Marginal seal evaluation of different resin sealants used in pits and fissures. An in vitro study

M. MONTANARI, G. PITZOLU, C. FELLINE, G. PIANA

ABSTRACT: Aim Oral health is important for everyone, but specially for children and people requiring special care owing to impaired manual ability. Primary prevention, with correct oral hygiene, proper diet, use of fluoride and pit and fissure sealants, can reduce caries risk thereby improving patients’ quality of life. The first aim of this paper was to assess the marginal microleakage of different pit and fissure sealants after immersion in a cariogenic solution. The second aim was to evaluate the ability of the materials to penetrate into the bottom of the fissure. Methods 32 posterior teeth were divided into four groups based on the type of sealant tested: Concise, Clinpro, Fissurit, Fissurit F. Samples were immersed and stored in a lactic acid solution (pH 4.4, 0.1 M) at 37°C for different periods: 1 day; 3 days; 7 days; 10 days. Then each sample was stored in erythrosine solution for 24 hours at 37°C. Each sample was sectioned in a mesio-distal direction and evaluated at the stereomicroscope and subsequently at SEM. Results SEM analysis showed that Clinpro and Concise obtained a good penetration inside the fissure and a good adaptation to the enamel wall, while Fissurit and Fissurit F showed gaps at the sealant-enamel interface and voids. Conclusion Sealant application is an important means for caries prevention but, for a lasting effect, it must be associated with good oral hygiene and regular dental check-ups.

KEYWORDS: Pit; Fissures; Dental sealants; Cariogenic solution; Microleakage.

Introduction

Over the last three decades, caries prevalence has declined worldwide [Kidd et al., 1993]. However, the decline has not occurred uniformly on all dental surfaces. Decayed pits and fissures represent the majority of carious lesions, 93.4% and 79.8% in 12-year-old boys and girls, respectively [Al-Khateeb et al., 1991; Akpata et al., 1992]. The high tendency may be attributed to the complex morphology of pits and fissures which are considered to be ideal sites for the retention of bacteria and food remnants, making mechanical means of debris removal inaccessible [Feldens et al., 1994]. Other factors responsible for the high incidence of occlusal caries include the lack of salivary access to the fissures as a result of surface tension, effectively preventing remineralisation and reducing the effectiveness of fluoride [Salama and Al-Hammad, 2002]. Preventive techniques such as systemic and topical fluorides are thought to protect smooth rather than occlusal surfaces. The prevalence of caries on pit and fissure surfaces emphasizes the importance of sealants in the prevention of caries today.

The effectiveness of sealants hinges on their ability to isolate pits and fissures from the combination of bacteria, their nutrients and acidic metabolic products [Bevilacqua et al., 2007]. Sealants are considered to be the most effective caries preventive measure that may be offered to a patient, mainly in those with high caries risk such as children with Molar- Incisor Hypomineralisation (MIH) [Kotsanos et al., 2005; Welbury et al., 2004; Weerheijm, 2003]. Some studies suggested that the benefit provided by protecting pits and fissures is based on good retention and integrity of the sealant [Kilpatrick et al., 1996; Simonsen, 1996]. However, since the retention of sealants is not permanent, this physical effect could be enhanced if the material simultaneously releases fluoride [Westerman et al., 2000]. The success of the sealing procedure depends on the morphology of pits.
and fissures and on the adequate preparation of the enamel before sealant application. Pre-treatment of the enamel surfaces is mandatory to obtain access to the deepest areas of pits and fissures, remove stains and organic debris and increase surfaces roughness [Geiger et al., 2000]. Failure to pre-treat may lead to an increase in microleakage [Ansari et al., 2004]. Since its introduction, acid etching has become a crucial and indispensable step in sealant applications. Etching produces microscopic porosities in the enamel surface into which the unpolymerised sealant flows and hardens in tag-like projections that attach the material to the tooth structure [Gungor et al., 2003].

Since the surface should be clean and dry when the material is applied, placement of a rubber dam is recommended to eliminate salivary contamination. This means that sealant should be applied after cleaning and polishing the teeth [Mazzoleni et al., 2007]. To achieve maximum benefit, sealants should bond appropriately to the enamel surface [Sheila et al., 1998]. Adequate retention of a sealant will be achieved if the tooth has a wide surface area and irregular pits and fissures [Ansari et al., 2004]. The marginal sealing ability of these materials is extremely important for successful treatment. Lack of sealing will lead to marginal leakage (i.e., passage of bacteria, fluids, molecules and ions through the tooth-material interface), which can prompt caries lesion progression underneath the restoration [Pardi et al., 2006; Atash and Vanden Abbeele, 2005].

The first aim of the present study was to assess the marginal microleakage of different resinous pit and fissure sealants after immersion in a cariogenic solution. The second aim was to evaluate the ability of the materials to penetrate into the bottom of the fissure without forming voids.

**Materials and methods**

Thirty-two freshly extracted posterior caries- and filling-free human teeth were selected and employed for this study.

Teeth were stored at 4°C in water for no more than two weeks.

Occlusal surfaces were cleaned with a prophylaxis brush mounted on a slow-speed handpiece with a water-based slurry of pumice for 10 s and then rinsed for 30 s and treated with ultrasound for 15 min to remove debris. Then each tooth was dried with a gentle air flow and etched with 37% orthophosphoric acid gel (Scotchbond Etchant, 3M-ESPE, St. Paul, Minnesota, USA) for 60 s. Samples were rinsed with water for 30 s and dried for 15 s to dehydrate the enamel and allow sealant penetration inside pits and fissures.

The teeth were divided into four groups based on the type of sealant used:
- Group A (n. 8) Concise (3M-ESPE, St. Paul, USA);
- Group B (n. 8) Clinpro (3M-ESPE, St. Paul, USA);
- Group C (n. 8) Fissurit (Voco GmbH, Cuxhaven, Germany);
- Group D (n. 8) Fissurit F (Voco GmbH, Cuxhaven, Germany).

Subsequently, sealants were applied inside pits and fissures following the manufacturers’ instructions. To reduce the formation of air bubbles, the resin was applied using a small dental probe (diameter 0.5 mm). Then each sealant was polymerized for 60 s with a halogen light curing unit (XL-2005, 3M-ESPE, St Paul, MN USA) with output power intensity of 600 mW/cm². Hardening and retention of all sealants were confirmed with an explorer. Teeth apical foramen were sealed with sticky wax to prevent dye penetration. The external root surface was coated with a double layer of nail varnish to leave exposed only the occlusal surface where the sealant was applied.

Each sample was then immersed and stored in a cariogenic solution [Nucci et al., 2004] of lactic acid pH 4.4 0.1 M at 37°C for different periods to form four sub groups:
- Subgroup 1: 1 day of storage (2 samples per group);
- Subgroup 2: 3 days of storage (2 samples per group);
- Subgroup 3: 7 days of storage (2 samples per group);
- Subgroup 4: 10 days of storage (2 samples per group).

The solution was changed every 24 hours. After acid treatment, samples were rinsed under running water for 2 min and then immersed and stored in erythrosine solution for 24 hours at 37°C. They were then rinsed in tap water for 20 min and treated with ultrasound for 15 min to remove dye residues. Roots were sectioned at the cementum-enamel junction with a slow-speed water-cooled diamond saw (Remet, Ceretoto di Casalecchio, Bologna, Italy). Subsequently, each sample was sectioned into two halves in the mesial and distal pits.

Then each sample was investigated by stereomicroscope. The tooth-sealant interface was evaluated and a microleakage score from 0 to 3 was given to each section based on dye penetration [Ovrebo and Raadal, 1990]:
- score 0 was given to no dye penetration;
- score 1 was given to dye penetration limited to the outer half of the sealant;
- score 2 was given to dye penetration limited to the...
inner half of the sealant;
- score 3 was given to dye penetration into the bottom of the fissure.

This evaluation scale is the most widely used in leading studies on sealant microleakage [Zervou et al., 2000; Pardi et al., 2006; Bevilacqua et al., 2007]. Mean dye penetration was calculated for each fissure and data were analysed by Anova system and Student’s t-test. Subsequently samples were prepared for SEM analysis (Jeol JSM 5200). They were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer solution at pH 7.2 for 48 h, then each sample was rinsed several times with 0.1 M sodium cacodylate buffer, dehydrated in increasing concentrations of ethanol (50%, 70%, 80%, 90%, 95%, 100%) for 30 min each. Then samples were gold-coated and analyzed by SEM.

Results
Microleakage results are shown in figures 1-4. Stereomicroscope images of dye penetration are summarised in figures 5-8. Statistical analysis revealed significant differences between the four sealants (p<0.05). ANOVA test showed that Group A obtained the best dye penetration compared with the other groups, but Student’s t-test failed to disclose statistical differences from Concise at different storage times (p>0.05). Group C did not show statistical differences from Group D in the four subgroups (p>0.05). Both Group A and Group B showed statistical differences from Groups C and D (p<0.05). In the same group sealants showed an increase in dye penetration over time (Fig. 1-4).

Groups A, B and C presented statistical differences (p<0.05) from 1 to 10 days of storage in cariogenic solution, whereas Fissurit did not (p>0.05). SEM analysis showed that Clinpro and Concise obtained a good penetration inside the fissure and a good adaptation to the enamel wall (Fig. 5b, 5f, 6b), whereas Fissurit and Fissurit F showed gaps at the sealant-enamel interface (Fig. 8c).

![Fig. 1, 2 - Score of dye penetration at 1 and 3 days of storage in cariogenic solution.](image1)

![Fig. 3, 4 - Score of dye penetration at 7 and 10 days of storage in cariogenic solution.](image2)
Discussion

Pit and fissure sealants have been considered an outstanding adjunct to oral health care preventive strategies decreasing occlusal caries onset and/or progression. The properties of an ideal sealing material include biocompatibility, retention and

**FIG. 5 - Most representative Clinpro samples.**
A: stereomicroscopic image of Clinpro stored in lactic acid for 1 day (25.6 X). The sample shows a good marginal seal.
B: SEM micrograph of Clinpro stored in lactic acid for 1 day. It shows a good marginal seal.
C: stereomicroscopic image of Clinpro stored in lactic acid for 3 days (20.48 X). No dye penetration is shown.
D: stereomicroscopic image of Clinpro stored in lactic acid for 7 days (20.48 X).
E: SEM micrograph of Clinpro stored in lactic acid for 7 days. The sealant penetrates deep into the bottom of the fissure in spite of the unfavourable anatomy.
F: stereomicroscopic image of Clinpro stored in lactic acid for 10 days (64 X). Dye penetration can be seen on the bottom of the fissure.
resistance to abrasion and wear. Sealant penetration into etched enamel is important because microleakage at the tooth-material interface can lead to failure [Pere-Lajarin et al., 2003]. Microleakage can be defined as the passage of bacteria, fluids, molecules or ions between a prepared tooth surface and the restorative material applied [Srinivasan et al., 2005]. The evaluation of marginal leakage gives a good idea of a sealant’s ability to prevent bacterial invasion and consequently reduce the risk of secondary caries development [Lupi-Pegurier et al., 2004]. Microleakage is therefore a key parameter to explore the effectiveness of dental sealants [Hicks, 1984]. Even if the clinical meaning of in vitro microleakage tests must today be interpreted with caution, despite their known limits these studies offer an invaluable tool for the evaluation of new materials or technologies. Although tracer infiltration is not an absolute value, it can demonstrate the lack of adaptation between material and enamel walls and the absence of a perfect seal [Roulet, 1994]. Resin sealants of different composition are now available on the market including materials that can release fluoride. Fluoride release by sealants is responsible for the anticariogenic effect around the enamel and on the adjacent tooth [Lobo et al., 2005].

The present study evaluated the in vitro microleakage of four different sealants to identify the material with the best performance that can be used in routine clinical practice. Different storage times in

![Fig. 6 - Most representative Concise samples.](image1)

**A**: stereomicroscopic image of Concise stored in lactic acid for 7 days (20.48 X). No dye penetration is displayed.

**B**: SEM micrograph of Concise stored in lactic acid for 7 days. A fracture between sealants and enamel is visible on the sample probably because of the dehydration.

![Fig. 7 - Most representative Fissurit F samples.](image2)

**A**: stereomicroscopic image of Fissurit F stored in lactic acid for 3 days (20.48 X). Several voids inside the thickness of the sealant can be seen as well as dye penetration on the bottom of the fissure.

**B**: stereomicroscopic image of Fissurit F stored in lactic acid for 10 days (20.48 X). Dye penetration can be seen on the bottom of the fissure.
cariogenic solution (1, 3, 7 and 10 days) were chosen to simulate the different conditions of the oral environment. Clinpro showed the lowest microleakage at different storage conditions compared with the other resin sealants tested (Fig. 5a). Clinpro penetrated deep into the bottom of the fissures without forming voids (Fig. 5c, 5e). Concise demonstrated a similar behaviour to Clinpro (Fig. 6a) but microleakage results showed greater dye penetration (Figs. 1-4). The differences between these two sealants were not statistically significant (p>0.05). Fissurit demonstrated greater infiltration than the other sealants, both in the bottom of the fissures and along the margins (Figs. 7a). In addition this sealant presented several voids inside its thickness (Figs. 7a, 7b). Fissurit F presented a similar behaviour to Fissurit (Figs. 8a, 8b, 8c). Time negatively influenced the marginal microleakage of all sealants tested with an increase in eritrosine penetration in the samples at 7 and 10 days (Fig. 3, 4).

The presence and the release of fluoride may be responsible for the best results of Clinpro. Fissurit F also contains fluoride but its release may not be able to prevent microleakage. Concise showed good results probably because its very low viscosity, that is responsible for a deep penetration inside fissures and inside etched enamel. In addition, the presence of different resinous components (Bis-GMA, TEGDMA, BHY, diuretan-dimethacrylate) in Fissurit and Fissurit F may be responsible for the poor resin penetration inside fissures. Voids within the thickness of sealants may play an important role in favouring dye penetration and hence bacterial microleakage.
Conclusion

A cariogenic solution was used to simulate the acid environment of the oral cavity, which determined an increase in microleakage. All the sealants used in this study presented higher values of microleakage after 10 days of storage in the solution, demonstrating that in addition to sealant type and fissure anatomy, the acid environment had a decisive influence on the sealant’s durability. Sealant application is an important means of preventing caries but, for a lasting effect, it must be associated with good oral hygiene and regular dental check-ups.

References