In vitro antibacterial evaluation of flowable restorative materials

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Objectives: The microgap at the tooth-restoration interface is inevitable and may allow bacterial penetration that could lead to failure. The purpose of this in vitro study was to assess the antibacterial potential of 4 flowable composite restorative materials. **Method and Materials:** The antibacterial potential of Aeliteflo (Bisco), Filtek Flow (3M ESPE), Tetric Flow (Ivoclar Vivadent), and Dyract Flow (Dentsply) was tested against *Streptococcus mutans*. Agar diffusion test (ADT) and direct contact test (DCT) were the methods used. For ADT, wells were punched in *S mutans*–inoculated plates. The materials were placed in the wells and polymerized. Inhibition zones were measured after 48 hours' incubation at 37°C. In the DCT, 8 samples of each tested material were placed on the side walls of wells in a 96-microtiter plate and polymerized. A suspension of *S mutans* was placed on the surface of each sample. Bacterial growth was monitored by optical density changes at 650 nm every 30 minutes for 16 hours. The experiment was repeated after the samples were aged in phosphate-buffered saline for 1 and 7 days. **Results:** In both tests, only Dyract Flow showed inhibition of *S mutans* growth. Except for Dyract Flow samples, aged samples did not statistically differ in *S mutans* inhibition when compared to their 1-hour control counterparts. Dyract Flow samples lost their *S mutans* inhibitory potential after 24 hours. **Conclusion:** The flowable composites tested do not possess effective long-term antibacterial ability. *(Quintessence Int 2009;40:327–332)*

Key words: antibacterial, direct contact test, flowable compomer, flowable composite resins

A microgap at the tooth-restoration interface is inevitable. This major disadvantage of adhesive restoration can be observed clinically by marginal staining and demonstrated in vitro by microleakage.[1]

Microleakage allows bacterial penetration, which may result in secondary caries leading to pulp pathology and failure of a restoration.[2] Effective and long-lasting antibacterial property of composite resin–restorative material may eliminate bacterial biofilm formation at the interface and thus increase restoration longevity. Numerous scientific publications investigating the antibacterial property of restorative materials indicate the importance of this property.[3–15] Flowable composite resin materials are advocated for use in Class 5 restorations, open-margin repairs, fissure sealing, and provision of an elastic stress-absorbent layer underneath a restoration to prevent microleakage and postoperative sensitivity, as well as to cancel undercuts in crown, onlay, and inlay preparations.[16–23]

The most common technique for assessing antibacterial properties of dental materials is the agar diffusion test (ADT), which enables measurement of the activity of soluble ingredients of the tested material in the surrounding medium, indicated by an inhibition halo.[7,24] This, however, does not meet the

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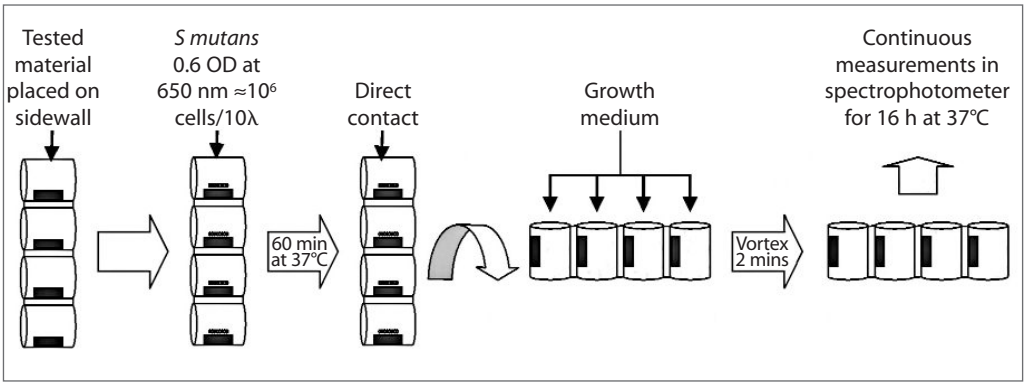

Fig 1 Experimental design of direct contact test (DCT) performed in a 96-well flat bottom microtiter plate. OD, optical density.

gold standard for nonsoluble, nondegradable, permanent dental restorative materials.[9,24]

Weiss et al developed a direct contact test (DCT) to overcome these limitations and further supply quantitative information by employing direct contact between the material and microorganism.[25] This technique is far more suitable for antibacterial assessment of nonsoluble, nondegradable dental materials.[9,12,25]

The purpose of this study was to conduct an in vitro quantitative assessment of the antibacterial properties of 4 commercial flowable composite resins by DCT and to compare the results with the most commonly used ADT.

METHOD AND MATERIALS

Tested materials

The following commercially available restorative materials were tested: Aeliteflo, flowable composite resin (Bisco); Filtek Flow, flowable composite resin (3M ESPE); Dyract Flow, flowable compomer (Dentsply); and Tetric Flow, flowable composite resin (Ivoclar Vivadent).

Tested microorganism

Streptococcus mutans is considered the primary etiologic agent of caries[26] and widely used to test the antibacterial properties of restorative materials.[4–6,26] Bacitracin-resistant *S mutans* #27351M was grown aerobically from a frozen stock culture in brain-heart infusion broth (BHI) (DIFCO) containing 0.5% bacitracin for 48 hours at 37°C.

Experimental design

The present study was conducted using ADT and DCT. The ADT was performed using *mitis salivarius* agar plates (Hy-labs). Three plates were inoculated with 400 μL of viable bacteria suspension using a Drigalski stick. Duplicate samples of each tested material were placed in 8 prepunched cylindrical holes, measuring 3 mm in diameter by 4 mm deep, and immediately polymerized from both sides of the plate using visible light cure (Elipar Trilight, 3M ESPE) for 20 seconds from each direction. The plates were then incubated for 48 hours at 37°C. The bacterial lawn was visually inspected for inhibition zones, and these were measured in 2 perpendicular axes using an electronic caliper (Mitutoyo).

The DCT was performed using a 96-well flat-bottom microtiter plate (Nunc). The antibacterial properties of the tested materials were examined immediately after polymerization. Similar experimental procedures were performed, allowing the tested materials to

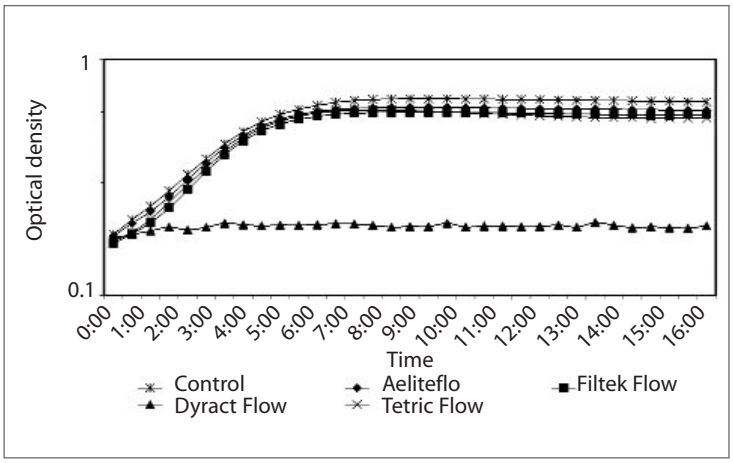

Fig 2 Bacterial outgrowth immediately after polymerization (1 hour) as measured by changes in optical density. The activity of the tested materials was compared to the positive controls. Dyract Flow presents inhibition of bacterial growth.

age in phosphate-buffered saline (PBS) and 0.5% bacitracin for 24 hours and 7 days after polymerization before being assayed by DCT. Each material was tested in octet.

The DCT relies on turbidimetric determination of bacterial growth in a 96-well microtiter plate and is described in detail by Matalon et al.[9]

The experimental setup is shown in Fig 1. A 96-well flat-bottom microtiter plate was held vertically so that the base of the well was perpendicular to the floor. Using a special template (surface area of 21 mm), the sidewalls of the 8-well test group were evenly coated with the tested materials. The samples were polymerized upon placement on the sidewall of the well in compliance with the manufacturers' instructions. Special care was taken to avoid the flow of the tested material to the bottom of the well (ie, the base of the well) so as not to interfere with optical readings later in the experiment. A suspension of 10 μL growth medium containing 106 viable bacteria, determined by serial dilution and viable count, was introduced in direct contact with the samples, with the plate remaining in the vertical position, and allowed to evaporate for 1 hour at 37°C. This assured direct contact between the tested material and the tested bacteria. Consequently, the plate was horizontally repositioned and 220 μL of BHI broth containing 0.5% bacitracin was added to each well and gently agitated for 2 minutes using a Gyrotory Shaker Model G2 (New Brunswick Scientific).

The positive control consisted of 3 wells such that identical bacterial inoculum was placed on the sidewall of the uncoated wells and processed as were the experimental wells. The negative control consisted of 4 wells such that only uninoculated fresh medium was added (with or without the tested material). The plates were then incubated at 37°C in a temperature-controlled spectrophotometer (Versamax, Molecular Devices) so that bacterial growth could be monitored for 16 hours at 650 nm in 30-minute intervals.

Calibration experiments were performed simultaneously in each microtiter plate to obtain a comparative quantitative scale as follows: Bacteria (106 colony-forming units) were placed on each sidewall of 3 wells, and 250 μL of BHI broth was added. Consecutive 5-fold dilution transfer was performed into 6 sets of 3 wells. The mean OD (optical density) measured in 3 wells simultaneously was plotted on a growth curve.

In the DCT, each microtiter plate was set up as an independent investigation with positive controls, negative controls, and calibration experiments.

The recorded data were plotted as semilogarithmic growth curves. The linear portion of the curve, which correlates with the bacterial growth rate, was extrapolated to represent a linear function. The slope of the line represents the growth rate of the experimental bacteria, and the y-intercept represents the number of viable bacteria before incubation. These parameters were analyzed using 2-way analysis of variance (ANOVA) to examine the

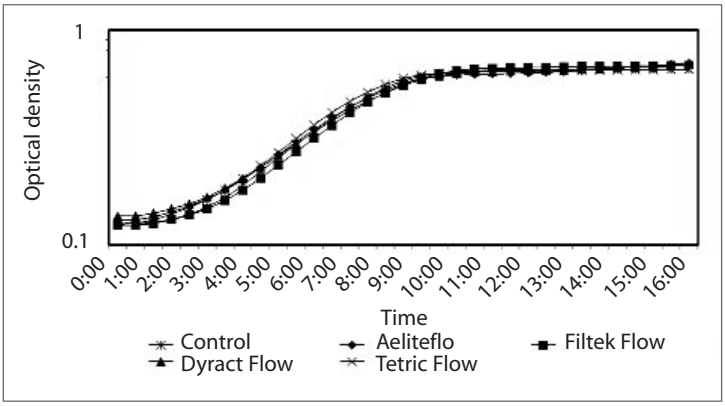

Fig 3 Bacterial outgrowth 1 day after polymerization as measured by changes in optical density. The activity of the tested materials was compared to positive controls. All samples present similar slopes and show no inhibition of bacterial growth.

Table 1 Bacterial growth rate after direct contact with tested samples

Material	Time		
	Immediately	1 day	7 days
Control	0.045 ± 0.002	0.040 ± 0.003	0.038 ± 0.002
Aeliteflo	0.043 ± 0.007	0.041 ± 0.003	0.039 ± 0.007
Filtek Flow	0.042 ± 0.007	0.038 ± 0.008	0.031 ± 0.002
Tetric Flow	0.047 ± 0.003	0.039 ± 0.002	0.042 ± 0.004
Dyract Flow	0.0001 ± 0.00001	0.036 ± 0.002	0.041 ± 0.003
1-way ANOVA	$P < .001$	No significant difference	No significant difference

Bacterial growth rate expressed by the slope of the linear portion of the growth curve. Values are the average of 3 experiments, 8 wells in each experiment. Vertical lines connect the values that do not differ significantly (Tukey comparison).

correlation between time and material, as well as 1-way ANOVA and Tukey multiple comparison tests by means of SPSS 14 for Windows (SPSS) ($P = .001$).

RESULTS

In the ADT, the only test material to demonstrate an inhibitory halo in the bacterial lawn was Dyract Flow. This halo measured 3.00 ± 0.12 mm in width in 2 perpendicular axes (beyond the diameter of the sampled material).

The DCT was performed on 8 specimens of each tested material. A regression line (R^2) was calculated on the linear portion of the growth curve, which represents the logarithmic growth phase. The R^2 of all growth curves ranged from 0.91 to 0.99. Two-way ANOVA showed a significant difference in bacterial growth rate, both on different adhesive materials ($P < .001$) and at different tested time points ($P < .001$).

Immediately after polymerization, Dyract Flow was the only material tested that demonstrated potent antibacterial properties compared with positive controls (Fig 2 and Table 1). After aging for 24 hours or 7 days, none of the tested materials possessed antibacterial properties, and all were similar to the controls (Fig 3 and Table 1).

Calibration growth curves are shown in Fig 4. The optical density at any given time correlates to the number of viable bacteria. Every point on the curve is the mean optical density of 3 wells. The curves indicate that the effect of reducing the inoculum size increases the lag until exponential growth but not the generation time nor the ultimate growth density.

DISCUSSION

This study evaluated the antibacterial properties of 4 commercially available flowable composite resins.

Two methods were used to evaluate the antibacterial properties: ADT and DCT. In this study, both methods produced similar

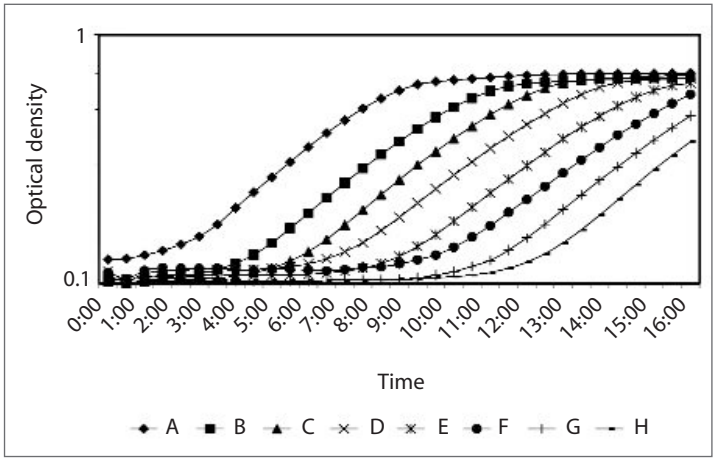

Fig 4 Bacterial growth as measured by changes in optical density. The optical density at any given time correlates to the number of viable bacteria. Every point on the curve is the mean optical density of a 3-well set, taken simultaneously. It is apparent that the constant decrease in the primary number of bacteria (due to dilution) does not affect the growth rate or the final amount of viable bacteria in the stationary phase. Dilution by a factor of 5 resulted in a lag of about 1 hour in the exponential phase. Starting with 10^6 (A) viable bacteria, the consequent curves represent the outgrowth of $2 \cdot 10^5$, $4 \cdot 10^4$, $8 \cdot 10^3$, $16 \cdot 10^2$, 320, 64, 12.8 bacteria per well, B to H respectively.

results. Dyract Flow, a flowable compomer, was the only one shown to have an antibacterial property.

The ADT test carried out for 48 hours after polymerization indicated that this material releases an antibacterial component into the aqueous milieu. This observation could be interpreted in part by the material's solubility, as indicated by the manufacturer's test values (2.71 ± 0.42 μg/mm³, Dentsply Dyract Flow portfolio). Nevertheless, this does not concur with the characteristics of an ideal restorative material.[24]

In the DCT, the same material was shown to have antibacterial ability only immediately after polymerization ($P < .001$), whereas after 24 hours and 7 days, no antibacterial effect was found. The combined conclusion from these methods of testing is that the flowable compomer Dyract Flow contains a short supply of a component with antibacterial ability that is released into the surrounding environment and disappears within a short period.

The combined methods allow differentiation between antibacterial properties dependent on the release of a component into the surrounding area, and antibacterial properties resultant on contact between the bacterium and the material, as shown in previous studies.[9,27,28] In this study, the DCT enabled an estimation of the time frame in which the antibacterial component was fully released and the material lost its antibacterial properties.

None of the other flowable composite resins tested showed any antibacterial capability. These results are in accordance with related findings on the effect that composite resins have on bacterial growth.[12,15,28–30]

Compomers (polyacid-modified composite resins), a group of dental restorative materials, were developed to improve clinical performance of conventional glass-ionomer restorative materials. The claimed distinction between composite resin materials and the compomers is the "glass ionomeric" inherent characteristics; dominant among them is the release and uptake of fluoride.[15,31] One could argue that this is a reasonable explanation for the results of the current study.

CONCLUSION

According to the results of this study, none of the flowable materials tested possesses effective long-term antibacterial ability.

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