

Original Article

Ions release evaluation and corrosion of titanium mini-implant surface in response to orthokin, oral B and chlorhexidine mouthwashes

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ABSTRACT

Background: The present study was performed to evaluate the effect of three types of mouthwash (orthokin, oral B and chlorhexidine [CHX]) on releasing of aluminum (Al), Titanium (Ti) and Vanadium (V) ions from titanium mini-implants (TMIs).

Materials and Methods: In this *in vitro*, experimental study, a total of 40 TMIs were divided equally into four groups (10 TMI in each group) and then were immersed into Orthokin, Oral B, CHX, and artificial saliva, as a control. The experiments were performed for 21 days as following groups 1–7 days, 8–14 days, and 15–21 days. The inductively coupled plasma-optical emission spectrometry method was used to assess releasing metal ions after immersion in the storage media. In addition, before and after each experiment, the corrosion of TMIs was assessed using a scanning electron microscope (SEM). All results were analyzed using Kruskal–Wallis, followed by Bonferroni-adjusted Mann–Whitney *U*-test at 0.05 level of significance.

Results: Our data showed that the maximum concentration of released Al was in the 1st week of exposure to Orthokin and Oral B (202.3 ± 68.5 and 72.3 ± 15.2 $\mu\text{g/L}$, respectively). Oral B exposure of TMI also caused to releasing of Ti to 128.1 ± 42.5 , 54 ± 19.4 and 22 ± 6 $\mu\text{g/L}$ for 1–7 days and 8–14 days and 15–21 days, respectively. Orthokin and CHX also induced the release of Ti more than artificial saliva ($P < 0.05$). In addition, there was no significant statistical difference between any types of mouthwashes and artificial saliva in releasing V. The results of SEM images also confirmed the corrosion effects of mouthwashes.

Conclusion: The factors of exposure time and mouthwash type influenced the pattern of releasing Al and Ti as well as corrosion level.

Key Words: Corrosion, mouthwashes, scanning electron microscopy, titanium alloy tial6 v4

Received: 03-Jun-2020
Revised: 13-Jul-2020
Accepted: 20-Sep-2020
Published: 24-May-2021

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INTRODUCTION

Mini-implants (MIs) as the most commonly used temporary anchorage devices have been paid lots of attention. This type of treatment has many advantages, including inexpensiveness as well as simplicity in both placement and removal. MIs also

have reduced the need for compliance of the patients without surgery. In addition, MIs have the ability to control appropriate anchorage in three spatial plans providing clinicians to improve orthodontic treatment. However, compared to endosseous implants, MIs have a reduced success rate, dependent

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How to cite this article: Alavi S, Ahmadvand A. Ions release evaluation and corrosion of titanium mini-implant surface in response to orthokin, oral B, and chlorhexidine mouthwashes. Dent Res J 2021;18:32.

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on some conditions ranging from 70% to >95%. MIs failure has been associated with some factors such as age, maintenance, bone density, root proximity, the screw design, the placement method, and chemical composition.^[1] Furthermore, peri-implant tissue inflammation has been associated with loosening of the miniscrews in clinical studies.^[2,3] The causes of the inflammation are multifactorial and include the level of patient health, surrounding tissue type, and design of the mini-screw head. Recently, corrosion has been considered as one of the factors related to peri-implantitis of TMIs with the release of titanium (Ti) ions. It is noted that macrophages surrounding failed dental implants have been found to be loaded with Ti from corrosion.^[4]

The chemical composition of TMIs also is of interest because it has been shown that corrosion of bracket, archwire, and other metallic fixed orthodontic appliances can occur in the oral environment.^[5-7] The typical composition of orthodontic MIs is Ti alloy, but certain amounts of aluminum (Al) and Vanadium (V) to provide stability, low toxicity, and resistance are added. However, these added ions make the alloy more susceptible to corrosion.^[8]

Practitioners recommend the daily use of fluoride mouthwashes to reduce the risk of development of white spots around orthodontic brackets during orthodontic treatment. However, some contents of mouthwashes such as fluoride ions, as well as the lower mouthwash pH, have been reported to enhance release TMIs ions causing corrosion and discoloration.^[9,10] Chlorhexidine (CHX) mouthwash is recommended also to prevent inflammation around the MI.^[11,12] These agents could lead to the corrosion of the implant head, which is exposed to the oral cavity.

Corrosion and releasing of metal ions from TMIs into biologic fluids appear to have a wide variety of harmful effects. Metal released from dental alloys has been reported to cause severe biological alterations. For instance, Natarajan *et al.* showed that the oral mucosal cells of healthy patients undergoing orthodontic treatment were damaged.^[13] The released ions can cause oxidative stress damaging to human cells. Oxidative stress seems to affect proteins, lipid, and nucleic acid, which consequently results in tissue damages and cancer progression.^[14,15] For instance, The released Nickel is appeared to has high uptake, transport, distribution, and retention at a cellular level.^[16,17] Cell culture studies have shown that Ti ions alter the phenotype and function of

T-lymphocytes, induce the differentiation of monocytes into active osteoclasts and increase the expression of cytokines in osteoclasts.^[18]

The current study is aimed to assess the levels of metal ions released from orthodontic TMIs in the presence of three types of OralB, Orthokin, and CHX mouthwashes. Indeed, these findings should guide practitioners to decide which mouthwash to prescribe for each patient.

MATERIALS AND METHODS

By the ethics and research committee of, Isfahan University of Medical Sciences (No.398893). Totally, 40 TMI (Jeil Medical, Seoul, Korea) were used for this *in vitro*, experimental study. The TMIs were divided randomly into four equal groups and immersed in artificial saliva (Kin Hidrat spray; Spain) and Oral B (Procter & Gamble, Weybridge, United Kingdom), Orthokin (Ortho-kin, Kin, Spain) and CHX (Iran Najo, Tehran, Iran) mouthwashes. These mouthwashes were chosen due to their commercial availability. MIs in each group were stored individually in a tube and incubated at 37°C for 21 days, which were divided into three separated immersion intervals as follows: 1–7 days, 8–14 days, and 14–21 days.

Inductively coupled plasma-optical emission spectrometry

Inductively coupled plasma-optical emission spectrometry (ICP OES) was generally applied for the determination of metals in body fluids and other liquids. For this purpose, multi-elemental calibrants were prepared from different standard solutions of the individual elements. Calibration curves were also prepared with matrix-matched calibration standards. For this experiment, the samples were prepared as described previously.^[19] Emission intensities of samples were measured at 308.215 nm, 334.936 nm, and 292.399 nm for Al, Ti and V, respectively. The samples were analyzed using ICP Perkin-Elmer 7300DV (USA) in Axial mode.

Scanning electron microscope observations

Qualitative analysis of the topography of the head of MIs was performed before and after their exposure to experimental media using a scanning electron microscope [SEM] (INCAx-sight, England) at ×5000 magnification. One representative sample of each of the four groups randomly were analyzed.

Statistical analysis

Statistical analysis of the results was performed using

SPSS 22 (SPSS Inc., Chicago, Illinois, USA). Normal distribution of data was assessed by the nonparametric Kolmogorov–Smirnov test. Since some variables presented abnormal distribution, nonparametric tests were employed (Kruskal–Wallis, followed by Bonferroni-adjusted Mann–Whitney *U*-test). All results were analyzed at a significance level of 0.05.

RESULTS

The concentration of the ions released in three experiment groups and control in a different time is shown in Tables 1-3. As analyzed by the Kolmogorov–Smirnov test, all ions had nonnormal distribution; therefore, the nonparametric test was used. As reported in Figure 1, the maximum concentration of Al was released in 1–7 days in the presence of Orthokin ($202.3 \pm 68.5 \mu\text{g/L}$, $P < 0.001$). The second higher concentration of Al was indicated in the OralB group in a time of 1–7 days ($72.3 \pm 15.2 \mu\text{g/L}$, $P < 0.001$). As indicated in Figure 1, there was no relationship between time of exposure to mouthwashes and released ion concentration. In addition, Oral B exposure of TMI caused to release of Ti to $128.1 \pm 42.5 \mu\text{g/L}$, $P < 0.001$ and $54 \pm 19.4 \mu\text{g/L}$, $P < 0.001$ and $22 \pm 6 \mu\text{g/L}$, $P < 0.05$ for 1–7 days and 8–14 days and 15–21 days, respectively. Orthokin and CHX also caused to release of Ti more than artificial saliva at various times ($P < 0.05$). Similar to Al, there was no relationship between time of exposure to mouthwashes and released Ti concentration [Figure 2]. Furthermore, V determination of samples showed that there was no significant statistical difference between any types of mouthwashes and artificial saliva at various times [Figure 3].

Before the immersion of TMI in mouthwash and artificial saliva, the SEM analysis was performed to detect probably manufacture defects Figure 4. After the indicated time of immersion period (1–7, 8–14, and 15–21 days) in the four testing solutions of Oral B, Orthokin, CHX, and artificial saliva SEM analysis were again performed. Representative SEM image of TMIs at various times is shown in Figure 5.

Dark spots, pits, and cracks and some corrosion products were observed on the surfaces of TMIs after immersion in mouthwashes. The results of SEM images showed a similar pattern of releasing ions. Orthokin caused corrosion more than Oral B, while CHX treatment resulted in minimal corrosion. Loss of gloss and increased corrosion in the surface of TMI

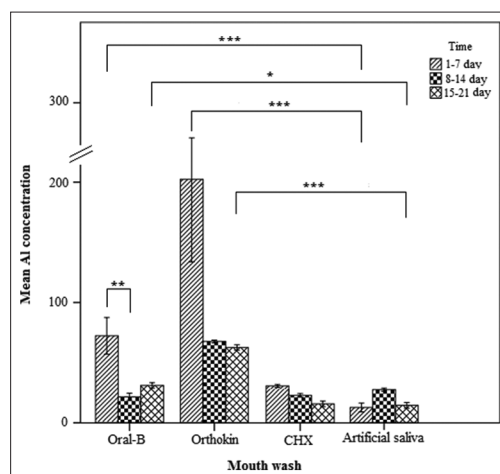


Figure 1: Released concentration of Al in response to three types of mouthwashes (orthokin, oral B and chlorhexidine) in different immersion times. *, ** and *** were stands for statistical significances of $P < 0.05$, $P < 0.01$ and $P < 0.001$.

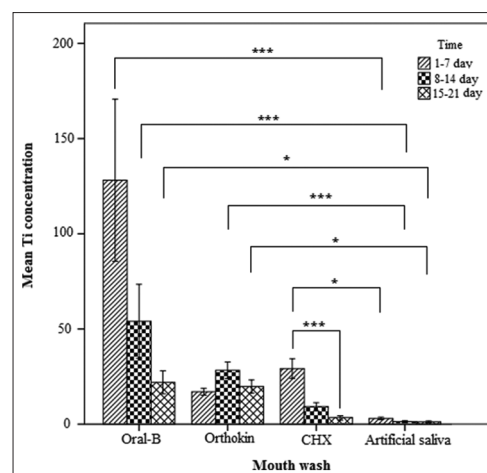


Figure 2 : Released concentration of Ti in response to three types of mouthwashes (orthokin, oral B and chlorhexidine) in different immersion times. *, ** and *** were stands for statistical significances of $P < 0.05$, $P < 0.01$ and $P < 0.001$.

Table 1: Al concentrations (µg/L) in the mouthwash solutions at 37°C from titanium mini-implants

Time (days)	Solution, mean±SD			
	Oral B (n=10)	Orthokin (n=10)	Chlorehexidin (n=10)	Artificial saliva (n=10)
1–7	72.300±15.275	202.300±68.559	29.500±1.459	12.700±3.683
8–14	21.600±2.951	67.600±1.074	22.800±1.475	27.500±1.269
15–21	31.100±2.330	62.600±2.270	15.700±2.287	14.400±2.412

SD: Standard deviation

by increasing the time of exposure were a significant result.

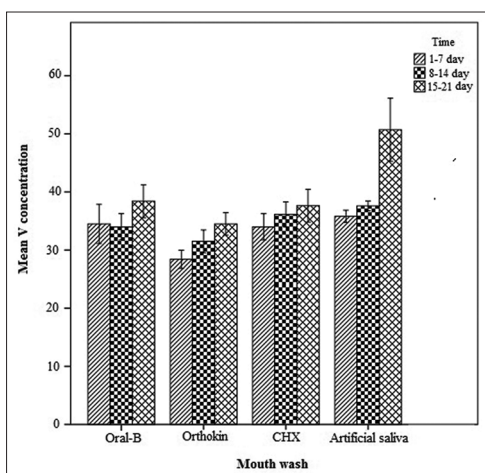


Figure 3: Released concentration of V in response to three types of mouthwashes (orthokin, oral B and chlorhexidine) in different immersion times. There is no statistically significant difference between each groups.

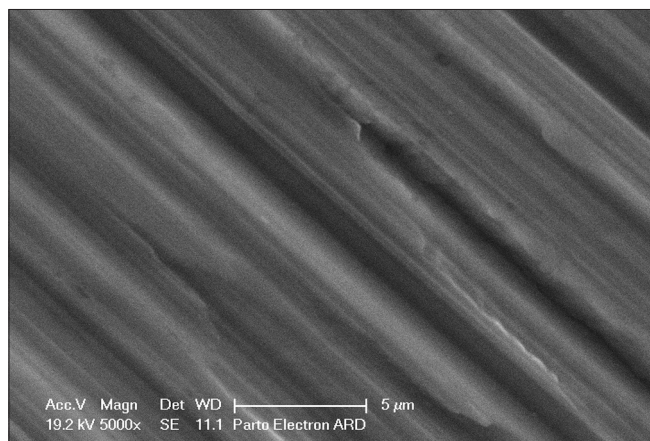


Figure 4: High-magnification SEM photomicrographs of titanium mini-implants without immersion ($\times 5000$).

DISCUSSION

Today during orthodontic treatment, practitioners recommend that their patients use mouthwashes daily to reduce the risk of dental caries. They also suggest that after using mouthwash, the patient should not eat, drink, and rinse; therefore, the chemical agents of mouthwash are exposed to TMIs for a long period that may lead to TMI corrosion.^[20] It is difficult to determine the exact contact duration between TMIs and mouthwash, but there is an assumption that in each 30–60 s, the mouthwash is present for 2–4 h. Orthodontic treatment is usually done in a long period, for about 1–2 years; therefore, 21 days is the arbitrary total duration assumption to immerse the TMIs into the mouthwashes.

Although, this is generally accepted that due to the stability of the metal oxide layer, Al, Ti, and V alloys are extremely resistant against corrosion. However, due to the chemical and mechanical properties of the oral cavity as well as microbial flora, enzymatic reaction, and thermal changes, some metal ions can be released from TMIs.

To date, the release of elements from implants has been indicated in different ways such as dental Atomic Absorption Spectroscopy,^[21,22] ICP-OES^[23] and ICP-Mass Spectrometry (MS).^[24] Among such methods, the best limits of detection, as well as higher sensitivity and specificity, are provided by ICP-MS.^[25] Due to the lower power of detection, ICP-OES is unable to determine the elemental background. However, this simple method is satisfying in the determination of Al, Ti, and V.^[26] In addition, corrosion assessment of TMI is commonly evaluated

Table 2: Ti concentrations ($\mu\text{g/L}$) in the mouthwash solutions at 37°C from titanium mini-implants

Time (days)	Solution, mean \pm SD			
	Oral B (n=10)	Orthokin (n=10)	Chlorehexidin (n=10)	Artificial saliva (n=10)
1–7	128.100 \pm 42.566	17.000 \pm 1.825	29.200 \pm 5.159	3.000 \pm 0.667
8–14	54.000 \pm 19.459	28.300 \pm 4.347	9.200 \pm 2.097	1.300 \pm 0.483
15–21	22.000 \pm 6.018	19.800 \pm 3.425	3.200 \pm 0.241	1.200 \pm 0.421

SD: Standard deviation

Table 3: V concentrations ($\mu\text{g/L}$) in the mouthwash solutions at 37°C from titanium mini-implants

Time (days)	Solution, mean \pm SD			
	Oral B (n=10)	Orthokin (n=10)	Chlorehexidin (n=10)	Artificial saliva (n=10)
1–7	34.500 \pm 3.374	28.400 \pm 1.577	34.00 \pm 2.260	35.800 \pm 1.032
8–14	34.000 \pm 2.260	31.500 \pm 1.957	36.300 \pm 2.162	37.600 \pm 0.843
15–21	38.400 \pm 2.836	34.500 \pm 1.957	37.400 \pm 3.241	50.700 \pm 5.417

SD: Standard deviation

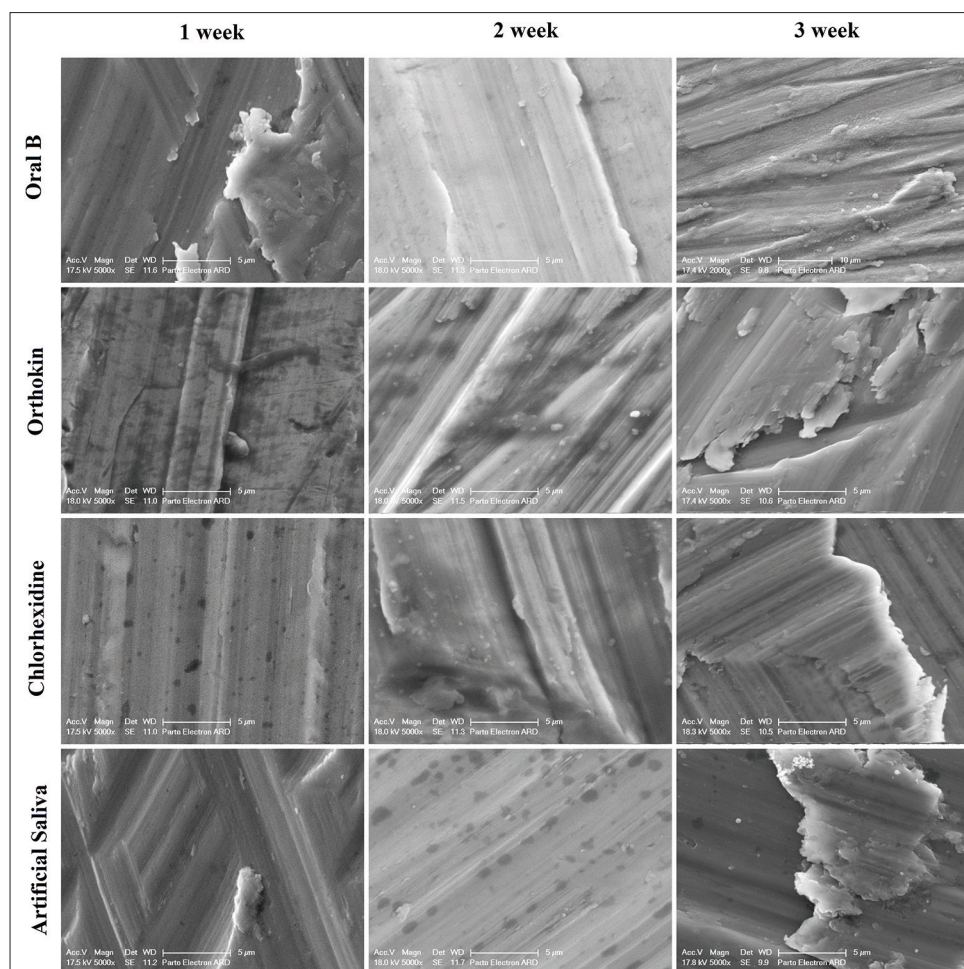


Figure 5: High-magnification scanning electron microscope photomicrographs of titanium mini-implants after immersion in different solutions ($\times 5000$).

by SEM This experiment was also validated by many studies.^[27-29]

In the present study, three types of mouthwash that commonly are recommended for patients were evaluated. Our findings showed that Al has a releasing peak in a time of 1–7 days in the presence of Orthokin and OralB. The World Health Organization (WHO) recommended that the aluminum tolerable daily intake is 1 mg/kg body weight/day.^[30] Due to the low concentration of Al (202.3 ± 68.5 and 72.3 ± 15.2 $\mu\text{g/L}$, for Orthokin and OralB, respectively) in comparison to approved daily intake, there was no matter for using Orthokin and OralB. Similarly, our data also indicated that Oral B exposure of TMI caused to release of Ti to 128.1 ± 42.5 , 54 ± 19.4 and 22 ± 6 $\mu\text{g/L}$ for 1–7 days, 8–14 days, and 15–21 days, respectively. Because of low Ti toxicity, it has no toxicological reference values. On the other hand, V determination of samples exhibited that there was no statistically significant difference between any

mouthwashes and artificial saliva. Among released ions, the lowest amount was for V. similarly Abboodi and Al-Dabagh showed that the least amount of release was V.^[27] This phenomenon may be associated with the composition and behavior of the passive oxide surface layer, which is composed mainly of TiO_2 , with small amounts of Al_2O_3 , hydroxylic groups, and water. As V is not present in the superficial oxide layer of Ti-6Al-4V, Ti and Al are the metal ions most likely to be released from the Ti-6Al-4V surface.^[31]

The released pattern of ions is more likely to be associated with fluoride concentration of mouthwashes. The concentration of fluoride was 500 ppm, 250 ppm, and 0 ppm for Orthokin, OralB and CHX, respectively. Because CHX that has no fluoride was lower in releasing ions. While the most ions were released by Orthokin that has a higher concentration of fluoride. This is consistent with previous studies that show in acidic fluoridated solutions, the F⁻ combines with H⁺ to form hydrofluoric acid (HF), which is

capable of destroying the oxide layer on Ti and its alloys. Soluble Ti-fluoride compounds form, leading to the dissolution of the metal.^[32] Kang *et al.* also evaluated the effects of acetic NaF solutions on Ti and Ti alloy brackets and noticed that the HF formed in acetic NaF solutions induced corrosion on the surface of Ti-based orthodontic brackets and with the increasing concentration of NaF the level of corrosion increased.^[33]

The concentration of all detected ions in response to all mouthwashes peak at day 7 and decrease in the next weeks of study. Such a reduction in the rate of ions release could be explained by the normal capacity of the passivation layer of the alloy to form a protective stable oxide film that inhibits the corrosion process in various environments; thus, the rate of corrosion will be decreased.^[34] Similarly, some of the previous studies have revealed that metal ions released from fixed orthodontic appliances have a peak of releasing on day 7.^[25,35,36]

On the other hand, the obtained results of released ions were also correlated with corrosion study, as indicated by SEM analysis.

Taken to gather our finding confirmed that although mouthwashes treatment of TMI resulted in releasing of Al and Ti in some period, according to the WHO standards, all types of mouthwashes are safe to be used. These data highlight that this type of MIs and CHX mouthwash is suitable for orthodontic purposes and meet the standard norms regarding the ingredient of CHX, and resistance of TMI against corrosion. However, other mouthwashes due to releasing the peak of Al and Ti at the time of 1–7 days must be paid attention.

Similarly, Pavlic *et al.* assessed corrosion of MIs in response to chlorhexidine and probiotic agents. They reported that for patients undergoing orthodontic TMIs, CHX could be recommended for oral-hygiene maintenance.^[37] Furthermore, Mandasaurwala, Mohammed *et al.* indicated that CHX-containing (HEXIDINE) mouthwash could be offered for patients who have orthodontic appliances rather than Na + alcohol-containing mouthwash (LISTERINE). They explained that greenish discoloration on the CHX sample occurs after 24 h, which constituted a type of passive surface.^[38] Although Danaei *et al.* reported that ion releasing from stainless steel brackets in response to deionized water was significantly higher than that

for OralB, CHX, and Persica mouthwashes. They indicated that Oral B and Persica mouthwashes might be better options than CHX for orthodontic patients.^[5] The difference between these results and ours could be due to the material of brackets that they used. Stainless steel is vulnerable against CHX, as Pavlic *et al.* also confirmed.^[37]

Whether the ion released from TMIs (despite the safety) will cause inflammation around TMI requires a separate study.

Although *in vitro* studies are valuable, the results should be evaluated cautiously. One of the limitations in analyzing the results of this *in vitro* study was using mouthwashes in a static condition while in a realistic situation; TMI is in contact with mouthwash several times per day. Furthermore, more metal could release in real life because of the fluidity of saliva in the mouth and because oxide layers are removed by tooth brushing.^[39]

CONCLUSION

The findings of our study showed that exposure time and mouthwash type influenced the pattern of releasing Al and Ti. However, released ions neither exceed the toxic levels nor the daily dietary intake of metal ions suggesting that OralB, Orthokin, and CHX are safe. However, these types of mouthwash were partially enhancing corrosion.

Financial support and sponsorship
Nil.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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