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ORIGINAL ARTICLE

# Effect of mouthwashes on the discoloration of bracket-bonded tooth surfaces: an in vitro study

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## Abstract

**Objectives** This in vitro study aimed to investigate the color changes of the bracket-bonded tooth surfaces after the use of 4 different mouthwashes.

**Materials and methods** A total of 100 human premolar teeth were randomly divided into 10 equal groups. Color values ($L^*a^*b^*$) of the buccal surfaces of each tooth were assessed using a digital spectrophotometer. Then the brackets were bonded. The groups were put either in sterile saline (4 test+1 control) or artificial saliva (4 test+1 control) solutions, and test groups were immersed in their mouthwashes (Colgate Plax, Listerine Cool Mint, Klorhex, and Tantum Verde) for 1 min each in the morning/evening to simulate the mouth washing for 21 days after the bonding. After the debonding and finishing procedures, final color measurements were performed. Color changes (ΔE) were calculated.

**Results** All of the parameters showed statistically significant differences among the groups. The least noticeable color changes were detected in the control groups. The most noticeable color change (ΔE) was observed in the Tantum Verde + artificial saliva group, followed by Tantum Verde + sterile saline and Klorhex + artificial saliva groups, all of which were significantly higher than the control groups.

**Conclusion** The use of mouthwashes during orthodontic treatment may cause noticeable changes in tooth color. Listerine Cool Mint may be the choice of mouthwash with its less discoloring effects. Artificial saliva should be preferred in similar in vitro studies to reflect the oral environment better.

**Clinical relevance** Mouthwash use during orthodontic treatment may result in different levels of enamel discoloration.

**Keywords** Discoloration · Mouthwashes · Orthodontic bonding · Spectrophotometer

## Introduction

With the orthodontic treatment, patients expect to have a beautiful smile with well-aligned teeth. However, the brackets which are bonded on the teeth during orthodontic treatment change the plaque accumulation levels and increase dental plaque on the tooth surfaces. These may cause unaesthetic results such as demineralization, white spot lesions and even dental caries around the brackets. Gingival inflammation may also be observed as a result of increased plaque accumulation due to the difficulty in maintaining oral hygiene during fixed orthodontic treatment. Therefore, maintaining good oral hygiene is an important part of achieving good esthetic results [1].

Mechanical methods such as brushing and flossing are referred to as the gold standard in maintaining good oral hygiene [2]. But, in patients receiving orthodontic treatment, these procedures may not be sufficient. For many years, mouthwashes have been in use as antiseptics and breath fresheners [3]. Due to their anti-plaque and anti-inflammatory effects, mouthwashes are often recommended in addition to the mechanical methods during orthodontic treatment. However, knowledge about the effects of mouthwashes on tooth color during orthodontic treatment is unclear.

Tooth color is affected by intrinsic and extrinsic factors. Light scattering, absorption, and reflection of enamel and dentine are the intrinsic factors. The absorption of substances such as iron salts, tea, and chlorhexidine onto the enamel structure induces the

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extrinsic staining [4]. Some studies reported that bonding and debonding procedures may cause enamel loss and make the underlying dentine more visible. Also, cleaning of the adhesives changes the enamel surface property which alters the reflection of light and affects the tooth color [5–7]. When all these factors are combined with the staining effects of the mouthwashes, patients may experience unpleasant esthetic results.

Different digital devices have been developed for the quantitative identification of tooth shade. Digital spectrophotometers are devices of objective tooth shade measurement, which measures the amount and spectral composition of reflected or transmitted light [8]. These devices generally demonstrate the color measurements in Commission Internationale de I'Eclairage (CIE) $L^*a^*b^*$ color system, which is the most commonly used color scale to describe the color difference numerically. Axis $L^*$ (luminosity) ranges from black (0) to white (100); axis $a^*$ indicates greenness (−a) and redness (+a); and axis $b^*$ displays blueness (−b) and yellowness (+b).This system locates an object in a 3D color space by its numerical values in 3 coordinates. The accuracy of the spectrophotometric methods in tooth shade measurement was demonstrated in different studies [9, 10].

The hypothesis tested in this study was that the daily use of the different mouthwashes after bracket bonding may cause tooth color changes. Therefore, this study aimed to evaluate the color changes of the bracket-bonded tooth surfaces after the use of common mouthwashes that have different compositions (Colgate Plax, Listerine Cool Mint, Klorhex, and Tantum Verde) by an in vitro procedure and to determine whether the use of sterile saline and artificial saliva solutions during this in vitro procedure cause any changes in these tooth color measurements.

## Material and methods

This present study was approved by the ethical committee of clinical research of the Bolu Abant Izzet Baysal University (No: 2018/300). A total of 100 human premolar teeth extracted for orthodontic purposes from patients between 12 and 18 years were collected. Each tooth was examined under light to check that there were no visible cracks, caries, demineralization, and discoloration on their buccal surface. Ten equal groups (8 test and 2 control) were created. The groups were Control + sterile saline, Colgate Plax + sterile saline, Listerine Cool Mint + sterile saline, Klorhex + sterile saline, Tantum Verde + sterile saline, Control + artificial saliva, Colgate Plax + artificial saliva, Listerine Cool Mint + artificial saliva, Klorhex + artificial saliva, and Tantum Verde + artificial saliva. The composition and manufacturer of each mouthwash were given in Table 1. For each group, 10 teeth were randomly selected. The sample size was determined from similar studies [2, 5, 11].

Before the initial color measurements (T0), all teeth were cleaned with oil-free pumice, then rinsed with running water and air-dried gently. Color values ($L^*$ $a^*$ $b^*$) of the buccal surfaces of each tooth were assessed using a hand-held digital spectrophotometer (Vita Easyshade Advanced, Vita Zahnfabrik, Germany) on a white background and were recorded according to the CIE L*a*b* system (Commission Internationale de l'Eclairage, $L^*$, $a^*$, $b^*$) [12]. As the middle third is where the bracket will be bonded and was previously identified as the best site to demonstrate the color, the middle third of the tooth was measured [13]. According to the manufacturer's instructions, the tip of the probe was placed perpendicular to the tooth surface. After the measurements are completed; the teeth in each group were etched with 37% orthophosphoric acid (Liquid etchant, Reliance, Itasca, IL, USA) for 30 s, rinsed thoroughly with water and air-dried. A thin layer of sealant (Light Bond™ light cure sealant resin, Reliance, Itasca, IL, USA) was applied to the enamel surface and light-cured. Then, adhesive material (Light Bond™ Light cure adhesive resin, Reliance, Itasca, IL, USA) was placed on a stainless-steel premolar bracket base (Mini Master®, American Orthodontics, Sheboygan, WI, USA). The brackets were bonded on the middle of the buccal tooth surfaces and

**Table 1** Information of the mouthwashes used in this study

| Mouthwash | Composition | Manufacturer |
|---|---|---|
| Colgate Plax | Sodium fluoride, cetylpyridinium chloride, water, glycerin, propylene glycol, 21.6% alcohol, sorbitol, poloxamer 338, poloxamer 407, potassium sorbate, sodium saccharin, citric acid, sucralose, blue dye | Colgate Palmolive, Istanbul, Turkey |
| Listerine Cool Mint | Thymol, eucalyptol, methyl salicylate, menthol, water, sorbitol solution, 30% alcohol, poloxamer 407, benzoic acid, sodium saccharin, sodium benzoate, green dye, mint essence | Johnson & Johnson, Istanbul, Turkey |
| Klorhex | 0.2% chlorhexidine gluconate, 20% glycerin, 0.2% lemon essence, 0.02% mint essence, distilled water | Drogsan, Ankara, Turkey |
| Tantum Verde | 0.15% Benzydamine hydrochloride, glycerin, saccharin, methyl paraben, quinoline yellow, patent blue, mint essence, polysorbate 20, 96% ethanol, distilled water | Angelini, Istanbul, Turkey |

pressed gently in place. After cleaning the excessive adhesive, samples were light-cured for 3 s using Valo Ortho LED curing light (Ultradent, South Jordan, UT, USA).

When the bonding procedures were completed, the groups were put in either sterile saline or artificial saliva solutions. Artificial saliva was prepared as described by Almqvist and Lagerlof [14]. The solution was at pH 7.0 and consisted of 1 mmol/L calcium in form of calcium chloride, 2mmol/L phosphate in form of monosodium phosphate, 0.01% sodium azide and 50 mmol/L potassium chloride [14]. Test groups were immersed in their mouthwash solutions for 1 min in the morning and 1 min in the evening to simulate the mouth washing for 21 days. Once the experiment is over, the brackets were debonded. Then the adhesive residues were removed with tungsten-carbide burs, and the tooth surfaces were polished by the slow-speed handpiece.

Since the brackets were bonded during the experiment, final color measurements (T1) were performed after the debonding and finishing procedures at the end of 21 days. All measurements were conducted by the same operator in the same environment. Color changes of each tooth (ΔE) were calculated using the following formula [12]:

$$\Delta E : \left[\left(L_1^*-L_2^*\right)^2 + \left(a_1^*-a_2^*\right)^2 + \left(b_1^*-b_2^*\right)^2\right]^{1/2}$$

## Statistical analysis

Statistical analyses were performed using SPSS (SPSS for Windows version 22.0; SPSS Inc., Chicago, IL, USA). Paired *t* test was used for the intragroup comparisons of *L*, *a*, and *b* parameters. Intergroup comparisons of Δ*L*, Δ*a*, Δ*b*, and Δ*E* parameters were established by using one-way ANOVA and Tukey's post-hoc tests. The level of statistical significance was set at $p < 0.05$.

## Results

Table 2 shows the intragroup comparisons of *L*, *a*, *b* parameters at T0 and T1 in 5 groups stored in sterile saline solution. While the control group showed no significant difference in any of the parameters, all mouthwashes had significant differences in different parameters. Listerine and Klorhex significantly increased the luminosity of the teeth, while Colgate Plax and Tantum Verde significantly stained the bracket bonded teeth yellow and green-blue, respectively.

Table 3 displays the intragroup comparisons of *L*, *a*, *b* parameters at T0 and T1 in 5 groups which were put in artificial saliva. There were no significant differences in any of the parameters in the control group. The luminosity of the bracket bonded teeth was significantly increased after the use of all mouthwashes. The teeth were significantly stained yellow and green-blue by Colgate Plax and Tantum Verde, respectively.

Intergroup comparisons of Δ*L*, Δ*a*, Δ*b*, and Δ*E* parameters were shown in Table 4. All parameters showed statistically significant differences among the groups. The most marked change in luminosity was detected in Klorhex + artificial saliva group, which was significantly higher than both of the control groups. The most noticeable changes in Δ*a* and Δ*b* parameters were detected in both Tantum Verde groups, which stained the teeth green-blue. The least noticeable color changes were detected in the control groups, showing that all of the mouthwashes used in this study resulted in color changes to some extent in bracket-bonded teeth, whether the teeth were put in sterile saline or artificial saliva. The most noticeable color change (Δ*E*) was observed in the Tantum Verde + artificial saliva group, followed by Tantum Verde + sterile saline and Klorhex + artificial saliva groups, all of which were significantly higher than the control groups.

## Discussion

Patients undergoing orthodontic treatment widely use mouthwashes for various reasons such as antimicrobial [15] and

**Table 2** Intragroup comparisons of *L*, *a*, *b* parameters at T0 and T1 in groups stored in sterile saline solution using paired *t* test

| Group | Parameter | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *L* | | | *a* | | | *b* | | |
| | T0 | T1 | *p* | T0 | T1 | *p* | T0 | T1 | *p* |
| Control | 88.83 (2.28) | 89.41 (2.77) | 0.270 | − 0.8 (1.14) | − 0.53 (1.08) | 0.265 | 30.73 (4.57) | 31.66 (3.48) | 0.202 |
| Colgate Plax | 82 (2.56) | 84.46 (2.76) | 0.067 | − 1.24 (1.12) | − 1.39 (1.2) | 0.213 | 27.88 (4.77) | 28.63 (4.3) | 0.033 |
| Listerine | 82.8 (4.35) | 84.9 (2.14) | 0.028 | − 0.67 (1.51) | − 1.27 (1.14) | 0.016 | 26.86 (3.03) | 26.24 (2.16) | 0.384 |
| Klorhex | 79.13 (2.25) | 82.37 (2.69) | 0.014 | − 1.41 (0.74) | − 0.97 (0.65) | 0.084 | 25.65 (3.34) | 25.99 (3.42) | 0.769 |
| Tantum Verde | 83.82 (6.75) | 85.73 (2.71) | 0.253 | − 0.01 (2.36) | − 1.68 (1.6) | < 0.001 | 30.65 (5.98) | 27.09 (4.75) | 0.002 |

**Table 3** Intragroup comparisons of *L*, *a*, *b* parameters at T0 and T1 in groups stored in artificial saliva solution using paired *t* test

| Group | Parameter | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *L* | | | *a* | | | *b* | | |
| | T0 | T1 | *p* | T0 | T1 | *p* | T0 | T1 | *p* |
| Control | 83.2 (2.72) | 84.04 (2.88) | 0.068 | − 1.89 (1.1) | − 1.62 (0.6) | 0.625 | 26.4(3.9) | 26.78(3.95) | 0.458 |
| Colgate Plax | 81.7 (3.67) | 84.56 (2.66) | 0.002 | − 1.56 (0.87) | − 1.83 (0.8) | 0.109 | 23.41 (4.57) | 25.11 (4.79) | 0.014 |
| Listerine | 82.31 (2.76) | 83.68 (2.51) | 0.003 | − 1.1 (0.61) | − 1.31 (1.11) | 0.390 | 24.05 (5.89) | 25.74 (7.59) | 0.194 |
| Klorhex | 81.14 (4.11) | 86.54 (2.61) | < 0.001 | − 1.63 (0.98) | − 1.85 (0.7) | 0.345 | 24.78 (3.68) | 23.76 (3.19) | 0.082 |
| Tantum Verde | 83.52 (4.64) | 86.92 (3.79) | 0.002 | − 1.67 (0.94) | − 2.63 (0.75) | 0.004 | 27.44 (2.21) | 24.14 (3.37) | 0.018 |

anti-plaque [16, 17] activity, prophylaxis on gingivitis [16, 17], prevention of halitosis [18], and treatment of traumatic oral ulcerations due to the appliances [19, 20]. Despite their extensive use, mouthwashes have several side effects which include taste loss, dryness of oral cavity, mucosal erosion, and discoloration of enamel [21]. The effect of mouthwash use on the discoloration of the teeth was previously investigated in several studies in the literature [2, 22]. However, no previous study analyzed the effect of mouthwashes on the color of bracket-bonded teeth. To our knowledge, this is the first study to assess the effects of daily mouthwash use on tooth shade during orthodontic treatment with fixed appliances.

Although the visual color perception is a subjective feature due to various factors like the position of the observer and object, the psychological state of the observer, ambient light [4], an objective evaluation can be obtained by using a digital device. Nowadays, there are various digital color-measuring devices for dental application. In the present study, we used the Vita Easyshade Advanced digital spectrophotometer due to its proven reliability in tooth color identification in vitro and in vivo [23]. Additionally, Vita Easyshade Advanced was found to be more accurate than Easyshade Compact in a previous study [8].

The CIE L*a*b* system is accepted as the standard color space, and the formulation of color changes is expressed as $\Delta E$ [24]. Different threshold values for $\Delta E$ were approved in different studies for visual detection of the color change. The $\Delta E$ values below 3.3 [25] and 3.7 [5] were reported to be visually undetectable. 3.7 was accepted as the threshold value for $\Delta E$ in the present study. $\Delta E$ values of all mouthwash groups except Listerine exceeded a $\Delta E$ of 3.7 in our study, showing that the color changes after these mouthwash applications were visually perceptible.

The effect of orthodontic treatment on teeth was previously investigated in various studies, and it was stated that visible tooth color alterations were observed after orthodontic treatment [6, 26]. As all the teeth in our study were bonded with the same adhesives and debonded with the same adhesive removal techniques, the effects of these were eliminated.

It is thought that the discoloration of the teeth in our study may have been caused by irreversible changes in the surface structure of natural teeth due to bonding and debonding, which resulted in alteration in the optical properties of the enamel, as well as pigmentation of the mouthwashes. The light scattering of the tooth surface enhances due to the increased microscopic roughness of the exposed enamel as a result of the dissolution of the apatite crystallites during acid etching [27]. Bracket debonding and adhesive removal techniques cause changes in enamel morphology which cannot be improved by the polishing methods, and these procedures were shown to be more invasive than etching in terms of enamel discoloration [28–30]. The retention and discoloration of resin tags into the enamel surface after cleaning could also result in enamel color changes after debonding [31]. This resin tag impregnation into the enamel is not reversible after debonding [32]. The color instability of resin composites may contribute to the discoloration of the resin-infiltrated enamel [6]. All of these factors may have affected the discoloration of the enamel in our study. Although no significant difference was detected in pairwise comparisons except Tantum Verde groups vs. control groups and Klorhex + artificial saliva group vs. control groups, all mouthwash groups showed higher color changes than the control groups which reflected the staining effects of the mouthwashes.

Previous studies have shown that periodontal diseases are observed after 10–21 days of plaque accumulation [33] and white spot lesions occur rapidly in 4 weeks [34]. However, mouthwash use for more than 5 weeks should be avoided because of their side effects [35]. Therefore, 3 weeks of daily use for twice a day was simulated in our study, as in a previous study [2]. However, the duration of treatment with Tantum Verde mouthwash is between 2 and 7 days, depending on the case, and in radiation-induced mucosal inflammation, the duration of treatment may be up to 15–20 days. The extended 21-day use of Tantum Verde may have caused increased pigmentation of the teeth in this study and resulted in the most prominent discoloration among the groups.

**Table 4** Intergroup comparisons of $\Delta L$, $\Delta a$, $\Delta b$, and $\Delta E$ parameters using one-way ANOVA and Tukey's post-hoc tests

| Parameter | Group | | | | | | | | | | $p$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sterile saline | | | | | Artificial saliva | | | | | |
| | Control | Colgate Plax | Listerine | Klorhex | Tantum Verde | Control | Colgate Plax | Listerine | Klorhex | Tantum Verde | |
| ΔL | 0.58 (1.55)$^{a}$ | 2.46 (3.74)$^{a,b}$ | 2.1 (2.53)$^{a,b}$ | 3.24 (3.36)$^{a,b}$ | 1.91 (4.95)$^{a,b}$ | 0.83 (1.27)$^{a}$ | 2.86 (2.04)$^{a,b}$ | 1.37 (1.06)$^{a,b}$ | 5.39 (2.57)$^{b}$ | 3.39 (1.86)$^{a,b}$ | 0.002 |
| Δa | 0.27 (0.71)$^{a}$ | − 0.15 (0.35)$^{a,b}$ | − 0.6 (0.64)$^{a,b,c}$ | 0.44 (0.58)$^{a}$ | − 1.67 (1.02)$^{c}$ | 0.27 (1.08)$^{a}$ | −0.27 (0.49)$^{a,b}$ | − 0.21 (0.74)$^{a,b}$ | − 0.22 (0.7)$^{a,b}$ | − 0.95 (0.79)$^{b,c}$ | < 0.001 |
| Δb | 0.93 (2.13)$^{a}$ | 0.75 (0.94)$^{a}$ | − 0.62 (2.14)$^{a,b}$ | 0.34 (3.55)$^{a,b}$ | − 3.56 (3.69)$^{b}$ | 0.38 (1.56)$^{a}$ | 1.7 (1.75)$^{a}$ | 1.69 (3.81)$^{a}$ | − 1.02 (1.65)$^{a,b}$ | − 3.3 (3.63)$^{b}$ | < 0.001 |
| ΔE | 2.43 (1.53)$^{a}$ | 4.22 (1.58)$^{a,b}$ | 3.53 (1.8)$^{a,b}$ | 5.32 (2.19)$^{a,b}$ | 5.94 (4.6)$^{b}$ | 2.27 (0.77)$^{a}$ | 4.05 (1.41)$^{a,b}$ | 3.61 (2.73)$^{a,b}$ | 5.86 (2.31)$^{b}$ | 5.97 (1.93)$^{b}$ | < 0.001 |

Different superscript letters indicate a significant difference between the groups

Although it was under the threshold value, the reason for discoloration in the Listerine groups in our study could be the low pH and high alcohol concentration of Listerine that was shown to promote the demineralization of the enamel after prolonged exposure [36]. It could also result in pigmentation of the enamel since pigmentation has been associated with low pH values and demineralization [37]. Despite the lower concentration of alcohol in Colgate Plax than Listerine, Colgate Plax caused higher levels of discoloration than Listerine in our study. This may be attributed to the different substances in their compositions or possible dissimilarities in enamel structure.

In our study, the second greatest color change was observed in both Klorhex groups. Chlorhexidine is frequently used in the treatment of oral diseases. However, it was shown to result in staining of the teeth due to three possible mechanisms which are non-enzymatic browning reactions (Maillard reactions) within the pellicle, the denaturation of pellicle resulting in the formation of pigmented sulphides of tin and iron, and precipitation of pigments present in diet onto adsorbed chlorhexidine [38, 39]. Similarly, our results showed that Klorhex use also resulted in discoloration of the bracket-bonded teeth. Even though there was not a significant difference, the enhanced discoloration of the Klorhex + artificial saliva group compared to Klorhex + sterile saline group may be attributed to the features of the pellicle that facilitate discoloration.

The samples were brushed before the color measurements in previous studies regarding discoloration due to mouthwashes [2, 40]. The samples were not additionally brushed in our study since the teeth were polished before bonding and after debonding of the brackets, which was coincident with pre-color measurements at T0 and T1.

Previous studies showed that $L^*$ values decreased after adhesive removal and increased after polishing, and there was no significant difference between pre-treatment and post-treatment [41]. In accordance with their results, the teeth in the control group in our study showed no significant difference in the $L^*$ parameter, as in all parameters. The positive $\Delta L$ values in our study indicated that the enamel became lighter. Unlike previous studies using mouthwashes, positive $\Delta L$ values in our study may have occurred due to the bonding of the brackets and following debonding and finishing procedures which may have altered the surface characteristics of the enamel or polishing of the enamel surface which may have resulted in a smooth enamel surface that allowed more specular and increased reflection of light.

The differences in the green-red axis were not as high as in the luminosity and blue-yellow axes except Tantum Verde groups, showing that the color alterations were not mainly occurred as a result of the changes in the a parameter. The greatest changes in the Δb parameters were detected in the Tantum Verde groups, which were significantly higher than both control, both Colgate Plax and Listerine + artificial saliva groups, indicating that the teeth became bluish.

In the previous studies investigating the effect of mouthwashes on the tooth color, either sterile saline [2] or artificial saliva [42] solutions were used to store the teeth in between mouthwash applications. Artificial saliva imitates the oral environment better than sterile saline, with its property of depositing a pellicle layer on tooth. Especially in dentition areas which are inaccessible to tooth brushing and the abrasive action of a dentifrice, pellicle was shown to have a tendency to develop extrinsic stain [43]. Saliva and the subsequent pellicle accumulation serve as a matrix for the stain formation, which will eventually result in the incorporation of chromogens into the pellicle layer and discoloration. To see if they have different effects on color change, we used sterile saline and artificial saliva solutions for the in vitro environment in our study. Although the difference was not significant, Listerine, Klorhex, and Tantum Verde groups which were put in artificial saliva showed higher color changes than their matching sterile saline groups. This increment in $\Delta E$ values may be the result of pellicle accumulation acting as a matrix for the stain deposition, which may have enhanced discoloration. The differences in measurements between sterile saline and artificial saliva solutions, which were thought to occur due to the formation of the pellicle, showed that the use of artificial saliva solution may be preferred in order to obtain results that reflect the oral environment better. However, in our study, lack of chromogens and dietary acid which has an impact on the pellicle layer should be noted.

The limitation of this study is its in vitro design that is not capable of fully reflecting complex oral conditions which include aging, food coloring, and toothbrush abrasion. Further in vivo studies are required to fully understand the effect of the oral environment on the color of the bracket-bonded teeth.

## Conclusion

Within the limitations of this in vitro study, it should be noted that the use of mouthwashes during orthodontic treatment may cause noticeable changes in tooth color. Nevertheless, Listerine Cool Mint may need to be selected among other mouthwashes used in this study because it does not produce clinically visible color changes. Considering the measurement differences in sterile saline and artificial saliva groups, the use of artificial saliva should be preferred in order to obtain more similar results to the oral environment in similar in vitro studies.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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