



EFFECT OF DIFFERENT FINISHING TECHNIQUES FOR RESTORATIVE MATERIALS ON SURFACE ROUGHNESS AND BACTERIAL ADHESION

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Statement of problem. The formation of biofilm and bacterial accumulation on dental materials may lead to the development of gingival inflammation and secondary caries.

Purpose. The purpose of this study was to examine the effect of different surface finishing and polishing methods on surface roughness and the adhesion of *S. mutans* bacteria to 2 new-generation indirect composite resins, 1 direct composite resin, and 1 ceramic material.

Material and methods. Forty specimens (10 × 10 × 2 mm) of each material, indirect composite resins (SR Adoro, Estenia), direct composite resin (Tetric), and a ceramic material (VITABLOCS Mark II), were fabricated. Specimens were divided into 4 groups (n=10) that were treated with 1 of the following 4 surface finishing techniques: diamond rotary cutting instrument, sandpaper discs (Sof-Lex), silicone-carbide rubber points (Shofu), or a felt wheel with diamond paste. Surface roughness was measured with a profilometer. Test specimens were covered with artificial saliva and mucin to produce pellicle. Bacterial suspension (10⁹ CFU/ml) was then added to the pellicle-coated specimens, and bacterial adhesion was determined using a confocal laser microscope and image analyzing program. Data were analyzed with 2-way ANOVA, followed by Tukey HSD test, Pearson correlation, and regression analysis ($\alpha=.05$).

Results. The highest surface roughness values were recorded in SR Adoro and diamond rotary cutting instrument groups. The lowest vital *S. mutans* adhesion was seen in the ceramic group and in SR Adoro indirect composite resin ($P<.05$).

Conclusions. Bacterial adhesion to indirect composite resin materials differed from that to ceramic material after surface treatments. A positive correlation was observed between surface roughness and the vital *S. mutans* adhesion. (J Prosthet Dent 2010;103:221-227)

CLINICAL IMPLICATIONS

Although sandpaper discs and polishing kits reduced the surface roughness and *S. mutans* adhesion, the composition of composite resin, indirect composite resin, and ceramic also affect surface roughness and *S. mutans* adhesion.

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The use of composite resins in restorative dentistry became routine with the improvement of bonding systems, polymerization systems, and mechanical and physical properties of the resin systems.¹ One of the primary reasons for replacement of composite resin restorations is secondary caries.² The formation of biofilm and bacterial accumulation on dental materials may result in gingival inflammation and secondary caries.³⁻⁵ The next step of biofilm formation involves the colonization of the microbial cells.⁶ Several previous studies refer to streptococci bacteria, since they belong to the group of so-called early colonizing bacteria. These bacteria, in particular, *Streptococcus mutans* (*S. mutans*), are known to have an important role in the pathogenesis of caries.^{3,7-9} The clinical performance of composite resin restorations is comparable to ceramic restorations, but the relatively low cost and the inherent low brittleness of composite resin have resulted in increased use of composite resin-based indirect restorations in the posterior region.¹⁰⁻¹² New-generation indirect resins (termed ceramic polymers) have a higher density of inorganic ceramic filler in comparison to traditional composite resins.⁹ Indirect composite resin restorations are thought to have superior mechanical properties compared to direct composite resin restorations.^{10,11} These enhanced properties are due to a higher degree of conversion obtained by the use of different polymerization procedures that involve photoactivation, heat between 90°C and 140°C, and/or vacuum or a nitrogen atmosphere.¹³

It has been reported that ceramics exhibit the least bacterial and glucan adhesion compared to other restorative materials.¹⁴⁻¹⁷ Some composite resins have been reported to stimulate bacterial adherence.^{2,18,19} The residual monomers released from polymerized composite resins might influence the growth of caries-associated microorganisms.¹⁸ However, little is known about bacterial adherence on the sur-

face of indirect composite resins such as Estenia and SR Adoro. Furthermore, surface roughness is thought to be an important factor in determining the amount of plaque accumulation.²⁰⁻²² Restorations may need some intraoral adjustment (occlusal adjustment, correction of overcontours, or trimming of excessive luting composite resin cement) after cementation. Such adjustments are usually done with fine-grained diamond rotary cutting instruments that break the polished layer and increase the surface roughness of the restorations.²³⁻²⁷ A poorly finished restoration might initiate biofilm adherence on the surface and its adjoining areas in the oral cavity.^{22,28} Many polishing kits are available to eliminate the grooves created during adjustments and to achieve a smooth surface.²⁹⁻³² Sandpaper discs, rubber wheels, and wheels with diamond paste are commonly used. Aside from the surface properties of resin materials, material components such as filler particles and the resin matrix, as well as polymerization conditions, might influence bacterial adhesion.³³⁻³⁵

This study evaluated the surface roughness of 4 restorative materials (2 indirect composite resins, a composite resin, and a ceramic) when modified by different intraoral finishing and polishing procedures, and also examined the effect of surface characteristics on bacterial (*S. mutans*) adhesion to restorative materials coated with artificial saliva and mucin using a confocal laser scanning microscope and image analysis method. The null hypothesis of this study was that there would be no difference in bacterial adhesion to indirect composite resin and ceramic materials when subjected to various surface treatments.

MATERIAL AND METHODS

Composition and manufacturing information for the dental restorative materials evaluated is presented in Table I. Resin-based specimens were

prepared with a custom stainless steel mold (10 x 10 x 2 mm). The composite resin was packed into the mold, and then both sides of the mold were clamped with a glass slide. Tetric EvoCeram specimens were polymerized with a light-polymerizing unit (bluephase; Ivoclar Vivadent AG, Schaan, Liechtenstein) from both sides for 20 seconds. Light intensity was 800 mW/cm², and the wavelength range was 430-490 nm. Estenia specimens were polymerized using the same unit for 180 seconds on both sides and then heated at 110°C for 15 minutes in an oven (KL 100; Kuraray Co Ltd, Osaka, Japan). SR Adoro specimens were prepolymerized with a hand-held unit (bluephase; Ivoclar Vivadent AG) for 20 seconds and then polymerized in an oven (Lumamat 100; Ivoclar Vivadent AG) at program 3 for 25 minutes. Ceramic specimens were cut to the same size as in a previous study²³ from VITABLOCS Mark II by using a diamond disc (Diamond Wafering Blade Series 15HC, No. 11-4244; Buehler Ltd, Lake Bluff, Ill) attached to a cutting machine (IsoMet Low Speed Saw II; Buehler Ltd) under water coolant. One side of each specimen was smoothed with ultra-fine 600-grit sandpaper (3M ESPE, St. Paul, Minn) for 60 seconds by a single operator.

The specimens of each material were divided into 4 groups of 10 specimens and then cleaned ultrasonically for 5 minutes. Each group received a different surface treatment. In the diamond rotary cutting instrument (DRCI) group, the surface was ground with fine (46 µm, No. 8837 KR.314.014; GEBR Brasseler GmbH, Lemgo, Germany) and then extra-fine (25 µm, No. 837 KREF.314.014; GEBR Brasseler GmbH) diamond rotary cutting instruments for 30 seconds each. In the Sof-Lex group, specimens were polished with a sequence of 3 sandpaper discs (Sof-Lex coarse: 100 µm, medium: 29 µm, and fine: 14 µm; 3M ESPE) for 60 seconds each. In the Shofu group, the surface was smoothed with a white stone (Dura-White Stone 0243; Shofu, Inc, Kyoto,

TABLE I. Materials used in this study

Brand	Type of Material	Manufacturer	Batch Number	Composition Provided by Manufacturer
SR Adoro	Indirect composite resin	Ivoclar Vivadent AG, Schaan, Liechtenstein	G16766	Aromatic aliphatic UDMA, inorganic filler (63%, SiO ₂)
Estenia	Indirect composite resin	Kuraray Co Ltd, Osaka, Japan	00216D	UTMA, other methacrylate monomers, inorganic filler (92.3%) (SiO ₂ , BaO, Al ₂ O ₃ , La ₂ O ₃)
Tetric EvoCeram	Composite resin	Ivoclar Vivadent AG	H06993	Bis-GMA, UDMA, TEGDMA, inorganic filler (48.5%, barium glass filler)
VITABLOCS Mark II	Ceramic	VITA Zahnfabrik, Bad Säckingen, Germany	7303	Feldspathic crystalline particles (SiO ₂ -Al ₂ O ₃ -Na ₂ O-K ₂ O) in glassy matrix

Bis-GMA: bisphenol-A glycidyl dimethacrylate; UDMA: urethane dimethacrylate; UTMA: urethane tetramethacrylate; TEGDMA: triethylene glycol dimethacrylate

Japan) for 60 seconds. Then, the surface was cleaned with air-pressured water and polished with a sequence of 3 silicone-carbide rubber points (Ceramide Standard: 48 µm, Ultra: 28 µm, and Ultra II: 6.3 µm; Shofu, Inc) for 60 seconds each. In the diamond paste group, specimens were polished using a felt wheel (Super-Snap Buff; Shofu, Inc) with diamond paste (Diamond Stick; Shofu, Inc) for 60 seconds each.

A profilometer (Mitutoyo SurfTest 402 Surface Analyzer; Mitutoyo Corp, Kawasaki Kanogawa, Japan) was used to measure the surface roughness. Three different regions (in the middle and sides) were evaluated in each specimen to determine the surface roughness (Ra) value, and averaged to determine the mean value. Before bacterial adhesion, specimens were cleaned for 15 minutes with an ultrasonic cleaner (BioSonic; Coltène/Whaledent, Inc, Cuyahoga Falls, Ohio) and then sterilized in an autoclave at 121°C for 15 minutes.

Test specimens were covered with artificial saliva and mucin suspension (M2378, Mucin from porcine stomach, Type II; Sigma-Aldrich, St. Louis,

Mo) (5 ml) in a petri dish and left for 1 hour to produce a pellicle. Type II mucin (140 mg) was added to 100 ml of artificial saliva. Artificial saliva was prepared as described in previous studies^{36,37}: 8.4 mg NaF, 2560 mg NaCl, 332.97 mg CaCl₂, 250.00 mg MgCl₂ (6H₂O), 189.48 mg KCl, 3015.00 mg CH₃COOK, 772.00 mg K₃PO₄ (3 H₂O), and 0.1 ml H₃PO₄ (85%) (Merck KGaA, Darmstadt, Germany). The specimens were washed with 5 ml of saline and placed into the sterilized petri dishes. The bacteria used in this study was *Streptococcus mutans* NCTC 10449 (*S. mutans*) (Selcuk University, Veterinary Faculty, Department of Microbiology, Konya, Turkey). The bacteria obtained from stock was plated onto Columbia agar (10455; Merck KGaA) and incubated at 37°C in a 10% CO₂ atmosphere for 24 hours. Bacteria from cultures was then transferred into tubes containing 5 ml of BHI (Brain-heart infusion, 10493; Merck KGaA) and incubated at 37°C in a 10% CO₂ atmosphere for 18 hours. The tube contents were mixed using a centrifuge for 5 minutes. Five ml of PBS (phosphate-buffered saline; Merck KGaA) suspension was

added into the tubes and mixed for 15 seconds by centrifuge (Firlabo, Emerainville, France). Bacterial suspension was concentrated as 10⁹ bacteria ml⁻¹ with a microplate reading (VersaMax; Molecular Devices, Sunnyvale, Calif). The mixture of the bacterial suspension (100 ml), including 10⁹ colony forming units (CFU)/ml, was added to each specimen surface, and the bacterial adhesion was provided for 15 minutes to the pellicle layer. BHI with 5% sucrose was added to each petri dish to cover all specimens, and dishes were placed into an incubator (Jouan GmbH, Unterhaching, Germany) at 37°C in a 5% CO₂ atmosphere for 24 hours. The specimens were then placed into tubes containing 2 ml of PBS and mixed with a centrifuge for 30 seconds to separate the free bacteria. The unpolished surfaces of the specimens were bonded with nail polish (Golden Rose Nail Lacquer; Erkul Cosmetics, Istanbul, Turkey) onto the microscope slide.

Before microscopic examination, 6 µl of fluorescein diacetate (FDA) (F7378; Sigma-Aldrich) stock solution and 3 µl of ethidium bromide (EB) (E1385; Sigma-Aldrich) were

mixed with 1 ml of cold saline. The final solution (5 μ l) was dropped onto the surface of each specimen and then covered with a glass slide. FDA is not fluorescent but is membrane soluble. In vital cells, it is metabolized to fluorescein, which fluoresces green and is no longer able to leave the cell, and the living cells are stained green. Dead cells are not able to metabolize the FDA. The EB only penetrates nonvital bacteria cells and stains them red.⁵⁻¹⁴

The specimens were transferred to a confocal laser scanning microscope (LSM 700; Carl Zeiss GmbH, Göttingen, Germany) and were examined with argon and HeNe (helium neon) lasers. The excitation wavelength was 488 nm for the argon laser and 543 nm for the HeNe laser. Confocal laser scanning photographs were made of the test specimens and counted with an image analysis program (Clemex Vision Lite 3.5; Clemex Technologies, Inc, Longueuil, Canada).

The data were evaluated with

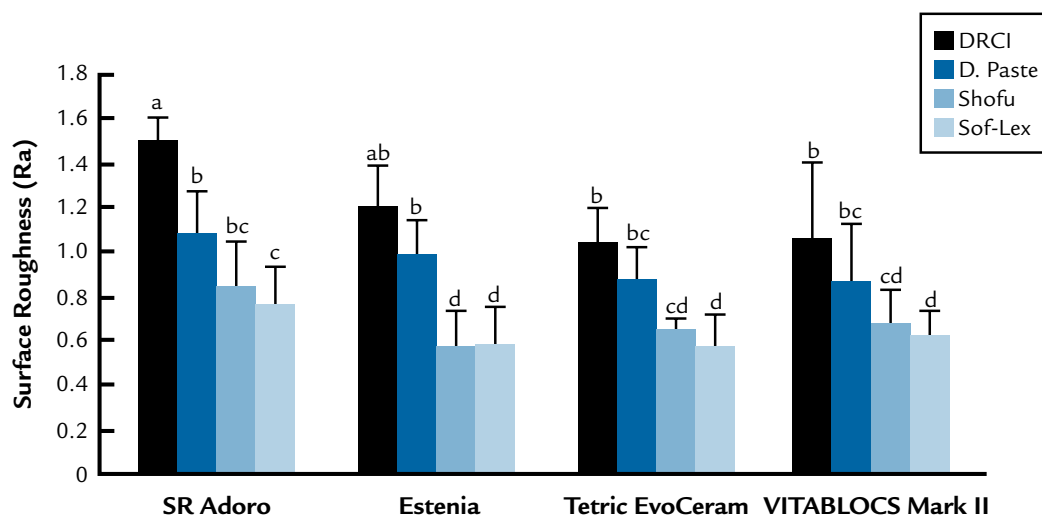
2-way analysis of variance (ANOVA), followed by Tukey Honestly Significantly Difference (HSD) tests, Pearson correlation, and regression analysis ($\alpha=.05$). Two-way ANOVA was performed to determine significant differences between surface treatments and restorative materials for surface roughness and bacterial adhesion. Pearson correlation and regression analysis were used to assess the relationship between the surface roughness and the amount of *S. mutans* adhered to the specimen surface.

RESULTS

The mean Ra values and SDs of the groups are presented in Figure 1. The results of 2-way ANOVA indicated that surface roughness values varied significantly depending on the restorative materials (SR Adoro, Estenia, Tetric EvoCeram, VITABLOCS Mark II) and surface treatments (DRCI, Sof-Lex, Shofu, diamond paste) ($P<.05$).

However, no significant interaction was found between restorative materials and surface treatments ($P=.103$) (Table II). The Tukey HSD test showed that the highest mean surface roughness value was obtained in the SR Adoro group (1.04 ± 0.3), followed by Estenia (0.83 ± 0.3), VITABLOCS Mark II (0.80 ± 0.3), and Tetric EvoCeram groups (0.78 ± 0.23), respectively. However, there were no significant differences among these 3 groups. (Fig. 1). The highest surface roughness value was obtained in the DRCI group (1.2 ± 0.3). The lowest surface roughness values were recorded for Sof-Lex (0.63 ± 0.2) and Shofu groups (0.68 ± 0.2), in all restorative material groups ($P<.05$). The diamond paste group (0.95 ± 0.2) showed higher surface roughness values than the Sof-Lex and Shofu groups ($P<.05$).

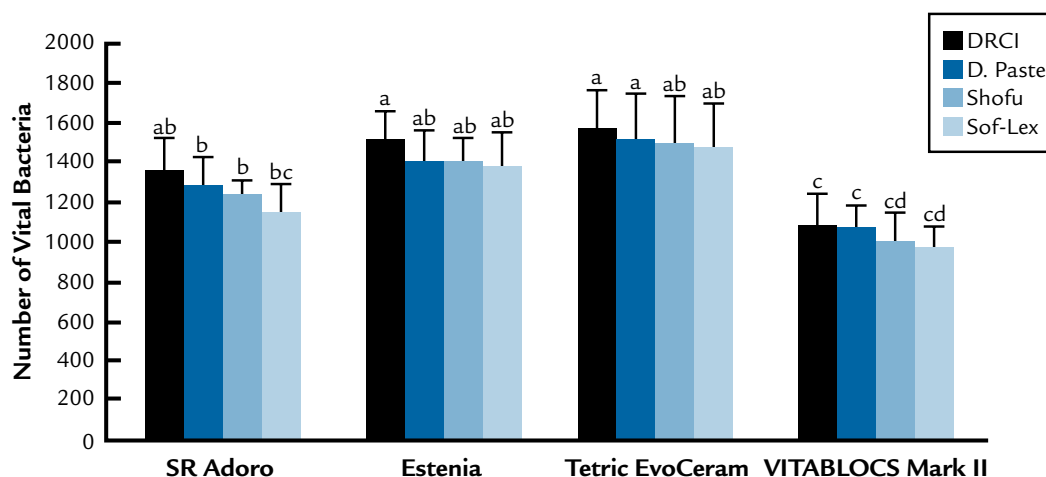
The mean and SDs of the number of vital bacteria for the groups are presented in Figure 2. The results of 2-way ANOVA indicated that the bac-



1 Mean and standard deviations of surface roughness values. Same lowercase letters indicate no significant difference at $P<.05$ using Tukey HSD.

TABLE II. Two-way ANOVA of surface roughness values

	Sum of Squares	df	Mean Square	F	P
Restorative material	1.73	3	0.57	15.82	<.001
Surface treatment	8.35	3	2.78	76.10	<.001
Restorative material \times surface treatment	0.54	9	0.06	1.66	.103



2 Mean and standard deviations of number of vital bacteria. Same lowercase letters indicate no significant difference at $P < .05$ using Tukey HSD.

TABLE III. Two-way ANOVA of bacterial adhesion values

	Sum of Squares	df	Mean Square	F	P
Restorative material	5383700	3	1794567	58.60	<.001
Surface treatment	212258	3	70753	2.31	.079
Restorative material × surface treatment	260412	9	28935	0.94	.488

terial adhesion values varied significantly, depending on the restorative materials (SR Adoro, VITABLOCS Mark II, Estenia, Tetric EvoCeram). However there were no significant differences among surface treatments (DRCI, Sof-Lex, Shofu, and diamond paste) and no significant interactions between restorative materials and surface treatments ($P = .49$) (Table III). The Tukey HSD test revealed that the lowest bacterial adhesion was observed on the VITABLOCS Mark II ceramic surface (1029.1 ± 142.7) ($P < .05$), followed by SR Adoro (1256 ± 155.7), Estenia (1418.4 ± 166.7), and Tetric EvoCeram (1512.9 ± 229.7) surfaces, respectively. There were no significant differences between Tetric EvoCeram and Estenia groups. The highest amount of bacterial adhesion was observed on the surface finished with a diamond rotary cutting instrument (1351.8 ± 282.4). Diamond paste (1314 ± 235.7), Shofu (1300.5 ± 232.3), and Sof-Lex (1250 ± 261.6)

groups followed the DRCI group. However, there were no significant differences among the groups ($P = .35$) (Fig. 2). The Pearson correlation and regression analysis showed that there was a significantly positive relationship between surface roughness and the amount of bacteria adhered to the material surface ($r = 0.594$).

DISCUSSION

Based on the results of the present study, the null hypothesis that there would be no difference in bacterial adhesion on indirect composite resin and ceramic materials when subjected to various surface treatments was rejected. A number of studies have investigated the rate or amount of plaque accumulation on various materials.^{14-17,21} These studies reported that ceramics demonstrated the least amount of plaque adhesion, irrespective of surface treatments. Similar to

previous reports, the current study showed that the amount of viable *S. mutans* (CFU) varied depending on the materials tested, and the lowest viable *S. mutans* counts occurred on ceramic (VITABLOCS Mark II) specimens, irrespective of surface treatments. The chemical composition of the surface is important for bacterial adhesion, particularly when the surface possesses components which are either beneficial or detrimental to the adhering population.⁵ Hansel et al¹⁸ reported that some monomers released from composite resin stimulated the growth of caries-associated bacteria *S. sobrinus* and *L. acidophilus*. Kawai and Takaoka¹⁹ found a higher amount of *S. sobrinus* on Tetric composite resin compared with compomers and resin-modified glass ionomer cements. Similar to the findings of these previous studies, in the present study, the composite resin Tetric EvoCeram demonstrated a higher bacterial adhesion compared

to the other restorative materials. The amount of bacteria retained on indirect composite resins (SR Adoro and Estenia) was greater than that found on the ceramic material (VITABLOCS Mark II). Although these materials have enhanced mechanical properties due to high ceramic filler contents and a different polymerization procedure,^{9,13} they are still composed of a polymer resin matrix. One of the indirect composite materials, Estenia, showed more vital bacterial adhesion than Tetric composite resin. However, the other indirect composite resin, SR Adoro, exhibited lower bacterial adhesion than both. The reason may be that bacterial accumulation varies according to filler size and matrix monomer.^{34,35} Estenia contains approximately 92 wt% of filler particles and a urethane monomer-based resin matrix.⁹ As purported by the manufacturer, SR Adoro contains approximately 65 wt% of filler particles and a newly developed aromatic-aliphatic urethane dimethacrylate that replaces the bis-GMA and TEGDMA. In contrast to bis-GMA and TEGDMA, this monomer does not consist of a hydroxyl group and, therefore, allows the development of a composite resin that is less susceptible to water absorption and solubility, as purported by the manufacturer.

Chairside polishing of the restoration is important to obtain an esthetically pleasing appearance and to prevent a roughened surface from abrading an opposing tooth. Another reason for polishing may be that less plaque can accumulate on a smooth surface.²¹ Several authors have investigated and described different polishing techniques and supported the use of polishing as an alternative for glazing.^{23,24,31,32} The results of the current study demonstrate that smoother surfaces were obtained with Shofu and Sof-Lex polishing systems, compared with surfaces polished with a felt wheel and diamond paste. This finding is in agreement with several previous reports recommending the use of Shofu^{25,26,29,31} and Sof-Lex pol-

ishing kits for smoothing roughness created by a diamond rotary cutting instrument.^{9,23,27} SR Adoro indirect composite resin showed the roughest surface compared with the other materials, followed by Estenia indirect composite resin. These materials contain higher amounts of inorganic fillers, predominantly consisting of ceramic, silicon dioxide, or glass (Table I). The polishing of filled resins has been found to cause a higher fracture of inorganic components in the surface layer.³³

Another finding of the present study is that the amount of viable *S. mutans* was correlated with surface roughness. Although there was no statistical difference among groups, the highest bacterial adhesion was observed on the surface finished with diamond rotary cutting instruments. Diamond paste, Shofu, and Sof-Lex groups had less bacterial adhesion than the DRCl group, respectively. This finding supports results of previous studies that found increased dental plaque formation on rough surfaces.^{21,22,33} However, some studies have not found a correlation between bacterial adhesion and surface roughness.^{3,14}

The adhesion of a salivary pellicle layer on the tooth surface is the initial step for oral bacterial colonization. Oral bacteria adhere to the receptors of host origin in the salivary pellicle.⁹ In the present study, test specimens were coated with artificial saliva and mucin to simulate the oral environment. A limitation of this in vitro study is that only a single type of bacteria was used. Therefore, the study conditions do not fully reflect the range of oral microbial flora. The surface roughness and amount of bacterial adhesion were evaluated quantitatively. Furthermore, morphological observations with a confocal laser scanning microscope could not identify the bacteria adhered to the specimen surface. Therefore, further studies are needed to clarify the qualitative differences in surface roughness and bacterial adhesion on the material surfaces.

CONCLUSIONS

Within the limitations of this in vitro study, the following conclusions were drawn:

1. Surface roughness varied depending on surface treatment and composition of the restorative materials tested. Sof-Lex and Shofu polishing systems produced the smoothest surfaces. Indirect composite materials with a relatively high proportion of inorganic fillers showed the highest surface roughness.

2. After 24 hours of bacterial exposure, the amount of adhered *S. mutans* varied depending on surface treatments and chemical composition of the restorative materials examined. The lowest bacterial adhesion was found with Sof-Lex and Shofu polishing kits. Maximum vital bacterial adhesion was found on surfaces finished with the diamond rotary cutting instrument. Ceramic material (VITABLOCS Mark II) and indirect composite resin material (SR Adoro) showed lower bacterial adhesion than Estenia and Tetric EvoCeram.

3. A significant and positive correlation was found between surface roughness and vital bacterial adhesion.

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