In Vitro Microleakage Evaluation Around Three Types of Dental Sealants

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Dental sealants are a specific prophylactic alternative for fissures with an irregular morphology. The aim of this in vitro study was to evaluate dye penetration around three materials recommended for fissure sealing with a different composition. Thirty teeth were sealed according to manufacturer's instructions as follows (n=10): Group I, light-cured unfilled resin-based sealant, Admira Seal® (Voco GmbH); Group II, light-cured filled resin-based sealant, Fotoseal® (S.C.Remed Prodimpex S.R.L., Bucharest, Romania); Group III, resin modified glass ionomer sealant without varnish, GC Fuji Triage® (GC Corporation). The teeth were thermocycled, then immersed in 0.5% basic fuchsine dye for 24 h. The teeth were sectioned buccal-lingually in order to obtain one section per tooth in the middle of the occlusal surface and the sections were examined at 40x with an inverted microscope. Dye penetration was recorded (μm) using specific software and related to the total length of the sealed fissure. Average values for each group were subjected to statistical analysis by Student's t and Kolmogorov-Smirnov tests ($p<0.05$). Significantly less dye penetration was recorded for both resin-based sealants compared to the resin modified glass ionomer sealant, with no difference between the filled and the unfilled resin sealant ($p=0.34$). The resin-based sealants were more effective in sealing the fissures than the resin modified glass ionomer cement sealant.

Keywords: dental sealant, microleakage, fissure, prevention

Carious lesions develop 5 times more frequently in pits and fissures and 2 and 1/2 times more frequently in buccal and lingual fissures compared to smooth surfaces [1]. The occlusal surfaces of the first permanent molars are most susceptible to caries in children because these particular areas are difficult to clean at this age and parents often do not now that a permanent tooth is erupting in a distal dental arch area [1, 2].

In spite of the progress of preventive methods, dental decay represents a key problem for the children's oral health in Romania. The preventive properties of sealants are well documented, but they are not frequently used in daily practice in our country. The main concerns are the difficulty in obtaining isolation in children, multiple technical steps, dye penetration, uncertain retention, and cost [3].

Different materials are currently available on the market to be used for fissure sealing. A main prerequisite for a sealant to fulfil its function is the tight seal, which means the lack of microleakage. This will prevent bacteria from penetrating into a gap between the material and the fissure, eventually causing new caries. Furthermore, the diffusion of nutrients from the oral environment into an aperture between the material and the occlusal surface should be blocking in order to prevent any nutrition supply for bacteria that are potentially left under the fissure sealing [4].

The purpose of this study was to evaluate marginal microleakage for three types of dental sealants: two light-cured resin-based sealants (RBS), of which one filled and one unfilled, and a resin modified glass ionomer cement sealant (RMGI). The final objective was to investigate whether a Romanian product performs comparably to other products already available on the market.

Experimental part

This experimental in vitro study was conducted in Paediatric Dentistry Department, Faculty of Dental Medicine, University of Medicine and Pharmacy I.Hatieganu, Cluj-Napoca, Romania.

Tooth selection. Thirty human first maxillary premolars freshly extracted for orthodontic purposes were used. The teeth did not present anatomic abnormality, distinct crack and surface pigment and were clinically evaluated with an explorer, under standard lighting conditions, as caries free. The teeth were cleaned by removing calculus and soft tissue deposits with a hand scaler and then stored in 0.9% NaCl containing 0.02% sodium azide at 4^0C until used.

Material: The light-cured filled RBS Fotoseal®, is a Romanian product, manufactured by the Dental Materials Group, Raluca Ripan Chemistry Research Institute Cluj-Napoca, and produced by Remed Prodimpex SRL, Bucharest, Romania. It has 60% (wt) dimethacrylate monomer mixture and 40% (wt) hybrid inorganic filler containing colloidal silica, titanium dioxide and eutectic fluoride.

The light-cured unfilled RBS is Admira Seal® (Voco GmbH) a light-curing mixture of different dimethacrylates,

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silicate fillers, ormocers and additives. The RMGI sealant is GC Fuji Triage® (GC Corporation).

Sealing procedures and microleakage assessment: The teeth were cleaned using pumice and Klint Paste® (Voco GmbH) and randomly distributed into three groups (n=10). The sealants were placed on the mesial-distal groove following the manufacturers' instructions, as follows:

Group I: the occlusal mesio-distal grooves were etched with 37% phosphoric acid gel for 30 s, rinsed with water spray for 20s and dried with a gentle air stream for 10s. One layer of light-cured unfilled RBS (Admira Seal®) was applied using a special applicator with a light brushing motion, and then light cured for 20 s using Optilux 501 curing unit (Kerr Corp.).

Group II: the occlusal mesio-distal grooves were etched with 37% phosphoric acid gel for 30s, rinsed with water spray for 20s and gently dried for 10s. One layer of light-cured filled RBS (Fotoseal®) was applied using a special applicator with a light brushing motion, and then light cured for 20 s using Optilux 501 curing unit (Kerr Corp.).

Group III: the occlusal mesio-distal grooves were conditioned with GC Cavity Conditioner (20% polyacrylic acid and 3% aluminum chloride hexahydrate) for 10s, then washed for 20s and dried but not desiccated with a gentle air stream for 3s, in order to obtain a moist surface. The resin modified glass ionomer sealant (RMGI) (GC Fuji Triage®) was prepared according to the manufacturer's recommendations and one layer was applied with an explorer, and then light cured for 20s using Optilux 501 curing unit (Kerr Corp).

The light-curing unit, Optilux 501(Kerr Corp), was operated in the standard mode at a light intensity of 740 ± 36 mW/cm^2. The light curing unit output was measured after every five procedures using a Kerr LED hand-held radiometer (Kerr Corp) as specified by the producer.

The teeth were thermocycled between 50 and 55^{0}C for 1000 cycles with a dwell time of 25s. The apices of the teeth were sealed with resin composite and the tooth surfaces were covered with two layers of nail varnish with the exception of 1 mm around the tooth-sealant interface. The teeth were immersed in 0.5% basic fuchsine dye for 24 h. They were removed, washed, dried and their roots were mounted in self-curing acrylic resin. Each premolar was sectioned in a buccal-lingual direction using a water-cooled microtome (Isomet Low Speed Saw, Buehler Ltd, USA) in order to obtain a 1.5 mm thick section in the middle of the occlusal surface.

Each section was examined by a single examiner with an inverted microscope (Olympus KC301, Olympus America Inc.) at 40x and microleakage values were recorded (µm) using the QuickPhoto Micro 2.2 software (Olympus Inc).

Microleakage values (I) were obtained measuring infiltration length (dye penetration Li) and the total length sealant-enamel interface (Lf) using the following formula: I=Li/Lf (figs 1-3).

Data were collected and the statistical analysis was carried out by using Statistical Packages for Social Sciences (SPSS 13.0, Chicago, IL, USA) for Windows. The average values for each group were subjected to statistical analysis by Student's t and Kolmogorov-Smirnov tests at a $p<0.05$ level of significance.

Results and discussions

In our study microleakage outcomes positive, for all three experimental groups, but in significantly different rates. For resin based sealants microscopic evaluation revealed less dye penetration compared with the resin modified glass ionomer cement.

Fig.1. Dye penetration measurement for Group I observed at the sealant-enamel interface

Fig.2. Dye penetration measurement for Group II observed at the sealant-enamel interface

Fig.3. Dye penetration measurement for Group III observed at the sealant-enamel interface

For every tooth infiltration values and the total length of the fissure sealant interface were recorded (µm) using the QuickPhoto Micro 2.2 software (Olympus Inc.) (table 1).

We observed significantly less dye penetration for Group I (average 0.03±0.04) than for Group III (average 0.18±0.08). The same statistically significant difference was observed between Group II (average 0.02±0.03) and Group III (average 0.18±0.08). No statistically significant differences in dye penetration were found between Group I (average 0.03±0.04) and Group II (average 0.02±0.03) (table 2).

Marginal microleakage at the enamel sealant interface is an inevitable phenomenon [4].The preventive effect of sealants is mainly mechanical, as long as it remains intact and bonded on enamel surface [5, 6].

In our study, no anatomical distinction was made between groove depths. The reason was that several studies have shown that there is no significant difference in microleakage in anatomically different grooves [7, 8].

In the present study, basic fuchsine penetration was used to evaluate the presence of microleakage. No protocol regarding one specific dye was recommended for dental sealants.

Furthermore, we performed dye penetration after thermocycling at specific temperature ranges because sealants have one of the highest thermal expansions among dental materials [9].

Table 1

INFILTRATION LENGTH AND SEALANT-ENAMEL INTERFACE (MEASURED VALUES)

Material	Tooth (number)	Infiltration (µm)	Interface (µm)
Group I *Admira Seal*	1	311	5185
	2	0	4738
	3	678	5904
	4	0	3963
	5	0	4232
	6	0	4233
	7	309	5234
	8	0	4012
	9	412	5567
	10	0	4823
Group II *Fotoseal*	1	291	4153
	2	0	5280
	3	0	5358
	4	0	6637
	5	0	3730
	6	0	5123
	7	0	4889
	8	122	5223
	9	0	4566
	10	233	3987
Group III *GC Fuji Triage*	1	454	3654
	2	2268	6374
	3	549	4377
	4	1125	4961
	5	1144	4590
	6	445	3778
	7	643	4234
	8	655	4779
	9	877	5442
	10	991	5321

Microleakage can be assessed in different ways, for example by scoring dye penetration; a more qualitative method that can be subject to individual variation between evaluators [7-9]. In our study, we evaluate microleakage through a quantitative method using specific software (Quick Photo Micro 2.2 software, Olympus Inc.) in order to minimized individual variability.

The RMGI sealant used in this study showed less sealing capacities than the two RBS. This is in line with research paper that measured microleakage associated with different types of pit and fissure sealants. In these studies, RMGI revealed higher microleakage. The authors attributed the results to the fact that the enamel was not etched and that this type of material has a resin component [6-10].

The adhesive capacity of a dental sealant is also related to and can be affected by the difference between the elastic module and the contraction and expansion coefficient of the RBS and those of the enamel. These differences may have an effect on the marginal integrity and retention of RBS in time [9-10]. Filled RBS should act better with respect to the statement above. However, in our study, both light-cured RBS showed similar sealing capacities, with no statistically significant improvements for the filled RBS. We assume that the filled resin-based sealant has a higher viscosity, so it was not capable of penetrating into the microscopic undercuts of the etched enamel as well as the unfilled sealant [11-12].

Table 2

MEAN VALUES OF PROPORTIONS BETWEEN THE LENGTH OF THE ENAMEL-SEALANT INTERFACE AND THE LENGTH OF DYE PENETRATION FOR EACH TYPE OF SEALANT USED IN THIS STUDT

Group	Mean value	SD	p
I	0.03	0.04	**0.34**
II	0.02	0.03	
I	0.03	0.04	**0.00003**
III	0.18	0.08	
II	0.02	0.03	**0.00003**
III	0.18	0.08	

One of the limitations of this study was the fact that he was developed in vitro with an uncomplicated access and moisture control.

Three clinical studies showed a low retention of the RMGI cement used as a sealant compared to resin-based sealants, with a minimal difference in caries increment. Despite reduce mechanical proprieties RMGI remains a routine alternative in high risk patients with incomplete erupted molars [13-15].

Microleakage was also correlated with the specific mode of preparing occlusal surfaces prior to the sealing procedure. In our study, we cleaned the surfaces with pumice and a professional paste without enameloplasty or any specific procedure for surfaces (laser, air-abrasion). Our results were in accordance with those of other studies that recommend professional cleaning and the acid-etched technique in order to acquire appropriate adhesion [16-18]. Even if enameloplasty or other procedures may improve adhesion between the sealant and enamel, we demonstrated that usual professional cleaning procedures are sufficient to obtain a good sealing capacity, highlighting the advantage of the significantly reduced working time, which is very important in pediatric dentistry [19-22].

Sealant use in occlusal surfaces decay prevention is a common practice in pediatric dentistry and their effectiveness has been proven in numerous studies. Meticulous application is the key point for an adequate retention on enamel surface. Sealant integrity and retention at the dental material-enamel interface are the element intrinsic related with preventive role of a fissure sealant [23-25].

Conclusions

Within the limits defined in the experimental design, the following conclusions may be drawn:

- the objective evaluation of dye penetration allows a better characterization;
- significantly less microleakage was associated with the use of resin-based sealants compared to the resin modified glass ionomer sealant;
- resin modified glass ionomer sealant could be used as a transitional sealant in specific condition;
- the filled resin-based sealant did not perform better than the unfilled resin-based sealant;
-Fotoseal® demonstrates comparable proprieties with a sealant already on the market.

The above results are viewed as the theoretical level of leakage which may or may not occur in vivo but may be accepted as an aid for selection of a good sealant material before placement of a fissure sealant.

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