

Cold Atmospheric Plasma for Dental CAD/CAM Materials: Bleaching Efficacy and a Preventive Approach to Discoloration

Gizem Dilara Özdemir,^{a,b} Şeyma Ecem Irmak,^b Büşra Kiş,^b Bora Bağış,^c & Utku Kürşat Ercan^{a,*}

^aDepartment of Biomedical Engineering, Faculty of Engineering and Architecture, Izmir Katip Celebi University, Izmir, Turkey; ^bDepartment of Biomedical Engineering, Graduate School of Natural and Applied Sciences, Izmir Katip Celebi University, Izmir, Turkey; ^cDepartment of Clinical Sciences, Faculty of Dentistry, Istanbul Health and Technology University, Istanbul, Turkey

*Address all correspondence to: Utku Kürşat Ercan, Department of Biomedical Engineering, Faculty of Engineering and Architecture, Izmir Katip Celebi University, Izmir, Turkey, Tel.: +90 232 329 3535 (3747), E-mail: utkuk.ercan@ike.edu.tr

ABSTRACT: Tooth bleaching is a common aesthetic dental procedure, traditionally performed using hydrogen peroxide (H_2O_2). With the growing use of dental prosthetic materials, their discoloration and aesthetic maintenance have become increasingly important. However, conventional bleaching methods may be ineffective or may compromise their surface properties. This study evaluates the bleaching efficacy of cold atmospheric plasma (CAP) on stained computer-aided design/computer-aided manufacturing (CAD/CAM) prosthetic materials: CEREC (a feldspathic glass ceramic) and Cerasmart (a nanohybrid ceramic in a resin matrix). Samples were stained with coffee to simulate clinical discoloration. Optimization studies identified 10 min as the optimal CAP exposure time. Three bleaching protocols were tested: 35% H_2O_2 alone, CAP alone, and CAP co-activated with 35% H_2O_2 . Results showed that CAP alone achieved comparable bleaching to the combined method for Cerasmart, while CEREC showed slightly better results with the combined treatment. CAP treatment also reduced restaining compared with H_2O_2 alone. CAP pretreatment had a preventive effect against discoloration, particularly Cerasmart. Surface analyses revealed minimal roughness changes in Cerasmart, whereas CEREC showed increased roughness. Bacterial adhesion was not adversely affected by CAP. Overall, CAP presents a promising alternative to conventional bleaching methods, offering effective whitening with minimal impact on surface properties and microbial behavior.

KEY WORDS: plasma medicine, dental materials, bleaching, discoloration, pretreatment

I. INTRODUCTION

Nowadays, demand for dental applications is on the rise due to health problems such as fractures, caries, and inadequate restorations.¹ Prosthetic restorations play a crucial role in reinstating the functionality of lost tooth and tissues while addressing aesthetic and phonological concerns.² Dentures, crowns, and bridges can be crafted from a variety of materials, including alloys, resins, ceramics, and hybrids.³ Preferred materials are expected to have properties such as wear and fracture resistance, biocompatibility, low roughness, and resistance to plaque adhesion.⁴ Additionally, prosthetic materials

are chosen based on their ability to closely match the natural tooth color, with ceramic emerging as the most favored choice.⁵

Traditionally, prostheses were crafted manually by technicians who took impressions from the patient.⁶ However, contemporary methods involve digital computer-aided design/computer-aided manufacturing (CAD/CAM) systems.⁷ CAD/CAM technology is a widely adopted and clinically established workflow in prosthetic dentistry, particularly for the fabrication of indirect restorations. In subtractive digital workflows, patient-specific prosthetic components are typically milled from prefabricated CAD/CAM blocks composed of materials such as zirconia, lithium disilicate, or polymethylmethacrylate.⁸ This approach offers enhanced efficiency and speed in producing prostheses with optimal durability and a natural appearance, thanks to CAD/CAM technology and industrially prepared materials featuring superior mechanical properties.⁹ In *in vitro* materials research, however, these CAD/CAM materials are commonly prepared as standardized specimens (e.g., discs or flat sections) to provide uniform test surfaces and to minimize geometry-related variability during surface and bonding analyses.¹⁰ While initial CAD/CAM-produced materials were characterized by low resistance, recent developments cater to diverse restoration needs by offering materials with varied strength, flexibility, and aesthetic properties.¹¹

The consumption of tobacco, caffeine, and colored beverages in daily life is known to alter tooth color, influenced not only by dietary habits but also by the inherent structural properties of teeth.¹² Concerns about the aesthetic impact of tooth discoloration have led to a surge in tooth bleaching applications, with various methods gaining widespread popularity in recent years.¹³ Tooth bleaching techniques, categorized as in-office and at-home, involve the breakdown of peroxides in the bleaching agent into free radicals. This oxidative process aims to eliminate stains by interacting with the tooth surface.¹⁴ Similar to the natural tooth, materials employed in prosthetic and restorative applications may undergo discoloration over time when exposed to substances like cigarettes, coffee, tea, red wine, and mouthwashes.¹⁵ Discoloration of CAD/CAM prosthetic materials can result from incomplete polymerization, water absorption, chemical reactivity, plaque accumulation, surface staining, and superficial deterioration.¹⁶ Although surface discoloration can be fixed by polishing, discoloration in deeper layers is often irreversible.¹⁷ Since most prosthetic materials do not have color stability, prostheses are renewed. During restoration, the discolored prostheses are removed from the mouth and the natural teeth are bleached using conventional tooth bleaching methods to restore aesthetic integrity.¹⁸ After waiting for the new tooth color to settle and for the requirements of the adhesive processes to be fulfilled, the color of the CAD/CAM prosthesis to be fabricated is decided and the process continues. This long period of prosthetic replacement can be a major disadvantage, as it reduces the patient's quality of life for some time.¹⁹

Researchers have explored the impact of traditional tooth bleaching methods on CAD/CAM materials with natural tooth color and translucency to address patients' aesthetic concerns and enhance their overall quality of life.²⁰ These investigations have involved varying concentrations of bleaching agents to different prosthetic materials and analyzing both their bleaching effects and their adverse impacts on the material surface.

Findings indicate that peroxide concentrations commonly used in clinical settings potentially have a negative effect on surface roughness and may compromise overall surface integrity.^{21,22} Diminished surface smoothness in CAD/CAM restorative materials may elevate the risk of bacterial adherence,²³ potentially leading to increased surface staining.²⁴ Recognizing that surface roughness can contribute to a lackluster and unappealing appearance, it becomes crucial to polish anterior restorations.²⁵ Hence, ongoing research is exploring novel methods with better bleaching efficacy but without surface damage.

Plasma, defined as the fourth state of matter, is formed under an electrical field, which leads to ionization of the gas via removal of electrons from orbitals due to electrical energy.²⁶ Cold atmospheric plasma (CAP) is a partially ionized gas produced at atmospheric pressure and approximately room temperature, demonstrating a thermal nonequilibrium between the removed electrons and the heavy particles. Thus, it can be safely applied to biological tissues and biomaterials without causing thermal damage.²⁷ In the dental field, CAP contributes positively to material performance, durability, and longevity by strengthening the bond between dentin and composite resins in restorative dentistry.²⁸ Its versatility extends to biofilm removal, osseointegration enhancement, management of intraoral diseases, adhesive processes, and root canal disinfection.²⁹ The establishment of effective adhesion between prosthetic material and supporting dental tissues is crucial for the sustained clinical success of restorations.³⁰ Moreover, CAP has been used for the bleaching of stained teeth.³¹ Therefore, CAP might be an alternative method for the bleaching of stained dental prosthetic materials.

This study evaluated the bleaching effect of CAP treatment on two CAD/CAM prosthetic materials. Furthermore, it assessed CAP pretreatment on CAD/CAM prosthetic materials to evaluate its efficacy in preventing discoloration.

II. MATERIALS AND METHODS

A visual representation of the proposed methodological framework is given in Fig. 1. A comprehensive explanation of each stage is provided in the following sections.

A. Preparation of CAD/CAM Materials

Two commonly employed CAD/CAM prosthetic blocks in dentistry—Dentsply-Sirona CEREC Block 14 A1C (S1-M, feldspathic, glass-ceramic material) and GC Cerasmart 270 A1 HT 14 (nanohybrid-ceramic material), with a size of 18 × 12 × 14 mm—were used for the study. A low-speed cutting device (Isomet, Buehler, Lake Bluff, IL) was employed to cut the blocks into 2-mm-thick samples. The samples underwent manual polishing using sandpaper with grits of 400, 600, 800, and 1,200 to eliminate all cutting-induced surface defects.³² Following the polishing process, the samples were meticulously cleaned and dried. Subsequently, the experimental groups were organized, and the ready-to-use CAD/CAM materials were placed in 24-well plates supplemented with 3 mL of deionized water (DIW), with the treatment surface facing upward, and maintained at 37°C for 24 h. Images were captured using a custom-made imaging setup.

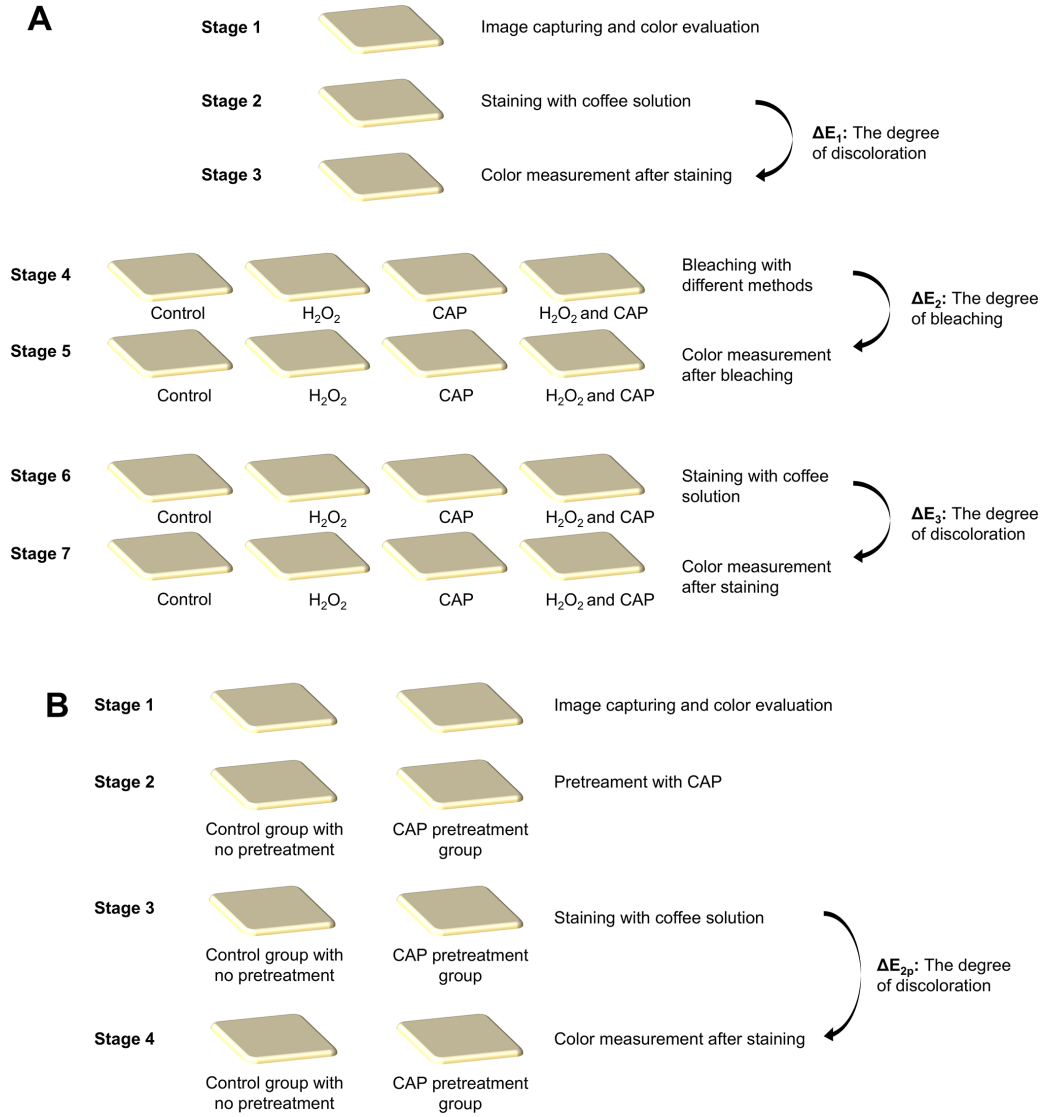


FIG. 1: Proposed methodological structure of the study. Investigating the effects of (A) different bleaching methods; and (B) CAP pretreatment on discoloration prevention in prosthetic materials.

B. Imaging Setup

A custom setup was arranged for capturing images to assess the color characteristics of prosthetic materials. A cardboard box with dimensions of $38.5 \times 11.5 \times 26.5$ cm was prepared to minimize external influences on the processed images and to ensure standardization. The box's inner surface was entirely coated with Munsell N7 ($L^* = 72.18$, $a^* = -1.74$, $b^* = -0.62$) neutral gray dye, serving as a background for assessing color

changes in the samples.³³ A 5,500-K color-corrected light source, simulating midday sun conditions for optimal color determination,³⁴ was affixed as a ring-shaped source to the large inner surface of the cardboard box. A hole, matching the lens size of a Nikon Coolpix L340 camera (Nikon, Shinagawa, Tokyo), was drilled through the center of the same surface to prevent shadows, as shown in Fig. 2B.

Data from the CIE Lab [Commission Internationale de l'Eclairage (International Commission on Illumination)] were predominantly used for determining changes in tooth color. The CIE Lab color space, relying on three parameters (L^* for lightness, a^* for redness-greenness, and b^* for yellowness-blueness), is preferred for its accuracy and reproducibility, providing not only object color but also details about light and the observer.³⁵ Following image capture, a specific MATLAB toolbox (the code is available online) was utilized for image processing and determining L^* , a^* , and b^* values, representing coloration in different experimental groups. Overall color change after each procedure (staining, bleaching, and pretreatment with different methods) was defined as ΔE and was calculated using Eq. (1).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

ΔL , Δa , and Δb represent the change in each color component after staining or bleaching.

C. Staining of CAD/CAM Materials

Following image capture, the samples underwent staining using a solution of 2 g instant coffee dissolved in 100 mL of boiling water without additives. Each well received 3 mL of the staining solution, which was renewed every 24 h for 7 days.³⁶ Subsequently, the stained samples underwent a rinse with 10 mL of DIW to eliminate residual coffee on the surface before imaging. The color of the samples was assessed before and after

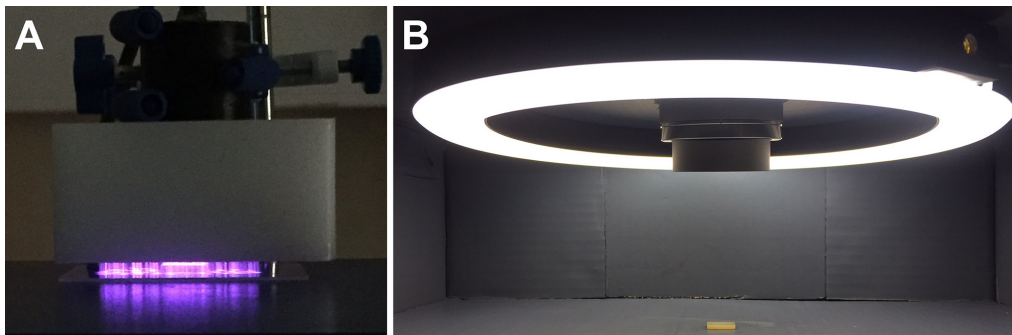


FIG. 2: (A) Representative image of CAP treatment on CAD/CAM materials; and (B) inner part of imaging setup taken after application

staining using the described imaging setup, and the color values before and after staining were recorded as L_0 , a_0 , b_0 , and L_1 , a_1 , b_1 , respectively. The degree of discoloration in the samples was defined as ΔE_1 and calculated using Eq. (1). The ΔE_1 values determined for CEREC and Cerasmart were 9.45 and 12.98.

D. Optimization of CAP Parameters

An optimization experiment was conducted to determine the optimal CAP treatment time for the bleaching experiments. A microsecond dielectric barrier discharge plasma power supply with a 31.6-kV peak-to-peak voltage and 2.5-kHz frequency settings operated in ambient air with a 10- μ s pulse duration and an output power of 13.5 W. All treatments were performed with a 2-mm discharge gap, as previously employed by our group in another study²⁸ where the effect of CAP treatment on shear bonding strength was evaluated. The CAD/CAM samples were stained as explained above. 50 μ L of 35% hydrogen peroxide (H_2O_2) bleaching agent was spread on the stained samples and treated with CAP for 0, 1, 3, 5, 7, and 10 min. Three replicates ($n = 3$) were carried out for each group in both CAD/CAM materials.³¹ The colors of the samples were determined before and after treatment. The colors of stained and bleached samples were recorded as L_{10} , a_{10} , b_{10} , and L_{20} , a_{20} , b_{20} , respectively. Bleaching efficacy was calculated using Equation 1 and defined as ΔE_{20} (o defines the optimization experiments).

E. Bleaching of CAD/CAM Materials

Stained CAD/CAM samples designated for bleaching underwent three distinct bleaching treatments: 35% H_2O_2 , CAP, and 35% H_2O_2 co-activated with CAP. For the first group, 100 μ L of DIW was spread on stained samples and subjected to CAP for 10 min. For the second treatment group, 50 μ L of 35% H_2O_2 was applied according to the manufacturer's recommendations and left on the surface for 10 min. For the third group, 50 μ L of 35% H_2O_2 was evenly distributed on the surface and immediately treated with CAP for 10 min. The colors of the stained and bleached samples were recorded as L_1 , a_1 , b_1 , and L_2 , a_2 , b_2 , respectively. Bleaching efficacy was calculated using Equation 1 and defined as ΔE_2 .

To determine the stability of different bleaching methods, the bleached samples underwent restaining following the previously outlined procedure. After restaining, images of the samples were taken and recorded as L_3 , a_3 , b_3 . The degree of discoloration in the samples was calculated using Eq. (1) and defined as ΔE_3 .

F. Evaluation of Discoloration Prevention via CAP

A pretreatment investigation was carried out to assess the potential of CAP in preventing discoloration. Prior to CAP pretreatment, the samples were immersed in DIW for 24 h, and images of the samples were captured and recorded as L_{1p} , a_{1p} , and b_{1p} . Subsequently, 100 μ L of DIW was spread on the surface of the materials, which underwent a 10-min

pretreatment. Following pretreatment, the samples underwent staining with coffee, as previously detailed, lasting for 7 days. After staining, images of the samples were captured and recorded as L_{2p} , a_{2p} , and b_{2p} . Bleaching efficacy was calculated using Equation 1 and defined as ΔE_{2p} (p defines the pretreatment experiments).

G. Surface Analysis

Scanning electron microscopy (SEM) imaging and a profilometer were used to assess alterations in the surface properties of the samples caused by the three bleaching procedures. In the images, the stained samples underwent coating with gold (QUORUM Q150R Plus, Sussex, UK) with an approximate thickness of 2 nm before being placed in the SEM device (300VP, Carl Zeiss, Oberkochen, Germany). Images were captured at various magnifications with 5-kV acceleration. The impact of the bleaching methods on surface roughness was investigated using a profilometer (Mitutoyo SurfTest, SJ-210, Kanagawa, Japan). Following cleaning, a 5- μ m diamond tip with a scanning length of 500 μ m and a scanning speed of 100 μ m/s measured surface roughness in five randomly selected areas on each treated surface.

Contact angle measurements were taken from the two CAD/CAM materials. Four microliters of distilled water droplets were dispensed over the 10-min CAP-treated samples, and images were obtained. The ImageJ tool was used for analyzing the contact angle of the droplets over the samples.

H. Bacterial Adhesion on CAD/CAM Materials

The impact of various bleaching methods on bacterial adhesion to the surfaces of the CAD/CAM materials was assessed using *Staphylococcus aureus* (ATCC 25923). Bleached materials were placed in 12-well plates, and 2 mL of biofilm culture was introduced. It was prepared by combining 100 μ L of an overnight-incubated *S. aureus* suspension with 9.9 mL tryptic soy broth (TSB). The samples underwent incubation for 6 and 24 h. Following incubation, samples were taken out of the wells and subjected to a triple wash with 1X sterile phosphate-buffered saline (PBS) to eliminate unattached bacteria. Subsequently, the samples were transferred into 2 mL PBS, where vortexing was performed for the quantification of adhered bacteria using a colony-counting assay.

I. Statistical Analysis

Statistical analysis was conducted utilizing GraphPad Prism software (Prism v10.1.2). The results were presented as mean $\pm$ standard deviation. The Shapiro-Wilk normality test was employed to ascertain the normal distribution of numerical variables. Parametric data underwent analysis with ANOVA, while nonparametric data were assessed using the Kruskal-Wallis test (as specified by $n = 8$). A p -value of $p < 0.05$ was considered in determining statistical significance.

III. RESULTS

A. Optimization

Cerasmart samples were initially stained to optimize CAP treatment time for subsequent experiments. Following discoloration, 100 μL H_2O_2 was spread on the stained surface and subjected to treatment for durations of 1, 3, 5, 7, and 10 min. As illustrated in Fig. 3B, ΔE_{20} exhibited an increasing trend with prolonged CAP treatment, peaking at approximately 10.5 after 10 min and subsequently plateauing for longer durations. Consequently, the optimal treatment duration was determined to be 10 min, a parameter used in subsequent experiments. Cerasmart samples before and after staining, as well as after bleaching, are given in Fig. 3A.

B. Bleaching

Bleaching of CEREC and Cerasmart involved three methods: 35% H_2O_2 , CAP, and 35% H_2O_2 co-activated with CAP (hereafter 35% $\text{H}_2\text{O}_2/\text{CAP}$). Optimal bleaching efficacy was observed with 35% $\text{H}_2\text{O}_2/\text{CAP}$ for CEREC, while Cerasmart exhibited superior bleaching with CAP alone. Fig. 4A shows that all bleaching methods had a more

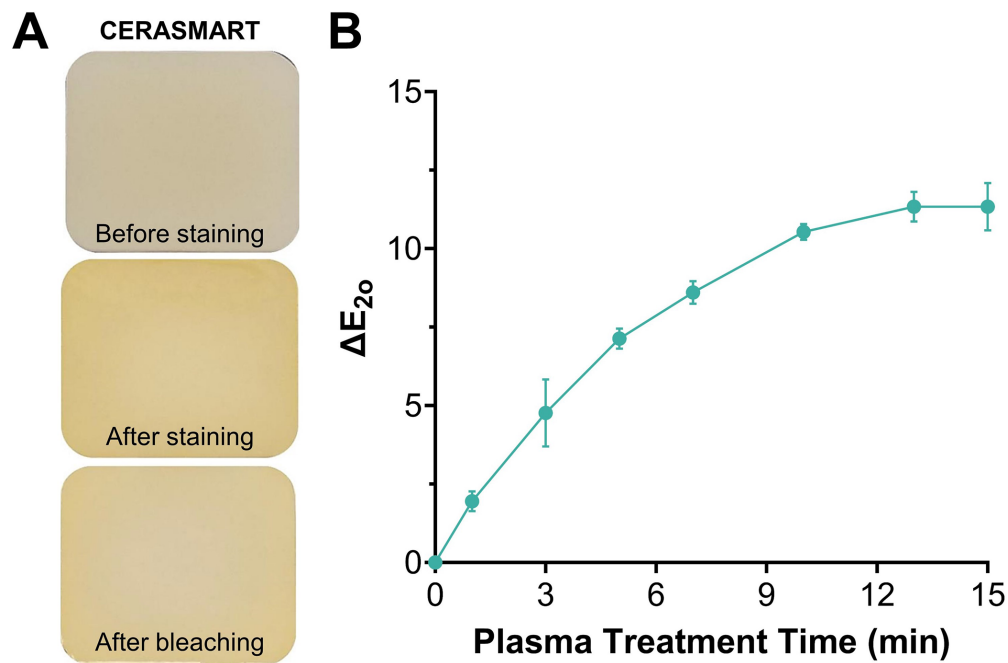


FIG. 3: (A) Discernibility of bleaching effectiveness of Cerasmart via 35% $\text{H}_2\text{O}_2/\text{CAP}$ for 10 min before bleaching, after staining, and after bleaching; and (B) determination of optimal CAP treatment time with data obtained from bleached Cerasmart with 35% $\text{H}_2\text{O}_2/\text{CAP}$

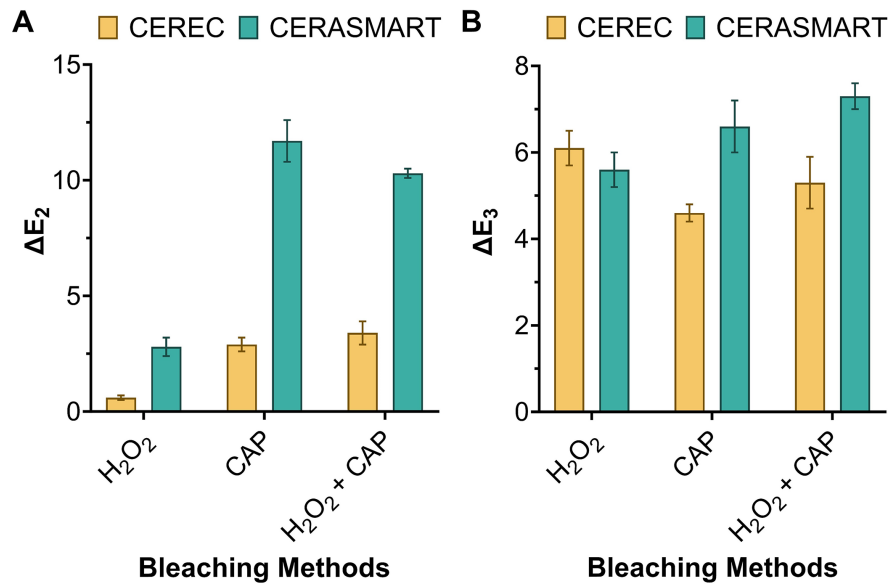


FIG. 4: (A) Bleaching efficacy among experimental groups for CEREC and Cerasmart; and (B) discoloration of experimental groups after 1-week bleaching for CEREC and Cerasmart

pronounced effect on Cerasmart than on CEREC. In both materials, activation of 35% H₂O₂ by CAP notably enhanced bleaching efficacy compared with H₂O₂ alone, with ΔE_2 values increasing from 0.6 to 3.4 for CEREC and from 2.8 to 10.3 for Cerasmart. Although CAP treatment alone had a bleaching effect superior to that of the 35% H₂O₂/CAP group, the bleaching effects of both methods were comparable and did not exhibit statistical significance.

Subsequent to the bleaching experiments, samples were restained for one week to assess bleaching stability. Consequently, the color change represented as ΔE_3 was measured. As depicted in Fig. 4B, CEREC showed less discoloration than Cerasmart across all bleaching groups. Despite observable discoloration in all bleaching methods for both materials, the discoloration was less than that for the unbleached samples, suggesting that 35% H₂O₂/CAP not only effectively bleaches but also contributes to the color stability of the CAD/CAM materials.

C. Prevention via CAP

The intent of pretreatment was to assess CAP's mitigation of discoloration in the CAD/CAM samples. Figure 5 shows that samples subjected to CAP pretreatment were less discolored than the control samples. Specifically, the discoloration values for the controls were measured at 12.5 for CEREC and 11.5 for Cerasmart, whereas the pretreated groups had values of 9.7 and 8.3, respectively. The disparity in discoloration between the control and pretreated groups was statistically significant.

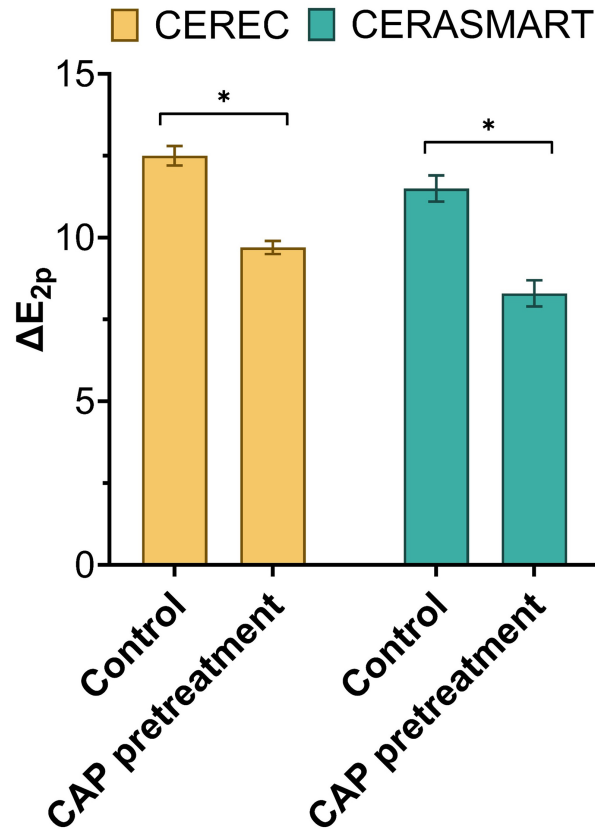


FIG. 5: Pretreatment of CEREC and Cerasmart with CAP to evaluate CAP prevention efficacy

D. Surface Characteristics

Figure 6B shows the alterations in surface roughness for the two materials following 35% H_2O_2 , CAP, and 35% H_2O_2 /CAP. While the surface roughness of CEREC increased across all treatment methods, there was no statistically significant variance in the final surface roughness, which remained approximately $0.37 \mu m$ across all groups. Conversely, for Cerasmart, although H_2O_2 alone led to a slight decrease in roughness, this change was not statistically significant. Furthermore, neither 35% H_2O_2 nor 35% H_2O_2 /CAP induced any noticeable alteration in surface roughness. These results were consistent with the SEM images shown in Fig. 6A, which revealed no discernible changes on the surface of Cerasmart, while irregularities and increased porosity were observed for CEREC across all treatment groups. Additionally, the findings from contact angle measurements indicated a decrease in θ for both CEREC and Cerasmart after undergoing 10 min of CAP treatment, as presented in Fig. 6C. Specifically for CEREC, θ decreased from 42.423 to 11.356, while for Cerasmart, it decreased from 41.647 to 11.969. These results signified the development of a more hydrophilic surface for both materials.

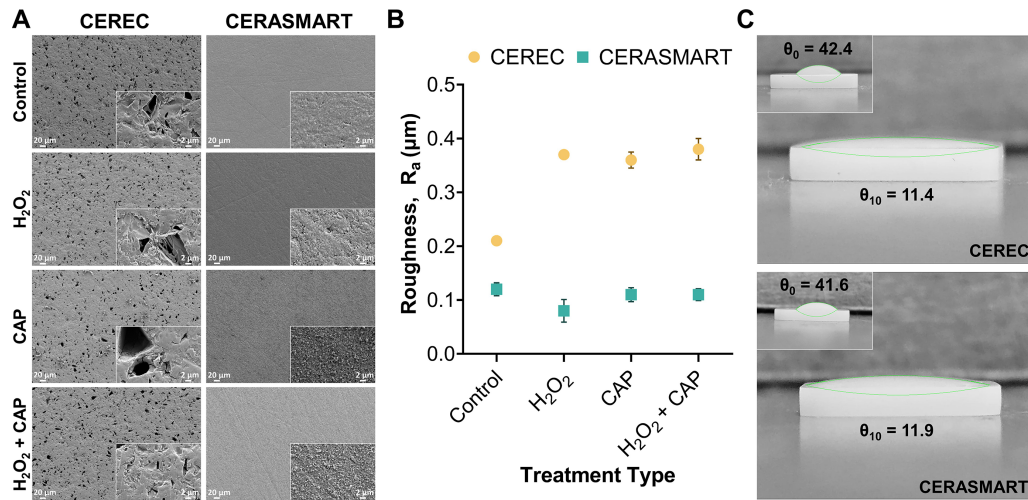


FIG. 6: Surface characterization assessment with (A) SEM imaging for 500 $\times$ and 10,000 $\times$; (B) surface roughness measurements; and (C) contact angle measurements at 0 and 10 min after CAP treatment for CEREC and Cerasmart

E. Bacterial Adhesion

As bacterial adhesion is seen after the bleaching of prosthetic materials in a clinical setting, the effect of CAP and 35% H_2O_2 /CAP on the prevention of bacterial adhesion was investigated. As seen in Fig. 7A and 7B, bacterial adhesion 6 h after the various treatments was evident for all experimental groups of both CEREC and Cerasmart and was around 6 to 7 logs. For Cerasmart, the amount of bacterial adhesion after 24 h of incubation was almost similar to that of 6 h of incubation for all treatment groups. On the other hand, CEREC, in all experimental groups, a decrease in bacterial adhesion for 24 h of incubation was observed. Moreover, such a decrease was superior for CAP treatment compared with other groups, where it dropped from 7 to about 5 logs, which was statistically significant.

IV. DISCUSSION

Aesthetic dentistry has been attracting attention for a couple of decades due to the demands from patients who seek a better smile.^{37,38} Accordingly, various techniques and methods have emerged in clinical dental practice. Among them, CAD/CAM prosthetic materials hold an important place as they can provide reliable and predictable restorations and can be adapted to different clinical scenarios.³⁷ Similarly, due to a desire for a bright and better smile, tooth bleaching has become one of the most common practices in aesthetic dentistry.³⁹ Numerous studies have shown the effectiveness of CAP treatment on the bleaching of vital and nonvital teeth.^{29,31} Dental prosthetic materials are expected to have appropriate physical properties and should provide good aesthetic

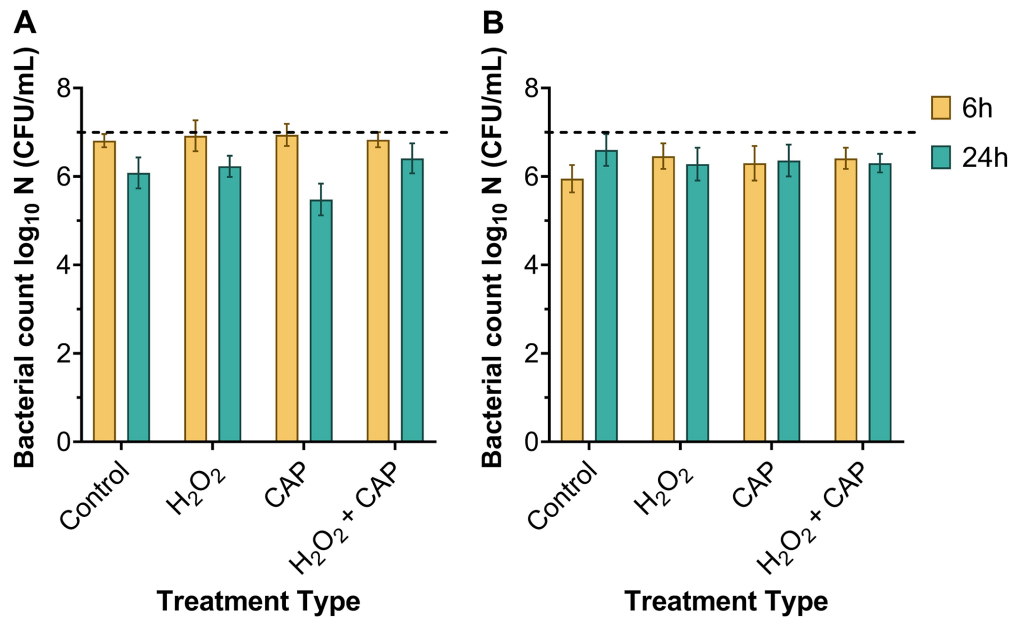


FIG. 7: Bacterial adhesion evaluation at 6 and 24 h for (A) CEREC and (B) Cerasmart

characteristics. Similar to natural teeth, they can also be stained depending on the patients' hygiene and nutritional habits.⁴⁰ Stained prosthetic materials are the cause of aesthetic worries for patients and require bleaching. Bleaching of dental prostheses is usually performed at the dentist's office using 35%–40% H₂O₂ preparations. However, 35%–40% H₂O₂ preparations may be insufficient for the desired bleaching and also may cause unwanted alterations on the surface and mechanical properties of the prosthetic materials.⁴¹

Novel techniques are required to improve the bleaching performance of dental prosthetic materials without altering material properties. Although CAP treatment is widely considered in the literature to increase the bonding strength of dental prosthetic materials, the use of CAP for bleaching of dental prosthetic materials has yet to be addressed.^{42,43} The present study investigates the bleaching effect of CAP on stained dental CAD/CAM prosthetic materials—namely, CEREC and Cerasmart. In the present study, two CAD/CAM dental prosthetic materials were selected, as they have gained popularity due to their capabilities to provide for personalized needs of patients.⁴⁴

The materials were kept in coffee for one week for the initial staining, where ΔE_1 was measured as 9.45 and 12.98 for CEREC and Cerasmart, respectively. A higher initial staining value for Cerasmart compared with CEREC was due to the different composition of the two materials. In detail, CEREC is a feldspathic, glass-ceramic material, while Cerasmart is a nanohybrid-ceramic in a resin matrix.⁴⁵ Materials with a resin matrix have higher water absorption capacity, as the resin matrix is mostly composed of hydrophilic polymers. Thus, during the staining procedure, more water along with the coffee

stain can be absorbed by Cerasmart, which leads to a higher initial staining value.^{46,47} To determine the required CAP treatment time for the bleaching experiments, an optimization study was carried out using Cerasmart, as it was expected to produce more staining, as explained above. The study showed that the bleaching efficacy of Cerasmart increased with increasing plasma treatment time and reached a plateau after 10-min of treatment, where an ΔE_2 of 10.5 was obtained. A higher bleaching effect with increasing treatment time could be attributed to a higher concentration of the plasma-generated ROS levels that increased with CAP treatment time.³¹ Numerous previous studies investigating bleaching of natural teeth with CAP treatment associated the bleaching effect to plasma-generated ROS—more specifically to the hydroxyl radical ($\text{OH}\cdot$).^{31,48} $\text{OH}\cdot$ is considered a primary ROS for bleaching as it carries an unpaired electron, which makes it unstable and highly reactive. Such reactivity and instability are why $\text{OH}\cdot$ readily reacts with chromophores that cause staining of the tooth or the prosthetic material, as in the present study. The reaction of $\text{OH}\cdot$ with chromophores causes the removal of electrons from chromophore molecules, which leads to the breaking of the chromophores and eventually bleaching of CAD/CAM prosthetic materials.^{49,50}

In the present study, three methods were used for the bleaching of dental prosthetic materials—namely, 35% H_2O_2 , CAP, and 35% $\text{H}_2\text{O}_2/\text{CAP}$. For all treatment groups, statistically significant higher bleaching values were obtained for Cerasmart, most likely due to its resin matrix, greater water absorption, and greater initial staining.⁴⁷ For CEREC, 35% $\text{H}_2\text{O}_2/\text{CAP}$ led to a slightly higher bleaching value compared with CAP, but the difference was not statistically significant. Inversely, for Cerasmart, CAP led to a slightly higher bleaching value compared with 35% $\text{H}_2\text{O}_2/\text{CAP}$, and the difference was also statistically insignificant. Previous studies of the bleaching effect of CAP itself and in combination with bleaching agents on extracted teeth reported a superior bleaching effect with CAP in combination with bleaching agents as opposed to what was observed in the present study.^{31,51} In the present study, the bleaching capacity of 35% H_2O_2 was limited, and that of CAP alone was remarkably higher and comparable to that of 35% $\text{H}_2\text{O}_2/\text{CAP}$, which suggests that the materials used in the present study reached a maximum bleaching capacity with CAP only; the addition of 35% H_2O_2 could not further contribute. However, further detailed studies where staining is performed (using varying dental prosthetic materials) with different chromophores might be needed to prove such speculation. Our findings suggest that CAP treatment could be sufficient for the bleaching of dental prosthetic materials, contributing to the elimination of unwanted effects from bleaching agents, such as interference with the materials' adhesive properties.⁵² The stability of the bleaching was investigated by means of restaining the materials after bleaching. As shown in Fig. 4B, CEREC restaining after CAP and 35% $\text{H}_2\text{O}_2/\text{CAP}$ was less than that after 35% H_2O_2 . However, the opposite was true for the Cerasmart. Such a finding for Cerasmart could be attributed to its significantly higher bleaching by different bleaching applications. Moreover, although Cerasmart might be seen as remarkable, the final staining of the material was still less than the initial staining for CAP and 35% H_2O_2 co-activated with CAP. In detail, the average ΔE_1 (the initial staining value) was 12.98 for Cerasmart.

After bleaching, the measured ΔE_2 value was an indicator of how much stain was removed. Thus, the subtraction of ΔE_2 from ΔE_1 could be considered as the remaining staining of the materials after bleaching, which was determined as 10.18, 1.28, and 2.18 for the 35% H_2O_2 treatment, CAP treatment, and 35% H_2O_2 co-activated with CAP treatment groups, respectively. After the determination of these values, samples were restained, and ΔE_3 values were calculated. In other words, the remaining staining after bleaching could resemble a background color before restaining. Thus, adding ΔE_3 to $\Delta E_1 - \Delta E_2$ could be used to compare initial staining with restaining. The $\Delta E_3 + \Delta E_1 - \Delta E_2$ values for Cerasmart were determined as 15.78, 7.88, and 9.45 for 35% H_2O_2 treatment, CAP treatment, and 35% H_2O_2 co-activated with the CAP treatment groups, respectively. In summary, despite the restaining of Cerasmart, which might be seen as remarkably high in Fig. 4B, the discoloration after restaining was still less than the initial staining, which might be indicative of CAP as a method for preventing discoloration of dental prosthetic materials. Thus, we evaluated the possible prevention effect of CAP treatment on the discoloration of CEREC and Cerasmart CAD/CAM prosthetic materials. CAP pretreatment showed a significant preventative effect. That finding could be related to the increased hydrophilicity of the tested materials. As shown in Fig. 6C, the contact angles of both materials dropped significantly after plasma treatment, which makes the surfaces highly hydrophilic. One of the main pigments in coffee that is responsible for staining teeth (and dental prosthetic materials) is melanoidin, a hydrophobic molecule.⁵³ Thus, the increased hydrophilicity of materials after CAP pretreatment might be effective on the adhesion of staining pigment on the materials' surface and subsequently cause less discoloration.

Effects of CAP treatment on the surface properties of dental prosthetic materials have been widely reported in the literature.^{28,54,55} Thus, in the present study, we evaluated the surface characteristics of CEREC and Cerasmart after bleaching to ensure that CAP bleaching would not disrupt the surface of materials compared with bleaching with 35% H_2O_2 alone. Our results revealed that the surface roughness of CEREC and Cerasmart after CAP alone and 35% H_2O_2 /CAP was comparable to that of 35% H_2O_2 alone. Moreover, such findings were in line with SEM micrographs of the samples. The SEM micrographs and surface roughness measurements showed that the roughness of CEREC increased after all bleaching procedures compared with control samples, and final roughness was not statistically significant according to the bleaching method. On the other hand, none of the bleaching methods led to a significant alteration in the surface roughness of Cerasmart. Such a difference in the surface roughness changes between CEREC and Cerasmart could be related to the composition of the tested materials. As mentioned before, Cerasmart is a nonhybrid ceramic in a resin matrix. In general, resin materials are more resistant to etching. In fact, hydrofluoric acid etching is not recommended for the bonding of resin-based materials in clinical applications.^{56,57} Thus, it can be concluded that the increased roughness of CEREC was due to etching, whereas Cerasmart was not affected.

The bacterial adhesion on CEREC and Cerasmart was also investigated after various bleaching procedures. On CEREC, in the first 6 h, bacterial adhesion was around

the initial bioburden and then dropped about 1 log for all groups. On the other hand, the bacterial adhesion on Cerasmart was around 6 log (1 log less) for all treatment groups after both 6 and 24 h. Based on the data we obtained, the bleaching methods did not seem to have a significant effect on bacterial adhesion. At least overall CAP treatment and 35% H₂O₂/CAP did not negatively affect bacterial adhesion on the tested materials compared with 35% H₂O₂ and even the control group. More specifically, because the most prominent reduction in bacterial adhesion was determined on CAP-treated CEREC after 24 h of incubation, however, further detailed investigations are needed to explain the reason for such findings.

V. CONCLUSIONS

To the best of our knowledge, this study was the first to investigate the bleaching efficacy of CAP treatment on dental CAD/CAM prosthetic materials, particularly in contrast to conventional bleaching agents. Our findings demonstrate that CAP treatment, either alone or in conjunction with 35% H₂O₂, delivers a markedly superior bleaching outcome compared with the use of 35% H₂O₂ alone, all while maintaining the integrity of surface characteristics and preventing bacterial adhesion. For these reasons, CAP pretreatment shows promise in the prevention of discoloration of CAD/CAM prosthetic materials.

ACKNOWLEDGMENT

This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) under Grant SBAG-120S278.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

Conceptualization: Özdemir GD, Bağış B, and Ercan UK.

Formal analysis: Özdemir GD, Irmak ŞE, Bağış B, and Ercan UK.

Investigation: Özdemir GD and Kış B.

Methodology: Kış B and Bağış B.

Writing—original draft: Özdemir GD, Irmak ŞE, and Ercan UK.

Writing—review & editing: Özdemir GD, Irmak ŞE, Bağış B, and Ercan UK.

REFERENCES

1. Sakaguchi RL, Powers JM. Craig's Restorative Dental Materials e-Book. (Elsevier Health Sciences, 2011).

2. Srimaneepong V, Heboyen A, Sohail Zafar A, Khurshid Z, Marya A, Fernandes GVO, Rokaya D. Fixed prosthetic restorations and periodontal health: A narrative review. *J Funct Biomater*. 2022; 13(15).
3. Ionescu RN, Totan AR, Imre MM, Tâncu AMC, Pantea M, Butucescu M, Farcașiu AT. Prosthetic materials used for implant-supported restorations and their biochemical oral interactions: A narrative review. *Materials*. 2022;15(1016).
4. Dobrzański LB. Mechanical properties comparison of engineering materials produced by additive and subtractive technologies for dental prosthetic restoration application, in Dobrzański LA, editor. *Biomaterials in Regenerative Medicine*. InTechOpen; 2018. p. 111–140.
5. Zitzmann NU, Hagmann E, Weiger, R. What is the prevalence of various types of prosthetic dental restorations in Europe? *Clin Oral Implants Res*. 2007;18:20–33.
6. Steinmassl P-A, Klaunzer F, Steinmassl O, Dumfahrt H, Grunert I. Evaluation of currently available CAD/CAM denture systems. *Int J Prosthodont*. 2017;30:116–22.
7. Canciglieri MB, de Moura Leite AFCS, Szejka AL, Canciglieri Júnior O. An approach for dental prosthesis design and manufacturing through rapid manufacturing technologies. *Int J Comput Integr Manuf*. 2019;32:832–47.
8. Cioloca Holban C, Tatarciuc M, Vitalariu AM, Vasluianu R-I, Anthohe M, Diaconu DA, Stamatina O, Dima AM. Three-dimensional printing and CAD/CAM milling in prosthodontics: A scoping review of key metrics towards future perspectives. *J Clin Med*. 2025;14:4837.
9. Srinivasan M, Gjengedal H, Cattani-Lorente M, Moussa M, Durual S, Schimmel M, Müller F. CAD/CAM milled complete removable dental prostheses: An in vitro evaluation of biocompatibility, mechanical properties, and surface roughness. *Dent Mater J*. 2018;37:526–33.
10. Gehrt M, Wolfart S, Rafai N, Reich S, Edelhoff D. Clinical results of lithium-disilicate crowns after up to 9 years of service. *Clin Oral Investig*. 2013;17:275–84.
11. Rexhepi I, Santilli M, D'Addazio G, Tafuri G, Manciocchi E, Caputi S, Sinjari B. Clinical applications and mechanical properties of CAD-CAM materials in restorative and prosthetic dentistry: A systematic review. *J Funct Biomater*. 2023;14:431.
12. Bazzi JZ, Bindo MJF, Rached RN, Mazur RF, Vieira S, de Souza EM. The effect of at-home bleaching and toothbrushing on removal of coffee and cigarette smoke stains and color stability of enamel. *J Am Dent Ass*. 2012;143:e1–e7.
13. Alqahtani, MQ. Tooth-bleaching procedures and their controversial effects: A literature review. *Saudi Dent J*. 2014;26:33–46.
14. Mounika A, Mandava J, Roopesh B, Karri, G. Clinical evaluation of color change and tooth sensitivity with in-office and home bleaching treatments. *Indian J Dent Res*. 2018;29:423.
15. Ceci M, Viola M, Rattalino D, Beltrami R, Colombo M, Poggio C. Discoloration of different aesthetic restorative materials: A spectrophotometric evaluation. *Eur J Dent*. 2017;11:149–56.
16. Alharbi A, Ardu S, Bortolotto T, Krejci I. In-office bleaching efficacy on stain removal from CAD/CAM and direct resin composite materials. *J Esth Restor Dent*. 2018;30:51–8.
17. Rutkunas V, Sabaliauskas V, Mizutani H. Effects of different food colorants and polishing techniques on color stability of provisional prosthetic materials. *Dent Mater J*. 2010;29:167–76.
18. Deligeorgi V, Mjör IA, Wilson NH. An overview of reasons for the placement and replacement of restorations. *Prim Dent Care*. 2001;1:5–11.
19. Duong H, Roccuzzo A, Stähil A, Salvi GE, Lang NP, Sculean A. Oral health-related quality of life of patients rehabilitated with fixed and removable implant-supported dental prostheses. *Periodontol*. 2022;88:201–37.
20. Karakaya İ, Cengiz-Yanardag E. Changes in optical characteristics and surface topography of CAD/CAM materials after bleaching applications: An AFM evaluation. *J Prosthodont*. 2020;29:226–36.
21. Karakaya İ, Cengiz E. Effect of 2 bleaching agents with a content of high concentrated hydrogen peroxide on stained 2 CAD/CAM blocks and a nanohybrid composite resin: An AFM evaluation. *Biomed Res Int*. 2017:1–11.

22. Bahannan SA. Effects of different bleaching agent concentrations on surface roughness and micro-hardness of aesthetic restorative materials. *Saudi J Dent Res.* 2015;6:124–8.
23. Özarlan M, Bilgili Can D, Avcioglu NH, Çalışkan S. Effect of different polishing techniques on surface properties and bacterial adhesion on resin-ceramic CAD/CAM materials. *Clin Oral Invest.* 2022;26:5289–99.
24. Silva ALF, Geng-Vivanco R, Tonani-Torrieri R, Pires-de-Souza FCP, Carvalho Panzeri F. Stain resistance and surface roughness of CAD/CAM processed hybrid ceramic. *Color Res Appl.* 2021;46:901–8.
25. Türkün LŞ, Türkün M. Effect of bleaching and repolishing procedures on coffee and tea stain removal from three anterior composite veneering materials. *J Esth Restor Dent.* 2004;16:290–301.
26. Laroussi M. Plasma medicine: A brief introduction. *Plasma.* 2018;1:47–60.
27. Braný D, Dvorská D, Halašová E, Škovierová H. Cold atmospheric plasma: A powerful tool for modern medicine. *Int J Mol Sci.* 2020;21:2932.
28. Adımcı P, İbiş F, Ercan UK, Bağis B. Evaluation of effects of non-thermal plasma treatment on surface properties of CAD/CAM materials. *J Adhes Sci Technol.* 2019;33:35–49.
29. Silva N, Marques J, da Cruz MB, Luis H, Sério S, Mata A. The applications of cold atmospheric plasma in dentistry. *Plasma Process Polym.* 2013;20:e2300067.
30. Zhao Z, Wang Q, Zhao J, Zhao B, Ma Z, Zhang C. Adhesion of teeth. *Front. Mater.* 2021;7:615225.
31. Çelik B, Çapar İD, İbiş F, Erdilek N, Ercan UK. Deionized water can substitute common bleaching agents for nonvital tooth bleaching when treated with non-thermal atmospheric plasma. *J Oral Sci.* 2019;61:103–10.
32. Demir N, Karci M, Ozcan M. Effects of 16% carbamide peroxide bleaching on the surface properties of glazed glassy matrix ceramics. *Biomed Res Int.* 2020;1:1864298.
33. Nogueira AD, Della Bona A. The effect of a coupling medium on color and translucency of CAD–CAM ceramics. *J Dent.* 2013;41:e18–e23.
34. Caglar A, Yamanel K, Gulsahi K, Bağis B, Özcan M. Could digital imaging be an alternative for digital colorimeters? *Clin Oral Invest.* 2010;14:713–8.
35. Green P. Colorimetry and colour difference, in Green P, editor. *Fundamentals and Applications of Colour Engineering.* Wiley;2023. p. 27–52. doi: 10.1002/9781119827214.ch2.
36. Pecho OE, Ghinea R, Ionescu AM, de la Cruz Cardona J, Paravina RD, del Mar Pérez M. Color and translucency of zirconia ceramics, human dentine and bovine dentine. *J Dent.* 2012;40:e34–e40.
37. Blatz MB, Chiche G, Bahat O, Roblee R, Coachman C, Heymann HO. Evolution of aesthetic dentistry. *J Dent Res.* 2019;98:1294–304.
38. Spear FM, Kokich VG. A multidisciplinary approach to aesthetic dentistry. *Dent Clin North Am.* 2007;51:487–505.
39. Kwon SR, Cortez E, Wang M, Jagwani M, Oyoyo U, Li Y. Systematic review of in vitro studies evaluating tooth bleaching efficacy. *Am J Dent.* 2020;33:17–24.
40. Bitencourt SB, Kanda RY, de Freitas Jorge C, Barão VAR, Sukotjo C, Wee AG, Goiato MC, Pesquiera AA. Long-term stainability of interim prosthetic materials in acidic/staining solutions. *Esth Restor Dent.* 2020;32:73–80.
41. Popescu, AD, Tuculina MJ, Dianconu OA, Gheorghită LM, Nicolicescu C, Cumpata CN, Petcu C, Abdul-Razzak J, Rica AM, Voinea-Georgescu R. Effects of dental bleaching agents on the surface roughness of dental restoration materials. *Medicina.* 2023;59:1067.
42. Kim JH, Lee, MA Han GJL, Cho BH. Plasma in dentistry: A review of basic concepts and applications in dentistry. *Acta Odontol Scand.* 2014;72:1–12.
43. Awad MM, Alhalabi F, Alshehri A, Aljeaidi Z, Alrahlah A, Özcan M, Hamama HH. Effect of non-thermal atmospheric plasma on micro-tensile bond strength at adhesive/dentin interface: A systematic review. *Materials.* 2021;14:1026.
44. Skorulska CA, Piszko P, Rybak Z, Szymonowicz M, Dobrzyński M. Review on polymer, ceramic and composite materials for CAD/CAM indirect restorations in dentistry—Application, mechanical characteristics and comparison. *Materials.* 2021;14:1592.

45. Alfouzan AF, Alnfaity SM, Alsaleh LS, Bawazir NH, Al Otaibi HN, Al Taweel SM, Alsehri HA, Labban N. Effects of background color and thickness on the optical properties of CAD-CAM resin-matrix ceramics. *J Prosthet Dent.* 2022;128:497e1–497.e9.
46. Stawarczyk B, Sener B, Trottmann A, Roos M, Ozcan M, Hammerle CHF. Discoloration of manually fabricated resins and industrially fabricated CAD/CAM blocks versus glass-ceramic: Effect of storage media, duration, and subsequent polishing. *Dent Mater J.* 2012;31:377–83.
47. Sağlam G, Cengiz S. The evaluation of the effect of coffee staining and 16% carbamide peroxide bleaching on the color change of chairside CAD/CAM ceramics. *J Dent Mater Tech.* 2021;10.
48. Pan J, Sun P, Tian Y, Zhou H, Wu H, Bai N, Liu F, Zhu W, Zhang J, Becker KH, Fang J. A novel method of tooth whitening using cold plasma microjet driven by direct current in atmospheric-pressure air. *IEEE Trans Plasma Sci.* 2010;38:3143–51.
49. Lee HW, Kim GJ, Kim JM, Park JK, Lee JK, Kim GC. Tooth bleaching with nonthermal atmospheric pressure plasma. *J Endod.* 2009;35:587–91.
50. Sulieman M, Addy M, Macdonald E, Rees JS. The bleaching depth of a 35% hydrogen peroxide based in-office product: A study in vitro. *J Dent.* 2005;33:33–40.
51. Yang X, Sun K, Zhu W, Li Y, Pan J. Time-dependent efficacy and safety of tooth bleaching with cold plasma and H₂O₂ gel. *BMC Oral Health.* 2022;22:535.
52. Goldberg M, Grootveld M, Lynch E. Undesirable and adverse effects of tooth-whitening products: A review. *Clin Oral Invest.* 2010;14:1–10.
53. Moreira ASP, Nunes FM, Domingues MR, Coimbra MA. Coffee melanoidins: Structures, mechanisms of formation and potential health impacts. *Food Funct.* 2012;3:903.
54. Dede DÖ, Ercan UK, Küçükkekenci AS, Kahveci Ç, Özdemir GD, Bağış B. Influence of non-thermal plasma systems and two favorable surface treatments on the shear bond strength of PAEKs to composite resin. *J Adhes Sci Technol.* 2022;36:748–61.
55. Altuntas M, Colgecen O, Ercan U, Cukur E. Nonthermal plasma treatment can eliminate sandblasting procedure for zirconia–resin cement bonding. *Int J Prosthodont.* 2022;35:752–60.
56. Muhammed HA, Mahmoud EM, Fahmy AE, Nasr DM. The effect of sandblasting versus acid etching on the surface roughness and biaxial flexural strength of CAD/CAM resin-matrix ceramics (In vitro study). *BMC Oral Health.* 2023; 23:169.
57. Kaban EŞ, Özdemir GD, İlgenli I, Ercan UK. Effect of cold atmospheric plasma treatment on the bond strength of glass fiber posts. *Plasma Med.* 2024;14:1.