
Article

The influence of modified experimental dental resin composites on the initial in situ biofilm – A triple-blinded randomized controlled split-mouth trial

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Abstract: The purpose of the study was to investigate the bacterial viability of the initial biofilm on the surface of experimental modified dental resin composites. Twenty-five healthy individuals with good oral hygiene were included in this study. In a split-mouth design, they received acrylic splints with five experimental composite resin specimens. Four of them were modified with either a novel polymeric hollow beads delivery system or methacrylated polymerizable Irgasan (Antibacterial B), while one specimen served as unmodified control (ST). The delivery system based on Poly-Pore® was loaded with one of the active agents Tego® Protect 5000 (Antiadhesive A), Dimethicone (Anti-adhesive B) or Irgasan (Antibacterial A). All study subjects refrained from toothbrushing during the study period. Specimens were detached from the splints after 8h and given a live/dead staining before fluorescence microscopy. Friedman test and post-hoc Nemenyi test were applied with significance level at $p < 0.05$. In summary all materials but Antibacterial B showed a significant antibacterial effect compared to ST. In conclusion dental resin composites with Poly-Pore loaded active agents show antibacterial effectiveness in situ.

Keywords: Antibacterial composites; Antiadhesive composites, Poly-Pore; Split-mouth; Clinical trial; Live/dead staining; Bacterial viability

1. Introduction

A vast majority of dental fillings fail due to recurring carious lesions on the existing filling margins [1]. The development of this so-called secondary caries, in contrast to primary carious lesions without existing dental restorations, seems to depend on the filling properties to a large extent [2].

On one hand, it is comprehensible that the surface structure in aspects of surface roughness or surface free energy of a dental filling influences the bacterial adhesion and consequently the development of secondary caries [3-9]. On the other hand, it has been reported that the specific material itself can influence the caries formation. Accordingly, amalgam is considered to be an effective filling material to modify the biofilm formation because of its bacteriostatic features [10]. In comparison composite resin fillings show an increased plaque accumulation over the course of wearing [11] and fail more often than amalgam because of the development of secondary caries at the filling margins [12-14].

One strategy to prevent secondary caries could be to diminish or even inhibit bacterial adhesion [15-17] not only on the natural oral hard tissues, but also on the incorporated

dental materials [18-21]. Therefore, innovative composite fillings with antiadhesive or antibacterial properties could play a key role to counteract the risk of secondary caries.

For this purpose, our team developed and produced experimental resin composite materials, which can release antibacterial or antiadhesive substances. The delivery process of these active substances as such is linked to comonomers or carrier substances and is driven through abrasion processes [17,22-24]. Antiadhesive and antibacterial properties of these abrasion responsive "smart materials" have already been observed in extensive in vitro studies [22-26].

To test the smart materials' effect on the initial biofilm, some of these studies used the early colonizers as described by Kolenbrander et al. [27] in the form of mono species cultures to show the influence on the number and viability of these bacterial strains with fluorescence microscopy examination [28,29]. As a result the modified test materials were able to reduce the number of adherent bacteria in total and the proportion of vital to non-vital microorganisms [17,28].

The present study continues the aforesaid investigations in a randomized and triple-blinded in situ split-mouth trial. This time, bacterial viability on the most promising four experimental modified resin composites (Antiadhesive A, Antiadhesive B, Antibacterial A, Antibacterial B) were compared with an unmodified experimental composite resin (ST). Hence the aim of the present study was to clinically examine the effects known from in-vitro studies in an in-situ setting with subsequent fluorescence microscopy examination. The null hypothesis was that the modified materials did not differ from the control or among each other in the total bacterial counts or in the respective bacterium's viability after 8 hours.

2. Materials and Methods

2.1. Participants

The study was conducted in full accordance with the World Medical Association Declaration of Helsinki with the approval from the Ethics Committee (Ethics Committee of the Medical Faculty of Heinrich-Heine-University, Dusseldorf, Germany, internal study number: 2912). Written informed consent was obtained before each subject's participation in the trial. The medical history was recorded and a dental report with tooth hard tissue status, periodontal condition and oral hygiene was collected. The participants were evaluated for eligibility with the following inclusion criteria:

1. Age from 25 to 40 years
2. Healthy dental condition
3. No signs of periodontitis following the Periodontal Screening and Recording Index (PSR) [30]
4. Good oral hygiene within the limits of the Silness-Loe Plaque Index (PLI) [31]
5. No systemic diseases

Subjects who did not meet the oral health parameters were offered to participate in a prophylaxis program and to have their carious lesions treated if any present. Participants were excluded if they did not meet the inclusion criteria.

2.2. Intervention

Each participant received a removable custom-made acrylic splint, that held the five specimens for simultaneous testing (Figure 1 (a)). The specimens had to be inserted into depressions and fixed with sticky wax facing towards the buccal teeth surfaces at the level of the approximal spaces of the first three posterior teeth. This prevented the disruption of the biofilm caused by contact with tongue or cheek on one hand, whereas the space between specimens and teeth remained free over the distance of 3 mm allowing undisturbed biofilm growth and unhindered salivatory function on the other hand (Figure 1 (b)).

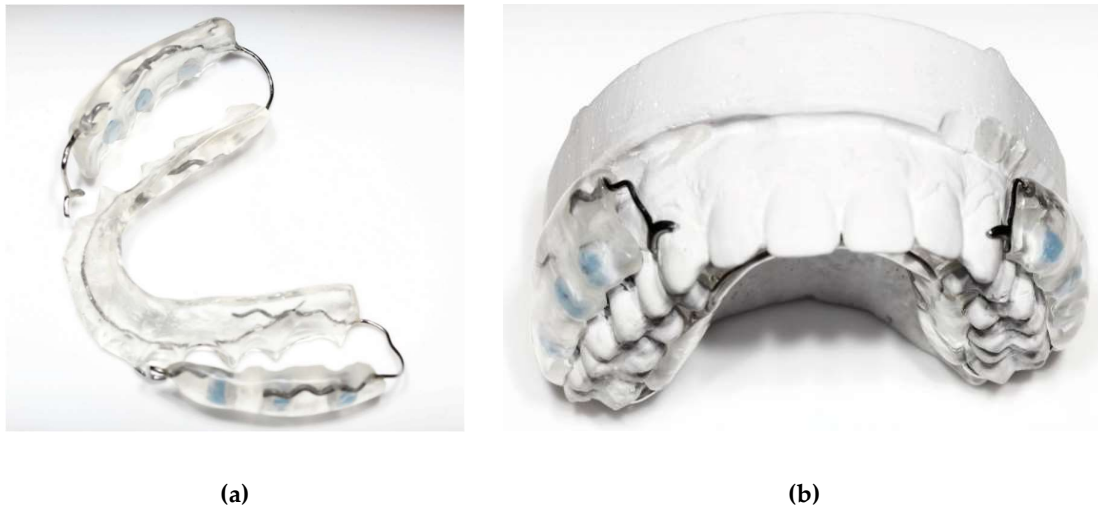


Figure 1. Custom-made removable acrylic splint (a) Specimens facing towards the buccal side of the first three approximal spaces of the posterior teeth; (b) Placed onto dental cast with outward shielding element towards the cheeks and free space between specimens and teeth allowing salivatory flow.

2.3. Trial Design

The split-mouth design allowed the five specimens to be tested simultaneously in one run per subject. One specimen from the experimental unmodified composite material served as control, while the other four specimens were either antiadhesive or antibacterial modified experimental composite materials.

The specifications of the experimental resin-based composites have been previously published with the standard composite corresponding to material ST [17,22,24], Antiadhesive A or Antiadhesive B corresponding to Material A or Material C, respectively [22,24] and Antibacterial A or Antibacterial B corresponding to Material A or C, respectively [17]. The material specifications can be found in Table 1 and Table 2.

Table 1. Formulations of the experimental resin-based restorative materials. ST served as control (wt. %).

Raw material	ST	Antiadhesive	Antiadhesive	Antibacterial	Antibacterial
		A	B	A	B
Glass	73.0	68.0	68.2	68.0	73.0
Poly-Tego	-	5.0	-	-	-
Poly-Dimeth	-	-	5.0	-	-
Poly-Irga	-	-	-	5.0	-
Methacryl-Irga	-	-	-	-	8.0
Matrix	27.0	27.0	26.8	27.0	19.0
Active agent	0	4.0	4.0	4.0	8.0

Matrix: UDMA, 44.1; Bis-GMA, 30.0; TTEGDMA, 25.0; photoinitiator, 0.3; CQ, 0.2; amine, 0.1; stabilizer, 0.1.

The specimens' labels were encrypted by a third person, so that participant, clinical investigator and laboratory evaluator were blinded throughout the study. In addition, the specimens' assignment to the splint depressions by the clinical investigator and the later assessment of the specimens by the laboratory evaluator were randomized. The labels were only revealed again for statistical analysis

Table 2. Raw materials. The information is based on the manufacturers' technical data sheets.

Code	Product/properties	Batch	Company
Photoinitiator	α,α -dimethoxy- α -phenylacetophenone	0066162S	Ciba Speciality Chemicals, Basel, Switzerland
Stabilizer	Pentaerythrityl-tetrakis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)-propionate	26099IC3	Ciba Speciality Chemicals
TTEGDMA	Tetraethyleneglycole dimethacrylate, standard monomer, functionality = 2, MW = 330 g mol ⁻¹ , good chemical and physical properties, very low viscosity (14 Pa s, 25 C), diluting	J1620	Cray Valley, Paris, France
UV stabilizer	2-Hydroxy-4-methoxy-benzophenone	411351/ 143302	Fluka, Buchs, Switzerland
UDMA	7,7,9-Trimethyl-4,13-dioxo-3,14-dioxo-5,12-diaza-hexadecan-1,16-diol-dimethacrylate, standard monomer, functionality = 2, MW = 471 g mol ⁻¹ , flexible, tough, very good chemical resistance, medium viscosity(10,000 m Pas,25 C)	330503057	Rahn A.G, Zürich, Switzerland
Bis-GMA	Bis-GMA, standard monomer, functionality = 2, MW = 513 g mol ⁻¹ , rigid, very good chemical resistance, very high viscosity (4500 m Pas, 60 C)	2008218303	Rahn A.G
CQ	D,L-Camphorquinone	0148990002	Rahn A.G
Amine	Ethyl-4-(dimethylamino)-benzoate	310170	Rahn A.G
Glass	Strontium borosilicate glass (GO 18-093, d50 = 0.7 μ m). silaned (3-methacryloyloxypropyl trimethoxy silane), D = 2.6 g cm ⁻³ ,	Lab14701	Schott Electronic Packaging, GmbH. Landshut. Germany
Poly-	Poly-Pore, cross-linked polyallyl methacrylate, adsorber, hollowbeads, diameter 20–40 μ m	L07070303AB	AMCOL Health & Beauty Solutions, Arlington Heights, IL, USA
Tego	TegoProtect 5000, hydroxyfunctional polydimethylsiloxane, hydro- and oleophobic, D = 1.05 g cm ⁻³	ES57608918	Evonik Tego Chemie, Essen, Germany
Dimeth	Dimethicone 200/350 cst, polydimethylsiloxane, D = 0.965 g cm ⁻³	4962250	Dow Corning Corp., Midland, MI, USA
Irga	Irgasan, 5-chloro-2-(2,4-dichlorophenoxy)phenole	1124816	Sigma Aldrich GmbH, Steinheim, Germany
Poly-Dimeth	Poly-Pore loaded with 80% Dimethicone, D = 1.0 g cm ⁻³	Experimental product	University laboratory
Poly-Tego	Poly-Pore loaded with 80% Tego Protect 5000, D = 1.0 g cm ⁻³	Experimental product	University laboratory
Poly-Irga	loaded with 80% Irgasan, D=1.0 cm ⁻³	Experimental product	University laboratory
Methacryl-Irga	5-chloro-2-(2,4-dichlorophenoxy)phenyl methacrylate	Experimental product	University laboratory

2.4. Specimen preparation

Twenty-five disc shaped specimens (diameter: 3 mm \pm 0.1 mm, thickness: 1 mm \pm 0.1 mm) from five experimental light-curing resin-based composites were made. Unmodified material ST, representing a common formulation of dental resin composites served as the control. All materials met the ISO 4049 criteria [33]. The specimens were cured for 40 s on each side (Spectrum 800, Model No.703EU, Dentsply DeTrey GmbH, Constance, Germany). The output of the curing device has been checked routinely (Bluephase Meter, Ivoclar Vivadent AG, Schaan, Liechtenstein). Irradiances of 884 \pm 53 mW/cm² were measured and no significant decrease of the output was observed.

The cured specimens were polished on the test side with SuperSnap-Finishing & Polishing Disks (Schofu Dental GmbH, Ratingen, Germany) green (20 μ m grit) and red (7 μ m grit) subsequently for one minute each with 10,000 rpm and a grinding pressure of 40 – 50 g.

2.5. Cell viability determination

After 8 hours the worn acrylic splints were removed and the specimens were placed in 500 μ l sterile 0.9 % sodium chloride solution (Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany). Afterwards vital and non-vital cells were determined with live/dead staining (LIVE/DEAD[®] BacLight Bacterial Viability Kit, Thermo Fisher Scientific GmbH, Dreieich, Germany) by measuring the fluorescence emission (BZ-X700E fluorescence microscope, Keyence Deutschland GmbH, Neu-Isenburg, Germany). Ten predetermined locations were examined on each disc surface and fluorescent microscopic images were captured (400-fold magnification) with fluorescent filter sets for both fluorescent dyes (SYTO9 480nm, emission 500nm; PI 490nm, emission 635nm).

The absolute number of vital and non-vital cells and the sum of both were counted with the Hybrid Cell Count Software (Keyence Deutschland GmbH). The bacterial cell viability ratio (BV) was reported as the percentage of vital cells from the total cell count.

2.6. Sample size

Sample size of n = 25 was calculated based on the results of the preliminary in vitro study [24] using a power of 80% and significance level of 0.05.

2.7. Statistical analysis

The medians and interquartile ranges were calculated and are presented as whisker-box plots with Tukey's fences. Extreme values were considered for statistical analysis but are not shown in the plots for reasons of clarity. The mean and standard deviation are also provided to compare the results with the results of previous studies. Normal distribution was tested using the Shapiro-Wilk test. As the data was not normally distributed, all statistical comparisons were performed using non-parametric methods. Friedman test was applied to find differences between the composite groups. Post-hoc pairwise comparisons were made using the conservative Nemenyi test, which already accounts for a familywise error [34]. Although no direct measure of effect size for the Friedman test is generally recognized, an indirect measure was obtained using the Kendall's W-statistic, computed from the Friedman Q value [35]. Effect sizes were interpreted using Cohen's interpretation guidelines [36]: Small $W < 0.3$; moderate $0.3 \leq W < 0.5$; large $W \geq 0.5$. Statistics and randomization processes were carried out with R Software Version 4.0.5. The statistical significance level for all tests was set at $p < 0.05$.

3. Results

3.1. Participants

From the catchment area of a German dental clinic, a total of 25 participants were selected for this split-mouth study. The participants' characteristics are presented in Table 3.

Table 3. Participants' characteristics.

	n	%
Participants	25	100
Female/ Male	19/ 6	76/ 24
Age (mean \pm SD) (years)	29.5 \pm 3.3	-
Tooth hard tissue		
Decay	0	-
Oral hygiene (PLI)		
Excellent (0)	16	64,0
Good (0.1 - 0.9)	9	36,0
Fair (1.0 – 1.9)	0	-
Poor (2.0 – 3.0)	0	-
Periodontal Screening and Recording Index (PSR)		
Grade 0	104	69.3
Grade 1	27	18
Grade 2	19	12.7
Grade 3	0	-
Grade 4	0	-

Abbreviations: n, number; SD, standard deviation

In particular, the mean age of the included participants was 29.5 \pm 3.3 years (median 29; range 25 – 39 years). They had no decayed teeth or signs of periodontitis (PSR \leq 2). No participant showed a compromised oral hygiene (PLI \leq 0.9).

3.2. Cell viability

There were statistically significant differences in cell counts depending on the composite material tested. The effect sizes were moderate for the vital and total cell counts and the bacterial cell viability ratio BV (all $p < 0.0001$). The non-vital cell count showed a small effect ($p = 0.00096$).

The detailed results and the significances of the post-hoc comparisons are shown in Table 4. The bacterial counts are additionally graphically presented in Figure 2 and Figure 3.

Table 4. Cell count medians (and interquartile ranges) of vital, non-vital and total cells and bacterial cell viability ratio (BV). Means \pm standard deviations are provided in square brackets. Values are rounded to valid digits. Equal subscript numbers within the columns indicate *non*-significant differences between the materials ($p > 0.05$)

Material	n	Vital	Non-vital	Total	% BV
ST	25	137.7 (251.4) ₁ [463.7 \pm 913.2]	108.5 (163.4) ₁ [274.4 \pm 540.0]	276.2 (506.4) ₁ [738.1 \pm 1434.2]	57.6 (19.4) ₁ [55.2 \pm 18.7]
Antiadhesive A	25	2.7 (37.0) ₂ [66.6 \pm 235.3]	44.0 (53.9) ₂ [135.4 \pm 286.1]	57.7 (84.9) ₂ [202.0 \pm 507.6]	6.4 (19.7) ₂ [15.3 \pm 20.8]
Antiadhesive B	25	10.8 (27.0) ₂ [141.9 \pm 604.2]	48.1 (89.2) ₂ [132.8 \pm 345.6]	54.1 (113.0) ₂ [274.7 \pm 946.9]	20.6 (18.9) ₂ [23.6 \pm 22.2]
Antibacterial A	25	5.0 (26.9) ₂ [105.0 \pm 321.7]	50.9 (70.6) ₂ [182.0 \pm 429.0]	53.3 (96.3) ₂ [287.0 \pm 712.2]	13.2 (33.6) ₂ [22.2 \pm 23.0]
Antibacterial B	25	41.6 (160.4) ₁ [298.7 \pm 926.5]	52.5 (138.3) _{1,2} [206.6 \pm 620.0]	111.6 (286.9) _{1,2} [505.3 \pm 1545.3]	55.6 (18.4) ₁ [51.9 \pm 17.7]

Abbreviations: n, number; ST, unmodified material (Control)

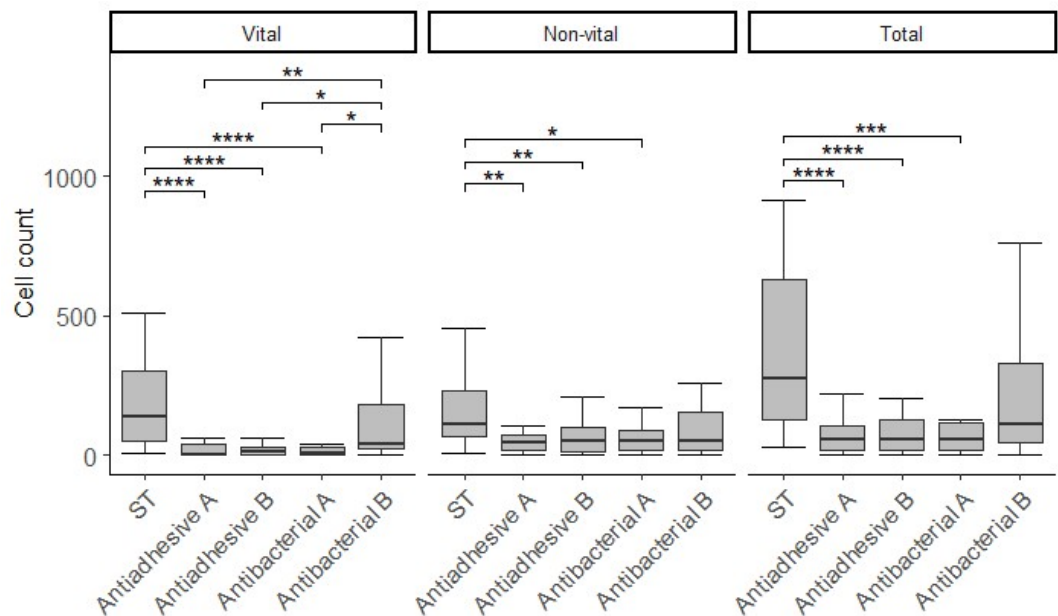


Figure 2. Tukey box plots of vital, non-vital and total bacteria cell count without outliers. Significant differences are bracketed with asterisks (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

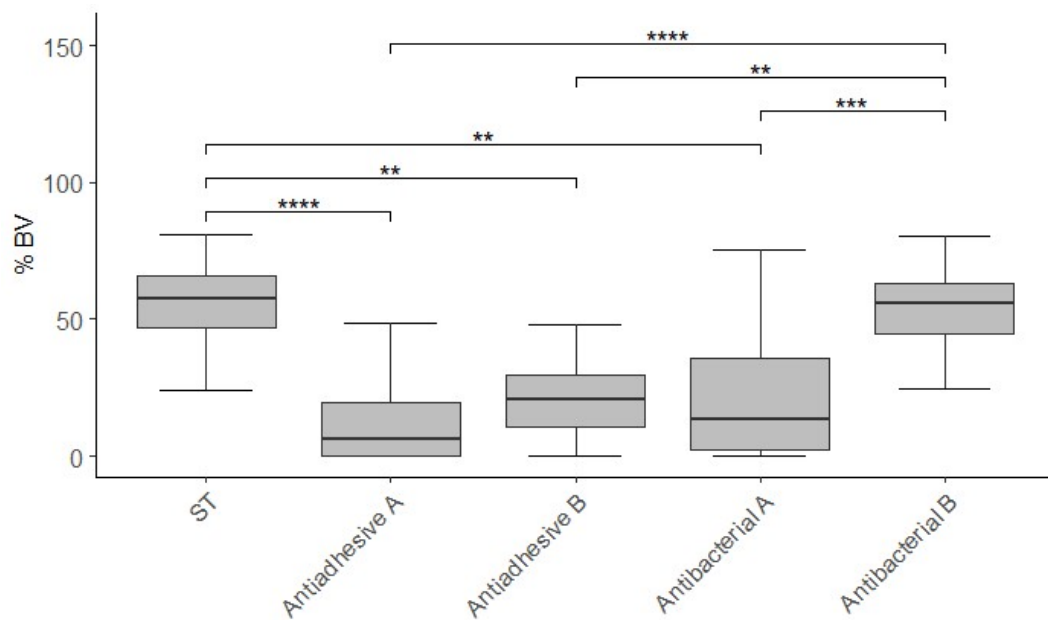


Figure 3. Tukey box plots of BV without outliers. Significant differences are bracketed with asterisks (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

All materials but Antibacterial B showed significant fewer vital bacterial cells than ST (all $p < 0.0001$) (Figure 4). The Antibacterial B material had significant more vital bacterial cells than the other modified materials (all $p < 0.05$).

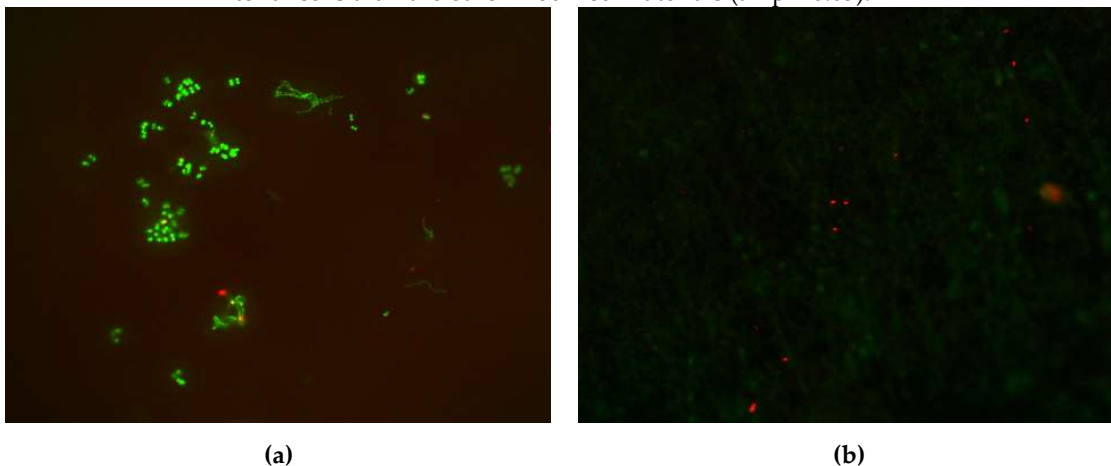


Figure 4. Comparison of representative superimposed fluorescence microscopic images (magnification 400-fold) of vital (green) and non-vital (red) bacterial cells from a single participant (a) ST accumulated many vital and a few non-vital bacterial cells (b) Antiadhesive A shows no vital but some non-vital bacterial microorganisms.

Considering the non-vital bacterial cells, all test materials had significant fewer cells than ST (all $p < 0.05$) except Antibacterial B. The same could be observed for the total cell count, where all materials had fewer cells than ST except Antibacterial B (all $p < 0.001$).

A lower ratio of vital to total cells (BV) could be demonstrated for all materials but Antibacterial B in comparison to ST (all $p < 0.01$). The Antibacterial B material had a higher BV than the other modified test materials (all $p < 0.01$).

Discussion

The determination of cell viability by live/dead staining and subsequent measurement of the fluorescence emission is a common and established method [5,15,28,29,32,37-41].

ST and four modified experimental dental resin composites with appropriate flexural strength, flexural modulus, polymerization shrinkage, water sorption, solubility, contact angle θ , surface free energy (SFE) and biocompatibility of the author's previous in-vitro studies were selected for their promising antibacterial effects [28,29,32,42,43]. All test materials are in accordance with the standard requested by EN ISO 4049 [33]. The preparation of ST and the modified materials by substituting ST's glass filler with the delivery system based on Poly-Pore [44] or by substituting the monomer matrix of ST with Methacryl-Irga [45-47] corresponds to the previously described procedure [28,29,32,42,43].

Consequently ST and the modified materials Antiadhesive A, Antiadhesive B or Antibacterial A do not differ in the type of matrix but only in the substitution of filler parts by loaded Poly-Pore to release the active agents TegoProtect 500, Dimethicone or Irgasan. The monomer matrix of material Antibacterial B contained the polymerizable Methacryl-Irga as the only additive compared to ST. Due to the high irradiance of the light curing device [28,29,48-50] and a very low reported solubility (0.2 ± 0.8 to $1.0 \pm 1.0 \mu\text{g mm}^{-3}$) of all modified test materials and ST [29,32] an optimal polymerization can be expected [28,29,32,48-52]. Therefore an antibacterial effect of the residual monomers is very unlikely although the degree of polymerization was not measured [28,29]. In addition there was no difference in polymerization shrinkage between the modified test materials and ST reported, which also indicated a good degree of conversion [29,32,53-57].

The surface roughness of all specimens was altered standardized by polishing the surface to simulate oral abrasion processes as reported in previous studies [28,29]. The significance of the surface roughness R_a on bacterial adherence was discussed thoroughly in the literature [4-8,20,58,59] and by the authors [28,29,32]. In summary $R_a \leq 0.2 \mu\text{m}$ was judged to have a negligible effect [5,8,58,59]. In consequence R_a is assumed not to be a relevant factor based on the results of the author's previous studies [28,29,32].

As we expected our materials to have the most interesting effect at the beginning of bacterial colonization, the splint wearing time was limited to 8h. The investigation of the test materials effect on cell viability at a very early stage of colonization is in accordance with the author's previous in-vitro studies [28,29].

The results presented in

Table 4 demonstrate the antibacterial effects of both antiadhesive materials and Antibacterial A in comparison to ST. Although the previous in-vitro studies with the investigation of antibacterial effects of the test materials on individual bacterial strains of the early colonizers *A. naeslundii*, *A. viscosus*, *S. mitis*, *S. oralis* and *S. sanguinis* were sophisticated [28,29], they could only be partially observed clinically.

In the present study both antiadhesive materials and Antibacterial A showed significant fewer vital, non-vital and total cells in comparison to ST. This effect could not be observed at all on the vital bacteria cells in previous in-vitro studies for *S. mitis* and hardly any for *A. naeslundii* [28,29]. Consequently the role of *S. mitis* and partly of *A. naeslundii* in the early colonization of the mentioned materials can be questioned in the present study, given the fact that *S. mitis* with low total SFE γ_S was reported to adhere better to low γ_S -materials like both antiadhesive test materials ($\gamma_S \leq 29.9 \pm 2.7 \text{ mJ m}^{-2}$) than to high γ_S -materials like ST and Antibacterial B ($\gamma_S \geq 42.9 \pm 1.3 \text{ mJ m}^{-2}$) [24,60].

Regarding the in-vitro results of non-vital and total cells, none of the three materials showed fewer cells than ST [28,29]. This demonstrates, to some degree, the antibacterial effects of the test materials modified surfaces on the bacterial cell adherence in the presence of saliva. The effect is very likely due to strong repulsive forces between the active agents and the aqueous oral medium, which quasi-force the active agents to form a new thin, floating hydrophobic surface layer [28,32]. Under the given circumstances, the bacteria might not have been able to adhere directly to the materials surfaces but only to the floating layer and therefore they might have been washed off by saliva, which was missing in-vitro. The lack of improvement of Antibacterial B with polymerized Methacryl-Irga in the present study compared to the other modified test materials with Poly-Pore loaded agents supports this hypothesis. On one hand Antibacterial B showed a clear antibacterial effect in-vitro on the cell viability for most of the early colonizers compared to ST [28], which could not be observed in the present study. On the other hand there were no differences between the in-vitro results regarding non-vital and total cells for most of the colonizers [28] and the results of the present study.

In addition, the lack of correlation between the reported contact angle θ [29,32] and the test materials total bacterial counts in the present study supports the assumption that the material chemistry dominates cell adhesion [28]. The association of θ and bacterial adhesion has already been extensively discussed in previous studies [28,29,61-63]. Overall composite resins are assumed to be more resistant against attack by water or water-soluble species with higher hydrophobicity [63-65]. Contrarily it was also hypothesized that hydrophobic surfaces support the cell adhesion by removing water more easily between bacterial cells and the material and thus allowing a closer approach with stronger adhesive forces between cell surface and hydrophilic material [61]. However, compared to ST both antibacterial materials did not show statistically significant different contact angles θ [29]. Nevertheless Antibacterial A had significant lower bacterial counts in the present study, allowing the conclusion of the materials chemistry influence. It should also be noted that the two antiadhesive test materials were the only ones with previously measured significant lower total SFE γ_S than ST (both $\gamma_S < 30 \text{ mJ m}^{-2}$) [32] and thus according to Vogler's interpretation hydrophobic by definition [66], which currently resulted in fewer cells for these materials. This coincided with in vivo studies, which show low supragingival plaque formation and thus low adhesion and biofilm formation for low γ_S substrata [5,9].

Furthermore taking the reported polar γ_S^{AB} values of the SFE in account all materials (γ_S^{AB} between $-2.4 \pm 1.3 \text{ mJ m}^{-2}$ and $-0.8 \pm 0.7 \text{ mJ m}^{-2}$) but Antibacterial B ($\gamma_S^{AB} 4.3 \pm 1.7 \text{ mJ m}^{-2}$) were reported to have significantly lower values than ST ($\gamma_S^{AB} 3.7 \pm 2.0 \text{ mJ m}^{-2}$) [29,32]. High polar term γ_S^{AB} was found to create strong bacterial adhesion, which implies that the low γ_S^{AB} might have reduced bacterial adhesion for all the modified test materials but Antibacterial B [19,28,67].

All in all biofilm formation is very complex and does not include only bacterial interaction. Therefore, protein adhesion on pellicle-coated surfaces should also be investigated

in further studies. In addition the comparison of previous in-vitro results with the present results is limited because numerous interactions may have occurred in the oral cavity that may have influenced the results and were not followed up.

5. Conclusions

The present study demonstrated the protective effect of experimental dental resin composites modified with small amounts of novel antiadhesive or antibacterial loaded delivery system. The sorption material being part of the delivery system might be used as a vehicle for any other, perhaps an even more effective, active agent. Based on the results of the study the null hypothesis has to be rejected for all test materials but Antibacterial B, as they showed significant differences to the unmodified control composite resin ST.

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