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Effect of different polishing techniques for composite resin materials on surface properties and bacterial biofilm formation

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ABSTRACT

Objectives: Both direct and indirect techniques are used for composite resin material (CRM) restorations. Polishing processes are needed in both techniques after intraoral adjustment. However, it is unclear as to which polishing technique should be preferred with respect to decreasing biofilm. The purpose of this*in vitro* study was to evaluate the surface properties and *Streptococcus mutans* biofilm formation on direct and indirect CRMs after using different polishing techniques.

Methods: Two CRMs (direct and indirect) and four polishing techniques (aluminium oxide discs, diamond polishing paste, aluminium oxide polishing paste, and silicon carbide brush) were evaluated. The specimens were prepared for taking scanning electron microscopy images ($n = 2$) and determining surface roughness, surface free energy, and bacterial biofilm formation (BBF) with colony-forming unit counting and confocal laser scanning microscopy assays ($n = 7$). The data were analysed using two-way analysis of variance with Bonferroni as a post hoc test and Pearson's correlation ($p < .05$).

Results: The surface roughness values in the control group were higher than those in the diamond polishing paste group ($p = 0.025$), but the values in the aluminium oxide polishing paste and silicon carbide brush groups were comparable with those in the control group ($p = 0.156$ and $p = 1.000$, respectively). The highest surface free energy values were recorded in the silicon carbide brush group ($p < 0.001$), whereas there were no differences found among the other groups ($p > 0.05$). The highest BBF was seen in the silicon carbide brush ($p < 0.001$) and direct CRM ($p < 0.001$) groups.

Conclusion: BBF on the surface of direct CRMs differed from that on indirect CRMs after polishing the surface. The tested polishing techniques significantly influenced surface properties and BBF.

Clinical significance: In situations that require the intraoral adjustment of CRMs, polishing with a diamond polishing paste seems to be a good option to polish the surface of both direct and indirect CRMs because the diamond polishing paste results better in terms of decreasing biofilm formation and improving surface properties.

1. Introduction

The use of composite resin materials (CRMs) in restorative dentistry has increased considerably over the last few decades because of improvements in their physical and mechanical properties [1]. These materials are a good alternative to metallic restorative materials and have been developed to be used both directly and indirectly. In the direct technique, a CRM is placed directly into the prepared cavity. This technique has some advantages such as a single treatment appointment and relatively low costs. However, the indirect technique is superior in terms of polymerization shrinkage and wear resistance when compared with the direct technique, because the polymerization of indirect CRMs is performed outside of the mouth, with exposure to light, heat, and pressure [2,3].

Studies have shown that the surface of CRMs is susceptible to bacterial adhesion, which is one of the stages of biofilm formation in the marginal areas of restorations. Especially, the adhesion of *Streptococcus mutans* plays a role in cariogenic biofilm formation [4–6]. The cariogenic biofilm formation is an important aetiological issue in secondary caries, which is one of the main reasons for the failure of CRM restorations [7]. Changes in surface roughness [8–11] and surface free energy [12,13], which are two of the surface properties of CRMs, may

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influence the failure of CRMs caused by biofilm formation. Some of the studies on CRMs reported that a decrease in surface roughness also caused a decrease in bacterial adhesion [8–11]; in other studies, it was emphasized that there is no relationship between bacterial adhesion and surface roughness [14,15]. Additionally, the surface of CRMs with high surface free energy values is known to increase bacterial adhesion [12,13]. Improving the surface properties of CRMs can contribute to the success of restorations by preventing biofilm formation.

The surface of CRMs may require intraoral adjustments after its placement in the mouth because of reasons such as removing excess cement [6], improving occlusion, or reducing over-contour [16]. After such adjustments, various finishing and polishing techniques are used to improve the surface properties of CRMs [6,16]. The finishing technique helps to obtain the desired anatomical contour, whereas the polishing technique allows to create a smooth surface by decreasing the scratches produced by the finishing technique [17]. Aluminium oxide discs can used in both finishing and polishing of CRM restorations; however, the shape of these instruments is inefficient for polishing of occlusal surfaces. Silicon carbide brushes and wheels with various polishing pastes, such as diamond and aluminium oxide, are commonly used for better polishing [6,16,18,19].

The purpose of this *in vitro* study was to analyse *S. mutans* biofilm formation on the surface of direct and indirect CRMs after different intraoral polishing techniques. The null hypotheses tested were that (1) there would be no difference in the surface properties of direct and indirect CRMs when subjected to different polishing techniques and (2) there would be no difference in *S. mutans* biofilm formation on the surface of direct and indirect CRMs when subjected to different polishing techniques.

2. Materials and methods

2.1. Specimen preparation

The two CRMs used in the current study are provided in Table 1 (GRADIA; GC Corporation, Tokyo, Japan). A total of 240 specimens were prepared for measuring surface roughness and surface free energy, determining bacterial adhesion and biofilm formation ($n = 7$), and taking scanning electron microscopy (SEM) images of biofilm formation on the surface of CRMs ($n = 2$). The disc specimens were prepared by packing of uncured CRMs into custom-made polytetrafluoroethylene moulds, with a diameter of 8 mm and thickness of 2 mm. Then, the CRMs were covered with Mylar strips on the top and bottom surfaces of the mould and pressed between two glass slides. The direct CRMs were cured for 20 s on each side with a light-curing unit (VALO; Ultradent, South Jordan, UT, USA) in direct contact with the Mylar strips. The light-curing unit had a power density of more than 1000 mW/cm^2 verified by a light-emitting diode (LED) radiometer (Demetron LED Radiometer 910726; Kerr Corporation, Middleton, WI, USA). The indirect CRMs were polymerized for 3 min with a light-curing unit (Labolight DUO; GC Corporation, Leuven, Belgium). The prepared specimens were subjected to a standardized polishing protocol with aluminium oxide discs (OptiDisc; KerrHawe, Karlsruhe, Germany) from extra-coarse to extra-fine grain size according to the manufacturer's instructions. Then the specimens were randomly divided into four groups according to the polishing techniques which were performed:

1 *Control (C):* The surface remained unaltered after the polishing protocol with aluminium oxide discs, and no polishing techniques were applied.
2 *Diamond polishing paste (DP):* The specimens were polished using a polishing brush (Hawe miniature cleaning and polishing brushes; KerrHawe, Bioggio, Switzerland) with a DP (GRADIA DiaPolisher; GC Corporation, Tokyo, Japan).
3 *Aluminium oxide polishing paste (AOP):* The specimens were polished using a polishing cup (Enhance Polishing System, Prisma Gloss; Dentsply, Konstanz, Germany) with Prisma Gloss polishing paste and Prisma Gloss extra-fine polishing paste according to the manufacturer's instructions.
4 *Silicon carbide brush (SCB):* The specimens were polished with fibre bristles impregnated with silicon carbide particles (Occlubrush; Kerr Hawe, Bioggio, Switzerland).

All finishing and polishing techniques were achieved dry with a low-speed handpiece at 10,000 rpm for 10 s in a slight, uniform, intermittent pressure and one direction by a single operator. A new polishing material was used for each specimen and then discarded after each use. The specimens were cleaned with distilled water and air-dried before starting the next finishing or polishing technique [19].

2.2. Surface roughness

The surface roughness of the specimens was measured using a profilometer (Surtronic S128; Taylor Hobson, Leicester, UK), with a tracing length of 5.6 mm and cut-off value of 0.8 mm. A reading was obtained by a diamond stylus moving at 0.5 mm/s, and arithmetic roughness (R_a) was recorded. This technique was repeated for four positions on the same specimen, and three readings for each position were obtained for each specimen [20]. Measurement data were facilitated by pooling, and the mean value (μm) was calculated.

2.3. Surface free energy

The surface free energy of the specimens was calculated from contact angle measurements, using the sessile drop method [21]. The contact angle values of the specimens were measured using Theta Optical Tensiometer (KSV Instruments, Helsinki, Finland), equipped with an automated droplet dispenser, high-speed (60 fps) digital camera, and image analysis software (One Attension; Biolin Scientific, Stockholm, Sweden). One dispersive (diiodomethane) and two polar (ultrapure

Table 1
Details of the materials tested in the study.

Composite Resin Materials	Fillers	Monomers
Gradia direct, shade A2 (Micro-hybrid composite)*	In total: 73 wt%, 64-65 vol% Silica: 38 wt %, 22 vol %, (850 nm) prepolymerized filler- 35 wt%, 42 vol%	UDMA (Urethane dimethacrylate) Dimethacrylate co-monomers
Gradia Plus indirect, HB/EL (Nano-hybrid composite)**	In total: 80 wt% Silica Nano filler (16–40 nm) Barium Glass Filler (300 nm) HPC Filler (10 μm)	na

na = no further/detailed information from the manufacturer available.
* [36].
** Data obtained from the manufacturers.

Table 2
Mean ± Standard Deviation (SD) for Surface Roughness (μm), Surface Free Energy (mJ m^{-2}), Colony forming unit (x10^5 ml/Cfu), Biomass ($\mu m^3/\mu m^2$) and the number of vital cells in biomass ($\mu m^3/\mu m^2$).

Groups	Surface Roughness	Surface Free Energy	Colony forming unit	Total biomass	Viable biomass
C	0,68 ± 0,22 ^{A}	34,15 ± 1,51^{a}	110,71 ± 56,90 ^{i}	101,32 ± 30,7^{+}	59,64 ± 17,53
DP	0,56 ± 0,05 ^{BC}	35,73 ± 6,89 ^{a}	299,5 ± 100,44 ^{ii}	106,42 ± 28,8^{+}	59,86 ± 15,96
AOP	0,58 ± 0,09 ^{AC}	39,87 ± 1,61 ^{a}	142,07 ± 81,59 ^{i}	113,5 ± 36,94^{+}	61,4 ± 18,62
SCB	0,66 ± 0,08 ^{AC}	48,96 ± 8,8 ^{b}	474,85 ± 131,3^{iii}	149,35 ± 35,56^{-}	74,32 ± 22,41

Different symbols in same column mean statistical difference. (Capital Latin letters for the surface roughness; lower Latin letters for the surface free energy; Roman numerals for the colony forming unit; mathematical symbols for total biomass). C: Control, DP: Diamond polishing paste, AOP: Aluminum polishing paste, SCB: Silicon carbide brush.

water and formamide) liquids were used. In total, two drops of each liquid (2.5 μl) were examined on 16 specimens for each group at room temperature. The images of the drops were recorded automatically during 60 s. The contact angle measurements were performed at room temperature (25 ± 3 °C), and the mean values were used to calculate the components of surface free energy. The value obtained from the sum of the dispersive and polar components was expressed as the total surface free energy [22].

2.4. Biofilm assays

2.4.1. Biofilm formation

The disc specimens were packed separately and then sterilized in an autoclave at 121°C for 15 min before being tested with bacteria. Using aseptic culture techniques, the test was performed on sterilized 24-well plates, and each sterilized disc was covered with 500 μl sterile human saliva prepared according to Baffone et al. [23] and incubated by shaking at 37 °C for 1 h to stimulate pellicle formation. The pellicle-coated discs were then rinsed with 2 ml phosphate-buffered saline and transferred to new sterilized 24-well plates. The discs were covered with 1.6 ml brain heart infusion (BHI) broth supplemented with 5% saccharose and were inoculated with 200 μl bacterial suspension of *S. mutans* ATCC 25175 (final concentration of 1.5×10^8 colony-forming unit (CFU)/ml). The plates were incubated anaerobically at 37 °C for 24 h. Following incubation, the discs were gently dip-washed three times in physiological saline to remove the loose bacteria.

2.4.2. Analysis by CFU counting

The specimens for each group were transferred into a sterile tube containing 1 ml physiological saline and then were vortexed for 1 min to harvest the adherent bacteria. The suspensions were sonicated at 30 W for 5 s to disrupt bacterial aggregates and were then 10-fold serially diluted in sterile physiological saline and plated onto a BHI agar. The plates were incubated anaerobically for 48 h at 37 °C, and the numbers of CFUs were then determined.

2.4.3. Analysis by confocal laser scanning microscopy

The specimens for each group were transferred to new sterilized 24-well plates, stained using the LIVE/DEAD BacLight Bacterial Viability and Counting Kit (Invitrogen, Merelbeke, Belgium), and allowed to sit for 15 min under light protection. Three fields randomly selected on each of the specimens were analysed by confocal laser scanning microscopy analysis (CLSM; Leica Lasertechnik, Heidelberg, Germany). Excitation wavelengths of 488 and 532 nm were used, and the specimens were observed using optical lenses with magnifications of 10 × / 1.0. The COMSTAT software was used to quantify the total and viable biomass ($\mu m^3/\mu m^2$) properties [24].

2.5. Analysis by SEM

To observe the biofilm on the surface of the specimens by SEM, two specimens of each group were fixed for 1 h in 2.5% glutaraldehyde and then dehydrated in several ethanol washes (10%, 25%, 50%, 75%, and 90% for 20 min and 100% for 1 h) and dried overnight in a bacteriological incubator at 37 °C. The specimens were coated with gold and observed carefully under an SEM (Zeiss EVO LS 10; Carl Zeiss SMT, Cambridge, UK) at 20 kV, with magnifications of 500 ×, 1000 ×, and 2000 ×. The representative micrographs of the biofilm on the surface of the specimens were recorded and their descriptive analysis was performed.

2.6. Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (v.15.0; SPSS, Chicago, IL, USA) and presented as means ± standard deviations (SDs). Two-way analysis of variance (ANOVA) with Bonferroni as a post hoc test was performed to determine significant differences between polishing techniques and CRMs for surface properties and biofilm formation. Pearson's correlation analysis was used to assess the relationship between the parameters ($p < .05$).

3. Results

3.1. Surface roughness

Table 2 summarizes the surface roughness results of the specimens. Two-way ANOVA revealed that R_a values were influenced by the polishing techniques ($F = 4.206$, $p = 0.01$). The DP group showed significantly lower R_a values when compared to the C group ($p = 0.025$), but the R_a values in the AOP and SCB groups were comparable with those in the C group ($p = 0.156$ and $p = 1.000$, respectively). Considering CRMs, no significant effect ($F = 2.639$, $p = 0.111$) on surface roughness could be found. No significant interaction between polishing techniques and CRMs was found ($F = 1.017$, $p = 0.393$). Pearson's correlation showed that there was no positive correlation between surface roughness and other parameters (surface free energy, $r = 0.178$; total biomass, $r = -0.082$; viable biomass, $r = -0.124$; CFU, $r = -0.002$).

3.2. Surface free energy

The mean surface free energy values and SDs of the groups are presented in Table 2. The surface free energy values were influenced by the polishing techniques ($F = 19.110$, $p < 0.001$). The surface free energy values in the SCB group were higher than those in the C ($p < 0.001$), AOP ($p = 0.002$), and DP ($p < 0.001$) groups. No significant differences were found among the other groups ($p > 0.05$). Considering CRMs, no significant effect on surface free energy could be found ($F = 0.093$, $p = 0.763$). There was a significant interaction between the polishing techniques and CRMs ($F = 8403$, $p = 0.001$). There was a positive correlation between surface free energy values and biofilm analysis (total biomass, $r = 0.575$; viable biomass, $r = 0.527$; CFU, $r = 0.548$).

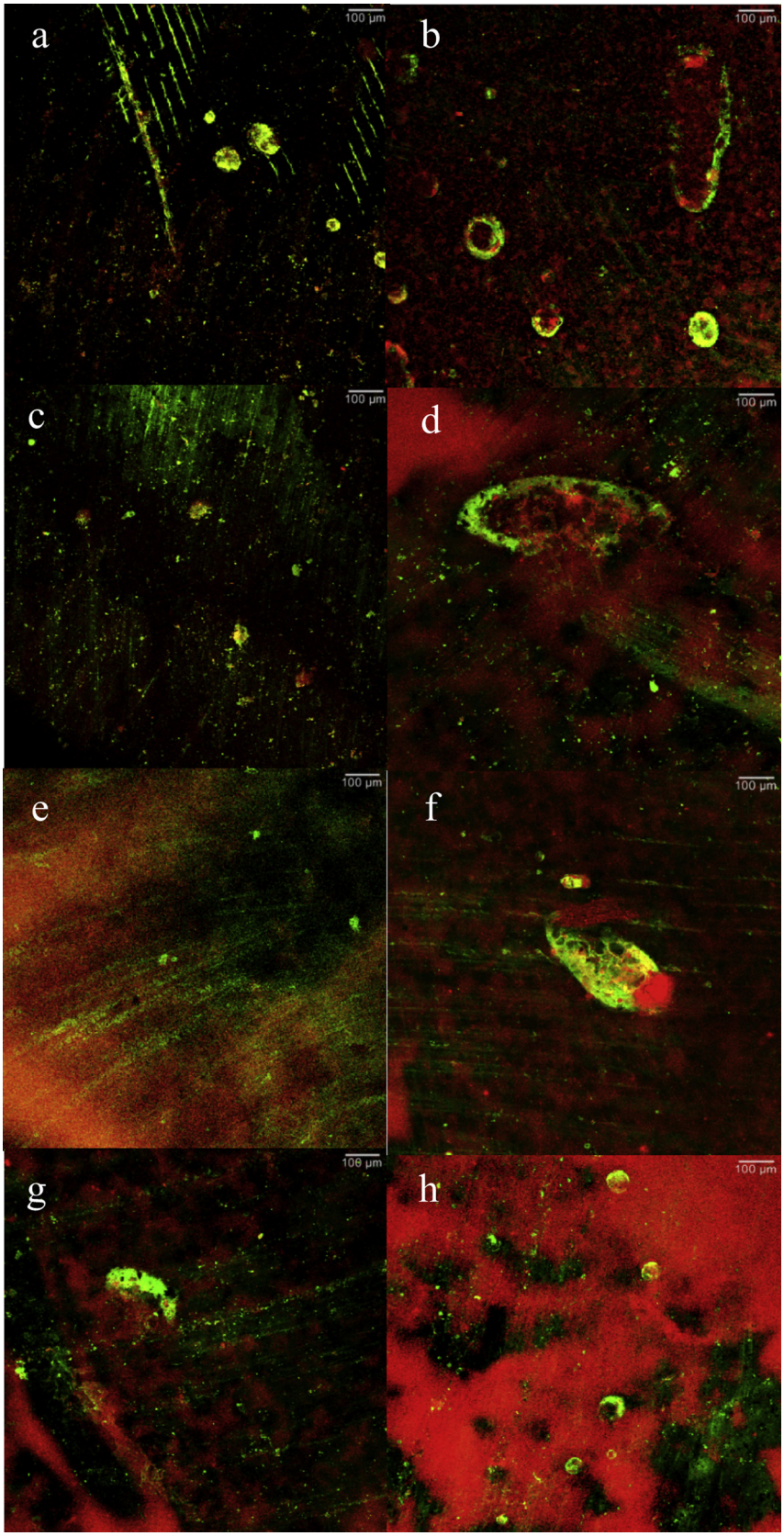

Fig. 1. Representative confocal laser scanning microscopy analysis (CLSM) images (4 ×) of the biofilm formed on the specimens in direct and indirect composite resin materials (CRMs) of the control (a,b), diamond polishing paste (c,d), aluminium oxide polishing paste (e,f), and silicon carbide brush (g,h) groups. The green colour indicates live *Streptococcus mutans* cells. The red colour defines non-viable *S. mutans* cells.

3.3. Biofilm assays

3.3.1. Analysis by CFU counting

Table 2 provides the values of mean and SDs for CFU/ml. The CFU values were influenced by the polishing techniques ($F = 42.488$, $p < 0.001$). The highest CFU value was measured in the SCB group ($p < 0.001$). No significant differences were found between the AOP and C groups ($p = 1.000$). Considering CRMs, no significant effect ($F = 0.76$, $p = 0.388$) on the CFU values could be found. There was no significant interaction between the polishing techniques and CRMs ($F = 1.263$, $p = 0.298$). There was a positive correlation between the values measured with CFU and CLSM (total biomass, $r = 0.392$; viable biomass, $r = 0.290$).

3.3.2. Analysis by CLSM

The representative CLSM images of the *S. mutans* biofilm formation on the surface of the specimens are shown in Fig. 1(a–h). The green colour in the images refers to live bacteria and the red colour to non-viable bacteria. In a descriptive analysis, the balance between live and dead *S. mutans* cells is observed for all groups. Considering CRMs, the biofilm in direct CRMs with higher biofilm formation values seemed to present a greater amount of painted area. The same relation could also be seen between the polishing groups. In the SCB group, the biofilm seemed to cover the surface of CRMs, but the groups with lower quantitative biofilm formation values (C, DP, and AOP) appeared to present a less painted area.

3.3.2.1. Total biomass. The means and SDs of the total biomass data are given in Table 2. The total biomass was influenced by the polishing techniques ($F = 10.902$, $p < 0.001$). The SCB group had higher total biomass values when compared to the C ($p < 0.001$), AOP ($p = 0.002$), and DP ($p < 0.001$) groups. No significant differences were found among the other groups ($p > 0.05$). The total biomass was also influenced by CRMs ($F = 52.580$, $p < 0.001$). The total biomass value in direct CRMs (135.11 ± 32.08 $\mu m^3/\mu m^2$) was higher than that in indirect CRMs (93 ± 25.42 $\mu m^3/\mu m^2$; $p < 0.001$). No significant interaction between the polishing techniques and CRMs was found ($F = 0.259$, $p = 0.855$). There was a positive correlation between the total and viable biomass ($r = 0.912$).

3.3.2.2. Viable biomass. The results of the viable biomass analysis are presented in Table 2. Viable biomass was not influenced by the polishing techniques ($F = 2.593$, $p = 0.06$), but there was an interaction between CRMs ($F = 22.997$, $p < 0.001$). Direct CRMs showed a higher viable biomass value (70.71 ± 117.50 $\mu m^3/\mu m^2$) than did the indirect ones (51.69 ± 15.48 $\mu m^3/\mu m^2$, $p < 0.001$). No significant interaction between the polishing techniques and CRMs was found ($F = 1.49$, $p = 0.230$).

3.4. Analysis by SEM

The representative SEM images of the *S. mutans* biofilm formation on the surface of the specimens are shown in Fig. 2(a–h). After 24 h of *in vitro* biofilm formation, the presence of colonies and isolated *S. mutans* was noted in all groups, but biofilm formation time (24 h) was not sufficient to coat the surface entirely with *S. mutans* colonies. In a descriptive analysis, according to the polishing techniques, large adherent aggregates were observed on the SCB group, which had the highest quantity of bacteria on its surface (Fig. 2g and h), whereas isolated streptococci and their small aggregates were found on the C, DP, and AOP groups, with lower biofilm formation values (Fig. 2a and f). As regards the CRMs, biofilm formation seemed to be higher on direct CRMs when compared to indirect CRMs (Fig. 2a–d).

4. Discussion

On the basis of the results of the current study, the first null hypothesis that there would be no difference in the surface properties of direct and indirect CRMs when subjected to different polishing techniques was not rejected. However, the second null hypothesis that there would be no difference in the *S. mutans* biofilm formation on the surface of direct and indirect CRMs when subjected to different polishing techniques was partially rejected.

In the current study, the same brand was intended for direct and indirect use. Two different types of CRMs were evaluated in terms of surface properties after using different polishing materials. A polishing technique reproduced from a standard clinical protocol using four silicon carbide papers with steadily decreasing grain size from 80 to 10 μm was used to prepare the surface of standardized CRMs [13]. In addition, the control group was not taken additional polishing treatment, whereas the experimental groups were treated with polishing materials with a particle size of less than 10 μm: DP, 1 μm grain size; AOP, 1.0–0.3 μm grain size; and SCB, 5 μm grain size. The results of the current study demonstrate that the additional polishing treatment on the surface of CRMs helped reduce surface roughness, irrespective of the type of the CRM, but statistically significant differences were obtained only for the DP group (Table 2). The hardness of particles in a polishing material should be enough for a homogeneous reduction in both the resin matrix and the filler particles of CRMs during polishing [25,26]. The diamond particles may create smoother surfaces of CRMs compared to aluminium particles, thanks to their hardness [25]. In addition, the surface roughness values in the DP and AOP groups were lower than the baseline value of the enamel-to-enamel surface roughness in occlusal contact areas ($R_a = 0.64$ μm) [27]. Taking this into account, both polishing materials can be considered clinically acceptable. On the contrary, the SCB group had the highest surface free energy values among all groups in the current study, irrespective of the type of the CRM (Table 2). The polishing technique may indirectly affect surface free energy by modifying the chemical composition of the surface of CRMs [13]. In the current study, the silicon carbide abrasive particles may be creating more modification in the chemical composition of CRMs compared to other polishing techniques.

According to the manufacturer's data, the direct CRMs used in the current study contained microhybrid fillers, whereas the indirect ones had nanohybrid fillers (Table 1). The nanohybrid CRMs exhibited similar surface features to microhybrids after being subjected to different polishing techniques. In the literature, there are studies suggesting that the surface properties of CRMs were not dependent on the size of filler particles [19,28,29]. The similarity of surface features between nano- and microhybrid CRMs may be related to the similarity in the composition of these materials. The exact content of these materials is generally proprietary and inaccessible. For this reason, the conclusions concerning the relationship between the evaluated parameters and the content of CRMs may remain speculative.

To perform quantitative and qualitative measurements of bacterial biofilm formation, CFU counting, SEM, and CLSM after staining of the bacteria can be used [5,6,8,30,31]. However, these techniques have certain limitations and disadvantages. In CFU counting, there is a series of stages on agar plates which can cause false quantitative results with respect to the structure of live, adherent bacterial aggregates [31]. In qualitative SEM, bacterial biofilm is fixed and dehydrated, which can cause alterations in the characteristics of the biofilm. Also, the evaluation of a selected area on the substrate may not be impartial in this analysis [7,32]. Using CLSM after staining of the bacteria is a simple method for quantitative and qualitative measurement of bacterial biofilm formation without removal from their substrate [5,6,8,31]. Furthermore, dead and live bacteria can be evaluated separately depending on their staining, using CLSM. But, discrimination between adherent and loose bacteria on the surface may not be possible with CLSM. Also, as in SEM, a selected area on the substrate is evaluated with CLSM

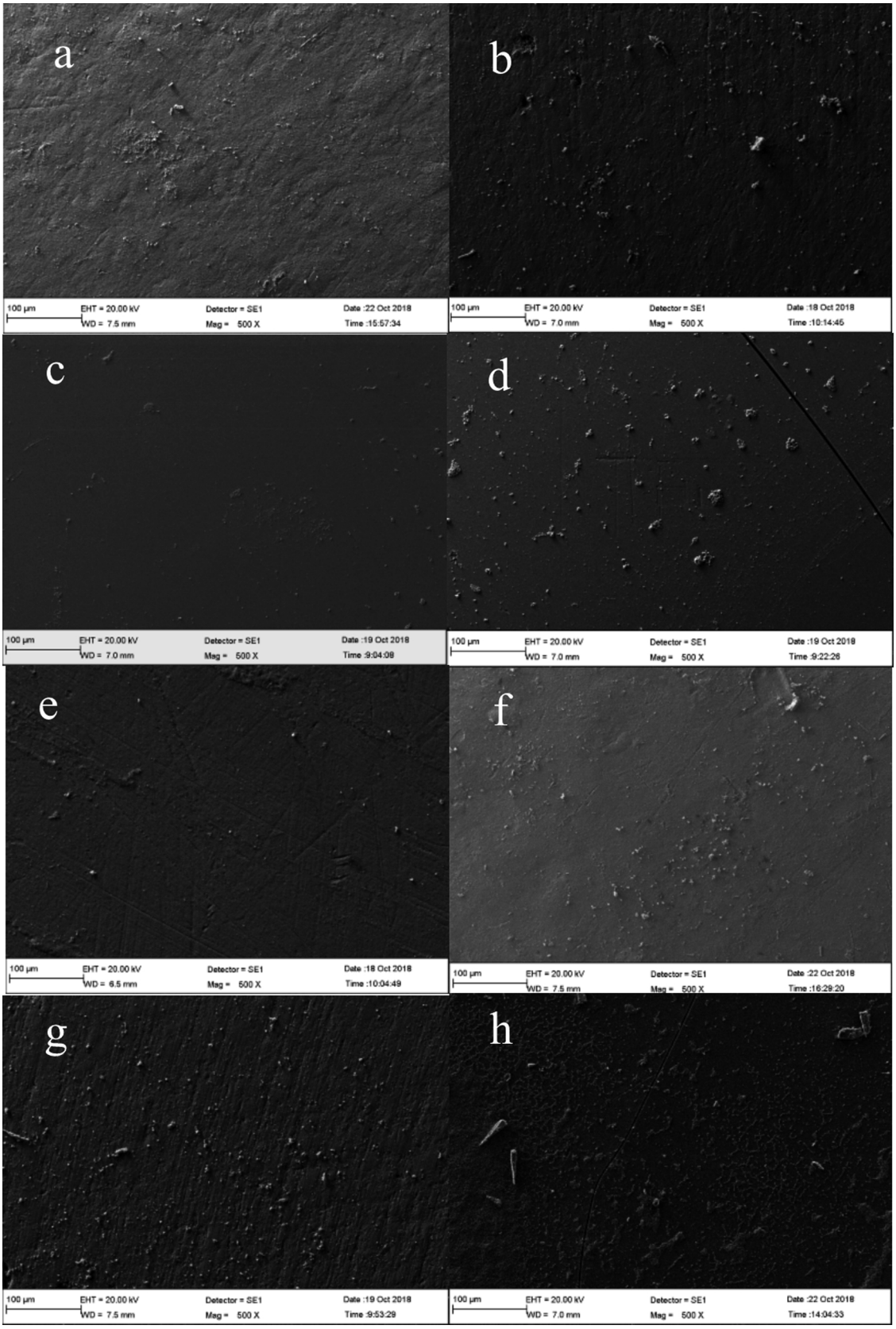

Fig. 2. Representative scanning electron microscopy (SEM) images (500×) of the biofilm formed on the specimens in direct and indirect composite resin materials (CRMs) of the control (a,b), diamond polishing paste (c,d), aluminium oxide polishing paste (e,f), and silicon carbide brush (g,h) groups.

[5,8]. Therefore, the current study evaluated bacterial biofilm formation by quantitative CLSM and CFU assay and qualitative SEM and CLSM assay.

In the current study, the specimens were evaluated in terms of 24-h bacterial biofilm formation. Irrespective of the polishing techniques, in biofilm formation with CLSM, direct CRMs formed more total and viable biomass in their surfaces when compared to indirect ones. These quantitative results were confirmed by the qualitative result of CLSM (Fig. 1) and SEM (Fig. 2) images. Although there were no differences in the CFU values of indirect and direct CRMs, a positive correlation was found in the results of both CFU and CLSM bacterial biofilm formation assays for all groups. Similarly, a previous study [30] found lower bacterial adhesion in indirect CRMs than in direct CRMs after 48-h incubation. The bacterial adhesion on CRMs depends on the chemical and physical properties of CRMs. These properties are influenced by several material factors, such as the type, size, and amount of inorganic fillers, as well as the type of the monomer [9]. In the current study, according to the manufacturer's data, direct CRMs contained microhybrid fillers, whereas indirect CRMs had nanohybrid fillers (Table 1). There are controversial results in terms of biofilm formation and filler size in the literature [9,14,18,33]. Several studies reported that microhybrid CRMs have a higher value of bacterial adhesion than do nanohybrid CRMs [9,18], but other studies showed that a difference could not be observed between the materials [14,33]. These results suggest that characteristics of the material other than filler size, such as the type of monomer and the amount of filler (by volume), may be more important for biofilm formation.

Polishing of CRMs after intraoral adjustment is important for alteration in the surface properties which affect bacterial adhesion [18]. The SCB group with the highest total surface free energy had the highest values of bacterial adhesion and biofilm formation in the current study (Table 2; Figs. 1 and 2). These results are consistent with the results of other studies, indicating that CRMs with high surface free energy values enhance the adhesion of *S. mutans* [12,13]. However, a positive correlation between *S. mutans* biofilm measurements and the surface roughness values was not found with different polishing techniques used in the current study. Although several studies present that adhering more bacteria on rougher surfaces of CRMs [8–11], surface roughness is more important for early stages of biofilm formation and least affects the amount of the biofilm formed in the last stages [14,34]. The results of this study which evaluated the 24-h bacterial biofilm formation agree with the studies that observed no relationship between the surface roughness values and *S. mutans* biofilm formation in polished CRMs after 24 [14] and 20 h [15]. In addition, the surface roughness values were not correlated to the surface free energy values. Nevertheless, the interpretation of the relationship between surface roughness and surface free energy parameters is complicated by the heterogeneous nature of CRMs, i.e., hard filler particles embedded in a relatively soft matrix [12,21].

Clinically, in situations that require intraoral adjustment of direct and indirect CRMs, polishing with a DP seems to be a good option to polish the surface of direct and indirect CRMs because DP results better in terms of decreasing biofilm formation and improving surface properties. However, the current study had a number of limitations: First, only one type of indirect and one type of direct CRMs were evaluated instead of all CRMs. The results cannot be applied to other CRMs with different structural characteristics. Second, although saliva-coated specimens were evaluated to simulate the oral environment, only one type of bacteria and static technique were used for biofilm measurement. Therefore, the study conditions do not fully reflect the mimetic intraoral conditions. Third, the correlation between biofilm measurement and chemical surface characteristics of CRMs, which give information about the amount of resin and filler on the surface [13], was not evaluated after polishing. Fourth, the releasing residual monomer from CRMs, which may affect the biofilm formation values [35], was not measured after polymerization. Therefore, future studies should focus on the diversion of the monomer and chemical surface characterization of different CRMs after polymerization and polishing; also, the relationship between these parameters and biofilm measurement should be obtained with a multispecies biofilm and dynamic artificial mouth system.

5. Conclusion

Within the limitations of the current study, the following can be concluded:

1

1 There was no difference in the surface properties of direct and indirect CRMs when subjected to different polishing techniques.
2 Significantly lower biofilm formation was found in the indirect CRM groups than in the direct CRM group regardless of the polishing techniques tested.
3 Surface roughness varied depending on the polishing techniques tested. The DP group produced the smoothest surfaces. No positive correlation could be established between surface roughness and other parameters.
4 Biofilm formation and surface free energy varied depending on the polishing techniques tested. The highest biofilm formation and surface free energy were found with the SCB group.

Declaration of Competing Interest

There are no conflicts of interest.

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