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RESEARCH AND EDUCATION

Effect of composite resin containing antibacterial filler on sugar-induced pH drop caused by whole saliva bacteria

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ABSTRACT

Statement of problem. Secondary caries around restorations is a major problem and can be attributed to bacteria invading microgaps formed at the tooth-restoration interface. An antibacterial composite resin containing quaternary ammonium silica (QASi) filler has been reported to inhibit enamel demineralization in situ. However, whether the prevention of enamel demineralization by QASi-containing composite resin is because of the reduced metabolic activity of acid-producing saliva bacteria is unclear.

Purpose. The purpose of this study was to compare the effects of QASi-containing composite resin and 2 other restorative materials on the viability of salivary bacteria and sugar-induced acid production.

Material and methods. Whole saliva from each of the 30 study participants, 14 at high risk and 16 at low risk for caries, was brought into contact with quadruplicate specimens of 3 restorative materials, Infinix Flowable Composite, an anti-bacterial composite resin containing 1.5% QASi filler (Nobio), Filtek Supreme Flowable Restorative (3M), a conventional flowable composite resin, and dental amalgam (Silmet). Bacterial growth and sugar-induced acid production on each restorative material were measured every 20 minutes for 18 hours. Caries risk groups were compared using the *t* test and repeated measures analysis of variance (α=.05). When significant, Bonferroni multiple comparisons were used.

Results. On average, the saliva with the QASi-containing composite resin specimens maintained a near-neutral pH, not dropping below pH 6.0. The saliva associated with both conventional restorative materials exhibited a pH drop below 5.5 ($P<.001$), the critical threshold for tooth demineralization according to the Stephan curve. Virtually no growth was measured on the surface of the antibacterial composite resin, whereas bacteria grew on the conventional composite resin and dental amalgam ($P<.001$). No differences were observed between participants at high and low risk of caries.

Conclusions. Unlike amalgam and conventional composite resin, the QASi-containing composite resin showed a near-complete shutdown of the metabolic activity of salivary bacteria upon contact and virtually no bacterial viability. This suggests that the prevention of tooth demineralization by QASi-containing restoratives is associated with a significant reduction in bacterial metabolic activity. (J Prosthet Dent xxxx;xxx:xxx-xxx)

Caries, the most prevalent noncommunicable human disease, affects all ages and socio-economic levels.[1–3] During recent decades, posterior cavitated lesions have been predominantly restored with composite resin, even though it has double the risk of failure compared with amalgam restorations, with secondary caries being the most common reason for failure.[4] Thus, the percentage of replaced restorations because of secondary caries may exceed 50% of estimates.[5] The high risk of secondary caries associated with composite resin restorations can be attributed to microgap formation at the tooth-restoration interface because of polymerization shrinkage and unstable bonding to dentin. A gap several micrometers wide is readily filled by salivary bacteria, nutrient leakage, and percolation, often leading to

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Clinical Implications

Composite resins containing antibacterial QASi filler are among the first restorative materials that have addressed the etiology of caries. They significantly inhibited sugar-induced acid formation by salivary bacteria. Broad-spectrum and potent antibacterial restoratives can reduce the recurrence of secondary caries and shift the balance toward a healthier oral ecosystem.

secondary caries.[6,7] As available composite resins do not possess antibacterial properties, restorative materials with sustained antibacterial properties are an unmet need.[7,8]

Incorporating low concentrations of quaternary-ammonium polyethyleneimine (QPEI) particles into a commercially available composite resin has been reported to result in a potent and broad-spectrum bactericidal restorative material.[9–12] When a composite resin with QPEI (1% wt/wt) was aged for 6 months, all inoculated *S. mutans* bacteria, over 1 million, were reported to be killed, as effectively as in a newly polymerized composite resin.[13] Like QPEI, quaternary ammonium silica dioxide (QASi) particles developed as nanosized or microsized composite resin fillers have been demonstrated to have a broad-spectrum and long-term antibacterial effect without compromising mechanical properties.[14,15] The ability of the antibacterial composite resin to reduce enamel demineralization was tested in situ in volunteers wearing removable partial dentures. An enamel slab facing a gap adjacent to the QASi-composite resin in the denture showed significantly lower demineralization than contralateral enamel adjacent to the commercially available composite resin.[16]

The research hypothesis was that the prevention of enamel demineralization by QASi-containing composite resin would be because of the reduced metabolic activity of the bacteria in the gap and the pH would remain close to or above a critical pH value. Therefore, whole salivary bacteria sampled from 30 volunteers were tested for their ability to grow on surfaces of 3 restorative materials and produce acid in the presence of sugars. A QASi-containing composite resin was compared with a conventional composite resin and a dental amalgam by using these 2 tests.

MATERIAL AND METHODS

Thirty dental students with a mean ±standard deviation age of 27.2 ±2.4 years, 18 men and 12 women, volunteered to participate in the study. Individuals who took antibiotics in the 14 days before the test were excluded. The study was approved by the university ethics committee (#0002568–1), and the participants signed a consent form. Each participant underwent a clinical and bitewing radiograph examination, and a complete caries risk assessment based on Caries Management by Risk Assessment (CAMBRA) as described by Featherstone et al.[17] One milliliter of whole saliva was collected from each participant in a sterile test tube. As the concentration of viable bacteria in the saliva is estimated to be 10^8 per mL, a 10 µL volume of saliva was used to inoculate about 1 million bacteria on each of the tested specimens.[10,15] Brain Heart Infusion (BHI) Broth (Sigma Aldrich LTD) diluted in saline at a ratio of 1:5 and supplemented with 2.5% dextrose was used as the medium in the growth experiment performed in quadruplicate wells. Acid production, indicated by pH drop, was monitored in a separate set of quadruplicate wells. For this, a pH color-indicator stock solution (Bromocresol green [BCG]; Sigma Aldrich), at concentration of 34.72 µM was prepared. Five microliters of pH indicator stock solution were added to the growth medium to allow continuous pH monitoring in parallel with bacterial growth measurements in the 96-well plate. In a separate calibration experiment, the growth medium with BCG was titrated with 1 M hydrochloric acid, and the pH was determined using a pH meter probe (pHenomenal, pH1100L; VWR) to generate a calibration curve; this allowed the convergence of the experimental optical values obtained in the 96-well plate readings into absolute pH values.

Three restorative materials were tested simultaneously on the same plate (Fig. 1). A flowable antibacterial composite resin (Ab composite) containing 1.5% QASi filler particles (Infinix Flowable Composite; Nobio). The QASi filler particle is a silica filler core that

Figure 1. Materials tested. A, Conventional composite resin (C composite) (Filtek Supreme Flowable Restorative; 3M). B, QASi-containing composite resin (Ab composite), (Infinix Flowable Composite; Nobio). C, Amalgam (admix alloy, high copper, nongamma II amalgam; Spherodon-M, Silmet).

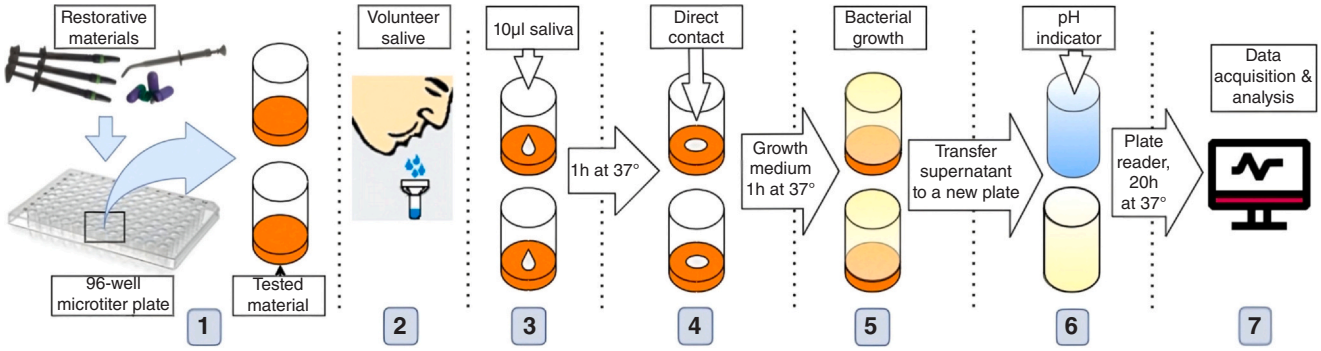

Figure 2. Bacterial growth and pH change experiments in 96-well microtiter plate. Quadruplicate wells for each experiment and for each tested material: (1) Coating bottom of wells with materials. (2) Collecting saliva. (3) Placing 10 µL saliva droplet (ca. 10^6 bacteria/mL) from one participant on all tested material specimens; incubation (1 hour at 37 °C) allows fluid to evaporate, ensuring direct contact of bacteria with materials. (4) Adding growth medium to wells and incubating plate (1 hour at 37 °C), allowing bacteria to multiply and grow. (5) Transferring 200 µL media from each well onto replica 96-well plate; adding pH indicator (BCG) to half of wells for testing pH changes. (6) Incubating replica plate in temperature-controlled spectrophotometer at 37 °C, measuring absorbance every 20 minutes at 2 wavelengths, A_{650} for bacterial growth and A_{616} for pH changes. (7) Analyzing data.

covalently binds dimethyl-octyl ammonium functional groups, the same active group as in the previously described QPEI particle.[9,10] In parallel, 2 conventional restorative materials were tested, a flowable composite resin (C composite) (Filtek Supreme Flowable, Restorative; 3M) and amalgam (admix alloy, high copper, nongamma II amalgam, Spherodon-M; Silmet).

Experiments were conducted in a 96-well, flat-bottom polystyrene microtiter plate (Thermo Scientific Nunclon Delta Surface; Fisher Scientific). The bottom of 8 wells, 2 sets of quadruplicate wells, were coated with each tested restorative material. In 1 set of quadruplicate wells, the changes in absorbance were monitored indicating bacterial growth; in the other quadruplicate set, changes in color were monitored indicating pH change. Composite resin specimens were light polymerized according to the manufacturers' instructions and stored for at least 24 hours. Before use, all wells were rinsed twice with phosphate-buffered saline (PBS). Saliva bacteria from a single participant were used as the inoculum for all wells of the 3 tested materials and the polystyrene controls. Figure 2 depicts the step-by-step experimental setup of 1 plate. In brief, the test tube with collected saliva was mixed by vortex (Vortex-Genie 2; Scientific Industries) for 5 seconds and rested at room temperature for 1 minute to allow aggregates to sediment; 10 µL of saliva was then placed at the center of the tested specimen in each well. Uncoated wells (polystyrene) served as a positive control, and 10 µL of saline on restorative material specimens was used for the negative control. On each plate, saliva obtained from 2 participants was tested. The 96-well plate was incubated for 1 hour at 37 °C to evaporate liquids, ensuring direct contact between all bacteria and the tested material surfaces. Thereafter, 220 µL of growth medium was added to each well and incubated for 1 hour at 37 °C with 5 seconds of mixing every 20 minutes in a microplate temperature-controlled spectrophotometer (VersaMax ELISA microplate reader; Molecular Devices). This second incubation period allowed all viable bacteria on the tested specimens to grow, shed, and disperse into the growth medium. Then, 200 µL of supernatant from each well was transferred to a new sterile 96-well plate, duplicating and preserving the original 96-well plate plan. Next, 5 µL of BCG was added to those quadruplicate wells in which the pH change would be monitored. The plate was incubated at 37 °C in the microplate temperature-controlled spectrophotometer, and optical absorbance at 650 nm for bacterial growth and 616 nm, for pH changes was recorded continuously every 20 minutes with 5 seconds premixing for at least 18 hours.

Data were exported to a software program (Excel; Microsoft Corp), and bacterial growth and pH decrease were plotted for each participant. A regression line from the linear portion of the bacterial growth curve (Fig. 3A) using the equation y=ax+b was drawn to provide the growth rate (a) and the growth onset (b), where the exponential growth phase began. Measurements obtained at each time point in the wells with the BCG indicator were corrected by reducing the optical density value measured simultaneously in the corresponding bacterial growth well to obtain the pH values. The curve that depicts the pH decrease (Fig. 3B) was analyzed similarly to the bacterial growth curve by using the equation y=ax+b; the negative slope corresponded to the pH decrease rate (a), and the intercept line with the neutral pH line corresponded to the pH decrease onset (b).

The differences between the caries risk levels and caries-free groups in bacterial growth rate, growth onset, pH decrease rate, and pH decrease onset were compared using *t* tests. Data were further analyzed using

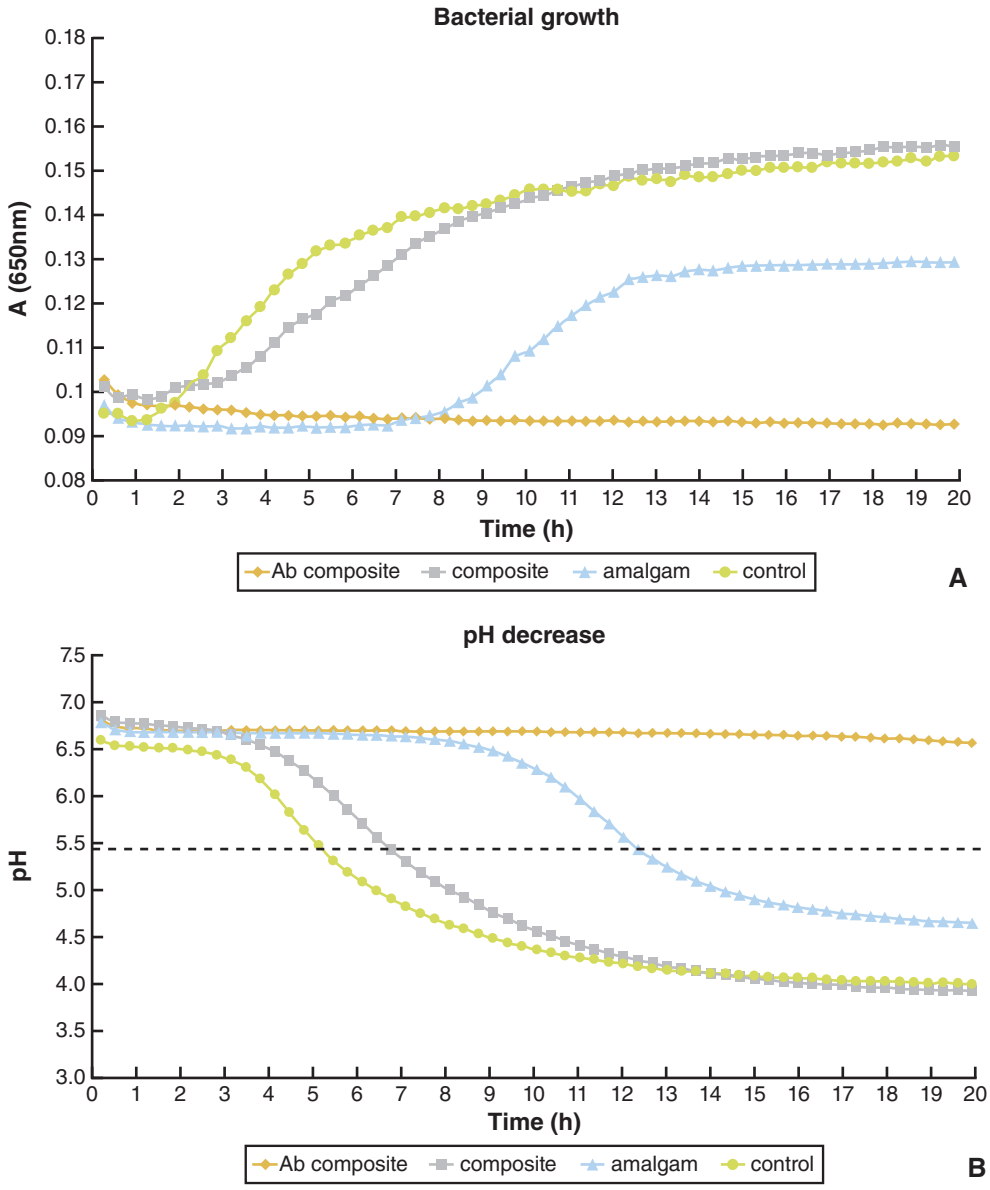

Figure 3. Results of saliva bacteria from one high-caries-risk participant. Plots of bacterial growth and pH change after contacting material surfaces. A, Bacterial growth curve. Each point represents average absorbance (A_{650}) in quadruplicate wells. B, Sugar-induced pH decreases. Each point represents average absorbance (A_{616}) measured simultaneously in quadruplicate wells. *Dashed line* indicates critical pH for enamel demineralization according to Stephan curve.

repeated measures analysis of variance, where the within-subjects factor was the tested material and the between-subjects factor was the caries risk level (α=.05). When significant differences were found for the material, Bonferroni multiple comparisons were used to compare the materials.

RESULTS

Of the 30 participants, 14 were at high risk for caries, and 16 were at low risk. None of the participants were at medium risk for caries. Bacterial growth curves and pH decrease curves of a high-caries-risk participant are shown in Figure 3. In contrast with the 2 conventional restoratives, the Ab composite resin wells showed a horizontal line indicating virtually no bacterial growth (Fig. 3A). Bacteria grew similarly on the conventional composite resin (C composite) and on the control surface. On amalgam, bacterial growth was partially inhibited; the onset of the bacterial growth started nearly 6 hours after the growth onset on the control. Both conventional restorative materials exhibited a pH drop below the critical pH of 5.5, the threshold for enamel

Table 1. Mean ±standard deviation values of bacterial growth and pH decrease of study participants. Values from line-equation of each study participant plots (one example in Fig. 2)

			Tested Materials			
	Participants	**n**	**Ab Composite Resin[c]**	**Composite Resin[c]**	**Amalgam[c]**	**Control[c]**
Growth onset (hours)	average[c]	28*	19.33 ±7.05	8.56 ±8.51	12.14 ±7.56	2.45 ±1.48
	high risk	12[a]	21.33 ±5.07	8.72 ±8.25	11.48 ±8.14	2.43 ±1.79
	low risk	16[a]	17.83 ±8.05	8.44 ±8.98	12.64 ±7.33	2.46 ±1.27
	caries free	10[b]	18.83 ±7.85	9.38 ±9.47	12.23 ±7.51	2.57 ±1.44
	caries experienced	18[b]	19.611 ±6.78	8.11 ±8.19	12.09 ±7.8	2.38 ±1.54
Growth rate	average[c]	30	0.0027 ±0.004	0.0132 ±0.0127	0.0104 ±0.009	0.0295 ±0.0173
	high risk	14[a]	0.0024 ±0.0042	0.0115 ±0.0122	0.0108 ±0.0092	0.0285 ±0.0168
	low risk	16[a]	0.003 ±0.0039	0.0147 ±0.0133	0.0102 ±0.0092	0.0304 ±0.0182
	caries free	10[b]	0.0032 ±0.0036	0.016 ±0.0164	0.0129 ±0.0105	0.0295 ±0.0146
	caries experienced	20[b]	0.0024 ±0.0041	0.0118 ±0.01	0.0092 ±0.0081	0.0295 ±0.0188
pH decrease onset (hours)	average[c]	28*	18.03 ±6.81	5.38 ±3.3	10.9 ±6.78	2.79 ±1.37
	high risk	12[a]	19.97 ±5.61	6.61 ±4.23	11.22 ±7.88	2.67 ±1.16
	low risk	16[a]	16.57 ±7.42	4.46 ±2.1	10.67 ±6.08	2.88 ±1.54
	caries free	10[b]	17.31 ±7.25	4.43 ±1.9	10.74 ±6.3	3.07 ±1.74
	caries experienced	18[b]	18.42 ±6.73	5.91 ±3.82	10.99 ±7.2	2.63 ±1.14
pH decrease rate	average[c]	30	-0.1 ±0.11	-0.44 ±0.23	-0.28 ±0.16	-0.65 ±0.24
	high risk	14[a]	-0.07 ±0.09	-0.35 ±0.2	-0.28 ±0.19	-0.67 ±0.25
	low risk	16[a]	-0.12 ±0.12	-0.51 ±0.24	-0.27 ±0.14	-0.63 ±0.25
	caries free	10[b]	-0.1 ±0.1	-0.46 ±0.22	-0.26 ±0.14	-0.61 ±0.23
	caries experienced	20[b]	-0.09 ±0.113	-0.42 ±0.24	-0.28 ±0.17	-0.67 ±0.26

* no results for two participants due to technical failure.

[a] no differences between groups in *t* test ($P<.05$).

[b] no differences between groups in *t* test ($P<.05$).

[c] ANOVA with repeated measures within participants and materials ($P<.001$).

demineralization, whereas Ab composite resin specimens maintained a near-neutral pH in the range of normal saliva and did not drop below a pH of 6.0 (Fig. 3B).

The average values of all participants for bacterial growth onset and growth rate are shown in Table 1. As no interaction was found between materials and the caries risk groups or the caries experience groups in the tested variables ($P>.05$), the results of all participants were further analyzed as 1 group. The differences in growth onset between the Ab composite and C composite and between the Ab composite and amalgam were statistically significant ($P<.001$ and $P=.003$, respectively) (Table 2). The differences in growth rate between the Ab composite and C composite and between the Ab composite and amalgam were significant ($P=.001$ and $P=.001$, respectively). The differences between amalgam and C composite in growth onset and growth rate were not significant ($P>.05$) (Table 2). The differences in pH decrease onset between the Ab composite and C composite and between the Ab composite and amalgam were significant (both $P<.001$). The differences in pH decrease rate between the Ab composite and C composite and between the Ab composite and amalgam were also significant ($P<.001$). The differences between amalgam and C composite in pH decrease onset and pH decrease rate were also significant ($P<.001$ and $P=.001$, respectively).

DISCUSSION

The QASi-containing composite resin demonstrated a significantly higher bactericidal effect on ex vivo saliva bacteria compared with the commonly used restorative materials, composite resin and amalgam. Virtually no saliva bacteria survived on the surface of QASi-containing composite resin. As an antibacterial material might affect a variety of oral microorganisms but not necessarily acid-producing bacteria, the 2 distinct tests performed simultaneously on the same saliva sample demonstrated that acid production was also significantly

Table 2. Pairwise comparison analysis of differences between tested materials

	Pairwise Comparisons					
	Ab composite / C Composite	**Ab composite / Amalgam**	**Ab composite / Control**	**C composite / Control**	**Amalgam / Control**	**Amalgam / C composite**
Growth onset	$P<.001$	$P=.003$	$P<.001$	$P=.003$	$P<.001$	$P=0.12$*
Growth rate	$P=.001$	$P=.001$	$P<.001$	$P<.001$	$P<.001$	$P=1$*
pH decrease onset	$P<.001$	$P<.001$	$P<.001$	$P<.001$	$P<.001$	$P<.001$
pH decrease rate	$P<.001$	$P<.001$	$P<.001$	$P=.001$	$P<.001$	$P=.001$

Bonferroni multiple comparisons of significant data in Table 1 (ANOVA with repeated measures; $P<.001$).

* not significant

affected compared with the restorative materials. Even in the presence of sugar, the pH values of QASi-containing composite resin remained considerably higher than the critical pH values that initiate caries lesions, according to the Stephan curve.[18] Keeping pH above the critical value for caries initiation has been reported to tilt the demineralization-remineralization balance toward health.[19] On the contrary, conventional composite resin or amalgam showed a rapid drop below the critical pH value. These findings were consistent with the in vivo enamel demineralization prevention shown in a clinical study.[16] It appears that QASi-containing restorative materials can be beneficial even for dentin or cementum, where the pH threshold for demineralization is higher than that of enamel.[18]

Contemporary dentists use different composite resin materials with improved esthetic qualities. While recently developed composite resins attempt to overcome polymerization shrinkage and other material physical shortcomings, a successful attempt to address the acid-producing microbiome, the primary etiology of caries, is lacking. Secondary caries around restorations has been attributed to bacteria invading the microgaps at the tooth-restoration interface. It follows that eliminating acid production and fluctuation at these sensitive sites by using QASi-containing composite resin will interrupt the demineralization-remineralization cycles, preventing secondary caries.

The comparative findings in the present study also show that amalgam is better than conventional composite resin, supporting the bactericidal hypothesis in the occurrence of secondary caries and showing that fewer caries can be found around amalgam than around composite resin restorations.[4] Interestingly, no significant differences between caries-risk groups were found in bacterial growth or pH decrease. The similar findings were consistent with those of Dong et al, who compared the Stephan curve values between caries-active and caries-free individuals and reported no significant differences, except for a faster pH rise in caries-free individuals at the end of the curve.[20]

Regular methods for measuring antibacterial properties, such as the agar diffusion test, minimal inhibitory concentration (MIC), or biofilm assays, are not suited for water-insoluble materials.[21] Measurement would be straightforward if water-soluble antibacterial compounds were used and released from a restorative material, but the effect, in that case, cannot be permanent. The bactericidal effect will fade, and the restorative material will eventually decompose, compromising its physical and optical properties. Nonetheless, insoluble antibacterial compounds such as QASi filler in a dental material are advantageous and will affect only those bacteria contacting the material and therefore, have a limited effect on the entire oral microbiome. Identifying those microorganisms that grew or survived on amalgam, composite resin, or QASi-containing composite resin was beyond the scope of the present study and merits further investigation.

Recurrent caries is a major complication in fixed partial dentures: secondary caries was found in 18.4% of patients with compromised oral hygiene and accounts for over 8% of failures in all patients after 7 years.[22] Composite resins containing antibacterial QASi filler are among the first restorative materials that have addressed the etiology of caries. They significantly inhibited sugar induced acid formation by salivary bacteria. Broad-spectrum and potent antibacterial restorative materials can reduce the recurrence of secondary caries and shift the balance toward a healthier oral ecosystem, especially when treating patients with a high risk for caries. The findings of the present study call for randomized longitudinal studies to determine the extent of secondary caries reduction and caries balance shifting by antibacterial materials, as proposed by Featherstone et al.[17]

CONCLUSIONS

Based on the findings of this study, the following conclusions were drawn:

1. Antibacterial restorative materials containing quaternary ammonium silica (QASi) filler can protect the tooth from demineralization and, subsequently, from secondary caries by shutting down bacterial metabolic activity and acid production.
2. In the presence of carbohydrates, the pH values on QASi-containing composite resin remained above the critical pH, avoiding the cariogenic demineralization remineralization cycles, whereas conventional composite resin, without bactericidal activity, allowed the metabolic fluctuation to initiate caries.
3. The composite resin containing antibacterial QASi filler was among the first restoratives that attempted to cope with the etiology of caries while shifting the balance toward a healthier oral ecosystem. This is of utmost importance in treating patients at risk, such as those with reduced salivary flow or other bacteria-induced oral pathosis.

REFERENCES

1. Wen PYF, Chen MX, Zhong YJ, Dong QQ, Wong HM. Global Burden and Inequality of Dental Caries, 1990 to 2019. *J Dent Res*. 2022;101:392–399.
2. Fleming E, Afful J. Prevalence of total and untreated dental caries among youth: United States, 2015-2016. *NCHS Data Brief*. 2018;307:1–8.
3. Dye B, Thornton-Evans G, Li X, Iafolla T. Dental caries and tooth loss in adults in the United States, 2011-2012. *NCHS Data Brief*. 2015;197:1–7.
4. Worthington HV, Khangura S, Seal K, et al. Direct composite resin fillings versus amalgam fillings for permanent posterior teeth. *Cochrane Database Syst Rev*. 2021;8:1–47.
5. Mjör IA. Clinical diagnosis of recurrent caries. *J Am Dent Assoc*. 2005;136:1426–1433.

6. Nedeljkovic I, Teughels W, De Munck J, Van Meerbeek B, Van Landuyt KL. Is secondary caries with composites a material-based problem? *Dent Mater*. 2015;31:e247–e277.
7. Ferracane JL. Resin composite-state of the art. *Dent Mater*. 2011;27:29–38.
8. Beyth N, Domb AJ, Weiss EI. An in vitro quantitative antibacterial analysis of amalgam and composite resins. *J Dent*. 2007;35:201–206.
9. Shvero DK, Zatlsman N, Hazan R, Weiss EI, Beyth N. Characterisation of the antibacterial effect of polyethyleneimine nanoparticles in relation to particle distribution in resin composite. *J Dent*. 2015;43:287–294.
10. Beyth N, Yudovin-Farber I, Perez-Davidi M, Domb AJ, Weiss EI. Polyethyleneimine nanoparticles incorporated into resin composite cause cell death and trigger biofilm stress in vivo. *Proc Natl Acad Sci U S A*. 2010;107:22038–22043.
11. Zaltsman N, Ionescu AC, Weiss EI, Brambilla E, Beyth S, Beyth N. Surface-modified nanoparticles as anti-biofilm filler for dental polymers. *PLoS One*. 2017;12.
12. Beyth N, Yudovin-Farber I, Bahir R, Domb AJ, Weiss EI. Antibacterial activity of dental composites containing quaternary ammonium polyethylenimine nanoparticles against Streptococcus mutans. *Biomaterials*. 2006;27:3995–4002.
13. Beyth N, Yudovin-Fearber I, Domb AJ, Weiss EI. Long-term antibacterial surface properties of composite resin incorporating polyethyleneimine nanoparticles. *Quintessence Int*. 2010;41:827–835.
14. Featherstone JDB. Dental restorative materials containing quaternary ammonium compounds have sustained antibacterial action. *J Am Dent Assoc*. 2022;153:1114–1120.
15. Dekel-Steinkeller M, Weiss EI, Samovici TL, Abramovitz I. Antibacterial performance of composite containing quaternary ammonium silica (QASi) filler - A preliminary study. *J Dent*. 2022;123:1–6. 104209.
16. Rechmann P, Le CQ, Chaffee BW, Rechmann BMT. Demineralization prevention with a new antibacterial restorative composite containing QASi nanoparticles: an in situ study. *Clin Oral Investig*. 2021;25:5293–5305.
17. Featherstone JDB, Alston P, Chaffee BW, Rechmann P. Caries management by risk assessment (CAMBRA): An update for use in clinical practice for patients aged 6 through adult. *J Calif Dent Assoc*. 2019;47:25–34.
18. Bowen WH. The Stephan Curve revisited. *Odontology*. 2013;101:2–8.
19. Selwitz RH, Ismail AI, Pitts NB. Dental caries. *Lancet*. 2007;369:51–59.
20. Dong YM, Pearce EI, Yue L, Larsen MJ, Gao XJ, Wang JD. Plaque pH and associated parameters in relation to caries. *Caries Res*. 1999;33:428–436.
21. Weiss EI, Shalhav M, Fuss Z. Assessment of antibacterial activity of endodontic sealers by a direct contact test. *Endod Dent Traumatol*. 1996;12:179–184.
22. Alenezi A, Alkhudhayri O, Altowaijri F, et al. Secondary caries in fixed dental prostheses: Long-term clinical evaluation. *Clin Exp Dent Res*. 2023;9:249–257.

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CRediT authorship contribution statement
Ervin Weiss: Conceptualization, Methodology, Supervision, Writing - review and editing; **Michal Steinkeller-Dekel:** Methodology, Validation, Resources, Writing - review and editing; **Omer Enoch:** Methodology, Formal analysis, Investigation, Writing - original draft, visualization.

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