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An experimental *in vivo* procedure for the standardised assessment of sealants retention over time

ABSTRACT

Aim *The elaboration of an experimental system to obtain reproducible and comparable photographs of the occlusal surface to monitor sealants retention.*
Materials and methods *An intraoral camera connected to a computer was used to obtain photos of the occlusal surfaces. A specific software was utilized to perform measurements on archived pictures. An experimental two-part system, consisting of a dental arch support and a camera support, connected to each other through holes and pins, was made to obtain a standardised and reproducible placement of the camera in the mouth. In the first part, to test the degree of reliability of the procedure and the percentage of image distortion, 120 first molars were sealed and for each molar ten photographs were taken, using the intraoral camera connected with the dental arch support, the camera support and the dedicated software. In the second part, 165 first molars were sealed and photographed, as above described, immediately after sealing (T0), 6 months (T1) and 1 year later (T2). With the software, the sealed areas were measured. The comparison of the selected sealed areas between T0 and T1, T0 and T2, T1 and T2 determined the percentage of sealant loss.*
Results *In the first part, the experimental procedure showed a reliability of 96.85%. In the second part, the difference in the rate of lost sealant between T0-T1 and T1-T2 was statistically significant (p<0.001). Statistics: ANOVA analysis was made.*
Conclusions *Photographs, obtained through the experimental two-part system, allowing a reproducible positioning of the intraoral camera in oral cavity, could represent a standardised and useful method to monitor sealants retention over time.*

Keywords *Dental caries prevention; Pit and fissure sealants; Sealants retention.*

Introduction

Despite an increased awareness of the importance of oral health and the progress of preventive measures [Giannattasio et al., 2015], such as improved oral hygiene [Ferrazzano et al., 2008; Re et al., 2015], use of fluoride-containing toothpaste, fluoride content in drinking water, prevalence of dental caries remains unacceptably high in many industrialised countries [Corrêa-Faria et al., 2016; Ferrazzano et al., 2006; Ferrazzano et al., 2016] and in people affected by systemic diseases [Ferrazzano et al., 2012]. Dental caries is particularly common on the pits and fissures of occlusal surfaces, whose complex morphology allows for plaque accumulation and makes mechanical cleaning difficult [Oba et al., 2012]. Pit and fissure sealants are a proven and effective method for these surfaces, since they act as a physical barrier that restrains the exchange of metabolic products between fissure microorganisms and the oral environment [Braz et al., 2011; Haznedaroglu et al., 2014]. A number of clinical studies indicate that the success of fissure sealants depends on the level of sealant retention, maintenance of sealant integrity, and the properties of the sealant material. Over the past 30 years, various materials and techniques have been studied to enhance the longevity of pit-and-fissure sealants. Definitely, the type of sealant material, its viscosity and flow, as well as the wear resistance of the material, are major factors contributing to retention and caries prevention. Most of the sealants used today are resin-based composite adhesives with a main component of Bis-GMA, which allows the addition of filler particles to the sealant composition, considerably increasing their wear resistance [Erdemir et al., 2014]. Mechanical preparation has been suggested to provide better access to the deeper fissure areas, thus enabling removal of debris, deeper sealant penetration, and improved retention [Geiger et al., 2000].

Many studies have suggested that bur preparation and air abrasion [Bevilacqua et al., 2007; Mazzoleni et al., 2007] enhance sealant penetration and adaptation, providing a greater surface area for retention as well as an increase in the bulk of sealant that improves wear resistance.

It has also been suggested that a combination of these measures could lead to increased clinical longevity. On the other hand, a number of investigators have reported no significant difference between conventional acid etch alone, and bur preparation followed by acid etching of pits and fissures [Ansari et al., 2004].

Another important factor to consider in the success of sealants is the prevention of microleakage: it has been shown that the application of the intermediate adhesive bonding agent between enamel and sealant could increase sealant retention and contribute to prevent the development of microleakage [McCafferty and O'Connell, 2016]. Therefore, the retention rate becomes a determinant of sealant effectiveness as a caries prevention measure. Otherwise, a partial loss of the sealant material leads to the occurrence of marginal leakage and, hence, to caries development underneath the sealant [Vijayaraghavan et al., 2012].

The most commonly used method to assess sealant retention is a visual clinical examination, in which the sealant is recorded as intact, partially lost, or completely lost. Despite being conducted by qualified and calibrated examiners, using artificial light and mirrors, visual clinical examination is far from being an optimal technique, depending on visual and memory skills of the operator and being poorly reproducible [Hu et al., 2014]. Other procedures consist of the standard color photograph and the replica: these methods may be more sensitive than visual clinical examination in measuring the levels of retention and degradation, but reflect the difficulty in judging clinically the retention of degraded or worn-out sealant. Furthermore, no studies can demonstrate the efficacy and precise reliability of these procedures [Braz et al., 2011].

Researchers also suggest the use of the optical coherence tomography (OCT) in monitoring sealant application and retention in the long term. OTC is a non-invasive imaging technique that produces high resolution, cross-sectional images of biological tissue at a micrometer scale, but it is widely far from being clinically used as a conventional method to assess sealant retention [Ito et al., 2016]. Therefore, the aims of the present study were: the elaboration of an experimental system to obtain reproducible and comparable photographs of the occlusal surfaces, and the use of this experimental system to monitor sealants retention over time.

Materials and methods

Subjects recruitment

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the "Federico II" University of Naples (Italy). The subjects were recruited from patients of the Department of Neuroscience, Reproductive and Oral Sciences, Section of Paediatric Dentistry, University of Naples, "Federico II", Naples, Italy. Parents or guardians of all included subjects had received and signed the informed consent form that explained the nature and the aim of the study.

Ninety seven patients aged 12 to 16 years in good general health with caries-free, completely erupted first permanent molars with deep and retentive pits and fissures were included in the study.

Exclusion criteria were: known allergy to any of the resins used; previously placed sealants or restorations; presence of orthodontic bands over the first permanent molars; bruxism; unwillingness to return at fixed study intervals for follow-up examinations.

Sealing procedures

Three specially trained and calibrated researchers performed the operative procedures. For fissure sealant application, teeth were isolated with rubber dam and clamp. The occlusal surfaces were cleaned with pumice powder, applied with a bristle brush to remove salivary pellicles and any remaining plaque. After prophylaxis, teeth were washed to remove pumice residues. Occlusal fissures were slightly opened using a fissurotomy diamond bur (D1 Intesiv, Dental Trey, Italy). Then, teeth were acid-etched using Scotchbond etchant (3M ESPE, St. Paul, MN, USA) for 30 s, rinsed for 15 s and dried until the surface was chalky white. A layer of bond (Adper™ Scotchbond™ 1 XT, 3M ESPE, St. Paul, MN, USA) was placed on occlusal surfaces and photopolymerised.

The next step consisted of applying a resin-based sealant (Clinpro, 3M ESPE, St. Paul, MN, USA). The material was carefully placed in the pits and fissures, manipulated with an explorer in order to prevent voids, air entrapment or bubbles and cured for 30 s with a LED curing light.

Experimental standardised system development

A high definition intraoral camera connected to a computer was used to obtain photos of the occlusal surfaces. A dedicated software (VixWin Platinum 3.3, Digital Imaging Software, Dentsply, Gendex Division, Milan, Italy) was used to perform measurements on archived pictures.

In order to obtain a stable, standardised and reproducible placement of the camera in the oral cavity, an experimental two-part system (a dental arch support and a camera support) was made. The dental arch support was constructed in Plexiglas (Fig. 1). It consists of a central body, which is the most mesial portion, and two wings, which extended mesio-distally. The central body is characterised by a wide hollow area, in which the incisor group is placed, while the two wings are positioned buccally and lingually to the occlusal surfaces of premolars and molars. On each wing there are two lines of 10 holes of 1 mm diameter, distant 3 mm from each other. This support can be used both for the upper and for the lower arch. The camera support was constructed in resin. It consists of a stabilisation casing in which the head of the camera is placed and locked (Fig. 2, 3). On the stabilisation casing there are two lines of 5 pins, having the same size of the holes present on the dental arch support. In this way, the camera support and the dental arch support can be connected to each other through the holes and pins (Fig. 4).

Experimental procedure (Part 1) Degree of reliability and images distortion assessment

To test the reliability of the experimental procedure in performing standardised and comparable photographs of the occlusal surfaces and the percentage of image distortion, 120 first molars (30 first maxillary right permanent molars, 30 first maxillary left permanent molars, 30 first mandibular right permanent molars, 30 first mandibular left permanent molars) from 42 patients were sealed as described above. For each molar ten photographs were taken (from T1 to T10), using the intraoral camera connected with the dental arch support and the camera support, once a month for 10 months.

The experimental two-part system described above, thanks to the connection between pins and holes, allowed to take photographs in the oral cavity in the same spatial position. To calculate the degree of reliability, on the occlusal surface of each molar 3 anatomical fixed identifiable reference points (called A, B, C) were detected. With the software, the lengths of the three segments (defined AB; AC; BC), connecting the 3 anatomical fixed reference points in the photos, performed at different times on the same molars, were measured. These lengths were calculated at T1 (AB1; AC1; BC1) and compared with the lengths obtained at T2 (AB2; AC2; BC2) up to the last control at T10 (AB10; AC10; BC10) (Fig.5). The comparison between the values of linear measurements allowed the calculation of the degree of reliability of the experimental procedure.

Experimental procedure (Part 2) Sealant retention assessment

In this part of the study the system was used to monitor sealants retention over time. In this stage, 165 teeth, 82 maxillary (40 right and 42 left) and 83 mandibular (45 right and 38 left) first molars from 55 patients, were sealed and photographed. For each tooth, one photo was taken after sealing (T0), 6 months (T1) and 1 year later (T2). With the software, the sealed area on each occlusal surface was measured (Fig. 6). The comparison of the identified sealed areas between T0 and T1, T0 and T2, T1 and T2 determined the percentage of sealant loss after 6 months, after 1 year, and between six months and 1 year, respectively (Fig. 7, 8). Data were submitted to statistic elaboration through SPSS (Statistical Package For Social Science, Inc, Chicago IL). ANOVA analysis was made and the kappa statistic was used to evaluate inter-examiners calibration.

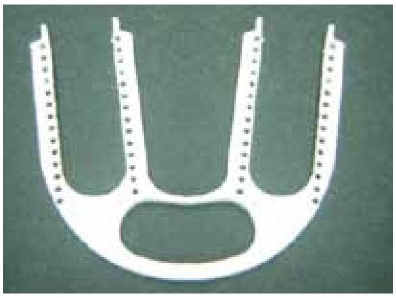

FIG. 1 Dental arch support. FIG. 2 Stabilisation casing. FIG. 3 Stabilisation casing with the camera located and locked. FIG. 4 Connection between the camera support and the dental arch support.

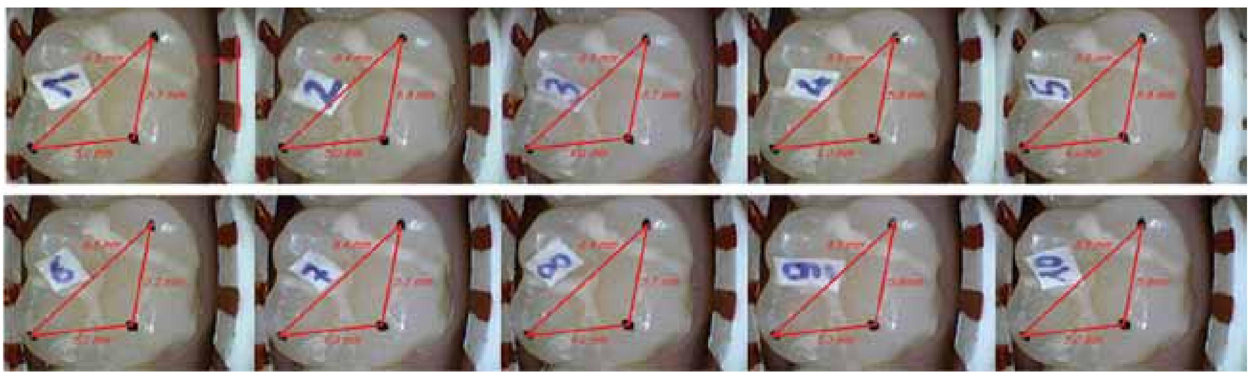

FIG. 5 Illustrative sequence: linear measurements from T1 to T10.

Results

Inter-examiners calibration

For the use of the intraoral camera connected with the dental arch support, the kappa score was 0.82 when comparing examiners 1 and 2, 0.89 when comparing examiners 2 and 3, and 0.87 when comparing examiners 1 and 3. The kappa score for sealing procedure was 0.74 when comparing examiners 1 and 2, 0.72 when comparing examiners 2 and 3, and 0.79 when comparing examiners 1 and 3.

Degree of reliability and images distortion assessment

In the first part of study 1200 images were obtained by photographing 120 sealed molars at 10 different times.

Therefore, in the pictures taken on the upper dental arch, the mean values of image distortion were 3.67±0.06%, and 2.57±0.08% for the right and left maxillary first molars, respectively. The maximum and minimum values of image distortion were 6.78% and 1.58%, respectively.

For the lower dental arch the mean values of image distortion were 3.33±0.04% and 3.01±0.05% for the right and left mandibular first molars, respectively. The maximum and minimum values of image distortion were 4.49% and 2.14%, respectively. The experimental procedure showed a reliability of 96.85%.

Sealant retention assessment (Part 2)

In the second part of study 495 images were obtained by photographing 165 sealed molars at T0, T1, and T2. In maxillary first molars the rate of lost sealant at T0-T1 was 5.17±3.10% with maximum and minimum values of 12.87% and 0.12%, respectively; at T1-T2 it was 2.38 ± 1.78% with maximum and minimum values of 6.77% and 0%, respectively; at T0-T2 it was 5.52 ± 3.33% with maximum and minimum values of 14.50% and 0.36%, respectively. In mandibular first molars the rate of lost sealant at T0-T1 was 4.26 ± 3.03% with maximum and minimum values of 14.5% and 0%, respectively; at T1-T2 it was 2.55±2.49% with maximum and minimum values of 10.9% and 0%, respectively; and at T0-T2 it was 6.63±3.05% with maximum and minimum values of 14.75% and 0.49%, respectively (Fig. 9). In addition, Table 1 shows the different rate of lost sealant at T0-T1, T1-T2 and T0-T2 for first left and right maxillary and mandibular molars, respectively. The difference in the rate of lost sealant at T0-T1, at T1-T2 and at T0-T2 between the maxillary and mandibular teeth is not statistically significant. Furthermore, there is no statistical difference between left and right molars for the rate of lost sealant.

Finally, the difference in the rate of lost sealant between T0-T1 and T1-T2 is statistically significant ($p<0.001$).

Discussion

Occlusal sealants are considered an effective caries-preventive measure, particularly among young patients; their benefits in protecting teeth from caries have been demonstrated in several systematic reviews [Ahovuo-Saloranta et al., 2008; Courson et al., 2011; Simonsen and Neal, 2011]. The effectiveness over time depends on the correct execution of the clinical protocol [Muller-Bolla et al., 2006]. For this reason, in the present study a resin-based sealant was used, being the first choice material for

FIG. 6 Sealed areas computer measurements at T0, T1 and T2, respectively. FIG. 7 Comparison of the sealed areas between T0 – T1. FIG. 8 Comparison of the sealed areas between T0 – T2.

	T0-T 1	T1-T 2	T0-T 2
Right mandibular molars	3.94 ± 3.07 maximum value 11.50 minimum value 0.0	2.65 ± 2.55 maximum value 10.90 minimum value 0.0	6.31 ± 3.09 maximum value 12.98 minimum value 0.49
Left mandibular molars	4.65 ± 3.01 maximum value 10.87 minimum value 0.0	2.45 ± 2.27 maximum value 8.70 minimum value 0.0	7.01 ± 2.99 maximum value 14.75 minimum value 1.18
Right maxillary molars	5.17 ± 3.17 maximum value 12.87 minimum value 0.23	2.50 ± 1.82 maximum value 6.77 minimum value 0.0	7.26 ± 3.34 maximum value 14.50 minimum value 0.36
Left maxillary molars	5.17 ± 3.07 maximum value 11.70 minimum value 0.12	2.26 ± 1.74 maximum value 6.73 minimum value 0.0	7.40 ± 3.23 maximum value 13.35 minimum value 1.03

TABLE 1 Rate of lost sealant a T0-T1, T1-T2 and T0-T2 for first left and right maxillary and mandibular molars.

the sealing procedure [Kühnisch et al., 2012]. Furthermore, in order to avoid saliva contamination, which can lead to a reduction of the mechanical retention [Locker et al., 2003], a rubber dam was utilised to provide the best isolation. In addition, a bond layer was put on the occlusal surfaces between the previously acid-etched enamel surface and the sealant material with the aim of enhancing sealant retention [Crall and Donly, 2015]. Moreover, this innovative experimental study showed interesting points for discussion. In fact, even if researchers suggested various methods to assess the sealant retention on teeth [Braz et al., 2011; Hu et al., 2014; Ito et al., 2016], none of those demonstrated a valid reproducibility and a statistical significance. Presently, traditional methods for evaluating the integrity of the dental sealants, such as visual and probing inspection, are unable to identify gaps, failures into the internal structure of sealants, which can cause infiltration and loss of material [Kubo et al., 2004]. In addition, even if color photographs method has demonstrated to ensure a higher level of validity than that performed through the use of the visual clinical examination and replica methods for the assessment of sealant retention [Hu et al., 2014], it is far from representing the best procedure, not being a standardised technique. By contrast, the first evidence of the present study demonstrates that it is possible to obtain a reproducible and stable placement of a camera device in the oral cavity, using this innovative dental support system; pictures taken using this new device, on maxillary and mandibular right and left first molars, show values of distortion not statistically significant: this could possibly be due to the connection between pins and holes of the camera device, allowing to take photographs in the oral cavity in the same spatial position. Therefore, using 3 anatomical fixed identifiable reference points on the occlusal surface of each molar, it is possible to obtain a photographic analysis with a reliability of 96.85%, after 10 months, demonstrating that this first part of the experimental procedure is well reproducible and statistically powerful. Concerning the second part of the study, the results demonstrate that it is possible to monitor in a statistically significant manner the quantity of lost sealant 1 year after treatment: particularly, there is not statistical difference in the rate of lost sealant between the maxillary and mandibular teeth and between left and right molars; these results show that the position of the sealed tooth in the oral cavity does not influence the quality and retention of the sealant. Moreover, the study shows that the difference in the rate of lost sealant between T0-T1 and T1-T2 is statistically significant, indicating that the greater loss of sealant occurrs in the first six months. These results are in contrast with other studies, reporting that most sealing failures take place within 1 year from application [Erdemir et al., 2014]. Finally, concerning both the sealing method and the use of the intraoral camera connected with the dental arch support, although these procedures are operator-dependent, the kappa scores show high inter-examiners agreement.

Conclusion

The above mentioned points of discussion demonstrates that photographs, obtained through the experimental two-part system (a dental arch support and a camera support, which permits the reproducible positioning of the intraoral camera in the oral cavity, could represent a standardised and useful method to observe and monitor dental occlusal sealants retention over time.

References

- Ahovuo-Saloranta A, Hiiri A, Nordblad A, Mäkelä M, Worthington HV. Pit and fissure sealants for preventing dental decay in the permanent teeth of children and adolescents. Cochrane Database Syst Rev 2008;(4):CD001830.
- Ansari G, Oloomi K, Eslami B. Microleakage assessment of pit and fissure sealant with and without the use of pumice prophylaxis. Int J Paediatr Dent 2004; 14(4):272-8.
- Bevilacqua L, Cadenaro M, Sossi A, Biasotto M, Di Lenarda R. Influence of air abrasion and etching on enamel and adaptation of a dental sealant. Eur J Paediatr Dent 2007;8(1):25-30.
- Braz AK, Aguiar CM, Gomes AS. Evaluation of the integrity of dental sealants by optical coherence tomography. Dent Mater 2011;27(4):e60-4.
- Corrêa-Faria P, Paixão-Gonçalves S, Paiva SM, Pordeus IA. Incidence of dental caries in primary dentition and risk factors: a longitudinal study. Braz Oral Res 2016 20; 30(1).
- Crall JJ, Donly KJ. Dental sealants guidelines development: 2002-2014. Pediatr Dent 2015;37(2):111-5.
- Courson F, Velly AM, Droz D, Lupi-Pégurier L, Muller-Bolla M. Clinical decision on pit and fissure sealing according to the occlusal morphology. A descriptive study. Eur J Paediatr Dent 2011;12(1):43-9.
- Erdemir U, Sancakli HS, Yaman BC, Ozel S, Yucel T, Yıldız E. Clinical comparison of a flowable composite and fissure sealant: a 24-month split-mouth, randomized, and controlled study. J Dent 2014; 42(2):149-57.
- Ferrazzano GF, Sangianantoni G, Cantile T, Ingenito A. Relationship between social and behavioural factors and caries experience in schoolchildren in Italy. Oral Health Prev Dent 2016;14(1):55-61.
- Ferrazzano GF, Scaravilli MS, Ingenito A. Dental and periodontal health status in Campanian children and relation between caries experience and socio-economic behavioural factors. Eur J Paediatr Dent 2006; 7(4):174-8.
- Ferrazzano GF, Sangianantoni G, Cantile T, Amato I, Orlando S, Ingenito A. Dental enamel defects in Italian children with cystic fibrosis: an observational study. Community Dent Health 2012;29(1):106-9.
- Ferrazzano GF, Cantile T, Sangianantoni G, Ingenito A. Effectiveness of a motivation method on the oral hygiene of children. Eur J Paediatr Dent 2008;9(4):183-7.
- Geiger SB, Gulayev S, Weiss EI. Improving fissure sealant quality: mechanical preparation and filling level. J Dent 2000; 28(6):407-12.
- Giannattasio A, Poggi E, Migliorati M, Mondani PM, Piccardo I, Carta P, Tomarchio N, Alberti G.The efficacy of Italian guidelines in promoting oral health in children and adolescents. Eur J Paediatr Dent 2015;16(2):93-98.
- Haznedaroglu E, Sozkes S, Mentes AR. Microhardness evaluation of enamel adjacent to an improved GIC sealant after different enamel pretreatment procedures. Eur J Paediatr Dent 2014;15(4):397-400.
- Hu X, Fan M, Rong W, Lo EC, Bronkhorst E, Frencken JE. Sealant retention is better assessed through colour photographs than through the replica and the visual examination methods. Eur J Oral Sci 2014; 122(4):279-85.
- Ito S, Shimada Y, Sadr A, Nakajima Y, Miyashin M, Tagami J, Sumi Y. Assessment of occlusal fissure depth and sealant penetration using optical coherence tomography. Dent Mater J 2016; 35(3):432-9.
- Kubo S, Yokota H, Yokota H, Hayashi Y. The effect of light-curing modes on the microleakage of cervical resin composite restorations. J Dent 2004; 32:247–54.
- Kühnisch J, Mansmann U, Heinrich-Weltzien R, Hickel R. Longevity of materials for pit and fissure sealing--results from a meta-analysis. Dent Mater 2012; 28(3):298-303.
- Locker D, Jokovic A, Kay EJ. Prevention. Part 8. The use of pit and fissure sealants in preventing caries in the permanent dentition of children. Br Dent J 2003; 195:375-8.
- Mazzoleni S, De Francesco M, Perazzolo D, Favero L, Bressan E, Ferro R, Stellini E. Comparative evaluation of different techniques of surface preparation for occlusal sealing. Eur J Paediatr Dent 2007;8(3):119-23.
- McCafferty J, O'Connell AC.A randomised clinical trial on the use of intermediate bonding on the retention of fissure sealants in children. Int J Paediatr Dent 2016; 26(2):110-5.
- Muller-Bolla M, Lupi-Pégurier L, Tardieu C, Velly AM, Antomarchi C. Retention of resin-based pit and fissure sealants: A systematic review. Community Dent Oral Epidemiol 2006; 34(5):321-36.
- Oba AA, Sönmez IŞ Ercan E, Dülgergil T. Comparison of retention rates of fissure sealants using two flowable restorative materials and a conventional resin sealant: two-year follow-up. Med Princ Pract 2012; 21(3):234-7.
- Re D, Augusti G, Battaglia D, Giannì AB, Augusti D. Is a new sonic toothbrush more effective in plaque removal than a manual toothbrush? Eur J Paediatric Dent 2015; 16(1):1318.
- Simonsen RJ, Neal RC. A review of the clinical application and performance of pit and fissure sealants. Aust Dent J 2011;56 Suppl 1:45-58.
- Vijayaraghavan R, Arun Prasad Rao V, Venugopal Reddy N, Krishnakumar R, Sugumaran D and Mohan G. Assessment and comparison of microleakage of a fluoride-releasing sealant after acid etching and Er: YAG laser treatment – An in vitro study. Contemp Clin Dent 2012; 3(1): 64–68.