



BIOFILM FORMATION ON TWO POLYMER-BASED CAD/CAM ESTHETIC RESTORATIVE MATERIALS: AN IN-VITRO STUDY

Engy Adel Ahmed Farag ^{1*}, Sara Moataz Zayed ²

ABSTRACT

Objectives: The surface characteristics of dental ceramics can be altered by intraoral adjustment and polishing, potentially leading to increased biofilm growth. This research focused on assessing roughness and bacterial adhesion to two polymer-based CAD/CAM ceramic materials. **Materials and methods:** Twenty discs were fabricated using two CAD/CAM resin-based composite blocks, Brilliant Crios (BC) and Tetric CAD (TC), each with a diameter of 10 mm. Ten discs were derived from each material. The surfaces of these discs were finished and polished to mimic typical intraoral procedures. Subsequently, surface roughness analysis (Ra) was conducted using contact profilometry. The study then examined biofilm formation and its correlation with surface roughness for the materials under investigation. **Results:** Brilliant Crios showed significantly higher roughness values of 0.25 ± 0.04 and endorsed significantly greater biofilm growth streptococcus mutans bacterial adhesion. **Conclusion:** Intraoral polishing methods simulation resulted in increased biofilm accumulation. The material chemical composition with its surface roughness influences bacterial accumulation.

KEYWORDS: Biofilm activity, Polymer based CAD/CAM restorative materials, Streptococcus mutans.

INTRODUCTION

The emergence of innovative materials and techniques, coupled with advancements in CAD/CAM (computer-aided design/computer-assisted manufacturing) technology, has substantially transformed the clinical processes within dentistry. This shift has introduced novel concepts for assessment and dental treatment. The CAD/CAM technique offers significant advantages, notably in reducing production time and ensuring the creation of precisely adapted structures ⁽¹⁾.

Among the array of CAD/CAM materials utilized for dental restorations, ceramic and composite resin stand out as the two extensively researched categories. Over the past decade, numerous enhanced materials with superior mechanical characteristics have been developed for CAD/CAM technology. The introduction of novel nanomaterials has further elevated the standards of dental care by modifying the properties of biomaterials. In the context of long-term treatment strategies, dental clinicians prioritize biocompatible materials that not only offer aesthetic appeal but also exhibit

1. Lecturer, Fixed Prosthodontics Department, Faculty of Dentistry, The British University in Egypt

2. Assistant lecturer, Medical Sciences Department, Faculty of Dentistry, The British University in Egypt.

• **Corresponding author:** engy.farag@bue.edu.eg

robust mechanical attributes ⁽²⁾. The smaller size of nanoparticles allows for better penetration into deeper lesions and minimizes porosities, resulting in enhanced mechanical strength. Moreover, the increased surface area-to-volume ratio of nanoparticles enables greater bioactivity, including improved bonding, integration, and heightened antimicrobial efficacy ⁽³⁾.

To make informed material selections, clinicians must consider several crucial attributes such as material composition, surface roughness, mechanical properties, biofilm retention capability, and interaction with the oral environment ⁽⁴⁾.

Oral biofilms exhibit organization assemblies of microorganisms enclosed within a polysaccharide-derived framework that incorporates nucleic acids, proteins, and water. These biofilms attach to teeth, dental restorations, or soft tissues within the mouth. As a result, pH levels within the mouth commonly vary, frequently dropping to low levels after consuming acidic substances or due to the acids released from microbial metabolism in the oral environment. Additionally, oral temperature experiences temporary variations during the consumption of hot or cold foods. Furthermore, oxygen levels within the oral cavity vary, with areas beneath the gingival margin exhibiting low oxygen content or even anaerobic conditions ⁽⁵⁾. Consequently, microbial colonization in the oral cavity is influenced by fluctuations in oxygen and pH levels, favoring the growth of aerobic or anaerobic microorganisms accordingly. The mouth harbors a distinct microbiota that generally fosters a symbiotic interaction with host tissues in a healthy state. Nonetheless, a disruption in the equilibrium between microorganisms and host tissues can precipitate oral conditions like gingivitis and periodontitis ⁽⁶⁾.

The development of biofilms in the oral cavity advances through four discernible phases: (a) acquisition of a pellicle; (b) initial colonization; (c) secondary colonization and co-aggregation; and (d)

formation of a mature biofilm. Human saliva acts as the main nutrient reservoir for microorganisms, aiding in the adherence and coating of hard or soft surfaces with a thin (5-10 μm thickness), diverse, non-cellular film referred to as an acquired pellicle or conditioning film. Subsequently, early colonization commences as primary bacteria bind to the acquired pellicle. Streptococcus species account for 60-80% of all primary colonizers. Secondary colonization takes place within a span of 3 to 5 days following acquired pellicle deposition, during which microorganisms commence proliferation and coalesce with other species, resulting in the structural organization of the biofilm. Biofilm maturation typically occurs within 2 to 3 weeks ^(6,7).

Increased surface roughness aids in microbial adhesion by expanding the interface between the organisms and the surface of the restoration, simultaneously reducing shear forces induced by saliva flow. A Ra value (arithmetic mean height) below 0.2 μm has been universally recognized as a threshold, indicating that further polishing beyond this point does not significantly decrease biofilm accumulation ⁽⁸⁾. This study aims to assess surface roughness and biofilm adhesion between the tested materials. The null hypothesis stated that there will be no differences between the two nanohybrid ceramics.

MATERIALS AND METHODS

Sample Size Calculation

Based on data extracted from Aydin N et al⁽⁹⁾ study results, using alpha (α) level and beta (β) level. A minimum sample size of six samples (n=6 in each group) will result in 95% power when the significant level is 0.05. The number of specimens per group was raised to ten. Statistical power analysis software (R statistical analysis software version 4.3.2 for Windows) was applied for sample size calculation. This study was approved by the ethics committee at the Faculty of Dentistry of the British University in Egypt (FD BUE REC #24-014).

Sample preparation

The CAD/CAM restorative materials examined in this study are outlined in Table 1, along with their material classification, composition, and manufacturer details. Each CAD/CAM block material was sectioned into discs ($n = 10$), measuring 2 mm in thickness and 10 mm in diameter, employing the IsoMet 4000 Linear Precision saw (Buehler Ltd., Lake Bluff, IL, USA), with consistent water irrigation.

The samples were randomized into two equal groups according to the type of material. (Group I) was manufactured from Brilliant Crios (BC) material, while (Group II) was manufactured from Tetric CAD (TC) material.

To complete the finishing process, a diamond-impregnated system (EVE Ernst Vetter GmbH, Neureutstr. 6, 75210 Keltern, Germany), was utilized. This system is renowned for its high abrasion rate and minimal heat generation, negating

the requirement for a water-cooling setup. For the polishing stage, a three-step diamond-impregnated polishing disc system from the same manufacturer was employed for a duration of 60 seconds. This system comprises coarse, medium, and fine grits, ensuring the attainment of a smooth surface finish suitable for a diverse range of ceramic materials.

The surface roughness of the samples was assessed using a contact profilometer (SJ-210 surface roughness tester, Mitutoyo, Japan) with a cutoff length of 0.25 mm and stylus speed set at 1 mm/s. Profilometric analysis of each sample was conducted by obtaining measurements from three distinct regions. The Ra values, representing the average surface roughness for each sample, were recorded by calculating the arithmetic mean of these measurements.

After conducting surface roughness testing, the samples underwent a cleaning process for 10 minutes using distilled water in an ultrasonic cleaner, followed by drying.

TABLE (1) Characteristics of the investigated materials.

Product	Type	Organic Matrix	Inorganic Filler	Manufacturer
Brilliant CRIOS	CAD/CAM composite	Cross-linked methacrylates (Bis-GMA, Bis-EMA, TEGDMA) (30% wt.)	barium glass with particle size < 1 μ m and amorphous silica SiO ₂ with particle size, 20 nm (70.7% wt.)	Coltène/Whaledent, Altstätten, Switzerland
Tetric CAD	CAD/CAM composite	Bis-GMA, Bis-EMA, TEGDMA, UDMA	barium aluminium silicate glass with a mean particle size of <1 μ m and silicone dioxide with an average particle size of <20nm (71.1 %wt)	Ivoclar-Vivadent, Schaan, Liechtenstein

Bis-GMA = bisphenol A glycol dimethacrylate; Bis-EMA = ethoxylated bisphenol A dimethacrylate; TEGDMA = Triethylene glycol dimethacrylate; UDMA = Urethane dimethacrylate

Preparation of Streptococcus mutans bacterial culture

The standard bacteria strain of Streptococcus mutans (ATCC 25175) was used in this study . The bacterial inoculum of S.mutans was prepared by selecting one pure single colony obtained from culture on brain heart agar (Lab M Ltd., United Kingdom) plate in test tubes containing 5 ml aliquots of brain heart infusion broth (Oxoid, USA) supplemented with 2% sucrose (Sigma-Aldrich, USA). The test tubes containing prepared bacterial inoculum of S. mutans were then incubated at 37 °C for 48 hours ⁽¹⁰⁾.

Bacterial adhesion on different ceramic materials

The optical density (OD) of the pre-prepared bacterial suspension was calibrated to 0.09 at 600 nm using spectrophotometer (Unicam, UK) to obtain standard bacterial suspension containing 108 CFU/ml. The discs were then split into two groups (Ten discs per group). The tested discs under investigation were sterilized and autoclaved in autoclave (Tomy, Japan) at 121°C for 15 minutes. The samples of each material were coded. The discs of Brilliant Crios and Tetric CAD materials were placed separately into wells of 24 well culture plates by sterile forceps. For bacterial adhesion, aliquots of 2 ml of prepared standardized bacterial inoculum were pipetted in the wells of microtiter plate containing sterile samples. The plates were incubated for 48 h at 37 °C in incubator (Binder, Germany)⁽¹¹⁾.

Assessment for bacterial adhesion on different ceramic materials by colony forming unit

After 48 hours, the samples underwent three gentle washes with 0.9% saline to remove loosely bound bacteria from the samples. After that the washed samples were transferred to new falcon tubes containing 5 ml of sterile physiological saline (El-Nasr Chemicals Co) 0.9%. The 50 ml falcon tubes were sonicated with vortex (Acculab, USA) at 30 g for 3 min to detach microorganism of formed

biofilm from the surface of samples. Serial dilutions of each sample were performed in triplicates. Aliquots of 100 µl of the bacterial suspension of each sample was two fold serially diluted up to 108. Then colony forming per unit was determined by plating 10 µl of each diluted suspension on brain heart agar plates. The plates were incubated at 37°C for 48 hours. Following incubation, the colony-forming units (CFU) in plates containing 30 to 300 typical colonies of S.mutans, were counted then reported in CFU/ml ⁽¹²⁾ .

RESULTS

Statistical Analysis

The data underwent analysis using SPSS version 22.0 statistical software (SPSS, Inc., Chicago, IL, USA). Quantitative data are presented as the mean ± standard deviation (SD). Data showed parametric distribution and was analyzed statistically by unpaired t-test. The (p-value < 0.05) was considered significant. Statistical analysis was conducted with R statistical analysis software version 4.1.3 for Windows.

Surface roughness

For surface roughness, mean and standard deviation (SD) values of surface roughness (Ra) for Brilliant Crios was (0.25±0.04) which is significantly higher than the mean value of Tetric CAD (0.18±0.06) (p<0.001) (Table 2).

Surface roughness (Ra) (mean±SD)		p-value
Brilliant Crios	Tetric CAD	<0.001*
0.25±0.04	0.18±0.06	

* Significant ($p \leq 0.05$)

Bacterial adhesion and assessment of colony forming unit (CFU Counting)

The count of colonies in each sample was determined using the subsequent equation: number

of colonies \times dilution factor /original volume of culture plate. The CFU/ml of each sample per group was determined. Representative images of number of *S.mutans* adherent on surface of Tetric CAD and Brilliant crios discs of serial dilution up to 108 on brain heart agar were shown in Figure 1. Comparison between colony forming unit developed by *S. mutans* on different materials of (Tetric CAD and Brilliant Crios) samples per group

is shown in Figure 2. The mean value of CFU/ml of sample for each group showed considerable variability between both groups. It was found that the Brilliant Crios discs have a higher number of *S.mutans* species compared to the Tetric Cad discs indicating higher frequency of bacterial adhesion on Brilliant Crios material than that of Tetric CAD. (Figure 3)

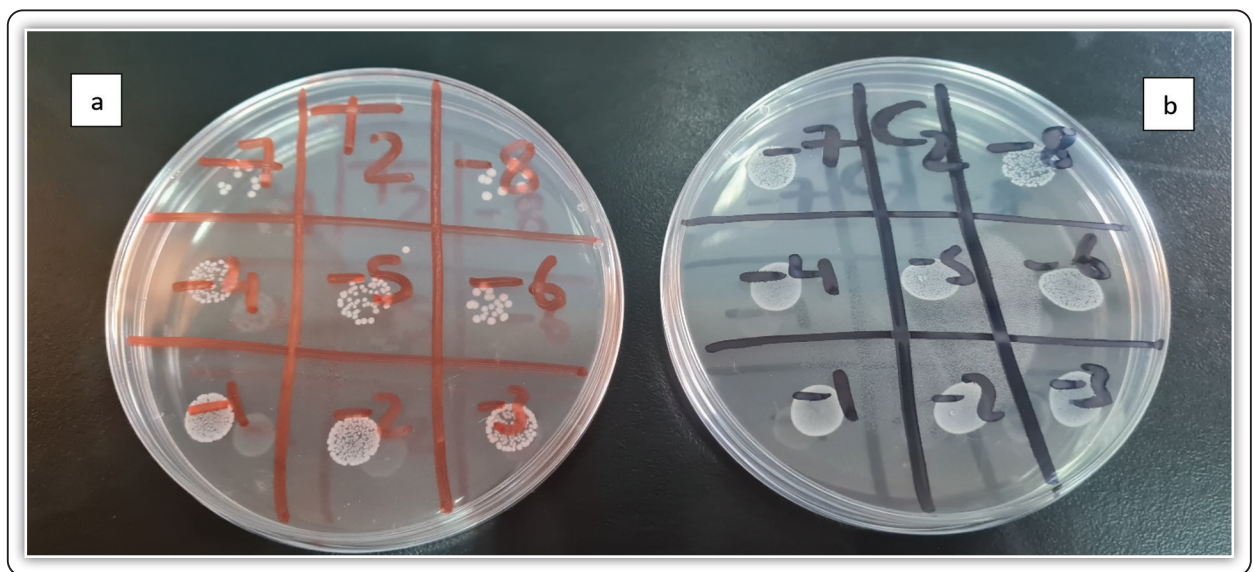


FIG (1) Bacterial adhesion on representative crown samples (T2 and C2) on brain heart agar where: (a) *S.mutans* adhesion on surface of Tetric CAD (b) *S.mutans* adhesion on surface of Brilliant Crios.

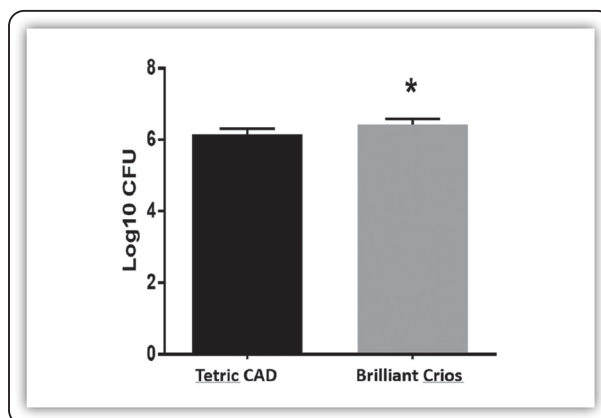


FIG (2) Bar chart showing the comparison between colony forming unit developed by *S. mutans* on different materials of (Tetric CAD and Brilliant Crios) samples per group

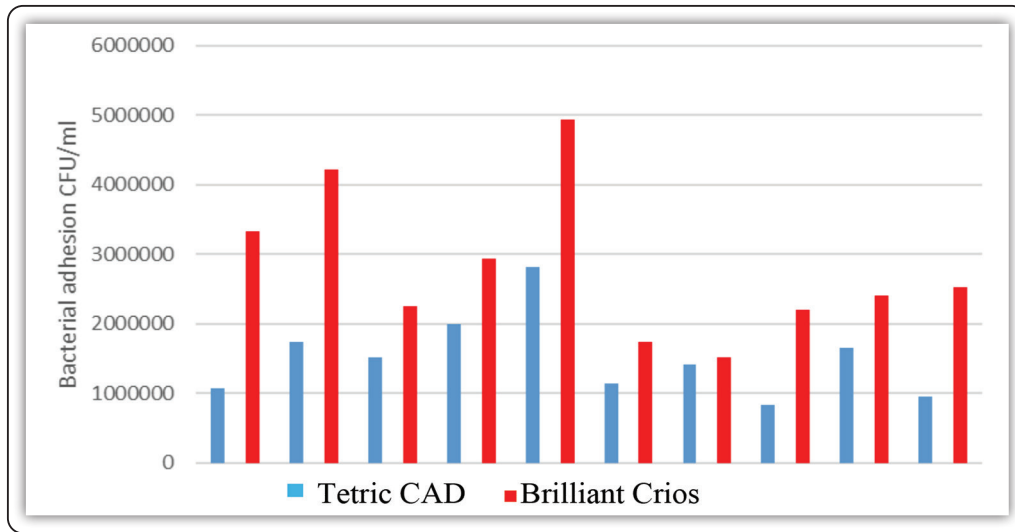


FIG (3) Bar chart showing bacterial count on each tested material

DISCUSSION

Investigations into resin-based hybrid ceramic CAD/CAM blocks signify the beginning of a new era in dental fixed restoration. In clinical settings, these materials are favored for their simplicity in preparation, polishing and repairability. Consequently, it is crucial to assess these materials regarding their physical attributes, antibacterial traits, and biocompatibility. The optimal materials should demonstrate minimal surface roughness, minimal biofilm formation on their surfaces, and diminished cytotoxic and genotoxic impacts ⁽¹³⁾.

The null hypothesis of this study, which proposed no difference between the tested materials, was disproven. Results indicated a significant disparity between the surface roughness values of Brilliant Crios samples and Tetric Cad samples. Additionally, Brilliant Crios exhibited higher bacterial adhesion values compared to Tetric Cad, which displayed lower values.

To ensure consistency, a single investigator completed the finishing and polishing of all samples within each group using identical procedures to achieve a uniform smooth surface ⁽¹⁴⁾. Polishing can be likened to a micro-grinding process on the material's surface. As for micro-mechanism of

polishing may vary, it typically entails material removal through abrasive wear, ductile flow, and to some extent, micro-fracturing ⁽¹⁵⁾.

This study examined how simulated intraoral adjustment and polishing techniques influence surface ultrastructural characteristics, surface roughness, and biofilm formation for two recently introduced hybrid materials available in the market. Polishing is essential to attain the ultimate smoothness of the restoration surface while minimizing alterations to its shape ⁽¹⁶⁾.

Surface roughness was assessed utilizing the (Ra) parameter, which remains a valuable standard for general surface topography assessment. This parameter offers a practical and easily comprehensible value, facilitating the comparison of surface roughness among different materials.¹⁴ It was chosen for its representativeness and ease of calculation. Profilometry, employing a surface roughness tester, was utilized for this purpose. Tactile profilometry, a reliable and representative method, was selected as it provides quantitative measurements of surface profile ⁽¹⁷⁾. Consistent with our findings, research on intraoral polishing techniques commonly indicates an increase in the roughness of nanohybrid ceramic surfaces ^(16,18).

Research focused on refining finishing techniques has documented surface roughness values as low as $0.171 \mu\text{m}$ ⁽¹⁹⁾. However, attaining such low values usually demands extensive polishing procedures employing diamond pastes. Conversely, in our study, we restricted polishing to mimic clinical conditions, resulting in heightened roughness.

The composition of the acquired pellicle is influenced by various surface properties, including surface energy, surface roughness, and material composition. Numerous studies have highlighted surface roughness as a crucial determinant for acquired pellicle deposition and plaque formation. Greater surface roughness is associated with enhanced bacterial adhesion, as it enlarges the surface area available for bacterial attachment ⁽²⁰⁾.

While surface roughness plays a significant role, bacterial attachment is also influenced by elements like chemical composition, surface topography, free energy, and hydrophobicity. The heterogeneous composition resulting from a combination of hydrophobic resin matrix and hydrophilic filler particles of varying dimensions, masses, and chemical composition may account for the varied tendencies of *S. mutans*. Additionally, polishing can uncover filler particles on the surface of heterogeneous materials, potentially impacting plaque accumulation ⁽¹¹⁾.

In the microbiological in vitro test, *Streptococcus* species were selected due to their prevalence in the initial stages of biofilm formation, laying the foundation for subsequent adherence of anaerobic and more pathogenic microorganisms, which become predominant in mature biofilms after 48 hours. Furthermore, *Streptococcus* mutants are frequently utilized for assessing bacterial accumulation because of their notable ability to adhere and form biofilms ⁽¹⁷⁾.

The current study found increased biofilm formation for Brilliant Crios. The discrepancy in biofilm formation was challenging to attribute solely to surface roughness, given the close similarity

between the two materials tested. However, Brilliant Crios exhibited surface roughness exceeding the threshold of $0.2 \mu\text{m}$. It is noteworthy that previous reports have highlighted the significant impact of surface topography on bacterial attachment. SEM images from the study of Hassan et al ⁽¹³⁾ demonstrated that Brilliant Crios exhibits soft grooves on its surface, together with conspicuous circular gaps or depressions, which could potentially enhance biofilm adhesion.

The chemical composition of dental material surfaces is another crucial factor that influences biofilm formation and microbial adhesion. A prior study noted a positive correlation between biofilm formation and the quantity of resin matrix, while an inverse relationship was observed with the filler content on the specimen's surface.²¹ Notably, Brilliant CRIOS contains a higher resin matrix content (30 wt %) compared to Tetric CAD.

Additionally, Bis-GMA, a component, contributes to the production of bis(hydroxypropoxy)phenyl propane (BisHPPP), a biodegradation byproduct known to enhance the activity of *S. mutans* biofilms.¹³ Studies have demonstrated that the water absorption capacity of the BisGMA monomer surpasses that of UDMA, TEGDMA, and BisEMA monomers. Furthermore, the UDMA monomer exhibits a more hydrophobic structure compared to the BisGMA monomer ⁽²²⁾.

Bis-EMA serves as an ethoxylated counterpart to Bis-GMA, lacking a secondary functional (-OH) group and combining it with TEGDMA leads to increased conversion rates and reduced water solubility and sorption. Additionally, due to its molecular structure and greater solubility compared to BisGMA, TEGDMA tends to leach more readily into the medium ⁽²³⁾.

This investigation had several limitations. Firstly, in the interest of consistency and standardization, the authors employed only one finishing and polishing method, potentially impacting the surface

properties of chemically diverse materials. Additionally, the study did not simulate oral conditions, thus limiting the direct applicability of their findings to clinical settings. Furthermore, all in vivo surface alterations occur due to the gradual development of biofilms, a process not replicable in experimental testing. Additionally, factors such as pH fluctuations, mechanical stresses from chewing, tooth brushing, or parafunctional habits were not accounted for in this study. Hence, further clinical research is needed to clarify the intraoral degradation mechanisms affecting the surface and optical characteristics of CAD/CAM composite resins.

CONCLUSIONS

Not only does the surface roughness impact biofilm adhesion, but also the chemical composition of the hybrid ceramic material, particularly the percentage of the matrix. Brilliant Crios surfaces could attain bacterial biofilm more than Tetric CAD attributed not only to its surface roughness but also to its matrix composition. As new dental materials are developed further, it will be essential to consider these studied characteristics to minimize bacterial adhesion and the occurrence of secondary caries.

REFERENCES

1. Ille CE, Moacă EA, Suci M, Barbu-Tudoran L, Negruțiu ML, Jivănescu A. The Biological Activity of Fragmented Computer-Aided Design/Manufacturing Dental Materials before and after Exposure to Acidic Environment. *Medicina (Kaunas)*. 2023 Jan 3;59(1):104.
2. Al-Odayni AB, Alfotawi R, Khan R, Sharaf Saeed W, Al-Kahtani A, Aouak T, Alrahlah A. Synthesis of chemically modified BisGMA analog with low viscosity and potential physical and biological properties for dental resin composite. *Dent Mater*. 2019 Nov;35(11):1532-1544.
3. Mok ZH, Proctor G, Thanou M. Emerging nanomaterials for dental treatments. *Emerg Top Life Sci*. 2020 Dec 17;4(6):613-625.
4. Holban AM, Farcasiu C, Andrei OC, Grumezescu AM, Farcasiu AT. Surface Modification to Modulate Microbial Biofilms-Applications in Dental Medicine. *Materials (Basel)*. 2021 Nov 18;14(22):6994.
5. Belibasakis GN, Charalampakis G, Bostanci N, Stadlinger B. Peri-implant infections of oral biofilm etiology. *Adv Exp Med Biol* 2015;830:69-84 .
6. Souza JC, Mota RR, Sordi MB, Passoni BB, Benfatti CA, Magini RS. Biofilm Formation on Different Materials Used in Oral Rehabilitation. *Braz Dent J*. 2016 Mar-Apr;27(2):141-7.
7. Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. *Clin Oral Implants Res* 2006;17: 68-81
8. Fuchs F, Schmidtke J, Hahnel S, Koenig A. The influence of different storage media on Vickers hardness and surface roughness of CAD/CAM resin composites. *J Mater Sci Mater Med*. 2023 Mar 18;34(3):13.
9. Aydın N, Karaoglanoglu S, Oktay E. Evaluating Cytotoxic Effects of Resin Based CAD/CAM Blocks, *J Res Med Dent Sci*, 2020, 8 (3):131-136
10. Zayed SM, Aboulwafa MM, Hashem AM, Saleh SE. Biofilm formation by *Streptococcus mutans* and its inhibition by green tea extracts. *AMB Express*. 2021 May 25;11(1):73.
11. Giti R, Shima D, Mohammad M, Reza D. 2021. "Surface Roughness, Plaque Accumulation, and Cytotoxicity of Provisional Restorative Materials Fabricated by Different Methods." *PLoS ONE* 16.
12. Kozmos M, Virant P, Rojko F, Abram A, Rudolf R, Raspor P, Zore A, Bohinc K. Bacterial Adhesion of *Streptococcus mutans* to Dental Material Surfaces. *Molecules*. 2021 Feb 21;26(4):1152.
13. Hassan SA, Beleidy M, El-Din YA. Biocompatibility and Surface Roughness of Different Sustainable Dental Composite Blocks: Comprehensive In Vitro Study. *ACS Omega*. 2022 Sep 15;7(38):34258-34267.
14. Albani R, Habib SR, AlQahtani A, AlHelal AA, Alrabiah M, Anwar S. The Surface Roughness of Contemporary Indirect CAD/CAM Restorative Materials That Are Glazed and Chair-Side-Finished/Polished. *Materials (Basel)*. 2024 Feb 21;17(5):997.
15. Yu P, Luo H, Yap AU, Tian FC, Wang XY. Effects of polishing press-on force on surface roughness and gloss of CAD-CAM composites. *J Oral Sci*. 2023;65(2):131-135.
16. Kim KH, Loch C, Waddell JN, Tompkins G, Schwass D. Surface Characteristics and Biofilm Development on Selected Dental Ceramic Materials. *Int J Dent*. 2017;2017:7627945.

17. Khalifa F., hassanein A., Hamza T. Biofilm adhesion and surface roughness of PEEK VS Monolithic Zirconium: Comparative “An In-Vitro Study”. *Journal of Fundamental and Clinical Research*, 2022; 2(2): 157-169.
18. Haralur S. B. Evaluation of efficiency of manual polishing over autoglazed and overglazed porcelain and its effect on plaque accumulation. *Journal of Advanced Prosthodontics*. 2012;4(4):179–186.
19. Boaventura J. M. C., Nishida R., Elossais A. A., et al. Effect finishing and polishing procedures on the surface roughness of IPS Empress 2 ceramic. *Acta Odontologica Scandinavica*. 2013;71(3-4):438–443.
20. Engel AS, Kranz HT, Schneider M, Tietze JP, Piwowarczyk A, Kuzius T, Arnold W, Naumova EA. Biofilm formation on different dental restorative materials in the oral cavity. *BMC Oral Health*. 2020 Jun 3;20(1):162
21. Lourenço B. N.; Marchioli G.; Song W.; Reis RL.; van Blitterswijk CA.; Karperien M.; van Apeldoorn A.; Mano J. F. Wettability influences cell behavior on super-hydrophobic surfaces with different topographies. *Biointerphases* 2012, 7, 46.
22. Recep Kara. Examination of Streptococcus Mutans Adhesion in Current Hybrid Ceramics and Composites. *International Journal of Dental Sciences and Research*. Vol. 8, No. 5, 2020, pp 138-142.
23. Colotta, F.; Allavena, P.; Sica, A.; Garlanda, C.; Mantovani, A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcin* 2009, 30, 1073–1081