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Bacterial colonization of resin composite cements: influence of material composition and surface roughness

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So-called secondary caries may develop in the cement gap between the tooth and the bonded restoration. Cement materials with a low susceptibility to biofilm formation are therefore desirable. In the present study, the adhesion of *Strepococcus mutans* onto three adhesive (Multilink Automix, RelyX Ultimate, and Panavia V5) and three self-adhesive (Multilink Speed Cem, RelyX Unicem 2 Automix, and Panavia SA plus) resin composite cements was evaluated. Previous studies have failed to evaluate concomitantly the effect of both the composition of the cements and their surface roughness on biofilm formation. The presence of *S. mutans* on cement surfaces with differing degrees of roughness was therefore recorded using fluorescence microscopy and crystal violet staining, and the composition of the cements was analyzed using energy-dispersive X-ray spectroscopy mapping. Biofilm formation on resin composite cements was found to be higher on rougher surfaces, implying that adequate polishing of the cement gap is essential. The use of copper-containing cements (Multilink Automix, Panavia V5, and Panavia SA plus) significantly reduced biofilm formation.

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The use of esthetic ceramic materials in dentistry requires the application of resin-based luting cement to bond a restoration to the tooth structure. In comparison with conventional cements, resin composite cements provide improved esthetics, lower margin wear, and higher mechanical strength (1–4). Failures of indirect ceramic restorations are mainly related to core fractures (5) or secondary caries (6).

Resin composite cements consist of three components: a polymer matrix; organic and ceramic fillers; and silanes that connect the organic and inorganic phases (7, 8). These single components and their respective microstructure define the properties of the resin composite cement, such as elasticity, hardness, strength, and thermal as well as chemical stability (8, 9).

Bacteria may colonize all soft and hard oral tissues and form heterogenic well-established communities, commonly called biofilms. Biofilm can be defined as a sessile community of bacteria irreversibly attached to a substratum embedded in an extracellular polysaccharide matrix that they have produced (10, 11). Once a protein pellicle is formed, reversible adhesion, involving weak, long-range physiochemical interactions between the bacterial cell surface and the pellicle, is created, which can lead to a stronger attachment mediated by the adhesion receptor (12, 13).

Streptococcus mutans in the biofilm is often considered as the main etiological factor for dental caries (14–17). As a result of its acidogenic and aciduric properties, *S. mutans* is better able than other species to survive in caries lesions (14). The etiology of secondary caries is similar to that of primary caries, involving biofilms of the same cariogenic microorganisms. When secondary caries develops it mainly affects the gingival margins of restored teeth and this can be ascribed to patients' poor hygiene in the area (18, 19) rather than to microleakage.

An increased surface roughness at the tooth–restoration interface, mostly caused by excess cement, results in greater accumulation of biofilm in this area and is therefore associated with a higher incidence of secondary caries (20–24). A surface roughness (R_a) of <0.2 μm is desirable for dental materials because for surfaces with an R_a of <0.2 μm, plaque accumulation is significantly reduced (25).

The extensive plaque formation that may occur at the cement gap underlines the need for cement materials with low susceptibility to biofilm formation.

Therefore, the aim of the present study was to assess the adhesion of *S. mutans* to different resin composite cements and to assess the effect of the surface roughness of the cements and of their composition on the bacterial adhesion. Although the formation of oral biofilm is a very complex process involving different bacteria, only one species of bacteria was used to assess the effect of material composition and roughness, in order to eliminate the potential impact of bacterial interactions.

Our hypotheses were that more biofilm is formed on cement surfaces with higher roughness and that all the cements tested enable similar levels of biofilm formation under identical conditions.

Material and methods

The formation of biofilm by *S. mutans* on three adhesive (Multilink Automix, RelyX Ultimate, and Panavia V5) and three self-adhesive (Multilink Speed Cem, RelyX Unicem 2 Automix, and Panavia SA plus) resin composite cements (Table 1) was quantified by crystal violet (CV) staining (by measuring absorbance at 595 nm). The cement surfaces were wet-polished with silicon carbide paper (grit 180, 400, or 2400) to produce three different levels of roughness for each cement, and the presence of bacteria on the cement surfaces was detected using fluorescence microscopy. Moreover, cement compositions were analyzed using energy-dispersive X-ray spectroscopy (EDX) in a scanning electron microscope.

Microorganism

Streptococcus mutans (ATCC 20523; American Type Culture Collection, Manassas, VA, USA) was used throughout the study. A 100 μl inoculum of *S. mutans* in skim milk solution (stored at −20°C) was spread on Columbia blood agar (BBL, Becton Dickinson, Allschwil, Switzerland) and incubated aerobically at 37°C for 72 h. Thereafter, one colony was picked and suspended in 32 ml of Todd Hewitt broth (BBL, Becton Dickinson) supplemented with 0.5% sucrose and incubated aerobically at 37°C for 22 h. Then, the culture was ultrasonicated for 30 s (30 W, Vibracell; Sonics & Materials, Newtown, CT, USA), centrifuged at 5896 ***g*** for 5 min, and resuspended in simulated body fluid (SBF), consisting of 7.996 g of sodium chloride (NaCl), 0.35 g of sodium bicarbonate ($NaHCO_3$), 0.224 g of potassium chloride (KCl), 0.228 g of potassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$), 0.305 g of magnesium chloride hexahydrate ($MgCl_2.6H_2O$), 0.278 g of calcium chloride ($CaCl_2$), 0.071 g of sodium sulphate (Na_2SO_4), and 6.057 g of tris (hydroxmethyl)aminomethane [$(CH_2OH)_3CNH_2$], dissolved in 1 l of ultrapure water and pH-adjusted to pH 7.25 with 1 mol/l of hydrochloric acid (HCl) (Sigma-Aldrich, Buchs, Switzerland), supplemented with 1% sucrose.

Saliva and serum

A mix of saliva and serum was used to coat the specimens because it has previously been reported that adding 10% human serum to the material coating solution leads to better adhesion of bacteria (26, 27). Saliva was stimulated (by chewing with paraffin wax to augment production) for collection from three healthy volunteers. The saliva was ultrasonicated for 30 s (30 W, Vibracell; Sonics & Materials), filtered through a 70 μm filter (Cell Strainer; Becton Dickinson), and centrifuged at 22,000 ***g*** for 40 min at 4°C. The supernatant was filtered through two connected filters (0.45 μm and 0.22 μm; Millex-HV and Millex-GV, respectively; Millipore, Darmstadt, Germany) and frozen at −20°C in aliquots of 5 ml, corresponding to the volume required for each individual experiment.

The serum used was taken from a pool of samples from 10 subjects (Blutspendezentrum SRK, Basel, Switzerland). The pH of the serum/saliva mixture was adjusted to pH 7.2 by adding potassium and sodium phosphate buffers (0.067 mol l^{-1}). All samples were coated with buffered serum/saliva mixture for 15 min at room temperature before the flow chamber experiments.

Cement specimens

A Teflon mold was used to produce disks with a diameter of 14 mm and a thickness of 1 mm from each of the cements listed in Table 1. The cavity of the mold was filled with cement and kept in place with polyester foil and a glass plate on each side. Light curing was performed for 120 s in total with a polymerization lamp (Elipar; 3M ESPE, Landsberg am Lech, Germany). All specimens were stored at 37°C for 24 h to complete polymerization and they were then wet-polished with silicon carbide paper of grit 180, 400, or 2400 (Struers, Ballerup, Denmark). This produced three different levels of roughness of the cement specimens, which were used to simulate a clinical situation in which a cement gap is polished to different levels of smoothness. It was not possible to simulate the situation of a clinically unpolished cement surface because the cement gap in a clinical situation is potentially exposed to contact with soft tissue or blood and forms an oxygen inhibition layer at its surface.

The roughness of each cement specimen was recorded for each pretreatment (grit 180, 400, and 2400) using a profilometer (T1000/TKK50; Hommelwerke, Schwenningen, Germany). The clinical relevance of the roughness of the tested cement surfaces was estimated by determining the roughness of additional specimens pretreated with six polishing instruments: rough diamond bur (FG 305L/6 106 μm; Intensiv, Montagnola, Switzerland); Proxoshape red (PS2 40 μm; Intensiv); Proxoshape yellow (PS3 15 μm; Intensiv); Brownie (0403; Shofu Dental, Ratingen, Germany); Greenie (0404; Shofu Dental); and Supergreenie (404B; Shofu Dental).

Aerobic flow chamber

The flow chamber model has been previously described in detail (26, 28) and thus will be summarized only briefly here. The system comprised a flow chamber (MINUCELLS and MINUTISSUE; Vertriebs, Bad Abbach, Germany) containing the test specimens, a dispenser containing the bacterial suspension, and a peristaltic pump. The bacterial solution was made to flow at 0.8 ml min^{-1} and was stirred at 240 r.p.m. Circulating bacteria were allowed to adhere on the protein-coated cement specimens under aerobic conditions at 37°C for 24 h.

Table 1

Cement materials used in this study

Code	Name	Manufacturer	Type	Monomers	Fillers	Initiators
MLA	Multilink Automix	Ivoclar Vivadent	Adhesive resin composite cement	Base paste: Bis-GMA, HEMA, 2-dimethylaminoethyl methacrylate Catalyst paste: ethyoxylated bisphenol A dimethacrylate, UDMA, HEMA	40 vol% • Barium glass • Ytterbium trifluoride • Spheroid mixed oxide Particle size: 0.25–3.0 μm	Dibenzoyl peroxide
MSC	Multilink Speed CEM	Ivoclar Vivadent	Self-adhesive resin composite cement	Base paste: UDMA, TEGDMA, polyethylene glycol dimethacrylate Catalyst paste: polyethylene glycol dimethacrylate, TEGDMA, methacrylated phosphoric acid ester, UDMA	40 vol% • Barium glass • Ytterbium trifluoride Particle size: 0.1–7 μm	Dibenzoyl peroxide
RUL	RelyX Ultimate	3M ESPE	Adhesive resin composite cement	Base paste: methacrylate monomers containing phosphoric acid groups, methacrylate monomers Catalyst paste: methacrylate monomers	43 vol% • Silanated fillers • Alkaline (basic) fillers Particle size: 13 μm	Sodium toluene-4-sulphinate Disodium peroxodisulphate Tert-butyl 3,5,5-trimethylperoxyhexanoate
RUN	RelyX Unicem 2 Automix	3M ESPE	Self-adhesive resin composite cement	Base paste: phosphoric acid-modified methacrylate monomers, bifunctional methacrylate Catalyst paste: methacrylate monomers	43 vol% • Alkaline (basic) fillers • Silanated fillers Particle size: 12.5 μm	Sodium toluene-4-sulphinate, Sodium Persulfate, Tert-butyl 3,5,5-trimethylperoxyhexanoate
PV5	Panavia V5	Kuraray	Adhesive resin composite cement	Paste A: Bis-GMA, TEGDMA, hydrophobic aromatic dimethacrylate, hydrophilic aliphatic dimethacrylate Paste B: Bis-GMA, hydrophobic aromatic dimethacrylate, hydrophilic aliphatic dimethacrylate	38 vol% • Silanated barium glass filler • Silanated fluoroalminosilicate glass filler • Colloidal silica • Silanated alminium oxide filler Particle size: 0.01–12 μm	dl-Camphorquinone
PSA	Panavia SA plus	Kuraray	Self-adhesive resin composite cement	Paste A: 10-MDP, Bis-GMA TEGDMA, hydrophobic aromatic dimethacrylate 2HEMA Paste B: hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate	40 vol% • Silanated barium glass filler • Silanated colloidal silica Particle size: 0.02–20 μm	dl-Camphorquinone

10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; Bis-GMA, bisphenol A diglycidylmethacrylate; HEMA, 2-hydroxyethyl methacrylate; MLA, Multilink Automix; MSC, Multilink SpeedCem; PSA, Panavia SA plus; PV5, Panavia V5; RUL, RelyX Ultimate; RUN, RelyX Unicem 2 Automix; TEGDMA, triethyleneglycol dimethacrylate; UDMA, urethane dimethacrylate.

Quantification of biofilm formation

After 24 h in the flow chamber, the specimens were taken out and rinsed in 0.9% NaCl to remove any loosely attached cells. The specimens were air-dried at room temperature and embedded in paraffin, after which 300 μl of 0.5% CV (Sigma-Aldrich, Buchs, Switzerland) stain was added to each sample and incubated for 10 min at room temperature. Excess stain was discarded and the disks were bathed in series of 0.9% NaCl to remove all unbound CV. The samples were air-dried again at room temperature and 1 ml of absolute ethanol was added to destain the samples. Optical density (OD) was measured at 595 nm to quantify the amount of biofilm bound to the surface of the specimens ($n = 9$, for each combination of material and roughness).

Fluorescence microscopy

The presence of the biofilm on different samples was detected using 4′,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich) staining and confocal laser scanning microscopy (CLSM). The *S. mutans* biofilms grown for

24 h in the flow chamber were fixed in 4% paraformaldehyde (Sigma-Aldrich) for 1 h at 4°C and washed once with PBS. Then, the biofilm samples were placed in DAPI solution (200 ng/ml; Sigma-Aldrich) for 2 min at room temperature and rinsed with 0.9% NaCl. Thereafter, the disks were embedded upside-down in 10 μl of Mowiol mounting solution (Sigma-Aldrich) and stored in the dark for at least 6 h at room temperature. Biofilms were examined using a Zeiss LSM700 microscope (Carl Zeiss Microscopy, Jena, Germany) fitted with a diode laser at 405 nm. Confocal images were obtained using a 63x (numeric aperture 1.4) oil-immersion objective.

Cement composition

Additional specimens were produced representing each type of cement ($n = 2$ per cement). These specimens were then fixed (UHU plus; UHU, Bühl, Germany) on a slide and simultaneously wet-polished with silicon carbide paper grit P1200–4000 using a polishing machine (Type 401319; Exakt, Oklahoma City, OK, USA). The polished cement specimens were then removed from the slide and gold-sputtered for scanning electron microscopy imaging. Scanning electron microscopy backscatter images of cement structures at magnifications of 1000x, 2500x, and 5,000x were captured (Philips XL30 FEG ESEM; Philips Electron Optics, Eindhoven, the Netherlands). Energy-dispersive X-ray spectroscopy mapping (Genesis; EDAX, Mahwah, NJ, USA) was performed at 10 kV and 5,000x magnification to test for the content of aluminium (Al), barium (Ba), calcium (Ca), carbon (C), copper (Cu), iron (F), germanium (Ge), potassium (K), sodium (Na), nitrogen (N), oxygen (O), phosphorus (P), silicon (Si), titanium (Ti), ytterbium (Yb), zinc (Zn), and zirconium (Zr) in order to determine inorganic filler composition. The elements investigated were chosen on the basis of the composition data provided by the manufacturer in the instructions for use and safety data sheets of the cements.

Statistical analysis

Variables representing OD measurements for different cements with different surface roughness were first analyzed for normal distribution using the Shapiro–Wilk test. All data were normally distributed, and two-way ANOVA was therefore used to test for statistically significant differences in biofilm formation (using OD values), according to type of cement and surface roughness, and was followed by post hoc Fisher's LSD test to evaluate differences between the groups ($P < 0.05$).

Results

Cement roughness

The roughness of the cement surfaces finished with different dental polishing instruments is presented in Fig. 1. The silicon carbide papers chosen for the pretreatment of the cement specimens can be considered to correspond to well-polished (grit 2400, similar to greenie/supergreenie), medium-polished (grit 400, similar to brownie), or rough-polished (grit 180, similar to proxoshape red) cement gaps.

Energy-dispersive X-ray spectroscopy

The weight% of the measured elements is reported in Table 2 for all cements. Cements displaying no biofilm formation (PV5 and PSA) revealed a Ba content of 20 wt%. Small amounts of Cu were present in MLA, PV5, and PSA. Large fillers, up to 20 μm, were found for MSC. The fillers of the other cements corresponded to the specifications provided by the manufacturer. Scanning electron microscopy backscatter images at a magnification of 5,000x are displayed in Fig. 2. These images show the filler compositions that were identified with the EDX mapping on polished cement specimens.

Fluorescence microscopy

Streptococcus mutans was present on all cement surfaces, but biofilm was only formed on specimens of MSC, RUL, RUN, and on the roughest MLA surface (grit 180). Fluorescence microscopy images of the *S. mutans* adherent on cement surfaces (grit 180) are presented in Fig. 3. Background fluorescence was present in MSC, RUL, RUN, and PSA, and should not be mistaken for bacteria. In Fig. 3, background fluorescence is indicated with grey arrows.

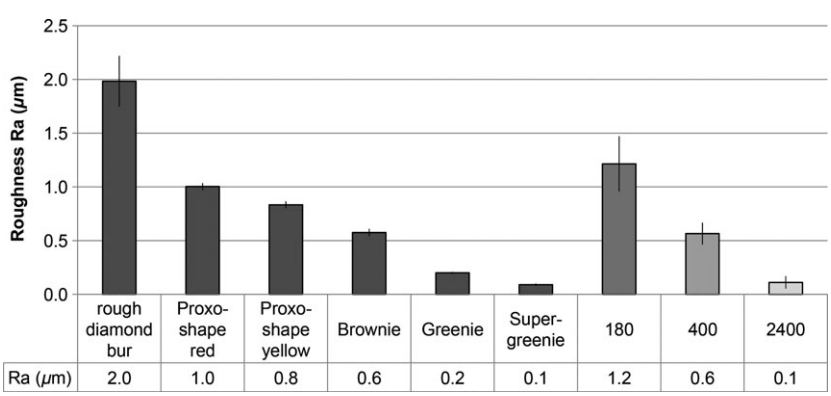

Fig. 1. Surface roughness (R_a) values of cements in comparison with polishing instruments used in the dental clinic. Silicon carbide papers of grit 180, 400, and 2400 were used for the pretreatment of the cement specimens.

Table 2

Energy-dispersive X-ray spectroscopy analysis of elements present in the different cements (measurements were taken at 5,000x magnification)

Element (wt%)	MLA	MSC	RUL	RUN	PV5	PSA
Al	3.45	4.99	9.66	8.3	6.01	3.91
Ba	11.72	4.32	0.18	0.58	20.83	20.97
Ca	0.38	3.28	0.06	0.14	0.00	0.00
C	11.38	19.02	22.57	17.72	26.33	23.95
Cu	0.04	0.00	0.00	0.00	0.07	0.08
F	4.04	7.11	4.68	2.94	0.49	0.21
Ge	0.72	0.85	0.00	0.00	0.00	0.00
K	0.00	0.00	0.00	0.00	0.00	0.00
Na	0.03	0.25	0.90	0.52	0.04	0.07
N	1.36	1.96	1.32	1.59	1.46	1.38
O	19.18	11.65	28.11	29.49	22.36	23.62
P	0.00	0.00	0.00	0.00	0.00	0.00
Si	18.74	11.42	24.38	29.38	17.02	20.52
Ti	0.00	0.00	0.06	0.00	0.00	0.00
Yb	21.86	25.15	0.00	0.00	0.00	0.00
Zn	0.14	0.11	0.24	0.19	0.48	0.27
Zr	6.96	9.87	7.84	9.15	4.92	5.02

Values are given as wt%.
MLA, Multilink Automix; MSC, Multilink SpeedCem; PSA, Panavia SA plus; PV5, Panavia V5; RUL, RelyX Ultimate; RUN, RelyX Unicem 2 Automix.

Biofilm formation

Figure 4 shows biofilm formation on the cement specimens, as quantified with OD at 595 nm (OD595), according to material type and roughness. A value of OD595 < 0.1, corresponding to the difference to the negative control with no bacteria in the flow chamber, was considered to represent no biofilm formation. Two-way ANOVA revealed that the biofilm formation was significantly influenced by the cement material ($P < 0.001$) and by the roughness of the cement specimens ($P = 0.018$).

Biofilm formation was affected by the material as follows: for all three levels of roughness, RUL/RUN and PV5/PSA were not statistically significantly different ($P > 0.05$). For the levels of roughness resulting from polishing with silicon carbide paper at grit 400 and 2,400, the biofilm formation on MLA, PV5, and PSA were not statistically significantly different ($P > 0.05$). The highest amounts of biofilm were found (in order of degree of biofilm formation) on: MSC > RUN = RUL > MLA ≥ PV5 = PSA.

The roughness of the cement surfaces affected the formation of biofilm as follows. For PV5, the surface roughness did not affect biofilm formation. For MSC and PSA, no statistically significant difference was found between specimens pretreated with silicon carbide paper grit 180 and 400 ($P = 0.119$). For RUN, no statistically significant difference was found in the biofilm formation of specimens polished with grit 400 and grit 2,400 ($P = 0.151$). All other cement specimens revealed increasing biofilm formation with increasing surface roughness. The correlation between the amount of biofilm formation (OD595) and roughness of cement specimen is shown in Fig. 5. Higher roughness of the cement surface was associated with more extensive biofilm formation. However, the association was not linear and differed between the cements. On cement surfaces that allow no biofilm formation (OD < 0.1), the surface roughness did not influence the bacterial adhesion.

Discussion

The adhesion of a cariogenic species of bacteria, *S. mutans,* was measured on different resin composite cements. Variations in roughness and composition of the cement significantly affected the formation of *S. mutans* biofilm. Increased roughness of the cement resulted in higher biofilm formation; hence, the first hypothesis was confirmed. The second hypothesis, that all tested cement materials display similar biofilm formation, was rejected because biofilm formation varied significantly between the cement materials.

The quantification of biofilm formation with OD measurements following CV staining provided reproducible results while, at the same time, allowing evaluation of bacterial growth on the total area of the specimens. The results of OD measurement were consistent with the images obtained from fluorescence microscopy, which indicates that no biofilm was lost as a result of handling the samples**.** As the formation of oral biofilm is a very complex process and involves many different bacteria, it is clear that the results obtained in this laboratory approach cannot be completely transferred to a clinical setting. The possible effects of interactions between different bacterial species (27) on biofilm formation, according to cement type and surface roughness, should be assessed in a further study.

Previously, a positive correlation between surface roughness values and bacterial adhesion has been reported for composite (21, 29), ceramic (30), as well as cement (24) materials. These results corroborate the findings of this study in which bacterial biofilm formation was increased on rougher surfaces although the increase was not linear. It is known that rougher surfaces promote bacterial adhesion (21, 22, 24, 29, 30) to an extent that exceeds the influence of other surface properties, such as surface free-energy (31). Recent studies also suggest that the surface composition and surface topography impact the formation of biofilms to a higher degree than does the surface free-energy (32–34). The roughness obtained by different cement-removal techniques without polishing has been observed to range from 1.0 to 1.7 μm (24). The present study additionally evaluated how polishing the cement, providing roughness values of 0.1, 0.6, or 1.2 μm, would affect the formation of *S. mutans* biofilm. An $R_a < 0.2$ μm is recommended to avoid rapid bacterial colonization on intra-oral surfaces (25). However, it has to be considered that the surface roughness of previously polished surfaces tend to increase over time owing to degradation of the polymer matrix (35). The present study revealed that bacterial biofilm formation over 24 h can be reduced by polishing surfaces up to $R_a = 0.1$ μm, although it cannot be entirely avoided for

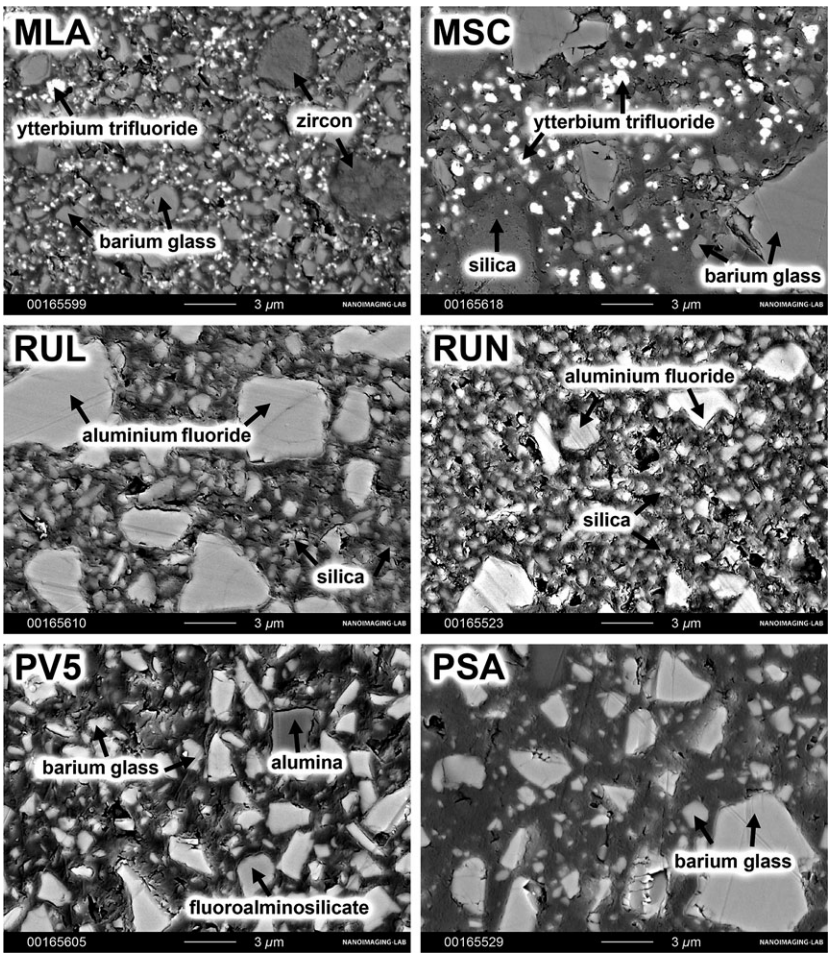

Fig. 2. Scanning electron microscopy backscatter images of polished cement surfaces (5,000x magnification). Compositions of the ceramic fillers are indicated with arrows. MLA, Multilink Automix; MSC, Multilink SpeedCem; PSA, Panavia SA plus; PV5, Panavia V5; RUL, RelyX Ultimate; RUN, RelyX Unicem 2 Automix.

some cements (MSC, RUL, and RUN). Two cements (PV5 and PSA) displayed no biofilm formation at all, irrespective of their surface roughness. In contrast to previous assumptions (24, 25, 36), it can be concluded that the impact of the cement material itself on the formation of biofilm is stronger than the impact of the surface roughness. However, for clinical use, cement gaps should be polished as well as possible using appropriate rubber polishers (supergreenie) that provide R_a values up to 0.1 μm to limit plaque accumulation in this area. Polishing eliminates excess cement and with the decrease in roughness and exposed surface area, sorption may also be decreased. Additionally, insufficient polishing may lead to staining or gingival irritation (37, 38).

The cements evaluated in the present study contained a wide range of ceramic fillers and had different compositions of the polymer matrix. The effect of each component and their interaction on bacterial adhesion was not assessed in this study. Bacterial adhesion on ceramic was found to be lower than for other restorative materials (20, 21) although these results have to be interpreted with care. Adhesion to ceramic surfaces differed significantly between the materials used and on the type of bacteria. For *Streptococcus gordonii* the lowest adhesion, and for *Streptococcus sanguinis* the highest adhesion, was found on glass ceramic compared with lithium disilicate ceramic (39). However, it has to be considered that the tested ceramics also varied in surface roughness. The EDX analysis demonstrated the elements contained in the cements, which allows an estimation of the ceramic filler compositions. The composition of the polymeric matrix cannot be analyzed because of its organic structure.

Cement materials containing chlorhexidine, fluoride, or silver particles are considered as antibacterial-agent releasing (40, 41). Although fluorine was found in all cements, it is part of inert fillers such as ytterbium fluoride (MLA and MSC), fluoroaluminosilicate glass (PV5), or alumina fluoride (RUL and RUN) and therefore has no antibacterial effect. Zinc particles that are supposed to provide antibacterial effects (42) were found in all cements but did not seem to have an effect on the biofilm formation. A small amount of Cu was found in MLA, PV5, and PSA, all the cements that revealed low bacterial adhesion. The Cu content in PV5

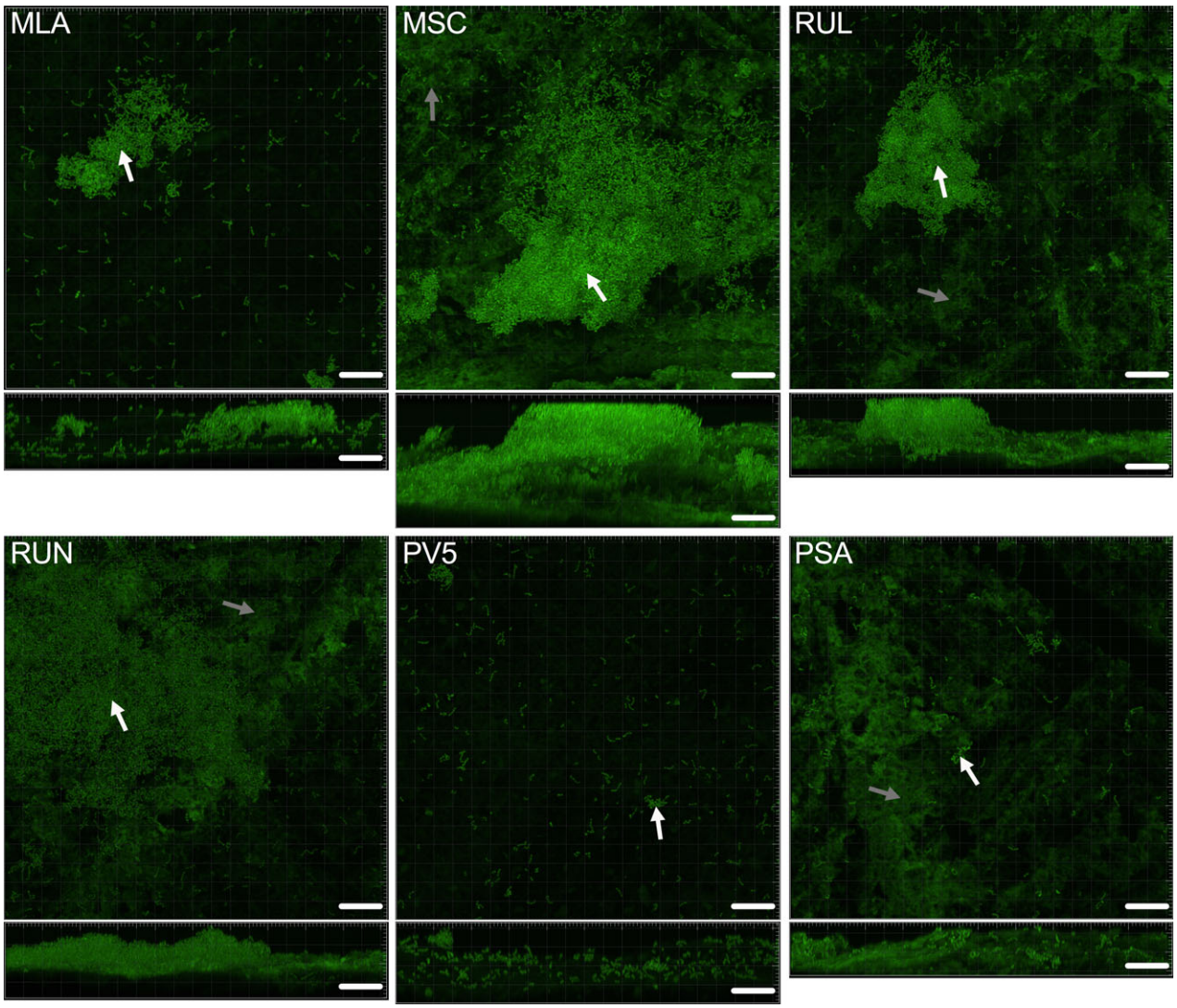

Fig. 3. Fluorescence microscopy images of the biofilm formed by *Streptococcus mutans* on the cement surfaces pretreated with silicon carbide paper grit 180. Bacteria are indicated with white arrows, and background fluorescence is indicated with grey arrows. MLA, Multilink Automix; MSC, Multilink SpeedCem; PSA, Panavia SA plus; PV5, Panavia V5; RUL, RelyX Ultimate; RUN, RelyX Unicem 2 Automix.

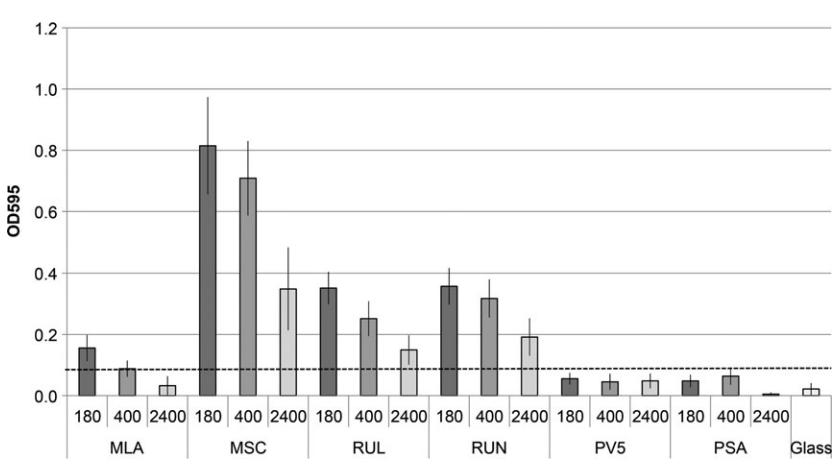

Fig. 4. Optical density at 595 nm (OD595) of formation of *Streptococcus mutans* biofilm on cements with different roughness (silicon carbide paper grit 180, 400, and 2400). Values of OD595 < 0.1 indicate no biofilm formation. MLA, Multilink Automix; MSC, Multilink SpeedCem; PSA, Panavia SA plus; PV5, Panavia V5; RUL, RelyX Ultimate; RUN, RelyX Unicem 2 Automix.

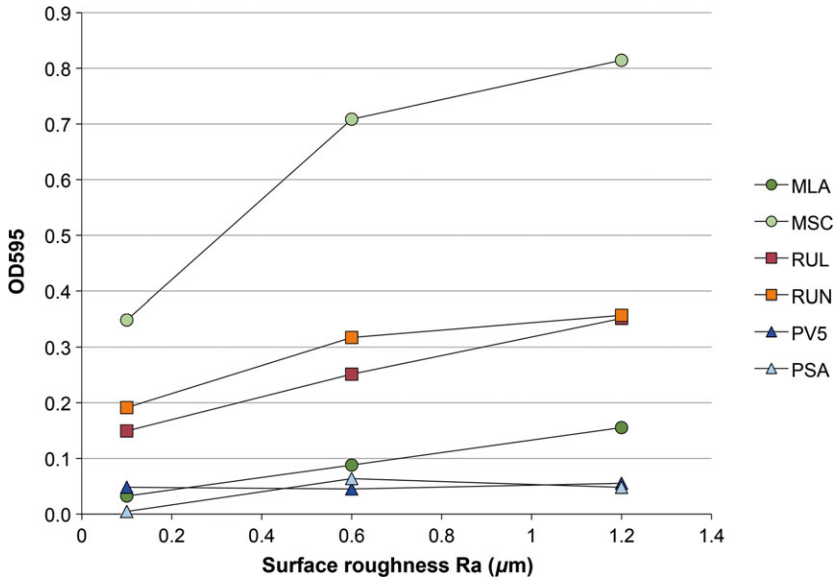

Fig. 5. Correlation between biofilm formation [determined by measuring the optical density at 595 nm (OD595)] of *Streptococcus mutans* and surface roughness (R_a) of the respective cements. MLA, Multilink Automix; MSC, Multilink SpeedCem; PSA, Panavia SA plus; PV5, Panavia V5; RUL, RelyX Ultimate; RUN, RelyX Unicem 2 Automix.

and PSA was higher than that in MLA, and those cements also displayed stronger anti-adhesive properties toward *S. mutans* than did MLA. According to the manufacturer's safety data sheet, RUL and RUN also contain Cu in the form of acetic acid copper salt monohydrate but only in a very small amount that could not be detected with the EDX analysis in the present study. Acetic acid copper salt is added to the cement as an accelerator. Antibacterial effects of Cu-containing glass-ceramic (43), phosphate cement (44), or gold-alloys (45) have been previously reported and corroborate the findings of the present study.No biofilm was formed on cement surfaces containing a high amount (around 20%) of Ba fillers (PV5 and PSA). However, although rather large fillers, up to 20 μm, were present in MSC, high amounts of biofilms were detected, as a homogeneous surface for bacterial adhesion was provided that might have increased the propensity to allow biofilm formation.

The polymer matrix of self-adhesive resin cements is generally composed of phosphoric and/or carboxylic acid methacrylate monomers that adhere to the tooth substance (3). The present study revealed no significant difference between self-adhesive and adhesive cements regarding bacterial adhesion. One adhesive (RUL) and one self-adhesive (RUN) cement from the same manufacturer revealed no statistically significant differences for bacterial adhesion, irrespective of their roughness. Other adhesive (PV5) and self-adhesive (PSA) cements from the same manufacturer presented similar composition of elements but different filler morphologies.

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Conflicts of interest – The authors declare no conflicts of interest.

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