

Examination of Streptococcus Mutans Adhesion in Current Hybrid Ceramics and Composites

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Abstract This study evaluated the surface topography and bacterial adhesion of a different hybrid ceramics and resin composites after optimal surface finishes. A total of disk samples were prepared using two different CAD/CAM blocks [Vita Enamic (VE), Shofu HC (SHC)] and two different composites [Tetric N Ceram bulk-fill (TNC), Estelite Sigma Quick (ESQ)] with diameter 10 mm and thickness 4.0 ± 0.2 mm. Fifteen disc samples were produced for each group (n=15). Surfaces of the sample disks were polished according to the manufacturer's recommendations with the specially produced polish sets. The surface roughness of specimens was analyzed parameter (Ra), sterilized, and subjected to bacterial adhesion. Data were submitted to one-way ANOVA and Tukey's test ($\alpha=0.05$). TNC and ESQ groups had lower surface roughness than VE and SHC groups ($p<0.05$). There was no difference in bacterial adhesion between hybrid CAD/CAM blocks and composite materials. The material type and surface finishing system did not significantly interfere with surface roughness parameters and bacterial adhesion. The surface polishing of nanocomposite resins performed is better than hybrid ceramic after polishing. An adequate finishing/polishing technique should always be applied after any adjustments made to indirect restorations with these materials tested. There is no difference between bacterial adhesions of the tested materials. If appropriate and sufficient, polishing is performed.

Keywords: bacterial adhesion, *S. mutans*, CAD/CAM hybrid ceramics, composite resins

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1. Introduction

A wide variety of restorative materials can be used in the construction of fixed and removable prostheses applied to restore the lost functionality and aesthetics due to dental deficiencies. They are an alternative to metal-ceramic systems and have more aesthetic results [1]. Especially in the front area restorations; A wide variety of full ceramic systems have been developed to create alternatives to metal-ceramic systems and to achieve more aesthetic results. Some of the systems; feldspathic ceramics, lithium disilicate based ceramics, hybrid ceramics, resin-modified nanoceramic, lithium silicate ceramics reinforced with zirconia and monolithic zirconia can be listed [2]. Due to their superior aesthetic properties and biological compatibility; Inlay, onlay, laminate veneer, crown prosthesis, especially in the production of anterior region implant-supported restorations, are widely used [3]. In addition to the aesthetic and mechanical properties of the restorative materials used, their biological compatibility and adhesion of microorganisms are important [4].

The biofilm layer, which is located on the tooth and restoration surfaces and cannot be cleaned by saliva flow,

tongue, lips, and cheeks, is called "dental plaque." This layer consists of bacteria, metabolic residues, and saliva components. It has been reported that 10^8 mg of bacteria in 1 mg mature bacterial plate [5]. These bacteria, dental caries, gingivitis, periodontitis, periimplantitis, and stomatitis are the main etiological factors. The earliest colonized in bacterial plaque and numerous microorganism is Streptococcus mutans (*S. mutans*) [6]. The virulence of *S. mutans* is due to its superior ability to produce a polymeric matrix that adheres well to solid surfaces such as teeth and restorative materials [7]. Dental materials surface properties have been reported to play an important role in the initial adhesion and retention of microorganisms in the bacterial plaque. Besides, it has been stated that the surface roughness increases the plaque formation rate [8].

Many devices and techniques are used to evaluate surface roughness. Research on surface roughness of dental materials can be done with quantitative methods such as scanning electron microscopy or quantitative methods such as surface profile analysis. Surface roughness measurements are electrically operated pointing devices that are frequently used and reported to give reliable results [9].

Recently, composite resins are used in the aesthetic restorations of both anterior and posterior teeth [10]. Bulk

fill composite resin materials have been produced in order to eliminate the disadvantages of conventional composite resin materials. This new composite resin type can be placed in a single layer up to 4 mm thickness. These composite resins contain monomers and a particle/filler structure similar to conventional nanohybrid composite resins. Differently, they contain polymerization regulators (modulators) and fluidizes to regulate polymerization kinetics. Besides, the filler content ratio has been reduced to facilitate light transmission to more in-depth, and the filler particle size has been increased to improve its mechanical strength [11]. Physicomechanical properties of these materials, such as fracture resistance, surface roughness, and monomer dissolution, have been extensively studied [12]. However, there is no much study on surface roughness and bacterial adhesions of bulk composite resins. Current composite resin technology has made progress with the development of nanofiller particles [13]. It has been reported that composite resins with nanofiller have better aesthetic and mechanical properties, better polishability, reduced polymerization shrinkage and reduced wear properties [14].

It is aimed to increase the degree of conversion of the material and to modify the content of the resin matrix by making some modifications in the physical and mechanical properties of composite resins. As a result of improvements in the degree of transformation, material blocks produced for CAD/CAM (computer-aided design/computer-aided manufacturing) technology have been introduced in dentistry. These blocks are industrially polymerized and have improved physical properties under parameters standardized at high temperature and pressure to provide optimal physical and mechanical properties. [15]. With the development of composite resin technology, CAD/CAM hybrid ceramics containing nanoparticle filler and containing the positive properties of ceramic and composite materials have been presented for clinical use. These materials can be produced and repaired more easily than restorations made from CAD/CAM ceramic materials [16,17].

It is desired that the surface conditions of the materials used in the field of dentistry be as smooth as possible [9]. For this purpose, various technologies that bring the surface properties of the materials to the standard desired by the clinician developed. Full ceramic systems with advanced features; It differs from traditional ceramic systems in many aspects such as surface properties, mechanical strength, biological compatibility, chemical stability, and aesthetics [2]. Few studies investigating the interaction of surface topography and bacterial adhesion in current all-ceramic systems and composites are minimal.

Comparison of surface roughness, *S. mutans* adhesion, and early biofilm formation in systems. Our null hypothesis is, "There is no difference in bacterial adhesion between hybrid CAD/CAM blocks and composite materials."

2. Material and Method

Two different CAD/CAM blocks and bulk-fill resin composite and nanocomposite were used. (Table 1). 4mm thick sections were obtained by using a precision cutting

machine (IsoMet 1000, Buehler; Illinois, ITW, USA) from standard block sizes. From the sections, 10 mm diameter CAD/CAM block samples were obtained with the help of a trepan burr (Hu-Friedy Mfg. Co., LLC, USA). Vaseline insulator was applied to prevent the adhesion of the composite resin to the inner sidewalls of a 4 mm high PVC mold with a 10 mm diameter gap. According to the manufacturer's instructions, composite resin placed in PVC mold was polymerized, and bulk-fill and nanoceramic resin samples were obtained. Fifteen disc samples were obtained from each test material, and a total of sixty discs were obtained

Surfaces of composite samples were polished with especially kit (Super-Snap Mini-Kit, Shofu INC; California, USA) for 10 sec. using low-speed contra-angle. Prepared CAD/CAM block sections were polished with a set clinical diamond polishing system (Vita Enamic, Vita Zahnfabrik, Germany) for VE material and Shofu Cadmaster HP Kit (Shofu Dental GmbH, Germany) for SH material according to manufacturer's recommendations. Samples were cleaned with distilled water for 15 minutes and air-dried for 10 sec. and made ready for surface roughness measurements.

The profilometer device (Marsurf PS 10, Mahr, Germany) was used for surface roughness measurement. Profilometric analysis of each sample was obtained with screening results from three different regions. By taking the arithmetic average of the values obtained from these measurements, Ra values which are the average surface roughness value for each sample was recorded. The samples were sterilized in an autoclave at 121 °C for 15 minutes.

For bacterial adhesion tests, firstly, a pH of 7.0, an artificial saliva solution with the following composition was prepared: 128 mg NaCl, 16.7 mg CaCl₂, 12.5 mg MgCl₂ (6H₂O), 9.5 mg KCl, 150.75 mg CH₃COOK, 38.6 mg K₃PO₄ (3H₂O) and 70 mg Type II mucin (Sigma Aldrich, USA). From the prepared artificial saliva, 1 mL was added to the sterile sample surfaces, and 1 hour waited for the formation of the pellicle. Adhesion tests for *S. Mutans* ATCC 25175 (Hemakim, Istanbul, Turkey) bacteria strain and brain-heart infusion (BHI) medium was used. Bacterial suspensions by 0.5 Mc Farland strain were prepared from the bacterial culture activated by 24 hours incubation in the BHI medium. 200 µl of bacterial suspension and 2.0 mL of BHI (containing 1% sucrose) medium were added to each sample surface placed in sterile glass containers for 24 hours at 37°C under anaerobic conditions. After incubation, the samples, whose bacterial adhesion was completed, were washed with the phosphate-buffered solution (PBS) for 60 seconds to remove the bacteria from the surface completely. For the detection and evaluation of adhering bacteria, samples were placed in glass tubes containing 1 mL of PBS each and mixed for 2 min on a Vortex device. After this process, BHI agar media were sown with the spreading technique to calculate the number of bacteria passing into PBS.

The data obtained were statistically evaluated using the SPSS package program (IBM SPSS Statistics v22; Chicago, IL, USA). After the Kolmogorov-Smirnov test determined the compatibility of the data to a normal distribution, statistical significance was compared with

one-way analysis of variance (ANOVA) and Tukey HSD post hoc tests ($p < 0.05$).

3. Results

The surface roughness values of the samples prepared from tested materials were compared statistically, and significant differences were found between the groups ($p < 0.05$). The surface roughness of different samples (VE, SHC, TNC, and ESQ) findings are seen in Table 2. The

highest average surface roughness value was determined in SHC group ($0.50 \pm 0.14 \mu\text{m}$), and the lowest average surface roughness value was determined in TNC group ($0.20 \pm 0.08 \mu\text{m}$). The surface roughness hybrid CAD/CAM block groups and composite groups were found statistically similar in themselves (Table 2) ($p > 0.05$). In this study, the roughness values of different materials are ranked from highest to lowest; SHC = VE > ES = TNC. While there was a significant difference in roughness values between groups, no statistical difference was found between bacterial adhesion values.

Table 1. Used materials

Material	Type	Manufacturer	Monomer	Filler	Mass%, (Vol%)
Estelite Sigma Quick (ESQ)	Conventional restorative composite resin	Tokuyama Dental Corporation, Tokyo, Japan	Bis-GMA, TEGDMA	Silica-zirconia filler, composite filler	82.0 (71.0)
Tetric N-Ceram Bulk-Fill (TNC)	Nano-hybrid Bulk-Fill composite	Ivoclar Vivadent, AG, Lichtenstein	Bis-GMA, Bis-EMA, UDMA	Barium glass, silicate glass,	81.0 (61.0)
Vita Enamic (VE)	Hybrid ceramic block	Vita Zahnfabrik H. Rauter GmbH, Bad Sackingen, Germany	UDMA, TEGDMA	Feldspar ceramic enriched with aluminum oxide	86.0 (75.0)
Shofu HC (SHC)	Hybrid ceramic block	Shofu Inc., Kyoto, Japan	UDMA, TEGDMA	Silica powder, micro fumed silica, zirconium silicate	61.0

Bis-GMA, bisphenol A diglycidylether methacrylate; UDMA, urethane dimethacrylate; TEGDMA, triethyleneglycol dimethacrylate; Bis-EMA, ethoxylated bisphenol-A dimethacrylate.

Table 2. Surface roughness values. Ra (μm)

Groups	N	95% Confidence Interval for Mean						
		Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
ESQ	15	0.31 ^a	0.19	0.04	0.20	0.41	0.11	0.76
TNC	15	0.20 ^a	0.08	0.02	0.15	0.25	0.11	0.38
VE	15	0.46 ^b	0.12	0.03	0.40	0.53	0.22	0.69
SHC	15	0.50 ^b	0.14	0.03	0.42	0.58	0.31	0.73
Total	60	0.37	0.183	0.02	0.32	0.41	0.11	0.76

* The same lower case letters show that there is no statistically significant difference between groups ($p > 0.05$, Tukey HSD post-hoc test).

Table 3. Adhesion values of *S. mutans*. CFU $\times 10^5$

Groups	N	95% Confidence Interval for Mean						
		Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
ESQ	15	234.20 ^a	99.58	31.49	162.96	305.44	131.03	447.56
TNC	15	225.82 ^a	48.22	15.24	191.32	260.31	149.77	286.49
VE	15	274.74 ^a	117.81	37.25	190.46	359.03	154.22	526.78
SHC	15	315.07 ^a	86.77	27.43	253.00	377.14	234.80	530.87
Total	60	262.46	95.20	15.05	232.01	292.91	131.03	530.87

* The same lower case letters show that there is no statistically significant difference between groups ($p > 0.05$, Tukey HSD post-hoc test).

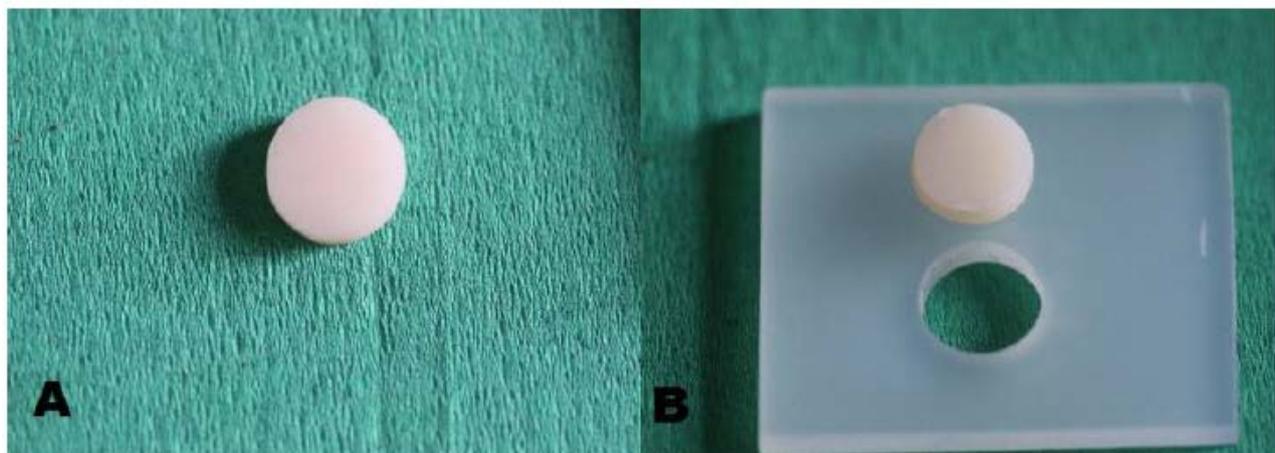


Figure 1. A= CAD / CAM block sample, B= PVC mold and composite sample

4. Discussion

Surface roughness and bacterial adhesion of current hybrid CAD/ CAM materials and bulk-fill resin composite and nano resin composite were examined in this study. The type of material used was determined to no effect the surface roughness and bacterial adhesion findings, and the null hypothesis tested was accepted. The surface properties and good polishability of all-ceramic systems used in the construction of prosthetic restorations are important in terms of easy removal of the biofilm layer and the prevention of potential bacterial colonization [18]. However, structural elements such as implant abutment joints in implant-supported prosthetic restorations that are suitable for bacterial adhesion and colonization are difficult to clean [19]. Surface roughness in dental materials has been reported to cause discoloration and plaque build-up. Surface roughness can vary depending on many factors, such as porosity in the structure of the material, inorganic filler content, filler type, and size [20]. In this study, the lowest surface roughness was obtained in the TNC group, and the highest surface roughness was obtained in the SHC group. Surface roughness values varying in the range were determined by 0.20-0.50 μm (Table 2). It is thought that the differences between the surface roughness values of the materials are due to microstructural and chemical differences between the materials used. The materials used in this study; differ in chemical content, amount of filler, and particle size (Table 1). The particle size of the resin-modified nanohybrid composites (TNC) is nanometer-size; It is believed that it can be polished better, and therefore, the surface roughness is lower compared to other groups [21]. In many studies examining the surface roughness in dental materials, large particle materials have been reported to have higher surface roughness values [9,20]. The particle size of the SHC block used in this study is thought to be effective in showing higher surface roughness values [22]. Mörmann et al., examined the surface properties of feldspathic ceramics and resin-modified nanoceramics, and reported that hybrid ceramic and resin-modified nanoceramics show similar surface roughness values [23]. Other studies examine the surface roughness of CAD/CAM materials and report similar results [20,24]. Kouzimi et al. examined the surface roughness values of resin matrix-based CAD/CAM materials (resin nanoceramic and hybrid ceramics) in their 2015 study and reported average surface roughness values ranging from 0.010-0.029 μm for the materials. In another study examined the effect of toothbrushing on the surface properties of CAD/CAM materials (resin nanoceramic, hybrid ceramic, feldspathic ceramic) before the brushing experiment; The surface roughness of the resin nano ceramic material was reported as $0.517 \pm 0.016 \mu\text{m}$, the surface roughness of the hybrid ceramic was $0.697 \pm 0.023 \mu\text{m}$ and the surface roughness of the feldspathic ceramic was reported as $0.246 \pm 0.017 \mu\text{m}$ [24]. While there was a difference between CAD/CAM block groups and composite groups as surface roughness, there was no

significant difference between materials related to bacterial adhesion in this study. Hybrid ceramic (SHC) had a lower roughness and lower bacterial adhesion than

the nanocomposite resin (TNC). These differences may be related to the material composition and microstructure.

Studies are showing that ceramics have lower roughness and bacterial adhesion than resin composites [25,26]. Awad et al. described the increasing surface roughness order for restorative materials as ceramics> feldspar ceramics> hybrid ceramics> resin-based composites> polymethylmethacrylate (PMMA) [27]. However, Fasbinder and Neiva observed a lower surface roughness in a nanoceramic resin material than a hybrid ceramic [28]. This result is consistent with the results of this study. Hybrid ceramics had a higher average surface roughness value. The difference in the studies is thought to result from variables such as surface preparation processes applied, surface roughness measurement method, and various measurements. The surface properties of materials, processing, and polishing processes affect the surface roughness. All this affects bacterial adhesion [29]. Different polishing materials are available; In this study, especially polishing systems suitable for material types were selected: Vita Enamic and Shofu Cadmaster HP Kit developed especially for CAD/CAM blocks and; Super Snap kit, which is widely used to polish composites. Each polish kit was also used according to the manufacturer's recommendations.

Among the tested materials, small but not significant differences were detected between the groups in terms of *S. mutans* adhesion and early biofilm formation. In studies, it has been reported that bacterial adhesion to restorative material is affected by factors such as the chemical content of the material, surface roughness, free surface energy, and whether the material is hydrophobic or hydrophilic [30]. Surface roughness is an important factor for bacterial adhesion, and it has been argued in many studies that it shows a positive correlation with bacterial adhesion values. However, in this study; A positive correlation between surface roughness and bacterial adhesion cannot be mentioned. It is thought that this finding can be explained by other factors effective in bacterial adhesion. For example; It is thought that *S. mutans*, which is more weakly attached to the zirconia surface, which is known to be hydrophobic, may be separated from the surface more easily than other ceramic surfaces during the experiment, resulting in low values in bacterial adhesion findings [31,32]. Researchers have shown that the water absorption potential of the BisGMA monomer is higher than the UDMA, TEGDMA, and BisEMA monomers [15]. The UDMA monomer has been shown to have a more hydrophobic structure than the BisGMA monomer [33]. It can be explained in this way that bacterial adhesion is not different in composite resin materials, although the BisGMA based organic matrix is more hydrophilic, although the roughness ratio is low. Also, different experimental conditions and bacterial strains can be used in other in vitro studies, as in this study.

These differences also affect the findings obtained in the studies. Studies are required in conditions similar to clinical conditions related to bacterial adhesion. Investigation of bacterial adhesion and roughness values on different restorative materials with different finishing methods may be beneficial.

5. Conclusion

When the tested materials are subjected to polishing in line with the recommendations of the manufacturer companies, they do not have a difference in bacterial adhesion, which is effective in the formation of caries.

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