COMPARATIVE EVALUATION OF THE EFFECT OF DIFFERENT CLEANING METHODS ON SEALANT PENETRATION AND NANOLEAKAGE

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FACULTY OF DENTISTRY
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ABSTRACT

Background: Fissure sealant prevents initiation and progression of occlusal caries by acting as a physical barrier that inhibits accumulation of microorganisms and food debris in pits and fissures. Proper cleaning and fissure preparation are necessary prior to sealant placement. Hence, the occlusal surface must be free from plaque, acquired pellicle, and debris that might interfere with the etching process or sealant penetration. The objectives of this study were to compare the effect of occlusal surface cleaning methods using either a toothbrush or a rotary brush prior to sealant placement on sealant penetration depth and nanoleakage occurrence, and to assess the influence of fissure type/morphology on sealant penetration. **Methods:** Thirty-three sound extracted human maxillary premolars were randomly divided into three groups of different cleaning methods prior to fissure sealant placement: (1) No cleaning of the occlusal surface (control), (2) occlusal surface cleaned with a toothbrush for 10 seconds, and (3) occlusal surface cleaned with a rotary brush attached to a slow-speed handpiece for 10 seconds. The teeth were mounted on epoxy resin, sectioned into halves before being stained with modified silver staining technique, and polished. Depth of sealant penetration was determined using scanning electron microscopy (SEM) according to the scoring method: (1) sealant penetrated one-third of the total fissure length, (2) sealant penetrated half of the total fissure length, and (3) sealant penetrated the total fissure length. Fissure type/morphology was classified into U-type, V-type; Y1-type and Y2-type. Nanoleakage expression along sealant-enamel interface was determined by measuring silver nitrate uptake using EDX. Data management and analysis were performed using SPSS version 23. Different cleaning methods were compared using Fisher‘s exact test (sealant penetration) and Kruskal-Wallis (nanoleakage). Association between fissure type/morphology and sealant penetration was determined using Fisher‘s exact test. The test was considered significant when $p<0.05$. **Results:** The occlusal surfaces cleaned with a rotary brush has the highest percentage (18%) of sealant penetration with score 3 followed by the occlusal surfaces cleaned with a toothbrush (15%) and no cleaning of the occlusal surface (12%). Higher mean rank of nanoleakage expression (91.4) was found in the control group followed by toothbrush group (81.0) and rotary brush group (76.5). However, no significant association was observed between the different cleaning methods (toothbrush and rotary brush); and the sealant penetration and nanoleakage expression ($p>0.05$). The only significant difference was observed between fissure type/morphology and sealant penetration depth ($p=0.025$). **Conclusions:** In conclusion, cleaning of the occlusal surface prior to sealant placement using a toothbrush is as effective as a rotary brush in relation to sealant penetration and occurrence of nanoleakage. Hence, toothbrush can be used as one of the cleaning methods on the occlusal surface prior to fissure sealant placement.

Keywords: fissure sealant, sealant penetration, nanoleakage, scanning electron microscopy (SEM)

PENILAIAN PERBANDINGAN TERHADAP KESAN-KESAN DARIPADA PROSES PEMBERSIHAN BERBEZA KE ATAS PENEMBUSAN PENGAPAN DAN KETIRISAN NANO

ABSTRAK

Latar belakang kajian: Pengapan fisur menghalang pembentukan dan perkembangan karies oklusal dengan berfungsi sebagai penghalang fizikal yang menghalang pengumpulan mikroorganisma dan sisa makanan dalam alur gigi yang dalam (fisur). Sebelum aplikasi selan, penyediaan alur dan pembersihan sepatutnya perlu dilakukan untuk memastikan permukaan oklusal bebas dari plak, *“pellicle”*, dan sisa makanan yang boleh mengganggu proses *“etching”* atau penembusan selan. Objektif kajian ini adalah untuk melihat kesan kaedah pembersihan permukaan oklusal dengan menggunakan berus gigi berbanding berus putar sebelum mengaplikasikan pengapan fisur ke atas penembusan pengapan dan ketirisan nano, dan untuk menilai pengaruh bentuk fisur pada penembusan pengapan. **Langkah-langkah kajian:** Tiga puluh tiga batang gigi premolar maksila manusia yang sihat dan telah dicabut dibahagikan kepada tiga kumpulan secara rawak untuk kaedah pembersihan yang berbeza; (1) Tiada pembersihan permukaan oklusal (kawalan), (2) permukaan oklusal dibersihkan dengan berus gigi selama 10 saat, dan (3) permukaan oklusal dibersihkan dengan berus putar pada *“handpiece”* kelajuan perlahan selama 10 saat. Pengapan fisur (Conseal f, SDI, Australia) diletakkan ke atas permukaan oklusal mengikut arahan pengeluar. Semua gigi dibenamkan ke dalam resin epoksi, dipotong kepada dua bahagian, diwarnakan mengikut teknik pewarnaan perak yang telah diubahsuai dan digilap. Kedalaman penembusan pengapan ditentukan menggunakan mikroskop elektron imbasan (SEM), mengikut kaedah pemarkahan: (1) Pengapan menembusi satu pertiga jumlah panjang fisur, (2) Pengapan menembusi separuh panjang fisur, dan (3) Pengapan menembusi panjang keseluruhan fisur. Bentuk fisur diklasifikasikan kepada jenis U, V, Y1, dan Y2.

Kejadian ketirisan nano ditentukan sepanjang sempadan enamel dan pengapan dengan mengira jumlah pengambilan perak nitrat menggunakan EDX. Pengurusan data dan analisis dilakukan menggunakan SPSS versi 23. Kaedah pembersihan yang berbeza telah dibandingkan dengan ujian *Fisher's exact* (penembusan pengapan) dan ujian *Kruskal-Wallis* (ketirisan nano). Kaitan antara bentuk fisur dan penembusan pengapan ditentukan dengan menggunakan ujian *Fisher's exact*. Ujian ini dianggap signifikan apabila $p<0.05$. **Keputusan:** Permukaan oklusal dibersihkan dengan kaedah berus putar mempunyai peratusan tertinggi (18%) pengapan menembusi panjang keseluruhan fisur (skor 3) diikuti oleh permukaan oklusal dibersihkan dengan kaedah berus gigi (15%) dan permukaan oklusal yang tidak dibersihkan (12%). *Mean rank* ketirisan nano yang lebih tinggi (91.4) didapati dalam kumpulan tiada pembersihan permukaan oklusal diikuti oleh kumpulan permukaan oklusal dibersihkan dengan kaedah berus gigi (81.0) dan kumpulan permukaan oklusal dibersihkan dengan kaedah berus putar (76.5). Walau bagaimanapun, tiada kaitan signifikan yang diperhatikan di antara kaedah pembersihan yang berbeza (berus gigi dan berus putar); dengan kedalaman penembusan pengapan dan ketirisan nano ($p>0.05$). Perbezaan yang signifikan hanya diperhatikan di antara bentuk fisur dan kedalaman penembusan pengapan ($p=0.025$). **Kesimpulan:** Kesimpulannya, pembersihan permukaan oklusal sebelum aplikasi pengapan menggunakan kaedah berus gigi memberi kesan sebaik kaedah berus putar dari segi penembusan pengapan dan ketirisan nano. Oleh itu, berus gigi boleh digunakan sebagai salah satu daripada kaedah-kaedah pembersihan permukaan oklusal gigi sebelum aplikasi pengapan fisur.

Kata kunci: pengapan fisur, penembusan pengapan, ketirisan nano, mikroskop elektron imbasan (SEM)

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LIST OF SYMBOLS AND ABBREVIATIONS

AAPD	:	American Academy of Paediatric Dentistry
EDX	:	Energy Dispersive X-Ray Spectroscopy
GIC	:	Glass ionomer cement
IL	:	Illinois, USA
mL	:	millilitres
mm	:	millimetres
µm	:	micrometres
nm	:	nanometres
NHANES	:	National Health and Nutrition Examination Survey
RMGIC	:	Resin-modified glass ionomer cement
SEM	:	Scanning electron microscope
SDI	:	Southern Dental Industries
SiC	:	Silicon carbide
SPSS	:	Statistical Package for the Social Sciences
USA	:	United States of America

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Background of the study

Untreated dental caries in permanent teeth is the most prevalent chronic oral diseases (Marcenes et al., 2013) which primarily cause oral pain and tooth loss (Selwitz et al., 2007). The occlusal surface of a tooth is more susceptible to dental caries than the smooth surface (Anderson, 2002; Demirci et al., 2010; Hopcraft & Morgan, 2006). The frequency of caries in pits and fissures on the first and second molars is very high, ranged between 52.7% and 66.3%. This may be due to the complicated surface morphology and limited access for effective oral hygiene (Demirci et al., 2010). Fissure sealant could prevent the initiation and progression of occlusal caries by acting as a physical barrier that inhibits accumulation of microorganisms and food debris in pits and fissures. Its application in children and adolescents has been proven to reduce the risk of developing new caries lesions by 76% as compared to those who did not received sealants (Wright et al., 2016). Therefore, application of fissure sealant is recognized as the most suitable and effective method (Simonsen & Neal, 2011) to prevent the development of caries lesion in pits and fissures.

Resin-based sealant is the most durable material and commercially available (Anusavice et al., 2013). It has better long-term caries-preventive effect and higher retention rate compared to other materials such as compomer or glass ionomer-based (Kühnisch et al., 2012; Muller-Bolla et al., 2006).

The success of fissure sealant in caries prevention is dependent on its ability to be retained in the occlusal fissure for a long time (Ahovuo-Saloranta et al., 2008; Beauchamp et al., 2008) by sealing off susceptible occlusal surfaces from the oral environment (Muller-Bolla et al., 2006). In addition, fissure sealant should be able to prevent microleakage at the sealant-enamel interface so that the caries process will not be initiated and progress underneath the sealant (Agrawal & Shigli, 2012). Nevertheless, Hariri et al. (2012) demonstrated that nanoleakage could occur in between resin-enamel interface (Hariri et al., 2012). Nanoleakage has been described as a kind of leakage that occurs because of penetration paths through porosities around 20-100 nanometres, even with the absence of marginal gap (Sano, 2006; Sano et al., 1995). It is large enough to allow the penetration of bacterial products such as acid and dentinal or oral fluid along the interface between restoration and tooth surface (Pioch et al., 2002).

Good retention and adaptation of resin-based fissure sealant have been related to several factors such as surface cleaning and fissure preparation, isolation technique, etching as well as drying techniques (Welbury et al., 2004). The presence of plaque, pellicle, debris and moisture in narrow fissures might interfere with the etching process and compromise the sealant penetration (Simonsen, 2002). Thus, various surface cleaning and fissure preparation methods are performed to achieve good sealant retention which include the use of toothbrush, rotary instrument (rubber cup or rotary brush), air abrasion, air polishing, hydrogen peroxide, laser irradiation and even invasive methods such as fissurotomy and enameloplasty (Simonsen & Neal, 2011). To date, there is no superior cleaning method to prepare pits and fissures of a tooth for fissure sealant placement that will reduce the microleakage and improve the long-term clinical retention of fissure sealants (Chaturvedy et al., 2013).

Ultimately, among all the cleaning methods used prior to sealant placement, toothbrushing and handpiece prophylaxis are still the least invasive methods and widely used (Gillcrist et al., 1998; Gray et al., 2009; Houpt & Shey, 1983; Mertz-Fairhurst et al., 1992). It has been practised since a few decades ago to mechanically remove the tooth surface debris using a rotary rubber cup or brush with pumice prior to sealant placement (Blackwood et al., 2002; M. Buonocore, 1970). However, cleaning of occlusal fissure prior to sealant placement using rotary instrument with pumice still remain controversial. It is believed that pumice particles easily entrapped in the fissures (Garcia-Godoy & Gwinnett, 1987; Garcia-Godoy & Medlock, 1988). Hence, the pumice particles incorporated into the sealant and altered its micromechanical bond that lead to microleakage (Agrawal & Shigli, 2012), and also causing removal of enamel surface at the range of 0.6-4.0 microns (Biller et al., 1980; Koch et al., 1975; Mellberg, 1979; Stookey, 1978; Vrbic et al., 1967). Whilst some researchers recommend clinicians to clean the surface prior to sealant placement, others claimed that it is unnecessary as acid etching alone can completely remove the acquired pellicle (Main et al., 1983) and besides, the rotary bristles would polish only the superficial areas (Donnan & Ball, 1989).

On the other hand, it has been suggested that occlusal surface cleaning with a toothbrush resulted in comparable retention rates of fissure sealant as those achieved by handpiece prophylaxis (Gillcrist et al., 1998; Gray et al., 2009; Houpt & Shey, 1983). Following to this, owing to lower cost and less equipment needed, toothbrushing may serve as a reasonable alternative to conventional handpiece prophylaxis, especially in school-based fissure sealant programme in remote area where handpiece may not be readily available (Gray et al., 2009).

Another factor that may influence the sealant penetration ability is fissure type/morphology (Duangthip & Lussi, 2003; Selecman et al., 2007). As this factor may also affect the quality of cleaning and sealant penetration, it is important to evaluate the influence of fissure association of fissure type/morphology on sealant penetration.

1.2 Clinical relevance

Fissure sealant is the most effective caries-preventive measure that can be given to a patient (Mertz-Fairhurst et al., 1992). Various studies were conducted to investigate and compare the effects of different cleaning methods of occlusal surfaces before receiving fissure sealant in relation to retention and microleakage (Agrawal & Shigli, 2012; Hegde & Coutinho, 2016). However, there is lack of *in vitro* study investigating the effects of toothbrushing on the depth of sealant penetration and nanoleakage at the sealant-enamel interface. Findings in this study would be useful to determine the importance of occlusal surfaces cleaning either by using a prophylactic handpiece with a rotary rubber cup/ brush or with just toothbrushing prior to sealant placement. Toothbrushing is a non-invasive method that can be recommended if it could effectively clean the occlusal surface as good as a rotary brush.

1.3 Purpose of the study

1.3.1 Aim

The aim of this study was to evaluate the effects of different cleaning methods prior to resin sealant placement on the penetration of resin sealant and nanoleakage expression.

1.3.2 Objectives

The objectives of this study were:

1. To compare the effect of cleaning the occlusal surface using a toothbrush to a rotary brush before resin sealant placement on the depth of sealant penetration using SEM.

2. To assess the influence of fissure type/morphology on sealant penetration.

3. To compare the effect of cleaning the occlusal surface using a toothbrush to a rotary brush before resin sealant placement on the occurrence of nanoleakage using SEM.

CHAPTER 2: LITERATURE REVIEW

2.1 Dental caries

2.1.1 Definition

Dental caries is the most prevalent disease worldwide ("The Challenge of Oral Disease - A call for global action. The Oral Health Atlas," 2015) and is the main cause of oral pain and tooth loss (Selwitz et al., 2007). It is defined as the localised destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates (Fejerskov & Kidd, 2009).

2.1.2. Caries experience

National Health and Nutrition Examination Survey (NHANES) 2011-2012 reported that 21% of children (six to eleven years old) and 58% of adolescents (12 to 19 years old) in the United States of America experienced dental caries in their permanent teeth (Dye et al., 2015).

2.1.3 Caries on occlusal surface

Dental caries predominantly occurs in pits and fissures (Anderson, 2002). Occlusal surfaces, especially those on permanent molars, contain pits and fissures which can easily trap debris and microorganisms, thereby increasing the risk of developing dental caries. Eklund and Ismail (1986) reported that 70% of all occlusal surfaces of molars would become carious within ten years of eruption (Eklund & Ismail, 1986). The first three years of eruption are the most critical period as the majority of caries lesions in occlusal surfaces would occur during this time (Reid & Grainger, 1955). Although the trend of prevalence of caries in adolescents and children is declining, the decrease in occlusal surface caries is less pronounced as compared to the decrease in the smooth surface caries (Macek et al., 2003). In permanent teeth, caries reduction is greater on the interproximal and smooth surfaces than on the occlusal surfaces (Brown & Selwitz, 1995).

2.1.4 Prevention of pit and fissure caries

The emphasis in the dental treatment has moved from restoration of carious fissures to the use of preventive measures such as fissure sealants and fluoride varnish (Simonsen, 2002). To maintain dental health, preventing a disease is recognised as a more economical option than treating an established disease. For prevention of pit and fissure caries, fissure sealant is the most suitable and effective method as compared to other preventive measures such as systemic and topical fluoride application (Ahovuo-Saloranta et al., 2016; Beauchamp et al., 2008; Simonsen & Neal, 2011).

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2.2 Fissure sealant

2.2.1 Definition

Fissure sealant is defined as:-

―a chemically-active liquid material that is introduced into the occlusal pits and fissures of caries-susceptible teeth, that after application, either cures chemically (autopolymerising), or is cured with a visible light source (light-cured), thus forming a micromechanically bonded protective layer that prevents the invasion of caries-producing bacteria, and simultaneously cuts off the access of surviving caries-producing bacteria from their source of nutrients" (Simonsen & Neal, 2011, p.45).

The first dental pit and fissure sealant was introduced in 1971 (Nuva-Seal by L.D. Caulk) (Simonsen & Neal, 2011) by Buonocore in 1955 (Buonocore, 1955; Cueto & Buonocore, 1967). Buonocore was the first person who discovers the acid etch technique (Buonocore, 1955) which later led to the development of technique of sealing pits and fissures for caries prevention (Cueto & Buonocore, 1967).

2.2.2 Use of fissure sealant

Fissure sealant serves as a physical barrier that prevents accumulation of microorganisms and food debris in pits and fissures, thus initiation and progression of dental caries do not occur (Azarpazhooh & Main, 2008; Beauchamp et al., 2008; Wright et al., 2016).

The application of resin-based sealants has led to the reduction in caries incidence in children and adolescents at about 86% after one year, 78.6% after two years, and 58.6% after four years of application (Beauchamp et al., 2008). Recent systematic review reported that after two years, the application of sealants in children and adolescents for caries prevention in their primary or permanent molars has led to a 76% reduction in the risk of developing new caries lesions than those who did not receive sealants (Wright et al., 2016).

2.2.3 Types of fissure sealants and the success rates

Sealant materials are classified into resin-based sealant, glass ionomer (GI) cement, polyacid-modified resin sealant, and resin-modified GI sealant (Anusavice et al., 2013). Resin-based sealant consists of urethane dimethacrylate, ―UDMA" or bisphenol A-glycidyl methacrylate (bis-GMA) monomers which are polymerised by either a chemical activator and initiator (autopolymerisation) or photopolymerisation using visible light or a combination of both processes. It comes either as unfilled, colorless, or tinted transparent materials or as filled, opaque, tooth-colored, or white materials (Anusavice et al., 2013). The retention rates for the material which is auto-polymerising resin-based after two years and five years were 84% and 65%, respectively. On the other hand, the material which is light-polymerising resin-based, has retention rates of 77.8%, 80.4% and 83.8%, after two years, three years and five years, respectively (Kühnisch et al., 2012).

Glass ionomer (GI) sealant is a type of material that has been widely recognised and known for its fluoride-releasing property. The acid-base reaction between a fluoroaluminosilicate glass powder and an aqueous-based polyacrylic acid solution resulted in the release of fluoride (Anusavice et al., 2013). The retention rates for GI sealant were reported as 12.3% after two years, 8.8% after three years and 5.2% after five years (Kühnisch et al., 2012).

Resin-modified glass ionomer cement has also been used as sealant materials. Essentially, it is a glass ionomer with resin component that reacts under light polymerisation (Beauchamp et al., 2008). The sealant does not only have fluoride-releasing property, but also has longer working time and less water sensitivity than that of traditional GI sealant (Anusavice et al., 2013). Yengopal and Mickenautsch (2010) found that both RMGIC and resin-based sealants appear to be equally efficient

(Yengopal & Mickenautsch, 2010). Another type of sealant is polyacid-modified resin sealant (also known as compomer). It is a resin-based material with adhesive and fluoride-releasing properties of GI sealant (Anusavice et al., 2013). The data from a meta-analysis showed that the corresponding retention rates of compomers after two years and six years were 52% and 17.9%, respectively (Kühnisch et al., 2012).

2.2.4 Resin-based sealants versus glass ionomer sealants

The most commonly used sealant material is resin-based as it was proven to have a better long term caries-preventive effect owing to its higher retention rate (Kühnisch et al., 2012; Muller-Bolla et al., 2006). Resin-based sealant appears to be more effective than glass ionomer cement at caries reduction, between 24 to 44 months after its placement in permanent teeth of children and adolescents (Beauchamp et al., 2008). In contrast to this view, a systematic review with a meta-analysis reported that both GIC and resin-based sealants exhibited similar significant caries preventive effects and not superior to the other in the prevention of dental caries (Yengopal et al., 2009).

The caries-preventive effect of the resin-based fissure sealant depends on its ability to seal pits and fissures by formation of tags after enamel acid etching which creates micro-retention (Yengopal et al., 2009). However, its application is highly technique-sensitive, in which saliva contamination would lead to reduction of micro-retention and caries-preventive effect (Bishara et al., 2002). On the contrary, glass ionomer cement (GIC) is not as moisture sensitive as hydrophobic resin. Therefore, GIC serves as an alternative in certain condition where the moisture control is difficult, such as when treating an uncooperative child or partially erupted first permanent molars (Yengopal et al., 2009). Furthermore, it has been speculated that the opening of the fissures would remain sealed even if GI sealant is clinically lost (Yengopal et al., 2009).

The presence of the sealant in the fissural opening would block the bacterial access to fermentable substrates and with its fluoride-releasing property, GIC would help to efficiently prevent caries (Oong et al., 2008). On the other hand, resin-based sealant may lose almost all of their protective effect once its retention is lost (Williams et al., 1996).

2.2.5 Factors affecting success of fissure sealant

2.2.5.1 Retention

The success of fissure sealant in preventing caries depends on its ability to retain itself on the occlusal fissure for a long duration of time (Ahovuo-Saloranta et al., 2008; Beauchamp et al., 2008). This is because the main function of sealants is to alter pit and fissure morphology and efficiently seal the susceptible enamel surface from oral environment (Muller-Bolla et al., 2006). The ultimate protection is only assured if all pits and fissures are completely covered with sealants (Williams et al., 1996). For a resin-based sealant, the retention is achieved through micromechanical interlocking between resin and enamel. The resin will penetrate into the porous enamel and subsequently forming tags (Harris & Garcia-Godoy, 2004). It is also proclaimed that longer resin tags lead to lesser microleakage. This will provide a better cariostatic action of the fissure sealant (Prabhakar et al., 2011).

2.2.5.2 Fissure type/morphology

Sutalo et al. (1989) described the variations in fissure type/morphology as type V, U, Y1, and Y2 (Sutalo et al., 1989) (Figure 2.1). Selecman and his co-workers (2007) found that all fissure types significantly affect the penetration of sealant. U-type and V-type fissures exhibit greatest penetration and Y2-type fissure demonstrated the lowest degree of penetration (Selecman et al., 2007). Other study showed better penetration of sealant to the total length of fissures in the shallow fissures than the deep fissures (Duangthip & Lussi, 2003).

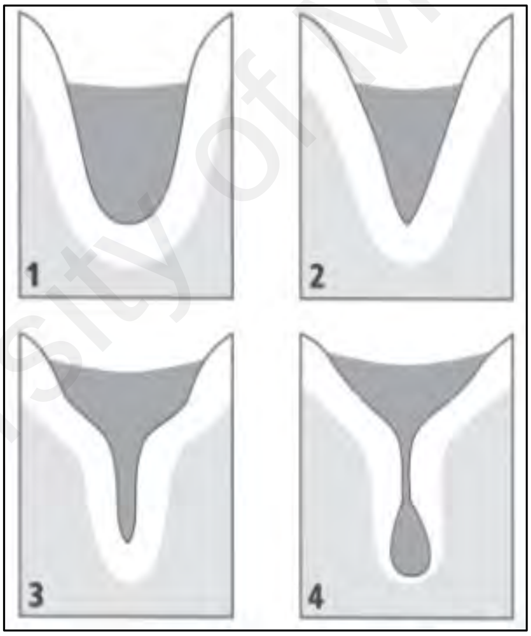


Figure 2.1: Fissure morphology classification: (1) U-type; (2) V-type; (3) Y1- type; (4) Y2-type.

(Adopted from Duangthip and Lussi, 2003)

2.2.5.3 Sealant penetration

The ability of fissure sealant to permeate into narrow and deep part of fissures is one of the most desired properties that would influence its caries preventive effect (Markovic et al., 2011). Variable amounts of organic remnants in deep fissures would limit complete penetration of fissure sealant. As a result, the area available for bonding will be decreased and further affecting the sealant efficiency. In improving the sealant adequacy, many pre-treatment procedures have been described to maximise sealant penetration.

2.2.5.4 Preventing microleakage

Apart from retention, another important requirement of a fissure sealant is its ability to hinder microleakage at the interface, so that the caries process will not be supported and progress underneath the sealant (Agrawal & Shigli, 2012). Numerous studies have been carried out worldwide to assess the interface between restoration and dentin and/or enamel for microleakage (Blackwood et al., 2002; Cooley & McCourt, 1990; Gopinath, 2017; Hernandes et al., 2014; Nandana et al., 2016; Tabari et al., 2016). Microleakage is defined as a clinically undetectable passage of bacteria, fluids, molecules or ions between a cavity wall and the restorative material (Kidd, 1976). It is known to cause secondary caries, marginal staining, postoperative sensitivity, failure of restoration and up to the extent of causing pulpal pathology or pulpal necrosis (Fabianelli et al., 2007; Pioch et al., 2001).

2.2.5.5 Cleaning and preparation of occlusal surface

Narrow morphology of occlusal pits and fissures can be a perfect site for the retention of bacterial and food debris. It has been reported that an average toothbrush bristle of 0.2 mm is too large to penetrate most of the fissures resulting in difficulty of mechanical debridement (Prabhakar et al., 2011). Proper cleaning of fissure before sealant placement is of the utmost importance to ensure occlusal surface of a tooth is free from dental plaque, acquired pellicle, debris and moisture that might interfere with the etching process or sealant penetration (Simonsen, 2002). In addition, sealant penetration may be obstructed by residual material in the fissure, air entrapment, and fissure geometry (Hegde & Coutinho, 2016). To ensure caries-preventive effect of fissure sealant, the tooth surface must be well cleaned, isolated and completely dried during the application process; and received sufficient time of polymerisation. In addition, routine periodic evaluation must be done and sealant must be repaired as required (Waggoner & Siegal, 1996).

There are various ways to prepare a tooth surface prior to etching such as brushing tooth surface with or without toothpaste, polishing using rubber cup with or without prophylaxis paste, polishing using rotary brush with or without pumice slurry, performing fissurotomy or enameloplasty, using air abrasion or air polishing (Prophy-Jet or Cavi-Jet units), applying hydrogen peroxide and to prolong the etching time (Simonsen & Neal, 2011). To date, it is inconclusive whether certain technique of tooth preparation is effective at reducing microleakage and improving long term clinical retention of fissure sealant (Chaturvedy et al., 2013).

2.2.5.5 (a) Handpiece prophylaxis

Prophylaxis and etching are undertaken to remove deposits from tooth surface and to widen enamel micropores in order to receive resin sealant (Simonsen, 2002). Cleaning method using slow-speed handpiece either with non-fluoridated paste, fluoridated paste, pumice slurry or water and bristled brush has been described before (Bogert & Garcia-Godoy, 1992; Garcia-Godoy et al., 1991).

Pumice prophylaxis was introduced by Buonocore to provide a clean enamel surface for etching (Buonocore, 1970). It was reported that pumice prophylaxis improved bond strength for orthodontic brackets on smooth surfaces of premolars in an *in vitro* study (Miura et al., 1973). In comparison to enameloplasty and air abrasion, pumice prophylaxis prior to sealant application led to reduced microleakage (Blackwood et al., 2002).

However, this protocol also has its own shortcomings. Back in 1983, Main et al. (1983) indicated that thorough prophylaxis of the fissures prior to etching may not be necessary as acquired pellicle is completely removed during acid-etching (Main et al., 1983). This is supported by Donnan and Ball (1989) who stated that rotating bristle brush is unlikely to clean pellicle from fissure embrasures because the bristles sweep across the inaccessible regions and polish only the more exposed superficial areas (Donnan & Ball, 1989). Pumicing would only remove organic material on smooth enamel surfaces and is unable to clean the deeper part of the fissures (Chan et al., 1999; Taylor & Gwinnett, 1973). In addition, pumice particles would be lodged in the fissures after prophylaxis and are not removed after rinsing with a stream of water (Garcia-Godoy & Gwinnett, 1987; Garcia-Godoy & Medlock, 1988). The impacted pumice particles in the fissures would become incorporated into the sealant, thus altering its micromechanical bond and causing microleakage (Agrawal & Shigli, 2012). Moreover, dependent on the speed of a handpiece, paste abrasivity, and cleaning duration, rubber cup prophylaxis with pumice paste can remove up to 0.6-4.0 microns of the outer enamel which include the fluoride-rich layer (Biller et al., 1980; Koch et al., 1975; Mellberg, 1979; Stookey, 1978; Vrbic et al., 1967). An *in vitro* investigation demonstrated that there was no significant difference in bond strength when various pastes (non-fluoridated or fluoridated pastes, a pumice slurry or water and bristle brush) used to clean occlusal surfaces were compared (Bogert & Garcia-Godoy, 1992; Garcia-Godoy et al., 1991). In addition, similar retention rates were reported after cleaning the fissures with a rotary brush and pumice or by gently running a probe (Donnan & Ball, 1989).

2.2.5.5 (b) Toothbrushing

According to Gray and co-workers (2009), surface cleaning with a toothbrush is as effective as handpiece prophylaxis at providing sealant retention (Gray et al., 2009). Houpt and Shey (1983) reported that clinical retention rates of fissure sealant placed after fissures cleaned with toothbrush and fluoridated toothpaste at four and six years were 73% and 58%, respectively (Houpt & Shey, 1983). In a study where the tooth surface was cleaned by toothbrushing without toothpaste prior to sealant application, it was found that retention of clinical sealant at 12 months was rather high (99.2%) which was comparable to that observed with rotary instrumentation (97.6%) (Gillcrist et al., 1998). There was no difference in microleakage whether the tooth surface was cleaned with a pointed-bristled toothbrush or pumice prophylaxis (Chan et al., 1999). Pumice prophylaxis removes certain amount of outer enamel which can be considered slightly invasive. It is suggested that the least invasive technique fulfills the goals of the procedure and minimizes the loss of enamel should be selected (AAPD, 2014). For this reason, the use of toothbrushing as an alternative to conventional handpiece prophylaxis is recommended. Other benefit includes lower costs for materials, equipment and personnel (Gray et al., 2009).

2.2.5.5.(c) Air polishing and air abrasion

Air polishing with special device can be used to prepare occlusal surface prior to sealant application (Goldstein & Parkins, 1994; Strand & Raadal, 1988). An air polisher was first introduced to the dental profession in the late 1970s (Gutmann, 1998). The unit includes a light handpiece like an ultrasonic scaler that generates slurry of pressurized air, abrasive powder and water to remove plaque biofilm and stains (Graumann, Sensat, & Stoltenberg, 2013). Air polishing of the occlusal surface before acid etching demonstrated greater penetration in an *in vitro* study (Brocklehurst, Joshi, & Northeast, 1992), produced high number of resin tags for micromechanical retention (Brockmann, Scott, & Eick, 1990), and showed higher bond strengths (Brockmann, Scott, & Eick, 1989) as opposed to that achieved when fissures were cleaned with rotary instrumentation and pumice. In addition, higher mean bond strength was reported for air polishing compared to no cleansing prior to acid etching (De Craene et al., 1989).

However, some contraindications to air polishing have been discussed, which were related to a variety of systemic medical conditions and medications especially in sodium-containing products. Air polishing procedure should not be used in patients with hypertension, renal insufficiency, Addison‘s disease, Cushing‘s disease, sodium–restricted diet, metabolic alkalosis, infectious disease, respiratory illness, or taking medications such as antidiuretics, potassium supplements or mineral corticoid steroids due to the possible absorption of sodium bicarbonate powder through the oral mucosa (Gutmann, 1998).

Another alternative method is air abrasion. It utilizes a high-speed stream of purified aluminium oxide particles conveyed by air pressure. Tooth preparation by air abrasion could improve patient‘s comfort and minimize the need for local anaesthesia (Myers, 1994). Air abrasion creates a roughened enamel surface, therefore, the need for acid etching prior to pit and fissure sealants application may not be relevant (Goldstein & Parkins, 1995; Myers, 1994).

Wright et al. (1999) found that preparing tooth surface with air abrasion yielded less microleakage than conventional acid etch method although it was not statistically significant. However, air abrasion resulted in significantly higher microleakage compared to bur preparation. The tensile bond strength of resin composite to air-abraded enamel with acid etching were significantly greater than that of air-abraded enamel surface without acid etching (Berry & Ward, 1995). This finding is supported by an *in vivo* study that reported no significant difference observed when combining air abrasion pre-treatment followed by acid etching as compared to acid etching only after three and six months follow up (Bhushan & Goswami, 2017).

2.2.5.5 (d) Laser irradiation

Recently, caries removal and cavity preparation by laser irradiation has become more popular (Borsatto et al., 2001). Enamel will absorb the laser energy resulting in superficial modification (Moshonov et al., 2005).

In a study comparing the retention of fissure sealants placed after pre-conditioning with carbon dioxide (CO_2) laser and acid etching, it was found that the retention rate after 14.5 months follow-up for CO_2 laser conditioning was greater (97.9%) than that of acid etching (94.6%), however the difference was not significant

(Walsh, 1996). In contrast, it was reported that preparation of pit and fissure with Er:YAG laser demonstrated inferior marginal sealing than that of acid etching alone (Borsatto et al., 2001).

2.3 Nanoleakage

2.3.1 Definition

Nanoleakage demonstrated that even though the marginal gap between sealant and tooth surface is non-existent, specific substances may be able to penetrate through the hybrid layer (Pashley et al., 1994; Sano et al., 1995). This leakage occurs because of the penetration paths through porosities of 20-100 nm, as compared to microleakage of 10-20 µm (Sano, 2006). This phenomenon was termed as ‗nanoleakage' by Sano and co-writers (1995).

It is believed that uptake of substance such as silver along the interfacial layer and in the adhesive layer is attributable to incomplete resin infiltration and residual water or solvent (Tay et al., 2002), poor polymerisation (Tay et al., 2002), or phase separation (Spencer & Wang, 2002). The presence of residual water within the adhesive may interfere with the polymerization of the adhesive (Tay et al., 2002).

In spite of very small spaces to allow bacterial migration, this type of leakage may allow penetration of bacterial products such as acid and dentinal or oral fluid along the interface (Pioch et al., 2002). The water movement within the dentin-adhesive interface may extract unconverted monomers from adhesive resins which then result in decreasing bond strength (Hashimoto et al., 2004; Tay et al., 2003). In addition, there was a significant inverse relationship between bond strength and nanoleakage (Reis et al., 2007).

Nanoleakage is believed to be uncommonly detected at the resin-enamel interface (Dörfer et al., 2000; Pioch et al., 2001; Sano et al., 1995). This may be due to a strong bond between resin and enamel surface created by resin tag formation following phosphoric acid-etching (Van Meerbeek et al., 2003; Yuan et al., 2007). However, Hariri et al. (2012) demonstrated that nanoleakage could also occur in between resin-enamel interface.

2.3.2 Nanoleakage Evaluation Methods

Nanoleakage can be observed by various qualitative or quantitative observation methods such as light microscopy (LM) (Saboia et al., 2008), scanning electron microscopy (SEM) (Hariri et al., 2012) and transmission electron microscopy (TEM) (Tay et al., 2002), and confocal laser scanning microscopy (CLSM) (T. Pioch et al., 2001).

2.3.3 Silver Nitrate Staining Technique

Owing to its exceptionally small diameter (0.059 nm), silver nitrate is the most popular material used for nanoleakage evaluation (Al-Agha & Alagha, 2015; Hashimoto et al., 2004; Reis et al., 2007; Sano et al., 1995; Tay et al., 2002; Yang et al., 2015). Due to the small size, silver nitrate can easily penetrates the interfacial zone, after which it may be immobilised and hindered from penetrating in much deeper during staining process (Pioch et al., 2001). In addition, silver nitrate generates a strong optical contrast that the degree of penetration can be easily detected and measured microscopically. Nevertheless, some argued that by using an acidic conventional silver nitrate (pH = 3.4), dentin may undergo demineralization. This will result in the formation of artificial paths along the interface and it may interfere with the nanoleakage assessment (Li et al., 2001). In response to this, Tay and co-workers developed a basic ammoniacal version of silver nitrate that yield a 50 weight percent solution (pH = 9.5) (Tay et al., 2002). In this study, the alternate hypothesis that nanoleakage is an artifact caused by mineral dissolution from the conventional acidic silver nitrate was rejected. Nevertheless, the 50 weight percent ammoniacal silver nitrate solution has become the most common method to detect nanoleakage (Al-Agha & Alagha, 2015; Coutinho et al., 2011; Hashimoto et al., 2004; Öznurhan & Ölmez, 2013; Sachdeva et al., 2016; Selvaraj et al., 2016; Yuan et al., 2007). Moreover, this method is able to identify additional pattern (spotted) of nanoleakage expression (Tay et al., 2002).

2.4 Scanning Electron Microscopy (SEM) for Nanoleakage Evaluation

SEM is a well-established technique to examine the interface between dental hard tissues and restorative materials (Hariri et al., 2012; Li et al., 2000; Makishi et al., 2010; Yamazaki et al., 2008). It produces very high-resolution images of a sample surface and reveals details about less than one to five nm in size. In this respect, silver nitrate ($AgNO_3$) was proven to be suitable as the dye for SEM due to it easily migrates within interface zone to induce a measurable electron microscopic contrast (Pioch et al., 2001).

In addition, the silver uptake can also be detected by energy dispersive X-ray (EDX)-microanalysis. Nanoleakage evaluation performed using SEM in conjunction with EDX, enables distinct images to be produced with both sensitive and accurate analysis. Inevitably, EDX analysis can identify the existence of metallic silver particles, as well as quantify the distributions of the elements objectively (Yang et al., 2015; Yuan et al., 2007). Inaccurate interpretations could be prevented (Pioch et al., 2001) and the credibility of nanoleakage results is likely to be increased (Yuan et al., 2007) when SEM is used with EDX-microanalysis.

Though SEM is available in two modes, backscattered electron mode of SEM is believed to be more suitable for nanoleakage observation than the secondary electron mode (Yang et al., 2015). It is because the backscattered mode can display additional information and produce image with better contrast because of its atomic number-dependent element characteristics (Hashimoto et al., 2004; Tay et al., 2000; Yang et al., 2015).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study design

This was a single-centre experimental study conducted at Paediatric Dentistry Specialist Clinic, Faculty of Dentistry, University of Malaya.

3.2 Sample size calculation

Sample size calculation was based on alpha error set at 5% and the power of the study was at 80% (P = 0.8, B = 0.2). This was based on an *in vivo* study which evaluated the method of cleaning and preparation of occlusal fissure surface before placement of sealant in pit and fissure (Hegde & Coutinho, 2016). The sample size was calculated using G Power software 3.0.10. The total number of teeth needed was 33, with a minimum of 11 teeth per group (Table 3.1).

Table 3.1: Sample size calculation

Article	α	Power	σ	No of teeth per group	Total number of teeth
Hegde and Coutinho, 2016	0.05	0.80	0.840	11	33

3.3 Conduct of the study

3.3.1 Pilot study

A pilot study was conducted on six extracted teeth. A few problems were identified, such as difficulty to mount and to section the teeth. These problems were resolved by obtaining assistance from the technical experts in the laboratory and by undergoing adequate training.

3.3.2 Intra-examiner and inter-examiner reliability

Standardization and calibration to determine various fissure type/morphology and depth of sealant penetration were performed between the trainee paediatric dental specialist and a resident paediatric dental specialist (Department of Paediatric Dentistry and Orthodontics, University of Malaya). The calibration was done on 30% of the total sample size. Both clinicians evaluated the samples for fissure type/morphology and scored the depth of sealant penetration on the same day. The scores between the trainee specialist and the resident specialist were compared for inter-examiner reliability. The same samples were evaluated by resident specialist two weeks later and the intra-examiner reliability was calculated. The kappa value for inter-examiner and intra-examiner are displayed in Table 3.2 and Table 3.3 respectively.

Table 3.2: Cohen's kappa values for inter-examiner reliability

	Sealant penetration	Fissure type/morphology
Value of κ	0.795	0.768

Table 3.3: Cohen's kappa values for intra-examiner reliability

	Sealant penetration	Fissure type/morphology
Value of κ	0.813	0.815

The kappa values were interpreted based on Altman (1999) (Table 3.4). The results in Table 3.2 and 3.3 suggested that there was a good inter-examiner agreement between the trainee specialist and the resident specialist. The trainee specialist had a very good intra-examiner agreement. The Cohen's kappa values were all above 7.0 for all measurements.

Table 3.4: Interpretation of kappa values (Altman, 1999)

Value of κ	Strength of agreement
<0.20	Poor
0.21 – 0.40	Fair
0.41 – 0.60	Moderate
0.61 – 0.80	Good
0.81 – 1.00	Very good

3.3.3 Ethical approval

This study was approved by the Ethics Committee, Faculty of Dentistry, University of Malaya (Approval period 5 Dec 2016 – 30 April 2018) Ref No: DF CD1615/0082 (P).

3.3.4 Research funding

This research was funded by the Dental Research Postgraduate Grant (DRPG) (DRPG/23/17).

3.3.5 Teeth collection, eligibility criteria and sampling

A total of 33 maxillary premolar teeth extracted for orthodontic reasons from patients who attended Orthodontic Clinic, Faculty of Dentistry, University of Malaya were collected and stored in distilled water. The teeth were inspected and only sound teeth were included. Carious teeth, restored teeth, cracked teeth, and teeth with developmental anomalies (e.g.: hypomineralization/ hypoplasia/ fluorosis/ dens evaginatus) were excluded. The selected premolars were randomly assigned to three groups (11 teeth in each group) which represented different surface cleaning method (Table 3.5).

Table 3.5: Grouping of different surface cleaning method

Group	Surface Cleaning Method
1	No cleaning of the occlusal surface (control)
2	Occlusal surface was cleaned using toothbrush for 10 seconds (a soft-bristled children toothbrush (Colgate®, Colgate-Palmolive, USA) was used using back and forth scrubbing motion)
3	Occlusal surface was cleaned with a brush in a slow-speed handpiece for 10 seconds

3.3.6 Pit and fissure sealant placement

Following cleaning as described in Table 3.5, the occlusal surface was dried with clean, dry, oil-free air. The occlusal surface was etched with 37% phosphoric acid (Super Etch Low Viscosity (LV), SDI, Australia) for 15 seconds. The surface was washed thoroughly with water and completely dried with clean, dry, oil-free air for 15 seconds until a matt white appearance was evident. Fissure sealant (Conseal f, SDI, Australia) (Figure 3.1) was applied into the fissures using a dispensing tip and polymerised for 20 seconds using a light emitting diode (LED) (Kerr Demi™ Plus) light curing model (921638) with an output intensity of 450 mW/cm^2 with 450 nm wavelength.

Figure 3.1: Fissure sealant (bottom) and etchant (top) used in this study

3.3.7 Sectioning of teeth

The teeth were embedded in acrylic resin up to cemento-enamel junction. They were sectioned longitudinally into halves in a bucco-lingual direction using a slow-speed saw (Micracut 125, Metkon). One-half of each tooth was randomly chosen for sealant penetration, fissure type/morphology, and nanoleakage evaluation.

3.3.8 Staining of teeth

Entire surface of the slab were coated with two layers of nail varnish except for the 1 mm surrounding sealed fissural surfaces to allow contact between the tracing agent and the sealant-enamel interface. The apical region was covered with sticky wax.

Next, ammoniacal silver nitrate (Tay et al., 2002) solution was prepared by dissolving 25 grams of silver nitrate crystals (Sigma-Aldrich®, USA) in 25 mL of distilled water. The black solution was titrated with concentrated (28%) ammonium hydroxide (Sigma-Aldrich®, USA) until it became clear as ammonium ions complexed the silver into diamine silver ions ($[Ag(NH_3)_2]^+$). The solution was diluted with distilled water to 50 mL, to obtain 50% solution (pH = 9.5). The slab was immersed in the ammoniacal silver nitrate solution in total darkness for 24 hours.

Following staining, the slab was thoroughly rinsed under running distilled water for five minutes. The stained slab was placed in a photo-developing solution for eight hours under fluorescent light to reduce the diamine silver ions into metallic silver grains within the voids along the bonded interface. The slab was removed out of the developing solution and placed under running distilled water for five minutes.

The surfaces of the slab were polished using grit SiC paper (400, 600, 800, 1200) and followed with fine diamond pastes at increasing coarseness (6, 3, 1 µm; Buehler, Lake Bluff, IL, USA). The specimens were then cleaned ultrasonically and air-dried before being placed in a desiccator for 24 hours and sputter-coated with gold.

3.3.9 SEM evaluation

The silver-stained specimens were observed under SEM (Quanta™ FEG 250, FEI Company, USA) (Figure 3.2) in a secondary electron mode at accelerating voltage of 10 kV and sealant penetration, fissure type/morphology and nanoleakage were evaluated.

The specimens were first viewed at magnification ranging between x100 and x200. The depth of sealant penetration was evaluated according to scoring described by Kane et al. (2009) (Table 3.6). The fissure type/morphology of the specimens was assessed according to the classification described by Sutalo and co-workers (Sutalo et al., 1989): (1) U-type; (2) V-type; (3) Y1- type; (4) Y2-type (Figure 2.1).

For nanoleakage expression, five fields of view along the sealant-enamel interface were captured and further divided into three regions (upper, middle, lower) (Figure 3.3). Using EDX (Figure 3.4), the amount of silver nitrate within the sealant-enamel interface in each field of view was measured at ×5000 magnification. The silver nitrate uptake was expressed and evaluated as a weight percentage of the total area evaluated.

Figure 3.2: SEM used in this study

Table 3.6: Sealant penetration scores (Kane et al. 2009)

Score	Description
1	Sealant penetrated 1/3 of the total length of the fissure
2	Sealant penetrated 1/2 of the total length of the fissure
3	Sealant penetrated the total length of the fissure

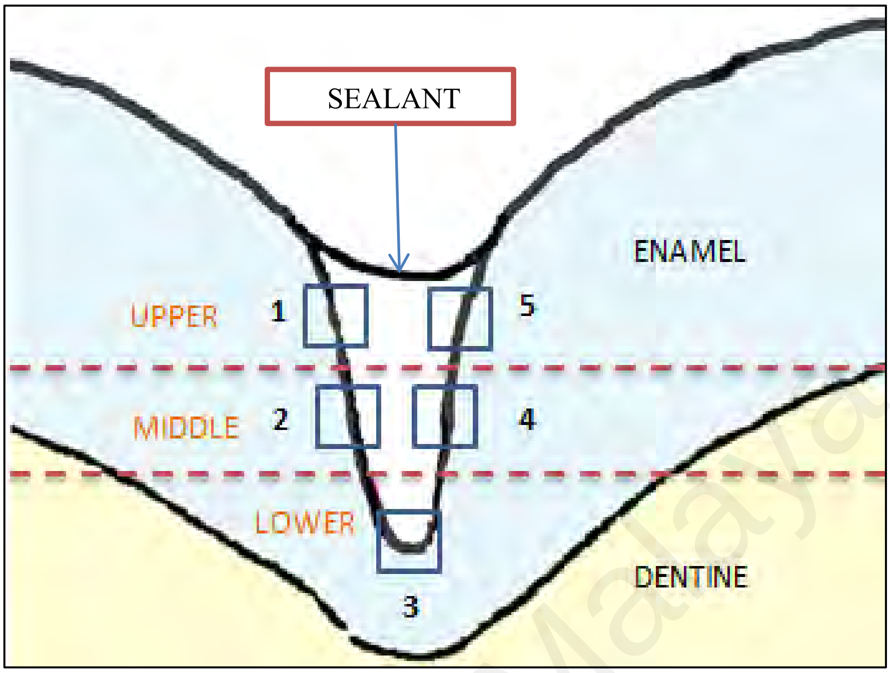


Figure 3.3: Fields of view (1-5) along the sealant-enamel interface (upper, middle, lower) of a specimen.

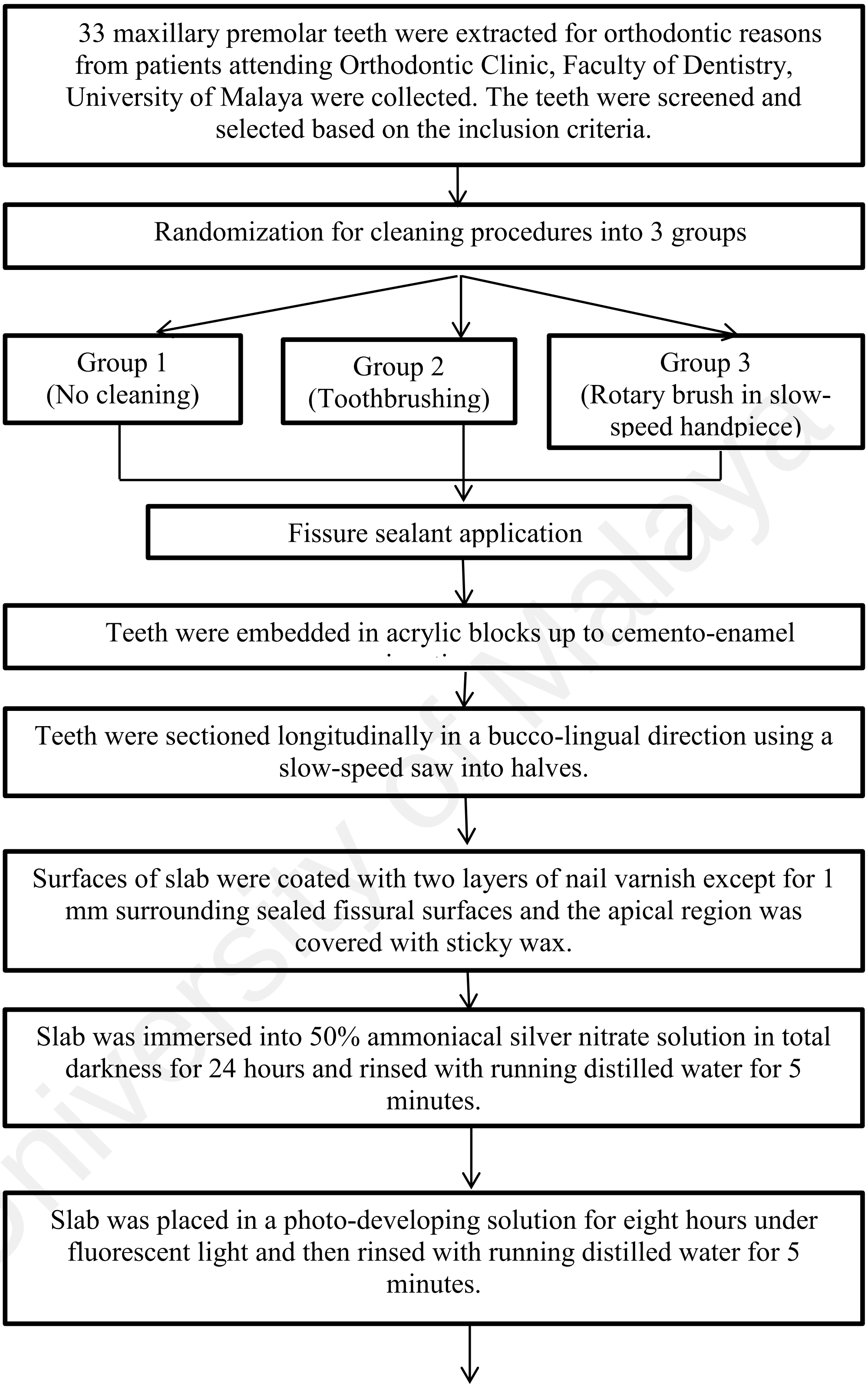
Figure 3.4: EDX

3.4 Data management and analysis

Data management and analysis for fissure type/morphology, depth of sealant penetration, and nanoleakage expression were performed using IBM Statistical Package for Social Sciences (SPSS) Data Editor version 23.0 (SPSS Inc. Chicago, USA). The tests were considered significant when $p<0.05$. The Fisher's exact test was used to determine (1) the association between different cleaning methods and the depth of sealant penetration, and (2) the association between fissure type/morphology and the depth of sealant penetration. The distribution of the data for nanoleakage study was first explored using Kolmogorov-Smirnov test and they exhibited violation of normality. Therefore, Kruskal–Wallis test was used to compare the quantity of silver uptake that implies the nanoleakage expression between the three groups.

3.5 Flowchart of the research

Summary of the events during experimental study is described in a flowchart (Figure 3.5).



(Continue next page)

Figure 3.5: Flowchart of the research

Continued from previous page

↓

The cut surface was polished using grit SiC paper at increasing coarseness and followed with fine diamond pastes.

↓

The specimen was cleaned ultrasonically and air-dried

↓

The specimen was placed in a desiccator for 24 hours and then sputter-coated with gold.

↓

Examination was carried out in a scanning electron microscope (SEM) (Quanta™ FEG 250, FEI Company, USA) using secondary electron images. The depth of sealant penetration was evaluated according to the scoring described by Kane et al. (2009), fissure morphology was assessed using classification described by Sutalo et al. (1989), and the nanoleakage value was evaluated by measuring the amount of silver nitrate uptake expressed as weight percentage at five field of view.

Figure 3.5, continued: Flowchart of research

CHAPTER 4: RESULTS

4.1 Depth of sealant penetration

The SEM images and scoring methods for the evaluation of depth of sealant penetration in each group are shown in Figure 4.1. The total score for each group is presented in Table 4.1 and Figure 4.2. Group 3 (occlusal surface cleaned with a brush attached to a slow-speed handpiece) demonstrated the highest frequency of sealant penetration to the total length of the fissure (score 3), followed by Group 2 (occlusal surface cleaned with a toothbrush) and Group 1 (no cleaning of the occlusal surface). Based on the Fisher‘s exact test, there was no association observed between different cleaning methods and the depth of sealant penetration (*p*=0.615).

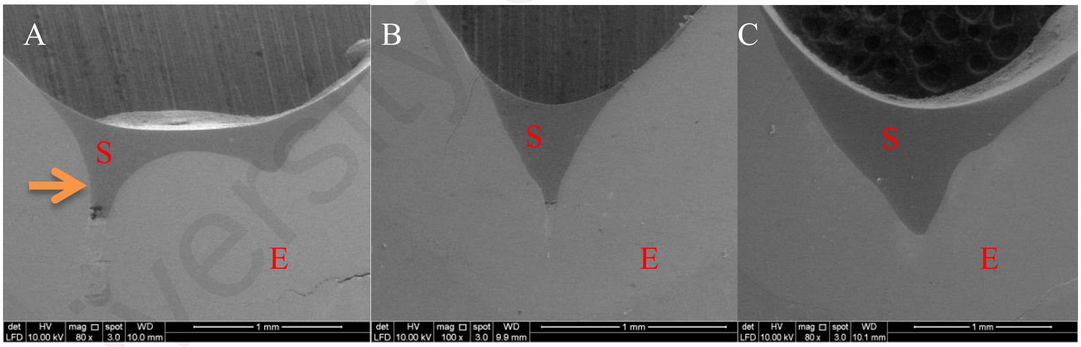


Figure 4.1: Representative SEM images showing the depth of sealant penetration. Score 1 (sealant penetrated 1/3 the the total length of the fissure) (Image A), score 2 (sealant penetrated ½ the total length of the fissure) (Image B) and score 3 (sealant penetrated the total length of the fissure) (Image C). In case there were two fissures in a section, the worst score will be chosen (Orange arrow).

S, fissure sealant; E, enamel.

Table 4.1: Inter-group comparison of depth of sealant penetration

Group	Score			Total	*p*-value
	1 (Sealant penetrated 1/3 of the total length of the fissure)	2 (Sealant penetrated ½ of the total length of the fissure)	3 (Sealant penetrated the total length of the fissure)		
	n	**n**	**n**	**N**	
1= No cleaning of the occlusal surface	2	5	4	11	0.615
2= Occlusal surface cleaned with toothbrush for 10 seconds	0	6	5	11	
3= Occlusal surface cleaned with a brush attached to a slow-speed handpiece for 10 seconds	0	5	6	11	
Total	2	16	15	33	

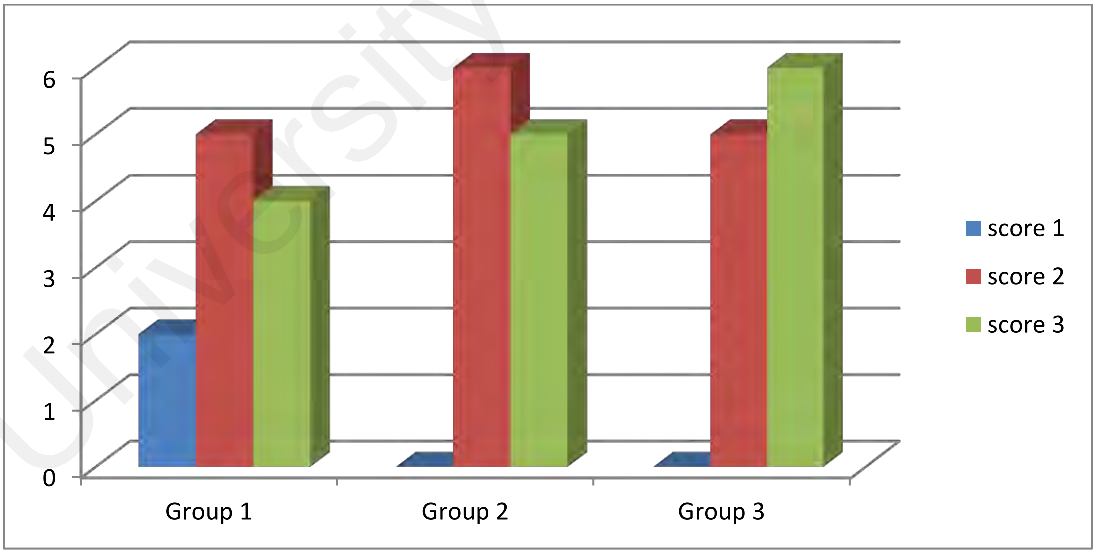


Figure 4.2: Column chart of depth of sealant penetration presented as score 1, 2 and 3.

4.2 Fissure type/morphology

The distribution of fissure type/morphology and its association with the depth of sealant penetration were shown in Table 4.2. Sixteen of the samples exhibited Y1-type fissure whereas another ten, five and two samples showed V-type, U-type and Y2-type fissures, respectively. Based on the Fisher‘s exact test, a significant difference was observed between fissure type/morphology and depth of sealant penetration (p=0.025).

Table 4.2: Association between fissure type/morphology and depth of sealant penetration

Depth of sealant penetration	Fissure Type/Morphology				Total	*p*-value
	U	V	Y1	Y2		
	N	N	N	N	N	
SCORE 1 (Sealant penetrated 1/3 of the total length of the fissure)	0	0	2	0	2	0.025
SCORE 2 (Sealant penetrated 1/2 of the total length of the fissure)	0	4	10	2	16	
SCORE 3 (Sealant penetrated the total length of the fissure)	5	6	4	0	15	
Total	5	10	16	2	33	
Significant: p<0.05						

4.3 Nanoleakage expression

Line-like homogeneous silver deposits were observed in Group 1 and 2 (Figure 4.3)

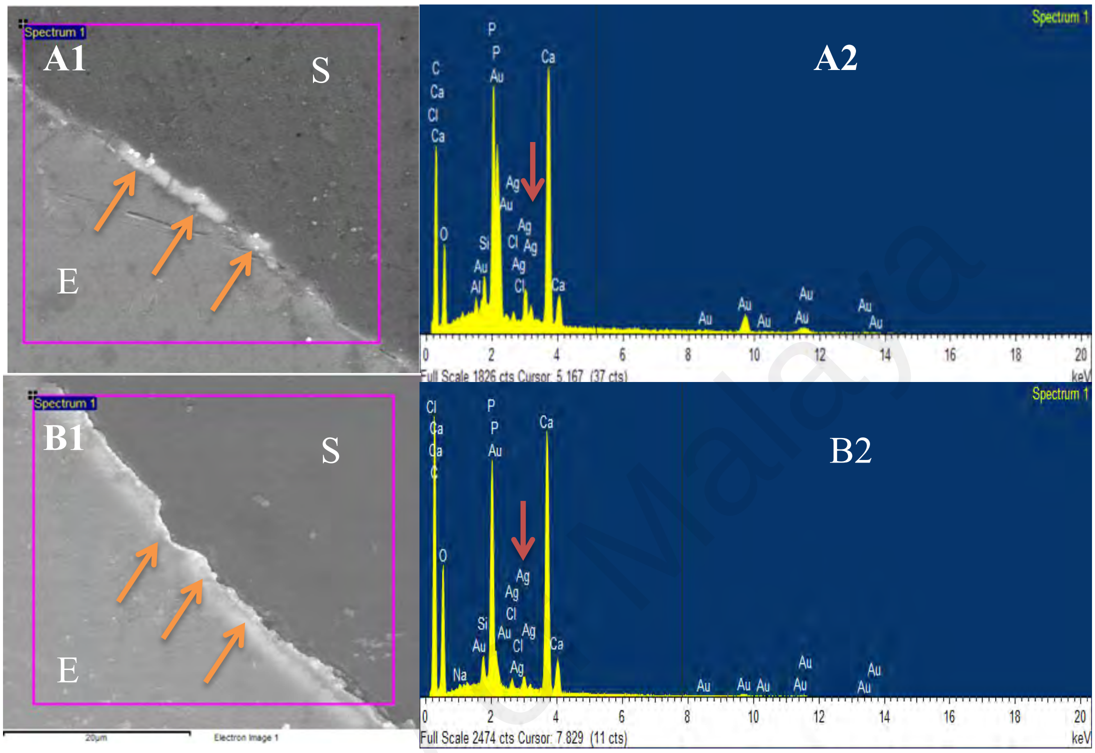


Figure 4.3: SEM images showing the interfacial silver uptake. Images A1 and B1 indicate the silver uptake (orange arrow) in the secondary electron mode of SEM. Distinct line-like homogenous silver deposits were observed through the interface in both images. Images A2 and B2 indicate the amount of several dominant element, especially metallic silver (red arrow) through the elemental energy spectra.

4.3.1 Comparison of the percentage of silver uptake between groups

The percentage silver penetration for all three groups is presented in Table 4.3. Group 1 (no cleaning of the occlusal surface) exhibited higher nanoleakage expression followed by Group 2 (occlusal surface was cleaned with a toothbrush) and Group 3 (occlusal surface was cleaned with a brush attached to a slow-speed handpiece). Overall, there was a significant difference in the percentage of silver uptake between the groups (p=0.027). A pair-wise comparison revealed that the difference was significant between Group 1 and Group 3 (*p*=0.026) (Table 4.4). In relation to the occurence of nanoleakage, rotary-brushed and toothbrushed occlusal surfaces exhibited no difference. Similar finding was observed between toothbrushed and non-cleaned occlusal surfaces (*p*>0.05).

Table 4.3: Percentage of silver uptake between groups

Group	N	Median	IQR	Mean rank	*p*-value
1	55	0.00	0.55	91.43	**0.027**
2	55	0.00	0.00	81.05	
3	55	0.00	0.00	76.52	
Significant (*p*<0.05), non significant (*p*>0.05), IQR: interquartile range					

Table 4.4: Pair-wise comparison of the percentage of silver uptake between groups

Group Comparison	***p*-value**
Group 1 and Group 2	0.204
Group 1 and Group 3*	**0.026**
Group 2 and Group 3	1.00
**p*-value < 0.05	

4.3.2 Comparison of percentage of silver uptake between regions

The percentage of silver penetration concerning different regions is presented in Table 4.5. The lower region showed a trend towards higher nanoleakage expression as compared to upper and middle regions, however, the difference was not significant (*p*=0.986).

Table 4.5: Percentage of silver uptake in different regions

Region	N	Median	IQR	Mean rank	*p*-value
Upper	66	0.00	0.00	82.55	0.986
Middle	66	0.00	0.00	83.19	
Lower	33	0.00	0.00	83.52	
Significant ($p<0.05$), non-significant ($p>0.05$), IQR: interquartile range					

4.4 Effects of different cleaning methods on sealant penetration and nanoleakage

The null hypothesis is rejected. The results suggested that neither toothbrushing nor rotary brushing cleaning methods demonstrated increased effect on the depth of sealant penetration and nanoleakage expressions of fissure sealant.

CHAPTER 5: DISCUSSION

5.1 Discussion

The average fissure depth of human premolars varies from 120 to 1,050 μm (Fejerskov et al., 1973). Due to the huge variations in fissure depth, sealant penetrability is unpredictable. It is widely accepted that the success of fissure sealant depends on its ability to prevent caries on the sealant-enamel interface (Ahovuo-Saloranta et al., 2013; Beauchamp et al., 2008). For resin-based sealants, the caries-preventive effect is assured when sealant adequately penetrates through the depth of the fissure and able to remain there for a long time (Al-Jobair, 2013). In this study, the depth of sealant penetration was evaluated using SEM after fissures were cleaned with different methods prior to sealant placement. The use of SEM enables direct visual observation of the penetration of sealant materials into enamel walls, as it produces very high-resolution images of a sample surface.

Findings in this study indicated that there is no difference in the depth of sealant penetration in either group. However, Group 1 with no cleaning of occlusal surface exhibit poorer penetration as compared to toothbrushing and rotary-brushing group. It is crucial to ensure deeper sealant penetration because it provides retention within the fissure in case it becomes worn or fractured at the cuspal inclines. By occluding the deeper fissure surface, fissure sealant can still maintain its caries-preventive effect (Brocklehurst et al., 1992). Additionally, toothbrushing and handpiece prophylaxis exhibit similar sealant penetration which was in accordance with previous study which found that dry toothbrushing procedure prior to sealant placement yielded higher (99.2%) clinical sealant retention compared to rotary brushing (97.6%) (Gillcrist et al., 1998).

The fissure type/morphology and its content affect the successful placement and retention of a sealant. In this study, fissure type/morphology exhibited a significant effect on sealant penetrability which is in agreement with previous studies (Duangthip & Lussi, 2003; Selecman et al., 2007). The V-type fissures demonstrated the greatest penetrability and Y2-type fissures exhibited inferior penetrability as compared to others. Results from the present study support the evidence reported by previous studies where they found that greatest sealant penetration to the base of the fissure is observed in the shallow fissures as compared to the deep fissures (Duangthip & Lussi, 2003; Marks et al., 2009). In addition, the present study found that there was no complete penetration of sealant into Y2-type fissures. This may be affected by the flowability of the sealant material which limited by the fissure constriction in a deeper and complex type of fissure such as Y1 and Y2. Cleaning and etching procedures are not able to reach beyond the region of fissure constriction and thus affecting the sealant flow (Garcia-Godoy & Gwinnett, 1987; Taylor & Gwinnett, 1973). Although we found that there was no significant difference in the depth of sealant penetration between different cleaning methods, it is important to consider the influence of fissure type/morphology in relation to the sealant penetrability.

The limitation of sealant penetration by fissure type/morphology has been shown by other studies (Garcia-Godoy & de Araujo, 1994; Hatirli et al., 2018). The pits and fissures constriction area may need to be widened using a bur that can increase the surface area for sealant adhesion (Garcia-Godoy & de Araujo, 1994; Hatirli et al., 2018). This method will also increase sealant adaptation and allow deeper penetration as a result of better elimination of organic material (Chaitra et al., 2011; Garcia-Godoy & de Araujo, 1994). However, this technique denies the principle of minimally invasive dentistry and should be restricted to deep and narrow discoloured fissures, suspected of being carious (Garcia-Godoy & de Araujo, 1994).

Another important criterion that should be looked into in determining the success of fissure sealant is the presence of microleakage and nanoleakage. Potential noxious effects of microleakage and nanoleakage could not be taken lightly because they may result in infiltration of bacteria and their by-products at the interface leading to development of secondary caries (Marks et al., 2009; Pioch et al., 2002). This study investigated the interfacial nanoleakage expression of the fissure sealant after the occlusal surface was cleaned with different cleaning methods. Evaluation of nanoleakage revealed that the line-like homogeneous silver deposits were observed in Group 1 and 2 (Figure 4.3). The findings of line-type silver deposits are in conjunction with that reported by previous studies (Pioch et al., 2001; Yang et al., 2015). However, the spotted pattern (Tay et al., 2002) and the reticular pattern (Tay et al., 2003) observed in other studies were not detected in this study. This may be due to the conventional nanoleakage evaluation such as SEM/TEM is heavily influenced by direction, position, and inclination of field of view (Coutinho et al 2011).

There was no difference in nanoleakage expression of the applied fissure sealant either after the enamel surface was cleaned with toothbrush or a rotary brush. This result indicates that the efficiency of toothbrush (manual) and rotary brush (power-driven) as a cleaning tool is comparable. However, the control group exhibited higher nanoleakage expression, probably because the plaque, acquired pellicle, debris and moisture were not sufficiently removed by means of acid etching. The presence of organic materials might interfere with etching process and subsequently result in incomplete resin infiltration (Simonsen, 2002). This may explain why there was a significant difference in nanoleakage expression between the control group and the rotary brush-cleaning group. This finding is also in agreement with Arhakis and co-workers (2007) who reported that all heavy stains, deposits, and debris must be eliminated from the occlusal surface prior to sealant placement (Arhakis et al., 2007). The findings, however, did not reach statistical significance between the control group and the toothbrush group, most likely due to too little samples.

Examination of the interface of different regions demonstrated no difference in nanoleakage expession of the fissure sealant. Nevertheless, the lower region expressed higher nanoleakage as compared to the upper and middle regions. The most likely explanation for this is insufficient drying at the deepest area which could have resulted in residual water entrapment in the region. Residual water may lead to incomplete polymerisation of fissure sealant which could cause nanoleakage.

Findings in this study also proved that although to a lesser extent, nanoleakage could occur at sealant-enamel interface. This result supports the study done by Hariri and co-workers (2012) who demonstrated that nanoleakage could occur in between resin-enamel interface (Hariri et al., 2012). In contrast, other findings reported that nanoleakage was not a common occurrence at the resin-enamel interface (Dörfer et al., 2000; Pioch et al., 2001; Sano et al., 1995). This phenomenon may be due to incomplete resin infiltration at the base of phosphoric-acid-etched enamel (Hariri et al., 2012) or incomplete polymerisation as a result of presence of residual water.

Several visual methods to evaluate the extent of nanoleakage qualitatively and quantitatively have been described before (Hariri et al., 2012; Makishi et al., 2010; Saboia et al., 2008; Tay et al., 2003). EDX-microanalysis seems to be more credible than the scoring methods (Yuan et al., 2007). With EDX-microanalysis inaccurate interpretations could be prevented (Pioch et al., 2001) as it enables distinct images to be produced with both sensitive and accurate analysis. Backscattered electron mode of SEM is believed to be more suitable for nanoleakage observation than that of the secondary electron mode (Yang et al., 2015). It is because the backscattered mode can display additional information and produce a better image contrast because of its atomic number-dependent element characteristics (Hashimoto et al., 2004; Tay et al., 2000; Yang et al., 2015). This study used secondary electron mode for nanoleakage observation as backscattered mode was not readily available at our centre. Though the backscattered mode may produce a better image contrast, images produced by the secondary mode were still acceptable and able to be analysed by EDX. However, SEM is not without a disadvantage. SEM evaluation may cause specimens to dry-out and resulted in cracks (Yuan et al., 2007). Care should be taken not to use very thin section for the evaluation because the specimens might end up being fractured during processing.

Handpiece prophylaxis is the established method in removal of dental plaque and stain on tooth surfaces since decades ago. However, taking into consideration the deep and narrow nature of fissure morphology, the use of rotary brush with pumice or prophylaxis paste prior to fissure sealant placement would be more detrimental as the materials can become impacted in the fissures and affecting the sealant penetration and subsequently the success of the sealant. The ability of the rotary bristles or rubber cup to penetrate the length of the fissure was also limited (Chan et al., 1999; Taylor & Gwinnett, 1973). Furthermore, a rotary brush would only brush across and polish the outer surface areas (Donnan & Ball, 1989).

In addition, due to the abrasive nature of the procedure, it is contraindicated to use a handpiece prophylaxis on hypoplastic and hypomineralised enamel (Sawai et al., 2015). The abrasive effect exerted by handpiece prophylaxis on the demineralised enamel which is already weak and less resistant to mechanical actions has been described before (Honorio et al. 2006). The demineralised enamel of bovine origin exhibited more wear than the sound bovine enamel when subjected to pumice prophylaxis in an *in vitro* study (Honorio et al., 2006). Even for tooth prophylaxis purpose, dentists are recommended to do selective prophylaxis (AAPD, 2011) because the procedure would remove the outer layer of tooth enamel, following which the fluoride-rich layer would take three months to reform (Sawai et al., 2015). Thus, it was advocated that without stain or calculus, a manual toothbrush alone is sufficient to achieve the aim of prophylaxis (AAPD, 2011).

At our centre, patients are normally asked to bring their own toothbrushes at every visit. They would be asked to brush their teeth themselves under dentist supervision. This is to promote positive behaviour towards good oral hygiene practice and to make sure that they learn the right technique. Taking advantage of this situation, occlusal fissures would be clean and no prophylaxis by means of rotary instruments should be required. This method would be suitable for anxious and uncooperative patients who refuse to have a handpiece in their mouth. In addition, cost of treatment can be reduced without the need to use materials such as pumice or prophylaxis paste. Toothbrush may serve as a reasonable alternative to a rotary brush especially in school-based fissure sealant programme in remote area where handpiece is not easily available.

5.2 Limitations of the study

Due to the time restriction and minimal financial support, the present study could only include 33 teeth resulting in a small sample size. The sample preparation and SEM evaluation are costly. Thus, it is believed that if more time and budget were allocated for this study, a larger sample size with a more representative result could be generated.

The present study is also limited by the unavailability of backscattered electron mode of SEM. Backscattered electron mode of SEM is more suitable for nanoleakage observation because it displays additional information and produces better image contrast due to its atomic number-dependent element characteristics (Hashimoto et al., 2004; Tay et al., 2000; Yang et al., 2015).

This study focused on sealant penetration depth and nanoleakage expression when occlusal surfaces were cleaned with a toothbrush in comparison to a rotary brush. However, time and budget restriction only allowed a small number of teeth collected; therefore, the teeth with different cleaning methods were not grouped into a different fissure type. The best results could be retrieved by dividing the samples into different fissure types. Fissure type is important to minimise bias in assessing the depth of sealant penetration and to determine the point of penetration measurement.

CHAPTER 6: CONCLUSIONS

6.1 Conclusions

Within the limit of this study:

1. Under the SEM evaluation, both toothbrush and rotary brush cleaning method had a similar effect on sealant penetration (p=0.615).
2. However, the depth of sealant penetration is influenced by the fissure type/morphology. Sealant penetrated better in shallower fissure type/morphology as compared to deeper fissure type/morphology.
3. Occlusal surface cleaned with a toothbrush prior to sealant placement is as effective as the occlusal surface cleaned with a rotary brush in relation to nanoleakage expression (p=1.00).

6.2 Recommendations

The recommendations proposed are:-

1. To obtain a more representative and accurate results, it is beneficial to conduct a similar study with a larger sample size.
2. Future study also should consider to evaluate sealant penetration depth and nanoleakage within similar fissure type groups
3. Apart from nanoleakage and sealant penetration, the effects of different cleaning methods prior to sealant placement on shear bond strength of a fissure sealant should also be evaluated.
4. Toothbrushing should be recommended as one of the method to clean occlusal surface before fissure sealant application.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

1) **Title: Comparative Evaluation Of The Effect Of Different Cleaning Methods On Sealant Penetration**

- Presented at the 17th Annual Scientific Meeting & 19th Annual General Meeting of International Association for Dental Research (IADR) Malaysian Section on 24th February 2018 (Appendix B).

2) **Title: Comparative Evaluation Of The Effect Of Different Cleaning Methods On Sealant Penetration**

- Presented at the 11th Postgraduate Conference 2018, Faculty of Dentistry, University of Malaya on 24.7.2018 (Appendix C)