



## BASIC RESEARCH:

### *In Vitro* Evaluation of Color Changes in CAD/CAM Materials Due to Long-Term Water Aging Evaluación *In Vitro* de los cambios de color en materiales CAD/CAM debido al envejecimiento prolongado por exposición al agua

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Received: 19-IX-2025

Accepted: 15-XI-2025

**ABSTRACT:** The aim of this study is to examine the discoloration in CAD/CAM materials used in dentistry after 4-years of contact with water, which is the environment to which they are most exposed in the oral cavity. Three different A2-colored resin-based CAD/CAM blocks (Cerasmart, Coltene Brilliant Crios and Shofu Block HC) were used in our study. A total of 180 specimens, 60 of each CAD/CAM material, were prepared with a thickness of 2-mm. Then, each material was divided into three different groups based on the polishing systems (Coltene Diatech, Sof-Lex Diamond, Identoflex Diamond Ceramic) applied in accordance with the recommendations of the manufacturing companies (n:20). The discoloration of the materials on a white background and under natural light sources at the beginning and after being stored in distilled water for 4-years were measured with the VITA EasyshadeV spectrophotometer. All the obtained data were examined using the Kolmogorov Smirnov and Shapiro Wilk Normality Test values. After the water aging process, no statistically significant difference was found between the Cerasmart ( $p=1.51$ ;  $p>0.05$ ) and Shofu ( $p=0.95$ ;  $p>0.05$ ) CAD/CAM material groups using three different polishing systems. A statistically significant difference was found between all polishing system groups of Coltene CAD/CAM materials ( $p=0.00$ ). All CAD/CAM materials showed color changes above the  $\Delta E_{00} \leq 0.8$  perceptibility threshold after being aged in distilled water for 4 years. Cerasmart CAD/CAM blocks (Coltene  $\Delta E_{00}=1.33$ , Sof-lex  $\Delta E_{00}=1.43$ , Identoflex  $\Delta E_{00}=1.69$ ) showed color changes below the  $\Delta E_{00} \leq 1.8$  acceptability threshold in all groups where different polishing systems were applied, while Coltene (Coltene  $\Delta E_{00}=3.7$ , Sof-lex  $\Delta E_{00}=3.81$ , Identoflex  $\Delta E_{00}=4.69$ ) and Shofu (Coltene  $\Delta E_{00}=2.33$ , Sof-lex  $\Delta E_{00}=1.74$ , Identoflex  $\Delta E_{00}=1.91$ ) blocks showed color changes above the acceptable threshold value.

**KEYWORDS:** CAD/CAM block; Color stability; Polishing systems; Spectrophotometry; Water aging; Dental materials.

**RESUMEN:** El objetivo de este estudio fue examinar la decoloración en materiales CAD/CAM utilizados en odontología tras cuatro años de contacto con agua, que constituye el medio al que están más expuestos en la cavidad oral. Se utilizaron tres bloques CAD/CAM de resina de color A2 (Cerasmart, Coltene Brilliant Crios y Shofu Block HC). Se prepararon un total de 180 especímenes, 60 de cada material, con un espesor de 2 mm. Posteriormente, cada material se dividió en tres grupos diferentes según los sistemas de pulido aplicados (Coltene Diatech, Sof-Lex Diamond, Identoflex Diamond Ceramic), siguiendo las recomendaciones de los fabricantes ( $n=20$ ). La decoloración de los materiales, colocados sobre un fondo blanco y bajo luz natural, se midió al inicio y después de haber sido almacenados en agua destilada durante cuatro años, utilizando el espectrofotómetro VITA Easyshade V. Todos los datos obtenidos se analizaron mediante las pruebas de normalidad de Kolmogorov-Smirnov y Shapiro-Wilk. Tras el proceso de envejecimiento en agua, no se encontraron diferencias estadísticamente significativas entre los grupos de materiales CAD/CAM Cerasmart ( $p=1.51$ ;  $p>0.05$ ) y Shofu ( $p=0.95$ ;  $p>0.05$ ) al utilizar los tres sistemas de pulido. Se encontró una diferencia estadísticamente significativa entre todos los grupos de sistemas de pulido del material CAD/CAM Coltene ( $p=0.00$ ). Todos los materiales CAD/CAM mostraron cambios de color superiores al umbral de perceptibilidad  $\Delta E_{00} \leq 0.8$  tras cuatro años de envejecimiento en agua destilada. Los bloques CAD/CAM de Cerasmart (Coltene  $\Delta E_{00}=1.33$ , Sof-lex  $\Delta E_{00}=1.43$ , Identoflex  $\Delta E_{00}=1.69$ ) presentaron cambios de color por debajo del umbral de aceptabilidad  $\Delta E_{00} \leq 1.8$  en todos los grupos en los que se aplicaron diferentes sistemas de pulido, mientras que los bloques de Coltene (Coltene  $\Delta E_{00}=3.7$ , Sof-lex  $\Delta E_{00}=3.81$ , Identoflex  $\Delta E_{00}=4.69$ ) y Shofu (Coltene  $\Delta E_{00}=2.33$ , Sof-lex  $\Delta E_{00}=1.74$ , Identoflex  $\Delta E_{00}=1.91$ ) mostraron cambios de color superiores al valor de aceptabilidad.

**PALABRAS CLAVE:** Bloques CAD/CAM; Estabilidad del color; Decoloración; Sistemas de pulido; Espectrofotometría; Envejecimiento en agua; Materiales dentales.

## INTRODUCTION

Computer-aided design/computer-aided manufacturing (CAD/CAM) technology was first used in dentistry in the 1970s and since then has been rapidly developed to produce oral restorations, with many different materials being offered to the market by many manufacturers. Currently, there are two main types of materials available for aesthetic CAD/CAM restorations: glass-ceramic/ceramic and resin composites (1). The flexural modulus and hardness of glass-ceramic/ceramics are significantly higher than resin composites. However, resin composite blocks can be repaired

and restored more easily than ceramic blocks. Furthermore, using resin based composite blocks instead of ceramic blocks for CAD/CAM procedures can considerably decrease production time and cause less wear on cutting equipment (2, 3). Considering their less abrasive effect on the opposing tooth and their mechanical and optical properties, polymerized resin based blocks can be regarded as alternative materials to ceramic blocks (4, 5).

Mechanical and optical properties and color stability are important criteria for the clinical preference of restorative materials (4, 6). Many studies have been conducted on the high quality

of CAD/CAM materials and their color compatibility with natural teeth and positive results have been obtained (7, 8).

Conditions such as humidity, temperature differences, chewing forces that restorative materials are exposed to in the oral environment are among the factors that cannot be controlled and affect the clinical life of the material (9). *In vitro* studies are preferable to clinical trials, considering operator variability, patient non-compliance, failure to recall patients, and the fact that clinical trials are more costly and time-consuming (10, 11). Although *in vitro* studies cannot fully simulate oral cavity conditions such as the clinical environment, humidity and stresses on teeth and restorations, the oral cavity environment can be reproduced to some extent through aging procedures (12).

Artificial aging of clinically applied materials affects their color stability and mechanical properties. Limited research has been published on the strengths and weaknesses of dental materials and how the properties of materials change with aging. Most of these published studies have shown that aging in thermocycles and distilled water significantly changes mechanical properties (13-16). However, only a limited number of studies have been conducted to examine the aging effect on the color change of materials (17).

Inadequate finishing and polishing of materials can cause rough surfaces in ceramic restorations. Rough surfaces are more susceptible to external coloration than smooth surfaces (18, 19). The discoloration of dental materials may depend on the nature of the restorative materials selected, the finishing and polishing system used, or the duration of exposure to the coloring agent (20-23).

One of the procedures used to imitate the physiological aging of dental materials in clinical practice is thermal cycling (12, 23). The longevity and aesthetic appearance of dental restorations

depend on the material's resistance to staining (24). Studies have shown that aging by thermal cycles and exposure to staining solutions have a significant effect on the optical properties of resin-containing polymerized materials and affect the aesthetic result (25, 26). Discoloration due to artificial aging are generally attributed to degradation of the polymer matrix, unreacted monomers of polymerization agents and exogenous staining agents (6). The research has shown that thermally-cycled materials vary in color due to temperature changes (25, 26). However, there are no studies showing how the materials will change in color after being exposed to distilled water for many years.

Therefore, the aim of this *in vitro* study was to investigate the color changes of resin-containing CAD/CAM materials polished with different polishing systems after long-term exposure to distilled water. The first null hypothesis of this study is that long-term exposure of resin-based CAD/CAM blocks to distilled water will not have a significant effect on their color stability. The second null hypothesis is that the use of different finishing and polishing systems for different CAD/CAM materials will not affect the color change after aging of the materials.

## MATERIALS AND METHODS

Three resin composite materials designed for CAD/CAM processing were used in this study: a hybrid ceramic [Shofu Block CAD/CAM Ceramic-Based Restorative (Shofu Dental Corp., Kyoto, Japan)], a hybrid nano-ceramic [Cerasmart Force Absorbing Hybrid CAD/CAM Block (GC Corp., Tokyo, Japan)] and a reinforced composite [Coltene Brilliant Crios (Coltene, Altstätten, Switzerland)]. To ensure standardization, all specimens were prepared in a single ceramic shade (A2).

The specimens were fabricated with a water-cooled slow saw at 2.3-mm intervals, considering that the thickness of the diamond cutting

disk (Buehler, Lake Bluff, IL, USA) was also 0.3 mm (Isomet 1000, Buehler, Lake Bluff, IL, USA). Each CAD/CAM block was cut to obtain specimens with a final thickness of 2-mm. Each group contained 20 specimens, with section measuring 12×10×2 mm (n=20) cut from the CAD/CAM blocks (27, 28). Each CAD/CAM block was used to prepare a total of 60 specimens, resulting in a total of 180 specimens (N:180). Following preparation, all specimens were stored in an incubator at 37°C for 24 hours and subsequently polished with 600- to 1000-grit silicon carbide paper under running water for 60 seconds.

The specimens were then divided into three groups based on the finishing and polishing method used: Group 1 (G1): Coltene Diatech Shape Guard Polishing System (Coltene, Altstätten, Switzerland); Group 2 (G2): Sof-Lex Diamond Polishing System (3M ESPE, St. Paul, USA); Group 3 (G3): Identoflex Diamond Ceramic Polishing System (KerrHawe SA). A new finishing and polishing system was used for every four specimens. Finishing was performed using a contra-angle handpiece (Kavo INTRAmatic 20CN, Kavo, Biberach, Germany) under water cooling. The Coltene Diatech Shape Guard Polishing System was applied to the G1 specimens: the polishing process was made with a purple spiral followed by a blue spiral for 15 sec under water cooling with a slow-speed handpiece (10000 to 12000 rpm). The Sof-Lex Diamond Polishing System was applied to the G2 specimens: a beige spiral was first applied for 15 sec, followed by a pink spiral for 15 sec under water cooling at with a slow-speed contra-angle handpiece operating within 15000 to 20000 rpm. The Identoflex Diamond Ceramic Polishing System was also applied to the G3 specimens: polishing was performed with a slow-speed handpiece under water cooling for 30 sec. The materials used in this study, the manufacturers, types and their contents are listed in Table 1.

Initial color measurements of the specimens were performed using a VITA Easyshade V (VITA Zahnfabrik, Bad Säckingen, Germany) spectrophotometer on a white background under natural light sources. After initial measurements, the specimens were placed in opaque plastic containers and each container was numbered according to the CAD/CAM block and polishing system. All storage containers were filled with distilled water at room temperature and the distilled water in each container was changed weekly to avoid bacterial contamination. This process was repeated for 4 years. At the end of 4 years of aging, color measurements of the specimens were measured again with VITA Easyshade V spectrophotometer.

The discoloration of composite CAD/CAM blocks at baseline and after 4 years of water aging was evaluated using the CIEDE2000 formula ( $\Delta E_{00}$ ), which takes into account the color parameters  $L^*$ ,  $a^*$  and  $b^*$ .  $\Delta L'$  is the change in lightness,  $\Delta C'$  is the change in chroma,  $\Delta H'$  is the change in hue, RT is the rotation factor, S is the weighting function. KL, KC and KH are the parametric factors (29).

Considering the parametric factors of the CIEDE2000 color difference formula, a  $\Delta E_{00} \leq 0.8$  was accepted as the perceptibility threshold, while a  $\Delta E_{00} \leq 1.8$  was considered the acceptability threshold (30).

## STATISTICAL ANALYSIS

The values of the obtained data were calculated using the IBM SPSS Statistic 25.0 package program. All the obtained data were examined using the Kolmogorov Smirnov and Shapiro Wilk Normality Test values. The numerical variables showing normal distribution were given as mean  $\pm$  standard deviation, and the median value was given to the ones not showing normal distribution.

In order to determine the difference between the groups, the One-Way ANOVA test was applied to the data showing normal distribution. Multi-

ple comparisons were made using the Tukey and Benferroni tests.  $p < 0.05$  was considered sufficient for statistical significance.

**Table 1.** The materials used in this study.

| Material   | Manufacturer                           | Type                 | Content   |
|--|--|----------------------|---|
| Shofu Block CAD/CAM Ceramic-Based Restorative (A2 LT)  | Shofu Dental Corporation, Kyoto, Japan | Hybrid ceramic       | UDMA, TEGDMA, Barium glass, Silica powder, Micro-fumed silica, Zirconium silicate |
| Cerasmart Force Absorbing Hybrid CAD/CAM Block (A2 LT) | GC Corporation, Tokyo, Japan           | Hybrid Nano-ceramic  | Bis-MEPP, UDMA DMA, SiO <sub>2</sub> , Barium glass                               |
| Coltene Brilliant Crios (A2 LT)                        | Coltene AG, Altstätten, Switzerland    | Reinforced Composite | Bis-MEPP, UDMA, DMA, Amorphous SiO <sub>2</sub> , Barium glass                    |
| Identoflex Diamond Ceramic Polishing System            | KerrHawe SA                            | -                    | Diamond abrasive particles  |
| Sof-Lex Diamond Polishing System                       | 3M ESPE, St. Paul, USA                 | -                    | Al <sub>2</sub> O <sub>3</sub> , Diamond abrasives                                |
| Coltene Diatech Shape Guard Polishing System           | Coltene, Altstätten, Switzerland       | -                    | Diamond abrasive particles  |

UDMA: Urethane dimethacrylate; TEGDMA: Triethylene glycol dimethacrylate; Bis-MEPP: 2,2-Bis (4-methacryloxy polyethoxy phenyl) propane; DMA: Dimethacrylate; SiO<sub>2</sub>: Silicium oxide; Al<sub>2</sub>O<sub>3</sub>: Aluminium oxide.

## RESULT

Table 2 shows the  $\Delta E_{00}$  values and statistical differences (p-values) between groups for CAD/CAM materials after 4 years of water aging of different CAD/CAM blocks polished with different polishing systems. All CAD/CAM materials used in all polishing systems showed color changes higher than the perceptibility threshold of  $\Delta E_{00} \leq 0.8$  after distilled water aging, when the parametric factors of the CIEDE2000 color difference formula were taken into account. Cerasmart CAD/CAM blocks showed color changes below the  $\Delta E_{00} \leq 1.8$  acceptability threshold in all groups where different polishing systems were applied. The  $\Delta E_{00}$  value for Cerasmart blocks in group G1 is 1.33, while the  $\Delta E_{00}$  value for group G2 is 1.43 and for group G3 it is 1.69. In Shofu CAD/CAM

blocks, color change below the  $\Delta E_{00} \leq 1.8$  acceptability threshold was observed only in the group polished with Sof-Lex Diamond Polishing polishing systems (G2) ( $\Delta E_{00} = 1.74$ ). Color changes above the acceptability threshold were observed in groups polished with the Coltene Diatech Shape Guard Polishing system (G1) ( $\Delta E_{00} = 2.33$ ) and Identoflex Diamond Ceramic Polishing system (G3) ( $\Delta E_{00} = 1.91$ ). Coltene CAD/CAM blocks showed color changes above the acceptability threshold in all groups (G1, G2, G3). After 4 years, the color changes between the Coltene block groups are as follows:  $\Delta E_{00} = 3.70$  for group G1,  $\Delta E_{00} = 3.81$  for group G2, and  $\Delta E_{00} = 4.69$  for group G3.

After Cerasmart CAD/CAM materials were polished using three different polishing systems and then aged with distilled water, the discolorsi-

ton of the materials was examined, and no statistical difference was found between the groups ( $p>0.05$ ).

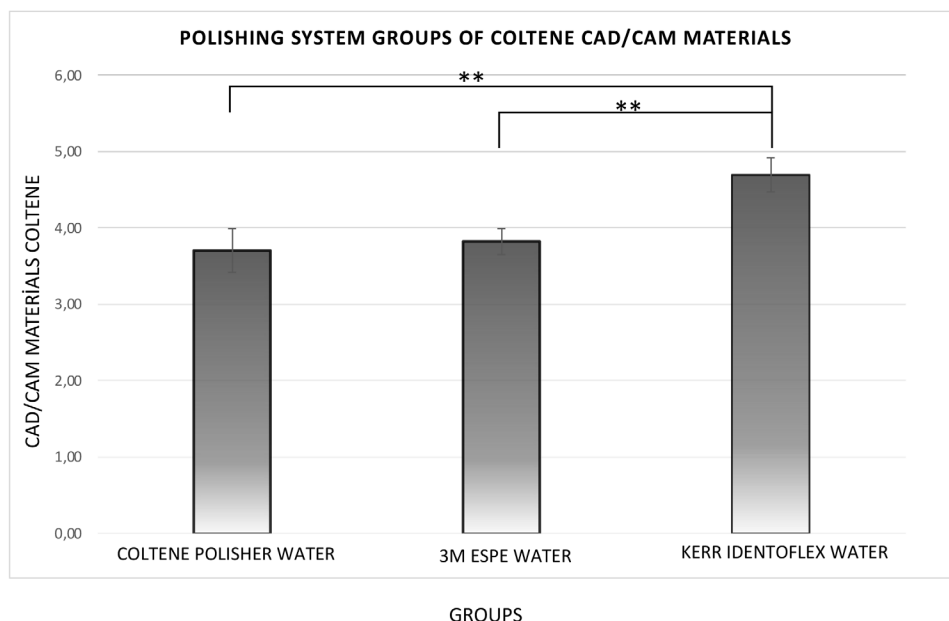
When the discoloration of Coltene CAD/CAM blocks in groups G1, G2, and G3 was examined after aging with distilled water, there was statistical difference between the groups ( $p=0.00$ ). In the dual examination of Coltene CAD/CAM material polishing system groups, there was no statistical difference between groups G1 and G2 ( $p=0.495$ ;  $p>0.05$ ), while group G3 showed statistical differences from the other groups in terms of color

changes ( $p<0.05$ ), corresponding to a large-to-moderate effect size (Cohen's  $d = 4.47$ , 95%).

When the discoloration of Shofu CAD/CAM materials in groups G1, G2, and G3 was examined after aging with distilled water, no statistical difference was found between the group ( $p=0.95$ ;  $p>0.05$ ). Figure 1 shows a comparison of average values for CAD/CAM material groups (Cerasmart, Coltene, Shofu) polished with the Coltene Diatech Shape Guard, Sof-Lex Diamond, and Identoflex Diamond Ceramic polishing systems. Data are presented as mean  $\pm$  standard deviation.

**Table 2.** Comparison of median, mean  $\pm$  standard deviation (mean  $\pm$  SD) values and statistical differences ( $p$ -values) between groups for CAD/CAM materials polished with Coltene Diatech Shape Guard, Sof-Lex Diamond and Identoflex Diamond Ceramic polishing systems.

| Polishing system / material | Cerasmart |                 |         | Coltene |                 |         | Shofu  |                 |         |
|-----------------------------|-----------|-----------------|---------|---------|-----------------|---------|--------|-----------------|---------|
|                             | median    | mean $\pm$ SD   | p-value | median  | mean $\pm$ SD   | p-value | median | mean $\pm$ SD   | p-value |
| Coltene Diatech Shape Guard | 1.33      | 1.33 $\pm$ 0.15 |         | 3.70    | 3.70 $\pm$ 0.07 |         | 2.33   | 2.33 $\pm$ 0.62 |         |
| Sof-Lex Diamond             | 1.43      | 1.43 $\pm$ 0.33 | 1.51    | 3.81    | 3.81 $\pm$ 0.17 | 0.00    | 1.74   | 1.74 $\pm$ 0.77 | 0.95    |
| Identoflex Diamond Ceramic  | 1.69      | 1.69 $\pm$ 0.18 |         | 4.69    | 4.69 $\pm$ 0.22 |         | 1.91   | 1.91 $\pm$ 0.31 |         |



**Figure 1.** Comparison of mean values for CAD/CAM material groups (Cerasmart, Coltene, Shofu) polished with Coltene Diatech Shape Guard, Sof-Lex Diamond and Identoflex Diamond Ceramic polishing systems. Data are presented as mean  $\pm$  standard deviation. Statistical analysis was performed using One-Way ANOVA for normally distributed data, with significance set at  $p < 0.05$ .

## DISCUSSION

In this study, our first null hypothesis that “long-term exposure of resin-based CAD/CAM blocks to distilled water will not have a significant effect on their color stability” is rejected. Cerasmart CAD/CAM blocks have less color change than Shofu and Coltene blocks and show color change below the threshold of  $\Delta E_{00} \leq 1.8$  acceptability. Our second null hypothesis that “the use of different finishing and polishing systems for different CAD/CAM materials will not affect the color change after aging of the materials” is rejected. The group polished with the Identoflex Diamond Ceramic Polishing System (G3) for Coltene CAD/CAM blocks showed more color change than the G1 and G2 polishing system groups. Additionally, the group polished with the Sof-Lex Diamond Polishing System (G2) for Shofu CAD/CAM blocks showed discoloration below the acceptable threshold value when compared to G1 and G3, indicating less color change than the other groups.

The surfaces of dental restorative materials should be as smooth as possible to prevent plaque accumulation and discoloration of the material (31, 32). Therefore, restorative materials applied to patients should be polished in parallel with the manufacturer's recommendations in order to prevent discoloration. Chemical degradation of restorative materials following contact with water and food can lead to increased plaque adhesion, discoloration, and secondary caries (33). Water is a component that can negatively affect resin-based restorative materials and plays an important role in the human diet (31, 32). In our study, we examined the changes in color that occurred in CAD/CAM restorative blocks polished with different polishing systems after long-term aging with distilled water.

Projan *et al.* (17) studied the color changes in modified and unmodified PEEK (semicrystalline linear polycyclic aromatic polymer) CAD/CAM materials after immersing them in distilled water at 37 °C for 28 days and then subjecting them

to thermal cycling (10000 cycles). The study utilized three different PEEK CAD/CAM materials. As a result of their studies, researchers found that after 4 weeks of exposure to water, all materials underwent a very slight to slight color change.  $\Delta E$  values measured on a white background at the end of the 4-week period ranged from 0.10 to 0.59. After thermocycling, there was again a slight color change. The results of this study show differences from our study. This can be attributed to the differences in the materials used in the study and the duration of exposure to water.

Papathanasiou *et al.* (34) conducted a study using six different CAD/CAM blocks (Brilliant CRIOS, Cerasmart, Lava Ultimate, Tetric CAD, Shofu Block HC, Grandio Blocs) and subjected the materials to 5000 cycles of water thermal cycling at temperatures ranging from 5 to 55 °C. As a result of the aging processes they applied, noticeable but acceptable color changes occurred in the materials. In our work, Cerasmart blocks showed acceptable discoloration above the perceptible threshold value in a similar manner to this study. However, while only the G2 group of Shofu blocks showed acceptable discoloration above the perceptible threshold value, the G1 and G3 groups showed discoloration above the acceptable threshold value. This difference may be due to the difference in the duration of exposure to water. The thermal aging protocol consisting of 5000 thermal cycles corresponds to a clinical period of 6 months (35). In our study, however, 4 years of aging with water was performed. Vasiliu *et al.* (25) used six different glass ceramics obtained by pressing and milling in a 10000-cycle thermal cycle in their study. Among the ceramics, the one least affected in terms of changes in optical properties was the heat-pressed and polished lithium disilicate ceramic. However, all specimens used in the study were affected by the aging process. This shows that similar results were obtained in our study. Restorative materials absorb water over time,

which causes an increasing change in their color over time.

In their study, Mourouzis *et al.* (36) examined the discoloration and surface roughness of the resin ceramic blocks Vita Enamic, lithium disilicate materials IPS e.max CAD and premium zirconium oxide material IPS e.max ZirCad MT Multi after acid exposure and aging in 10000 thermal cycles. As a result of their studies, discoloration exceeding the clinically acceptable threshold value occurred in the materials due to acid exposure. After thermal cycling, color changes above the clinically acceptable threshold value ( $\Delta E_{00} \leq 1.8$ ) were observed in zirconium oxide materials ( $\Delta E_{00} = 2.04$ ), while no color changes above the acceptable threshold value were observed in lithium disilicate ( $\Delta E_{00} = 1.12$ ) and resin ceramic materials ( $\Delta E_{00} = 1.49$ ). Unlike this study, the fact that most of the materials used in our study have undergone color change may be related to the use of a different color measurement device and differences in exposure times to water. Most studies have shown that measuring color changes in dental materials using spectrophotometers is more reliable than visual inspections (37). Therefore, in our study, we measured the colors of the initial specimens and the specimens exposed to water for 4 years using a VITA Easyshade V spectrophotometer.

The limitations of this *in vitro* study may be considered to be that the specimens were not exposed to the pH changes present in the oral environment and the complexity of the coloring agents, and that the protective effect of saliva was absent. The color change of the materials was examined in a simply established *in vitro* environment. Although our study is an important study evaluating CAD/CAM materials, which have an important place among aesthetic restorations, in terms of long-term water exposure, future studies will include *in vitro* models that more closely mimic the oral environment.

## CONCLUSION

All CAD/CAM blocks used in this study showed a discoloration above the perceptible threshold value after prolonged exposure to water.

In our study, only Cerasmart blocks showed discoloration below the clinically acceptable threshold value, while Coltene blocks showed discoloration above the clinically acceptable threshold value.

**CONFLICT OF INTERESTS:** The authors declare that they have no potential conflict of interest regarding the investigation, authorship, and/or publication of this article.

**ETHICAL APPROVAL:** This article does not contain any studies with human participants or animal performed by any of the authors.

**AUTHOR CONTRIBUTION STATEMENT:** Conceptualization and design: E.İ.S. and S.B.B.; Literature Review: E.İ.S. and S.B.B.; Methodology and validation: E.İ.S. and N.T.; Formal analysis: K.K.S.; Investigation and data collection: E.İ.S. and S.B.B.; Resources: E.İ.S., S.B.B. and N.T.; Data analysis and interpretation: K.K.S.; Writing-original draft preparation: E.İ.S. and S.B.B.; Writing-review & editing: E.İ.S., S.B.B. and N.T.; Supervision: E.İ.S. and N.T.; Project administration: E.İ.S. and S.B.B.; Funding acquisition: E. İ.S. and S.B.B.

**ACKNOWLEDGMENTS:** This research was carried out by Kocaeli Health and Technology University Scientific Research Projects Coordinators. Supported by project number 2023/23.

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