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An Evaluation of the Effect on Streptococcus Mutans Adhesion of Surface Roughness in Different Aesthetic Restorative Materials

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Research Article

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ABSTRACT

Aim: The aim of this study was to evaluate the effect on Streptococcus mutans (S. mutans) adhesion of the surface roughness in different aesthetic restorative materials after the application of different finishing and polishing disc systems to tooth-coloured restorative materials.
Material-Method: A total of 126 disc-shaped samples were prepared as 21 for each of the restorative test materials used in the study. The samples were separated into 3 groups of 7 for the application of a different finishing and polishing disc set to each group. Pellicle formation on the surface of the materials was obtained by leaving the samples for one hour in artificial saliva containing mucin. The samples were then incubated in solutions containing S. mutans, and after 24 hours, the number of S. mutans showing adhesion were counted.
Results: A statistically significant difference was determined between the materials in respect of surface roughness values and bacteria adhesion ($p<0.05$), and no significant difference was found between the finishing and polishing disc sets ($p>0.05$). A positive correlation was determined at the rate of 26.2% between the surface roughness of the materials and S. mutans adhesion ($p<0.05$).
Conclusion: Materials show different surface roughness and adhesion values and the chemical content and physical properties of the material have an impact on this. The reasons of bacteria adhesion on material surface is clarified with in-vitro and in-vivo studies.

Keywords: Composite Resin, Glass Ionomer Cement, Streptococcus Mutans.

Farklı Estetik Restoratif Materyallerde Yüzey Pürüzlülüğünün Streptokok Mutans Adezyonuna Etkisinin Değerlendirilmesi

Research Article

Süreç

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ÖZ

Amaç: Bu çalışmanın amacı, diş rengindeki restoratif materyallere farklı bitirme ve polisaj disk sistemlerinin uygulanmasından sonra, farklı estetik restoratif materyallerdeki yüzey pürüzlülüğünün Streptokok mutans (S. mutans) adezyonuna etkisini değerlendirmektir.
Gereç-Yöntem: Çalışmada kullanılan restoratif test materyallerinin her biri için 21 adet olmak üzere toplam 126 adet disk şeklinde örnek hazırlandı. Her gruba farklı bitirme ve polisaj diski seti uygulanması için numuneler 7'şerli 3 gruba ayrıldı. Örnekler müsin içeren yapay tükürük içerisinde bir saat bekletilerek malzemelerin yüzeyinde pelikıl oluşumu sağlandı. Örnekler solüsyonlarda inkübe edildi ve 24 saat sonunda adezyon gösteren S. mutans sayısı belirlendi.
Bulgular: Malzemeler arasında yüzey pürüzlülük değerleri ve bakteri adezyonu açısından istatistiksel olarak anlamlı farklılık tespit edilirken ($p<0,05$), bitirme ve cilalama disk setleri arasında ise anlamlı bir fark bulunamadı ($p>0,05$). Malzemelerin yüzey pürüzlülüğü ile S. mutans adezyonu arasında %26,2 oranında pozitif korelasyon belirlendi ($p<0,05$).
Sonuç: Materyallerin farklı pürüzlülük ve adezyon değerleri göstermesinde; materyallerin kimyasal içerik ve fiziksel özelliklerinin etkili olduğu; materyal yüzeyine gerçekleşen bakteri tutulumunun nedenlerinin in-vitro ve in-vivo araştırmalarla aydınlatılabileceği düşünülmektedir.

Anahtar Kelimeler: Kompozit rezin, cam iyonomer siman, streptokok mutans

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Introduction

Composite restorative materials were developed both mechanically and physically until today. Also these materials are currently the most preferred aesthetic filling material with its wide are of use. However, it has been shown that microleakage, which develops due to polymerization shrinkage of composite resins, causes secondary decay under the material. Bonding problems of composite restorations are also encountered when they do not be sufficiently isolated from saliva.[1-4] All these reasons led to a preference for glass ionomer cements (GIC), which have the ability to express fluoride, as a restoration material in patients with poor oral hygiene. As GIC achieve chemical bonding through chelation with calcium of the hard dental tissues, express fluoride, and have a low pH, they have an important place in dentistry with these antimicrobial properties.[5]

Even after brushing the teeth, saliva proteins and macromolecules settle on the dental surface, which is covered with a non-cellular, clear film layer, known as the pellicle.[6] Together with the settling of bacteria on the pellicle, the accumulation of food remnants, and saliva glycoproteins, leukocytes, macrophages, and epithelial cells on the pellicle form a soft, semi-transparent, adhesive, microbio dental plaque.[7] Conducted researches show that surface roughness plays a role directly in generation of microbial dental plaque and critical surface roughness value is 0,2 µm. According to research reports if this value is exceeded, microbial dental plaque generation also will be rised.[8]

Bacteria where are located in oral cavity, necessitate to adhere to surface and have the ability to reproduce for maintaining their presence. At the forefront of microorganisms with this capability is Streptococcus mutans (S. mutans), which is accepted as the most cariogenic and is predominant in enamel decay, and Lactobacilli, predominant in dentin decay, and Actinomyces in root decay. However, these bacteria need to be able to easily adhere to the plaque structure on hard surfaces such as teeth and restorations and to be able to overcome colonization resistance.[6,7] Conducted studies show that bacteria adhesion is directly correlated with materials' surface characteristics and chemical ingredients.[3]

The surface properties of dental restorations are extremely important in respect of both oral health and restoration life, and aesthetic properties. Finishing and polishing procedures are beneficial in the shaping of the restoration surface and making it as smooth as possible. In this way, plaque retention and discoloration in restorations, and the associated patient complaints overcome, and the formation of gingival irritation and secondary decay are prevented.[9] The materials produced for this purpose are primarily diamond and carbide finishing mills, finishing and polishing discs of different abrasive sizes, polishing pastes, and metal-plastic bands used for the interfaces.[10] Previous studies were shown that finishing and polishing disc sets provide the best results in anterior region restorations, and the form of these discs is more appropriate for use on straight or outward curved surfaces in particular.[9-11]

The main goal of this research was to discuss the effect on S. mutans adhesion of the surface roughness in different aesthetic restorative materials after the application of different finishing and polishing disc systems to tooth-coloured restorative materials. The null hypothesis of the study is built that the surface roughness of different aesthetic restorative materials have effect on the adhesion of S. mutans. According to study findings null hypothesis was approved and crucial difference was notice between the materials with respect to bacterial adhesion.

Methods

The kind of restorative materials used in this research, the manufacturer's information, the content and LOT numbers are remarked in Table 1.

The finishing and polishing disc materials used in the study, the manufacturer's information, the content, LOT numbers, and application stages are shown in Table 2.

Table 1. The restorative materials used in the study, the content, and the manufacturer's information

Materials	Type	Lot No	Content
Filtek™ ultimate universal restorative (3m espe, St. Pau, MN, USA)	composite (nanofil)	NA16279	Bis-GMA, UDMA, TEGDMA, PEGDMA, Bis-EMA, silica and zirconia. Filler weighted 72.5%, volume weighted 63.3%.
Filtek™ z250 (3m espe, St. Pau, MN, USA)	composite (microhybrid)	NA53191	Bis-GMA, UDMA, Bis-EMA, Zirconia and silica. Filler weighted 82%, volume weighted 60%.
Estelite asteria (Tokuyama, Tokyo, Japan)	composite (nanohybrid)	W128	Bis-GMA, UDMA, Bis-MPEPP, TEGDMA, mequinol, dibutyl hidroxy toluene, UV absorber, glass particles, silicone dioxide, zirconium dioxide. Filler weighted 82%, volume weighted 71%.
Ceram. x duo (Dentsply, Konstanz, Germany)	composite (nanoceramic)	1905000953	Bis-GMA, UDMA, TEGDMA, modified methacrylate polysiloxane, glass particles, iron oxide, titanium oxide. Filler weighted 76%
Photac fil quick aplicap (3m espe, St. Pau, MN, USA)	resin-modified glass ionomer	5725992	Na-Ca-Al fluorosilicate glass, polyacrylic acid, maleic acid, HEMA.

Fuji 2 lc capsule (Gc, Tokyo, Japan)	resin-modified glass ionomer	1910041	Fluoro aluminium silicate glass, distilled water, polyacrylic acid, 2-hydroxyethyl methacrylate, urethane dimethacrylate.
Polystyrene (SEPAR plastic)	plastic (control group)	-	Styrene monomer.

Table 2. The restorative materials used in the study, the content, and the manufacturer's information

Materials	Content	Lot no	Application stages
Sof-lex (3m espe, St. Pau, USA)	Finishing and polishing discs coated with aluminum oxide urethane	NA54445	Disc set in 4 stages; decreasing height and contouring discs used at 10,000 rpm. Finishing and polishing discs usedat 30,000 rpm.
Optidisc (Kerr, orange, CA, USA)	Contains aluminum oxide, synthetic polymer, polyethylene, epoxy resin adhesive	7053476	A 4-stage application sequence of extra thick (decreasing height and contouring), thick (finishing), fine (polishing) and extra fine (high polishing)
Super-snap (Shofu, Inc, Kyoto, Japan)	Finishing and polishing discs containing aluminum oxide particles	0719020	A 4-stage finishing and polishing system containing no metal parts: leveling, finishing, polishing, and super polishing stages.

Preparation of the Restorative Samples

A total of 126 disc-shaped samples were prepared as 21 for each of the restorative test examples used in the research. The 7 samples of polystyrene material used as the control group were purchased from SEPAR Plastik. Using an mouth spatula, all the materials were placed in plastic molds, 8 mm in diameter and 2 mm in depth, and were then compressed between a clear band and two glass plates. After removing the glass plates, polymerization was applied with an LED light device (Woodpecker LED-G, China) in related with the manufacturer's instructions.

Finishing and Polishing procedures applied to the Prepared Samples

The 21 prepared samples for each material were divided into 3 groups of 7 for the application of the finishing and polishing plans according to the producer's instructions, implementing a different polishing disc set for each group. After completion of the finishing and polishing procedures, the samples were cleaned under running tap water for 20 seconds, and were after rested in distilled water until the bacterial adhesion stage. The polystyrene material used as the control group was not included in these procedures.

Dimensions of the Surface Roughness of the Samples

Surface roughness dimensions of the edited samples were made using a profilometer (Veeco Dektak 6M, NY, USA). The measurement was performed by applying 5mg force with the recording end of the profilometer deviceto record a distance of 2 mm at a fixed speed of 10 seconds. The measurements were taken from 3 different regions of each sample and the average of these was taken for the computing of the surface roughness (Ra) values.

After completion of the surface roughness values, the samples were sterilized by washing and then left for 15 minutes in an autoclave at 121°C and 1 atmosphere pressure (Newmed Kronos B, İzmir, Turkiye) before the bacteria adhesion stage.

Preparation of the Synthetic Saliva

At the stage of preparation of the synthetic saliva in which the samples were to be placed, a solution was prepared of 2 lt of distilled water with the addition of 2560 mg sodium chloride (NaCl), 332.97 mg calcium chloride ($CaCl_2$), 250 mg magnesium chloride hexahidrate ($MgCl_2(6H_2O)$), 189.48 mg potassium chloride (KCl), 3015 mg potassium acetate (CH_3COOK), 772 mg tripotassium phosphate trihydrate ($K_3PO_4(3H_2O)$) and 0.1 ml 85% phosphoric acid (H_3PO_4). The solution was mixed until it became clear.[10] The synthetic saliva was prepared to have pH of 6.5-7, with the addition of 140 mg Type II mucin (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) to every 100 ml of synthetic saliva, sterilized in the autoclave.

S. mutans Adhesion to the Restorative Material Samples

For the adhesion test in this study, S. mutans ATCC® 25175 (RSHM NO:7038) isolate was used, purchased from the Turkish Public Health Institute culture collection. The isolate was revitalized by adding Tryptic Soy Broth (TSB) to the lyophilised isolate. A 5% sheep blood agar (SBA) subculture was made to a solid medium from the suspension of the isolate in TSB and left for 24 hours incubation at 35-37°C in jars containing 5-10% CO_2. The bacteria produced from the colonies were activated by again making a subculture of the SBA medium. After sterilization in the autoclave, the restorative material samples were placed in sterile petri dishes 90 mm in diameter. Synthetic saliva containing 20 ml mucin was added to the petri dishes to completely cover each sample, and then after waiting 1 hour for the pellicle formation, the synthetic saliva was removed from the petri dish.

From the activated S. mutans bacteria, a bacteria suspension at 0.5 McFarland turbidity (1 x 10^8 CFU/ml) was obtained using a Nephelometer and Vortex device within the TSB, and was added to the petri dishes to cover the samples.

The petri dishes containing the samples and bacteria suspension in jars containing 5-10% CO_2 were incubated for 24 hours at 35-37°C. Following the incubation, the

samples were removed from the petri dishes and washed 3 times with sterile phosphate buffer solution (PBS) to remove the bacteria not showing full adhesion to the surface. Each washed sample was placed in a tube containing 1 ml sterile PBS, and these were left for 5 mins in an ultrasonic bath operating at 285 W and 50/60 KHz (Gen-Probe, San Diego, CA, USA). Thus the bacteria showing adhesion were transferred from the sample surface to the PBS.

To not cause a change in the number of bacteria, a sample was taken with 0.01 ml extract without being removed from the restorative material within the PBS and was seeded in 5% SBA and left for incubation for 24 hours. The colonies formed in the medium after 24 hours of incubation were determined with the bacteria count method and recorded as Colony-Forming Units (CFU/ml).

Statistical Analysis

The data get from this study were analyzed statistically using SPSS 25 software (Statistical Package for the Social Sciences).Identified statistics were indicated as mean ± standard deviation values. To investigate the effect on a dependent variable of two different factors formed from multiple groups, Two-Way Variance Analysis was applied, and to decide from which group the discrepancy originated, the Tukey Multiple Comparison test was used. Outcomes were indicated in a 95% confidence interval. When interpreting the results, the level of statistical significance was accepted as 0.05.

Results

Examination of the Surface Roughness Values of the Materials

The mean surface roughness values obtained using 6 different aesthetic filling materials and 3 different finishing and polishing disc sets are shown in Table 3.

The materials showing the greatest surface roughness were Photac Fil Quick Aplicap (3m espe, St. Pau, MN, USA), a resin-modified glass ionomer cement (RMGIC), followed by Fuji 2 LC Capsule (Gc, Tokyo, Japan) another RMGIC material. The material with the least surface roughness, excluding polystyrene used as the control group, was the nano-hybrid-based Estelite Asteria (Tokuyama, Tokyo Japan) composite resin material.

Two-way variance analysis is used for compare the effects of the polishing and finishing disc sets on the surface roughness values. In terms of surface roughness there is a significant difference between materials ($p<0.05$) However there is no meaningful difference exist between the polishing and finishing materials ($p>0.05$)

According to the Tukey Multiple Comparison test to determine from which material the difference originated, the nanofil-based Filtek™ Ultimate Universal Restorative (3m espe, St. Pau, MN, USA) material showed significantly lower roughness compared to only the two RMGIC materials ($p<0.05$) and the nano-hybrid-based Estelite Asteria (Tokuyama, Tokyo Japan) showed significantly lower roughness compared to the two RMGIC materials and the nano-ceramic-based Ceram.x Duo (Dentsply, Konstanz, Germany) from the composite resins ($p<0.05$). No statistically curicial difference was observed between the other materials in respect of surface roughness ($p>0.05$). Tukey Multiple Comparision test results are shown in Table 4.

Table 3. Surface roughness values (Ra)

Materials	Finishing and polishing disc sets	Mean value	Standard deviation
Polistren (control group)	-	0.23739	0.120210
3m filtek™ ultimate universal restorative	3m sof-lex	2.07388	1.113412
	Kerr optidisc	2.44061	0.913932
	Shofu super-snap	2.19690	1.190862
3m filtek™ ultimate z250	3m sof-lex	2.34977	0.663118
	Kerr optidisc	2.77105	0.943048
	Shofu super-snap	2.68842	1.282877
Tokuyama estelite asteria	3m sof-lex	1.93706	0.924018
	Kerr optidisc	1.82675	0.861827
	Shofu super-snap	1.56323	0.494112
Dentsply ceram.x duo	3m sof-lex	2.86446	0.722355
	Kerr optidisc	2.93628	1.214530
	Shofu super-snap	2.61183	0.756362
3m photac fil quick aplicap	3m sof-lex	3.55144	1.134469
	Kerr optidisc	3.39085	1.587742
	Shofu Super-Snap	3.15430	0.755827
Gc fuji 2 lc capsule	3m sof-lex	3.21044	0.529651
	Kerr optidisc	3.57197	0.838714
	Shofu super-snap	3.24643	0.783736

Table 4. Surface roughness values (Ra)

Test materials		p
Polistren (control group)	3m filtek™ ultimate universal	0.0*
	3m filtek™ ultimate z250	0.0*
	Tokuyama estelite asteria	0.003*
	Dentsply ceram.x duo	0.0*
	3m photac fil quick aplicap	0.0*
	Gc fuji 2 lc capsule	0.0*
3m filtek™ ultimate universal	3m filtek™ ultimate z250	1.0
	Tokuyama estelite asteria	1.0
	Dentsply ceram.x duo	0.774165
	3m photac fil quick aplicap	0.002295*
	Gc fuji 2 lc capsule	0.003*
3m filtek™ ultimate z250	Tokuyama estelite asteria	0.073197
	Dentsply ceram.x duo	1.0
	3M Photac fil quick aplicap	0.139681
	Gc fuji 2 lc capsule	0.173326
Tokuyama estelite asteria	Dentsply ceram.x duo	0.007859*
	3m photac fil quick aplicap	0.0*
	Gc fuji 2 lc capsule	0.0*
Dentsply ceram.x duo	3m photac fil quick aplicap	0.809669
	Gc fuji 2 lc capsule	0.963323
3m photac fil quick aplicap	Gc fuji 2 lc capsule	1.0

* p<0.05

Examination of the S. mutans Adhesion Values of the Materials

The mean S. mutans adhesion values determined on the surface of the restorative materials are shown in Table 5.

The material with the greatest S. mutans adhesion was the nano-ceramic-based Ceram.x Duo (Dentsply, Konstanz, Germany), followed by the RMGIC (Fuji 2 LC Capsule, Photac Fil Quick Aplicap), and the nanofil-based Filtek™ Ultimate Universal Restorative (3m espe, St. Pau, MN, USA) material. The restorative material showing the least bacteria involvement, excluding polystyrene used as the control group, was the nano-hybrid-based Estelite Asteria (Tokuyama, Tokyo Japan) composite resin material.

As a result of the Two-Way Variance Analysis used to compare the effects of the finishing and polishing disc sets on the S. mutans adhesion values, a statistically crucial difference was observed between the materials (p<0.05) and no significant difference was get between the finishing and polishing disc sets (p>0.05).

According to the Tukey Multiple Comparison test to determine from which material the difference originated, there was determined to be no statistically crucial difference between the nano-hybrid-based Estelite Asteria (Tokuyama, Tokyo, Japan) and the micro-hybrid-based Filtek™ Ultimate Z250 (3m espe, St. Pau, MN, USA), which showed the lowest S. mutans adhesion values (p>0.05) and the difference between these two composite resins and all the other materials was found to be statistically significant (p<0.05). No statistically crucial difference was determined between the other materials in respect of bacteria adhesion (p>0.05). The values of bacteria adhesion's Tukey Multiple Comparision test results are shown in Table 6.

When the relationship between surface roughness and bacteria adhesion was evaluated, a positive linear correlation was determined at the rate of 26.2% between the surface roughness of the materials and the amount of S. mutans adhesion to the restoration surface (p<0.05). As the surface roughness increased, so there was an increase in bacteria involvement.

Table 5. The mean S. mutans adhesion values determined on the surface of the restorative materials (cfu/ml)

Materials	Finishing and polishing disc sets	Average value	Standard deviation
Polistren (control group)	-	5971.43	2114.01
3m filtek™ ultimate universal restorative	3m sof-lex	54714.28	30210.78
	Kerr optidisc	68042.86	24421.15
	Shofu super-snap	67428.57	14125.23
3m filtek™ ultimate z250	3m sof-lex	39571.43	9182.72
	Kerr optidisc	50814.29	11211.07
	Shofu super-snap	46714.28	18693.53
Tokuyama estelite asteria	3m sof-lex	35685.71	11809.66
	Kerr optidisc	29257.14	13218.15
	Shofu super-snap	32142.86	5365.27
Dentsply ceram.x duo	3m sof-lex	75742.86	14810.79
	Kerr optidisc	79428.57	13813.60
	Shofu super-snap	78600.00	15275.68

	3m sof-lex	66385.71	17157.35
3m photac fil quick aplicap	Kerr optidisc	67985.71	16977.47
	Shofu super-snap	69414.29	16926.35
	3m sof-lex	71828.57	16911.01
Gc fuji 2 lc capsule	Kerr optidisc	70371.43	17975.79
	Shofu super-snap	68957.13	10489.97

Table 6. The values of bacteria adhesion's tukey multiple comparision test results

Test materials		p
Polistren (control group)	3m filtek™ ultimate universal	0.0*
	3m filtek™ ultimate z250	0.0*
	Tokuyama estelite asteria	0.0*
	Dentsply ceram.x duo	0.0*
	3m photac fil quick aplicap	0.0*
	Gc fuji 2 lc capsule	0.0*
3m filtek™ ultimate universal	3m filtek™ ultimate z250	0.007*
	Tokuyama estelite asteria	0.0*
	Dentsply ceram.x duo	0.059
	3m photac fil quick aplicap	1.0
	Gc fuji 2 lc capsule	1.0
3m filtek™ ultimate z250	Tokuyama estelite asteria	0.119
	Dentsply ceram.x duo	0.0*
	3m photac fil quick aplicap	0.0*
	Gc fuji 2 lc capsule	0.0*
Tokuyama estelite asteria	Dentsply ceram.x duo	0.0*
	3m photac fil quick aplicap	0.0*
	Gc fuji 2 lc capsule	0.0*
Dentsply ceram.x duo	3m photac fil quick aplicap	0.679
	Gc fuji 2 lc capsule	1.0
3m photac fil quick aplicap	Gc fuji 2 lc capsule	1.0

* $p<0.05$

Discussion

New materials are continuously being introduced in restorative dentistry and studies are ongoing for the development of the ideal material. Of these materials, composite resins take first place.[12] Although there is currently great use of composite resins, because of negative properties such as the fact that they are not be used in cavities that are not be isolated, microleakage seen as a result of polymerization shrinkage and associated secondary decay, the use of GIC, which express fluoride, have antibacterial properties and chemically bonds to dental hard tissues, has come to the fore especially for patients with poor oral hygiene.[3,4]

To prolong the clinical life of restorations and to be able to obtain a more aesthetic appearance, finishing and polishing procedures are required. In restorations where the finishing and polishing procedures are unapplied correctly, surface discolouration associated with plaque accumulation and gingival irritation is occur.[13,14]

Although an anatomic form of the restoration is obtained with finishing procedures, a scratched and rough area is formed on the surface of the material, sopolishing of the restorative material surface is recommended to prevent this.[14] Well-applied finishing and polishing procedures increase the surface hardness of the restoration, increase colour stabilization, and prolong clinical life.[15]

Gauthier *et al.* (2005) examined the surface properties of composite resins and reported that the oxygen inhibition layer in the outermost layer formed during polymerization was very important for the surface property of the material. Incomplete polymerization in this layer causes a decrease in the restoration surface hardness. The idea has gained weight that because of the finishing and polishing processes the oxygen inhibition layer are removed, thereby obtaining a smoother surface that prevents bacterial adhesion to the material surface.[16]

Different filler particle dimensions and organic matrix hardness of the material shows an significant role in the degree of the effect of the finishing and polishing procedures on the material surface. The harder ones are preferred in abrasives used for the finishing and polishing systems with respect to other filling particles. When this is not the case, there is separation of the organic matrix, filler particles not abraded remain above, and thus there is the possibility of a surface of increased roughness. Moreover, the type, shape, size, amount, and distribution of the filler particles contained in the material are wise to have an effect on surface roughness. Following finishing and polishing procedures, greater roughnessis encountered on the surfaces of resin materials with large particles.[17,18]

In a study by Koh *et al.* (2008), comparisons were made of the effect of single-stage and multi-stage polishing systems on the surface roughness of microhybrid and nanofil composites. The results showed that the nano filler composites showed less surface roughness than the hybrid composites and the Optidisc and Sof-Lex systems were helpful in obtaining a better surface.[19]

The surface roughness values of GIC and composite resins were investigated in several studies, and GIC was reported to show greater surface roughness.[20,21] In a study by Eick *et al.* (2004), the surface roughness of composite, RMGIC, traditional GIC, compomer, ceramic, and amalgam materials was examined, and it was reported that traditional GIC showed the highest roughness value, followed by RMGIC.[22]

In the this study, when compared with polystyrene material used as a positive control group, the surface roughness of the other materials was established to be statistically meaningfully low ($p<0.05$). The nanohybrid-based Tokuyama Estelite Asteria (Tokuyama, Tokyo, Japan) composite material showed less surface roughness in all the finishing and polishing systems compared with the other restorative materials, and the two RMGIC (Photac Fil Quick Aplicap and Fuji 2 LC Capsule) were the restorative materials showing the worst surface roughness.

Previous studies demonstrated that the material's critical surface rougness value is 0,2 µm for bacteria that responsible for decay to shows adhesion. Moreover it is also reported that in the situation of exceeding 0,2 µm value, the microbiodental plaque formation was increased.[8] In this study, all the restorative materials used, including the control group, were seen to have surface roughness above the critical surface roughness value.

According to several studies, there is a positive relationship between surface roughness of the material and the number of bacteria showing adhesion to the surface, that plaque formation is increased on rough surfaces, and that bacterial colonization starts from rough areas such as a groove, crack, or wear defect in the restorative material.[23]

Tanner *et al.* (2003) reported that surface roughness affected the adhesion of S. mutans, and rough surfaces formed a retention area for bacterial involvement.[24]

In a study by Brambilla *et al.* (2005) using composite, compomer and GIC, it was concluded that the material with the highest S. mutans adhesion was compomer and the material with the lowest value was GIC.[25]

In different studies using traditional GIC and composite resin, Carlen *et al.* (2001) reported a lower number of S. mutans showing adhesion to the composite resin surface compared to GIC.[26] Eick *et al.* (2004) examined the relationship between bacteria adhesion and the surface roughness of amalgam, composite, compomer, ceramic, traditional GIC and RMGIC materials, and stated that the highest level of bacteria adhesion was to the traditional GIC material which had the highest roughness value.

It has also been claimed that fluoride, which has an antibacterial property, in the content of GICs does not prevent the adhesion of S. mutans to the material surface.[22] Montanaro *et al.* (2004) showed that fluoride inhibited the proliferation and metabolism of bacteria, but remained insufficient in preventing bacteria adhesion.[27] Similar studies confirmed that although fluoride strengthens the enamel surface against external factors and raises the plaque pH, it does not decrease S. mutans adhesion to the material surface.[28-31]

The results of the this study was confirmed the findings of other studies. When the polystyrene used as the control group was excluded, the restorative material showing the least S. mutans adhesion was determined to be the nanohybrid-based Estelite Asteria (Tokuyama, Tokyo, Japan) composite material, which had the best surface smoothness after polishing. The material with the second least bacteria adhesion was the microhybrid-based Filtek™ Ultimate Z250 (3m espe, St. Pau, MN, USA) composite material, and the difference between the two materials was not statistically significant ($p>0.05$). However, a statistically significant difference was determined between these materials and the others ($p<0.05$). The material showing the most S. mutans adhesion after polishing was the nanoceramic-based Ceram.x Duo(Dentsply, Konstanz, Germany), which is also a composite resin. This was followed by the RMGIC, Fuji 2 LC Capsule (Gc, Tokyo, Japan) and Photac Fil Quick Aplicap (3m espe, St. Pau, MN, USA), and then Filtek™ Ultimate Universal Restorative (3m espe, St. Pau, MN, USA) composite resin. However, no statistically significant difference was seen between these four restorative materials ($p>0.05$).

The possibility of a clinical follow-up study of patients followed up related to the study hypothesis was severely restricted because of the COVID-19 pandemic. A further limitation was that different bacteria species showing aerobic and anaerobic properties which are seen in plaque were not included in the study due to the need for specific environments and techniques. There is a need for further in-vivo and in-vitro studies on this subject.

Conclusions

Within the limitations of this study, it was concluded that there is a positive correlation between surface roughness and bacteria adhesion, as the surface roughness increases, the bacteria adhesion also increases. According to this study's findings $H_{(0)}$ hypothesis is supported and meaningful differences are observed in materials between in the context of bacterial adhesion.

In showing the different roughness and adhesion values of composite resin materials, the degree of the effect of the chemical content and physical properties of materials and the causes of bacteria adhesion to the material surface is needed to explain with further in-vitro and in-vivo studies. In the light of the findings in this study, bacteria adhesion is considered to increase in direct relation to the increase in surface roughness as a result of fluoride expression in RMGIC.

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Conflict of Interest

The authors indicate no financial support or financial conflict of interest. The authors have indicated they have no financial relationships with any company and no external funding.

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