CLINICAL TRIALS

Effect of Fluoridated Sealants on Adjacent Tooth Surfaces: A 30-mo Randomized Clinical Trial

M.G. Cagetti¹, G. Carta², F. Cocco^{1,3}, S. Sale², G. Congiu², A. Mura², L. Strohmenger¹, P. Lingström⁴, G. Campus^{1,2*}, and Italian Experimental Group on Oral Health[†]

Abstract: A double-blind randomized clinical trial was performed in 6- to 7-yr-old schoolchildren to evaluate, in a 30-mo period, whether the caries increment on the distal surface of the second primary molars adjacent to permanent first molars sealed with fluoride release compounds would be lower with respect to those adjacent to permanent first molars sealed with a nonfluoridated sealant. In sum, 2,776 subjects were enrolled and randomly divided into 3 groups receiving sealants on sound first molars: high-viscosity glass ionomer cement (GIC group); resin-based sealant with fluoride (fluoride-RB group); and a resin-based sealant without fluoride (RB group). Caries ($D_1 - D_3$ level) was recorded on the distal surface of the second primary molar, considered the unit of analysis including only sound surfaces at the baseline. At baseline, no differences in caries prevalence were recorded in the 3 groups regarding the considered surfaces. At follow-up, the prevalence of an affected unit of analysis was statistically lower ($p = .03$) in the GIC and fluoride-RB groups ($p = .04$). In the GIC group, fewer new caries were observed in the unit of analysis respect to the other 2 groups. Incidence

rate ratios (IRRs) were 0.70 (95% confidence interval: 0.50, 0.68; $p < .01$) for GIC vs. RB and 0.79 (95% confidence interval: 0.53, 1.04; $p = .005$) for fluoride-RB vs. RB. Caries incidence was significantly associated with low socioeconomic status (IRR = 1.18; 95% confidence interval: 1.10, 1.42; $p = .05$). Dental sealant high-viscosity GIC and fluoride-RB demonstrated protection against dental caries, and there was evidence that these materials afforded additional protection for the tooth nearest to the sealed tooth (clinical trial registration NCT01588210).

Key Words: pit and fissure sealants, fluorides, dental caries, primary teeth, community dentistry, primary prevention.

The use of fluoride is the main reason for the decrease in prevalence of caries seen lately in Western industrialized countries (Bratthall, 2005; Petersen and Christensen, 2006; Pitts *et al.*, 2006). Otherwise, recent surveys have described a reversal of this trend: a small but significant increase in caries prevalence was detected in primary and permanent dentition (Bagramian *et al.*, 2009). Socioeconomic status (SES)

of the family acts as a well-known risk factor in caries development (Campus *et al.*, 2007, 2009; Bagramian *et al.*, 2009). Children from families with a low SES—such as those living in southern Italy, where the mean *per capita* income is significantly lower than in other parts of the country—have less access to dental care services and consequently show a higher level of caries disease (Campus *et al.*, 2007, 2009). “Bad” dietary and behavioral habits—such as high-frequency consumption of sugary food and beverages and poor oral hygiene—are also associated to caries development (Harris *et al.*, 2004).

Low but slightly elevated levels of fluoride in the oral environment, derived from different sources, help to prevent enamel dissolution and reduce caries development (ten Cate & Featherstone, 1991). The use of fluoride-releasing dental materials was proposed to provide an additional benefit in caries prevention on adjacent teeth surfaces (Donly *et al.*, 1999). Different fluoride-releasing materials have been used as sealants, including glass ionomer cements (GICs), resin-modified glass ionomer cements, fluoride-releasing composite sealants, and adhesive systems (Lobo *et al.*, 2005; Barja-Fidalgo *et al.*, 2009; Bayrak *et al.*,

DOI: 10.1177/0022034514535808. ¹WHO Collaborating Centre of Milan for Epidemiology and Community Dentistry, University of Milan, San Paolo Hospital, Milan, Italy; ²Department of Surgery, Microsurgery, and Medical Science, School of Dentistry, University of Sassari, Italy; ³Department of Chemistry, University of Sassari, Italy; and ⁴Department of Cariology, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Sweden; [†]Italian Experimental Group on Oral Health: M. Bossù, G. Campus, M.G. Cagetti, G.P. Caria, G. Carta, F. Cocco, G. Congiu, G. Conti, D. Corridone, P. Lingström, S. Mastroberardino, A. Mura, A. Polimeni, S. Sale, G. Sanna, L. Strohmenger; *corresponding author, gcampus@uniss.it

© International & American Associations for Dental Research

2010). The benefit of dental sealants for pits and fissures is based on retention and integrity over time. However, since the retention is not permanent, this physical effect could be enhanced by the local release of fluoride from the sealant material (Lobo *et al.*, 2005). Low retention rates for GIC sealants have been described (Poulsen *et al.*, 2001; Kühnisch *et al.*, 2011). Although high percentages of lost GIC sealant have been observed, this loss does not seem to have a direct relationship with caries development (Antonson *et al.*, 2012); even when the sealant appears partially lost clinically, it may act as a source of fluoride.

No clinical data are available in the literature regarding the caries preventive effect of fluoride released by dental sealants on the adjacent tooth surfaces. The same research group (Campus *et al.*, 2013) reported that the concentration of fluoride in interproximal fluids increased after the use of a high-viscosity GIC as a sealant, compared with a resin-based (RB) sealant with and without fluoride content.

The null hypothesis tested in this study was that in a period of 30 mo, for distal surfaces of second primary molars adjacent to sealed first permanent molars, the increment of caries lesions is the same whether the first permanent sealing material contains fluoride-releasing compounds (high-viscosity GIC or RB sealant containing fluoride) or not.

Materials & Methods

Study Design and Sampling

This randomized clinical trial was carried out on a population of schoolchildren (age range, 6-7 yr) from a low socioeconomic areas with a medium caries rate (Campus *et al.*, 2007, 2009). The fluoride concentration in local tap water is low (0.3 mg/L). Baseline examinations were carried out from January 2008 to June 2010, and follow-up examinations were carried out between July 2010 and January 2013. The study was part of a preventive project (supported by the Italian Ministry of Health) aimed to assess the prevalence of

caries disease and gingivitis in children, to seal their first permanent molars, and to provide information on oral health (Campus *et al.*, 2013).

The study was designed as a randomized clinical trial (registered at <http://www.clinicaltrials.gov>). The total number of children aged 6 to 7 yr living in the studied area was 3,271. Parents/guardians were contacted by mail to provide consent for their children's participation in the trial. Inclusion and exclusion criteria were evaluated via an *ad hoc* prepared questionnaire, completed by parents together with the consent form.

The inclusion criteria were as follow: year of birth from 2002 to 2003, informed consent signed by parents/guardians, and the presence of at least 2 sound permanent first molars as soon after eruption as isolation could be achieved, as well as the presence of at least 1 sound distal surface of adjacent second primary molar (*i.e.*, to studied first permanent molars).

The exclusion criteria were as follows: carious and/or demineralized lesions, hypomineralized permanent molar, presence of filling or sealant on the occlusal surface of the first permanent molars, fixed orthodontic appliances, the use of topical antimicrobial agents (*e.g.*, chlorhexidine, triclosan and fluoride) except for fluoridated toothpaste. Children with systemic disease or a history of systemic antibiotic use during the 3 mo before the beginning of the trial were excluded.

All children ($N = 3,271$) were invited to participate: 2,975 (91.0%) agreed; 2,808 (85.8%) were eligible; and 2,776 (98.9%) of the eligible sample were enrolled. The participants, identified by serial number, were compiled into a list. Randomization was performed (G. Campus) with Excel 2003 in permuted blocks of 2 or 4 with random variation of the blocking number, and 3 groups were created: the first group received fissure sealants with a high-viscosity GIC (GIC group; $n = 926$); the second group received an RB sealant containing fluoride (the fluoride-RB group; $n = 923$); and the third group received an RB sealant without fluoride content (RB group; $n = 927$) (Figure 1).

Clinical Examination and Questionnaire

Clinical examinations were performed at baseline and 30-mo follow-up and were carried out in the Paediatric Dentistry Department at the University of Sassari by 2 *ad hoc* calibrated examiners (G. Carta, S.S.) who were kept blinded regarding the sealant materials used. Intra- and interexaminer reliability was assessed before the beginning of the 30-mo evaluation by examining and reexamining 20 random children the first day of the study and 72 hr afterward. Interexaminer reliability was evaluated using fixed-effects analysis of variance in comparison with benchmark values (G. Campus). Intraexaminer reproducibility was assessed as the percentage of agreement based on Cohen kappa statistic. Differences in scores were discussed with a benchmark (G. Campus) until consensus was reached. Good interexaminer reliability was found for initial and manifest lesions, with no significant difference from benchmark values ($p = .17$) and a low mean square of error (0.45). Intraexaminer reliability was also high (0.90) when analyzed with the Cohen kappa statistic.

The presence of carious lesions was assessed with a plain mirror and a World Health Organization periodontal probe, under optimal conditions. The caries score was recorded on the distal surfaces of the second primary molars. Both initial and manifest caries were scored ($D_1 - D_3$) (Beck *et al.*, 1997): D_1 was scored when a clinically detectable enamel lesion without cavitation was recorded; D_2 was scored when a cavity limited to enamel was found; and, finally, D_3 was scored when a cavity involving the dentine was detected (Warren *et al.*, 2006). Bitewing X-rays were taken and manually developed via standard conditions and standard processing times, and radiographs were examined according to O'Mullane criteria (O'Mullane *et al.*, 1997).

Participants were also examined to assess sealant retention 30 mo after the sealants had been placed. The examiners were blinded to the subject's allocation group, as the materials were the same

in appearance. The sealant retention rate was calculated with the Beirut criteria (Beirut *et al.*, 2006). An *ad hoc* prepared questionnaire was fulfilled by parents/guardians, investigating fluoride supplement use, brushing frequency, and some socioeconomic variables (Campus *et al.*, 2013).

Data Analysis

The distal surface of the second primary molar was considered the unit of analysis, so only surfaces that were sound at the baseline were included. An event was defined as the presence of a new carious lesion or a filling at the surface level that developed during the 30-mo follow-up period (Beck *et al.*, 1997). Differences among groups registered at baseline and at follow-up were evaluated with the nonparametric Mann-Whitney *U* test.

Systemic fluoride (tablets/drops) was categorized as *yes* when the parents stated that the child had used it for ≥ 1 yr. Brushing frequency was categorized as $\geq$ or $< 2/d$. The SES of the family was categorized according to the SocFam scale (Campus *et al.*, 2007) as a low and medium-low level based on the educational level of the parents.

Taking into account the large proportion of zero increments, the zero-inflated negative binomial regression was used to model the caries increment on distal surface of the second primary molar (Solinas *et al.*, 2009; Preisser *et al.*, 2012). The incidence rate ratios (IRRs) were calculated as the overall effect of GIC *vs.* RB, GIC *vs.* fluoride-RB, and fluoride-RB *vs.* RB. The relationships between new caries lesions and the following explanatory variables were evaluated: treatment group, age (in months), sex, systemic fluoride supplements, the socioeconomic level of the family (SES), brushing frequency, and examiner (G. Carta and S.S.). After 30 mo, the complete retention rate of the sealant was also calculated. The data were entered into a database, checked for errors, and analyzed by an epidemiologist who was blinded to the sealant procedure, using STATA 12.0 software (<http://www.stata.com>). A *p* value $< .05$ was considered statistically significant.

Figure 1.
CONSORT flowchart of the study.

Results

Table 1 reports the demographic characteristics of the 3 groups: GIC, fluoride-RB, and RB. No statistically significant differences were recorded between baseline and follow-up examination in the 3 groups regarding overall caries experience, fluoride supplement use, and SES. The majority of the lesions were registered as D1; therefore, all lesions were combined in the same category (D₁ - D₃).

Table 2 shows the baseline and 30-mo follow-up caries data of the distal surface of the second primary molars (D₁ - D₃). At baseline, no differences in caries prevalence and indices were recorded among groups. The dropout rate was similar in the 3 groups (*p* = .17). At the follow-up, a reversal in caries diagnosis $< 1\%$ was recorded. In the GIC group, the prevalence of affected surfaces was

significantly lower (*p* = .03) than the mean number of distal surfaces affected (0.94 ± 1.17 *vs.* 1.02 ± 1.34 in the fluoride-RB group and 1.28 ± 1.50 in the RB group; *p* = .04). No statistically significant difference was noted regarding the number of distal surfaces filled among groups. The distal surfaces of the second primary molars developed fewer new caries in the GIC group than in the other 2 groups. IRR was 0.70 (95% confidence interval [CI]: 0.50, 0.68; *p* $< .01$) between GIC and RB and 0.89 (95% CI: 0.89, 1.28; *p* = .10) between GIC and fluoride-RB. IRR between fluoride-RB and RB groups was 0.79 (95% CI: 0.53, 1.04; *p* = .005). Caries incidence was also significantly associated with low SES (IRR = 1.18; 95% CI: 1.10, 1.42; *p* = .02).

At the 30-mo examination, 76 molars in the GIC group (2.95%), 52 in the fluoride-RB group (1.41%), and 50 in the RB group (1.35%) had partial sealant loss

Table 1.

Demographic Characteristics of the 3 Groups at Baseline and at the Follow-up Examination

Groups	GIC	Fluoride-RB	RB	<i>p</i>
Baseline				
No.	926	923	927	
Sex				
Male	462 (49.89)	458 (49.62)	455 (49.08)	
Female	464 (50.11)	465 (50.38)	472 (50.92)	.91
dmfs ^a	1.43 ± 3.09	1.48 ± 2.92	1.45 ± 2.97	.64
Socioeconomic status				
Medium-low	582 (62.85)	576 (62.40)	580 (62.57)	
Medium	344 (37.15)	347 (37.60)	347 (37.43)	.98
Systemic fluoride use				
Yes	87 (9.40)	95 (10.29)	88 (9.49)	
No	839 (90.60)	828 (89.71)	839 (90.51)	.77
30-mo examination				
No.	851	856	850	
Sex				
Male	429 (50.41)	424 (49.53)	427 (50.24)	
Female	422 (49.59)	432 (50.47)	423 (49.76)	.93
dmfs ^a	1.49 ± 2.67	1.56 ± 3.05	1.52 ± 2.63	.57
Socioeconomic status				
Medium-low	534 (62.75)	552 (64.49)	521 (61.29)	
Medium	317 (37.25)	304 (35.51)	329 (38.71)	.39
Systemic fluoride use				
Yes	86 (10.11)	87 (10.16)	84 (9.88)	
No	765 (89.89)	769 (89.84)	766 (90.12)	.98

Values in no. (%) or mean ± SD.

GIC, glass ionomer cement; RB, resin based.

^aFor each group, median = 0.

($p = .03$). No statistical association was found between incomplete sealants and caries development ($p = .28$).

Discussion

This study sought to explore whether sealing the first permanent molar with a fluoridated sealant might affect the caries incidence of the adjacent distal surface of the second primary molar. The hypothesis was that the caries rate, evaluated in a 30-mo period, would be lower in teeth sealed with fluoride-releasing compounds (high-viscosity GIC or RB sealant

containing fluoride) with respect to those sealed with a nonfluoridated material (RB sealant). The null hypothesis was rejected; the distal surfaces of the second primary molar adjacent to a tooth sealed with GIC displayed a significantly lower level of caries with respect to surfaces near teeth sealed with a traditional RB sealant without fluoride.

The status of the distal surface of the primary second molars was chosen as the outcome because the presence of a caries lesion on this surface may significantly affect the development of the disease of the mesial surface of the permanent first

molars (Mejäre *et al.*, 2001; Vanderaas *et al.*, 2004). Moreover, caries experience in primary molars at the age of 5 is the best predictor of the future caries experience in the permanent molars (Gray *et al.*, 1991).

High-viscosity GIC sealants exhibited a caries-preventive effect, which was 3 times higher than that of the composite resin sealant after 5 yr (Beirut *et al.*, 2006). However, the caries-preventive effect was evaluated on the pits and fissures of the sealed tooth only.

Several randomized clinical trials have assessed the effectiveness of sealants in caries prevention at the pits and

Table 2.Baseline and 30-mo Follow-up Caries Data ($D_1 - D_3$ Level) of the Distal Surface From the Second Primary Molars

Groups	GIC	Fluoride-RB	RB	<i>p</i>
Baseline examination				
No.	926	923	927	
Surfaces, no.	3704	3692	3708	
d/f surfaces, no.	913	875	940	
d(_v Dist), %	23.19	21.99	23.91	NS
f(_v Dist), %	1.45	1.70	1.45	NS
df(_v Dist), %	24.65	23.70	25.35	NS
d(_v Dist), mean ± SD	0.82 ± 0.77	0.81 ± 0.72	0.82 ± 0.88	NS
f(_v Dist), mean ± SD	0.02 ± 0.11	0.02 ± 0.12	0.02 ± 0.13	NS
df(_v Dist), mean ± SD	0.84 ± 0.82	0.83 ± 0.79	0.84 ± 0.85	NS
30-mo examination				
No.	851	856	850	NS
Dropout rate, %	8.15	7.20	8.30	
Surfaces, no.	3404	3424	3400	
Reversal, no. (%)	12 (0.35)	15 (0.44)	9 (0.29)	NS
d/f surfaces, no.	1249	1253	1415	
d(_v Dist), %	23.75	22.64	28.38	.03
f(_v Dist), %	13.08	14.12	13.35	NS
df(_v Dist), %	36.82	36.76	41.73	.04
Δdf(_v Dist), %	12.17	13.06	16.38	.04
d(_v Dist), mean ± SD	0.94 ± 1.17	1.02 ± 1.34	1.28 ± 1.50	.04
f(_v Dist), mean ± SD	0.51 ± 0.76	0.53 ± 0.88	0.50 ± 1.02	NS
df(_v Dist), mean ± SD	1.45 ± 1.58	1.55 ± 1.52	1.78 ± 1.24	.03
Δdf (dfup+Dfupdbas)	0.61	0.72	0.94	.03

GIC, glass ionomer cement; RB, resin based; d/f surfaces, number of decayed and filled surfaces; d(_vDist), decayed distal surface of the second primary molar; f(_vDist), filled distal surface of the second primary molar; df(_vDist), decayed and filled distal surface of the second primary molar; reversal, misclassification of caries diagnosis at follow-up; NS, nonsignificant.

fissures level (Jokovic and Locker, 2001; Mejäre *et al.*, 2003). The application of sealants is recommended to prevent caries in children and adolescents' permanent molars, and the effectiveness of different types of sealants has yet to be established (Ahovuo-Saloranta *et al.*, 2013); nevertheless, the use of high-viscosity GICs as sealants has recently increased due to their growing retention rate with respect to traditional GIC and to their fluoride-releasing capacity (Beirut *et al.*, 2006). It has been hypothesized that, despite a high rate of macroscopic sealant loss, GICs remain at the bottom of fissures, acting as fluoride reservoir (Barja-Fidalgo *et al.*,

2009). In the present article, no statistical significant difference regarding the retention rate when comparing the GIC and RB sealants was observed, and no statistically significant association was detected between sealants' retention rate and caries development in the distal surface of the second primary molar. This finding may be related to the preventive action produced by the remaining material in the bottom of the fissures (Barja-Fidalgo *et al.*, 2009). One may also speculate whether sealant loss had occurred shortly before the examination.

The caries pattern differs in different tooth sites with varying level of disease.

In primary teeth, it was suggested that more caries arise in molars, especially at the interproximal level (Allison and Schwartz, 2003). Interdental plaque is more acidogenic than plaque covering other surfaces. This is most likely related to variations in bacterial composition and saliva access, especially in primary molars with a wider contact surface (Cagetti *et al.*, 2011). Strategies to prevent interproximal caries in primary teeth were proposed: the benefit of professional flossing with fluoride and chlorhexidine was reported (Allison and Schwartz, 2003), but this method requires funds for dental personnel and multiple follow-ups.

Previously (Campus *et al.*, 2013), a higher fluoride concentration was found in the interproximal fluid, collected between the first permanent molar and the second primary molar, in teeth treated with GICs when compared with those recorded near teeth sealed with a RB sealant or not containing fluoride. This higher fluoride concentration may explain the reduced caries increment detected on the distal surfaces of the primary tooth.

In the present study, caries increment on the distal surface of second primary molars was significantly associated with the SES of the family, confirming that a low SES is an important risk factor for childhood caries development and supporting the need for preventive measures centered on children belonging to disadvantaged groups. The application of sealants containing fluoride appears to be an effective preventive procedure, requiring a single application and low costs.

Some shortcomings of this study might be raised. First, the follow-up time can be considered to be quite short, but this period was selected to reduce the risk of too many dropouts. It is also necessary to underline that caries increment was recorded to be present in second primary molars, and it is common that these teeth may already be exfoliated at the age of 10 yr. Second, a bias may exist related to environmental and behavioral factors, such as dietary habits or changes in lifestyle conditions. However, no population-based professional caries preventive program was proposed to these patients. Otherwise, this study benefits from several strengths, such as the high number of participants, the random allocation of subjects, the parallel-group design, the blinding of examiners and participants, the use of standardized criteria for the assessment of sealant retention, and the choice of a true outcome measure ($D_1 - D_3$ lesion).

Conclusion

The results of the present randomized clinic trial offer new insight into caries

prevention. Fluoride-releasing sealants (high-viscosity GIC and fluoride RB) were demonstrated to provide protection against caries on the distal surface of second primary molar. This preventive strategy might contribute to maintain the integrity of the mesial surfaces of the first permanent molar.

Acknowledgments

The authors thank the manager of the Sassari school district and all the teaching staff. This study was funded by the Italian Minister of Health. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

References

- Ahovuo-Saloranta A, Hiiri A, Nordblad A, Mäkelä M, Worthington HV (2013). Pit and fissure sealants for preventing dental decay in the permanent teeth. *Cochrane Database Syst Rev* 3:CD001830.
- Allison PJ, Schwartz S (2003). Interproximal contact points and proximal caries in posterior primary teeth. *Pediatr Dent* 25:334-340.
- Antonson SA, Antonson DE, Brener S, Crutchfield J, Larumbe J, Michaud C, *et al.* (2012). Twenty-four month clinical evaluation of fissure sealants on partially erupted permanent first molars: glass ionomer versus resin-based sealant. *J Am Dent Assoc* 43:115-122.
- Bagramian RA, Garcia-Godoy F, Volpe AR (2009). The global increase in dental caries: a pending public health crisis. *Am J Dent* 22:3-8.
- Barja-Fidalgo F, Maroun S, de Oliveira BH (2009). Effectiveness of a glass ionomer cement used as a pit and fissure sealant in recently erupted permanent first molars. *J Dent Child* 76:34-40.
- Bayrak S, Tunc ES, Aksoy A, Ertas E, Guvenc D, Ozer S (2010). Fluoride release and recharge from different materials used as fissure sealants. *Eur J Dent* 4:245-250.
- Beck ID, Lawrence HP, Koch GG (1997). Analytic approaches to longitudinal caries data in adults. *Community Dent Oral Epidemiol* 25:42-51.
- Beirut N, Frencken JE, van't Hof MA, Taifour D, van Palenstein Helderman WH (2006). Caries-preventive effect of a one-time application of composite resin and glass ionomer sealants after 5 years. *Caries Res* 40:52-59.
- Bratthall D (2005). Estimation of global DMFT for 12-year-olds in 2004. *Int Dent J* 55:370-372.
- Cagetti MG, Campus G, Sale S, Cocco F, Strohmer L, Lingström P (2011). Association between interdental plaque acidogenicity and caries risk at surface level: a cross sectional study in primary dentition. *Int J Paediatr Dent* 21:119-125.
- Campus G, Solinas G, Cagetti MG, Senna A, Minelli L, Majori S, *et al.* (2007). National Pathfinder survey of 12-year-old children's oral health in Italy. *Caries Res* 41:512-517.
- Campus G, Solinas G, Strohmer L, Cagetti MG, Senna A, Minelli L, *et al.* (2009). National pathfinder survey on children's oral health in Italy: pattern and severity of caries disease in 4-year-olds. *Caries Res* 43:155-162.
- Campus G, Carta G, Cagetti MG, Bossù M, Sale S, Cocco F, *et al.* (2013). Fluoride concentration from dental sealants: a randomized clinical trial. *J Dent Res* 92(7):238-288.
- Donly KJ, Segura A, Wefel JS, Hogan MM (1999). Evaluating the effects of fluoride-releasing dental materials on adjacent interproximal caries. *J Am Dent Assoc* 130:817-825.
- Gray MM, Marchment MD, Anderson RJ (1991). The relationship between caries experience in the deciduous molars at 5 years and in first permanent molars of the same child at 7 years. *Community Dent Health* 8:3-7.
- Harris R, Nicoll AD, Adair PM, Pine CM (2004). Risk factors for dental caries in young children: a systematic review of the literature. *Community Dent Health* 21(1):71S-85S.
- Jokovic A, Locker D (2001). Evidence-based recommendations for the use of pit and fissure sealants in Ontario's Public Dental Health Programs. In: *Quality assurance report 21*. Locker D, editor. Toronto, ON: Community Dental Health Services Research Unit, University of Toronto.
- Kühnisch J, Mansmann U, Heinrich-Weltzien R, Hickel R (2011). Longevity of materials for pit and fissure sealing: results from a meta-analysis. *Dent Mater* 28:298-303.
- Lobo MM, Pecharki GD, Tengan C, da Silva DD, da Tagliaferro EP, Napimoga MH (2005). Fluoride-releasing capacity and cariostatic effect provided by sealants. *J Oral Sci* 47:35-41.
- Mejäre I, Stenlund H, Julihn A, Larsson I, Permert L (2001). Influence of approximal caries in primary molars on caries rate for the mesial surface of the permanent first molar in Swedish children from 6 to 12 years of age. *Caries Res* 135:178-185.
- Mejäre I, Lingström P, Petersson LG, Holm AK, Twetman S, Källestål C, *et al.* (2003). Caries-preventive effect of fissure sealants: a systematic review. *Acta Odontol Scand* 61:321-330.

- O'Mullane DM, Kavanagh D, Ellwood RP, Chesters RK, Schafer F, Huntington E, *et al.* (1997). A three-year clinical trial of a combination of trimetaphosphate and sodium fluoride in silica tooth-pastes. *J Dent Res* 76:1776-1781.
- Petersen PE, Christensen LB (2006). Dental health status and development trends among children and adolescents in Greenland. *Int J Circumpolar Health* 65:35-44.
- Pitts NB, Boyles J, Nugent ZJ, Thomas N, Pine CM (2006). The dental caries experience of 11-year-old children in Great Britain: surveys coordinated by the British Association for the Study of Community Dentistry in 2004/2005. *Community Dent Health* 23:44-57.
- Poulsen S, Beiruti N, Sadat N (2001). A comparison of retention and the effect on caries of fissure sealing with a glass-ionomer and a resin-based sealant. *Community Dent Oral Epidemiol* 29: 298-301.
- Preisser JS, Stamm JW, Long DL, Kincade ME (2012). Review and recommendations for zero-inflated count regression modeling of dental caries indices in epidemiological studies. *Caries Res* 46:413-423.
- Solinas G, Campus G, Maida C, Sotgiu G, Cagetti MG, Lesaffre E, *et al.* (2009). What statistical method should be used to evaluate risk factors associated with dmfs index? Evidence from the National Pathfinder Survey of 4-year-old Italian children. *Community Dent Oral Epidemiol* 37:539-546.
- ten Cate JM, Featherstone JD (1991). Mechanistic aspects of the interactions between fluoride and dental enamel. *Crit Rev Oral Biol Med* 2:283-296.
- Vanderas AP, Kavvadia K, Papagiannoulis I (2004). Development of caries in permanent first molars adjacent to primary second molars with interproximal caries: four-year prospective radiographic study. *Pediatr Dent* 26:362-368.
- Warren JJ, Levy SM, Broffitt B, Kanellis MJ (2006). Longitudinal study of non-cavitated carious lesion progression in the primary dentition. *J Public Health Dent* 66:83-87.