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Effect of Dental Sealants on Oral Microbial Burden of *Scardovia wiggsiae* within a Pediatric Population: A Pilot Study

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Authors' contributions

This work was carried out in collaboration between both authors. Authors KQ and KK designed the study, performed the statistical analysis, wrote the protocol, and wrote the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: Dental caries is among the most prevalence and pervasive of childhood infections, with prevention factors such as fluoride and dental sealants regarded as the most effective prevention and treatment modalities. Many studies of dental sealants have focused on traditional caries-causing microbial agents, such as *Streptococcus mutans* – although the lack of clinical information regarding the novel cariogenic pathogen *Scardovia wiggsiae* s makes the primary aim of this study an evaluation of the prevalence of this organism and the effect of dental sealants on this pathogen.
Study Design: This was a prospective, non-randomized experimental study design.
Place and Duration of Study: University of Nevada, Las Vegas – School of Dental Medicine pediatric clinic between July 2016 and March 2018.
Methodology: Using an approved protocol, saliva was collected from pediatric patients at a Nevada dental school, which was subsequently screened for the presence of *Scardovia*. If treatment

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included dental sealants, saliva was collected at follow up appointments and screened using Polymerase Chain Reaction or PCR to determine any effects on the oral microbial burden of this organism.

Results: Pre-treatment samples (n=39) were matched with post-treatment samples (n=26) for DNA isolation and screening using PCR primers specific for *Scardovia*. Placement of dental sealants was sufficient to reduce the levels of detectable *Scardovia* among those patients initially testing positive (23%). However, most samples were initially *Scardovia*-negative (77%) and this study revealed a subset of these *Scardovia*-negative patients were subsequently found to harbor *Scardovia* from their corresponding post-sealant samples (28%).

Conclusion: This may be among the first studies to evaluate the effects of dental sealants on *S. wiggsiae*, demonstrating dental sealants may be sufficient to reduce *Scardovia* levels in some patients, but also allowing some patients with very low (even undetectable) levels of *Scardovia* to exhibit rapid and detectable changes in these levels.

Keywords: Scardovia wiggsiae; pediatric; dental sealant.

ABBREVIATIONS

Scardovia wiggsiae (SW); Office for the Protection of Research Subjects (OPRS); Institutional Review Board (IRB); University of Nevada Las Vegas (UNLV); School of Dental Medicine (SDM); polymerase chain reaction (PCR); deoxyribonucleic acid (DNA); glyceraldehyde 3-phosphate dehydrogenase (GAPDH) years (yrs) degrees of freedom (d.f.).

1. INTRODUCTION

Dental caries is among the most prevalence and pervasive of childhood infections, with many efforts focused on the epidemiology and prevention of early childhood caries [1-3]. Many factors are known to influence childhood caries, including parental influences such as socioeconomic status, income, and general health knowledge [4-6]. However, many of these same studies also conclude that brushing, flossing, and diet remain critical factors that are known to influence the microbial composition and burden that directly contributes to caries development [7,8].

Although a growing number of studies have found that oral health education and modeling are important factors to reduce the oral health disparities and caries burden among children, evidence continues to suggest that other prevention factors such as fluoride and dental sealants remain highly effective prevention and treatment modalities [9-12]. Although many studies have demonstrated clinical benefit and risk reduction with the use of dental sealants and varnishes, most of these studies have been done to evaluate the reduction in traditional caries-causing microbial agents, such as *S. mutans* [13-15]. However, new evidence has uncovered a novel cariogenic pathogen *Scardovia wiggsiae* recovered from children and adolescents with both low- and high-caries risk and caries experience – and no evidence has yet evaluated the effect of prevention measures including dental sealants on the microbial burden in these patients [16-19].

Recent studies from this group have revealed the presence of *S. wiggsiae* among both pediatric and adult patients, although no evidence to date has been collected to evaluate the effect of caries prevention efforts such as the placement of dental sealants on oral microbial burden of this organism [20]. Based upon this paucity of evidence, the primary objective of this pilot study was to evaluate the microbial burden of *Scardovia* among pediatric clinical patients in a Nevada dental school – and to subsequently evaluate the effect of dental sealants on the oral microbial burden of this pathogen within this patient population.

2. METHODOLOGY

2.1 Study Approval

The protocol for this study was reviewed and approved by the Office for the Protection of Research Subjects (OPRS) and Institutional Review Board (IRB) OPRS#880427-1 “The Prevalence of Oral Microbes in Saliva from the University of Nevada Las Vegas (UNLV) School

of Dental Medicine (SDM) pediatric and adult clinical population".

2.2 Study Design

This prospective study involved the collection of saliva from a convenience sample of pediatric clinic patients, recruited at random the UNLV-SDM pediatric clinic between May 2016 and March 2018. Inclusion criteria included pediatric patients aged seven (7) years or older and their parents or guardians who agreed to participate. Pediatric assent and Parental permission to consent for voluntary participation were obtained at the time of study enrollment. Exclusion criteria included any child that was not a patient of record at UNLV-SDM, any child who declined to participate, any patient over the age of 18 years, and any parent or guardian that declined to let their child participate.

2.3 Saliva Collection (Initial)

In brief, patients enrolled in the study were given a sterile 50 mL container for saliva collection and asked to provide up to 5 mL for analysis. Samples were stored on ice until transfer to a biomedical laboratory for processing and screening. To prevent research bias and prevent any patient identifying information from being disclosed, a randomly generated, non-duplicated number was assigned to each sample and concurrently collected patient demographic information. No patient-specific identifying information was subsequently available to any research team member.

2.4 Sealant Placement Protocol

All patients who received dental sealants were standardized. Each patient had Isolite isolation or cotton roll isolation if this was not tolerated. All providers were standardized and calibrated for dental sealant placement with the 3m ESPE Clinpro Sealant technique guide - with a modification of placing bonding agent between etching and placing the sealant. Each tooth receiving a dental sealant was etched for 15 seconds with 37% phosphoric acid etch, bonded with Optibond solo plus, sealed with 3m ESPE Clinpro Sealant, and cured for 20 seconds. Sealants were placed on the occlusal surfaces of molars and pre-molars.

2.5 Saliva Collection (Follow Up)

If dental sealants were deemed appropriate for the clinical treatment plan, treatment was provided and the patient was scheduled for follow up appointments according to clinic protocol. At the recall or secondary appointment, patients were allowed to provide a secondary or follow up (post-sealant) sample for screening and analysis. The majority of patients given dental sealants were scheduled for 90 day recall appointments, with the majority returning within six months of the original saliva collection and dental sealant placement.

2.6 DNA Isolation

All samples (pre-sealant, post-sealant) were stored in a biomedical laboratory at -80C. To screen for the presence of *S. wiggsiae* (SW), DNA was subsequently isolated from each patient sample using the GenomicPrep DNA isolation kit from Amersham Biosciences (Buckinghamshire, United Kingdom) and the procedure recommended by the manufacturer. The DNA isolates were screened for quality and quantity using ratio measurements of absorbance at 260 and 280 nm (A260/A280 ratio).

2.7 Polymerase Chain Reaction (PCR)

Screening for the presence of SW was accomplish using PCR with specifications that included an initial incubation at 50C for two minutes, denaturation at 95C for 10 minutes and 40 cycles at 95C for 15 seconds and 60C for one minute. Positive DNA controls were derived from previously identified SW-positive samples. Primers synthesize from Eurofins MWG Operon (Huntsville, AL) were:

Forward primer-GAPDH,
ATCTTCCAGGAGCGAGATCC; 20 nt, 55% GC, Tm 66C
Reverse primer-GAPDH,
ACCACTGACACGTTGGCAGT; 20 nt, 55% GC, Tm 70C
Annealing temperature 67C
Optimal temperature T(opt): Lower temperature – 5C = 61C

Forward 16S rRNA universal primer,
ACGCGTCGACAGAGTTTGATCCTGGCT; 27 nt, 56% GC, Tm 76C
Reverse 16S rRNA universal primer,
GGGACTACCAGGGTATCTAAT; 21 nt, 48% GC, Tm 62C
Annealing temperature: 63C
Optimal temperature T(opt): Lower temperature – 5C = 58C

Forward primer-SW,
GTGGACTTTATGAATAAGC; 19 nt, 37% GC, Tm 55C
Reverse primer- SW,
CTACCGTTAAGCAGTAAG; 18 nt, 44% GC, Tm 56C
Annealing temperature: 56C
Optimal temperature T(opt): Lower temperature – 5C = 50C

2.8 Statistical Analysis

Basic demographic information regarding the study sample (age, sex, race or ethnicity) were compiled and presented using simple descriptive statistics (counts and percentages). To determine the appropriate sample size for this type of PCR screening for microbial composition using DNA extracted from saliva, the recovery rate from the sample-limited step of DNA extraction was used (90-95%) to establish the minimum expected difference of 0.10 or 10%. Using a significance level of alpha= 0.05 and a power p = 0.80, a minimum sample size of forty (N = 40) was calculated. Any statistical differences between the pre- and post-sealant prevalence were determined using Chi square analysis.

3. RESULTS

A total of thirty nine (n=39) patients participated in this study, which was nearly evenly distributed among females (53.8%) and males (46.2%) and not significantly different from the overall clinic population, p=0.07 (Table 1). In addition to sex, demographic information was also collected about patient race and/or ethnicity, revealing the majority of patients were non-White minorities (69.2%) which was similar to the overall clinic patient population (58.6%) but statistically different, p<0.0001. More specifically, the percentage of patients in the study sample that were Hispanic or Latino (61.5%) was greater than the overall patient population (41.3%), although fewer were Black (5.1% vs. 13.1%) or Asian (2.6% vs. 4.2%). Finally, the average age of the study sample was 11.3 yrs with a range of 7 – 15 yrs, while the average age of the overall clinic population was 13.5 yrs with a range of 2 – 17 yrs, which was statistically significant, p<0.0001.

An analysis of the demographic profile of pre-sealant and post-sealant study participants was also performed (Table 2). These results revealed that only two-thirds or 66.7% of participants returned for the post-sealant screening (n=26/39). Of these patients, the majority were females (65.4%), which was higher than the pre-sealant study sample (53.8%) and was statistically significant, p<0.0001. In addition, the majority of returning post-sealant samples were derived from minorities (61.5%), which was lower than the pre-sealant study sample (69.2%) and statistically significant, p<0.0001. More specifically, fewer post-sealant samples were derived from Hispanics or Latinos (53.8%) compared with the pre-sealant group (61.5%), p<0.0001.

DNA was extracted from all samples and analyzed for both quality and quantity (Fig. 1). In brief, the average DNA concentration from the pre-sealant samples was 647.6 ng/uL with a range between 259 and 994 ng/uL. This was slightly higher than the DNA concentration from the post-sealant samples of 615.3 ng/uL with a range of 275 – 937 ng/uL, but was not statistically significant, p=0.571.

The average quality of DNA was assessed using spectrophotometric analysis of the ratio of absorbances of 260 nm and 280 nm or A260:A280. This analysis revealed an average DNA concentration from the pre-sealant samples of 1.54 ng/uL (ranging between 1.21 and 1.78), with the average DNA concentration from the post-sealant samples of 1.69 (ranging between 1.56 and 1.82).

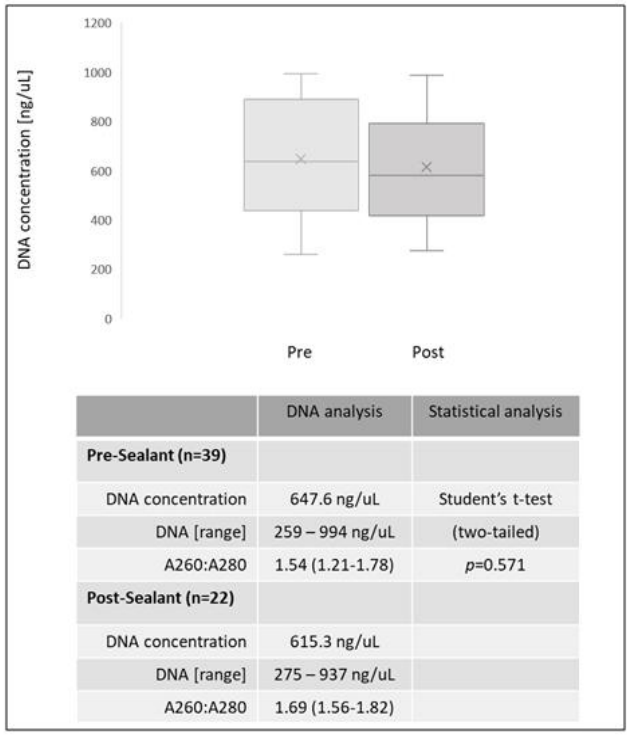
In order to provide positive controls for PCR and to determine any changes in overall levels between human and bacterial DNA pre- and post-sealants, DNA was screened using primers for the human glycolytic pathway enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and bacterial 16S rRNA (Fig. 2). These data clearly demonstrate that the relative fluorescence units (RFU) for GAPDH were similar in both the pre- and post-sealant samples (148.5 RFU, 144.2 RFU, respectively), which was not statistically significant, p=0.586. However, there were stark differences in the RFU for bacterial 16S rRNA, which was much higher in the pre-sealant group (101.2 RFU) than the post-sealant (74.2 RFU) group, which was statistically significant, p<0.0001.

To determine the effect of sealant placement on *S. wiggsiae* (SW) prevalence, DNA isolates were screened using primers specific for SW (Fig. 3). In brief, these data demonstrated that samples from SW-positive patients did not harbor

detectable levels of SW from the follow-up, post-treatment samples. Although the majority of samples that were found to be SW-negative in the pre-treatment samples group, a significant percentage of those patients were subsequently found to harbor small but detectable amounts of SW in the post-treatment samples – and were therefore considered SW-positive. Despite the change from SW-negative to SW-positive among some of the patients evaluated, these changes in the overall percentage of patients testing SW-positive were not large enough to be statistically significant, p=0.1594.

Table 1. Demographic analysis of study sample

	Study sample (n=39)	Pediatric clinic	Statistical analysis
Sex			χ2=3.365
Female	53.8% (n=21)	50.9%	d.f.=1
Male	46.2% (n=18)	49.1%	p=0.0667
Race / Ethnicity			
White	30.8% (n=12)	41.4%	χ2=180.889
Minority	69.2% (n=27)	58.6%	d.f.=3
Hispanic or Latino	61.5% (n=24)	41.3%	p<0.0001
Black	5.1% (n=2)	13.1%	
Asian / Other	2.6% (n=1)	4.2%	
Age			
Average age	11.3 yrs.	13.5 yrs	Two-tailed t-test
Age range	7 – 15 yrs	2 – 17 yrs.	p<0.0001



	DNA analysis	Statistical analysis
Pre-Sealant (n=39)		
DNA concentration	647.6 ng/uL	Student's t-test
DNA [range]	259 – 994 ng/uL	(two-tailed)
A260:A280	1.54 (1.21-1.78)	p=0.571
Post-Sealant (n=22)		
DNA concentration	615.3 ng/uL	
DNA [range]	275 – 937 ng/uL	
A260:A280	1.69 (1.56-1.82)	

Fig. 1. Analysis of DNA isolation. DNA isolated from the pre-sealant (n=39) and post-sealant (n=26) samples was analyzed to determine quality and quantity. Average DNA quantity from the pre-sealant samples was 647.6 ng/uL (range 259-994 ng/uL) compared with 615.3 ng/uL (range 275-937 ng/uL) from the post-sealant samples (p=0.571). Purity determined by A260:A280 absorbance ratio was 1.54 and 1.69 for the pre- and post-sealant samples, respectively

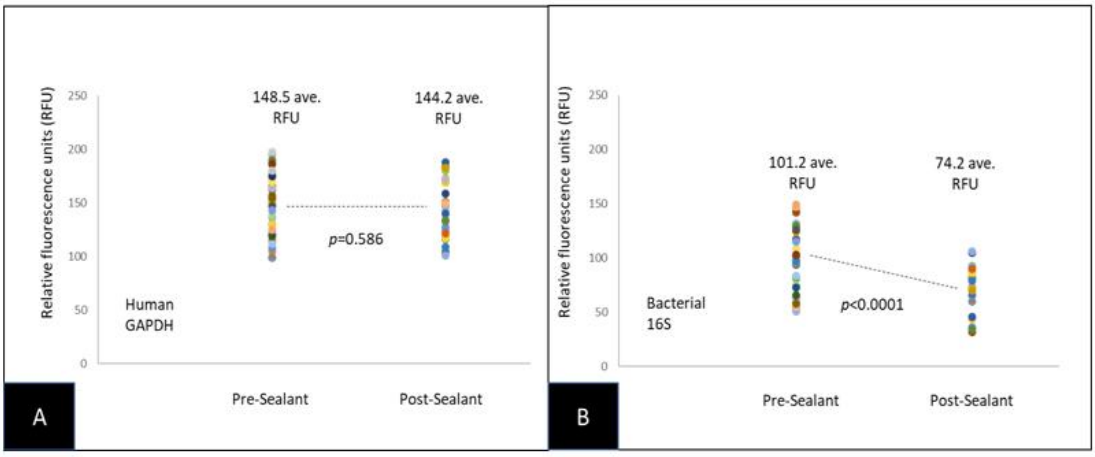
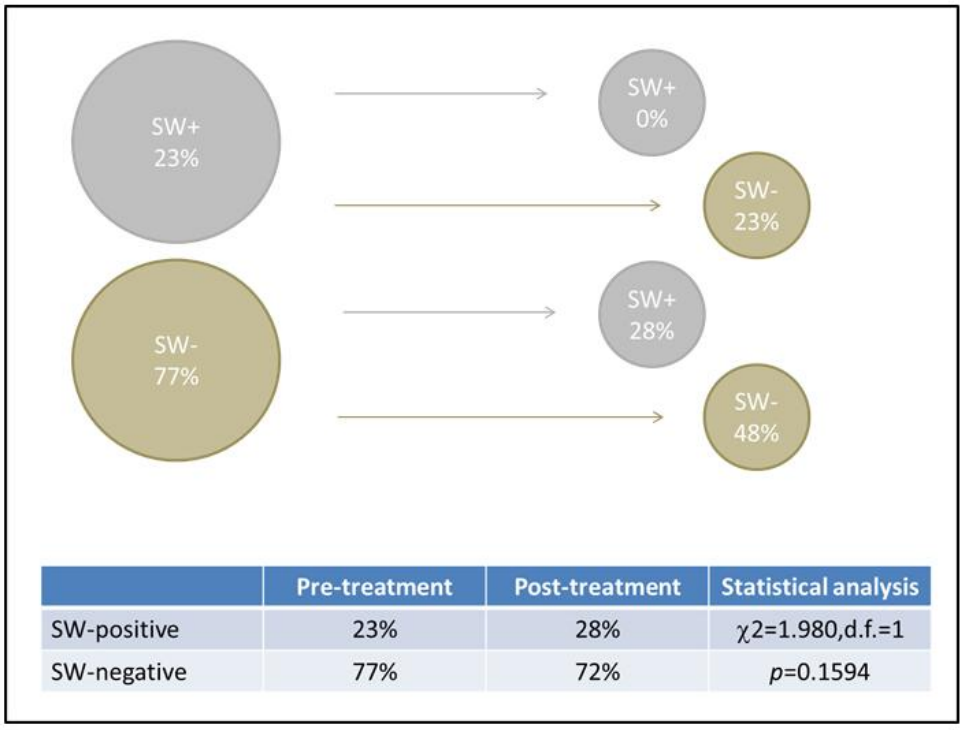


Fig. 2. PCR positive controls. Screening of isolates using both GAPDH and 16S rRNA primers revealed samples contained both human and bacterial DNA. A) No overall differences in human GAPDH RFU signals (representing DNA levels) between the pre- (148.5 RFU) and post-sealant (144.2 RFU) samples, p=0.586. B) Higher levels of bacterial 16S rRNA RFU signals were noted among the pre-sealant (101.2 RFU) samples compared with post-sealants (74.2 RFU), which was statistically significant, p<0.0001



	Pre-treatment	Post-treatment	Statistical analysis
SW-positive	23%	28%	$\chi 2$=1.980,d.f.=1
SW-negative	77%	72%	p=0.1594

Fig. 3. PCR analysis of *S. wiggsiae* (SW)-positive and negative samples. Screening of samples from the pre- and post-treatment group revealed differences in the prevalence of SW-positive and SW-negative samples that were not statistically significant, p=0.1594. However, more in-depth analysis revealed that some of the samples that initially tested SW-negative were subsequently found to harbor SW following the application of dental sealants. No differences in average age were found between the pre- and post-treatment SW-positive samples (11.6 yrs, 11.2 yrs yrs, p=0.221)

Table 2. Demographic analysis of pre- and post-sealant study participants

	Pre-sealant (n=39)	Post-sealants (n=26)	Statistical analysis
Sex			$\chi 2=59.465$
Female	53.8% (n=21)	65.4% (n=17)	d.f.=1
Male	46.2% (n=18)	34.6% (n=9)	$p<0.0001$
Race / Ethnicity			$\chi 2=25.041$
White	30.8% (n=12)	38.5% (n=10)	d.f.=1
Minority	69.2% (n=27)	61.5% (n=16)	$p<0.0001$
Hispanic or Latino	61.5% (n=24)	53.8% (n=14)	
Black	5.1% (n=2)	3.8% (n=1)	
Asian / Other	2.6% (n=1)	3.8% (n=1)	
Age			
Average age	11.3 yrs.	11.2 yrs.	Two-tailed t-test
Age range	7 – 15 yrs	7 – 15 yrs.	$p=0.7654$

4. DISCUSSION

The primary objective of this pilot study was to evaluate the microbial burden of *Scardovia* among pediatric clinical patients in a Nevada dental school – and to subsequently evaluate the effect of dental sealants on the oral microbial burden of this pathogen within this patient population. The results of this pilot study demonstrated that dental sealants may be an effective tool in reducing *Scardovia* in those patients already determined to have a significant oral microbial burden. However, this study also demonstrated that among the *Scardovia*-negative patients – the effect of sealant application may not be sufficient to stop the oral microbial composition from becoming *Scardovia*-positive in all patients.

Therefore, these data may have direct implications for clinicians interested in public health and prevention strategies for reducing cariogenic pathogens using dental sealants. The mechanisms that might explain this conversion are complicated, but may involve the competition of microbial constituents for scarce oral resources. For example, the placement of sealants may have negative impacted other biofilm and cariogenic organisms on the occlusal surfaces (such as *Streptococcus mutans*), which may then allow for competitive advantages to small populations of *Scardovia* on other non-occlusal surfaces. This working hypothesis may be possible to test using direct tooth biofilm sampling for comparison with overall salivary screening results to determine the relative abundance of these organisms on specific tooth surfaces.

Despite the significance of these results, there are limitations associated with this study that must also be considered. For example, this is the first pilot study of this organism involving sealants, which included only a limited number of pediatric patients at a non-profit dental school clinic. Previous research has demonstrated that this patient population has significant oral health challenges and lower levels of oral health literacy, which may influence the findings of this study [21,22]. If parents or children believe they are protected from cavities following the placement of dental sealants, any negative changes in oral hygiene (such as less frequent brushing) or diet (such as more frequent cariogenic food intake) could have affected the overall results. In addition, other variables that could have significantly affected microbial count, such as sealant microleakage, dietary patterns and brushing habits, were not measured in the current study but should be included in all future studies.

As more studies evaluate the biofilm, salivary and microbial profiles of children and adults with *S. wiggsiae*, new methods of identification, screening, prevention and treatment may be revealed [23,24]. In fact, recent evidence now suggests specific therapies that include sugar alcohols might be effective in reducing specific species of oral bacteria – including *S. wiggsiae* [25,26]. There may be many other factors that may influence the acquisition and prevalence of this organism, although more research will be needed to elucidate the role of these variables.

One additional complication for these types of studies may be the type of sealants used. For example, this study was completed at an academic dental school clinic, which utilizes one type of specific dental sealant for all patients [27,28]. Several types of dental sealants are available, including resin-based, glass ionomer,

and resin-modified glass ionomer sealants, which may also have influenced the outcomes of this study [29,30]. Future studies may include comparisons between different types of sealants and their effects on Scardovia prevalence, which may provide more thorough and comprehensive information for oral health researchers and clinicians.

5. CONCLUSIONS

Despite these limitations, these results demonstrated that significant and detectable changes can be observed in the oral microbial burden of this organism following placement of dental sealants. More research will be needed to determine the specific mechanisms that might be responsible for these observations, which should provide more detailed information regarding the benefits and risks of dental sealant placement for the reduction in overall cariogenic potential and risk in these patient populations. Finally, other potential methods for reducing the microbial burden of *Scardovia* must also be explored.

CONSENT

Pediatric assent and Parental permission to consent for voluntary participation were obtained at the time of study enrollment.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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