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RESEARCH AND EDUCATION

Effect of surface roughness and biofilm formation on the color properties of resin-infiltrated ceramic and lithium disilicate glass-ceramic CAD-CAM materials

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ABSTRACT

Statement of problem. Computer-aided design and computer-aided manufacturing (CAD-CAM) materials have become popular for dental restorations; however, which materials should be preferred in terms of surface properties after biofilm formation is unclear.

Purpose. The purpose of this in vitro study was to investigate the effect of biofilm formation on the discoloration properties of resin-infiltrated ceramic and glass ceramic CAD-CAM materials and human teeth and to examine the effect of the brushing procedure on color change.

Material and methods. One hundred and six 2-mm-thick specimens were prepared from IPS e.max CAD and Cerasmart, and a total of 53 intact human teeth were used. Five specimens from each group were used to measure the amount of live biomass in the biofilm. The remaining 48 specimens in each group were divided into 4 subgroups: kept in distilled water without the formation of dental biofilm (DW), kept in tea without the formation of dental biofilm (T), kept in distilled water after the formation of dental biofilm (DWB), and kept in tea after the formation of dental biofilm (TB) (n=12). After finishing and polishing the materials, initial color measurements were made using a spectrophotometer, and surface roughness measurements were made using noncontact profilometer. After creating a biofilm layer in DWB and TB, all specimens were kept in their solutions at 37 °C for 24 hours, and the color measurements were repeated. After the biofilm layer had been removed by brushing, a third color measurement was made. The data were statistically analyzed with one-way analysis of variance (ANOVA) and two-way ANOVA (α=.05).

Results. The lowest roughness value was observed in Cerasmart. Tooth-IPS e.max CAD gave similar results. The Cerasmart material had the most viable biomass, whereas the IPS e.max CAD material had the least. TB had the highest ΔE1 value for all materials and DW had the lowest (P<.05). The brushing procedure caused the materials to return to their initial colors or reduce the color change in most groups.

Conclusions. The presence of biofilm on CAD-CAM materials immersed in distilled water caused an unacceptable degree of discoloration (ΔE>1.8), and immersion in tea led to greater color change. The adhesion of biofilm to restorative dental materials plays an important role in the coloring of these dental materials. (J Prosthet Dent xxxx;xxx:xxx-xxx)

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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Clinical Implications

Adhesion of biofilm to materials plays an important role in the discoloration and surface roughness of the restorative dental materials. Cerasmart and IPS e.max CAD can provide clinically acceptable color stability and surface roughness.

A biofilm is a complex polymicrobial community surrounded by a polysaccharide matrix produced by microorganisms capable of containing different types of microorganisms, communicating among themselves and adhering to surfaces.[1] These biofilms confer properties such as adaptation to diverse environmental conditions and development of resistance mechanisms for the microorganisms in their content. The 3 main components necessary for a biofilm are microorganisms, a solid surface, and liquid flow.[2,3] Biofilms have been reported to adhere more easily to solid surfaces, especially unpolished surfaces; therefore, the amount of plaque accumulation is related to the surface structure and surface roughness.[4,5]

Cracks, grooves, and worn surfaces lead to greater surface roughness, creating suitable areas for bacterial adhesion. These surface irregularities protect bacteria from shear forces in the oral cavity and enable bacteria to bind more strongly to the substrate, also causing discoloration of the restoration over time.[6] The color stability of a material affects restoration success. Teeth and restoration materials are exposed to many factors such as biofilm, acidic by-products formed in the biofilm, and coloring foods and beverages.[6] The results of in vitro testing materials exposed to these conditions can be used to predict the clinical performance of restorations regarding longevity and esthetics. Previous studies have focused on bacterial adherence on materials used in restorative dentistry,[7–9] but studies conducted on biofilm formation in computer-aided design and computer-aided manufacturing (CAD-CAM) restorative materials are sparse. Therefore, this study aimed to investigate the color changes that occurred in CAD-CAM materials and human teeth by forming a biofilm layer and to determine the extent this color change was reversed. The null hypotheses were that increasing the degree of roughness of CAD-CAM materials would not increase the amount of live biomass formed on the material or cause more discoloration of the material, that the presence of biofilm on CAD-CAM materials would not cause further discoloration of the materials by coloring beverages, and that brushing would not influence discoloration.

MATERIAL AND METHODS

This study was approved by the Ethics Committee of Kocaeli University (KOU KAEK 2021/222). A power analysis was performed using an analysis program (G*Power 3.1.9.7.; Heinrich Heine University Düsseldorf). The analysis was performed with 95% power and a 5% error (effect size=0.40, α=.05) for a total specimen size of 102. This was increased to 144 specimens to increase statistical power.

The specimens were sliced into 2-mm-thick sections from the Cerasmart (GC Corp) and IPS e.max CAD (Ivoclar AG) blocks under water cooling using a low-speed diamond saw (Micracut; Metkon) (n=53), and 53 extracted intact caries-free third molar human teeth were collected for testing (Table 1). Five specimens from each group (IPS e.max CAD, Cerasmart, teeth) were divided for the measurement of live biomass in the biofilm. The remaining 48 specimens from each group were divided into 4 experimental subgroups (n=12), kept in distilled water without forming a dental biofilm (DW), kept in tea without forming a dental biofilm (T), kept in distilled water after the dental biofilm had formed (DWB), and kept in tea after the dental biofilm had formed (TB) (Fig. 1).

A glaze (IPS e.max CAD Crystall/Glaze Spray; Ivoclar AG) was sprayed on the IPS e.max CAD blocks specimens according to the manufacturer's instructions from a 10-cm distance to produce a uniform layer on the specimens. Then, they were crystallized and glazed in one step in a ceramic oven (Programat P300; Ivoclar AG). The Cerasmart blocks were finished and polished using a 2-stage polishing system (Sof-Lex Diamond Polishing system; 3M ESPE) with a slow-speed micromotor (handpiece; KAVO AG) running at 15 000 to 20 000 RPM. After the surface treatments had been completed, the specimens were cleaned in an ultrasonic cleaner (Aleks Makina Co) using deionized water for 10 minutes, and roughness (Nanovea 3D Non-Contact Profilometer PS50; Nanovea) and color measurements (VITA Easyshade Compact device; VITA Zahnfabrik) were made. The color measurements were made on a standard white background and under constant light to eliminate the background effect,[10] and the L* (lightness), a* (redness), b* (yellowness) values were recorded. The CIEDE2000 system

Table 1. Materials used

Material	Contents	Manufacturer	Lot Number	Color
Cerasmart CAD-CAM Bloc	71% silica and barium glass nanoparticles, 20 nm silica, 300 nm barium glass nanoparticles, 29% Bis-MEPP, UDMA and DMA polymers	GC Corporation	2012151	A2 HT
IPS e.max CAD CAD-CAM Bloc	(57–80%) SiO_2, (11–19%) Li_2O, (0–13%) K_2O, (0–11%) P_2O_5, (0–8%) ZrO_2, (0–8%) ZnO, (% 0–12) other oxides	Ivoclar AG	Z00FTN	A2 HT

Bis-MEPP, 2;2-bis (4-methacryloxy polyethoxy phenyl) propane; DMA, dimethacrylate; K_2O, potassium oxide; Li_2O, lithium oxide; P_2O_5, diphosphorus penta oxide; SiO_2, silicon dioxide; UDMA, urethane dimethacrylate; ZnO, zinc oxide; ZrO_2, zirconium oxide.

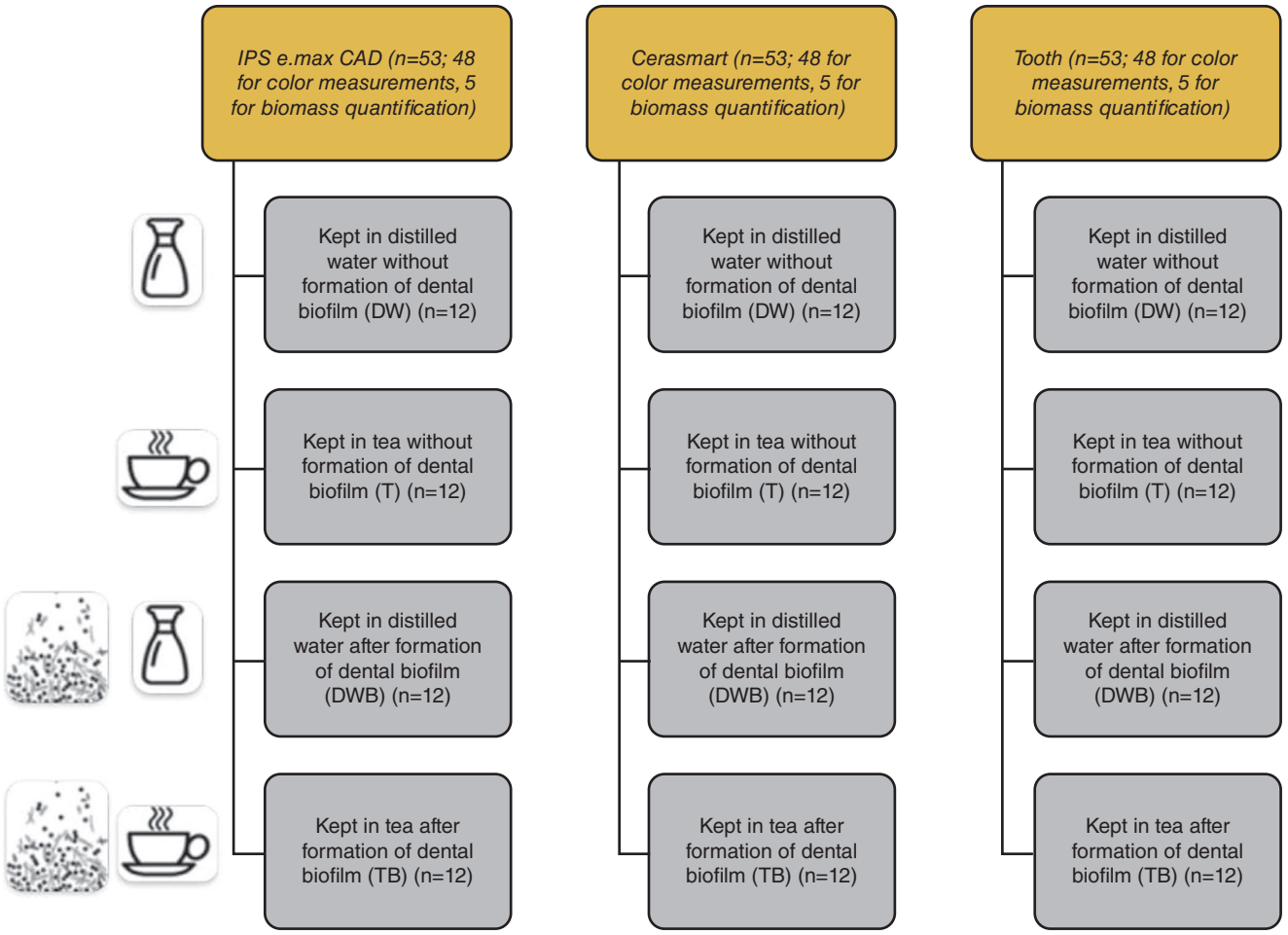

Figure 1. Distribution of materials in subgroups.

(ΔE_{00}) was used to calculate the color change. All specimens were then sterilized using an ethylene oxide gas sterilization system (Steris Amsco Eagle). The sterilization procedure was performed according to the stages of vacuum, heating, humidification, sterilization, and ventilation. Specimens were exposed to ethylene oxide gas at temperatures ranging from 37 °C to 54 °C using the cold vapor and dry systems of the ethylene oxide sterilization technique for 4 hours and then aerated for 12 hours.[11]

To ensure bacterial attachment and to create a pellicle layer on the specimens, artificial saliva was created.[12] For 2 L of artificial saliva, the following components were used: 8.4 mg NaF, 2560 mg NaCl, 332.97 mg $CaCl_2$, 250.00 mg $MgCl_2$ ($6\,H_2O$), 189.48 mg KCl, 0.1 mL H_3PO_4 (85%), and 0.1 mmol NaOH. All materials were mixed, and the pH of the resulting mixture was measured using a pH meter (pH meter; Hanna Instruments Inc). To adjust the pH of the mixture to between 6.5 and 7, 30 mL of 0.1 M NaOH was added to the saliva mixture.[9] The artificial saliva was sterilized by passing it through a 0.22-μm syringe polyethersulfone membrane filter (PES filter; BIOSORFA), and then, under aseptic conditions, Type II mucin (Sigma-Aldrich Chemie GmbH) was added to achieve a concentration of 140 mg per 100 mL.[9]

Streptococcus mutans (*S. mutans*) HF76, *Streptococcus sanguinis* (*S. Sanguinis*) ATCC 10556, and *Candida albicans* (*C. albicans*) ATCC 90028 strains were used for biofilm formation. *S. mutans* and *S. sanguinis* strains were removed from a freezer and inoculated on 5% sheep blood agar (SBA) medium and incubated at 37 °C in 5% CO_2. *C. albicans* was inoculated on Sabouraud dextrose agar (SDA) medium and incubated at 37 °C for 24 hours. After incubation, *S. mutans*, *S. sangiunis*, and *C. albicans* were subcultured. The subcultured tubes were centrifuged at 3000 *g* at 19 °C for 5 minutes and the supernatant was discarded. Then, sterile phosphate-buffered saline (PBS; VWR) (pH 7.0) was added and washed, and this process was repeated twice. For biofilm formation, the turbidity of bacteria and yeast was adjusted to 0.5 McFarland (1.5×108 cfu/mL) *S. mutans* and *S. sangiunis* was prepared by suspending brain heart infusion (BHI) broth with 3% sucrose, 0.5 McFarland *C. albicans* Sabouraud Dextrose Brouth (SDB).[13]

Before biofilm formation, previously sterilized IPS e.max CAD (n=29) and Cerasmart (n=29) CAD-CAM blocks were placed in 24-well plates, and human teeth (n=29) were placed in tubes. All specimens were covered with artificial saliva and incubated at 37 °C for 1 hour for pellicle formation. After incubation, artificial saliva was removed from the specimens. For biofilm formation, prepared bacteria and yeast solutions were inoculated onto all specimens in equal amounts and incubated for 48 hours at 37 °C in a 5% CO_2-supplemented environment.[8,14]

Biofilm formation was analyzed for 5 specimens from 3 different materials (CAD-CAM specimens and human teeth) by using an MTT-based assay based on previous studies.[14,15] The MTT stock solution was prepared by dissolving 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide in sterile PBS. PMS stock solution was prepared by dissolving 0.3 mg/mL N-methylphenazinium methyl sulfate in sterile PBS. The solutions were stored in light-proof vials at 2 °C until the day of the experiment. MTT solution was prepared freshly by mixing 1 mL of MTT stock solution, 1 mL of PMS stock solution, and 8 mL of sterile PBS. Five IPS e.max CAD specimens, 5 Cerasmart specimens, and 5 teeth were transferred to new 24-well plate and tubes, respectively. All specimens were gently washed 3 times with sterile PBS. Then, MTT solution was placed in each well and the tubes were incubated for 5 hours in darkness at 37 °C. After incubation, the MTT solution was gently removed by aspiration. Lysis solution (10% v/v sodium dodecyl sulfate and 50% v/v dimethylformamide in distilled water) was added to each well and tube and incubated in darkness for 1 hour at room temperature to allow the biofilm to disaggregate and the intracellular formazan crystals to dissolve. Subsequently, 80 µL of each well and tube was placed in a 96-well plate, and the optical density (550 nm) was measured using an ELISA microplate reader (Alisei). CAD-CAM specimens on which biofilm was formed were transferred to new 24-well plates, and the teeth were placed in new tubes and gently washed 3 times with PBS to remove nonadherent cells.[14,15]

Two different solutions, distilled water and tea, were prepared for coloring the specimens. Two 2-g tea bags (Yellow Label Tea; Lipton) were immersed in 300 mL of boiling distilled water for 10 minutes to create the tea solution.[16] After the specimens had been kept in solutions for 24 hours, the second color measurements were made.

To remove the plaque on the specimens, a rechargeable toothbrush (Oral B Ultra Plaque Remover D9; Braun AG) was used and fixed to a stand to simulate brushing according to the International Organization for Standardization (ISO) 14569–1 standard.[17,18] All specimens were brushed under a 2-N load for 10 seconds using toothpaste (ProNamel; Sensodyne) homogeneously mixed with distilled water in a 1:1 ratio to a slurry.[19] After the brushing procedure, the specimens were washed in distilled water and cleaned, and third measurements were made.

All statistical analyses were made with a statistical software program (IBM SPSS Statistics, v20.0; IBM Corp). The Shapiro-Wilk test was used to assess the normality assumption. Continuous variables are presented as mean ±standard deviation. One-way analysis of variance (ANOVA) was performed to determine differences between groups. A two-way ANOVA was conducted to investigate the main and interaction effects of factors (α=.05).

RESULTS

The initial surface roughness of the Cerasmart, IPS e.max CAD materials, and teeth were measured, and the Ra (arithmetic average roughness), Rz (average maximum height of the profile), and Sa (arithmetic mean height of the surface) values recorded (Table 2). Representative 3-dimensional images of the specimens are shown in Figure 2.

The mean Ra values of Cerasmart, IPS e.max CAD, and teeth were 0.181, 0.427, and 0.238, respectively, with a significant difference between Cerasmart and IPS e.max CAD specimens (P=.001), and the teeth and IPS e.max CAD specimens (P=.007). The Cerasmart and the tooth specimens were statistically similar (P=.602).

The mean Rz values of Cerasmart, IPS e.max CAD, and the teeth were 0.700, 2.209, and 0.838, respectively, with a significant difference between Cerasmart and IPS e.max CAD specimens (P<.001) and IPS e.max CAD and tooth specimens (both P<.001). The Cerasmart and the tooth specimens were statistically similar (P=.737).

The mean Sa values of Cerasmart, IPS e.max CAD, and teeth were 0.402, 4.827, and 4.811, respectively, with a significant difference between Cerasmart and IPS

Table 2. Ra, Rz, Sa (mean ±standard deviation), df, and f values

Material	Ra	Rz	Sa	*df*
Cerasmart	0.181 ±0.186 A	0.700 ±0.099 A	0.402 ±0.108 A	*33*
IPS e.max CAD	0.427 ±0.141 B	2.209 ±0.732 B	4.827 ±2.623 B	*33*
Tooth	0.238 ±0.080 A	0.838 ±0.261 A	4.811 ±1.553 B	*33*
df	2	2	2	
f	9.756	40.755	25.139	

Different uppercase letters indicate statistically significant differences in column (P<.05).

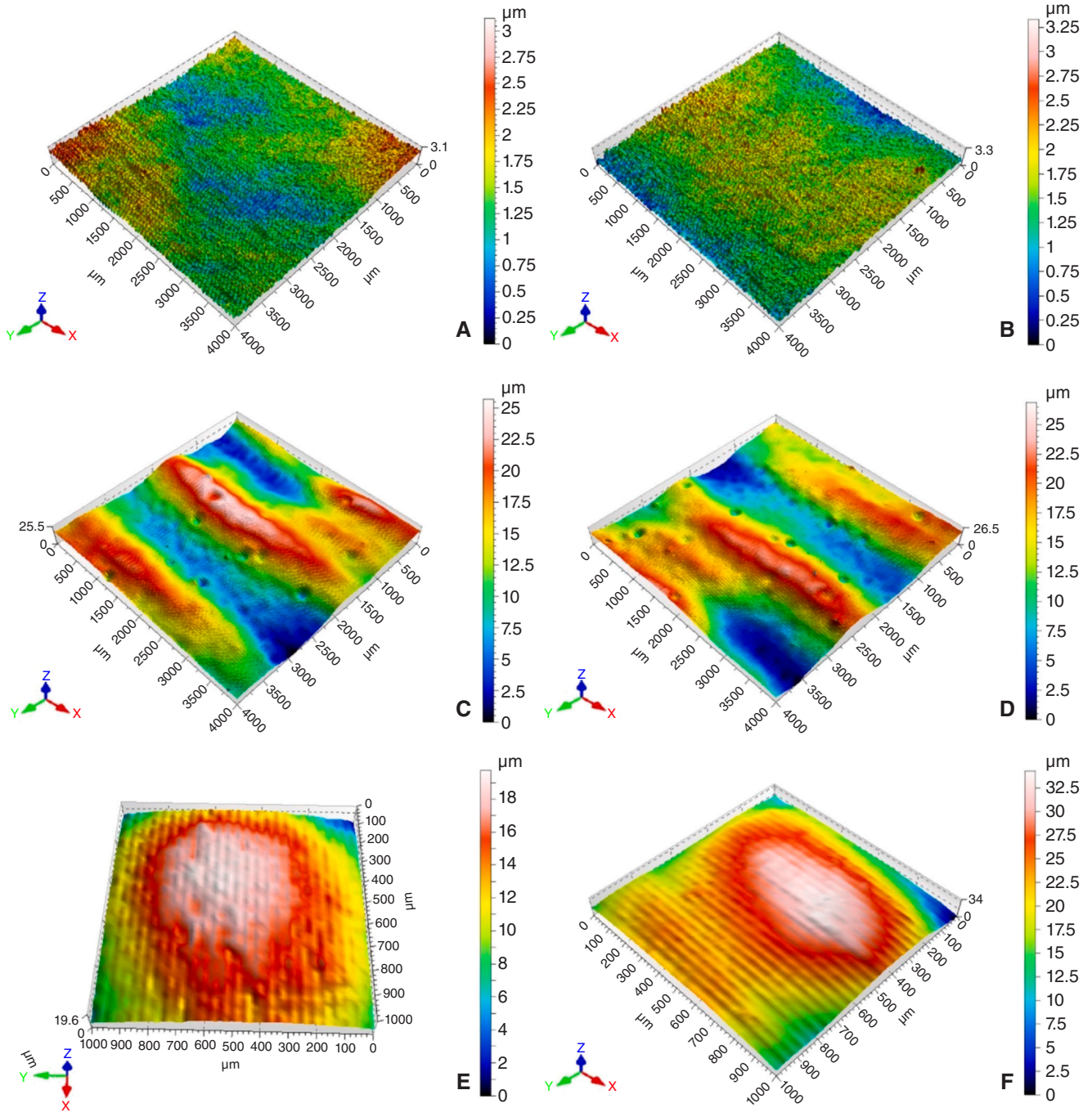

Figure 2. Representative three-dimensional images of specimens. A, B, 3D images of Cerasmart. C, D, IPS e.max CAD. E, F, Human tooth.

e.max CAD specimens ($P<.001$) and IPS e.max CAD and tooth specimens (both $P<.001$). The Cerasmart and the tooth specimens were statistically similar ($P=.999$). Cerasmart had the lowest roughness values in all parameters (Ra, Rz, Sa) and IPS e.max CAD had the highest.

The mean ±standard deviation color change values of the CAD-CAM materials and tooth specimens after dental biofilm formation are shown in Table 3. Color differences were calculated between baseline and after storage in solutions with or without biofilm formation (ΔE1), after storage and after brushing (ΔE2) and between baseline and after brushing (ΔE3). In the 4 groups stored in solution (DW, T, DWB, TB), the lowest value was obtained in DW and the highest in TB in IPS e.max specimens. After brushing, the materials' initial color was restored or the color change was less in most groups ($P<.05$).

Table 3. ΔE values (mean ±standard deviation)

Material	Solution	Biofilm Formation	ΔE1	*P*	ΔE2	*P*	ΔE3	*P*
Cerasmart	Distilled Water	DW: Without biofilm formation	0.893 ±0.634	<.001	0.832 ±0.332	<.001	0.696 ±0.509	.311
		DWB: With biofilm formation	2.265 ±0.780		2.244 ±0.843		0.496 ±0.231	
	Tea	T: Without biofilm formation	2.917 ±0.438	<.001	2.897 ±0.412	<.001	0.553 ±0.218	.905
		TB: With biofilm formation	6.153 ±1.365		6.214 ±1.325		0.479 ±0.322	
IPS e.max CAD	Distilled Water	DW: Without biofilm formation	0.444 ±0.103	.024	0.652 ±0.167	0.017	0.636 ±0.165	.451
		DWB: With biofilm formation	2.118 ±1.019		1.629 ±0.599		0.948 ±1.282	
	Tea	T: Without biofilm formation	2.722 ±0.883	<.001	3.009 ±0.940	<0.001	0.432 ±0.120	.030
		TB: With biofilm formation	7.787 ±4.563		7.863 ±4.508		0.347 ±0.217	
Tooth	Distilled Water	DW: Without biofilm formation	3.575 ±1.228	.557	1.567 ±1.033	0.951	3.725 ±1.003	.174
		DWB: With biofilm formation	6.373 ±5.492		3.731 ±5.076		3.996 ±2.594	
	Tea	T: Without biofilm formation	2.683 ±1.204	.891	1.496 ±0.957	0.889	2.680 ±1.290	.064
		TB: With biofilm formation	6.581 ±4.634		3.892 ±2.026		5.436 ±2.080	

ΔE1: Difference in color change between baseline and after biofilm formation.

ΔE2: Difference in color change between biofilm formation and after brushing procedure.

ΔE3**:** Difference in color change between baseline and after brushing procedure.

Table 4. Mean ±standard deviation values with live biomass evaluation

Material	Live Biomass Evaluation (Mean ±Standard Deviation)
Cerasmart	0.883 ±0.235 A
IPS e.max CAD	0.352 ±0.065 B
Tooth	0.496 ±0.054 B

Different uppercase letters indicate statistically significant differences in the column (*P*<.05).

The data for biofilm viability on the teeth, Cerasmart, and IPS e.max CAD specimens using the MTT method are shown in Table 4. The highest amount of viable biomass was observed in Cerasmart (0.883), and the lowest was in IPS e.max CAD (0.352) (*P*<.05).

DISCUSSION

Restorations should have a smooth surface to achieve optimal esthetics and function. Adhesion of oral bacteria to restorative materials is influenced by the bacteria and surface properties, including surface free energy, surface chemical composition, and roughness.[6,15,20] A relationship has been reported between decreasing roughness and a decline in bacterial adhesion,[6] although other studies have reported that roughness is independent of adhesion[9,21] or that there is no relationship between bacterial adhesion and roughness.[22,23]

Different roughness values have been reported for ceramics because roughness depends on the composition of the material, manufacturing method, measurement techniques, and surface treatment, which can affect bacterial adhesion and biofilm formation.[23,24] In the present study, the lowest roughness values were observed with the Cerasmart specimens, but they were associated with the highest amount of viable biomass. Therefore, the null hypothesis that increasing the degree of roughness of CAD-CAM materials would not increase the amount of live biomass formed on the material was not rejected. Despite the higher surface roughness values for IPS e.max CAD, a lower amount of live biomass was detected compared with the Cerasmart.

Glazing creates smooth undulations on the material surface, associated with minimal accumulation of plaque.[25] The polishing process removed superficial matrix-rich composite resin layers in the resin-infiltrated ceramic Cerasmart specimens, producing a chemically and physically different surface than its unpolished counterpart.[26] Additionally, e.max CAD may have improved surface properties associated with its oven finish, which may have impacted the results.

S. mutans and *S. sanguinis* were included in the present study because they are the main colonizers of hard surfaces in the oral cavity, functioning as a bridge between other bacteria that attach to and grow on the substrate surface.[27] Quantifying bacterial viability in the biofilm is important to evaluate the pathogenicity of dental plaque. In the present study, a correlation was found between the glass content of the materials and bacterial viability. The ceramic content has been reported to affect bacterial viability during adhesion with low *S. oralis* and *S. sanguinis* counts on tetragonal stabilized zirconia, zirconia-reinforced glass ceramics, and glass alumina ceramics.[28]

A direct relationship has been reported between surface roughness and color stability,[29,30] although other studies[31,32] could not establish such a relationship. In the present study, although Cerasmart had the lowest roughness values in all parameters (Ra, Rz, Sa); the ΔE1, ΔE2 and ΔE3 values were relatively higher compared with those of IPS e.max CAD. Therefore, the null hypothesis that increasing the degree of roughness of CAD-CAM materials would not cause more discoloration of the material was not rejected.

The null hypothesis that the presence of biofilm on CAD-CAM materials would not cause further discoloration of the materials by coloring beverages was rejected. In the present study, tea, containing tannins or

tannic acid with protein denaturation activities, was used as the staining solution. Tannic acid has been reported to support the formation of a brownish-stained pellicle on teeth.[33] In the present study, the discoloration was statistically similar between the 2 materials with the formation of biofilm.

Tooth brushing has been associated with reduced retention of external pigments,[29,34,35] but it depends on how deeply the pigment molecules penetrate the material. Mozzaquatro et al[36] concluded that, although the materials had significant discoloration depending on the duration of exposure to red wine and brushing, short-term brushing reduced the color change and did not increase roughness. The present study supported these conclusions, and that brushing mostly restores the initial color. Therefore, the null hypothesis that brushing does not influence discoloration was rejected.

Limitations of this study included the lack of simulation of the oral environment. The color and roughness of materials can be affected by factors that include the soft tissues, other dental structures, and diet. Therefore, further studies are needed under oral conditions to simulate the aging procedure and to test different CAD-CAM materials such as zirconia.

CONCLUSIONS

Based on the findings of this in vitro study, the following conclusions were drawn:

1. The adhesion of biofilm to restorative dental materials plays an important role in the coloring of dental materials.
2. The coloring and biofilm adhesion was less with IPS e.max CAD than with Cerasmart.

PATIENT CONSENT

All participants were freely invited, and those who accepted signed an informed consent approved and stamped by the local ethics committee.

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