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Clinical Technique/Case Report

Clinical/Photographic/Scanning Electron Microscopy Analysis of Pit and Fissure Sealants After 22 Years: A Case Series

D Sundfeld • LS Machado • LM Franco • FM Salomão
NIP Pini • MLMM Sundefeld • CS Pfeifer • RH Sundfeld

Clinical Relevance

Resin-modified glass ionomer cements and polyacid-modified resin composite had similar clinical performance as pit and fissure sealants and successfully prevented dental caries lesions after 22 years.

SUMMARY

Pit and fissure sealant is a clinical technique adopted to prevent caries lesion development. Ionomeric and/or resin-based materials are commonly used for this purpose. This article presents a case series of sealed teeth with 22-year follow-up evaluated by clinical, photographic, and microscopic analysis. In 1992, sixteen patients (9-14 years of age) had at least three teeth sealed with one of the following materials: resin-modified glass ionomer cement (RMGIC, Vitrebond or Fuji II LC) or polyacid-modified resin composite (PMRC, VariGlass VLC), totaling 86 sealed permanent teeth. After 22 years, 10 patients were recalled, representing 41 teeth. The retention of sealants was assessed by three methods: clinical analysis by visual inspection; photography;

Daniel Sundfeld, DDS, MDS, PhD student, Department of Restorative Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, Brazil

Lucas Silveira Machado, DDS, MDS, PhD, professor, Department of Conservative Dentistry, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

Laura Molinar Franco, DDS, MDS, PhD student, Department of Restorative Dentistry, Araçatuba Dental School, State University of São Paulo, Araçatuba, Brazil

Fabio Martins Salomão, DDS, MDS, PhD student, Department of Restorative Dentistry, Araçatuba Dental School, State University of São Paulo, Araçatuba, Brazil

Núbia Inocência Pavesi Pini, DDS, MDS, PhD, temporary professor, Department of Restorative Dentistry, Araçatuba Dental School, State University of São Paulo, Araçatuba, Brazil

Maria Lúcia Marçal Mazza Sundefeld, DDS, MDS, PhD, professor, Department of Pediatric and Community Dentistry, Araçatuba Dental School, State University of São Paulo, Araçatuba, Brazil

Carmem Silvia Pfeifer, DDS, PhD, professor, Department of Restorative Dentistry, Division of Biomaterials and Biomechanics, Oregon Health and Science University, Portland, OR, USA

*Renato Herman Sundfeld, DDS, MDS, PhD, professor, Department of Restorative Dentistry, Araçatuba Dental School, State University of São Paulo, Araçatuba, Brazil

*Corresponding author: 1193 José Bonifácio St, Araçatuba, SP, Brazil; e-mail: sundfeld@foa.unesp.br

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and scanning electron microscope (SEM) images and classified as retained (pits and fissures filled by sealant material); partially retained (pits and fissures partially filled by sealant material); or totally lost (no material was found in pits and fissures). The SEM images provided a higher number of retained sealants when compared with the clinical and photographic evaluations. Also, no totally lost scores were found with SEM analysis, regardless of the sealing material. No caries lesions were found. A fully or partially retained sealant in pits and fissures was capable of preventing caries lesions after 22 years within the patient pool analyzed.

INTRODUCTION

Pit and fissure sealants were introduced in the 1960s as a clinical technique for preventing dental caries,[1,2] the most prevalent oral disease among children.[3] Sealants act as a physical barrier that prevents food/biofilm from accumulating in pits and fissures and therefore prevents the growth of bacteria that can lead to dental decay.[4] Since the first clinical report[5] on the application of pit and fissure sealants by Cueto and Buonocore in 1967, this extraordinary preventive technique has been applied all over the world, and hundreds of reports have demonstrated its effectiveness in hindering dental caries lesion development.

The most susceptible site for developing a caries lesion is the occlusal surface—in about 67%-90% of children 5-17 years old[3,6]—due to the shape, depth, and narrowness of fissures.[7,8] This anatomical configuration increases the difficulty of self-cleaning by the food bolus, tongue, cheeks, and lips while also making cleaning by other methods difficult.[7,8] Although dental caries is a preventable disease,[9] some populations all over the world still experience a high incidence,[10,11] in contrast to others where the prevalence of caries lesions is declining.[11,12] However, because oral hygiene is highly behavior-dependent, dental caries is still a problem, even in populations where its prevalence is low.[4]

Three main types of materials are available as pit and fissure sealants: glass ionomer cements (GICs), polyacid-modified resin composite (PMRCs), and resins.[2] GICs can chemically bond to calcified tissues and are able to release and take up fluoride.[13,14] Due to the properties of GICs, many clinical research studies have applied them as pit and fissure sealants but with poor retention rates through the years,[15,16] which has been explained by their inherent brittle characteristics, with low mechanical strength and wear resistance.[17-19] To overcome this condition, resin-modified glass ionomer cements (RMGICs) and PMRCs were developed in the late 1980s, combining components of GICs and composite resins,[2,20] in order to provide better esthetic properties, less sensitivity to the application technique, and a decrease in imbibition and synerisis.[21] On the basis of previous findings,[15,16] lower retention rates have been reported after clinical evaluation of RMGICs. However, these studies showed clinical analyses of no longer than one year. PMRCs, also known as "compomers," have been reported to perform as well as composites,[22] with even better resistance to wear than composite resin[23] but still with controversial retention rates for fissure sealants.[20,21,24]

Since the 1980s, the ability of pit and fissure sealants to protect pits and fissures has been assessed using sealant retention as the relevant end point, which also provides a quantification of the longevity of various sealing materials.[25] A well-retained material in the pits and fissures exercises its preventive effect for much longer than any other intervention.[26] The retention can be evaluated by clinical, digital photographic, replica, and microscopic analysis.[8,27]

Little scientific literature data are available regarding the use of compomers as pit and fissure sealants.[2] Thus, the aim of this case series study is to report the retention rate and characteristics of pit and fissure sealants performed with two RMGICs and one PMRC after 22 years of clinical use.

CASE SERIES

The patients had their teeth sealed in 1992 at the Araçatuba Dental School–São Paulo State University (UNESP), Brazil. Sixteen patients (9-14 years old; eight girls, eight boys) participated and had 86 permanent posterior teeth sealed (upper and lower molars and premolars)——on average, five to six teeth were sealed in each patient. All patients were given an initial clinical evaluation using visual inspection with adequate light, a dental mirror/probe, and bitewing radiographs to assess the presence or absence of caries lesions. The patients received oral hygiene instructions regarding tooth brushing and routine flossing. The general population of 9- to 14-year-olds in Araçatuba was previously deemed as being "moderate to high caries-risk" according to the 1994 decayed-missing-filled index. Fluoride was available in the city's water supply. The patients presented at least three sound, unsealed permanent premolars and molars. Teeth with

Table 1: *Composition of the Materials Used in This Study*

Commercial Sealant	Clinical Indication	Composition	Powder to Liquid Ratio
Vitrebond (3M ESPE) Resin-modified glass ionomer	Liner/base	Powder: fluoroaluminosilicate glass. Liquid: copolymer of acrylic and maleic acid; HEMA; photoactivator, water.	1:1
Fuji II LC (GC) Resin-modified glass ionomer	Restorative material	Powder: fluoroaluminosilicate glass. Liquid: aqueous solution of polycarboxylic acid, TEGDMA, and HEMA.	1:2
VariGlass VLC (Dentsply) Polyacid-modified resin composite	Restorative material	Powder: fluoroaluminosilicate glass. Liquid: polyacrylic acid, HEMA, TEGDMA, 2,6-Di-tert-butyl-4-methylphenol.	1:2

Abbreviations: HEMA, 2-hydroxyethylmethacrylate; TEGDMA, triethyleneglycol dimethylmethacrylate.

active decay or previously placed restorations were not included in the study. All the procedures were explained to the parents, who gave consent for the study.

Clinical Procedures

One operator (last author) performed all of the occlusal sealing procedures. First, all teeth subjected to the sealing procedures were isolated with a rubber dam and were cleaned with pumice and water. Then, acid etching using 37% phosphoric acid was performed for one minute on the entire occlusal surface, under vibration, inside the pits and fissures with a tapered-end explorer probe. After washing and drying, one of the following sealing materials was applied: RMGIC (Vitrebond, 3M ESPE, St Paul, MN, USA); RMGIC (Fuji II LC, GC, Tokyo, Japan); PMRC (VariGlass VLC, Dentsply, Milford, DE, USA) (Table 1). Each patient had at least one type of each sealant material. The materials were hand-mixed and inserted into pits and fissures using a No. 5 explorer probe, also under constant vibration. Each material was light-cured for 40 seconds using a halogen light-curing device (Fibralux, Dabi Atlante, Ribeirão Preto, Brazil). Occlusal contacts were checked and adjusted when necessary using fine-tapered diamond finishing burs. No sealants required replacement.

Clinical, Photographic, and SEM Analysis

After 22 years, 10 patients attended the recall appointment (five women, five men), representing 50 teeth subjected to the present analysis. Four sealed teeth were extracted due to orthodontic reasons, and five were restored: three due to interproximal caries and two to be used as retainers for fixed partial dentures. Therefore, 41 teeth (18 lower premolars, 11 upper premolars, seven lower molars, and five upper molars) were evaluated for sealant retention through clinical analysis, photography, and SEM images.

Sealed teeth were evaluated by three methods in order to assess the sealant retention: 1) clinical analysis: visual assessment using adequate light, drying the occlusal surface and dental mirror; 2) photographic images: sealed teeth were recorded using a digital photographic camera (Nikon D 300 Digital Camera–Macro Lens 105 mm f/2.8G, Nikon Corp, Japan); and 3) SEM images: impressions of the sealed teeth were made using a silicone impression material (Express XT Putty and Light body, 3M ESPE). Then, epoxy resin (Epo-Thin Resin, Buehler Lake Bluff, IL, USA) was used to cast the impressions and produce replicas. The replicas were positioned on aluminum stubs and sputter-coated with gold and subjected to SEM analysis (JSM 5600LV, JEOL, Tokyo, Japan) operated under 15 kV. Both photographic and SEM images were shown on an LED screen that had a resolution of 1366×768 pixels. The clinical analysis and photographic/SEM images were assessed by two double-blind authors who were previously calibrated and unaware of the type of sealant material used (only the last author knew which material was being evaluated). Intraexaminer reproducibility was assessed by the Cohen κ test with a reliability of 81% for clinical analysis, 96% for photographic images, and 100% for SEM images.

The criteria adopted to rank the three methods of evaluation were 1) retained (R): pits and fissures filled by sealant; 2) partially retained (PR): pits and fissures partially filled by sealant; and 3) totally lost (T): no sealant material was found in pits and fissures.

Clinical, Photographic, and SEM Analysis Results

The retention rate according to the clinical analysis and photographic/SEM images is represented in

Table 2: *Retention Rate (% in Parentheses) According to the Evaluation Method and Sealant Material After 22 Years*

Sealant Materials	Clinical Analysis			Photographic Images			SEM Images		
	R (%)	PR (%)	T (%)	R (%)	PR (%)	T (%)	R (%)	PR (%)	T
Vitrebond	0	5 (38.5)	8 (61.5)	1 (7.7)	6 (46.15)	6 (46.2)	10 (76.9)	3 (23)	0
Fuji II LC	1 (7.14)	9 (64.3)	4 (28.6)	2 (14.3)	7 (50)	5 (35.7)	11 (78.6)	3 (21.5)	0
VariGlass VLC	0	11 (78.6)	3 (21.4)	3 (21.4)	9 (64.3)	2 (14.3)	10 (71.4)	4 (28.5)	0

Abbreviations: R, retained; PR, partially retained; T, totally lost.

Table 2. Fuji II LC and VariGlass VLC had similar retention behavior when clinical and photographic analysis was compared; however, the SEM analysis showed the same clinical behavior for all three sealing materials. The SEM images provided a higher number of retained sealants when compared with the clinical and photographic evaluations (Figure 1), regardless of the sealing material. Also, no "totally lost" scores were found when using SEM analysis.

Figures 2-7 represent clinical cases in which sealed teeth were photographed and subjected to SEM analysis. It was not possible to recover all initial photographic images; however, retained images between 1992 and 2014 are presented as representative. No caries lesions were found on sealed teeth.

DISCUSSION

In 1992, when the present study began, the materials used were not primarily indicated to be pit and fissure sealant materials. Therefore, in an effort to find available ionomeric materials with better retention and mechanical properties, VariGlass VLC, Fuji II LC, and Vitrebond were chosen. In general, the three materials underwent chemical/physical degradation after 22 years of analysis.

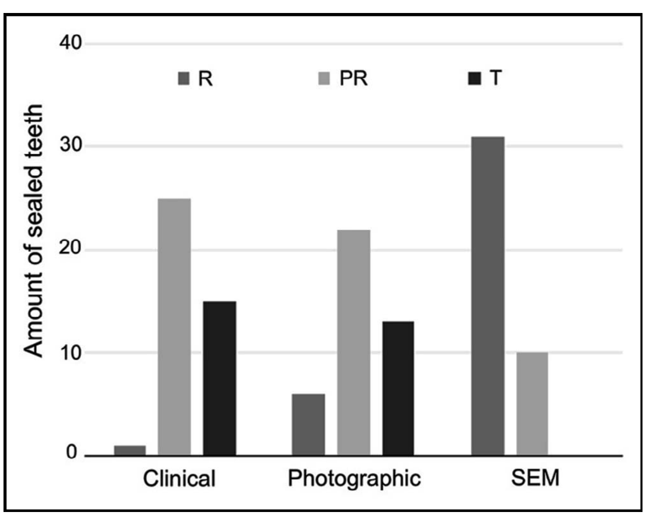

Figure 1. *Comparison between methods of evaluation and retention rate scores of sealants after 22 years. R, retained; RP, partially retained; T, totally lost.*

The three methods applied in this study successfully accomplished the objective of evaluating sealant retention. Clinical analysis resulted in predominantly partially retained scores, considerable totally lost scores, and low retained scores due to the difficulty of the naked eye to properly distinguish the anatomic form of sealants in deeper parts of pits and fissures. With photographic image analysis, a slight decrease in partially retained and totally lost and an increase in retained was found when compared with clinical analysis because it was possible to observe the images on an LED screen with higher magnification and better definition. Although the most common method for evaluating the clinical performance of a pit and fissure sealant is by visual clinical examination, the assessment of sealant retention from photographic images has a higher reproducibility[27] and enables retrospective analysis of the effectiveness in everyday dental practice.[8,28] However, it was not possible to retrieve all baseline images; therefore, precise comparison of the baseline (right after sealants were applied) with the present analysis was not possible.

SEM is considered a reference standard of sealant retention[27] because clinical analysis and photographic images do not present the same magnification and definition as SEM. This difference was quite evident, given that SEM analysis presented elevated retained scores and eliminated the totally lost scores for pit and fissure sealants, given that remnants of sealant material were clearly observed at deeper regions that were scored as totally lost when using clinical and photographic analyses (Figure 6).[26,27] Although some reports have found low retention rates for compomers and resin-modified glass ionomers applied in pits and fissures,[20,21,24,29] the assessment methods used in those studies was visual clinical analysis, which could not properly describe the presence of the sealant material at deeper parts of pits and fissures.

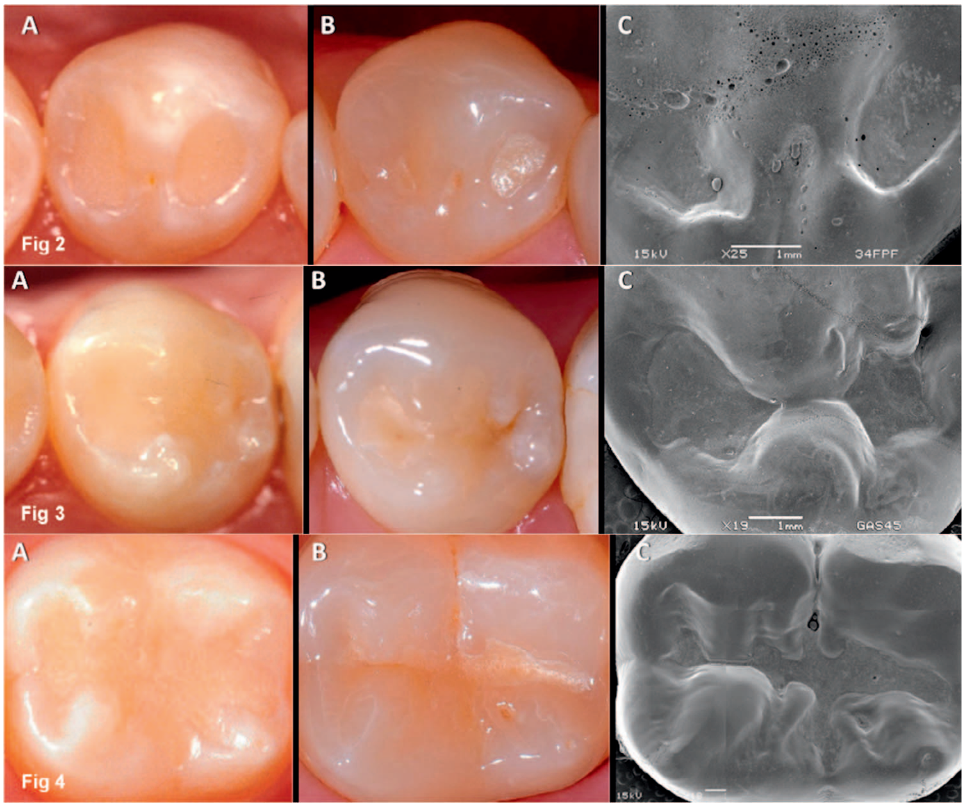

Figure 2. *(A): Photo six months after sealing with VariGlass VLC. (B): Photo after 22 years. (C): SEM showing the presence of sealing material. Evaluation scores: clinical analysis: partially retained; photographic image: retained; SEM image: retained.*

Figure 3. *(A): Photo two years after sealing with Fuji II LC. (B): Photo after 22 years. (C): SEM showing the presence of sealing material. Evaluation scores: clinical analysis: partially retained; photographic image: partially retained; SEM image: retained.*

Figure 4. *(A): Photo six months after sealing with VariGlass VLC. (B): Photo after 22 years. (C): SEM showing the presence of sealing material. Evaluation scores: clinical analysis: partially retained; photographic image: partially retained; SEM image: retained.*

Better wear resistance of the compomer was expected due to its improved mechanical properties over RMGICs[30] and because they contain no water and have components similar to composite resins.[31] However, compomers contain hydrophilic components that cause water to be drawn into the material following cure and inevitably lead to a decline in certain physical properties.[31] This might explain why VariGlass VLC presented a similar wear behavior when compared with Fuji II LC and Vitrebond. Even though the wear was not measured in the replicas at baseline and at 22 years, photographic evidence suggests that VariGlass presented clinically comparable wear patterns in relation to Fuji II LC and Vitrebond. Cehreli and others[32] reported marked occlusal wear for PMRCs, albeit with a good retention after three years. It is noteworthy that no resin material would present the same baseline shape/volume after 22 years of clinical performance because they all inevitably undergo physical/chemical degradation over time due to masticatory forces, articular accommodation, movements of eruption, and abrasion/erosion processes.

To achieve long clinical success with dental sealants, the maintenance of a satisfactory retention to enamel is primary.[33] In the case of teeth with partially retained sealants, the risk of developing caries lesions is higher than in a fully covered area[27] because food/biofilm may still accumulate in pits and fissures. However, the present case series report did not find any carious lesions on sealed teeth, regardless of their scores. Even if small portions of the sealant material are found in deeper parts of pits and fissures (Figures 5-7), their protective/preventive effects against caries lesions still goes on as a physical barrier, even as resin tags embedded in the etched enamel.[20] In 1992, the manufacturer did not recommend phosphoric acid etching before placing a resin-modified glass ionomer. However, the acid-etching procedure was adopted in the current study because it was hypothesized at that time that it would benefit the retention rate of the sealing material. Later reports have shown that etching with phosphoric acid improves the bond strength of sealing materials to enamel[34-36] by creating tags,[36] which may have greatly extended the retention and protection of pit and fissure materials against caries lesions. Horowitz and others[37] reported that teeth with partially retained sealants had lower incidences of caries lesions when compared with an unsealed group, confirming the positive effect of partially retained sealants.

Fluoride released from the tested materials may have played an important role as a protective/

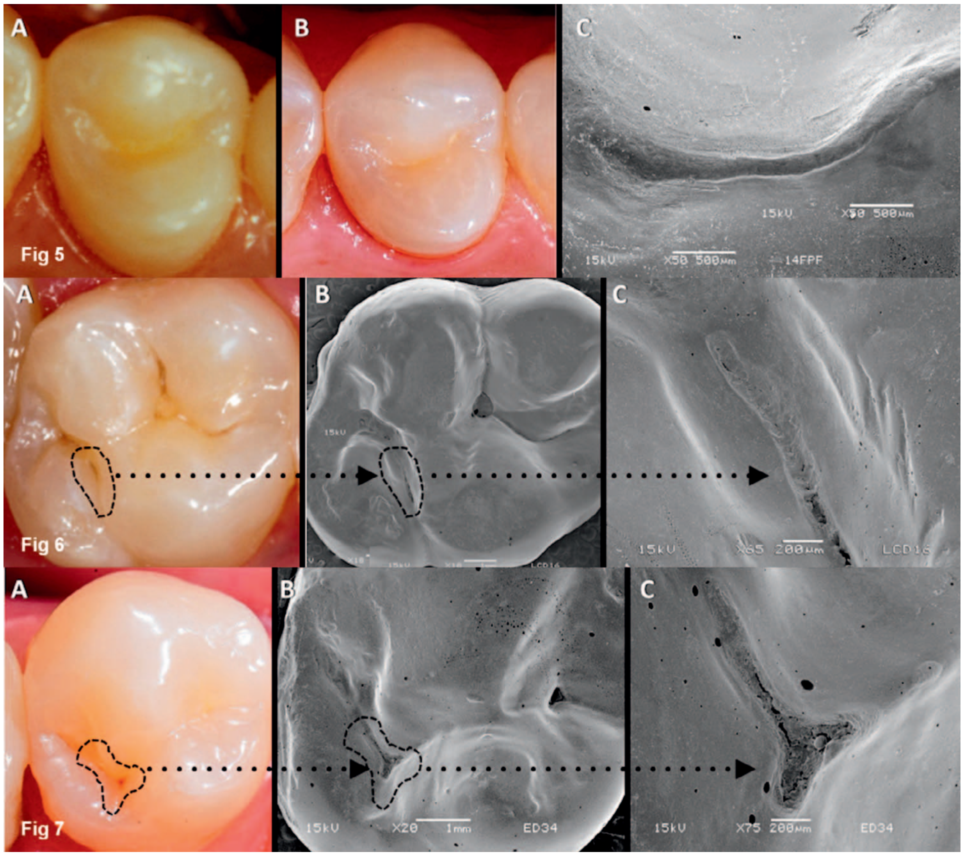

Figure 5. *(A): Photo five years after sealing with Vitrebond. (B): Photo after 22 years. (C): SEM showing the presence of sealing material. Evaluation scores: clinical analysis: totally lost; photographic image: retained; SEM image: retained.*

Figure 6. *(A): Photo 22 years after sealing with Vitrebond. (B): SEM showing all occlusal surfaces. (C): SEM showing the presence of sealing material in the deep part of pits. Evaluation scores: clinical analysis: totally lost; photographic image: totally lost; SEM image: retained.*

Figure 7. *(A): Photo 22 years after sealing with VariGlass VLC. (B): SEM showing all occlusal surface. (C): SEM showing the presence of sealing material in the deep part of pits. Evaluation scores: Clinical analysis: partially retained; photographic image: partially retained; SEM image: retained.*

preventive agent against caries lesions. Fluoride acts as a cariostatic agent, inhibiting demineralization and favoring remineralization.[38] Furthermore, it has been indicated that fluoride-releasing materials reduce the amount of enamel demineralization adjacent to the material.[39] However, polyacid-modified resin materials have no fluoride recharging capability[40] and release less fluoride when compared with conventional cements and RMGICs,[32,41-45] which may affect its caries preventive abilities.[41] It is not possible to state that fluoride is still being released from the sealing materials after 22 years for modified-glass ionomer materials that present a high initial fluoride release; on the contrary, a decrease during the aging period must have certainly occurred.[42]

Due to the multifactorial nature of caries lesions, it is challenging to determine the most effective manner of preventing tooth decay: sealants, proper dental hygiene, proper diet, fluoridated mouth rinses, supply water, toothpaste, or sealant. Definitely, when the four strategies are combined, the possibility of teeth to decay considerably decreases. Given that it is not possible to ensure that all people around the world are supplied with fluoridated water and/or toothpastes[46] and an anticariogenic diet may not be practiced by all levels of the population, sealants remain as a clinical technique that may greatly prevent tooth decay (Figure 8). One other important aspect that may have influenced the absence of caries lesions is that permanent enamel

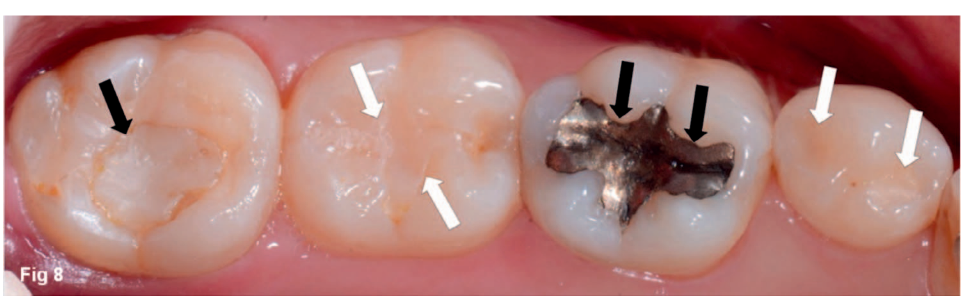

Figure 8. *Sealed No. 18 and No. 20 showing nondecayed teeth after 22 years (VariGlass VLC) (white arrows). In contrast, No. 19 was already restored when the sealing procedures were performed (black arrows). No. 17 was not sealed, and at the recall the tooth was restored, probably due to caries (black arrows).*

undergoes posteruptive maturation, accumulating fluoride, becoming harder, less porous, and less caries-prone[47] when compared with recently erupted teeth, which are known to be at higher risk for decay than old teeth.[48]

The present report corroborates previous research, with a minimum of 11 years of follow-up,[8,49-52] that sealed teeth had a reduction in caries lesions and restorations, associated with a long-lasting caries-prevention effect. There were no clinical failures of any sealant after 22 years, given that no occlusal restoration was performed and no cavitated caries lesions were found at any clinical examination.[53]

CONCLUSIONS

All sealing materials suffered similar physical degradation after 22 years. Thus, the full retention of sealants is not of crucial concern because pit and fissure sealants showed remnants of material in deeper parts. Sealing pits and fissures continues to be a suitable and cost-effective clinical technique for preventing dental caries throughout life.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Araçatuba School of Dentistry–UNESP, Brazil.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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