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The Remineralizing and Desensitizing Effect of Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) Versus Fluoride Cream Application Following the Use of Lumacool (Teeth Whitening Pen) on Premolars: An Ex-Vivo Trial

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ABSTRACT

Aim: This study sought to compare the remineralizing and desensitizing capabilities of case in phospho-peptide-amorphous calcium phosphate (CPP-ACP) and fluoride cream when applied after Lumacool (a teeth whitening pen) on premolars. **Subjects and methods:** Fifty-six stained sound human premolars were divided into four equal groups and bleached with different agents. Patient tooth sensitivity was assessed using the Visual Analogue Scale (VAS) at 1, 7, 14, and 28 days post-application. Teeth were subsequently extracted and prepared for scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and Vickers micro-hardness testing. Morphological analysis was conducted using SEM, and statistical analysis of paired (matched) data was performed with the Wilcoxon signed rank test. **Results:** highest mean values were observed at day 28 in Groups 3 and 4 (6.14 ± 1.86 and 6.14 ± 2.04, respectively), while the lowest was in Group 1 at day 1 (0.43 ± 0.54). SEM examination of the enamel surface revealed surface irregularities, cracks, and scratches in the hydrogen peroxide groups, contrasting with the relatively smooth surface in the placebo group. Enamel micro-hardness significantly decreased ($p < 0.05$) in all groups, with Group 2 and Group 4 being the most affected ($p = 0.018$). Group 1 showed a moderate decrease ($p = 0.043$), while Group 3 exhibited minimal change ($p = 0.866$). **Conclusion:** Hydrogen peroxide is effective for bleaching. Calcium supplementation improves tooth properties and reduces sensitivity. Fluoride is effective for desensitization. Calcium and fluoride combined offer limited benefits compared to individual use.

KEYWORDS

Enamel, hydrogen peroxide, calcium, fluoride, color change, teeth sensitivity, VAS, micro-hardness.

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INTRODUCTION

Dental bleaching has witnessed a significant increase in popularity among patients, clinicians, and researchers in recent years.[1,2] Due to its minimally invasive nature, affordability, and high patient satisfaction rates, bleaching is often the preferred treatment for extrinsic tooth discoloration.[3,4] Compared to more invasive procedures like crowns and ceramic veneers, bleaching offers a conservative, safe, and effective approach to whitening teeth. [5]

The American Dental Association (ADA) recognized the growing popularity of teeth whitening in 2010, highlighting it as the most controlled methodology in cosmetic dentistry.[6] This trend was further supported by market data, with over 100 million Americans reportedly using various teeth whitening procedures in 2010, generating a market size of $15 billion. A 2008 survey by Krupp (2008) also confirmed this popularity, with 32% of respondents identifying teeth whitening as the most popular aesthetic dental treatment. [7]

Teeth whitening techniques vary widely, with three primary methods: dentist-supervised at-home bleaching, in-office bleaching, and combined techniques. While at-home bleaching offers advantages such as affordability, convenience, and minimal invasiveness, it may also lead to enamel damage, tooth sensitivity, and compromised physical properties.[8,9] In-office bleaching, on the other hand, provides more rapid results but can increase the risk of tooth sensitivity and structural defects.[10] Combined techniques, which involve both in-office and at-home treatments, aim to balance the benefits of both approaches, offering faster results while minimizing potential side effects.

Hydrogen peroxide (H_2O_2) plays a crucial role in successful tooth bleaching due to its diffusion capacity within the tooth structure. Research by Wang et al. (2015) and Woodhouse (2021) highlights the importance of concentration and application time control to minimize potential negative effects of this potent chemical.[11,12] Tooth sensitivity remains a common side effect, typically lasting 4-7 days post-treatment. [13] This highlights the concerns raised by the UK General Dental Council in 2010 regarding patient safety risks associated with unsupervised or poorly administered whitening procedures.[12] The whitening effect itself stems from H_2O_2 decomposition, generating free radicals that interact with color pigments (chromophores) within the dentin. The low molecular weight of H_2O_2 and its derivatives, combined with the porous nature of enamel and dentin, facilitate their diffusion throughout the tooth structure, contributing to the bleaching process. [14]

The bleaching agent penetrates the tooth structure, releasing reactive oxygen species (ROS) that can diffuse to the pulp chamber, causing tooth sensitivity.[15] Factors such as peroxide concentration, contact time, enamel and dentin thickness, and the presence of restorations influence the extent of penetration. To minimize sensitivity, a primary adverse effect of bleaching, it is crucial to use low-concentration oxidizing agents. [16, 17]

Tooth sensitivity is a common side effect of teeth bleaching, often hindering treatment.[18] Casein milk protein has been suggested as a potential remedy. Studies have shown that higher bleaching gel concentrations and hydrogen peroxide can increase sensitivity. [19] The aim of this study is to investigate the remineralizing and desensitizing effects of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) versus fluoride cream application following the use of Lumacool (teeth whitening pen) on premolars.

PATIENTS AND METHODS

Fifty-six sound human premolars were obtained from patients aged 18-50 at the Orthodontic Department, Faculty of Dentistry, Kafr El-Sheikh University, Egypt, with their informed consent.

1. Shade Recording and Drying

Baseline tooth shade was documented under a standardized light source to minimize color discrepancies (metamerism) using a shade guide. Patient's mouths were then dried using cotton rolls around the stained tooth. Buccal surfaces were dried for 10-15 seconds with a brush in a circular motion, followed by a 30-minute period with no eating or drinking. This drying procedure was applied to treated and placebo teeth in Group 1, as well as treated teeth with calcium and fluoride cream in Groups 2, 3, and 4. The cream application in these groups lasted 3 minutes, followed by a 30-minute period without brushing, repeated twice daily (9 am and 9 pm).

2. Tooth Sensitivity and Shade Evaluation

After 14 days, the investigator (blinded) recorded clinical tooth sensitivity using a visual analogue scale (VAS) and documented the final tooth shade with the shade guide.

3. Sample grouping

The twenty-eight volunteers with 56 stained premolars were randomly divided into four groups. All groups received lumacool whitening pen twice daily for 28 days. Group 1 served as a control, receiving a placebo on the contralateral side. Groups 2, 3, and 4 received additional treatments on the contralateral side: calcium phosphate (CPP-ACP) in group 2, fluoride cream in group 3, and both CPP-ACP and fluoride cream in group 4. Teeth sensitivity was recorded using a Visual Analogue Scale (VAS) at 1, 7, 14, and 28 days. At each time point, the treated teeth were assessed using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), Vickers' microhardness testing, and VAS.

4. Randomization and allocation

The study employed simple randomization. A random sequence generator program from random.org was used to randomly assign each patient to one of the four groups. Two independent observers, blinded to the treatment groups, scored the data. Participants and personnel responsible for blinding included: Assessor of scanning electron microscope results Assessor of energy dispersive X-ray spectroscopy results.

5. Sample preparation

Thirty-two teeth were prepared for analysis using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX).[20] Additionally, 24 teeth were subjected to microhardness testing using a Vickers microhardness tester.

6. Data collection

The study employed several methods to assess the effects of the bleaching treatments.

1. ***Color Measurement:*** Baseline tooth color was recorded under a standardized light source using a shade guide to minimize the influence of metamerism and facilitate evaluation of bleaching results and patient satisfaction.
2. ***Surface Micro-Hardness Measurement:*** The micro-hardness of 24 specimens was measured using a Vickers Micro-Hardness Tester (Figure 1). A 200g load was applied for 20 seconds at three locations on each sample surface. The diagonal lengths of the indentations were measured, and Vickers hardness values were calculated. The mean of the three measurements represented the enamel surface micro-hardness for each specimen. Readings were taken from the middle third of each specimen.
3. ***Visual Analogue Scale (VAS):*** Participants used a 10-point VAS to report their pain levels (0 = no pain, 10 = worst pain) along with the type of sensitivity (hot, cold, or other). The duration of pain was recorded throughout the 28-day follow-up period (days 1, 7, 14, and 28). For analysis, pain severity was categorized as follows: 0 = none, 1-3 = mild, 4-6 = moderate, and 7-10 = severe.
4. ***Scanning Electron Microscopic Examination (SEM):*** To prevent interference during scanning, 32 samples were gold-coated. The enamel surface was examined at magnifications

of 1000x and 2000x using a Scanning Electron Microscope (SEM) (Figure 2) at the Faculty of Agriculture, Mansoura University. Qualitative changes in surface topography were assessed.

5. ***Energy Dispersive X-ray Analysis (EDX):*** The elemental composition of the enamel surface was quantitatively analyzed using EDX (Figure 3). This technique involves bombarding the sample with a high-energy electron beam, causing the emission of characteristic wavelengths for each element. Changes in the emitted wavelengths reflect alterations in the elemental concentration on the sample surface. The EDX system is an integrated component of the SEM and cannot function independently.

7. Sample size calculation

Sample size was estimated as 7 volunteers with 14 teeth per each group & total 28 volunteers with 56 teeth for all research groups.

Statistical analysis

Data were statistically analyzed using descriptive statistics, including mean ± standard deviation (SD) or median and range as appropriate. To compare the active and control groups, a Wilcoxon signed rank test for paired (matched) data was employed. Two-sided p-values less than 0.05 were considered statistically significant. All statistical calculations were performed using IBM SPSS (Statistical Package for the Social Sciences; IBM Corp, Armonk, NY, USA) version 22 for Microsoft Windows.

Ethical consideration

This study was conducted at the Laboratory of the Dental Department, Kafr El-Sheikh University, with ethical approval from the Ethics Committee of the Faculty of Dentistry, Cairo University No: 9720 **(Supplementary file 1)**.

RESULTS

SEM

Scanning electron microscopy (SEM) was employed to compare the surface morphology of normal and treated enamel in four experimental groups. Specimens were examined at magnifications of 1000x and 2000x. The placebo group exhibited a relatively smooth enamel surface with an aprismatic layer, characterized by few rod ends, scratches, and pores (Figs. 4A & 4B). Luma cool gel treatment resulted in a loss of the aprismatic layer, with increased rod ends, scratches, irregularities, and microporosities (Figs. 4C & 4D). The application of luma cool alone revealed surface cracks, scratches, pores, and aprismatic areas with numerous depressions (Figs. 4E & 4F). Finally, luma cool with calcium showed aprismatic areas, pores, and small concave depressions (Figs. 4G & 4H).

Following the application of luma cool alone, SEM examination revealed surface irregularities, depressions, micropores, cracks, and areas of aprismatic enamel (Figs. 5A & 5B). When fluoride was added to luma cool, similar findings were observed, including surface irregularities, depressions, cracks, and aprismatic enamel (Figs. 5C & 5D). Luma cool application in Group 4 resulted in surface scratches, depressions, irregularities, microporosities, and aprismatic enamel (Figs. 5E & 5F). The addition of calcium and fluoride to luma cool led to the presence of scratches, cracks, rod ends, few microporosities, and aprismatic enamel areas (Figs. 5G & 5H).

EDX

A comparative analysis of calcium (Ca) and phosphorus (P) weight percentages (wt%) was conducted among the four experimental groups. The results demonstrate that Group 2 (H_2O_2+Ca) exhibited the highest mean Ca wt% (38.24±2.75), while Group 2 (H_2O_2 Only) recorded the lowest (21.79 ± 2.80). Wilcoxon signed rank tests revealed statistically significant differences in Ca wt% among all groups ($P<0.05$). Similarly, Group 2

(H2O2 + Ca) had the highest mean P wt% (18.70 ± 2.03), followed by Group 2 (H2O2 Only) with the lowest (12.81 ± 1.89). Wilcoxon signed rank tests also indicated statistically significant differences in P wt% among the groups ($P < 0.05$).

Surface Micro-hardness

Analysis of surface micro-hardness (VMH) revealed significant differences ($P < 0.05$) among the groups. Group 1 (H2O2 only) exhibited the highest mean VMH (426.5 ± 8.33), while Group 4 (H2O2 + Ca + F) displayed the lowest (333.8 ±8.37).

VAS Score

VAS scores exhibited significant changes within each group over time ($p < 0.05$). Across all groups, the lowest mean scores were observed at day 1 with placebo treatment (Group 1: 0.43±0.54, Group 2: 0.57±0.54, Group 3: 0.86±0.69, Group 4: 0.71±0.76), indicating minimal initial sensitivity. Conversely, the highest mean scores within each group were recorded at day 28, suggesting a potential increase in sensitivity following treatment (Group 1: 5.71 ± 1.80, Group 2: 4.29±2.50, Group 3: 6.14 ± 1.86, Group 4: 6.14 ± 2.04). Interestingly, Group 4 displayed the same mean VAS score at day 1 for both placebo and H2O2 treatments, suggesting no initial difference in sensitivity between these conditions. Further investigation is warranted to elucidate the specific effects of each treatment on VAS scores over time.

DISCUSSION

Premolar teeth extracted from orthodontic crowding cases (free of caries and restorations) were used for this study. To ensure uniform enamel thickness, measurements were taken at the middle third of the buccal surface. Following Poggio et al. (2009), specimens were cleaned with 5.25% sodium hypochlorite for one hour, a method confirmed not to alter the enamel surface. [21] Teeth were then stored in distilled water, adhering to Ribeiro et al.'s (2019) recommendation for maintaining tooth structure integrity. [4]

This study aimed to compare the remineralizing and desensitizing effects of CPP-ACP versus fluoride cream following Lumacool (at-home bleaching pen) application on premolars. We evaluated the impact of these agents on color change, micro-hardness, mineral content, and tooth sensitivity, with a focus on the influence of calcium or fluoride addition to the H2O2 bleaching gel on sensitivity levels.

Our results align with Sulieman et al. (2006), Meireles et al. (2010, 2012), and Cvikl et al. (2017), demonstrating noticeable whitening effects with various peroxide concentrations. [22-25] However, unlike most in vitro studies investigating bleaching effects (e.g., roughness, micro-hardness), we incorporated an in vivo element to assess tooth sensitivity. Our findings revealed significant tooth sensitivity during and after bleaching with 7.5% hydrogen peroxide (highest VAS scores in Groups 3 and 4 at day 28). This contradicts Renato Herman et al. (2014) who reported effective bleaching with 10% hydrogen peroxide and minimal sensitivity. [26] Similarly, Alexandra et al. (2022) and Gousalya et al. (2023) suggested a trade-off between higher bleaching efficacy and sensitivity with increased peroxide concentration.[27,28] We propose further investigation into this discrepancy. Notably, we concur with K. Chemin et al. (2018) that at-home bleaching is effective with 4% and 10% hydrogen peroxide, but the latter increases sensitivity risk as in Ghidaa Yahya et al., 2022. [26, 29]

Furthermore, our study supports the addition of fluoride to mitigate sensitivity. Fluoride application significantly reduced sensitivity compared to the placebo group, aligning with Wang et al. (2015), ZYF Alkhateeb et al. (2023), and Armenio et al. (2008).[11,30,31] While Elize Bonafé et al. (2014) found no decrease in sensitivity incidence with pre-bleaching fluoride gel, our results suggest a substantial reduction in both incidence and severity (VAS scores mostly zero or one). [32]

Commercial bleaching gels often incorporate calcium or fluoride to mitigate potential adverse effects. As home bleaching becomes increasingly popular, evaluating the effectiveness of these remineralizing and desensitizing agents is crucial.[33,34] Prior studies support their benefits: Borges et al.[35] demonstrated increased enamel hardness with CPP-ACP combined with bleaching agents, while Alexandrino et al. (2017) observed reduced sensitivity when using CPP-ACP post-bleaching. [36] We concur with these findings, suggesting CPP-ACP addition to H2O2 improves hardness and reduces sensitivity.

Desensitizing agents have been shown effective in reducing in-office bleaching sensitivity,[37] but their efficacy in home bleaching, our focus, remains unclear. Michael G Jorgensen et al. (2002) reported significant post-bleaching sensitivity with 15% carbamide peroxide home bleaching without desensitizers.[38] Our findings partially align, with nearly 50-60% of participants experiencing mild sensitivity (lowest in placebo) and a small percentage experiencing moderate or severe sensitivity (highest in the group using H2O2 gel only). Notably, calcium and fluoride incorporation significantly reduced sensitivity, as evidenced by VAS scores indicating mostly mild sensitivity with minimal moderate cases.

This study employed SEM and EDX analyses to evaluate surface changes and elemental composition.[16] While the effects of bleaching agents on mineral loss and surface morphology remain somewhat controversial[39,40], our SEM observations ranged from unchanged to slight pitting and porosity, potentially influenced by the bleaching agent and application regime.[6,41-43] Further investigation is needed to elucidate the precise mechanisms by which these agents influence sensitivity and surface morphology.

This study evaluated surface changes and mineral composition in enamel following exposure to various bleaching agents. The placebo group displayed no morphological alterations, while groups treated with 7.5% hydrogen peroxide exhibited dose-dependent effects. Longer treatment durations (28 days) resulted in more prominent features like small pores, depressions, irregularities, cracks, and enamel erosion. These findings align with Orilisi et al. (2021), who demonstrated similar surface modifications on enamel with high H2O2 concentrations. [44]

Our observations contradict Oltu and Gürgan (2000) who reported minimal structural changes with low carbamide peroxide concentrations.[40] This discrepancy may be attributed to differences in storage media (artificial saliva vs. ex vivo teeth) as suggested by Vilhena et al. (2019). Fearon et al. (2007) further support the notion that high peroxide concentrations can lead to structural changes and increased tooth sensitivity. [45,46]

Mineral content analysis using EDX revealed a significant decrease in calcium and phosphorus in all treatment groups except placebo. These results are consistent with Cavalli et al. (2011, 2018) and Soares et al. (2017), who observed similar reductions following 10% hydrogen peroxide bleaching.[16,47] This decrease can be explained by the dissociation of hydrogen peroxide into free radicals.[48] Interestingly, Do Amaral et al. (2012) found no differences in mineral content between home and in-office bleaching, highlighting the need for further investigation. [49]

Surface micro-hardness analysis confirmed the observed morphological and compositional changes. All treated groups exhibited a significant decrease in micro-hardness, with the hydrogen peroxide and calcium/fluoride combination group being the most affected. However, we disagree with their findings on the combined effect of CPP-ACP and fluoride, as we observed no changes in hardness with this combination. Our results support Loguercio et al. (2017), Grazioli et al. (2018), and Rodríguez-Martínez et al. (2019) who reported increased hardness after remineralization with CPP-ACP and fluoride.[18,50,51] This discrepancy might be due to variations in application protocols. Maia et al.[52] also reported no significant differences in microhardness with home bleaching agents, suggesting that application strategy (time and frequency) might be more influential than peroxide concentration alone.

CONCLUSION

Hydrogen peroxide is effective for bleaching but can cause adverse effects with excessive use. Calcium supplementation improves tooth properties and reduces sensitivity. Fluoride is effective for desensitization. Calcium and fluoride combined offer limited benefits compared to individual use. Further research is needed to optimize their application.

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تأثير إعادة التمعدن وإزالة الحساسية لفوسفات الكازين الفوسفو ببتيد-فوسفات الكالسيوم غير المتبلور (CPP-ACP) مقارنةً بتطبيق كريم الفلورايد بعد استخدام لوماكول (قلم تبييض الأسنان) على الضواحك: تجربة خارج الجسم الحي

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الملخص :

الهدف: سعت هذه الدراسة إلى مقارنة قدرات إعادة التمعدن وإزالة التحسس لـ CASE في فوسفات الكالسيوم الفوسفوري الببتيدي غير المتبلور (CPP-ACP) وكريم الفلورايد عند تطبيقه بعد استخدام LUMACOOL (قلم تبييض الأسنان) على الضواحك.

المواد والاساليب: قُسّمت ستة وخمسون ضرسًا بشريًا سليمًا مصبوغًا إلى أربع مجموعات متساوية، وطُبِّخَت بمواد مختلفة. قُيِّمت حساسية أسنان المريض باستخدام مقياس التناظر البصري (VAS) بعد 1 و7 و14 و28 يومًا من الاستخدام. بعد ذلك، استُخرجت الأسنان وجُهِّزت للفحص المجهري الإلكتروني الماسح (SEM)، ومطيافية الأشعة السينية المشتتة للطاقة (EDX)، واختبار صلابة فيكرز الدقيقة. أُجري تحليل مورفولوجي باستخدام المجهر الإلكتروني الماسح، وأُجري تحليل إحصائي للبيانات المقترنة (المتطابقة) باستخدام اختبار رتبة ويلكوكسون الموقعة.

النتائج: لوحظت أعلى القيم المتوسطة في اليوم 28 في المجموعتين 3 و4 (6.14 ± 1.86 و6.14 ± 2.04 على التوالي)، بينما كانت أقل قيمة في المجموعة 1 في اليوم 1 (0.43 ± 0.54). كشف فحص المجهر الإلكتروني الماسح لسطح المينا عن وجود مخالفات سطحية وشقوق وخدوش في مجموعات بيروكسيد الهيدروجين، على عكس السطح الأملس نسبيًا في مجموعة الدواء الوهمي. انخفضت صلابة المينا الدقيقة بشكل ملحوظ ($P < 0.05$) في جميع المجموعات، وكانت المجموعة 2 والمجموعة 4 الأكثر تأثرًا ($P = 0.018$). أظهرت المجموعة 1 انخفاضًا معتدلًا ($P = 0.043$)، بينما أظهرت المجموعة 3 تغيرًا طفيفًا ($P = 0.866$).

الخلاصة: بيروكسيد الهيدروجين فعال في التبييض. تعمل مكملات الكالسيوم على تحسين خصائص الأسنان وتقليل الحساسية. الفلورايد فعال في إزالة التحسس. يقدم الكالسيوم والفلورايد معًا فوائد محدودة مقارنة بالاستخدام الفردي

الكلمات المفتاحية : مينا الأسنان، بيروكسيد الهيدروجين، الكالسيوم، الفلورايد، تغير اللون، حساسية الأسنان، VAS، صلابة دقيقة