Bacterial Viability and Physical Properties of Antibacterially Modified Experimental Dental Resin Composites

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Abstract

Purpose: To investigate the antibacterial effect and the effect on the material properties of a novel delivery system with Irgasan as active agent and methacrylated polymerizable Irgasan when added to experimental dental resin composites.

Materials and Methods: A delivery system based on novel polymeric hollow beads, loaded with Irgasan and methacrylated polymerizable Irgasan as active agents were used to manufacture three commonly formulated experimental resin composites. The non-modified resin was used as standard (ST). Material A contained the delivery system providing 4 % (m/m) Irgasan, material B contained 4 % (m/m) methacrylated Irgasan and material C 8 % (m/m) methacrylated Irgasan. Flexural strength (FS), flexural modulus (FM), water sorption (WS), solubility (SL), surface roughness Rₐ, polymerization shrinkage, contact angle Θ, total surface free energy γₛ, polar γₛ⁺, Lewis acid γₛ⁺, Lewis base γₛ⁻, and its apolar γₛ₋₋ term as well as bacterial viability were determined. Significance was p < 0.05.

Results: The materials A to C were not unacceptably influenced by the modifications and achieved the minimum values for FS, WS and SL as requested by EN ISO 4049 and did not differ from ST what was also found for Rₐ. Only A had lower FM than ST. Θ of A and C was higher and γₛ⁺ of A and B was lower than of ST. Materials A to C had higher γₛ⁺ than ST. The antibacterial effect of materials A to C was significantly increased when compared with ST meaning that significantly less vital cells were found.

Conclusion: Dental resin composites with small quantities of a novel antibacterially doped delivery system or with an antibacterial monomer provided acceptable physical properties and good antibacterial effectiveness. The sorption material being part of the delivery system can be used as a vehicle for any other active agent.

Citation: Rüttermann S, Trellenkamp T, Bergmann N, Beikler T, Ritter H, et al. (2013) Bacterial Viability and Physical Properties of Antibacterially Modified Experimental Dental Resin Composites. PLoS ONE 8(11): e79119. doi:10.1371/journal.pone.0079119

Editor: Ali Al-Ahmad, University Hospital of the Albert-Ludwigs-University Freiburg, Germany

Received June 12, 2013; Accepted September 19, 2013; Published November 1, 2013

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Funding: This study was supported by the DFG Deutsche Forschungsgemeinschaft, Bonn, Germany (Project No. RU 825/3-1). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

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Introduction

Several attempts have been made to modify dental resin composites to avoid or at least to diminish pellicle and bacterial adherence which is considered to be important in early plaque formation [1-7]. Mainly two concepts were investigated to reduce bacterial adherence: (a) alteration of the resin matrix by adding fluoride-releasing materials [8], silver nanoparticles [9], fluorne polymers [10], antimicrobial monomers, polymers or additives [11-14] or quaternary ammonium polyethylenimine nanoparticles [15,16], (b) reduction of the materials’ wettabilities since some evidence was found that materials with low wettability, meaning low surface free energy (SFE), resulted in significantly less bacterial adherence and thus less plaque [10,17-23]. But solely quaternary ammonium polyethylenimine nanoparticles of concept (a) were reported to have strong antibacterial activity without affecting flexural strength and modulus of dental resin composites [15,16]. Also concept (b) was challenged by other studies reporting no strong correlation of streptococcal adhesion and substratum surface roughness or SFE, respectively [23-25], and found that SFE’s influence on bacterial adhesion significantly decreased after saliva coating [23,26].

More recent literature presented an entirely new approach to obtain dental resin composites with low SFE, antimicrobial effect and acceptable physical properties [4,5]. Novel polymer
The material's SFE.

were always present in the outer material's surface, (b) quantities to experimental resin-based restorative materials. It (2,4-dichlorophenoxy)phenyl methacrylate, Table 1) and the Methacryl-Irga 5-chloro-2-(2,4-dichlorophenoxy)phenyl methacrylate

Methacryl-Irga 5-chloro-2-(2,4-dichlorophenoxy)phenyl methacrylate

Poly-Irga loaded with 80% Irgasan, D=1.0 [g cm⁻³]

experimental products

university laboratory

Table 1. Raw material.

| Code       | Product / properties                                                                 | Batch      | Company                           |
|------------|--------------------------------------------------------------------------------------|------------|-----------------------------------|
| Photoini   | α,α-dimethoxy-a-phenylacetoephonene                                                   | 0066162S   | Ciba Specialties Chemical Inc.    |
|            |                                                                                      |            | Basel, Switzerland                |
| Stab       | pentaerythritol-tetakis[3-(3,5-di-tert.-butyl-4-hydroxyphenyl)-propionate              | 26099C3    | Ciba Specialties Chemical Inc.    |
|            |                                                                                      |            | Basel, Switzerland                |
| TTEGDMA    | tetraethylenglycole dimethacrylate, standard monomer, functionality=2, MW=330 [g mol⁻¹]. good chemical and physical properties, very low viscosity (14 Pa s, 25 °C), diluting |            |                                    |
| UV-Stab    | 2-hydroxy-4-methoxy-bezophenone                                                        |            |                                    |
|            |                                                                                      |            |                                    |
| UDMA       | 7.7,9-trimethyl-4,13-dioxo-3,14-doxia-5,12-diaza-hexadecon-1,16-diol-dimethacrylate, standard monomer, functionality=2, MW=471 [g mol⁻¹], flexible, tough, very good chemical resistance, medium viscosity (10000 mPa s, 25 °C) |            |                                    |
| Bis-GMA    | Bis-GMA, standard monomer, functionality=2, MW=513 [g mol⁻¹], rigid, very good chemical resistance, very high viscosity (4500 mPa s, 60 °C) | 2008218303 | Rahn AG. Zürich. Switzerland       |
| CQ         | D,L-camphorquinone                                                                   | 0148990002 | Rahn AG. Zürich. Switzerland       |
| Aminie     | ethyl-4-(dimethylamino)-benzoate                                                       | 310170     | Rahn AG. Zürich. Switzerland       |
| Glass      | strontiumborosilicate glass (Glass G0 18-093, 0.7 µm), silaned (3- methacryloyloxypropylmethoxy silane), D=2.6 [g cm⁻³]  | Lab14701   | Schott Electronic Packaging GmbH. Landschul Germany |
| Poly-      | Poly-Pore, cross-linked polyallyl methacrylate, adsorber, hollow beads, diameter 20 - 40 µm | L07070303AB | AMCOL Health & beauty Solutions, Arlington Heights, IL, USA |
| Irga       | Irgasan, 5-chloro-2-(2,4-dichlorophenoxy)phenole                                       | 1124816    | Sigma Aldrich GmbH, Steinheim, Germany |
| Methacryl-Irga 5-chloro-2-(2,4-dichlorophenoxy)phenyl methacrylate | university laboratory |            |                                    |
| Poly-Irga  | loaded with 80% Irgasan, D=1.0 [g cm⁻³]                                              |            |                                    |

Information is based on the manufacturers' technical data sheets

doi: 10.1371/journal.pone.0079119.001

hollow beads (Poly-Pore, Table 1) used as a carrier material, were highly loaded with different types of low surface tension agents resulting in a delivery system which was added in small quantities to experimental resin-based restorative materials. It was hypothesized and found that (a) single delivery particles were always present in the outer material's surface, (b) occurring abrasion processes, simulated by polishing procedures, destroyed the delivery particles, and (c) the low surface tension agents flushed the surface and thus reduced the material's SFE.

The goal of the present investigation was, based on the new concepts of the aforesaid literature [4,5], to examine the material properties (flexural strength, modulus, water sorption, solubility, surface roughness, polymerization shrinkage, contact angle, surface free energy) of four antibacterially modified experimental dental resin composites and the bacterial viability (A. naeslundii, A. viscosus, S. oralis, S. mitis, S. sanguinis) after 8 or 24 hours, respectively, on these materials. One experimental dental resin composite was partly replaced by polymerizable Methacryl-Irga (Table 1 and 2, Figure 2) to obtain materials B and C. Flexural strength, flexural modulus, water sorption, solubility and surface roughness Rₐ were determined. Curing was done with a quartz-tungsten halogen device (Spectrum 800, Dentsply De Trey GmbH, Constance, Germany) performing an irradiance of 931 ± 90 mW cm⁻² which was checked periodically with the bluephase meter (Ivoclar Vivadent AG, Schaan, Liechtenstein).

Materials and Methods

Four experimental resin-based restorative materials were prepared (Tables 1 and 2) using a laboratory vacuum planet kneader (Herbst Maschinenfabrik GmbH, Buxtehude, Germany). The standard ST represented a common formulation for resin-based restorative materials. Poly-Pore sorption material loaded with Irgasan (5-chloro-2-(2,4-dichlorophenoxy)phenole) as active agent (Tables 1 and 2, Figure 1) was the delivery system. ST was modified by replacing parts of the glass filler with the delivery system resulting in material A. The matrix of ST was partly replaced by polymerizable Methacryl-Irga (Table 1 and 2, Figure 2) to obtain materials B and C. Flexural strength, flexural modulus, water sorption, solubility and surface roughness Rₐ were determined. Curing was done with a quartz-tungsten halogen device (Spectrum 800, Dentsply De Trey GmbH, Constance, Germany) performing an irradiance of 931 ± 90 mW cm⁻² which was checked periodically with the bluephase meter (Ivoclar Vivadent AG, Schaan, Liechtenstein).
Manufacturing of the delivery system

Irgasan (Table 1) was dissolved in great excess of butanone (Lot 244238, Brenntag GmbH, Mülheim, Germany) and the Poly-Pore sorption material (Table 1) was added while stirring. The obtained thin slurry was stirred for approximately 10 minutes to optimally wet the sorption particles. Next, while stirring, the mixture was slightly warmed to evaporate the solvent. Stirring was stopped when the mixture became too stiff and it was put in a drying closet at 50 °C until constant weight was obtained (generally 24 hours). After this treatment the loaded sorption material, representing the delivery system, appeared totally dry and powdery.

Analytical data

\[ \text{\textsuperscript{1}H-NMR (CDCl}_3\text{): } \delta = 1.94 (t, \text{J(H,H) = 1.28 Hz, 3H, methyl -CH}_3\text{);} 5.69 (q, \text{J(H,H) = 1.47 Hz, 1H, acryl =CH}_2\text{);} 6.18 (t, \text{J(H,H) = 1.05 Hz, 1H, acryl =CH}_2\text{);} 6.86 (dd, \text{J(H,H) = 8.73 Hz, 1H, aryl}); 6.89 (d, \text{J(H,H) = 8.73 Hz, 1H, aryl}); 7.14 (dd, \text{J(H,H) = 8.73 Hz, J(H,H) = 2.47 Hz, 1H, aryl}); 7.41 (J, \text{J(H,H) = 2.46 Hz, 1H, aryl}) \text{ppm.} \]

\[ \text{\textsuperscript{13}C-NMR (CDCl}_3\text{): } \delta = 18.21 (\text{methyl -CH}_3\text{);} 119.91 (\text{aryl}; 120.66 (\text{aryl}); 124.59 (\text{aryl =CH}_2\text{);} 125.69 (\text{aryl C-Cl}; 126.93 (\text{aryl); 128.03 (aryl); 128.1 (aryl); 129.23 (aryl C-Cl); 129.46 (aryl C-Cl); 130.33 (aryl); 134.81 (aryl C=C); 142.06 (aryl -ether C-O); 146.5 (aryl -ester C-O); 151.05 (aryl -ether C-O); 164.61 (ester C=O) ppm.} \]

\[ \text{FT-IR (diamond): } \nu = 3092 (\text{w, =CH}_2\text{)}, 2983 (\text{w, CH}), 2875 (\text{w, CH}), 1741 (\text{ss, C=O}), 1636 (\text{ss, C-C}), 1098 (\text{w, C-O-C}) \text{cm}^{-1}. \]

\[ \text{GC/MS m/z (%): 361 (3), 360 (20), 358 (56), 357 (8), 356 (60) [M]+, 252 (20), 189 (10), 69 (100), 41 (14).} \]

| Experimental resin-based filling materials, formulations [weight-%] |
|-----------------|---|---|---|---|
| ST  | A   | B   | C   | Raw material |
| 73.00 | 68.00 | 73.00 | 73.00 | Glass |
| ---   | 5.00  | ---  | ---  | Poly-Irga |
| ---   | ---   | 4.00  | ---  | Methacryl-Irga |
| 27.00 | 27.00 | 23.00 | 19.00 | Matrix |

Matrix: UDMA=44.10, Bis-GMA=30.00, TEGDMA=25.00, photoinitiator=0.30, CO=0.20, amine=0.10, stabilizer=0.10

doi: 10.1371/journal.pone.0079119.t002

Flexural strength and flexural modulus

Ten specimens (25 ± 2 x 2 ± 0.1 x 2 ± 0.1 mm) were made from each material according to ISO 4049 [33] and cured in five 40 s increments from each side (400 s in total). Testing was done after 24 hours of water storage at 37 °C with the three-point-bending test (universal testing machine, crosshead speed of 0.75 mm min\(^{-1}\), Model 106.L, Test GmbH, Erkrath, Germany).

Flexural strength was calculated by \( \sigma = (3FL) / (2bh^2) \) and flexural modulus by \( E = (L/4bh^3) x (F/Y) \) both expressed in MPa with \( F = \) maximum strength, \( L = \) distance between the rests, \( b = \) width of the specimen, \( h = \) height of the specimen, and \( F/Y = \) slope of the linear part of the stress-strain curve.

Water sorption and solubility

Ten disks (thickness: 1 ± 0.1 mm, diameter: 15 ± 0.1 mm) were made from each material according to ISO 4049 [33] and cured in eight overlapping steps of 40 s each side (320 s in total). The specimens were dried and weighed (analytical balance, Toledo XS, Mettler Toledo GmbH, Greifensee, Switzerland) until mass \( m_1 \) was constant. Their volumes \( V \) were determined by measuring their diameters and thicknesses.
After 7 days water storage at 37 ± 1 °C mass \( m_2 \) was weighed. Then the specimens were totally re-dried to obtain mass \( m_3 \). Water sorption was calculated by \( W_{sp} = (m_2 - m_3) / V \) and solubility by \( W_{sl} = (m_1 - m_3) / V \) both expressed in \( \mu g \) mm\(^{-3}\).

**Surface roughness \( R_a \)**

After contact angle measurement the specimens were polished again and their surface roughness \( R_a \) was determined (Surftest SJ-210 profilometer, diamond pick-up, tip radius: 5 μm, load: 4 mN, Mitutoyo Corporation, Kawasaki, Japan). Each specimen was measured five times at different distances and in different directions (evaluation length of 0.8 mm, stylus speed 0.5 mm s\(^{-1}\)) from its center. For each measurement, the stylus was automatically moved five times forward and backward along the same path. The data were filtered with a cut-off (\( \Lambda_c \)) of 0.8 mm (Gauss profile-filter) following DIN EN ISO 4288 [34].

**Polymerization shrinkage**

Polymerization shrinkage was determined according to the Archimedes’ principle (Density Determination Kit, Mettler Toledo GmbH, Gießen, Germany). After weighing the specimens in air and in water, their densities were calculated in g cm\(^{-3}\) by \( D = (A/(A-B)) \times (D0-DL) + DL \) with \( D \) = density of sample, \( A \) = weight of sample in air, \( B \) = weight of sample in water, \( D0 \) = density of water, and \( DL \) = air density (0.0012 g cm\(^{-3}\)). Firstly the density \( D1 \) of the uncured material (sphere-shaped, approximately 0.1 g) was determined. Next, disks (diameter 10 ± 0.1 mm, thickness 1 ± 0.1 mm) were prepared, polymerized for 40 s from each side and the density \( D2 \) was measured. The polymerization shrinkage in % was calculated by \( \Delta V = ((1 / D^2) - (1 / D1)) \times (1 / D1) \times 100 \).

**Contact angle, SFE of solids and surface tension of active agents**

Contact angles were determined with the sessile drop method (Phoenix-Alpha contact angle goniometer, Surface Electro Optics - SEO - Corporation, Suwon-City, Korea). Ten pictures of each drop were photographed (magnification 25-fold) in one second (CCD-camera: FireDragon, Toshiba-Teli Corporation, Tokyo, Japan) and analyzed (Image XP-software, Version 6.0 FW 012108, SEO Corporation). To determine contact angles and SFE, ten discs (diameter 10 ± 0.1 mm, thickness 1 ± 0.1 mm) of each material were prepared and polymerized for 40 s from each side. Their surfaces were polished with fine and superfine polishing-discs (Super-Snap mini, Shofu Inc., Kyoto, Japan) for 1 minute each with a grinding pressure of 40 - 50 g and 10000 rpm (Endo-Mate TC, Nakanishi Inc., Tochigi, Japan). Contact angle Θ was measured one time on each specimen. Measurements with aqua dest. (laboratory product) were done before and after three weeks water storage at 37°C. Measurements with glycerol (LOT II1097071, Thermo Scientific, Rockford, IL, USA), ethylene glycol (LOT 3289749, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) and diiodomethane (LOT S82251,
Sigma Aldrich GmbH, Taufkirchen, Germany) were solely done after three weeks water storage at 37°C. SFE was calculated from the Θ measured after water storage. Apolar Lifshitz-van der Waals $\gamma_{LW}$, polar Lewis acid $\gamma_{L}^+$, and Lewis base $\gamma_{L}^-$ terms of the test liquids were taken from the literature [32] and the specimens' total SFE $\gamma_{S}$, their apolar term $\gamma_{S}^{AB}$, acid term $\gamma_{S}^+$ and base term $\gamma_{S}^-$ were calculated according to the equation of van Oss et al. [35] by $\cos \Theta = 1 \times \gamma_{S} = 2(\sqrt{\gamma_{LW}^2 + \gamma_{L}^+ \gamma_{L}^-}) + \sqrt{(\gamma_{LW}^2 + \gamma_{L}^+ \gamma_{L}^-)}$ and with $\gamma_{S}^{AB} = 2(\gamma_{S}^+ \gamma_{S}^-)$ (Image XP, Version 6.0 FW 072809, SEO Corporation).

**Bacteria preparation**

*Streptococcus sanguinis* (strain 20068), *Streptococcus oralis* (strain 20627), *Streptococcus mitis* (strain 12643), *Actinomyces viscosus* (strain 43329), and *Actinomyces naeslundii* (strain 17233, all strains from DSMZ GmbH, Braunschweig, Germany) were used to determine cell viability. Bacteria were exposed on agarplates and incubated for 48 hours at 37 °C (actinomyces under anaerobic conditions). Overnight cultures of the streptococci were established in sterile trypticase soy broth (Sigma-Aldrich Inc., St. Louis, MO, USA) supplemented with yeast extract (Carl Roth GmbH, Karlsruhe, Germany) at 37°C. Single colonies of the actinomyces were cultivated in sterile actinomyces broth (Vegiton, Sigma Aldrich GmbH) for 24 hours at 37 °C. The bacteria were harvested by centrifugation (1000 rpm, 10 min, 18 °C), washed twice with a sterile 0.9 % NaCl solution and re-suspended again in sterile 0.9 % NaCl solution. Optical density of the suspensions was adjusted to 1 at 600 nm (Smart Spec plus, Biorad Laboratories Inc., Hercules, CA, USA), which corresponded to a microbial concentration of 5 x 108 cells ml$^{-1}$.

**Salivam preparation**

Unstimulated human saliva was collected from ten healthy non-smoking subjects (age 28 - 58, mean 37.7 years). Approval from the ethics committee was obtained (Ethics committee of the Medical Faculty of Heinrich-Heine-University, Düsseldorf, Germany, internal study number: 2912). The participants gave their consent verbally, as they all were part of the academic faculty staff of the department. Documentation of the oral consent was done by making a list of prospective saliva donors. All donors who subsequently gave their oral consent were check-off on this list. The oral consent was explicitly judged as adequate, and the need for written informed consent from the participants was waived by the ethics committee. The saliva samples were all mixed with each other, so that they could not be identified anymore after sampling. The saliva was centrifuged (30 min, 4500 rpm, 4 °C U, Universal 16R, Hettich GmbH, Tuttingen, Germany) and the supernatant was sterile filtered (Millex-GV, 0.45 μm, PVDF, 3 mm, Millipore Inc., Billerica, MA, USA) and subsequently heated to 56 °C for 30 minutes (NeoBlock Heizer Mono I, neoLab Migge Laborbedarf-Vertriebs GmbH, Heidelberg, Germany). Finally the samples were pooled and stored at -20 °C. Prior to incubation samples were defrosted and 1:1 diluted with PBS (Dulbecco’s Phosphate Buffered Saline modified, Sigma-Aldrich Inc.).

**Specimen preparation and incubation**

Thirty disks (thickness: 1 ± 0.1 mm, diameter: 10 ± 0.1 mm) of each material were made and cured 40 s each side (Spectrum 800, Dentsply deTrey GmbH, Constance, Germany). The output of the curing device was routinely checked (bluephase Meter, Ivoclar Vivadent AG, Schaan, Liechtenstein). Irradiances of 931 ± 90 mW cm$^{-2}$ were measured and no significant decrease of the output was observed. The cured specimens were stored for 14 days in water at 37°C, disinfected (Bacillol AF®, Bode Chemie, Hamburg, Germany) and then one side was wet-polished (sterile water) with fine and superfine polishing-discs (Super-Snap mini, Shofu Inc., Kyoto, Japan) for 1 min, with a grading pressure of 40 - 50 g and 10000 rpm (Endo-Mate TC, Nakanishi Inc., Tochigi, Japan). Afterwards they were placed with the polished side up in a well (TC Test Plate 24 wells, Orange Scientific INC., Braine-l’Alleud, Belgium), incubated with 250 μl of the diluted saliva for 2 hours at 37 °C and finally washed twice with a sterile 0.9 % NaCl solution.

**Determination of cell viability (live/dead staining)**

Three specimens (polished sides) for each material / bacterium / measurement point were used and incubated with 350 μl bacterial suspension for 8 hours or 24 hours, respectively, at 37 °C (streptococci at 5 % CO$_2$, actinomyces at anaerobic conditions), then washed with 0.9 % NaCl solution and air-dried. Vital and non-vital cells were determined (LIVE/DEAD BacLight bacterial viability kit, Molecular Probes Inc., Eugene, OR, USA) by measuring the fluorescence emission (fluorescence microscope DM2500, Leica Microsystems Ltd., Wetzlar, Germany) on the blinded specimens. Fluorescent microscopic images of four randomly selected sites of each specimen (magnification 400 fold) were captured (digital camera DFC420C, Leica Microsystems Ltd.) with different fluorescence filter sets (N2.1 and I3, Leica Microsystems Ltd.). Vital and non-vital cells were calculated by counting pixel per colour (pixel count software, written by a stuff member of the department).

**Statistical analysis**

Means and standard deviations were calculated. Normal distribution was tested by Kolmogoroff-Smirnoff-Test. Univariate ANOVA and post hoc Scheffé’s test were performed separately for each tested property and for each test material and bacterial subgroup (total, vital, non-vital). T-Test was used to identify differences of the contact angle between dry and wet storage and to find differences between the 8 hour and 24 hour bacteria counts. Multivariate ANOVA and post hoc Scheffé’s test were calculated to identify differences between the test materials’ SFEs (SPSS 15.0, SPSS, Chicago, IL, USA). Statistical significance for all tests was considered as p < 0.05.

**Results**

Table 3 shows that material A had the lowest flexural modulus of all materials (all p > 0.0005). The water sorption decreased from ST to A, B and C (all p > 0.0005). The shrinkage of test materials did not differ from ST. The surface
Bacterial viability after 8 hours observation time

For the overall bacteria the total count of material A was higher but of B and C lower than of ST (all p < 0.0005). Materials A to C had less vital cells and A had more non-vital cells than ST (all p < 0.0005). Considering the individual species the total bacteria count was lower for A. naeslundii on material B (p = 0.015) and for A. viscosus and S. sanguinis on materials B and C than on ST (p < 0.0005 to p = 0.002). Higher total bacteria counts were observed for A. viscosus and S. mitis on material A (all p < 0.0005). Less vital cells were found for A. viscosus, S. oralis and S. mitis on materials A to C (p < 0.0005 to p = 0.031) but for A. naeslundii only on A (p = 0.011) and B (p = 0.003) and for S. mitis only on B and C than on ST (all p = 0.010). More non-vital bacteria were counted for each individual species on material A (all p < 0.0005). Materials A, B and C did not differ among each other in the vital cells of S. sanguinis after 8 hours. S. oralis had less vital cells (p = 0.002) on material A than on C.

Bacterial viability after 24 hours observation time

For the overall bacteria consideration the total bacteria count decreased only on material C compared with 8 hours (p = 0.012). Materials B and C showed less vital (p < 0.0005 to p = 0.048), ST and B showed more non-vital (P = 0.001 and p = 0.017) but A showed less non-vital cells (P = 0.005). Considering the individual species less total actinomyces were found on materials B and C compared with ST (P = 0.006 to p = 0.014) This was also observed for S. mitis and S. sanguinis (p < 0.0005 to p = 0.039), but not for S. oralis. Less vital actinomyces also existed on A, B and C (p < 0.0005 to p = 0.003) but for S. mitis and S. sanguinis less vital cells existed only on B and C (p = 0.002 to p = 0.011) and for S. oralis only on A (p = 0.044). Less vital actinomyces lived on material C (p < 0.0005 to p = 0.002) but more total (p = 0.033) and more non-vital (p = 0.005) A. naeslundii were on material B. Total and non-vital S. mitis cells were only influenced by material A (p = 0.006 and p = 0.009) resulting in a decrease. The vital cells of S. oralis decreased on ST, B and C (p = 0.016 to p = 0.046) but of S. sanguinis only on ST and C (P = 0.024 and p = 0.002).

Materials A, B and C did not differ among each other in the vital cells of the overall bacteria, of the actinomyces species, of S. mitis and S. oralis S. sanguinis showed more vital cells (p = 0.009) on materials A and C. Two examples of the vital fluorescence pictures can be seen in Figures 3 and 4.

Discussion

The preparation of ST and the experimental dental composites [4,5], the determination of the materials properties [17,18,21,33,35-38] and of the cell viabilities [4,18,39-44] followed well-established methods and are exactly in accordance with the authors earlier publications [4,5]. It could be criticized, that the CFUs were not counted, but instead the number of pixels was assessed. Doing this only enables to compare the data between the same bacterial species, as the bacteria have different sizes. Nevertheless, we chose a computer-based assessment, as this method is more objective.
and less sensitive for mistakes. ST of the previous studies [4,5] was used and the experimental dental resins were formulated by substituting ST’s glass filler with the Poly-Irga delivery system, based on Poly-Pore, a novel highly effective sorption material [45], or by substituting the monomer matrix by Methacryl-Irga representing an antibacterial monomer [30-32]. An antibacterial effect of residual monomers was very unlikely because ST and the experimental materials did not differ in the type of the matrix or the filler but only in the addition of the Poly-Irga delivery system or the Methacryl-Irga. Although the degree of polymerization was not measured an antibacterial effect of residual monomers is very unlikely. ST and material A did not differ in the type of monomers but only in the amount of the loaded Poly-Pore replacing parts of the filler. The materials B and C contained the polymerizable Methacryl-Irga but due to the high irradiance of the light curing device and the very low solubility (0.2 ± 0.8) to 0.4 ± 0.3 µg mm-3) of all test materials and ST an optimal polymerization can be expected [4,5,37,38,46]. Furthermore, the test materials and ST did not differ in polymerization shrinkage what also indicated a good degree of conversion. These assumption are based on the literature which proved the relation between degree of conversion on solubility [47,48] or polymerization shrinkage [49-53]. The significance of surface roughness R\text{a} on bacterial adherence was discussed thoroughly in the literature [18,36,54-59] and by the authors [4,5] and R\text{a} ≤ 0.2 µm was judged to have negligible effect [18,56-58], therefore, based on the results of this investigation (Table 3), R\text{a} is assumed not to be a factor. To mimic the salivary pellicle [60] the specimens were incubated in preparations of human saliva which was centrifuged and filtered to eliminate bacteria and heated to destroy lytic enzymes. Since the SFE of a saliva-coated bacterium was found to be same for original or heated (60 °C) saliva the heating process is considered to be allowed [26]. As already reported formerly [4] the typical protein spectrum appeared after gel electrophoresis which indicated the realistic simulation of in vivo pellicle formation. To investigate the experimental materials effect on cell viability at a very early stage of colonization, this investigation focused on the typical early colonizers of the oral biofilm [61]. As we expected our materials to have the highest impact at the beginning of bacterial colonization, we decided to investigate the early colonizers at first. It would be interesting for a following study to determine their effects on later colonizers like S. mutans or Lactobacilli, as these bacteria can also attach to the tooth/ restoration surface directly. Based on the literature [62-64] and the found highly significantly results (see Results section) a.
reduced number of micrographs and an area of 0.99 mm² were assumed to be appropriate for the present study.

According to Table 3 the modifications of ST with the PolyPore delivery system or with Methacry-Irga did not influence the material properties unacceptably. All test materials achieved the minimum values for flexural strength, water sorption and solubility as requested by EN ISO 4049 [33]. Interestingly the water sorption even decreased from ST to material C. In this order the contact angle Θ increased what might explain this behaviour (Table 4). None of the materials showed total SFE \( \gamma_s < 30 \text{ mJ m}^{-2} \) so that they were not really hydrophobic according to Vogler’s interpretation [65]. The polar terms \( \gamma_s^{AB} \) of materials A and B were significantly lower than of ST which even suggests a higher hydrophilicity.

The results of Table 5 prove the antibacterial effect of the modified composite resins A to C which had significantly less vital bacteria for the overall consideration as well as for most of the individual species and observation times. It is remarkable that the immobilized polymerizable Methacryl-Irga seems to be more effective (less vital cells) than the Irgasan which was set free from the delivery system (S. mitis for 8 and 24 hours and S. sanguinis for 24 hours). The results also show that amounts of 4 % (m/m) were adequate to reduce the number of vital cells significantly (material A and B) because no increased cell death was observed with 8 % (m/m) active agent in material C. S. oralis was the most resistant bacterium which did not differ in the vital cells from ST after 24 hours for material B and C and only material A had less vital cells for this observation time. This is the only case where the delivery system (material A) is more effective than Methacryl-Irga in 4 % (m/m) concentration (material B). Since materials A and B did not differ in their surface properties (Table 4) the higher sensibility of S. oralis against free Irgasan is the only plausible explanation.

Considering the total bacteria counts it is very striking that significantly less cells were found on materials B and C than on ST and mostly also on material A. Again S. oralis was the only exception from this observation. This means that S. oralis was not only significantly more resistant against Irgasan but also has very strong adhesive forces which were not reduced by the materials’ chemistry. Material A which provides free Irgasan due to the PolyPore delivery system did not show less total bacteria for none of the tested species but sometimes even a higher total bacteria count was calculated. This behaviour connected with the powerful antibacterial effect (less vital cells than ST) strongly indicated that an antibacterial effect did not necessarily diminish bacterial adhesive forces. As shown by the results for materials B and C the chemistry of the antibacterial ingredient needs also to be considered. Although
Table 5. Means and (standard deviations) of total, vital bacterial and non-vital bacterial pixel-count after 8 h and 24 h observation time.

| Material | Overall (n=60) | A. naeslundii (n=12) | A. viscosus (n=12) | S. mitis (n=12) | S. oralis (n=12) | S. sanguis (n=12) |
|----------|----------------|----------------------|---------------------|----------------|-----------------|------------------|
|          | 8h             | 24h                  | 8h                  | 24h            | 8h              | 24h              |
| ST       | 5.1 (3.3)      | 6.9 (5.1)            | 5.5 (2.4)           | 6.9 (4.4)      | 4.7 (2.7)       | 6.6 (5.6)        |
|          | vit            | 3.8 (3.1)            | 3.7 (3.7)           | 2.8 (2.2)      | 3.9 (3.0)       | 3.5 (2.6)        |
| non      | 1.4 (1.6)      | 2.3 (2.4)            | 2.7 (2.3)           | 3.1 (1.9)      | 1.2 (1.3)       | 1.0 (0.9)        |
| A        | 7.9 (3.3)      | 7.1 (4.0)            | 7.4 (4.1)           | 7.1 (3.8)      | 10.0 (1.1)      | 9.1 (3.8)        |
|          | 1.0 (1.1)      | 1.5 (1.6)            | 0.8 (1.1)           | 1.1 (1.3)      | 0.5 (0.3)       | 0.6 (0.4)        |
| non      | 6.9 (3.4)      | 5.7 (3.4)            | 7.5 (3.7)           | 6.7 (2.8)      | 9.4 (1.2)       | 7.5 (3.5)        |
| B        | 1.7 (1.0)      | 1.9 (1.8)            | 1.9 (1.0)           | 3.1 (1.2)      | 1.2 (0.9)       | 1.3 (1.1)        |
|          | 0.8 (0.9)      | 0.6 (0.8)            | 0.5 (0.4)           | 0.4 (0.3)      | 0.6 (0.3)       | 0.7 (0.8)        |
| non      | 0.9 (1.0)      | 1.3 (1.3)            | 1.4 (0.6)           | 2.7 (1.1)      | 0.6 (0.7)       | 0.7 (0.6)        |
| C        | 2.9 (3.0)      | 2.2 (2.0)            | 4.7 (2.2)           | 2.6 (0.9)      | 2.0 (0.5)       | 1.8 (1.2)        |
|          | 1.4 (1.5)      | 0.7 (1.0)            | 1.9 (1.4)           | 0.4 (0.4)      | 1.3 (0.4)       | 0.3 (0.3)        |
| non      | 1.6 (1.7)      | 1.6 (1.3)            | 2.7 (1.1)           | 2.1 (0.7)      | 0.6 (0.3)       | 1.5 (1.1)        |

Values are rounded to valid digits. Underlined numbers within the rows indicate non significant differences between the 8 h and 24 h observation period of each bacteria group. The same superscript number within the columns indicates non significant differences between total, vital or non-vital bacterial pixel-count, respectively (p > 0.05).

tot = total, vit = vital, non = non-vital

doi: 10.1371/journal.pone.0079119.t005

Inspirable in water, the surface concentration of the free Irgasan provided by the delivery system might be reduced during the manipulation processes of the specimens. This could not happen with the immobilized polymerizable Methacrlyl-Irga.

An influence of SFE on bacterial adherence was not clearly found in this study. As already discussed previously [4] a high polar term $\gamma_{p}$ was reported to create strong bacterial adhesion [21,66], which implies that that low $\gamma_{p}$ might lessen bacterial adhesive forces. Considering the total bacteria counts which might be an indicator for bacterial adhesion no relation was detected between the number of total bacteria and $\gamma_{p}$. Table 4 in combination with Table 5 shows that material A and B did not differ in $\gamma_{p}$ but material B had significantly less total bacteria for all species with the exception of $S. oralis$ and material C with the same $\gamma_{p}$ than ST had less total cells than ST and did not differ from B with lower $\gamma_{p}$. The differences of materials B and C for $\gamma_{v}$ were not surprising because Irgasan is a very hydrophobic substance and its concentration was twice as much in material C than in material B. It is hardly to explain why $\gamma_{v}$ of material A and B was highly significantly lower than of ST. It might be possible that the polar groups of Irgasan (Cl- and O-atoms) turned away from the hydrophobic parts of the matrix and levelled to the hydrophilic environment.

The much higher Methacrlyg-Irga concentration of material C might have overcompensated this effect which explains the increased $\gamma_{v}$.

The present study is certainly limited by the fact that the antibacterial effects needs not to be investigated in-vitro but also in-vivo. Furthermore, other antibacterial substance or combinations thereof should be included in the delivery system. To test combinations of the present antibacterial monomers or delivery systems with the bacteria-repellent formulations of previous investigations [4,5] would certainly be of great interest.

Conclusion

Experimental dental resin composites modified with small quantities of a novel antibacterially doped delivery system or with an antibacterial monomer were described that provided acceptable physical properties and good antibacterial effectiveness. The sorption material being part of the delivery system can be used as a vehicle for any other, perhaps even a more effective, active agent. Based on the results of the study the null hypothesis has to be rejected in total.

Acknowledgements

Results are part of the thesis of Taina Trellekan.

Author Contributions

Conceived and designed the experiments: SR TT NB TB HR RJ. Performed the experiments: TT NB. Analyzed the data: SR NB RJ. Wrote the manuscript: SR NB RJ.
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