

# Effects of Microabrasion Prior to In-office Bleaching on Hydrogen Peroxide Permeability, Color Change, and Enamel Morphology

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## Clinical significance

A waiting time of 7 days after performing microabrasion (MA) before performing in-office (IO) bleaching decreases the potential for hydrogen peroxide to reach the pulp chamber.

## SUMMARY

**Purpose:** This study evaluated hydrogen peroxide (HP) diffusion within the pulp chamber, as well as color change and the surface morphology of teeth subjected to various microabrasion (MA) protocols associated or not with

**in-office (IO) bleaching. Methods:** Forty sound premolars were randomly divided into the following four groups (n=10): no treatment (NC); IO bleaching only; IO immediately after MA (IMA), and IO seven days after MA (7MA). After treatments, the HP concentration ( $\mu\text{g/mL}$ ) within the pulp chamber was determined using ultraviolet-visible (UV-Vis) spectrophotometry. The color change ( $\Delta E^*$ ) was evaluated using the digital spectrophotometer before and 1 week after bleaching. The surface morphology was evaluated by scanning electron microscope (SEM). Data from each test were submitted to one-way ANOVA and Tukey tests ( $\alpha=0.05$ ). **Results:** All experimental groups exhibited higher HP concentrations compared to the NC group ( $p<0.00001$ ). However, higher amounts of HP were observed for the IMA group compared to the IO and 7MA groups ( $p<0.00001$ ). No significant difference in color

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change was observed among the groups ( $p<0.001$ ). Pronounced grooves in enamel were found in the IMA and 7MA groups. However, enamel erosion areas were observed only in the 7MA group. **Conclusions:** The association between MA and IO bleaching could significantly affect the amount of HP inside the pulp chamber. Therefore, it is highly recommended to wait for 1 week after MA procedures before performing IO bleaching.

INTRODUCTION

Dental fluorosis is an enamel defect that occurs due to excessive chronic fluoride intake during the tooth formation process.<sup>1,2</sup> The structural changes caused by this anomaly generate porosity and reduced mineral content, causing visual effects on the enamel.<sup>3,4</sup> This effect can result in esthetic compromises ranging from thin white lines to extensive opaque areas. Indeed, depending on the severity of the porosity, these spots can acquire a yellowish or brownish appearance.<sup>5-8</sup> The negative esthetic effects of fluorosis can also impact patient quality of life.<sup>9,10</sup> Therefore, in the last decade, demand by patients seeking treatment to improve their smiles' esthetics has increased.

Many techniques can improve the esthetic appearance of tooth stains caused by fluorosis. A recent systematic review<sup>11</sup> examined the aspects of minimal intervention and established that the most effective current treatment options for dental fluorosis include tooth bleaching, microabrasion (MA), or a combination of both treatments.

MA is a conservative, simple treatment aimed at removing stains or defects restricted to the enamel through chemical and abrasive action.<sup>12,13</sup> The microabrasive agent facilitates the controlled removal of thin enamel layers (varying from 20 to 200  $\mu\text{m}$ ). In this sense, the amount of removal depends on the acid concentration, the abrasiveness of the particles, the application time, and the pressure exerted on the enamel during the procedure.<sup>13-15</sup> After enamel MA, teeth may develop a yellowish appearance due to the reduction of the enamel's thickness, which makes the underlying dentin more evident. Therefore, bleaching has become an option for improving the esthetic appearance of teeth subjected to MA. This is one of the main reasons several authors indicate a combined treatment (MA and bleaching).<sup>9,16-18</sup>

Unfortunately, when this combined treatment is required, tooth sensitivity is the most common adverse effect patients report.<sup>16,17</sup> Given that tooth

Table 1: Description of the Materials (Manufacturers) and Composition of the Products Used in This Study

Material (Manufacturers)	Composition
Opalustre [Ultradent, South Jordan, UT, USA]	6.6% Hydrochloric acid and silicon carbide microparticles with particle size from 20 to 160 $\mu\text{m}$
Opalescence Boost 40% [Ultradent, South Jordan, UT, USA]	40% hydrogen peroxide (HP), 0.11% Fluor ions (1000 ppm), and 3% potassium nitrate

sensitivity is related to the amount of hydrogen peroxide (HP) that reaches the pulp chamber,<sup>19</sup> several authors have hypothesized that the enamel thickness reduction after MA could be partly responsible for increased tooth sensitivity in patients submitted to bleaching procedures.<sup>9,16,17</sup> Hence, once the amount of mineralized structure and thickness is reduced,<sup>13-15</sup> it may be important to remineralize the enamel surface before applying an in-office (IO) bleaching gel to avoid an increased HP diffusion into the pulp chamber.

Therefore, this *in vitro* study was aimed at quantifying the permeability of HP within the pulp chamber and evaluating color change and surface enamel morphology in human teeth submitted to various protocols that combine MA and IO bleaching. The null hypotheses tested were that MA performed prior to IO bleaching (1) will not increase concentration of HP inside the pulp chamber, (2) will not change the surface morphology, and (3) will not interfere in the bleaching efficacy of an IO bleaching product.

METHODS AND MATERIALS

Forty sound upper premolars were donated by patients who underwent tooth extraction at the local university for orthodontic reasons. Specimen enamel analysis was performed using a stereomicroscope (Lambda LEB-3, ATTO instruments, Hong Kong, China) at 10 $\times$  magnification. Specimens with morphological alterations or the presence of enamel cracks were excluded. The products used are described in Table 1.

Sample size calculation

The study's primary outcome was the amount of HP that reached the pulp chamber. According to a previous study,<sup>20</sup> the amount of HP inside the pulp chamber in the groups submitted to IO bleaching was 0.28, whereas it was 0.004  $\mu\text{g/mL}$  for the control group (without IO bleaching). To achieve a power of 80% and detect significant differences at the 5%

Table 2: Data Points for the Calibration Curve

H <sub>2</sub> O <sub>2</sub> Data For Each Point		Solutions Required to Obtain 3000 µL for Each Point for the Calibration Line				
H <sub>2</sub> O <sub>2</sub> Weight (µg)	H <sub>2</sub> O <sub>2</sub> Concentration (µg/mL)	Acetate Buffer Solution (µL)	46.15 µg/mL H <sub>2</sub> O <sub>2</sub> Solution (µL)	Peroxidase (µL)	Leuco Crystal violet (µL)	Deionized Water (µL)
1.0704	0.356	75	25	50	100	2750
0.8563	0.285	80	20	50	100	2750
0.6422	0.214	85	15	50	100	2750
0.4281	0.143	90	10	50	100	2750
0.3425	0.114	92	8	50	100	2750
0.1710	0.057	96	4	50	100	2750
0.0855	0.029	98	2	50	100	2750
0.0000	0.000	100	0	50	100	2750

level, the minimal sample size was nine teeth in each group to detect differences of 0.2 µg/mL among the tested groups. However, considering the possible loss of samples, 10 teeth were used for each group.

### Specimen preparation

The roots of the teeth were removed approximately 3 mm apical to the cemento-enamel junction using a low-speed diamond disk (Isomet 1000, Buehler, Lake Bluff, USA). The pulp tissue was removed and rinsed with ultrapurified water. Access to the pulp chamber was expanded carefully with a round bur (#1014, KG Sorensen, São Paulo, SP, Brazil) to allow 25 µL of solution to be introduced into the pulp chamber using a micropipette (LabMate Soft, HTL Lab Solutions, Warsaw, Poland).

### Obtaining the study calibration curve

The study used analytical products without prior purification, and all solutions were prepared using ultrapurified water obtained from a Millipore Milli-Q system (MS2000, Gehaka, SP, Brazil). First, a standard reference line was plotted using a 5.000 µg/mL stock solution prepared from a concentrated solution (34%-36% HP, LABSYNTH, Diadema, SP, Brazil); this solution was diluted in an acetate buffer solution (pH=4.5) and calibrated using traditional methods. The solution was titrated with a potassium permanganate solution to determine its analytical grade and actual solution concentration.<sup>21</sup> Based on this verified initial concentration, serial volumetric dilutions of 0.000-0.356 µg/mL were performed to plot the line of work (Table 2).<sup>22</sup> The known HP concentrations were added to the glass tubes and placed in a Varian Cary 50 UV-Vis (ultraviolet-visible) spectrophotometer (Varian, Palo Alto, CA, USA). This procedure yielded a standard reference

line for the extrapolation of the study samples' results ( $R=0.998$ ).

### Experimental groups and treatment protocols

The forty sound premolars selected previously were allocated randomly into four groups (n=10) according to the treatments. In the group that received no treatment (NC), no bleaching or MA procedures were performed. In the IO bleaching group (IO), only an IO procedure (Opalescence Boost 40%; Ultradent Products, South Jordan, UT, USA) was applied. In the third and fourth groups, MA (Opalustre, Ultradent) was performed prior to IO bleaching (Opalescence Boost 40%; Ultradent). However, in the third group, IO bleaching was applied immediately after MA (IMA), whereas in the fourth group, IO bleaching was applied seven days after MA (7MA). In this latter group, specimens were stored in artificial saliva during this period.

For the third and fourth groups, the MA treatment was performed using Opalustre in accordance with the manufacturer's recommended protocol. The product was applied to the enamel surface for 60 seconds by active application with a rubber cup (OpalCups, Ultradent). The OpalCups were used in a low-speed handpiece with circular movements at 500 rpm and intermittently applied low pressure similar to a prophylaxis procedure. The procedure was performed twice per each tooth. Finally, it was washed and air dried. For all treatment protocols, a single calibrated and experienced operator, blinded to the assigned groups, was responsible for the material application.

### Hydrogen peroxide quantification inside the pulp chamber

For all groups, the specimens were vertically fixed to a wax plate with the occlusal surface toward the

plate. Before the bleaching agent was applied, the buccal surface of each specimen was isolated by applying a light-cured resin barrier enclosing an area of 8 mm<sup>2</sup> (OpalDam, Ultradent). A 25- $\mu$ L aliquot of acetate buffer (pH = 4.5) was inserted into the pulp chamber of each tooth to absorb and stabilize any HP that might penetrate the pulp chamber.

In the experimental groups, the bleaching treatment was performed using Opalescence Boost 40% (Ultradent). The gel was applied to the enamel in a single 20-minute session. Following the bleaching procedure (which was not performed in the NC group), the acetate buffer solution inside each specimen's pulp chamber was removed using a mechanical micropipette and transferred to a glass tube. This procedure was performed by rinsing each specimen's pulp chamber four times with 25  $\mu$ L of acetate buffer and transferring this solution to the same glass tube. Thereafter, more ultrapurified water (2.725  $\mu$ L) was added to the glass tube along with 100  $\mu$ L of 0.5 mg/mL (Leuco Crystal Violet, Sigma Chemical Co, St Louis, MO, USA) and 50  $\mu$ L of 1 mg/mL horseradish peroxidase enzyme (Peroxidase Type VI-A, Sigma Chemical). This procedure was repeated separately for each specimen. The resulting solution had a violet color with a maximum absorbance peak at 596 nm, which was measured using a Varian Cary 50 UV-Vis spectrophotometer (Varian, Palo Alto, CA, USA). According to Beer's Law, absorbance corresponds directly to concentration. Therefore, HP concentration ( $\mu$ g/mL) was determined by comparing it with the calibration curve already obtained.

### Color change evaluation

Color change was measured before and 1 week after bleaching treatments using a digital spectrophotometer (VITA Easyshade Advance 4.0, Vita Zahnfabrik, Bad Säckingen, Germany). During this period, specimens were immersed in artificial saliva, with daily changes at a controlled temperature of 37°C.

To standardize the position of the spectrophotometer, impressions were taken using a condensation silicone paste (Coltoflax and Perfil Cub Kit, Vigodent, Rio de Janeiro, RJ, Brazil). A 6-mm diameter window referring to the tip of the spectrophotometer was created in the middle-third of each specimen's buccal surface using a metal device. The color parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ) were recorded using the tip of the device, which was inserted into the impression guide. The  $L^*$  value represented the lightness, values for which varied from 0 (black) to

100 (white). The  $a^*$  value represented the color along the red–green axis, and the  $b^*$  value represented the color along the yellow–blue axis. Color change ( $\Delta E^*$ ) was calculated based on the difference between the specimen before (baseline) and 1 week after bleaching for each group, using the following formula from CIELab 1976:  $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ .

### Enamel surface morphology evaluation

After the previous test, three fragments were obtained from each group to evaluate enamel surface morphology. After being cut, each sample was cleaned in an ultrasonic bath with a frequency of 42 kHz (Cristófoli Ultrasonic Washer, Cristófoli, Brazil) and dehydrated in a desiccator containing colloidal silica for 24 hours at 37°C and sputter-coated with 10 nm of gold before being taken to a low-vacuum scanning electron microscope (SEM). To standardize image acquisition, three pictures of each specimen were acquired at a magnification of 10,000 $\times$  in the secondary electron mode at 15 kV (MIRA3, TESCAN ORSAY HOLDING, Warrendale, PA, USA). The first image was taken in the center, and the other two images were taken 0.3 mm to the left and right of the first image. A technician who was blinded to the experimental conditions evaluated all images and selected the most representative one.

### Statistical analysis

Before submitting the data for analysis using the appropriate statistical test, the Shapiro–Wilk test was performed to assess whether the data followed a normal distribution. Bartlett test for equality of variances was performed to determine whether the assumption of equal variances was valid. After evaluation of the normal distribution, the data obtained regarding HP concentration ( $\mu$ g/mL) and color change ( $\Delta E^*$  measurements) were statistically analyzed using one-way ANOVA and Tukey tests ( $\alpha=0.05$ ). The enamel surface morphology was only evaluated qualitatively.

## RESULTS

### Hydrogen peroxide quantification inside the pulp chamber

One-way ANOVA revealed statistically significant differences among groups for HP concentration ( $p=0.0001$ ; Table 3). A lower amount of HP was detected inside the pulp chamber of the NC group when compared to the bleaching groups ( $p<0.00001$ ; Table 3). However, the highest and only significant



Table 3: Means and Standard Deviations of HP Concentration ( $\mu\text{g/mL}$ ) Detected inside the Pulp Chamber and  $\Delta E^*$  Measurements after Treatment

Experimental Groups	Hydrogen Peroxide (HP) Concentration ( $\mu\text{g/mL}$ )	$\Delta E^*$ Measurements
No treatment (NC)	$0.0001 \pm 0.0001$ A	$2.5 \pm 0.7$ b
Only in-office (IO)	$0.32 \pm 0.04$ B	$7.0 \pm 2.7$ a
IO immediately after microabrasion (IMA)	$1.05 \pm 0.43$ C	$7.6 \pm 3.1$ a
IO 7 days after microabrasion (7MA)	$0.41 \pm 0.15$ B	$5.8 \pm 2.1$ a

*\*Identical uppercase, lowercase, or superscript letters in each column indicate statistically similar means (One-way ANOVA and Tukey test,  $\alpha=0.05$ ).*

amount of HP was observed for the IMA group compared to IO and 7MA groups ( $p < 0.00001$ ; Table 3).

### Color change evaluation

One-way ANOVA revealed statistically significant differences among groups for color change ( $p = 0.001$ ; Table 3). A lower, imperceptible color change was observed in the NC group when compared to the other groups ( $p = 0.001$ ; Table 3). All bleaching groups showed similar  $\Delta E$  values ( $p > 0.05$ ; Table 3).

### Enamel surface morphology evaluation

Differences in surface morphology were observed between the NC group and the bleaching groups (Figure 1). Mild superficial alterations, irregularities, and slight demineralization was observed in enamel in the IO group. Pronounced grooves were observed on the enamel surfaces in the groups that underwent MA. However, areas of erosion on the enamel surface were observed only in the IMA group (Figure 1).

## DISCUSSION

The presence of HP inside the pulp chamber of the groups submitted to bleaching was expected, because the active HP molecules used in the bleaching agents have a low molecular weight. HP penetrates and interacts with organic components present in the dental structure, thus promoting a color change.<sup>19</sup> This is why HP has the capacity to diffuse inside the tooth, reaching the pulp tissue within 15 minutes after application.<sup>23-25</sup>

However, the IMA group showed the highest HP values inside the pulp chamber when compared to other IO bleaching groups. This led us to partially reject the first null hypothesis. The MA product used on the enamel surface contains hydrochloric acid and abrasive particles. The former is responsible for causing significant erosion in the enamel surface, which is followed by wear due to the rubbing application of abrasive particles leading to a removal of a thin layer of enamel (20-200  $\mu\text{m}$ ).<sup>13-15</sup> Previous studies showed that the thickness of the enamel played an important role in the passage of HP into the pulp chamber.<sup>26-28</sup> Thus, at first glance, a reduction in enamel thickness where the MA procedure was applied might be responsible for the higher amount of HP inside the pulp chamber. However, similar enamel reduction should have been expected when the same MA procedure was applied, as for IMA and 7MA groups, and only the former showed higher HP values inside the pulp chamber.

As is known, the MA procedure promotes significant changes in the proportion of organic and inorganic components, mainly by modifying the interprismatic spaces at the enamel surface,<sup>29,30</sup> which may potentially alter the permeability of the enamel surface of dental tissues,<sup>26</sup> and, consequently

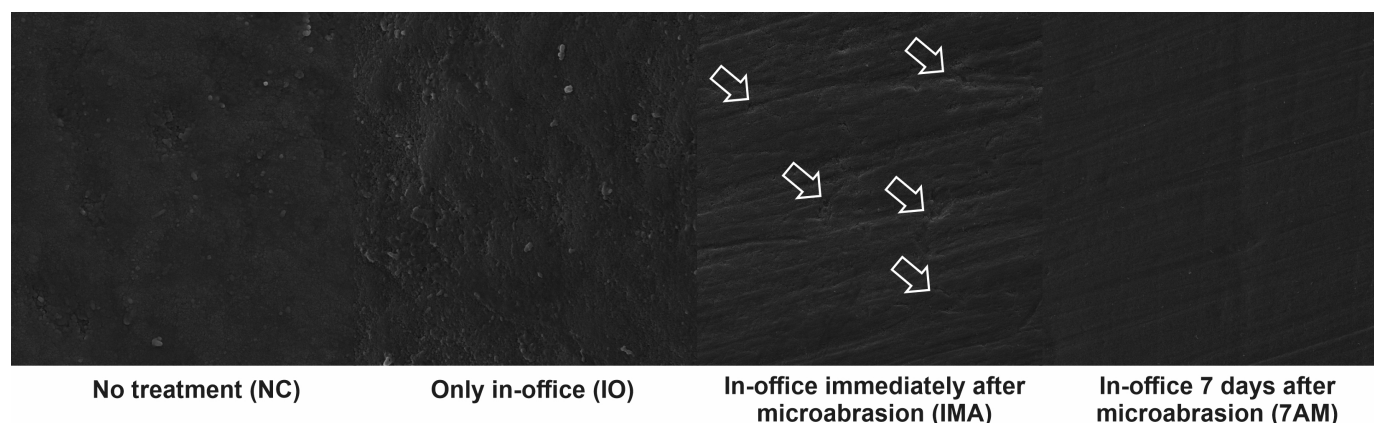


Figure 1. Morphology of the enamel surface obtained via scanning electron microscopy at 10,000 $\times$  magnification.

may facilitate HP diffusion along the tooth structure. For both groups in which MA procedures were applied (IMA and 7MA), it was possible to see pronounced grooves on the enamel surface when compared to the control, and only IO groups led us to reject the second null hypothesis.

There were substantial differences observed in the SEM images for both MA groups. The presence of more evident erosive zones in the enamel surface was observed for the IMA group. This indicated an increasing susceptibility of “freshly” microabraded enamel to greater surface demineralization, which produced eroded areas<sup>31-33</sup> primarily when IO bleaching was applied immediately after the MA procedure. Therefore, the more pronounced alterations in the enamel surface, combined with enamel permeability, could have made the enamel more susceptible to bleaching agent penetration in the IMA group.

On the other side, a considerable reduction in the amount of HP inside the pulp chamber in the 7MA group should be attributed to the remineralizing effect due to the specimen storage in artificial saliva at a controlled temperature for a week before performing IO bleaching. Saliva and its components might play an important role in maintaining the integrity of dental tissues when they are prone to mineral loss.<sup>34</sup> Some studies have speculated that the interaction of saliva with microabrasive components can be significant and might be related to the compression of silica particles in enamel when performing the MA. This results in tricalcium silicate ( $\text{Ca}_3\text{SiO}_5$ ), which indicates the formation of a new layer of apatite crystals in the microabraded enamel.<sup>35,36</sup>

Therefore, based on the results of the present study, the increased amount of HP inside the pulp chamber observed in the IMA group should be more related to increased permeability of the enamel outer layer after the MA than due to enamel thickness reduction observed after the MA procedure.

Although several changes occurred in the enamel surface, mainly when MA procedures were performed, no detrimental effects were observed in terms of bleaching effectiveness. All groups exposed to IO bleaching produced a color change of 5.8-7.0  $\Delta E^*$ , regardless of the surface treatment performed. Therefore, the third null hypothesis that the use of MA might affect color changes was rejected.

One of the current study's limitations was the use of only one IO bleaching and one microabrasive product. Several characteristics could influence the

final results of IO bleaching, including pH, application technique, viscosity, and chemical stability,<sup>22,25,26</sup> as well as the microabrasive system, the effects of which rely on the concentration and pH of the acid used, the type of abrasive included in its composition, instrumentation time, applied force, application mode, and revolutions per minute. All these situations are related to the changes observed in the microabraded enamel.

However, the decision to evaluate the IO bleaching technique was based on thinking in a scenario where a single appointment of MA followed by IO bleaching could be carried out.

Another limitation of the present study was the bleaching technique applied. Taking in consideration that at-home bleaching is a popular and safe technique, mainly because low concentration HP is used, at-home bleaching could be considered the more desirable when the MA procedure is performed. In the other side, the decision to evaluate IO bleaching, instead of at-home bleaching, was based on a single appointment where MA followed by IO bleaching could be carried out. Therefore, future studies using different MA products and techniques, as well as combined with at-home bleaching, need to be done to confirm the hypothesis.

## CONCLUSIONS

Based on the results obtained in this study, the three null hypotheses tested were rejected; using an MA product immediately before IO bleaching increased HP diffusion into the pulp chamber and produced significant alterations in the enamel surface. However, these effects did not interfere with the efficacy of the bleaching agent in terms of color changes. Clinicians should avoid the MA procedure immediately prior the use of an IO bleaching product.

Avoiding a combination of MA and IO bleaching in a single session is recommended due to increased HP levels inside the pulp chamber and a greater erosive potential on the enamel surface. Changes in the tooth enamel do not interfere with the color changes exhibited by teeth submitted to IO bleaching.

## Conflicts of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this manuscript.

## Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guide-

lines and policies of Institutional review board of National University of Colombia. The approval code issued for this study is HERMES 45686.

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## Departments

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### Errata

Operative Dentistry apologizes for the errors in the following manuscripts.

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AD Loguercio, LJC Vargas, MW Favoreto, HF Andrade, CP F Borges, A Dávila-Sánchez, A Reis, CP Mora; Effects of Microabrasion Prior to In-office Bleaching on Hydrogen Peroxide Permeability, Color Change, and Enamel Morphology. *Oper Dent* 1 November 2021 46(6) 661-668. doi: <https://doi.org/10.2341/20-179-L>

**There are errors in the author order and contact list. The correct author order and author affiliations list should read (corrections are underlined):**

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**Additionally, the legend in Table 3 should read:**

\*Identical uppercase or lowercase letters in each column indicate statistically similar means (one-way ANOVA and Tukey test,  $\alpha=0.05$ ).

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D Kaisarly, M ElGezawi, R Haridy, A Elembaby, A Aldegheishem, R Alsheikh, KS Almulhim; Reliability of Class II Bulk-fill Composite Restorations With and Without Veneering: A Two-year Randomized Clinical Control Study. *Oper Dent* 1 September 2021 46(5) 491-504. doi: <https://doi.org/10.2341/19-290-C>

**There are errors in the author order and contact list and corresponding author information. The correct author order, author affiliations list, and corresponding author information should read (corrections are underlined):**

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**There are errors in the author names and contact list, in the Summary, and in the Results. The correct author spelling and author affiliations list should read (corrections are underlined):**

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#### **In the Methods paragraph of the Summary:**

The sentence, “A comprehensive search was performed in PubMed, Bridge Base Online (BBO), Latin American and Caribbean Health Sciences Literature database (LILACS), Cochrane Library, Scopus, Web of Science, and grey literature without date and language restrictions on April 23, 2017 (updated on September 26, 2019).”

#### **Should read (correction is underlined):**

“A comprehensive search was performed in PubMed, Bibliografia Brasileira de Odontologia (BBO), Latin American and Caribbean Health Sciences Literature database (LILACS), Cochrane Library, Scopus, Web of Science, and grey literature without date and language restrictions on April 23, 2017 (updated on September 26, 2019).”

#### **In the Study Selection paragraph in the Results section:**

The sentence, “After title screening, 227 studies remained, and this number was reduced to 32 full texts that were assessed for eligibility (Figure 1).”

#### **Should read (correction underlined):**

“After title screening, 228 studies remained, and this number was reduced to 32 full texts that were assessed for eligibility (Figure 1).”