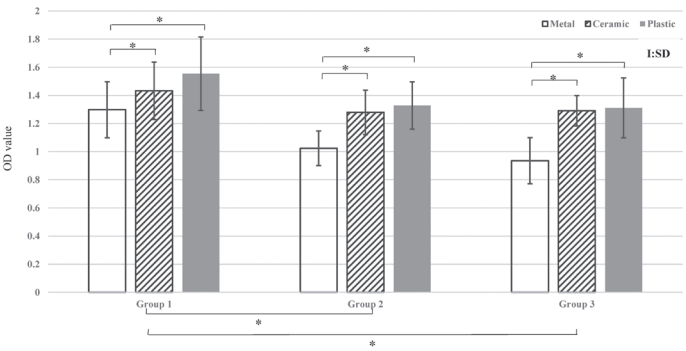

Fig. 5 OD values measured immediately after SRW treatment. ($n = 22$; $*P < 0.01$) Group 1, no SRW treatment; Group 2, treated with SRW for 5 min; Group 3, treated with SRW for 10 min. SD: standard deviation.

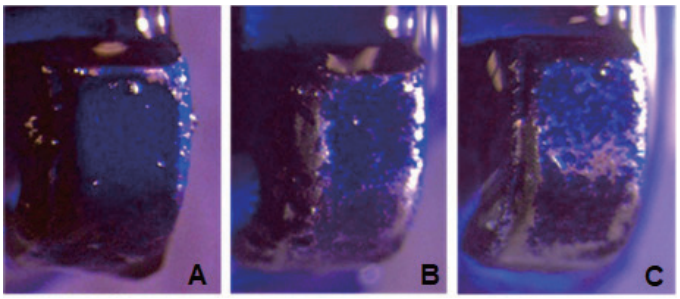

Fig. 6 Typical images of each type of metal bracket in each group stained with crystal violet. (A) metal bracket of group 1, (B) metal bracket of group 2, (C) metal bracket of group 3.

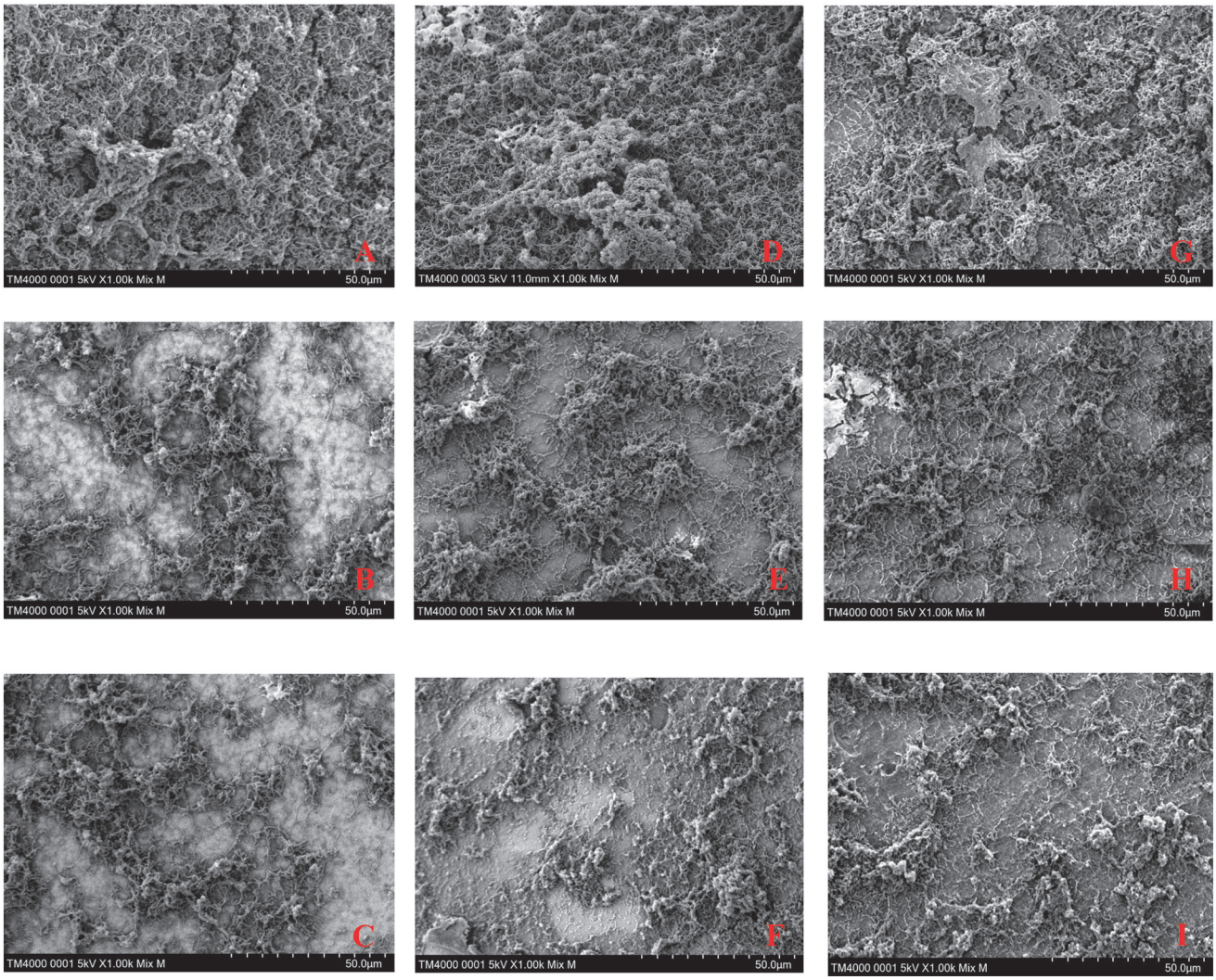

Fig. 7 SEM observations. *S. mutans* colonies and biofilm formation on the surfaces of the orthodontic brackets after treatment (1,000× magnification). The reticulated form represents the biofilm, which contains the bacterial colony. (A) metal bracket of group 1, (B) metal bracket of group 2, (C) metal bracket of group 3, (D) ceramic bracket of group 1, (E) ceramic bracket of group 2, (F) ceramic bracket of group 3, (G) plastic bracket of group 1 (H) plastic bracket of group 2, and (I) plastic bracket of group 3

brackets treated with and without SRW, thereby rejecting the null hypothesis.

In the absence of SRW, the metal brackets presented with the lowest amount of *S. mutans* adhesion, followed by the ceramic and plastic brackets, in ascending order (Figs. 3, 4). According to some studies, the amount of *S. mutans* adhered to the surface was significantly lower in metal brackets compared to those on ceramic and plastic brackets [24], whereas others have reported the opposite effect [25,26]. This discrepancy in the extent of adherence based on the bracket type may be caused by the surface free energy (SFE) of the bracket, which is reported to have a significantly positive correlation with the plaque-retaining capacity. In one study, metal brackets showed the lowest SFE, followed by ceramic and plastic brackets, in ascending order [26].

After the formation of the bacterial biofilms on the surfaces, the brackets treated with SRW (for 5 and 10 min) exhibited significantly lower total viable counts and ATP values (Figs. 3, 4) than those not treated with the water; no significant differences were observed between those treated for 5 and 10 mins. The reduction rates in the total viable counts after the 5 min application of SRW ranged from 97.9% in the metal bracket to 99.2% in the plastic bracket. The rates were slightly increased in each bracket after the 10 min application. The reduction rates in ATP values were about 98% in the three types of brackets after the 5 min application; a slight increase was observed in specimens treated with SRW for 10 min. The total viable counts measured after 10 min of treatment with saline (9.9 mL) + bacterial suspension containing *S. mutans* (0.1 mL) were compared with those measured after 10 min of treatment with SRW (9.9 mL) + the same suspension (0.1 mL). The results showed a reduction in the total viable count after treatment with SRW (2.9×10^5 CFU/mL) compared to treatment with saline (1.0×10^6 CFU/mL). These findings suggested that 5 min of treatment with SRW had a significant bactericidal effect on *S. mutans*, regardless of the bracket type.

Okajima et al. [13] reported the bactericidal effect of SRW against periodontopathic bacteria (*Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*) after 15 and 30 s of exposure. The difference in the action time of SRW between this study and the current study might be due to the thickness of the peptidoglycan layer of the Gram-positive and Gram-negative bacteria. Azuma et al. [27] showed that the peptidoglycan layer of the Gram-positive bacterium was thicker than that of the Gram-negative bacterium, which is resistant to mechanical attacks.

According to Lee et al. [12], the bactericidal effect of functional electrolyzed water (Puri water) was due to the presence of free chlorine and short-lived reactive oxygen species, such as singlet oxygen, superoxide free radicals (O^{2-}), and hydroxyl radicals (OH^-). Furthermore, Okajima et al. [13] attributed the bactericidal effect of SRW to the high negative oxidation-reduction potential (−344 mV) and OH^-. Taken together, these findings suggest that the bactericidal effect of SRW against *S. mutans* might be due to the OH^- in the water.

The application of SRW (pH, 12.3) resulted in a significant decrease in the attachment of biofilms to the brackets (groups 2 and 3 vs. group 1); no significant difference in the amount of biofilm was observed between the 5- and 10-min application groups (group 2 vs. group 3; Fig. 5-7), thus suggesting that a 5-min application of SRW was sufficient to have a biofilm removing effect. These results are in accordance with those reported by Sun et al. [14], wherein the amount of biofilm formed by *Staphylococ-*

cus aureus decreased following the application of functional electrolyzed water (pH, 10.8 and 11.6) for 120 s.

The SRW used in the current study consisted of many negative ions, which were formed during the manufacturing process. These negative ions ionize the positively-charged outermost surfaces of the biofilm and brackets through electric and intermolecular forces [13]. The negative ions are thought to gather around and cover the outermost surface layers, and the electric repulsion force caused by these ions removes the biofilms from the brackets.

Irrespective of the application or non-application of SRW, metal brackets presented with the least biofilm adherence, followed by the ceramic and plastic brackets in ascending order, with no significant difference between the ceramic and plastic brackets. Rabin et al. [28] reported that biofilms were communities of microorganisms attached to a surface, thus suggesting that low adherence of the biofilm to the surface might indicate low adherence of the bacteria (*S. mutans* in the current study). This decrease in the adherence of bacteria and biofilms might be due to the low SFE of the metal bracket compared to those of the ceramic and plastic brackets [26].

The results of the crystal violet assay demonstrated marked reductions in biofilm formation from the 5- to 10-min SRW application in the three types of brackets (9.9-27.9% after the 10-min application). However, these reduction rates were lower than those in the total viable counts and ATP values, thus suggesting that the biofilm removal effect of SRW was not as strong as its bactericidal effect. In this study, the electric repulsion forces caused by the negative ions in SRW removed only a part of the biofilm on the brackets; about 70% of the biofilm remained on the surfaces.

From a clinical perspective, SRW can be safely applied to the oral cavity. Merne et al. [29] showed that the oral mucosae of rats allowed to drink alkaline water (pH, 11.2 and 12.0) for 52 weeks remained unaffected by the high alkalinity without any specific morphologic alterations. However, the application of calcium hydroxide paste, which has a pH almost similar to that of alkaline electrolyzed water, to the oral mucosa of rats and hamsters resulted in cell atypia [30, 31]. The combined use of a mouthguard may prevent cell atypia and ensure the safety of the application of SRW in the oral cavity. Thus, the use of mouthguards containing SRW for 5 min, followed by mechanical cleaning, may aid in maintaining a safer and more hygienic oral environment in orthodontic patients.

One of the limitations of this study is that the effect of only one bacterial species (*S. mutans*) was evaluated. Additional studies investigating the effects of SRW on other dental caries bacteria (such as *Streptococcus sobrinus* and *Lactobacillus*) are warranted. Furthermore, the effectiveness of the vibration of a mouthguard containing SRW to improve the biofilm removal effect.

In conclusion, this study demonstrated the bactericidal effect of SRW and its effectiveness in removing the biofilm from the surfaces of three types of orthodontic brackets. The findings of the study might prove useful for maintaining the routine oral hygiene of the orthodontic patient, if used as a chemical cleansing method.

Conflicts of interest

The authors report no conflicts of interest.

References

1. Chen Y, Wong RW, Seneviratne CJ, Hägg U, McGrath C, Samaranayake LP (2011) Comparison of the antimicrobial activity of Listerine and Corsodyl on orthodontic brackets in vitro. Am J Orthod Dentofacial Orthop 140, 537-542.
2. Rosenbloom RG, Tinanoff N (1991) Salivary Streptococcus mutans levels in patients before, during, and after orthodontic treatment. Am J Orthod Dentofacial Orthop 100, 35-37.
3. Forsberg CM, Brattström V, Malmberg E, Nord CE (1991) Ligature wires and elastomeric rings: two methods of ligation, and their association with microbial colonization of Streptococcus mutans and lactobacilli. Eur J Orthod 13, 416-420.
4. Papaioannou W, Gizani S, Nassika M, Kontou E, Nakou M (2007) Adhesion of Streptococcus mutans to different types of brackets. Angle Orthod 77, 1090-1095.
5. Ho CS, Ming Y, Foong KW, Rosa V, Thuyen T, Seneviratne CJ (2017) Streptococcus mutans forms xylitol-resistant biofilm on excess adhesive flash in novel ex-vivo orthodontic bracket model. Am J Orthod Dentofacial Orthop 151, 669-677.
6. Marsh PD, Bradshaw DJ (1995) Dental plaque as a biofilm. J Ind Microbiol 15, 169-175.
7. Marsh PD (1992) Microbiological aspects of the chemical control of plaque and gingivitis. J Dent Res 71, 1431-1438.
8. Baehni PC, Takeuchi Y (2003) Anti-plaque agents in the prevention of biofilm-associated oral diseases. Oral Dis 9, 23-29.
9. Renton-Harper P, Addy M, Moran J, Doherty FM, Newcombe RG (1996) A comparison of chlorhexidine, cetylpyridinium chloride, triclosan, and C31G mouthrinse products for plaque inhibition. J Periodontol 67, 486-489.
10. Barnett ML (2003) The role of therapeutic antimicrobial mouthrinses in clinical practice: control of supragingival plaque and gingivitis. J Am Dent Assoc 134, 699-704.
11. Kim SS, Kim S, Kim E, Hyun B, Kim KK, Lee BJ (2003) Synergistic inhibitory effect of cationic peptides and antimicrobial agents on the growth of oral streptococci. Caries Res 37, 425-430.
12. Lee SH, Choi BK (2006) Antibacterial effect of electrolyzed water on oral bacteria. J Microbiol 44, 417-422.
13. Okajima M, Kanzaki M, Ishibashi Y, Wada Y, Hino F, Kitahara Y et al. (2011) In vitro bactericidal activity against periodontopathic bacteria by electrolyzed ion-reduced water. Drug Discov Ther 5, 306-310.
14. Sun JL, Zhang, SK, Chen JY, Han BZ (2012) Efficacy of acidic and basic electrolyzed water in eradicating Staphylococcus aureus biofilm. Can J Microbiol 58, 448-454.
15. Arya R, Bryant M, Degala HL. Mahapatra AK, Kannan G (2018) Effectiveness of a low-cost household electrolyzed water generator in reducing the populations of Escherichia coli K12 on inoculated beef, chevon, and pork surfaces. J Food Process Preserv 42, e13636.
16. Nelson Filho P, Macari S, Faria G, Assed S, Ito IY (2000) Microbial contamination of toothbrushes and their decontamination. Pediatr Dent 22, 381-384.
17. Yan P, Daliri EBM, Oh DH (2021) New clinical applications of electrolyzed water: a review. Microorganisms 9, 136.
18. Sueishi N, Ohshima T, Oikawa T, Takemura H, Kasai M, Kitano K et al. (2021) Plaque-removal effect of ultrafine bubble water: oral application in patients undergoing orthodontic treatment. Dent Mater J 40, 272-278.
19. Akama Y, Nagamatsu Y, Ikeda H, Naoko-Kuroishi K, Kometani-Gunjigake K, Kawamoto T et al. (2022) Applicability of neutral electrolyzed water for cleaning contaminated fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 161, e507-e523.
20. Okajima M, Shimokawa KI, Ishii F (2010) The healing effect of electrolytic-reduction ion water on burn wounds. Biosci Trends 4, 31-36.
21. Shu T, Okajima M, Shimokawa KI, Ishii F (2010) The treatment effect of the burn wound healing by electrolytic-reduction ion water lotion. Biosci Trends 4, 1-3.
22. Shu T, Okajima M, Wada Y, Shimokawa KI, Ishii F (2010) The treatment effect of the atopic dermatitis by electrolyticreduction ion water lotion. Drug Discov Ther 4, 499-503.
23. Leelanarathiwat K, Katsuta Y, Katsuragi H, Watanabe F (2020) Antibacterial activity of blue high-power light-emitting diode-activated flavin mononucleotide against Staphylococcus aureus biofilm on a sandblasted and etched surface. Photodiagnosis Photodyn Ther 31, 101855.
24. Fournier A, Payant L, Bouclin R (1998) Adherence of Streptococcus mutans to orthodontic brackets. Am J Orthod Dentofacial Orthop 114, 414-417.
25. Eliades T, Eliades G, Brantley WA (1995) Microbial attachment on orthodontic appliances: I. Wettability and early pellicle formation on bracket materials. Am J Orthod Dentofacial Orthop 108, 351-360.
26. Ahn HB, Ahn SJ, Lee SJ, Kim TW, Nahm DS (2009) Analysis of surface roughness and surface free energy characteristics of various orthodontic materials. Am J Orthod Dentofacial Orthop 136, 668-674.
27. Azuma Y, Kato H, Usami R, Fukushima T (2007) Bacterial sterilization using cavitating jet. J Fluid Sci Technol 2, 270-281.
28. Rabin N, Zheng Y, Opoku-Temeng C, Du Y, Bonsu E, Sintim HO (2015) Biofilm formation mechanisms and targets for developing antibiofilm agents. Future Med Chem 7, 493-512.
29. Merne ME, Syrjänen KJ, Syrjänen SM (2001) Systemic and local effects of long-term exposure to alkaline drinking water in rats. Int J Exp Pathol 82, 213-219.
30. Malhotra SL (1993) The Thesis of the alkaline milieu in oncology: a review. Med Hypotheses 40, 139-141.
31. Jin YT, Tsai ST, Wong TY, Chen FF, Chen RM (1996) Studies on promoting activity of Taiwan betel quid ingredients in hamster buccal pouch carcinogenesis. Eur J Cancer 32B, 343-346.