Effectiveness of In-office Hydrogen Peroxide With Two Different Protocols: A Two-center Randomized Clinical Trial

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Clinical Relevance

When an alkaline and desensitizing-containing in-office gel is used, it is recommended to be applied for one 40-minute application without gel refreshing.

SUMMARY

Objectives: The aim of this study was to compare the bleaching efficacy and tooth sensitivity (TS) of a 38% hydrogen peroxide bleaching agent used for in-office bleaching, applied under different time protocols: a 40-minute application or two 20-minute applications.

Methods and Materials: Forty-four patients from Brazil and Colombia, with right superior canines darker than C2, were selected for this multicenter, single-blind, randomized trial. The teeth were bleached in two sessions, with a one-week interval between them, in a split-mouth design. The bleaching agent was applied in two 20-minute (2×20) applications or one 40-minute (1×40) application in each session according to the manufacturer's instructions. The color changes were evaluated by using subjective (Vita Classical and Vita Bleachedguide) and objective (Easyshade Spectrophotometer) methods at baseline and 30 days after the second session. Tooth sensitivity was recorded up to 48 hours with a 0-10 visual analog scale. Also, the pH values during...
the application of bleaching were recorded. Color change in shade guide units and ΔE were analyzed by using the Student \( t \)-test (\( \alpha = 0.05 \)). The absolute risk and intensity of TS were evaluated with the McNemar test, the Wilcoxon signed-rank test, and the Friedman test, respectively (\( \alpha = 0.05 \)).

Results: Significant whitening was observed in both groups after 30 days of clinical evaluation. The use of a 40-minute application did not significantly influence the absolute risk of TS (68%, 95% confidence interval [CI] = 53-80) as well as the intensity of TS compared with the acid bleaching gel (absolute risk of 82%, 95% CI = 68-91). The pH values did not differ significantly between groups and at the different assessment periods (\( p = 0.42 \)).

Conclusion: The use of a 40-minute in-office bleaching agent gel application produced the same whitening degree and TS that the two 20-minute bleaching agent applications did. The former preferably should be applied because one 40-minute application does not require gel refreshing.

INTRODUCTION
Dental treatment patterns have changed due to patients’ interest in dental esthetics, and more emphasis has been placed on having a beautiful smile.\(^1\) When dealing with discolored teeth, dental bleaching should be the first choice for treatment, as it is an effective and conservative approach\(^2\) when compared with other treatment modalities, such as veneers, crowns, or composite bonding.\(^3,4\) Carbamide peroxide at 10% is most used as a standard product for at-home bleaching.\(^4\) However, high concentrations of hydrogen peroxide (HP), ranging from 25% to 40%, have been used in office to achieve faster bleaching effectiveness.\(^5,6\)

In-office bleaching with high concentration HP provides visible results after a single session, thus increasing the acceptance of the in-office protocol.\(^7,8\) Additionally, it is a treatment alternative for patients who do not feel comfortable using a bleaching tray at home and who require faster results. It may also be employed in some patients who require close attention due to the presence of tissue recession or nonrestored cervical lesions.\(^3\)

The wide range of in-office bleaching products in the dental market makes it difficult to choose one. Considerable variation exists in the products’ active concentrations of HP,\(^5,6\) additives, pH variations,\(^9-11\) and application modes.

With regard to application modes, the results from clinical studies that compared the bleaching efficacy of a single 45-minute application and three 15-minute applications are not in agreement in terms of color change and risk and intensity of tooth sensitivity (TS).\(^7,12-14\) For instance, Reis and others\(^7\) concluded that a single application of a 35% HP gel for 45 minutes decreased bleaching efficacy and slightly increased overall patient sensitivity compared with three 15-minute applications. On the other hand, Kossatz and others\(^14\) generated the opposite findings. The authors concluded that subjects who received three 15-minute applications each had more TS than did those who received a single 45-minute application each. The variations in compositions, mainly pH and additives, among the different brands of HP gels and the decreased pH stability of some products may explain such results.\(^7,12-15\)

Therefore, conducting randomized clinical trials that investigate the effect of a prolonged application time on the efficacy, risk, and intensity of TS for other bleaching gel brands is still required. Therefore, the aim of this single-blind, randomized controlled study was to evaluate the color change, risk, and intensity of TS of an in-office bleaching gel applied in a single 40-minute application or two 20-minute applications. We also investigated the in vivo pH stability of the gel for both application modes.

METHODS
Protocol Registration
This study was registered at the Brazilian registry of clinical trials under the identification number REBEC:RBR-3h6n6c.

Study Designs, Settings, and Locations of Data Collection
This was a randomized, single-blind (evaluators), split-mouth and equivalent multicenter trial with an equal allocation rate between groups. This study took place within the dental clinics of the UFAM and USC from September 2015 to February 2016.

Recruitment
The participants who took part in this study were those who sought dental treatment at the dental clinics at the universities. At this time, the participants were examined in a dental chair after dental
prophylaxis with pumice and water to check whether they met the study’s eligibility criteria.

**Eligibility Criteria**
To be included in this study, participants had to be age 18 years or older and be in good general and oral health. Participants had to have canines that were at least shade C2 or darker during assessments with the value-oriented shade guide (VITA classical, VITA Lumin, VITA Zahnfabrik, Bad Sackingen, Germany), and all anterior teeth without restorations. Participants with anterior teeth presenting noncarious cervical lesions, full crowns, or veneers; gingival recession; endodontically treated teeth; internal tooth discoloration; moderate to severe fluorosis; or spontaneous tooth pain were not included in the study. Additionally, pregnant or lactating women were also excluded from this study.

**Sample-Size Calculation**
The sample-size calculation was done for the color change, the primary outcome, measured with the spectrophotometer (ΔE). If no difference was found between the standard and experimental treatments, then 44 patients were required to be 90% sure that the limits of a two-sided 90% confidence interval would exclude a difference in means of three units in the ΔE values. Each center recruited 22 participants for the study (Figure 1).

**Random Sequence, Allocation Concealment, and Blinding**
A third operator, not involved in the research protocol, conducted the randomization procedure by using computer-generated tables. We used blocked randomization (block sizes of two and four) with an equal allocation ratio (www.sealedenvelope.com). The randomization sequence was placed in sequentially numbered, opaque, and sealed envelopes.

Once the participant was eligible for the procedure and completed all baseline assessments, the side of the patient’s arch where treatment was applied first was decided by tossing a coin. At this time, the operator could open the envelope to reveal the group where the experiment would be conducted first. Neither the participant nor the operator knew the group allocation before this stage. The other treatment was applied on the patient’s second side.

Participants and operators could not be blinded to the study groups, as they could easily identify the side in which the material had been refreshed once. However, the evaluator who performed the color assessments and the statistician was blinded to the treatments.

**Study Intervention**
The gingival tissue was isolated with a light-cured resin dam (Opal Dam, Ultradent Products Inc, South Jordan, UT, USA). The 38% HP gel Opalescence...
Boost (Ultradent Products Inc) was applied in a single 40-minute application or in two 20-minute applications in all maxillary incisors, canines, and premolars of the same patient following the description of each group (Table 1).

After seven days, this procedure was repeated using the same protocol. All participants were instructed to brush their teeth at least three times a day using a fluoridated toothpaste (Colgate, Colgate-Palmolive, São Paulo, Brazil).

### Color Evaluation

Color was recorded before the bleaching procedure, seven days later, and 30 days after the end of the bleaching treatment. For this purpose, we used an objective (Easyshade spectrophotometer, Vident, Brea, CA, USA) and a subjective instrument (value-oriented shade guide VITA classical). Color evaluation was completed in a room under artificial lighting conditions without interference from outside light. For both devices, color was checked at the middle third of the canine.

For calibration, the color assessments of 10 patients were conducted in both study centers three times with an interval of three days between times to check the intra- and interagreement. The same person, who visited both centers, performed the calibration process and coordinated the administration of the study. The operator was considered calibrated only when he could get a weighted kappa of 80% in two consecutive readings of the same teeth in 10 different patients.

For the objective shade evaluation, an impression of the maxillary arch with high-putty silicon paste (Clonage, Nova DFL, Rio de Janeiro, Brazil) was taken, and a window on the labial surface of the silicon guide was created by using a metal device with a 6-mm radius to standardize the area for color evaluation with the spectrophotometer. Color was determined using the parameters of the digital spectrophotometer, on which the following values were indicated: L*, a*, and b*, where L* represented luminosity (the value from 0 [black] to 100 [white]), and where a* and b* represented the color along the red-green axis and the color along the yellow-blue axis, respectively.

The difference between baseline and each recall period ($\Delta E^*$) was calculated by using the following formula: $\Delta E^* = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$. For the subjective evaluation, the 16 tabs of the shade guide (VITA classical, VITA Zahnfabrik) were arranged from lightest to darkest as follows: B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3.5, B4, C3, A4, C4. Color changes were calculated from the beginning of the active phase through to the individual recall times by calculating the change in the number of shade guide units ($\Delta$SGUs), which occurred toward the lighter end of the value-oriented list of shade tabs.

Two examiners at each center, blinded to the allocation assignment, scheduled these patients for bleaching and evaluated their teeth against the shade guide at the different time assessments. In

### Table 1: Products, Composition, and Application Regimens

<table>
<thead>
<tr>
<th>Products and Composition</th>
<th>Application Regimen for Both Groups</th>
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</table>
| Opalescence Boost Gel: 38% Hydrogen peroxide, 20% water and desensitizing agents (3% potassium nitrate and 1.1% fluoride) | 1. Dry teeth and apply Opal Dam to dental arch slightly overlapping enamel (building the barrier 4-6 mm high and 1.5-2.0 mm thick) and interproximal spaces.  
2. Light cure Opal Dam for 20 s per arch using a scanning motion. Carefully check the resin cure with an instrument.  
3. Attach both syringes before mixing. Press in the plunger of the red syringe, pushing all the contents into the clear syringe. Forcefully press the small clear stem completely into the larger clear stem. Then press the clear plunger completely into the red syringe. To activate, press the chemical from the red syringe into the clear syringe with thumbs. Reverse action and mix a minimum of 25 times on each side.  
4. Press all mixed gel into the red syringe. Separate the two syringes and attach the Micro 20ga FX tip onto the red syringe.  
5. Apply a 0.5-1.0 mm thick layer of Opalescence Boost to the labial surface of the tooth and slightly onto the incisal/occlusal surfaces.  
*Group 2 × 20 min*  
5.1 Leave gel on for 20 min.  
5.2 Suction off using a surgical aspirator tip. Do not use water.  
5.3 Repeat gel application for more 20 min (40 min total).  
*Group 1 × 40 min*  
6. Leave gel on for 40 minutes undisturbed  
7. After the last application, suction all the gel off, then wash and apply suction.  
8. Remove Gingival Barrier by lifting it from one end. |
| Boost (Ultradent Products Inc) was applied in a single 40-minute application or in two 20-minute applications in all maxillary incisors, canines, and premolars of the same patient following the description of each group (Table 1). |
the event of disagreements between the examiners during shade evaluation, a consensus was reached.

**Tooth Sensitivity Evaluation**

Patients were asked to record their perceptions of TS during the first and second bleaching sessions using a 0-10 visual analog scale (VAS) in three different assessment periods: during treatment and up to 1 hour after bleaching, from 1 hour to 24 hours after bleaching, and from 24 hours to 48 hours after bleaching.

This VAS scale is a 10-cm horizontal line with scores of 0 and 10 at their ends, where 0 = no sensitivity and 10 = severe sensitivity. The patient had to mark the intensity of the TS with a vertical line across the horizontal line of the scale. Then, the distance in millimeters from the zero ends was measured with the aid of a millimeter ruler.

If the patient scored zero (no sensitivity) during all time assessments from both bleaching sessions, this patient was insensitive to the bleaching protocol. In all other circumstances, the patients were sensitive to the bleaching procedure. This dichotomization allowed us to calculate the absolute risk of TS, or the percentage of patients who reported TS at least once during treatment. As two bleaching sessions were performed, the worst VAS score obtained was considered for statistical purposes.

**In vivo pH Analysis**

A pH meter with a 6-mm circular and flat surface pH electrode (Extech pH100: ExStik pH Meter; Extech Instruments, Nashua, NH, USA) was positioned directly on the middle tooth surfaces of canines, lateral incisors, and central incisors in both of the patient’s arches and held in position until the pH was stabilized on the screen. As the pH electrode is very sensitive, it was possible to make three measurements for each tooth. For both application protocols (two 20-minute applications or a single 40-minute application), pH was registered every 20 minutes.

**Statistical Analysis**

The analysis followed the intention-to-treat protocol and involved all of the participants who were randomly assigned. The statistician was also blinded to the study groups. The color change ($\Delta$SGU and $\Delta$E) between the baseline versus 30 days was calculated for each group. The $\Delta$E and ASGU data from both groups were subjected to a paired Student t-test.

The absolute risk of TS of both groups was compared using the McNemar test ($\alpha=0.05$). The relative risk and the confidence interval for the effect size were calculated. The comparison of the TS intensity of the two groups at two different assessment points was performed using the Wilcoxon signed-rank test. Comparisons between times within each group were performed using the Friedman test. Data from the pH values were compared using a two-way repeated measures analysis of variance.

In all statistical tests, the significance level was 5%. We performed all analyses by using the software SigmaPlot version 11.0 (Systat Software, San Jose, CA, USA).

**RESULTS**

A total of 129 participants were examined; 44 participants were selected (Figure 1). Patients were excluded mainly due to the presence of anterior restorations and color (shades lighter than C2). The mean age (years) of the participants and the baseline SGUs are described in Table 2. One can observe comparable data among treatment groups. None of the patients discontinued the intervention. No medication and/or desensitizer were necessary to prescribe/apply in the participants from this study for the relief of bleaching-induced TS. We did not observe statistical differences between the study centers for any of the outcomes ($p>0.05$; data not shown). Therefore, the data from these centers were merged for statistical evaluation.

**Color Change**

After 30 days, whitening of approximately 9 to 10 SGUs and a variation of approximately eight units in $\Delta$E were detected for the 2x20 and 1x40 groups (Table 3). No statistically significant difference was observed between the study groups.

**Tooth Sensitivity**

The absolute risks of TS were 82% and 68%, respectively, with no significant difference observed.

Table 2: Baseline Characteristics of the Participants Included in This Clinical Trial

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>2 x 20 Min</th>
<th>1 x 40 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD, y)</td>
<td>27.5 ± 6.2</td>
<td>26.1 ± 5.5</td>
</tr>
<tr>
<td>Baseline color (mean ± SD, SGUs)</td>
<td>12.3 ± 2.3</td>
<td>11.7 ± 2.1</td>
</tr>
</tbody>
</table>

Abbreviations: SD = standard deviation; SGU – shade guide units Vita Classical.
between them \((p=0.27; \text{Table 4})\). The TS intensity of both bleaching protocols was statistically similar \((p>0.42)\). The overall TS intensity at the different assessment points (Table 5) showed that most of the TS complaints occurred within the first 24 hours after bleaching.

**PH Analysis**

The pH values did not differ significantly between groups and at the different assessment periods \((p=0.42; \text{Table 6})\).

**DISCUSSION**

The results of this study indicated that both in-office bleaching protocols showed significant whitening after two bleaching sessions. The application of a 38% HP gel for 40 minutes, without being refreshed, maintained the bleaching efficacy compared with the two 20-minute applications that the manufacturer recommended.

HP is usually applied and left undisturbed on the tooth surface for up to 15 to 20 minutes, and then manufacturers recommend gel removal and reapplication twice or even five times according to the bleaching gel brand. This recommendation is probably based on the assumption that after 15 to 20 minutes, no HP will be available for the oxidation of the organic content of dentin.

Table 3: Color Change in Shade Guide Units (ΔSGUs for Vita Classical) and ΔE (Means ± Standard Deviations) Between Baseline Versus 30 Days After Bleaching for the Two Treatment Groups*

<table>
<thead>
<tr>
<th>Color Evaluation Tools</th>
<th>2 × 20 Min</th>
<th>1 × 40 Min</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔSGU</td>
<td>9.8 ± 2.8</td>
<td>10.4 ± 2.6</td>
<td>0.68</td>
</tr>
<tr>
<td>ΔE</td>
<td>8.9 ± 3.2</td>
<td>8.2 ± 3.0</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* Paired Student t-test.

Table 4: Comparison of the Number of Patients Who Experienced Tooth Sensitivity at Least Once During the Bleaching Regimen in Both Groups Along With Absolute Risk and Risk Ratio*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Number of Participants</th>
<th>Absolute risk* (95% Confidence Interval)</th>
<th>Risk Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>2 × 20 min</td>
<td>36</td>
<td>08</td>
<td>82 (68-91)</td>
</tr>
<tr>
<td>1 × 40 min</td>
<td>30</td>
<td>14</td>
<td>68 (53-80)</td>
</tr>
</tbody>
</table>

* McNemar test \(p=0.27\).

Table 5: Tooth Sensitivity Intensity (Means ± Standard Deviations) at the Different Assessment Points for Both Study Groups

<table>
<thead>
<tr>
<th>Time Assessments</th>
<th>Visual Analog Scale</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 × 20 Min</td>
<td>1 × 40 Min</td>
</tr>
<tr>
<td>Up to 1 h</td>
<td>1.3 ± 1.9 A**</td>
<td>1.0 ± 1.8 a</td>
</tr>
<tr>
<td>1 h to 24 h</td>
<td>1.1 ± 2.1 A</td>
<td>0.9 ± 1.8 a</td>
</tr>
<tr>
<td>24 h to 48 h</td>
<td>0.3 ± 0.9 B</td>
<td>0.3 ± 1.0 b</td>
</tr>
</tbody>
</table>

* Wilcoxon signed rank test \(p=0.42\); ** Friedman test \(p=0.01\); *** Friedman test \(p=0.03\). Within each column, means identified with the same letters are statistically similar.

Table 6: pH Variation (Means ± Standard Deviations) at the Different Assessment Points for Both Study Groups and the Statistical comparison*

<table>
<thead>
<tr>
<th>Time Assessments</th>
<th>pH Variation</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 × 20 Min</td>
<td>1 × 40 Min</td>
</tr>
<tr>
<td>Baseline</td>
<td>7.4 ± 0.3</td>
<td>7.5 ± 0.2</td>
</tr>
<tr>
<td>After 20 min</td>
<td>7.5 ± 0.6</td>
<td>7.5 ± 0.6</td>
</tr>
<tr>
<td>After 40 min</td>
<td>7.7 ± 0.4</td>
<td>7.8 ± 0.3</td>
</tr>
</tbody>
</table>

* Two-way repeated measures analysis of variance test \(p=0.42\).
bleaching gel does not need to be removed and reapplied within 40 to 50 minutes. This single application approach is of clinical interest, as it results in a shorter amount of chair time associated with a reduced cost of in-office bleaching because less material is spent per patient. Furthermore, it could reduce the risk of occasional soft tissue burns because the material would be handled only once.

When it comes to the whitening effectiveness, the bleaching product also showed significant color improvement; this was also true for other studies that used highly concentrated HP products for two clinical sessions.\(^7\),\(^14\),\(^19\)-\(^21\) A change of approximately 10 SGUs and 8.5 units of \(\Delta E\) were observed after two bleaching sessions, which is within the range reported by other investigators.

In terms of absolute risk and intensity of TS, no significant difference was observed between the study groups. Some earlier studies hypothesized that the risk and intensity of TS were higher in a single gel application, likely due to the reduction of the gel during bleaching.\(^7\),\(^14\) The current study evaluated the pH variations of the bleaching gel during in-office bleaching. In the present study, we observed a stable pH throughout the bleaching process, which may be the key factor for the similarity between the groups.

Indeed, alkaline pHs also seemed to be associated with a reduced risk and intensity of TS. In a previous study,\(^15\) the authors evaluated two in-office bleaching gels, with one of them being the same as that used in the present study (Opalescence Boost, Ultradent Products) and the other a product with acidic features (Pola Office, SDI, Victoria, Australia). A lower risk of TS for Opalescence Boost was observed compared with Pola Office. A very important difference between these two products was that the initial pH may prevent or minimize the passage of HP to the pulp chamber, consequently diminishing the pulp damage and TS. An in vitro study by Mena-Serrano and others\(^22\) showed reduced HP penetration into the pulp chamber for bleaching gels with alkaline pH. Also, it is worth mentioning that the effect of rheological properties of the gel could affect the HP penetration, and consequently TS.\(^23\),\(^24\) Opalescence Boost is a viscous gel while Pola office is mixed with HP liquid and a powder with inherent differences in viscosity.

Other studies that compared two bleaching gels from the same manufacturer also demonstrated a significantly lower risk and intensity of TS for the product with the highest pH.\(^14\),\(^25\) Obviously, other studies with different bleaching gel brands should be conducted, and variations in pH should be correlated with the risk and intensity of TS to detect if these two variables are indeed associated.

From a theoretical point of view, keeping the pH slightly alkaline and stable also brings advantages in terms of bleaching effectiveness. It is known that the HP dissociation constant (pKa) is around 11.5,\(^26\) meaning that the closer the pH is to the pKa, the higher the HP dissociation rate. Indeed, it was experimentally detected that the HP dissociation rate was 2.7 times higher in a pH of 9 than in an acidic solution (pH = 4.4). However, this higher whitening efficacy was not demonstrated in studies that evaluated bleaching gels with different pHs.\(^14\),\(^25\)

Another factor that may reduce bleaching-induced TS is the presence of additives in the bleaching gel, such as potassium nitrate, sodium fluoride, or amorphous calcium phosphate.\(^27\) Opalescence Boost contains two desensitizing agents (3% potassium nitrate and 1.1% fluoride, according to the manufacturer’s information). However, so far, no clinical studies have compared the risk and intensity of TS of in-office bleaching gels with and without desensitizers. This comparison was done for at-home bleaching gels, and controversial results were observed. Some authors reported a reduction of bleaching-induced TS when desensitizing-containing gels were used,\(^15\),\(^17\),\(^28\)-\(^32\) whereas others reported no significant difference between desensitizing-containing and desensitizing-free at-home bleaching.\(^33\),\(^34\)

Unfortunately, conflicting results have been published in the literature in terms of TS when in-office bleaching gels are applied.\(^35\) The high number of bleaching gels and protocols evaluated in different randomized clinical trials\(^7\),\(^15\),\(^21\),\(^36\) makes it difficult for clinicians to reach a clear conclusion regarding which protocol presents an increased risk and intensity of TS induced by bleaching.\(^35\),\(^37\) Future studies need to be done to evaluate the application protocols for different bleaching brands.

In summary, we can conclude that a simpler protocol (40-minute application) produces the same bleaching effectiveness with no increase in the risk and intensity of TS compared with two 20-minute applications.

**CONCLUSIONS**

The use of one 40-minute in-office bleaching agent gel produced the same whitening degree and TS
associated with two 20-minute applications. The former preferably should be applied because one 40-minute application does not require gel refreshing.

Acknowledgements

The authors of this study would like to thank Ultradent Brazil for donating the bleaching gels employed in this study. This study was partially supported by the National Council for Scientific and Technological Development (CNPq) under grants 304105/2013-9 and 305588/2014-1 from Brazil and the University of Santiago de Cali under grant 447.621.014.019 from Colombia. This study was developed during the Visiting Professor Scholarship of Dr Alessandro D. Loguercio in the Federal University of Amazonas (Edital 019/2013 à FAPEAM).

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Federal University of Amazonas and Santiago de Cali University. The approval codes for this study were 1.178.283 and 441.014.019, respectively.

Conflict 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 article.

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