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Research article

Streptococcus-mutans and Porphyromonas-gingivalis adhesion to glazed/polished surfaces of CAD/CAM restorations

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ABSTRACT

Purpose: Dental restorations fabricated using CAD/CAM require modification/adjustment before cementation. Streptococcus mutans *(S.mutans)* and Porphyromonas gingivalis *(P.gingivalis)* are prevalent bacterial species that may adhere to these materials and can cause caries, gingivitis/periodontitis. The purpose of this in vitro study was to evaluate the bacterial adherence of *S. mutans* and *P.gingivalis* to five different kinds of modern CAD/CAM restorative materials with different compositions following chairside finishing/polishing and glazing.

Materials and methods: Specimens (N = 75) from five test materials (n = 15 each) "Tetric-CAD®; IPS-e.max-CAD®; IPS-e.max-ZirCAD®; CELTRA-Duo® and Vita-Enamic®" were prepared in disc shape (10 × 3 mm) using CAD/CAM. The specimens underwent glazing and finishing/polishing using established procedures. The surface roughness was measured in micrometers (μm) using a profilometer. Bacterial adherence to test materials' glazed and finished/polished surfaces was tested using bacterial culture growth over the test materials. Data obtained was tabulated and statistical analysis performed using Kruskal Wallis test, post-hoc Conover test, Mann-Whitney *U* test and Tukey post hoc test.

Results: With the exception of IPS-e.max-ZirCAD®, which showed the contrary, the adherence of *S.mutans & P.gingivalis* was less on glazed surfaces compared to finished/polished surfaces for four test materials: "Tetric-CAD®, IPS-e.max-CAD®, CELTRA-Duo®, and Vita-Enamic®". On the glazed surfaces, the adhesiveness of *S.mutans and P.gingivalis* was not significant ($p = 0.099$; $p = 0.660$); however, on the finished/polished surfaces, it was significant ($p = 0.002$; $p = 0.004$). With the exception of 'IPS-e.max-ZirCAD®', which showed the reverse behavior, the adhesion of *S.mutans & P.gingivalis* to finished/polished surfaces was greater for each of the four ceramics under investigation "Tetric-CAD®, IPS-e.max-CAD®, CELTRA-Duo®, and Vita-Enamic®".

Conclusion: Glazed surfaces for majority of test materials demonstrated decreased adhesion from *S.mutans & P.gingivalis*, hence prior to final placement of restoration, it is advised to adhere to the minimal glazing criteria. Regardless of the chemical composition of the materials, the surface texture of the tested materials significantly influenced bacterial adhesion.

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1. Introduction

The oral environment, containing numerous enzymes and proteins, is complex and heterogeneous. Changes in pH, dietary habits, hygiene, teeth number, and health status can affect dental tissues and restorative materials, leading to biofilm formation and compromising oral health [1]. Disturbances can lead to dental caries, gingivitis, and periodontitis [2]. Bacteria in the oral cavity play varying roles in initiation and progression of these diseases [3]. Streptococcus mutans *(S.mutans)*, a gram-positive facultative anaerobe, is the main culprit for caries initiation [4]. Other bacterial species involved in periodontal diseases include Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis *(P.gingivalis)*, Prevotella intermedia, Forsythus, Campylobacter rectus, Eubacterium nodatum, Peptostreptococcus micros, and Streptococcus intermedius [5–9]. *P.gingivalis* is a gram-negative anaerobic bacterium, which affects the periodontium either directly or indirectly by regulating the host's response to inflammation and considered as a major factor contributing to the formation of chronic periodontitis [10–12].

Dental restorative materials have significantly advanced in recent years, focusing on repairing naturally impaired teeth to restore function and appearance [13]. Materials used include metals, ceramics, synthetic polymers, and combinations of these [14]. However, challenges like shrinkage, durability, and biological toxicity can be addressed through advanced technology and innovative breakthroughs [15]. Developments are needed to enhance mechanical strength, antibacterial properties, mineralization capacity, self-repair capabilities, and regenerative attributes [16]. Clinical barriers include dimensional accuracy, wear resistance, aesthetic appearance, and biocompatibility, requiring immediate investigation [13–16].

Dental restorative materials face challenges due to the presence of oral biofilm, a complex microbial community primarily originating from bacteria and saliva [17]. This biofilm can lead to undesirable side effects such as secondary caries, periodontal disease, peri-implantitis, stomatitis, and restoration failure [18]. The surface properties of these materials, including roughness, chemical makeup, and form, significantly influence the microbial flora. The particle sizes also influence the properties of restorative materials [19]. Assessing the level of bacterial colonization on restorative materials is crucial to determine the negative effects of bacterial biofilms at the tooth-restoration interface [20]. Surface alterations and enhancements, such as chairside polishing and glazing, can further enhance the evaluation of harmful infections [21,22].

Studies for bacterial adhesion to computer aided design/computer aided manufacturing (CAD/CAM) fabricated modern day restorative materials are scarce. Numerous chairside finishing and polishing kits on the market include wheels, fine diamond-burs, paste, rubber-cups and sandpaper discs [23]. In the literature, varying outcomes have been reported concerning consistent and advised chairside finishing and polishing systems, attributed to differences in materials utilized, evaluation methods, and diverse measurement parameters [24]. In order to compare and assess the bacterial adhesion of two different species of bacteria (*S.mutans & P. gingivalis*) to glazed and chairside finished/polished surfaces of five popular modern indirect CAD/CAM restorative materials "Tetric-CAD®, IPS-e.max-CAD®, IPS-e.max-ZirCAD®, CELTRA-Duo® and Vita-Enamic®" using bacterial culture growth, this in vitro study was conducted.

2. Materials and methods

2.1. Setting and ethical approval

This laboratory study was conducted at the College of Dentistry Research Center (CDRC) and Molecular and Cell Biology Laboratory (MCBL), Dental College, King Saud University, Riyadh, KSA. The CDRC (No. PR 0143) and Institutional Review Board (IRB) ethical committee (No. E−22-7201) at King Saud University Medical City, Riyadh, Saudi Arabia, granted ethical approval.

2.2. Test materials

Five types of CAD/CAM materials were selected: Composite, Lithium Disilicate Glass-ceramic, Zirconium Oxide ceramic, Zirconium

Table 1
Test materials and bacterial details.

Test Material	Abbreviation	Brand		Manufacturer
Composite	TC	Tetric® CAD		Ivoclar Vivadent, Schaan, Lichtenstein
Lithium-Disilicate-Glass-ceramics	LS_2	IPS e.max® CAD		
Zirconium Oxide ceramics	ZrO_2	IPS e.max® ZirCAD		
Zr-Reinforced, Lithium Silicate	ZLS	CELTRA® Duo		Dentsply-Sirona, Bensheim, Germany
Hybrid-Ceramic	HC	Vita Enamic®		VITA, Zahnfabrik, Germany
Bacterial Name	**Abbreviation**	**Type**	***ATCC**	**Company**
Streptococcus Mutans	*S. Mutans*	Gram- Positive (Facultatively Anaerobic)	25175	Streptococcus Mutans Clarke, ATCC® 25175™
Porphyromonas Gingivalis	*P. Gingivalis*	Gram-Negative (Obligately Anaerobic)	33277	Microbiologics, Kwik-Stok, 0912, Saint Cloud, MN, United States

*American Type Culture Collection.

Reinforced Lithium Silicate and Hybrid Ceramic. Under brands names of "Tetric-CAD®, IPS-e.max-CAD®, IPS-e.max-ZirCAD®, CELTRA-Duo® and Vita-Enamic®", respectively. Furthermore, two types of oral bacteria were selected: *S.mutans & P.gingivalis*. Table 1 provides information about the test materials and bacteria utilized.

The G*Power software, version 3.1.9.3 program (Heinrich-Heine-Universitat Dusseldorf, Germany) was used for power analysis. A power of 0.85 (85 %), an alpha threshold of 0.05, and a medium effect size of 0.5 were chosen. After computation, a total sample size of 75 (N = 75) was found, with 15 specimens in each group (n = 15).

2.3. Specimen preparation

Specimens in disc shape with a diameter of 10 mm and thickness of 3 mm were designed using ExoCad software and milled using the milling machine (Ceramill Motion 2). Glazing was performed on one surface following the manufacturer's instructions. Using diamond-impregnated systems, the other surface underwent chair-side finishing/polishing procedures. (DIASYNT®Plus, EVE, Germany) and (DIAPOL® Set HP 310, EVE, Germany).

2.4. Surface roughness measurements

A Profilometer (Contour-GT-X, 3D Optical Microscope, Bruker Nano Surfaces Division, San Jose, CA, USA) was used to evaluate the surface roughness of both surfaces for the five materials in micrometers (μm/m).

Bacteria	*S.Mutans*	*P.Gingivalis*
Agar	Brain Heart Infusion (BHI)	Brucella with Hemin and Vitamin K
Microbial Suspension	100 μL of bacteria streaked on an agar plate, Incubated at 37 °C in anaerobic jar with the use of anaerobic gas pack.	
	Incubation Period	
	2 days	1 week
Broth	BHI	Tryptic Soy
	1 swap of bacteria fused in a conical tube containing 50mL of broth and incubated at 37 °C in anaerobic jar with the use of anaerobic gas pack for bacterial growth.	
	Incubation Period	
	1 day	3 days
Bacterial Application	Sterilization	
	Ultraviolet (UV) germicidal irradiation for 1 hour in each surface.	
	Cell-well Preparation	
	Specimens placed in each well with the tested side facing upward following that, 100 μL of broth injected in each well and 50 μL of infused bacteria applied with volume pipettes.	
	Incubation Period	
	1 day	3 days
Serial Dilution	Specimens washed with distilled water to remove the unattached bacteria. Placed in conical tube filled with 1000 μL of distilled water. Agitated using a vortex agitator for 1 minute to detach the bacteria from the sample surface. Four microcentrifuge tubes assigned for each sample and filled with 900uL distilled water. 100μL of the first microcentrifuge tubes transferred to the second tube and mixed. The process repeated for all microcentrifuge tubes.	
Bacterial Colonization	1- Each agar plate was divided into two sections. 2- 50uL of solution contained in conical tubes was taken with the volume pipette and spread into the agar using a spreader.	
	Incubation	
	2 days	1 week
Colony forming unit (CFU)	Plates examined visually for CFU and the number of living bacteria calculated by multiplying the mean of CFU of each sample by the inverted coefficient of dilution.	

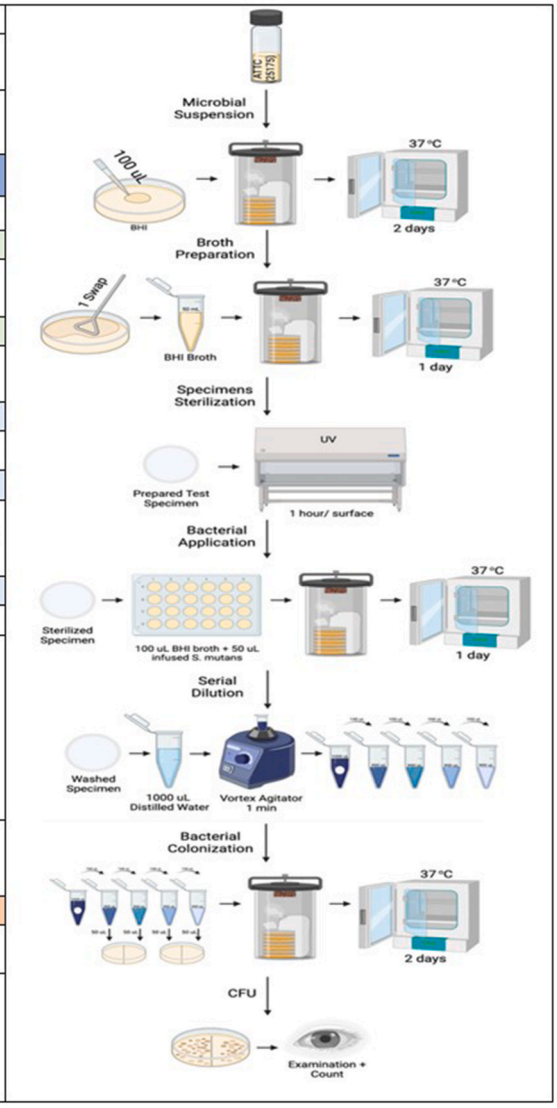

Fig. 1. Flow chart of the Bacterial Adhesion Process with detailed Illustration.

2.5. Bacterial adhesion process

Prior to the initiation of bacterial adhesion process, specimens were soaked in artificial saliva (Biotene® Dry mouth Oral Rinse, Biotene, Germany) and left for 1 h. Bacterial adhesion process was applied to each surface twice (one with *S.mutans*, the other with *P. gingivalis*). The complete process is described in detail in Fig. 1.

2.5.1. Agar plates preparation

Two types of agar were used in this study, Brain Heart infusion (BHI) agar for *S.mutans* (Fig. 2a) and Brucella agar with Vitamin K was used for *P.gingivalis* (Fig. 2b). Below are the details of the preparation.

A. BHI Agar Plates:

Agar plates were prepared by thoroughly mixing 37g of BHI (ThermoScientific CM1135B, Thermo Fisher Scientific, USA) with 1000 mL of distilled water until a homogeneous mixture was obtained. Following that, it was placed in an autoclave machine for 15 min at 15 psi and 121 °C (TR-24S, ALP), then poured in 90 mm × 14.2 mm plates (PD-900, Plasti Lab) and left to solidify overnight.

B. Brucella Agar with Vitamin K Plates:

Due to the difficulty and technique sensitivity of preparing this type of agar plates, it was purchased from an outside company (Brucella agar with vitamin K, Second Advanced Medical Company, Riyadh, Saudi Arabia).

2.5.2. Broth preparation

Two types of broth were used. BHI broth for *S.mutans* and Tryptic soy broth for *P.gingivalis*. The preparation were carried out by mixing the needed amount of water with the recommended amount of broth powder based on the manufacturer instructions, Autoclaved for 15 min at 15 psi and 121 °C.

2.5.3. Microbial suspension

The microbial suspension of *S.mutans* bacteria (*Streptococcus mutans* Clarke, ATCC 25175) and *P.gingivalis* bacteria (Porphyromonas gingivalis 2561, ATCC 33277) were prepared from the bacterial solutions obtained from "Molecular and Cell Biology Laboratory of the Prince Naif bin Abdulaziz Health Research Center at College of Dentistry, King Saud University".

100 μL of each bacterium was streaked on its corresponding agar plate, placed in an anaerobic jar system (anaerobic jar + anaerobic gas pack) and incubated at a temperature of 37 °C in an incubator for 2 days (*S.mutans*) and for 1 week (*P.gingivalis*). Fig. 3a and b shows the bacterial agar after incubation for *S.mutans* and *P.gingivalis* bacteria, respectively.

Following that, swap of bacteria was added to a conical tube containing 50 ml of broth and incubated in anaerobic jar system in an incubator at temperature of 37 °C for 1 day (*S.mutans*) and for 3 days (*P.gingivalis*).

2.5.4. Bacterial application

The specimens underwent washing with chlorine and sterilization through ultraviolet (UV) germicidal irradiation (UVP PCR3 HEPA Cabinet & Workstation, 849-00002-2, analytikjena) for a total of 2 h, with 1 h allocated for each side. 50 μL of the infused bacteria were dispensed onto the glazed and finished/polished surfaces of the test specimens using volume pipettes (10–100 μL, 409076A, Eppendorf Research). The specimens were then incubated in an anaerobic system within an incubator at 37 °C for 2 days (for *S.mutans*) and for 1 week (for *P.gingivalis*). Afterward, the samples were rinsed with distilled water to eliminate any unattached bacteria. Subsequently, the samples were transferred to a conical tube (Falcon Conical Tubes, 15 μL, 38009, STEMCELL Technologies) containing 1000 μL of distilled water and agitated for 1 min using a vortex mixer (Classic Vortex Mixer, F202A0173, VelpScientifica) to

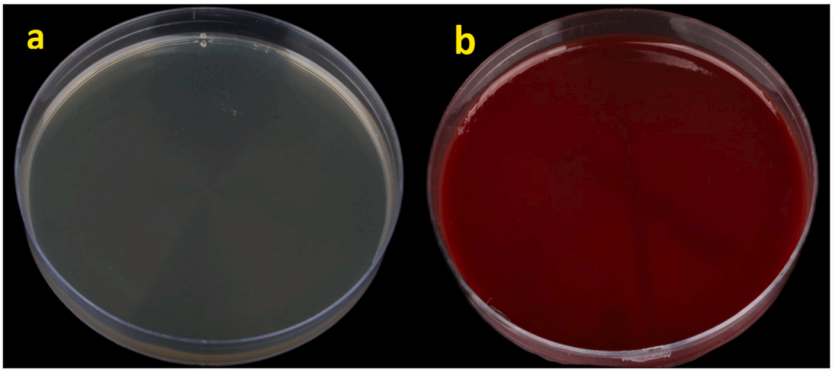

Fig. 2. Agar plates (a): BHI agar, (b): Brucella agar with vitamin K.

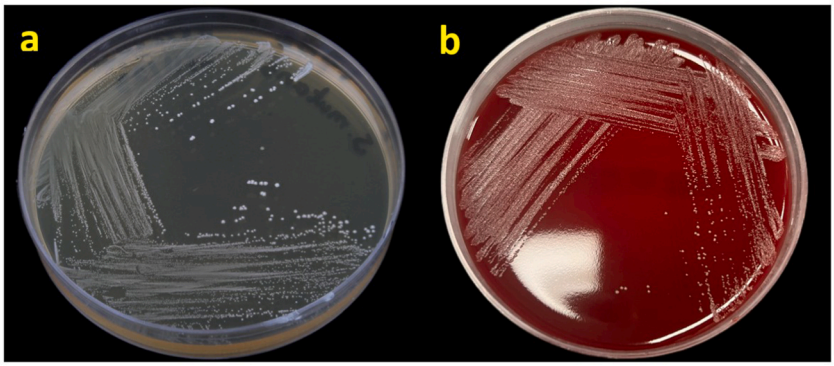

Fig. 3. Bacterial agar after incubation. (a): *S.mutans* bacteria (b): *P.gingivalis* bacteria.

dislodge the bacteria from the sample surface.

2.5.5. Serial dilution

Each sample was assigned to one of four microcentrifuge tubes (Easy-lock microtube 1.5 mL conical, 23043, FL Medical), which were then filled with 900 μL of distilled water each. Next, the first tube was filled with 100 μL of distilled water that contained unattached bacteria, and the vortex agitator (Classic Vortex Mixer, F202A0173, VelpScientifica) was used to mix the mixture. Subsequently, 100 μL from the initial tube were moved to the subsequent tube and mixed again. Every one of the four microcentrifuge tubes underwent the same procedure twice.

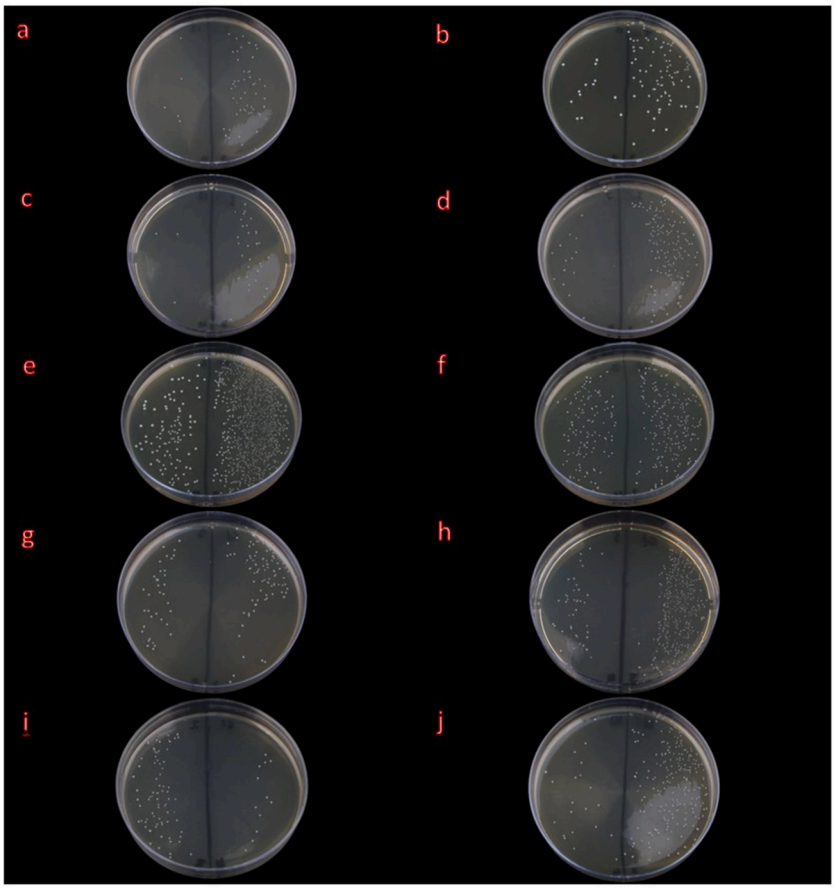

Fig. 4. CFUs of *S.mutans* glazed and finished/polished surfaces CFU. (a): glaze Tetric CAD, (b): finished/polished Tetric CAD, (c) glazed IPS e.max, (d): finished/polished IPS e.max, (e): glazed IPS e.max ZirCAD, (f): finished/polished IPS e.max ZirCAD, (g): glazed Celtra Duo, (h): finished/polished Celtra Duo, (i): glazed Vita Enamic and (j): finished/polished Vita Enamic.

2.5.6. Bacterial colonization

The agar plates prepared were utilized for culturing the detached bacteria collected from each sample. Agar plates were divided into two sections, each section assigned for different dilution, by doing this number of agars used was reduced. After that, with the use of volume pipettes, 50 μL of solution contained in each conical tubes was collected and spread onto agar plates using a T-Spreader (VWRI612-2653, VMR International).

2.5.7. Incubation and calculation of colony forming units (CFU)

The agar plates were placed in an anaerobic system and incubated at 37 °C for 2 days for *S.mutans* and 1 week for *P.gingivalis* to provide sufficient time for colony formation. Subsequently, the plates were visually inspected for CFUs formation of the *S.mutans* (Fig. 4a–j) and *P.gingivalis* (Fig. 5a–j) for the five test materials and manually counted. The mean CFU of each sample was multiplied by the reciprocal of the dilution coefficient to get the number of live bacteria.

2.6. Data analysis

IBM SPSS Statistical software for Windows version 26.0 (IBM Corp., Armonk, N.Y., USA) was used to analyze the data. To describe the log initial CFU numbers, descriptive statistics such as mean, standard deviation, median, and interquartile range were used. Non-parametric statistical tests were used since the log starting CFU levels did not follow a normal distribution: (i) The mean rankings of log initial CFU values between the five research materials—"Tetric-CAD®, IPS-e.max-CAD®, IPS-e.max-ZirCAD®, CELTRA-Duo®, and Vita-Enamic®"—in each of the two treatments (Finished/Polished and Glazed) were compared using the Kruskal-Wallis test and the post-hoc Conover test. In each of the five research materials "Tetric-CAD®, IPS-e.max-CAD®, IPS-e.max-ZirCAD®, CELTRA-Duo®, and

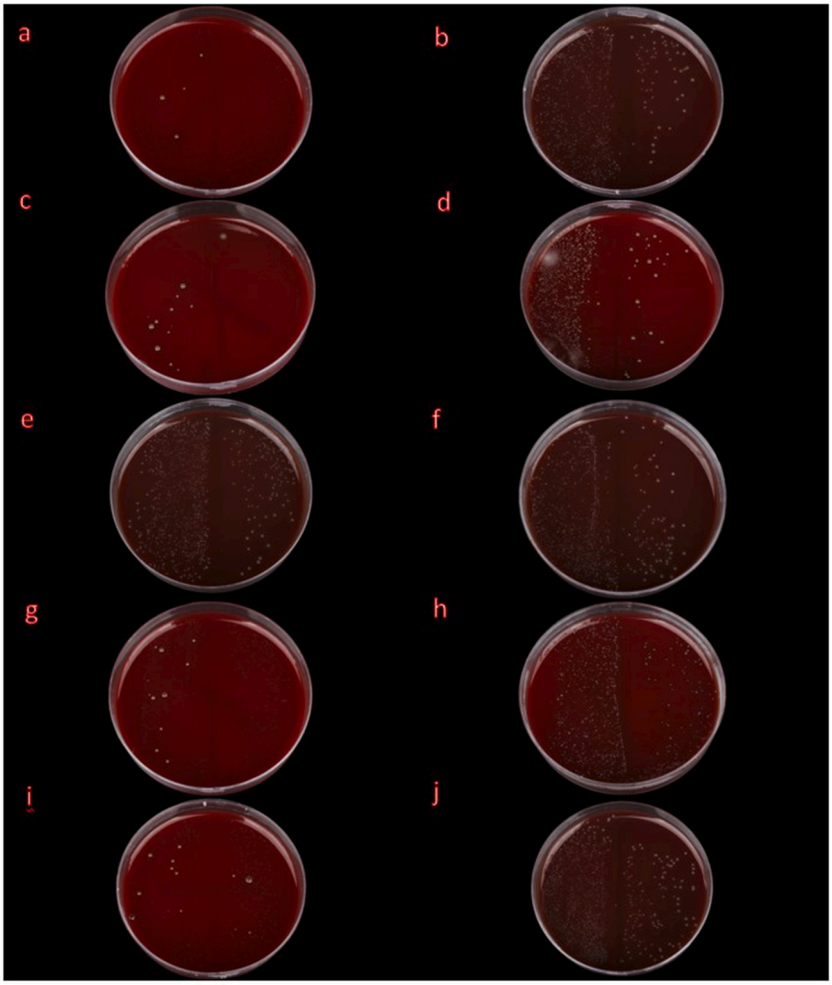

Fig. 5. CFUs of *P.gingivalis* glazed and finished/polished surfaces CFU. (a): glaze Tetric CAD, (b): finished/polished Tetric CAD, (c) glazed IPS e.max, (d): finished/polished IPS e.max, (e): glazed IPS e.max ZirCAD, (f): finished/polished IPS e.max ZirCAD, (g): glazed Celtra Duo, (h): finished/polished Celtra Duo, (i): glazed Vita Enamic and (j): finished/polished Vita Enamic.

Vita-Enamic®", the values of mean-rank of log initial CFU between the two types of treatments (Finished/Polished and Glazed) were compared using the Mann-Whitney *U* test (ii). A significance level of <0.05 was utilized to indicate the statistical significance of the data.

3. Results

Fig. 6 shows the surface roughness of the five test materials' surfaces that were finished/polished and glazed prior to the start of the bacterial adhesion process. There was a substantial statistical difference in the mean rankings of surface roughness between the two types of surfaces (Finished/Polished and Glazed) in each of the five research materials. For the four research materials "Tetric-CAD®, IPS-e.max-CAD®, CELTRA-Duo® and Vita-Enamic®", the mean-rank values of surface roughness using the glazed surface were significantly lower than the mean-rank values of surface roughness using the finished/polished surface ($p < 0.0001$). However, with the IPS-e.max-ZirCAD®, the mean-rank values of surface roughness of the glazed surface were significantly greater ($p < 0.0001$) than the mean-rank values of the finished/polished surface.

Table 2 shows the descriptive statistics of log initial CFU values of *S.mutans* & *P.gingivalis* across the five study materials for each of the 2 type of treatments. The comparison of mean ranks of log initial CFU values shows no statistically significant difference among the five study materials by using glazed treatment ($p = 0.099$) (Table 3). But there was highly statistically significant difference in the mean ranks of log initial CFU values among the five study materials by using finished/polished treatment ($p = 0.002$) (Table 3). The post hoc analysis revealed that Vita-Enamic® material's log initial CFU values were substantially greater than those of "Tetric-CAD®, IPS-e.max-CAD®, and IPS-e.max-ZirCAD® ($p < 0.001$)", but they were not different from CELTRA-Duo® values ($p > 0.05$). Furthermore, there was a significant difference ($p < 0.001$) between the Celtra-Duo® and IPS-e.max-ZirCAD® values. Additionally, Table 3 shows that the values of Tetric-CAD®, CELTRA-Duo®, and Vita-Enamic® were substantially higher than the values of IPS-e.max-ZirCAD® material ($p < 0.001$).

The comparison of mean ranks of log initial CFU values showed no statistically significant difference among the five study materials by using glazed treatment ($p = 0.660$) (Table 4). But the mean ranks of log initial CFU values were statistically significantly different across the five study materials by using finished/polished treatment ($p = 0.004$). The log initial CFU values of Tetric-CAD® and IPS-e.max-CAD® were substantially greater than those of the two materials, IPS-emax-ZirCAD® and CELTRA-Duo®, according to the post hoc test ($p < 0.001$). Additionally, the values of Vita-Enamic®, Tetric-CAD®, and IPS-e.max-CAD® materials were substantially higher than those of CELTRA-Duo® ($p < 0.001$) (Table 4).

The comparison of mean ranks of log initial CFU values between samples using glazed and finished/polished treatment of *S.mutans* & *P.gingivalis* bacteria within each of the five test materials demonstrated significant difference for the IPS-emax-ZirCAD® material where the values using glazed treatment were significantly greater than the values using finished treatment ($p = 0.026$). And there was no statistically significant difference in the log initial CFU values between the two types of treatments for *S.mutans* bacteria of the other four study materials (Table 5). The comparison of mean ranks of log initial CFU values between samples using glazed and finished/polished treatment of *P.gingivalis* bacteria within each of the five test materials showed no statistically significant variance in the log initial CFU values between the two types of treatments for *P.gingivalis* bacteria of all the five study materials (Table 5).

The comparison of mean ranks of log initial CFU values between samples of *S.mutans* & *P.gingivalis* within each of the five test materials using glazed treatment revealed statistically significant difference of IPS.e.max CAD test material where the number of *P.gingivalis* sample were significantly higher than the values of *S.mutans* ($p = 0.041$). And there was no statistically significant difference in the log initial CFU values between the samples of *S.mutans* & *P.gingivalis* of the other four study materials (Table 6).

Comparison of mean rankings of log initial CFU values between samples of *S.mutans* & *P.gingivalis* in each of the five study materials using finished/polished treatment showed statistically significant difference for the IPS-e.max-CAD® material where the values of *P.gingivalis* sample were significantly greater than the values of *S.mutans* ($p = 0.012$). And there was no statistically significant difference in the log initial CFU values between the samples of *S.mutans* & *P.gingivalis* of the other four study materials (Table 6).

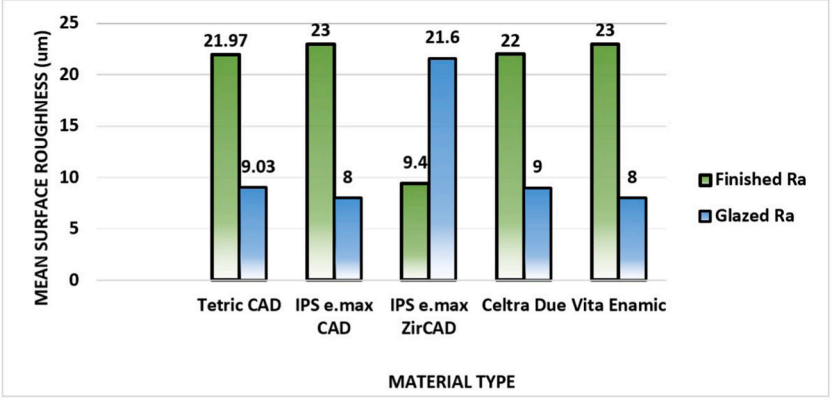

Fig. 6. The mean surface roughness in micrometers (μm) for two surfaces—finished/polished and glazed—for each of the five research materials.

Table 2
Descriptive statistics of Log (initial CFU/ml) values of S.Mutans & P.Gingivalis in each of the 5 study materials in relation to 2 types of treatments (Finished and Glazed).

Type of Bacteria	Test Materials	Type of treatment			
		Glazed		Finished	
		Mean (Sd.)	Median (IQR)	Mean (Sd.)	Median (IQR)
S.Mutans	Tetric CAD	7.08 (0.48)	6.86 (1.05)	7.32 (0.59)	7.23 (1.04)
	IPS e.max CAD	7.07 (0.65)	6.80 (0.61)	7.14 (0.39)	7.15 (0.88)
	IPS e.maxZir CAD	7.47 (0.69)	7.13 (1.49)	6.95 (0.50)	6.89 (0.33)
	CELTRA Duo	7.05 (0.46)	6.85 (0.60)	7.40 (0.61)	7.38 (1.43)
	Vita Enamic	7.46 (0.58)	7.38 (1.17)	7.70 (0.46)	7.50 (1.03)
P.Gingivalis	Tetric CAD	6.29 (1.30)	6.36 (2.53)	7.51 (0.17)	7.49 (0.33)
	IPS e.max CAD	7.34 (0.15)	7.32 (0.13)	7.48 (0.27)	7.50 (0.52)
	IPS e. maxZir CAD	7.24 (0.09)	7.25 (0.10)	6.83 (0.74)	7.13 (1.19)
	CELTRA Duo	6.41 (1.13)	6.35 (2.15)	7.24 (0.18)	7.29 (0.27)
	Vita Enamic	7.00 (0.90)	7.47 (0.91)	7.56 (0.07)	7.57 (0.14)

Table 3
Comparison of mean ranks of Log (initial CFU/ml) values of S. Mutans among the 5 study materials for the Glazed and Finished surfaces.

Surface Treatment	Type of material	Mean ranks (Glazed)	p-value
Glazed	Tetric CAD	33.73	0.099
	IPS e.max CAD	30.67	
	IPS e. maxZir CAD	45.50	
	CELTRA Duo	32.63	
	Vita Enamic	47.47	
Finished	Tetric CAD	39.40[a]	0.002
	IPS e.max CAD	32.40[b]	
	IPS e. maxZir CAD	22.67[c]	
	CELTRA Duo	41.73[d]	
	Vita Enamic	53.80[e]	

By post-hoc Conover test:.
[a] Significantly higher than IPS e.max Zir CAD & Lower than Vita Enamic.
[b] Significantly lower than Vita Enamic.
[c] Significantly lower than Tetric CAD, CELTRA Duo & Vita Enamic.
[d] Significantly higher than IPS e.max Zir CAD.
[e] Significantly higher than Tetric CAD, IPS e.max CAD and IPS e.max Zir CAD, but not different from CELTRA Duo.

Table 4
Comparison of mean ranks of Log (initial CFU/ml) values of P. Gingivalis among the 5 study materials for the Glazed and Finished surfaces.

Surface Treatment	Type of material	Mean ranks (Glazed)	p-value
Glazed	Tetric CAD	22.30	0.660
	IPS e.max CAD	28.65	
	IPS e. maxZir CAD	22.70	
	CELTRA Duo	23.85	
	Vita Enamic	30.00	
Finished	Tetric CAD	31.95[a]	0.004
	IPS e.max CAD	29.80[a]	
	IPS e. maxZir CAD	15.60[b]	
	CELTRA Duo	16.00[b]	
	Vita Enamic	34.15[a]	

By post-hoc Conover test.
[a] Significantly higher than IPSe.maxZir CAD & CELTRA Duo.
[b] Significantly lower than Tetric CAD, IPS e.max CAD & Vita Enamic.

4. Discussion

The current study's findings indicate that there were variations in *S.mutans* & *P.gingivalis* adherence to the five test materials. These variations only mattered, though, for the material specimens that underwent finishing. Observations revealed non-significant *S.mutans* & *P.gingivalis* adherence to the surfaces of specimens subjected to glazing. For the test group with glazed surfaces, the null hypothesis of identical *S.mutans & P.gingivalis* colonization/adhesion to the five test materials investigated in the study was accepted; however, for the test group with surfaces exposed to finishing, it was rejected. This indicated that if the glazing was completed appropriately and in

Table 5
Comparison of mean ranks of Log (initial CFU/ml) values between samples of Glazed and Finished treatment of S. Mutans & P. Gingivalis bacteria in each of 5 study materials.

Type of Bacteria	Type of Material	Type of sample treatment		[a]p-value
		Glazed	Finished	
S. Mutans	Tetric CAD	13.53	17.47	0.233
	IPS e.max CAD	14.00	17.00	0.367
	IPS e. maxZir CAD	19.07	11.93	0.026
	CELTRA Duo	14.17	16.83	0.412
	Vita Enamic	13.77	17.23	0.285
P. Gingivalis	Tetric CAD	8.00	13.00	0.063
	IPS e.max CAD	9.40	11.60	0.436
	IPS e. maxZir CAD	11.20	9.80	0.631
	CELTRA Duo	10.00	11.00	0.739
	Vita Enamic	8.35	12.65	0.105

[a] P-value was significant at $P \leq 0.05$.

Table 6
Comparison of mean ranks of Log (initial CFU/ml) values between samples of S. Mutans and P. Gingivalis using Glazed and Finished surface treatment in each of 5 study materials.

Type of Surface Treatment	Type of Material	Type of Bacteria		[a]p-value
		S. Mutans	P. Gingivalis	
Glazed	Tetric CAD	14.33	11.00	0.285
	IPS e.max CAD	10.53	16.70	0.041
	IPS e. maxZir CAD	12.87	13.20	0.935
	CELTRA Duo	14.07	11.40	0.397
	Vita Enamic	13.47	12.30	0.723
Finished	Tetric CAD	11.33	15.50	0.177
	IPS e.max CAD	10.00	17.50	0.012
	IPS e. maxZir CAD	12.93	13.10	0.978
	CELTRA Duo	13.73	11.90	0.567
	Vita Enamic	13.27	12.60	0.849

[a] P-value was significant at $P \leq 0.05$.

accordance with the manufacturer's instructions, *S.mutans & P.gingivalis* should generate similar intraoral biofilms on the glazed surfaces of the dental restorations under examination.

Colonization of *S.mutans* and *P. gingivalis* was assessed on chair side finished/polished and glazed surfaces for five modern CAD/CAM restorative materials with different compositions, namely "Tetric-CAD®, IPS-e.max-CAD®, IPS-e.max-ZirCAD®, CELTRA-Duo® and Vita-Enamic®", in the current in-vitro research study. The onset of dental caries and periodontitis has been linked to the representative bacterial strains of *S.mutans & P.gingivalis* [24] that were employed in the present investigation. The bacterial suspensions used in the investigation were made in accordance with earlier studies [19–21]; the bacteria were cultivated in the same culture medium and under the same circumstances to reduce the impact of environmental variations.

The bacterial adherence of *S.mutans & P.gingivalis* to five different dental restorative materials, each possessing unique compositions, physical variances, and aesthetic characteristics; "composite (Tetric-CAD®), Lithium Disilicate Glass-ceramics (IPS-e.max-CAD®), Zirconium Oxide Ceramics (IPS e.max-ZirCAD®), Zr Reinforced Lithium Silicate (CELTRA-Duo®), and Hybrid Ceramic (Vita-Enamic®)" were investigated in the present study. The compositions of the five materials that were tested, varied and this variation may have an impact on the bacterial adherence to their surfaces. An important metric that is used to evaluate the integrity of the outer surface of dental restorative materials and has the potential to influence bacterial adhesion is surface roughness [20,25]. The surface topography has a direct effect on plaque and bacterial adhesion to the restorative materials [26]. Most of these materials go through the glazing process, which is the final step before cementation and is done to give the restoration a glossy finish. The smooth surface is preferred for hygienic as well as aesthetic reasons. Plaque buildup can be avoided with the aid of glazing. Furthermore, there is reduced chance of bacterial adhesion to smooth restorations [20,27,28].

Defects and textures in the dental materials are the primary sources of greater surface roughness, and these factors can affect the longevity and performance of these materials [29]. Glazing these restorations before luting is the easiest way to have a smooth surface. Nevertheless, during delivery consultations, chairside clinical changes to these restorations can be necessary [21]. Indirect restorations usually require adjustments to be made on their occlusal surfaces in order to remove working or nonworking interferences. Indirect restorations that are over-contoured proximally or when proximal contacts are excessively tight can also be adjusted proximally to help with seating [30]. Lastly, indirect restorations may be modified to correct shapes for functional or cosmetic purposes. Usually, this procedure entails using a finishing bur, which unavoidably causes the ceramic surface to become rough, and then followed by polishing to restore the smoothness of the surface [30,31].

The five test materials' surfaces were glazed and finished/polished before the bacterial adhesion procedure began at present

investigation. CAD/CAM blocks offer an advantage in their industrial production, ensuring uniformity and eliminating the potential for processing errors [32]. The ideal surface roughness for all of these materials would have been similar [33], however, the results revealed that the specimens under study had different surface roughness levels and displayed different behaviours on their finished/polished and glazed surfaces. The studied materials' surface roughness values demonstrated that, with the exception of Zirconia ceramics (IPS e.max-ZirCAD®), which revealed opposite roughness values, the glazed surfaces were less rough than the finished/polished surfaces. The tested bacterial adhesion mechanism was impacted by these differences as well. With the exception of zirconia ceramics (IPS e.max-ZirCAD®), where bacterial colonization was greater on glazed surfaces than on finished/polished surfaces, all tested bacterial colonization on ceramic materials demonstrated higher colonization on finished/polished surfaces relative to glazed surfaces. This outlier can be explained by the fact that zirconia's glazed surface is rougher than its finished/polished surface. Zirconia has a high surface roughness that makes sense given its crystalline structure and material hardness; this has been documented in earlier research [34,35]. The materials used in this test group may have had a rougher surface due to the porosity and grain boundary fractures created during sintering, which weaken zirconia and impair its structural endurance.

The aforementioned result is consistent with several investigations that assessed bacterial adherence on various restorative materials and found no appreciable differences in biofilm formation [17–20]. Regardless of the kind of restorative material employed, a qualitative examination of the biofilm attached to the various materials revealed that they had comparable architectural traits. The numerous ions emitted from material surfaces have not been linked to the formation of biofilm, according to the authors, who also suggested that surface roughness is a component that influences biofilm and bacterial retention [19–21]. Comparing surface glazing to mechanical finishing/polishing, some research has revealed that surface glazing increases the likelihood of biofilm development and bacterial colonization [36,37].

Previous studies examining the level of bacterial adherence among various ceramic prosthetic materials have discovered that rough prosthetic surfaces and clinical adjustment heighten the degree of bacterial adhesion [38]. Habib SR et al., reported significantly higher *S.mutans* colonization on the rougher surfaces of the different dental ceramic materials [20]. Study by Contreras L et al., assessing the adherence of several bacterial species have revealed that *S.mutans* adheres to surfaces better than Candida albicans [39]. According to Poole et al. (2020), there was an increased adhesion of Prevotella intermedia to various ceramic surfaces [40]. Abdalla et al. found that polished zirconia-reinforced lithium disilicate exhibited the lowest bacterial adherence compared to other ceramic materials when comparing the degree of bacterial attachment to several types of ceramic prosthetic materials [38]. As far as the researchers are aware, very few have looked into how much *P.gingivalis* adheres to various composite materials, and none have looked into how much adhesion it has to ceramic materials. Park et al. came to the conclusion that variations in the surface roughness of various composite materials have no appreciable impact on *P.gingivalis* and other periodontal bacteria [41]. Nonetheless, further research is required to determine how finishing and polishing affect the ceramic materials' surface roughness.

The ceramic test materials used in this study varied in composition, came from different manufacturers, and were manufactured according to their instructions, all of which may have had an impact on the test materials' surface characteristics. Literature has shown us that a dental restoration's long-term performance is influenced by its fabrication technique as well as its various physical, chemical, and biological characteristics [42]. It's possible that variations in the surface characteristics of identical materials from other brands also affected the adhesion of germs. Crucially, it would have been beneficial for our study to compare the tested materials with the tested bacterial adherence to cementum, dentine, or enamel. Additionally, the present in-vitro study was conducted under specific conditions, at a pH of neutrality, and did not contain the necessary proteins, enzymes, or other salivary contents. Nevertheless, an attempt was made to expose the tested ceramics to artificial saliva prior to the bacterial adhesion process beginning. On the other hand, the investigation gave us insight into and evaluation of the intrinsic and extrinsic textural features of the materials under test, with respect to their propensity to cling *S.mutans* & *P.gingivalis* under ideal bacterial growth circumstances.

5. Conclusion

The assessed ceramic test materials' glazed and finished surfaces demonstrated a comparable vulnerability to *S.mutans* & *P.gingivalis* adherence. For each of four studied ceramics "Tetric-CAD®, IPS-e.max-CAD®, CELTRA-Duo® and Vita-Enamic®", adherence of *S.mutans* & *P.gingivalis* to finished surfaces was greater than glazed surfaces. Adhesion of *S.mutans* & *P.gingivalis* to glazed surfaces of Zirconia ceramics "IPS-e.max-ZirCAD®" was higher compared to its finished surface. Overall, glazed surfaces for majority of test materials demonstrated decreased adhesion of *S.mutans* & *P.gingivalis*. Surface texture of the tested materials significantly affected bacterial adhesion, regardless of their chemical composition.

CRediT authorship contribution statement

Ragad Albani: Writing – original draft, Resources, Methodology, Investigation, Data curation, Conceptualization. **Syed Rashid Habib:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Abdulaziz AlQahtani:** Writing – original draft, Methodology, Investigation, Data curation. **Abdulaziz A. AlHelal:** Writing – original draft, Methodology, Investigation, Data curation. **Mohammed Alrabiah:** Writing – original draft, Resources, Methodology, Data curation.

Data availability

Data will be made available on request.

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Declaration of competing interest

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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