

# The effect of dental sealants on bacteria levels in caries lesions

## A review of the evidence

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**S**trong evidence shows that sealants are effective in preventing caries in children at varying degrees of risk.<sup>1,2</sup> Despite this evidence of effectiveness, sealant prevalence among lower-income children (who are at higher risk of experiencing dental caries) remains at around 30 percent,<sup>3</sup> well below the Healthy People 2010 objective of 50 percent.<sup>4</sup> Survey data of dentists suggest that one of the major barriers to their providing sealants is concern about inadvertently sealing over caries.<sup>5,6</sup> This concern has become an obstacle to implementation of school-based sealant programs (Association of State and Territorial Dental Directors, unpublished data, 2005). Documenting the effectiveness of placing sealants over existing caries, thus, is important, because such documentation could remove a barrier to providing a proven intervention.

Dental caries is an infectious and transmissible disease, caused by cariogenic bacteria of the oral cavity, specifically those colonizing the surfaces of teeth.<sup>7-10</sup> Caries lesions may be caused by a range of bacteria, but principal among the cariogenic flora are the mutans streptococci and lactobacilli.<sup>7,10</sup> It long has been hypothesized that sealing an existing lesion from contact with the oral fluids should lead to eventual reduction and even death of these organisms and,

### ABSTRACT

**Background.** Concern about inadvertently sealing over caries often prevents dentists from providing dental sealants. The objective of the authors' review was to examine the effects of sealants on bacteria levels within caries lesions under dental sealants.

**Methods.** The authors searched electronic databases for comparative studies examining bacteria levels in sealed permanent teeth. To measure the effect of sealants on bacteria levels, they used the log<sub>10</sub> reduction in mean total viable bacteria counts (VBC) between sealed and not-sealed caries and the percentage reduction in the proportion of samples with viable bacteria.

**Results.** Six studies—three randomized controlled trials, two controlled trials and one before-and-after study—were included in the analysis. Although studies varied considerably, there were no findings of significant increases in bacteria under sealants. Sealing caries was associated with a 100-fold reduction in mean total VBC (four studies, 138 samples). Sealants reduced the probability of viable bacteria by about 50.0 percent (four studies, 117 samples).

**Conclusions.** The authors found that sealants reduced bacteria in carious lesions, but that in some studies, low levels of bacteria persisted. These findings do not support reported concerns about poorer outcomes associated with inadvertently sealing caries.

**Clinical Implications.** Practitioners should not be reluctant to provide sealants—an intervention proven to be highly effective in preventing caries—because of concerns about inadvertently sealing over caries.

**Key Words.** Pit-and-fissure sealants; caries; bacteria.

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thereby, should arrest the lesion's progress.<sup>11</sup> Accordingly, the fate of bacteria in caries lesions that are purposely sealed over has been of great interest to researchers and clinicians alike.

Therefore, we undertook a systematic review of the evidence regarding the effectiveness of sealants in stabilizing or reducing bacteria levels in caries lesions. This study is part of a larger systematic review that examined the effectiveness of sealants in managing caries in the pits and fissures of permanent teeth. Another report from this review found that dental sealants reduced the probability of caries progression by more than 70 percent compared with untreated control teeth.<sup>12</sup>

## METHODS

**Inclusion criteria.** This analysis was part of a broader systematic review of sealant effectiveness in known carious lesions in the pits and fissures of permanent teeth. Initially, we included all in vivo studies published in English that compared outcomes, such as caries progression or bacteria levels, in permanent teeth treated with sealants with outcomes in permanent teeth not treated with sealants. Comparisons could involve concurrent randomized controlled trials (RCTs), controlled trials or cohort studies (prospective or retrospective) or studies conducted across time (before-and-after, time series) in the same groups. In this analysis, we included comparative studies that examined bacteria viability in sealed carious lesions. There were no restrictions regarding study populations.

**Identification of studies.** Details of our search strategy and results have been described elsewhere.<sup>12</sup> Two reviewers (B.G. and S.G.) independently examined the titles and abstracts of the 1,905 unique records identified in our search for primary studies or systematic or narrative reviews of the effectiveness of sealants in preventing or treating caries. Of these records, we ordered 262 articles; from our examination of their references, we ordered an additional 49 articles, for a total of 311.

**Study selection.** Three reviewers (B.G., S.G. and W.K.) reached a consensus that of these 311 articles, 26 studies should be evaluated further. These three reviewers rejected seven studies for inclusion for the following reasons: they were case studies, lacked appropriate outcomes or did not include both baseline and follow-up examinations. Of the 19 studies included in the larger system-

atic review, nine included data on bacteria levels under sealed carious lesions; of these nine studies, six had sufficient data from which to calculate outcome measures. The Quality of Reporting of Meta-Analyses Flow Diagram for the original, larger study has been published elsewhere.<sup>12</sup>

### Data abstraction and quality assessment.

Two reviewers (S.G. and E.O.) abstracted studies by using a modified version of a form developed for the National Institutes of Health Caries Consensus Development Conference in 2001.<sup>12</sup> This form was used in a systematic review of methods to manage caries.<sup>13</sup> We made one notable modification to the form to collect detailed information about bacteria-sampling methodology. The abstractors collected information to document study quality (in terms of such characteristics as study design, dropout rate, examiner blinding and bacteria-sampling methodology).

**Outcome measures.** We used two outcomes—mean viable bacteria count (VBC) as measured with colony-forming units per milligram (CFU/mg) and percentage of samples with VBC greater than zero—to measure activity for total bacteria, *Streptococcus mutans* and lactobacilli. To evaluate the effect of sealants on mean VBC, we examined the change in  $\log_{10}$  mean VBC ( $= \log_{10}$  mean VBC<sub>SEALED</sub> –  $\log_{10}$  mean VBC<sub>NOT-SEALED</sub>, where a  $\log_{10}$  mean VBC value of 6 equals  $1 \times 10^6$ , or 1,000,000 CFU) and whether the difference in mean VBC for sealed and unsealed teeth was significant ( $P < .05$ ). To measure the effect of sealants on the percentage of samples with VBC greater than zero, we used the percentage change in proportion of samples having VBC greater than zero:

$$\left( \frac{\% \text{ samples VBC} > 0 \text{ SEALED}}{\% \text{ samples VBC} > 0 \text{ NOT SEALED}} - 1 \right) \times 100$$

**Synthesis of findings.** We report the overall median and mean effect measures across all studies. We did not calculate confidence intervals for these summary measures because we included multiple observations from the same study, so observations likely were not independent.

**ABBREVIATION KEY.** CFU: Colony-forming unit.

DEJ: Dentinoenamel junction. GIC: Glass-ionomer cement. RBS: Resin-based sealant. RCT: Randomized controlled trial. VBC: Viable bacteria count.

## RESULTS

**Description of studies.** Of the six studies<sup>14-19</sup> used to calculate outcome measures in this analysis (representing 303 bacteria samples), two studies were RCTs,<sup>17,18</sup> one was a subgroup analysis of an RCT of split-mouth design,<sup>14</sup> two were controlled trials that did not mention randomization<sup>15,16</sup> and one was of a before-and-after design (in which the same tooth was sampled before and after sealant placement)<sup>19</sup> (Table 1).

About 94 percent of sampled lesions were cavitated at baseline (that is, allowed explorer penetration, had visible cavitation or had radiographic evidence of lesion depth ranging from the dentinoenamel junction [DEJ] to the dentin-pulp border but without pulpal involvement). The remaining 6 percent of lesions most likely were noncavitated (that is, they permitted the explorer probe to catch without penetration or sticking). In four studies, unsealed teeth likely had been carious for a shorter time than had sealed teeth.<sup>14-17</sup> Bacterial samples from unsealed teeth were obtained at baseline while samples from sealed teeth were obtained at follow-up<sup>15-17</sup> or, for the one study in which all bacteria samples were obtained at follow-up, unsealed teeth were diagnosed as carious at follow-up while sealed teeth were diagnosed at baseline.<sup>14</sup> Three studies used polymerized, resin-based sealant (RBS),<sup>14,15,17</sup> two used autopolymerized RBS<sup>16,18</sup> and one used both glass-ionomer cement (GIC) and visible-light-polymerized RBS.<sup>19</sup> Study populations included children, adolescents and young adults, ranging in age from 6 to 25 years.

**Sealant effectiveness: total bacteria.** We used results from four studies (18 observation points across five years representing 254 samples) to examine the effect of sealants on VBC.<sup>14-16,19</sup> There were no findings of significant increases in total bacteria under sealants. The reduction in log<sub>10</sub> mean VBC at the last period in each study was approximately three in two studies<sup>15,16</sup> and two in the remaining two studies<sup>14,19</sup> (one of these two studies reported the median not the mean value). The overall median and mean reductions were 3.01 and 2.56 (138 samples), respectively (Table 2, page 275), and appeared to increase as time since sealant placement increased. Mean total VBC was lower for sealed teeth than for unsealed teeth in the three studies that tested for statistical significance.<sup>14-16</sup>

Four studies (nine observations across five

years representing 117 samples) reported the proportion of samples with viable bacteria from sealed and unsealed caries lesions.<sup>14,17-19</sup> The reduction in the proportion of samples with viable bacteria attributable to sealants ranged from zero percent to 100.0 percent, with a median value of 50.0 percent and a mean value of 51.6 percent (Table 3, page 276). In all but one study,<sup>17</sup> lesions were sealed with a maximum depth of one-half of the distance from the DEJ to the pulp. In that study, however, the researchers presented findings for both moderate dentinal lesions ranging in depth from the DEJ to one-fourth the distance from the DEJ to the dentin-pulp border and deep dentinal lesions ranging in depth from one-fourth the distance from the DEJ to the pulp to the full distance from the DEJ to the pulp. If we were to exclude the findings for deep dentinal lesions, then the median and mean reduction in percentage of samples having viable bacteria would increase to 87.5 percent and 71.8 percent, respectively.

**Sealant effectiveness: *S. mutans* and lactobacilli.** Three studies<sup>14,16,19</sup> provided data for mean and median *S. mutans* VBC counts (seven observations representing 130 samples with follow-up times ranging from one day to five years; data not shown). Two of the three studies showed a twofold reduction in the log<sub>10</sub> mean *S. mutans* VBC at the last sampling period.<sup>14,16</sup> In one of these two studies, however, the median count was 0 for both sealed and unsealed teeth.<sup>16</sup> The other study, the only one to test for statistical significance, showed that the reduction was indeed significant.<sup>14</sup> In the third study, the reduction in the log<sub>10</sub> median *S. mutans* VBC was -0.45; it should be noted that in this study, the mean VBC were very low at baseline ( $< 1 \times 10^1$ ) and at follow-up ( $< 6 \times 10^1$ ), so any difference likely represented normal microbiological sampling variability. Two studies presented data on the percentage of samples with *S. mutans*. In one study,<sup>14</sup> sealants reduced the probability of viable *S. mutans* by 63 percent, and in the study with very low *S. mutans* counts at baseline, sealants increased the probability of viable *S. mutans* by 38 percent.

Two studies<sup>14,19</sup> provided data on lactobacilli counts (two observations across time representing 68 samples; data not shown). The reduction in log<sub>10</sub> mean and median VBC was 1.75. The reduction was significant in the one study that tested for statistical significance.<sup>14</sup> In both studies, the

TABLE 1

**Description of included studies.**

CHARACTERISTIC	STUDY AUTHOR, YEAR, SITE AND DURATION (MONTHS)					
	Going and Colleagues, <sup>14</sup> 1978, United States, 60	Handelman and Colleagues, <sup>15</sup> 1976, United States, 24	Jensen and Handelman, <sup>16</sup> 1980, United States, 12	Jeronimus and Colleagues, <sup>17</sup> 1975, United States, 1	Mertz-Fairhurst and Colleagues, <sup>18</sup> 1979, United States, 12	Weerheijm and Colleagues, <sup>19</sup> 1993, Netherlands, 7
<b>Subjects' Age (Years) and Background Community Fluoridation Exposure</b>	10 to 14; no fluoridation	12 to 15; study location was fluoridated	8 to 25; study location was fluoridated	6 to 12; not reported (NR)	Children; NR*	7 to 18; NR
<b>Lesion and Sealant Method by which cavitation status was assessed at baseline</b>	Visual-tactile (VT) examination	VT/radiographs	VT/radiographs	VT/radiographs	VT/radiographs	VT
<b>Lesion classification</b>	Enamel (explorer catch) or dentinal (explorer stick/penetration)	Dentinal: no more than one-half the distance between dentinoenamel junction (DEJ) and pulp	Dentinal: no more than half the distance between DEJ and pulp	Dentinal: from DEJ to pulp	Dentinal: lesion aperture between 1 and 3 mm	Dentinal: visible lesion
<b>Material used† Retention rate (%)</b>	RB1 100§	RB1 100	RB2 100	RB1‡ 100	RB2 NR	GIC/RB3 0/100
<b>Study Design</b>	51	NR	NR	11	4	13
<b>No. of subjects at baseline</b>	59	89	97	41	8	17¶
<b>No. of teeth Design</b>	Subgroup of randomized controlled trial (RCT) of split-mouth design (in subgroup analysis, control and treatment teeth not necessarily in same subject)	Non-RCT	Non-RCT	RCT (parallel groups)	RCT (split-mouth design)	Before-after
<b>Dropout (DO) rate for teeth</b>	27% across 5 years	NR	NR	NR	NR	NR
<b>Examiner blinding</b>	Yes#	NR	NR	NR	NR	NR
<b>Laboratory Methods</b>	70	89	97	41	8	17
<b>No. of samples</b>	Rubber dam	Rubber dam	Rubber dam	Rubber dam	Rubber dam	Rubber dam
<b>Isolation</b>	Betadine solution followed by 70% isopropyl alcohol	7% tincture of iodine and 70% alcohol	70% ethyl alcohol	7% tincture of metaphen followed by 70% alcohol	Merbromin and 70% alcohol	NR
<b>Site sterilization</b>	1 mg	1 mg	1 mg**	1 cubic mm	Dentin sample by probe mixed with Todd Hewitt medium and then 0.1 milliliter of mixture plated	0.2 mg
<b>Sample size</b>	MM10 sucrose agar, mitis-sucrose-bacitracin (MSB) agar, and Rogosa agar	Baird Parker	Baird Parker and MSB agar	Sterile trypticase soy broth	Todd Hewitt agar	Blood agar, nitrocellulose blood agar, and Rogosa agar
<b>Medium</b>	3 to 4 days	4 days	4 days	4 days	5 days	4 days
<b>Culture time</b>	CFU/mg (plate)††	CFU/mg (plate)	CFU/mg (plate)	Culture turbidity (yes/no)‡‡	CFU/mg (plate)	CFU/mg (plate)
<b>Outcome</b>	<p>* The researchers were located in Augusta, Ga., which had a fluoridated water supply at the time the study was conducted.                      † RB1: Ultraviolet light-polymerized resin-based sealant. RB2: Autopolymerized resin-based sealant. RB3: Visible light-polymerized resin-based sealant. GIC: Glass ionomer cement sealant.                      ‡ Findings for EpoxyLight 9075 (Lee Pharmaceuticals, South El Monte, Calif.) and 3M Caries Preventive Treatment (3M, now 3M ESPE, St. Paul, Minn.) excluded because two-week retention was less than 50 percent. For 3M product, acid concentration for etching was below recommended norm.                      § Study states that researchers verified integrity of sealant at each examination period (three, six, 12, 24, 36, 48 and 60 months).                      ¶ Study had 13 subjects and 24 teeth. We excluded findings for seven resealed teeth because the baseline bacteria levels were lower than those in never-sealed teeth.                      # All bacteriologic samples were processed and interpreted without knowledge of which treatment group was involved or of the clinical findings.                      ** The researchers attempted to obtain representative samples for all teeth; thus, for slight caries penetration they sampled almost the entire lesion, and for deep lesions they sampled both superficial and deeper layers.                      †† CFU/mg: Colony-forming units per milligram.                      ‡‡ Cloudiness in liquid culture indicates bacterial activity.</p>					

**TABLE 2**

**The effect of sealants on mean total viable bacteria count (MTVBC\*) per milligram of carious dentin, by months since placement.**

STUDY	MONTHS SINCE SEALANT PLACEMENT	SEALED CARIES		CONTROL		EFFECT		
		No. of Bacterial Samples	MTVBC	No. of Bacterial Samples	MTVBC	Log <sub>10</sub> Reduction	Mean Difference	P Value Mean Difference
Jensen and Handelman <sup>16</sup>	0.03	11	455.6 × 10 <sup>4</sup>	9†	925.1 × 10 <sup>4</sup>	0.3	469.5 × 10 <sup>4</sup>	.398
Jensen and Handelman	0.10	8	320.8 × 10 <sup>4</sup>	9	925.1 × 10 <sup>4</sup>	0.5	604.3 × 10 <sup>4</sup>	.227
Jensen and Handelman	0.23	10	120.6 × 10 <sup>4</sup>	9	925.1 × 10 <sup>4</sup>	0.9	804.5 × 10 <sup>4</sup>	.060
Handelman and Colleagues <sup>15</sup>	0.35	8	5.0 × 10 <sup>4</sup>	29‡	115.5 × 10 <sup>4</sup>	1.4	110.5 × 10 <sup>4</sup>	.048
Jensen and Handelman	0.5	12	35.9 × 10 <sup>4</sup>	9	925.1 × 10 <sup>4</sup>	1.4	889.2 × 10 <sup>4</sup>	.024
Handelman and Colleagues	1	10	4.7 × 10 <sup>4</sup>	29	115.5 × 10 <sup>4</sup>	1.4	110.8 × 10 <sup>4</sup>	.027
Jensen and Handelman	1	12	12.1 × 10 <sup>4</sup>	9	925.1 × 10 <sup>4</sup>	1.9	913.0 × 10 <sup>4</sup>	.020
Handelman and Colleagues	2	10	2.9 × 10 <sup>4</sup>	29	115.5 × 10 <sup>4</sup>	1.6	112.6 × 10 <sup>4</sup>	.025
Jensen and Handelman	2	8	154.5 × 10 <sup>4</sup>	9	925.1 × 10 <sup>4</sup>	0.8	770.6 × 10 <sup>4</sup>	.110
Handelman and Colleagues	4	6	1.0 × 10 <sup>4</sup>	29	115.5 × 10 <sup>4</sup>	2.1	114.5 × 10 <sup>4</sup>	.076
Jensen and Handelman	4	10	6.7 × 10 <sup>4</sup>	9	925.1 × 10 <sup>4</sup>	2.1	918.4 × 10 <sup>4</sup>	.034
Handelman and Colleagues	6	8	0.6 × 10 <sup>4</sup>	29	115.5 × 10 <sup>4</sup>	2.3	114.9 × 10 <sup>4</sup>	.040
Jensen and Handelman	6	8	7.5 × 10 <sup>4</sup>	9	925.1 × 10 <sup>4</sup>	2.1	917.6 × 10 <sup>4</sup>	.058
Weerheijm and Colleagues <sup>19</sup>	7	17	1.5 × 10 <sup>3</sup>	17	1.0 × 10 <sup>5</sup>	1.8	9.9 × 10 <sup>4</sup>	NR§
Handelman and Colleagues	12	12	0.1 × 10 <sup>4</sup>	29	115.5 × 10 <sup>4</sup>	3.0	115.4 × 10 <sup>4</sup>	.012
Jensen and Handelman	12	9	0.9 × 10 <sup>4</sup>	9	925.1 × 10 <sup>4</sup>	3.0	924.2 × 10 <sup>4</sup>	.043
Handelman and Colleagues	24	6	0.1 × 10 <sup>4</sup>	29	115.5 × 10 <sup>4</sup>	3.3	110.5 × 10 <sup>4</sup>	.073
Going and Colleagues <sup>14</sup>	60	30	25.6 × 10 <sup>3</sup>	21	32,247 × 10 <sup>3</sup>	2.1	3,199.1 × 10 <sup>3</sup>	< .05
Mean (Last Follow-Up)						2.5		
Median (Last Follow-Up)						3.0		

\* Power represents inverse of dilution ratio; that is, a power of 4 indicated dilution ratio was 1:4.  
 † Samples from nine teeth obtained at baseline served as the control group in all follow-up periods.  
 ‡ Twenty-nine samples obtained at baseline served as the control group in all follow-up periods.  
 § NR: Not reported.  
 ¶ Median value per 0.2 milligrams of carious dentin.

**TABLE 3**

**Percentage reduction in proportion of samples having viable bacteria for sealed and unsealed caries lesions.**

STUDY	MONTHS SINCE PLACEMENT	SEALED LESIONS			UNSEALED LESIONS			% REDUCTION
		No.	With > 0 CFUs*		No.	With > 0 CFUs		
			No.	%		No.	%	
<b>Jeronomus and Colleagues<sup>17</sup> (I†)</b>	0.5	6	1	17	6‡	4	67	75.0
<b>Jeronomus and Colleagues (I)</b>	0.75	6	0	0	6	4	67	100.0
<b>Jeronomus and Colleagues (I)</b>	1	6	0	0	6	4	67	100.0
<b>Weerheijm and Colleagues<sup>19</sup></b>	7	17	16	94	17§	17	100	5.9
<b>Mertz-Fairhurst and Colleagues<sup>18</sup></b>	12	4	0	0	4	4	100	100.0
<b>Going and Colleagues<sup>14,1</sup></b>	60	30	15	50	21	21	100	50.0
<b>Jeronomus and Colleagues (MD#)</b>	0.5	5	5	100	5**	5	100	0.0
<b>Jeronomus and Colleagues (MD)</b>	0.75	4	4	100	5	5	100	0.0
<b>Jeronomus and Colleagues (MD)</b>	1	3	2	67	5	5	100	33.3
<b>Median (All Studies, All Observations)</b>				50			100	
<b>Mean</b>				47			89	
<b>Median (All Studies, Excluding Jeronomus MD)</b>				8			83	
<b>Mean</b>				27			83	
<b>Median (All Studies, All Observations)</b>								50.0
<b>Mean</b>								51.6
<b>Median (All Studies, Excluding Jeronomus MD)</b>								87.5
<b>Mean</b>								71.8
* CFUs: Colony-forming units. † I: Incipient dental caries, no more than one-quarter of the distance between the dentinoenamel junction and pulp. ‡ Samples obtained from six teeth at baseline served as controls in all follow-up periods. § Bacterial samples obtained before sealant placement served as the control group; bacterial samples obtained from the same teeth seven months after sealant placement served as the treatment group. ¶ Minimum level of detection in study was 50 organisms per sample. # MD: moderate-to-deep dental caries, more than one-half the distance between the dentinoenamel junction and the pulp. ** Samples obtained from five teeth at baseline served as controls in all follow-up periods.								

percentage of samples with lactobacilli was lower for sealed teeth than for unsealed teeth. The percentage reduction in probability of viable lactobacilli was 37 percent.

**DISCUSSION**

Sealants were effective in reducing total bacteria counts in caries lesions. The reduction increased with time since sealant placement. At the last follow-up, there was a 100-fold decrease in mean

bacteria counts in two studies<sup>14,19</sup> and a 1,000-fold decrease in the remaining two studies.<sup>15,16</sup> Sealants also reduced bacterial cultivability. On average, 47 percent of sealed lesions had viable bacteria (median = 50 percent) compared with 89 percent of unsealed lesions (median = 100 percent). When we excluded deep dental lesions, these values decreased to 27 percent for sealed lesions (median = 8 percent) and 83 percent in unsealed lesions (median = 83 percent) (Table 3).

These data suggest that a limited number of cultivable organisms may persist in some lesions but that their numbers are small. The effect of sealants on levels of *S. mutans* and lactobacilli, which have been suggested as primary cariogens in pit-and-fissure caries, also was strong in two of the three studies that examined this outcome.<sup>14,16</sup> These results provide more specific information about the preventive effects of sealants at the surface level.

Bacterial activity, as measured by a reduction in log<sub>10</sub> mean VBC or the percentage of cultivable samples, decreased with time in all studies that had multiple follow-up periods.<sup>15-17</sup> Results of one study showed a linear decrease in mean log<sub>10</sub> VBC across time.<sup>16</sup> Since bacteria decreased across time, the findings of this review suggest that retained sealants deprive bacteria of access to nutrients in the substrate. Furthermore, it appears that bacteria that persist under sealants cannot produce acid when isolated from the carbohydrate substrate and, thus, adequately sealed lesions are unlikely to progress. Another analysis of studies included in the larger systematic review that supported this report on bacteria levels under sealants found that sealing noncavitated lesions reduced the probability of lesion progression by more than 70 percent.<sup>12</sup>

The importance of adequately sealing a carious lesion is further supported by the finding that retained sealants regardless of material were effective. Studies included in this review used a variety of sealant materials: RBS polymerized by visible or ultraviolet light, autopolymerized RBS and GIC. Of the six studies that used RBS,<sup>14-19</sup> five reported retention rates,<sup>14-17,19</sup> and in these studies, retention was 100 percent. For the one study that also used GIC, full retention was 0 percent, but in all lesions, the opening remained sealed at follow-up.<sup>19</sup> Because the opening remained sealed, we cannot determine if the effectiveness of GIC was attributable to the isolation of bacteria from nutrients in the substrate, the release of fluoride into the dentin or a combination of both factors. It is hypothesized that release of fluoride from GIC contributes to primary caries prevention.<sup>20</sup> However, the clinical effect of fluoride release from GIC is not well-established; a systematic review showed insufficient evidence to recommend GIC for the primary prevention of dental caries.<sup>2</sup> Interestingly, one study reported that fissures with caries retained sealants better than did apparently intact fissures.<sup>14</sup>

The larger systematic review found two additional studies providing evidence that sealants are effective in reducing bacteria viability. The first study,<sup>21</sup> which was published in 1943, examined bacteria levels in caries sealed with base-plate gutta-percha packed down tightly and then in turn covered by zinc oxyphosphate cement. Results from this study showed that lactobacilli died out in all cases between two and 10 months after sealing and that streptococcus test results remained positive in more than one-third of the teeth studied after having been sealed for more than one year. Another study, an RCT, compared sealing bacteria in carious dentin with GIC restorative material with sealing bacteria with amalgam.<sup>22</sup> This study found that at six months, both materials inhibited caries progression as measured by total counts of bacteria, *S. mutans* and lactobacilli but that a larger decrease in *S. mutans* and lactobacilli resulted from GIC use.

Other studies document that at least two other species of bacteria can persist even when deprived of nutrients.<sup>23,24</sup> These species enter a starvation state, which allows bacterial long-term persistence in a nongrowing but cultivable state for at least two months. Further research is needed to determine how long cariogenic bacteria can persist when isolated from nutrients. The longest period for studies included in this review was five years; however, current data suggest that a sizable number of sealants are retained for almost twice that time.<sup>25</sup> One additional argument for the effectiveness of sealants in reducing bacterial activity is the fact that fissures in sound teeth harbor cariogenic bacteria and that, because these sealed teeth remain caries-free in most instances, these sealed-over bacteria either perish or are no longer metabolically active. Study results indicate that some teeth still have a considerable number of bacteria remaining even after acid etching.<sup>14,17</sup>

One limitation of this review was that all included studies were conducted before 2000. The sole criterion for bacterial viability in these studies was cultivability. Since that time, microbiological quantification and characterization have become DNA-based, obviating the need for cultivation, which captures only the cultivable minority of microorganisms present.<sup>26</sup> Another limitation was that one outcome measure reported in four studies, mean VBC, is sensitive to outlying values.<sup>14-16,19</sup> As a result, mean VBC typically are transformed to log<sub>10</sub> values, and the

mean then is calculated for these transformed values. However, investigators in two of the three studies that found that mean VBC were lower in sealed teeth performed their statistical testing on transformed values.<sup>15,17</sup> Further research is needed with studies that meet current standards in design and conduct.

Our findings do not support reported concerns about poorer outcomes associated with inadvertently sealing caries and should lessen practitioners' reluctance to provide sealants—an intervention proven to be highly effective in preventing caries. Indeed, although study conduct varied considerably, there were no findings of significant increases in bacteria under sealants.

## CONCLUSION

We found that sealants significantly reduced bacteria levels in cavitated lesions, but that in some studies, low levels of bacteria persisted. These findings support those of a recent meta-analysis that sealants prevented caries progression.<sup>12</sup> In combination, these two sets of findings suggest that when sealants are retained, and thus access to fermentable substrates is blocked, bacteria do not appear capable of exerting their cariogenic potential. ■

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