

EFFECT OF NEW MOUTHWASH ON COLOR STABILITY AND MICROHARDNESS OF CAD/CAM CERASMART HYBRID CERAMIC BLOCKS

Ahmed Fawzy Aboelezz*  and Ahmed Mohamed Hoseny Fayed** 

ABSTRACT

The purpose of this study was to evaluate color stability and microhardness of CAD/CAM Cerasmart hybrid ceramic blocks after different immersion times in chlorohexidine and new plant-based mouth wash.

Materials and Methods: A total of 120 square discs (10x12x1mm) were cut from 12 hybrid ceramic CAD/CAM blocks (shade A1\1M2, C12, 10x12x15mm, Cerasmart, GC) with a diamond blade (Isomet 15LC; Buehler) mounted in a cutting machine (Buehler). Specimens were divided into three equal groups (n=40) according to immersion media, either, distilled water (DW), chlorohexidine mouthwash (CM) (Orovex, Macro Group pharmaceuticals, Egypt) or Neem (Azadirachata indica) mouthwash (NM) (Neem Mouthwash, Theraneem Naturals, USA). Each group was subdivided into four equal subgroups (n=10) according to immersion time, either one hour and half, three hours, 18 hours or 36 hours. After each time interval, specimens were stored in DW at 37°C till time of evaluation of color change and micro-hardness.

Results: Two ways ANOVA showed that the immersion media had a statistically significant effect on the VHN and ΔE of Cerasmart at a p-value= 0.0001 for both, also significance was detected for the effect of immersion time on the VHN and ΔE of Cerasmart at a p-value= 0.0001 for both.

Conclusion: Long term usage of antimicrobial mouthwashes used in this study (more than one year) should be avoided due to its negative effect on surface microhardness of the used resin based CAD/CAM restorations. Prolonged use of Neem mouthwash had a minimal effect on color change on the used resin based CAD/CAM restorations compared to CHX containing mouth wash (Orovex).

KEYWORDS: Mouthwash, Color stability, Microhardness, Hybrid ceramics

* Assistant Professor of Operative Dentistry, Suez Canal University.

** Lecturer of Operative Dentistry, Misr International University.

INTRODUCTION

Computer-aided design / computer-aided manufacturing (CAD/CAM) has undoubtedly gained a wide popularity in the last years. It has been applied in various branches of engineering, science and art as a swift prototype way for accelerating the design process and facilitating its transition into manufacturing⁽¹⁾.

In the last years, a huge revolution in dental CAD/CAM materials and systems have been developed for manufacturing of different ceramic restorations. These new materials and systems have been established to fulfill the esthetic and functional demands for both patients and operators. Eliminating the need for many laboratory steps and obtaining a high quality of work, CAD/CAM has recently acquired a large popularity in the dental field and has nowadays been recommended rather than conventional esthetic indirect restorations, due to their premium esthetics and high mechanical characteristics^(2,3).

Recently, various restorative materials can be used with CAD/CAM systems. Glass ceramics were used to be the material of choice for the anterior region due to their high esthetic properties and different methods were been introduced to improve their mechanical properties. One of the most turning points was the introduction of nanoceramics and hybrid ceramics that combined the favorable characteristics of glass ceramics and resin composites^(4,5).

The concept of hybrid ceramics (polymer infiltrated glass ceramics) was to be fabricated by two intermingled phases of inorganic and organic components giving a higher flexural strength compared to single-phase materials. Organic and inorganic components consist of polymers and ceramics respectively⁽⁶⁾. Resin composite materials contain inorganic filler particles and organic matrix, while in hybrid ceramics the inorganic fillers and the organic matrix are intermingled with each other, which is the main reason for improving the mechanical properties of the material. A clear example for this im-

provement is the polymer component of the material preventing and/or halting any crack propagation in the ceramic phase. Moreover, the chemical structure of the hybrid ceramics directing the occlusal forces from the contact area to a wide area which leads to minimizing the stresses formed^(7,8).

Ceramics represent 86% of hybrid ceramics composition while polymer represent 14%. Ceramic composition includes, 9-11% Na₂O, 58-63% SiO₂, 4-6% K₂O, 20-23% AL₂O₃ and 0.5-2% B₂O₃. Polymer composition, on the other hand, includes urethane dimethacrylate (UDMA) and triethylene glycol dimethacrylate (TEGDMA)⁽⁹⁾. First, the pre-sintered porous structured feldspathic ceramic is formed as changing the size of the ceramic particles and firing temperature affects the porosity. Secondly, this porous ceramic structure is filled by the resin polymer after adding silane into the ceramic structure as a bonding agent⁽¹⁰⁾.

Cerasmart is one of the hybrid ceramic blocks which contains nano-hybrid resin composite and inorganic ceramic fillers which is mainly 71% silica and barium glass by weight. It was found that it has high fracture resistance, high strength under compressive load and higher wear potential than other popularly used CAD/CAM peers with respect to the mechanical performance⁽¹¹⁾.

No doubt that dental plaque, a biofilm, is one of the principal etiological factors for dental caries and periodontal diseases which are the most common diseases of the mouth. Effective control of this biofilm is very important to maintain oral health. Searching for easy, quick and effective methods to control and/or remove this biofilm has been a target for many researchers and clinicians⁽¹²⁾.

One of these methods are mouthwashes which can reduce microbial plaque. The most popular mouthwash, which showed effective way to reduce dental plaque and pathogenic microorganisms such as *Streptococcus Mutans*, is chlorohexidine (CHX). In literature, most of the recent researches, CHX represents the gold standard, taken as a positive control, to evaluate the efficacy of other products.

However, discoloration of teeth, unpleasable sensation such as mouth dryness and burning sensation are among the drawbacks which make patients avoid using CHX mouthwashes⁽¹³⁾.

Several studies investigated the effect of different plant extracts as anti-microbial agents. Herb, *Azadirachta indica* (Neem) which belongs to the Melicea plant family, has a component that showed an anti-bacterial property including the cariogenic bacteria. Neem extracts showed inhibition of the growth of *Streptococcus Mutans* through its anti-oxidant, anti-inflammatory and anti-microbial characteristics, hence, nowadays it is used for the treatment of periodontitis and for managing high caries risk patients⁽¹⁴⁾.

No doubt, the surface properties of restorations have an immense effect on their clinical durability in the oral environment, hence any chemical surface deterioration resulting from chemical stimulants will affect the surface properties of the material. This surface biodegradation will lead to plaque accumulation, wear and discoloration of the restoration⁽¹⁵⁾. Although oral hygiene products, such as mouthwashes, are very important for maintaining a healthy oral environment, however, these chemical-based products could negatively affect the surface properties of restorative materials. Excessive and prolonged usage of these mouthwashes may lead to damage of resin composite restorations because of alcohol and pH variations in the composition of most mouthwashes^(16,17).

Despite the different manufacturers' recommendations, mouthwashes are used by patients for a prolonged period. Their effect on esthetic restorative materials, especially CAD/CAM hybrid ceramic blocks is still controversial and few studies were held to investigate the effect of mouthwashes on these CAD/CAM blocks. Thus, the aim of this study was to evaluate color stability and microhardness of CAD/CAM Cerasmart hybrid ceramic blocks after different immersion times in chlorohexidine and new plant-based mouth wash.

MATERIALS AND METHODS

A total of 120 square discs (10x12x1mm) were cut from 12 hybrid ceramic CAD\CAM blocks (shade A1\1M2, C12, 10x12x15mm, Cerasmart, GC) with a diamond blade (Isomet 15LC; Buehler) mounted in a cutting machine (Buehler). Sample size calculation was done by power analysis which used ΔE as a primary outcome. The effect size $f = 0.6887$ was calculated based upon the results of Sasanya et al. 2021⁽¹⁸⁾. Assuming that the standard deviation within each group = 0.73, using alpha level of 5% and Beta level of 95% i.e. power = 95%. The minimum estimated sample size was a total of 108 samples (9 samples per group). Sample size calculation was done using G*Power version 3.1.9.2.

Each specimen was sequentially pre-polished with #180, 320, 400, and 600 silicon carbide papers (Struers, GmbH) under water cooling by using the EcoMet 6 grinding machine (Buehler). Laboratory polishing was performed by using the EcoMet 6 grinding machine at 250 rpm under light hand pressure. Specimens were sequentially polished under water cooling by using #800, 1200, 2400 and 4000 silicon carbide papers, 15 seconds per grit. To simulate the clinical scenario, chairside polishing was performed with the polishing kit recommended by the manufacturer (Ceramaster finishing and polishing kit, Shofu Dental Corp, Ceramaster CA-0123 at 20000 rpm, cotton buff and Diapolisher paste, GC at 15000 rpm) using a low-speed hand-piece and light hand pressure for 30 seconds per step. Specimens were cleaned for 10 minutes with deionized water in an ultrasonic cleaner (Branson Ultrasonics) and air-dried for 20 seconds for baseline color measurements (T_0) using intra-oral spectrophotometer (VITA Easyshade V® Vita Zahnfabrik, Germany).

Specimens were divided into three equal groups ($n=40$) according to immersion media, either distilled water (DW), chlorohexidine mouthwash (CM) (Orovex, Macro Group pharmaceuticals, Egypt) or Neem (*Azadirachata indica*) mouthwash

(NM) (Neem Mouthwash, Theraneem Naturals, USA) [Table (1)]. For each immersion, specimens were individually inserted in a container containing 15 mL of the respective immersion medium. Each group was subdivided into four equal subgroups (n=10) according to immersion time, either one hour and half, three hours, 18 hours or 36 hours. After each time interval, specimens were stored in DW at 37°C till time of evaluation of color change and micro-hardness. For evaluation of color change, intra-oral spectrophotometer (VITA Easyshade V® Vita Zahnfabrik, Germany) was used. T_0 was used as a reference point, representing the color before immersion. Before measurements, the spectrophotometer was calibrated as per the manufacturer's instruction. Color was assessed using the international equation De L'Eclairage. Internationale De L'Eclairage (International Commission on Illumination, denoted CIE)

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

Where L^* denotes lightness (achromatic), while a^* and b^* denote green-red and blue-yellow coordinates respectively, the difference between the two shades is ΔE .

As for micro-hardness evaluation, same samples after color change assessment, Vickers hardness numbers were determined using a micro-hardness tester (Wilson ® Hardness Tester, Model Tukon 1102, Buehler, Lake Bluff, IL, USA), where a 500 gram load was applied smoothly, without impact, forcing the indenter into the tested specimen. The indenter was held in place for 15 seconds. The physical quality of the indenter and the accuracy of the applied load were controlled in order to get the correct results. After the load was removed, the indentation was assessed with a magnifying eye piece and the two impression diagonals were measured to the nearest 0.1 μm with a micrometer and averaged. The Vickers hardness number (VHN) was calculated using the equation: $VHN = 1854.4 L/d^2$. Where the load L is in gf and the average diagonal

d is in μm (this produces hardness number units of $\text{gf}/\mu\text{m}^2$. In practice the numbers were reported without indication of the units).

TABLE (1) Composition of the products used in this study

Product	Composition	Manufacturer
Cerasmart	Filler: 71% SiO_2 and barium glass nanoparticle Polymer: BisMEPP, UDMA, DMA	GC Corp, Tokyo, Japan
Orovex Mouthwash	Thymol, Menthol, Glycerine, Sodium Saccharine, Sodium Fluoride, Chlorhexidine	Macro Group Pharmaceutical, Egypt
Neem Mouthwash	Deionized Water, Aloe Barbadensis gel, Sorbitol, Glycerine Vegetarian, Poloxamer 407, peppermint leaf, Spearmint leaf, Clove, Illicium verum oil, Azadirachta Indica (Neem) Bark, Thymus Serphyllum leaf oil, Ascorbic acid, Xylitol, Potassium Sorbate	Theraneem Naturals, USA

Statistical analysis

Numerical data from the experiment was collected, tabulated and checked for normality using test of normality (Shapiro–Wilk test). The data was found to be normally distributed and a parametric test, two way ANOVA, was used to compare between different groups, and post-hoc test was used to detect significance if present. The significance level was set a $p \leq 0.05$. IBM SPSS statistics for windows, was used for statistical analysis.

RESULTS

Two way ANOVA followed by Tukey's post hoc test was done to evaluate the effect of the two tested variables, the first was the effect of the type of immersion medium, with its three levels, either

distilled water (DW), chlorohexidine mouthwash (CM) and Neem mouthwash (*Azadirachata indica*) (NM), and the second was the effect of immersion time with its four levels, either 1.5 hours, 3 hours, 18 hours, or 36 hours, on ΔE and VHN of Cerasmart. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics Version 20 for Windows.

Two ways ANOVA showed that the immersion medium had a statistically significant effect on the ΔE and VHN of Cerasmart at $p = 0.0001$ for both, also significance was detected for the effect of immersion time on the ΔE and VHN of Cerasmart

at $p = 0.0001$ for both [Table (2) &(4)].

Statistical significant difference was detected between all tested groups after 1.5 hours as DW had a ΔE of (0.413), CM scored (1.311) and (1.05) for NM, also after 3 hours all immersion mediums was not able to cause any significant change in ΔE . But after 18 hours, all the tested groups showed a significant increase in ΔE , and DW was significantly superior to both CM and NM. At 36 hours only CM showed a significant increase in ΔE , unlike DW and NM didn't suffer any significant rise [Table (3) and Figures (1&2)].

TABLE (2) Two way ANOVA statistical evaluation for the effect of the type of immersion medium and time on ΔE of Cerasmart.

Variables	df	Sum of Square	Mean Square	F Statistic	P-value
Immersion medium	2	42.255	21.1275	1374.5818	0.0001
Time	3	29.3655	9.7885	636.8518	0.0001
Immersion medium x Time	6	14.5302	2.4217	157.559	0.0001

TABLE (3) Means, standard deviation and significance in ΔE for the tested groups.

Mouth wash	Symbol	1.5 hour	3 hours	18 hours	36 hours
Distilled water	DW	0.413±0.04 ^(g)	0.456±0.0 ^{9(gf)}	0.686±0.09 ^(fc)	0.91±0.09 ^(e)
Orovex	CM	1.311±0.16 ^(d)	1.49±0.16 ^(d)	2.669±0.16 ^(b)	4.41±0.17 ^(a)
Neem	NM	1.05±0.13 ^(e)	1.091±0.13 ^(e)	2.06±0.12 ^(c)	2.14±0.15 ^(c)

Different superscript show statistical significance $p \leq 0.05$

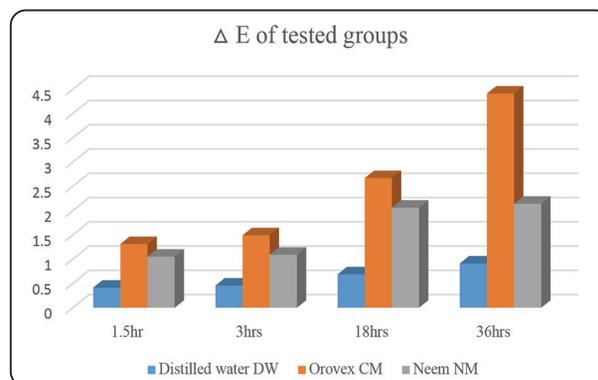


Fig. (1) Shade difference (ΔE) of Cerasmart stored at the three tested immersion media at different time intervals.

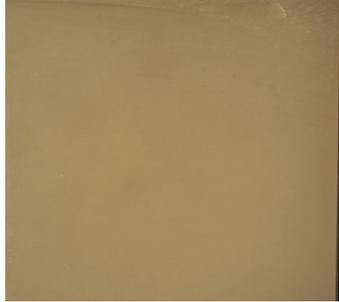
Mouth wash	Distilled water DW	Orovex CM	Neem NM
1.5 hour			
3 hours			
18 hours			
36 hours			

Fig. (2) Photographic images illustrating for the effect of the type of immersion medium and time on ΔE of Cerasmart.

Statistical significant difference was not detected between all tested groups after 1.5hours, but after 3 hours all the tested groups showed a drop in VHN, and CM group was significantly lower than NM group and DW showed the highest significant difference with a mean VHN of 79.96 ± 1.93 . After

18 hours, although the VHN of all the tested groups dropped, yet there were no statistical significant difference in between the groups, unfortunately all tested groups except DW showed a significant drop in VHN after 36 hours [Table (5) and Figure (3)].

TABLE (4) Two way ANOVA statistical evaluation for the effect of the type of immersion medium and time on VHN of Cerasmart.

Variables	df	Sum of Square	Mean Square	F Statistic	P-value
Immersion medium	2	65.7453	32.8726	22.0544	0.0001
Time	3	849.5049	283.1683	189.9785	0.0001
Immersion medium x Time	6	11.7181	1.953	1.3103	0.2666

TABLE (5) Means, standard deviation and significance in VHN for the tested groups.

Mouth wash	Symbol	1.5 hours	3 hours	18 hours	36 hours
Distilled water	DW	82.73 ± 1.88 ^(a)	79.96 ± 1.93 ^(b)	76.03 ± 0.55 ^(d)	75.03 ± 0.55 ^(d)
Orovex	CM	81.56 ± 0.92 ^(a)	76.86 ± 1.65 ^(d)	73.92 ± 0.59 ^(d)	72.45 ± 1.06 ^(e)
Neem	NM	81.96 ± 0.85 ^(a)	78.58 ± 1.62 ^(c)	73.93 ± 0.36 ^(d)	72.46 ± 1.21 ^(e)

Different superscript show statistical significance $p \leq 0.05$

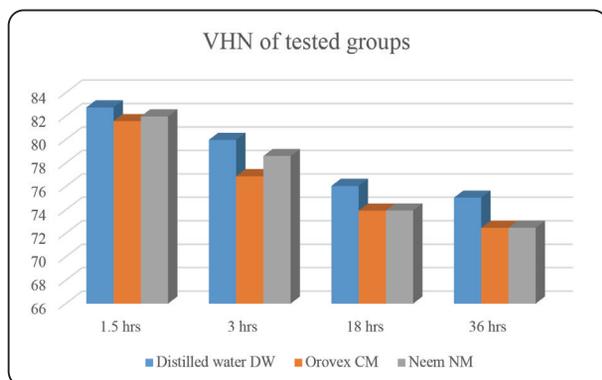


Fig. (3) Microhardness (VHN) of Cerasmart stored at the three tested immersion media at different time intervals

DISCUSSION

In our study, chlorohexidine mouthwash (Orovex) was selected as it represents the most popular antibacterial mouthwash used in preventive dentistry. On the other hand, neem mouthwash was selected to represent naturally introduced compounds with a claim of their capability in competing with other commercially available antibacterial mouthwashes in the market. Although chlorohexidine has gained the popularity as claimed in literature, yet its prolonged usage may lead

to deterioration of resin-based restorations^(19,20). Theraneem, on the other hand, was chosen to represent a newly introduced natural extract mouthwash, which has been claimed for its high antimicrobial effect with minimal shortcomings that were encountered with other artificially prepared chemical mouthwashes⁽²¹⁾.

The immersion time of our samples in this study was one and half hour, three hours, 18 hours and 36 hours. These time of immersion was calculated to simulate the manufacturer instructions of mouthwashes usage - 3 minutes per day- for one month, two months, one year and two years respectively⁽¹⁸⁾.

Vickers hardness number was selected to evaluate the performance of the material after being subjected to different immersion medias, as it is not only representative for resistance of the material to wear but will also have an impact on surface roughness, plaque accumulation and abrasion behavior of the material against opposing teeth. Furthermore, taking into consideration, its participation in maintaining occlusal anatomy and stability of the restoration under various intra-oral challenges⁽²²⁾.

In our current study, distilled water was the reference of choice to evaluate the effect of the immersion media on color change and VHN in accordance with studies conducted by Vichi et al., Erdrich et al., Yanikoglu et al⁽²³⁻²⁵⁾.

Cerasmart blocks are composed of 71% silicone dioxide (SiO_2) and barium glass nanoparticles embedded in a resinous matrix composed of various molecular weights of Bisphenole A ethoxylate dimethacrylate (Bis-MEPP), Urethane dimethacrylate (UDMA) and Dimethacrylates (DMA) contributing of the hydrophilic behavior of the material presented in water sorption and low color stability than other CAD/CAM ceramic blocks⁽²⁶⁻²⁸⁾.

Color change results in our study showed that there were a statistically significant difference between all the tested groups and there were

significant effect of the immersion media and time intervals, yet neither of them exceeded the threshold ΔE of 3.3 at which color differences can be distinguished in the oral environment⁽²⁹⁾, except after immersion in chlorohexidine mouthwash for 36 hours (which represent prolonged usage of CHX for two years). Regarding microhardness results, no difference between the immersion media appeared at 1.5 hours, yet both tested mouthwashes started to show significant drop in VHN number at 18 and 36 hours.

These results may be due to surface and bulk deterioration as a result of adding fluoride containing compounds (sodium fluoride) - which is present in Orovex mouthwashes - and antibacterial natural extracts (oil of clove) which is present in both mouthwashes - used in this current study - which may play a major role in surface deterioration and degradation of the resin based materials affecting their esthetics and surface properties^(30,31).

In addition, other different components in mouthwash formulations have affected the hydrogen ion concentration rendering the chlorohexidine mouthwashes more acidic in nature while the neem containing mouthwash has a pH shifted towards the alkaline nature, such variation in hydrogen ion concentration may have participated in the performance of the used mouthwashes, allowing them to have a varying degrading and plasticizing effect on resinous component^(32,33).

Such deterioration is attributed to both endogenous and exogenous factors. Regarding the endogenous factors, the link between organic and inorganic components of the resin based restorations play a major role in color stability and surface property due to hydrolytic sensitivity of such link. As for the exogenous factors, surface properties and surface energy affect the interaction with variant chromogens introduced in antiseptic mouthwashes⁽³⁴⁾.

Moreover, introduction of resinous component in the Cerasmart blocks may participate in raising the surface energy of the restorations⁽³⁵⁾. This is

facilitating the spreading of chromogenic and hydrolytic factors, which may have a negative impact on color stability and surface hardness⁽²⁶⁾. This may explain that, at the beginning of the immersion of samples in mouth wash, partial hydrolytic degradation of the surface may have occurred, which is manifested in only change in shade, and as the immersion time increase, the surface degradation increase leading to changes in surface properties manifested in shade and microhardness change⁽³⁶⁾.

One of the limitations of this study was the use of one type of resin-based CAD\CAM restorative block (Cerasmart) which didn't give us the full overview on the effect of antimicrobial mouthwashes on other types of resin-based CAD\CAM restorative materials which have variations in their chemical composition through different products and manufacturers.

According to our findings in this study, the following could be concluded:

1. Long term usage of antimicrobial mouthwashes used in this study (more than one year) should be avoided due to its negative effects surface microhardness of resin based CAD\CAM restorations.
2. Prolonged use of neem mouthwash had a minimal effect on color change on resin based CAD\CAM restorations compared to CHX.

According to our findings in this study, the following suggestions are recommended:

- a. Surface maintenance of resin based restoration is recommended to counteract the possible cumulative deteriorating effect of different antimicrobial mouthwashes.
- b. Further aging challenges, such as thermocycling, will be beneficial to test the performance of indirect resin based blocks and response to antimicrobial mouthwashes.

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