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Antibacterial activity of a triclosan-containing resin composite matrix against three common oral bacteria

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Abstract This study investigated the antibacterial effect of a resin composite matrix with or without incorporated triclosan (0.3 wt%) on *Streptococcus mutans*, *Actinomyces viscosus* and *Lactobacillus casei*. In the quantitative assay, bacterial suspensions were filled into 20-µl cavities within temporary restorative resins. After 0, 4, 8, 12, 24 and 48 h of incubation, the suspensions were removed from the restoratives and the numbers of viable bacteria were determined. Bacterial suspensions incubated without restoratives served as the controls. Ten replicates were carried out for each experiment. The resin composite containing triclosan demonstrated variable degrees of antibacterial activity against the microorganisms, revealing a significant inhibitory effect on *S. mutans* within 12 h compared to the control. The viable counts of *A. viscosus* significantly decreased after 24 h. A significant reduction of *L. casei* was observed after 48 h. The unloaded resin composite did not reveal a marked antibacterial effect. The resin composite loaded with triclosan might be beneficial in preventing cavity contamination and minimizing the risk of pulpal irritation in the short-term.

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1 Introduction

The surfaces of dental restorations that are exposed to the oral cavity are colonized by bacteria, which form a biofilm called dental plaque. Within the complex formation of such biofilms, *Streptococcus mutans*, *Actinomyces viscous* and *Lactobacillus casei* are common oral bacteria associated with dental caries [1–3] and adverse pulpal reactions [4, 5]. The amount and the vitality of the accumulated biofilm not only depends on environmental factors, but also on the applied underlying material [6–9]. Having no intrinsic antibacterial activity [10, 11], resin composites attract more vital bacteria deriving from the accumulating plaque mass and cariogenic streptococci than other restorative materials both in vitro [8] and in vivo [9]. In addition, the shrinkage during polymerization of resin-based materials leads to the formation of gaps along the tooth-restoration interface. From the time that a temporary resin-based restorations is placed to the point when the permanent restoration is seated, it is important to prevent a reinfection of the underlying dentin-pulp complex caused either by invading bacteria along the tooth-restoration interface or by residual bacteria [5, 12]. Moreover, the discoloration and bad odor that are often associated with short-term restorations may be of bacterial origin [13]. Therefore, temporary resin composites with antibacterial properties could help to reduce the degree of bacterial colonization and to protect the subjacent pulp of prepared tooth cavities.

A number of studies have focused on the incorporation of antibacterial agents such as chlorhexidine, quaternary ammonium or silver ions into the resin composite matrix [14–17]. All currently available antimicrobial agents possess specific strengths and limitations, which are most often related to the desire to minimize potential tissue toxicity, while maximizing antibacterial activity [14–17]. Triclosan

(2,4,4′-trichloro-2′-hydroxy-diphenylether) is a widely used, broad-spectrum biocide with a favourable safety profile. It is used in a large number of dermatological preparations [18] and oral hygiene products such as toothpastes and mouthrinses [19–21]. Research has shown that triclosan is a strong inhibitor, and only a small amount is needed to produce powerful antibacterial effects [22]. The effectiveness of dentifrices containing low concentrations of triclosan against dental plaque, gingivitis and bad breath odor is well-established [20, 21]. Because triclosan is effective against bacteria in general but particularly against gram-positive microorganisms [19], its incorporation into the resin composite matrix might be suitable for reducing the cariogenic microflora comprising mainly gram-positive bacteria. However, only a few studies have centred on the topic of triclosan-containing restorative materials [17, 23, 24]. Most of these investigators studied the antibacterial effect in vitro by using liquid culture or agar diffusion assays and by testing the minimum inhibitory concentrations. These methods are limited to testing water-soluble components of the restorative material instead of the whole material [8]. The direct contact test and its novel modifications, such as the BSWM (bacterial suspension within materials) assay, have gained increasing importance because the solid dental materials are allowed to act directly on the bacteria, which provides a more suitable approximation to the clinical situation [8, 25]. The BSWM assay has been successfully used for the evaluation of the antibacterial effect of different kinds of permanent and temporary restorative materials [25].

The aim of the present study was to assess the antibacterial potential of a resin composite matrix loaded with low concentrations of triclosan and that of unloaded samples against monocultures of *S. mutans*, *A. viscosus* and *L. casei* in the BSWM assay.

2 Materials and methods

2.1 Bacterial strains and culture conditions

The microorganisms used throughout this study were *S. mutans* (DSM#20523), *A. viscosus* (DSM#43798) and *L. casei* (DSM#20011). All bacterial strains were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and were routinely maintained aerobically (*S. mutans*) or anaerobically (*A. viscosus*, *L. casei*) on Mueller–Hinton blood agar plates (BAP) (Heipha Diagnostika, Heidelberg, Germany). For liquid precultures, one colony of a fresh grown culture from agar plates was used to inoculate 10 ml of Todd-Hewitt broth (Oxoid Ltd, Basingstoke, Hampshire, UK) supplemented with 0.5% (wt/vol) yeast extract (Difco Laboratories, Detroit, MI, USA) (THY). *A. viscosus* and *L. casei* were incubated anaerobically at 37°C in anaerobic jars (Anaerocult, Merck Eurolab GmbH, Darmstadt, Germany) supplied with BBL GasPak Plus envelopes (Becton–Dickinson Microbiology Systems, Sparks, USA). *S. mutans* was grown aerobically in 5% CO_2 and 20% O_2 atmosphere at 37°C. To obtain standardised aliquots for the inhibition assays, the numbers of the colony forming units (CFU)/ml of culture were determined for all three bacterial strains by viable plate counting at various optical densities at 600 nm (A_{600} nm) (Ultraspec 1000, Pharmacia Biotech, Freiburg, Germany).

2.2 Restorative materials

Two temporary light-curing resin composite restoratives, Fermit (Ivoclar Vivadent, Schaan, FL, lot number D08245) and Systemp.inlay (Ivoclar Vivadent, lot number C44504), were used. According to information provided by the manufacturer, the monomer matrix of both resin composites consists of high-molecular dimethacrylates that is filled with highly dispersed silicon dioxide and prepolymerized dimethacrylates as the copolymer. Catalysts and stabilizers are also components of the materials. The compositions of the two materials differ from one another in that Systemp.inlay additionally contains 0.3 wt% triclosan (Table 1).

2.3 Determination of viable counts after incubation in materials

The evaluation of the antibacterial activity of the restorative materials was performed as previously described [25]. The experimental set-up of the BSWM assay is schematically shown in Fig. 1. In brief, 0.5 g of unpolymerized resin composite was filled into sterile 0.5 ml Eppendorf reaction tubes. Prior to light-curing of the resin composites, a narrow tube-shaped cavity (5 mm in length) with a volume of 20 µl was created in the centre of the restorative material within the Eppendorf tube by pressing the tip of an Eppendorf pipette into the material. Afterwards, the resin

Table 1 Temporary resin composite restoratives used in the study

Composition	Fermit	Systemp.inlay
Polyester urethane dimethacrylate	49.6	49.4
Prepolymerized dimethacrylate	33.0	33.0
Highly dispersed silicon dioxide, silanized	16.5	16.4
Catalysts and stabilizers	0.9	0.9
Triclosan	–	0.3

Composition (wt%) according to information provided by the manufacturer

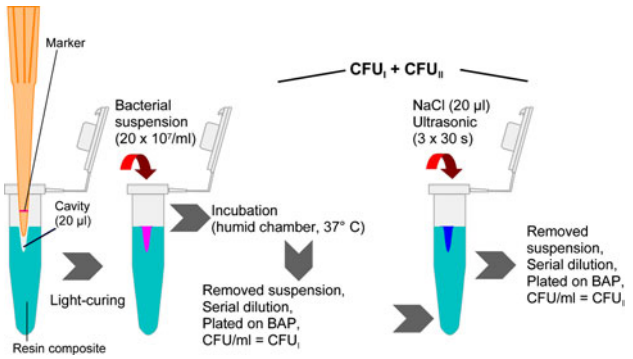

Fig. 1 Scheme of the BSWM (bacterial suspension within materials) model

composites were light-cured according to the manufacturer's instructions for 60 s by exposure to a halogen-light curing unit (Heliolux DLX, Ivoclar Vivadent) at a regularly measured light-intensity of 600 mW cm^{-2}.

Twenty-four hour cultures of all three bacterial strains were diluted 50-fold in a liquid medium (THY) and further incubated under the culture conditions described above. When A_{600} nm reached the level corresponding to 2×10^7 CFU/ml, which represents an early logarithmic growth phase, bacteria were sedimented by centrifugation at 6000×*g* and pellets were resuspended in a freshly prepared mixture of equal volumes of human serum and sterile 0.9% NaCl solution [25]. The serum was obtained from the same donor throughout the study. Twenty microlitres of the prepared bacterial suspension were filled into each test cavity within the materials. Bacterial suspensions in Eppendorf tubes without restorative material served as the growth control group. Incubation was performed at 37°C in a humid chamber that was equilibrated with 0.9% NaCl for 1 h before adding the specimens with capped Eppendorf tubes.

At selected time intervals (0, 4, 8, 12, 24 and 48 h) the suspensions were removed from the test cavities. At the baseline examination (t = 0 h), bacteria were immediately removed from the test cavities without incubation. The removed suspensions were serially diluted in 0.9% NaCl. One hundred µl aliquots of three dilution steps were plated onto BAP and incubated for 2 days (*S. mutans*) or 4 days (*A. viscosus*, *L. casei*) under the conditions described above for each bacterial strain. After the incubation, colonies were counted and the numbers of CFU/ml were calculated (CFU_I). After removal of the suspension, the test cavities were refilled with 20 µl of 0.9% NaCl and exposed to three ultrasonic pulses of 30 s each (Sonorex RK 102H, Bandelin Electronic, Berlin, Germany) in order to detach the bacteria which had remained on the walls of the test cavities. Following serial dilution of these suspensions, 100 µl aliquots were plated on solid media and the numbers of viable bacteria per ml were determined as described above (CFU_{II}). The total number of CFU/ml was calculated as $CFU_I + CFU_{II}$. For each restorative material including the controls, a series of ten experiments was carried out per bacterial strain and time interval.

2.4 Statistical analysis

The results of the measurements were depicted as mean values ($n = 10$) and standard deviations (SD). Non-parametric tests were chosen, since the data included several extreme values and were not normally distributed. For each set of data obtained at given incubation periods, the Kruskal–Wallis test was employed to detect significant differences in CFU/ml between the groups (restorative material, control). When the Kruskal–Wallis test confirmed significant effects ($P < 0.05$), pairwise comparisons between the groups were performed with the Mann–Whitney test. The resulting P values were corrected for multiple comparisons by using the Holm procedure. Zero counts were treated as 1 CFU/ml. All statistical analyses were performed with the statistical software SPSS (v. 14.0 for Windows, SPSS, Munich, Germany).

3 Results

The colony counts of the control groups were almost constant for all of the three tested bacterial strains over the incubation period of 48 h. The progression of the determined CFU/ml after incubation in the restorative materials is depicted for the three strains in Figs. 2, 3, and 4. The resin composite containing triclosan showed distinct inhibitory effects on the used bacteria. *S. mutans* was the most sensitive of the tested microorganisms, revealing a significant reduction in CFU/ml after incubation in the specimens for ≥12 h compared to the resin composite without triclosan and the control group ($P < 0.05$). An intensification of this effect was observed until the end of the incubation period ($P < 0.001$). The unloaded resin composite without triclosan showed no significant antibacterial activity inhibiting the vitality of the bacteria in the specimens at all time intervals ($P > 0.05$) (Fig. 2).

After incubation in the resin composite that contained triclosan, the determined colony counts of *A. viscosus* showed a significant decrease after ≥24 h compared to the cell numbers in the resin composite without triclosan and the control group ($P < 0.001$). After 48 h, the resin composite without triclosan inhibited the bacterial growth more successfully than the control ($P < 0.001$), but to a lower degree than the triclosan-containing resin composite ($P < 0.001$) (Fig. 3).

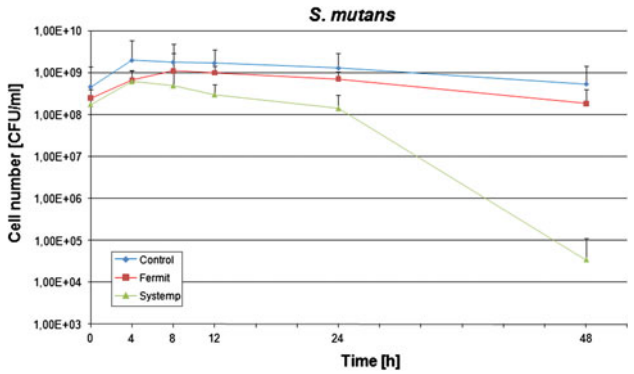

Fig. 2 Growth inhibition of *S. mutans* after incubation within resin composites compared to the growth control (incubation without restorative materials). The graphs show the mean values of ten independent experiments per time interval in the logarithmic scale. Error bars indicate SD. For explanation of the resin composites see Table 1

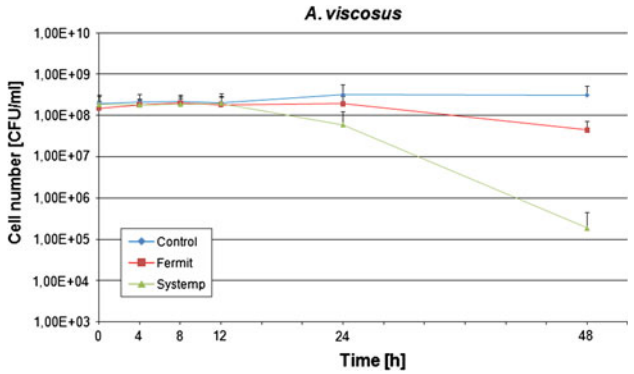

Fig. 3 Growth inhibition of *A. viscosus* after incubation within resin composites compared to the growth control (incubation without restorative materials). The graphs show the mean values of ten independent experiments per time interval in the logarithmic scale. Error bars indicate SD. For explanation of the resin composites see Table 1

L. casei revealed the highest degree of resistance against the inhibitory effects of triclosan-containing resin composite. The progression of the colony counts was comparable to that of the control group with no reduction of the cell numbers in the specimens of both materials. Compared to the control group, a significant decrease of the CFU/ml was observed in the triclosan-containing resin composite only after an incubation period of 48 h ($P < 0.05$). No significant difference between the two materials was detected at all determined times ($P > 0.05$) (Fig. 4).

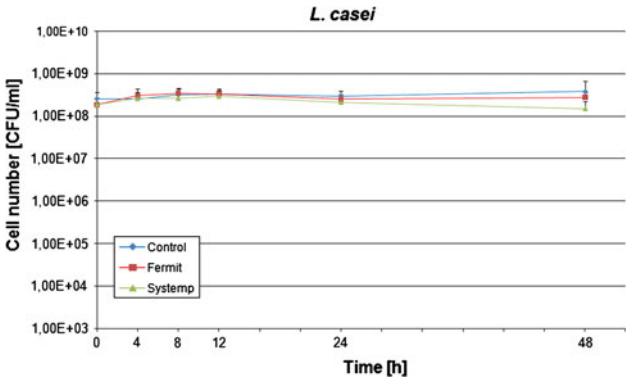

Fig. 4 Growth inhibition of *L. casei* after incubation within resin composites compared to the growth control (incubation without restorative materials). The graphs show the mean values of ten independent experiments per time interval in the logarithmic scale. Error bars indicate SD. For explanation of the resin composites see Table 1

4 Discussion

In vitro assays are considered to be helpful screening devices for assessing the antibacterial potential of dental materials. Most of the studies conducted to date have involved short-term evaluations, i.e. 24 or 48 h after polymerization of the material [7, 10, 16, 24–28]. In the present study a quantitative assay was used to determine the number of viable bacterial counts after incubation of a small amount of bacterial suspensions in tube-shaped cavities within material specimens. This BSWM model seems to reflect clinical conditions more reliably than liquid culture or agar diffusion assays, because (i) a relatively small volume of bacterial suspension is surrounded by a large surface of restorative material, (ii) the bacteria in suspension are allowed to come in direct contact with the tested material, regardless of the solubility and diffusivity of their components, and (iii) the material specimens are standardized by weight and surface area [25].

In the BSWM assay, the antibacterial effect of a resin composite that contains triclosan (Systemp.inlay) increased over the incubation period of 48 h. Within this time interval no recovery of the determined viable counts was observed for any of the tested microorganisms. This is in agreement with an in vitro study, where Systemp.inlay showed significant antibacterial properties when in direct contact with *S. mutans* for at least 1 week [17]. Furthermore, the results correlate with observations in vivo demonstrating the antiplaque and antigingivitis effectiveness of Systemp.inlay for at least 6 weeks [13]. In contrast, experiments with a triclosan-containing resin composite and liquid monocultures of *S. mutans* revealed an inhibitory influence on the adherence of the bacterial cells but only a transient effect on the growth behaviour [23]. In another study, the evaluation of triclosan-containing polymers on liquid cultures of the extraoral microorganisms *Escherichia coli* and *Bacillus thuringiensis* showed only some initial slowing of the bacterial growth and no antibacterial effect over longer incubation periods [24].

However, considering the differences in the study designs, one should not be surprised by these conflicting results.

For the mechanism of inhibition, it was speculated that only triclosan molecules on the surface would display an antibacterial efficacy by dissolution of unbound molecules into the surrounding medium or directly by contact inhibition of the adhering bacterial cells. But due to the low solubility product of triclosan in water or aqueous liquids, triclosan-containing polymers were found to release triclosan in concentrations under the minimum inhibitory concentrations for most bacteria [23, 26]. However, this low concentration already had an inhibitory influence on the growth of bacteria in liquid cultures [24]. Due to the lack of "turnover" of the solute as it would occur in swallowing saliva in vivo (clearance), the influence of unbound triclosan on the bacterial growth in the present study could not be excluded. This should be assessed in further experiments investigating the release kinetics and durability of the material under the influence of bacterial colonization.

Several investigations revealed different degrees of sensitivity of the microorganisms against the same antibacterial influence of restorative materials [8, 10, 17, 29]. In the present study, the triclosan-containing resin composite Systemp.inlay reduced the total number of three known-cariogenic bacterial species of which *L. casei* was the most resistant strain within the incubation period of 48 h. The determined CFU/ml of *A. viscosus* decreased about 4 log-units compared to the control group and 3 log-units compared to the resin composite matrix from which triclosan had been omitted (Fermit). Although Fermit caused a significant reduction of viable counts, the inhibitory effect was much lower than in the Systemp.inlay group. The strongest growth inhibition of Systemp.inlay was observed with *S. mutans*. The decrease of the colony counts was about 5 log-units compared to that of the control and the Fermit group. The antibacterial effectiveness against *S. mutans* was comparable to that of the polymer-reinforced zinc oxide eugenol cement IRM in a previous study using the same in vitro assay [25]. The powerful antibacterial effects of IRM are well documented and have also been confirmed in vivo by culturing bacteria derived from caries sealed with IRM for 5 months [30].

The different degrees of sensitivity against triclosan are based on the characteristic topology of the bacterial cell membrane [31] and the achievement of mechanisms for drug resistance [32, 33]. The antibacterial effect of triclosan is based on its interaction with a specific enzyme involved in the bacterial lipid biosynthesis. It acts as a competitive inhibitor of the enoyl acyl-carrier protein reductase (*Fab*I) of different gram-positive and gram-negative bacterial species [22, 34]. By interacting with the bacterial cell membrane, triclosan causes changes in the structural integrity and inhibits membrane-dependent processes, such as signal transduction and electron transport chains [31]. The extent of the inhibition depends on the strain-specific microstructure of the membrane that could vary within different groups of microorganisms and might lead to intrinsic insensitivity against triclosan. However, further experiments with microbial mixed cultures should be performed as triclosan seems to be more effective against gram-negative bacteria as shown in a continuous mixed culture model [35]. The gram-positive bacteria were affected to approximately the same degree unlike in the present study. Perhaps there might be an interspecies-mediated resistance against antimicrobials when bacteria are grown in mixed cultures.

The application of antibacterial additives in dental products should not lead to the disturbance of the ecological homeostasis of the oral microflora or favour the colonization by exogenous pathogens. Furthermore the material should not support the development of bacterial resistances to avoid the growth of super pathogens, which could lead to serious therapeutic failures in infectious diseases. Problems concerning the application of triclosan-containing restorative materials may arise from the elimination of only sensitive microorganisms. This provides the ideal setting for resistant bacterial strains to grow [24], resulting in an alteration of the microbial composition of the physiological plaque microflora. The problem of this selection is that the resistance against triclosan mediates cross-resistance against other important and systemic antibiotics by inducing an effective multi-drug efflux system [36]. A triclosan-induced multi-drug resistance efflux system has been previously described [33]. Further resistance mechanisms which might influence the susceptibility of oral bacteria against triclosan include mutation of the *Fab*I target site, increased target expression, and enzymatic inactivation or degradation [33]. However, the chronic exposure to triclosan, which would be sufficient to cause an approximately 400-fold increase in resistance in *E. coli* did not produce a significant increase in triclosan-resistance in ten oral bacteria, determined by minimum inhibitory and bactericidal concentrations, or any cross-resistance to antibiotics, such as tetracycline and metronidazole [37]. In addition, from the experience gathered with 0.3% triclosan-containing dentifrices [19–21] and triclosan-impregnated plastic storage boxes [38], one can conclude that the triclosan-modified resin composite used in the present study will not produce the mentioned changes in the oral microflora or cause an increase of triclosan resistance in the oral cavity.

5 Conclusions

The triclosan-containing resin composite reduced the total number of three known-cariogenic bacterial strains. In

contrast, the assessed resin matrix without triclosan did not reveal a marked antibacterial effect. The findings indicate that incorporation of the antibacterial compound triclosan into a resin-based material with a concentration of 0.3 wt% seems to be useful for the preventive desinfection of prepared cavities. If there are no remaining bacteria, pulpal irritation is less likely to occur. However, the question whether the level of antibacterial activity is sufficient in the clinical setting cannot be answered sufficiently and has to be further evaluated in vivo. Moreover, it remains speculative if longer incubation times or higher concentrations of triclosan would increase the antibacterial effects of triclosan-containing resin composite materials.

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