5. Discussion

5.1 Methodology

5.1.1 Tooth collection

In this study, sound premolar teeth that were extracted for orthodontic reason were collected and disinfected using 0.5% Chloramine Trihydrate solution for one week as recommended by ISO (ISO/TS 11405:2003).It has bactericidal and bacteriostatic effects and is recommended that the teeth to be stored in this solution for a maximum for one week, followed by their storage in distilled water in refrigerator (4°C) or freezed below -5°C. In this study all the collected teeth were stored in refrigerator (4°C).Distilled water was changed regularly every week in order to prevent deterioration. There are various types of storage medium for extracted teeth that had been used in other studies. These included formalin, thymol, sodium azide, saline and water (Hilton, 2002).

5.1.2 Tooth selection

The teeth were examined under a stereoscopic microscope (MEIJI) at x 10 magnification in order to eliminate teeth that displayed cervical caries, radicular cracks or craze lines from the study. This is important in order to ensure that the penetration of dye occurred is due to microleakage and not because of the damage to the tooth structure.

5.1.3 Cavity preparation

In this study, Class V cavity design was chosen to minimize the variable on the occlusal surface due to present of pits and fissures. The absence of sclerotic dentine on the buccal surface could reduce the variability while conditioning the tooth before placement of composite material in this study (Gonzalez, 1992). Class V restorations are more exposed to oral fluid, food and drinks that we take and more relevant to the objectives of the study. This type of cavity preparation was also commonly used by other researchers in their microleakage studies of dental restorations materials (Crim & Mattingly 1981; Tjan & Tan, 1991; Brackett et al, 1998; Chimello et al, 2002). Other than Class V restoration, Class II cavity has been seen in previous studies. In 2004, Aranha and Pimenta used bovine tooth to prepare Class II on the mesial and distal surfaces, whereas Arias et al (2004) prepared the same cavity design on the human molar.

In this study, cavity was prepared 2 mm above the cementoenamel junction to ensure all cavities were prepared only on the enamel. Cavities were bevelled in order to increase bonded area thus reduce microleakage (Saunders et al, 1990).

5.1.4 Etching and bonding system

In this study Scotchbond™ etching gel that contained 35-45% phosphoric acid was used to etch the cavity preparation. This procedure was followed by application of Adper™ Single Bond 2 which is a combination of primer and bonding. Tenniswood et al (2006) in their mocroleakage study found that the total etch system were performed best in areas of enamel closer to the occlusal surface where as the self-etch systems were best in enamel close to the CEJ and on dentine.

5.1.5 Restorative material

3M Filtek ™ Z 250 Universal Restorative Paste was selected and used in this study because the material can be used for both anterior and posterior restorations. It provides acceptable aesthetic, can be used with glass ionomer cement in sandwich technique, core and cusp build up and splinting purposes.

Z250 is a microhybrid composite which contains a blend of UDMA (urethane dimethacrylate) and Bis-EMA (bisphenol A polyethylene glycol diether dimethacrylate) in place of TEGDMA (triethyleneglycol dimethacrylate). This resin blend has a higher molecular weight that can reduce the polymerization shrinkage of Z250 as well as reduce the aging effects and increase the fracture toughness. Z250 is also purported to be quite hydrophobic and, therefore, less sensitive to atmospheric moisture. Z250 is filled to 60 percent by volume with zirconia/silica particles having a size range of 0.01 to 3.5 microns and an average size of 0.6 micron. These types of fillers were easier to light cure and provide excellent polishing surface.

5.1.6 Dye penetration study

Dye penetration is the most popular and the oldest technique used to assess microlekage of dental restorations. Some of the dyes used include basic fuchsin, methylene blue, eosin, aniline blue, crystal violet and erytrosin (Shortall, 1982). Methylene blue is a suitable tracer for microleakage study because it has inherent properties, which include solubility in water, low molecular weight, basic in reaction, no colour changes during the test and lack of high transmission of incident light (Gonzales, 1992).It has no tendency for bonding to tooth structure or restorative materials, unlike basic fuschsin. Methylene blue can be detected on the margin easily due to its colour contrast from the tooth structure, and it gives same evidence on enamel or dentine. Although the different colour gave advantages, it created problem due to staining of the equipment used. Other disadvantages are the subjective evaluation of the result (Alani & Toh, 1997), the low molecular weight of the dye which is smaller than that of bacteria, and poor standardization of the method. Test using dyes could detect leakage where bacteria could penetrate. However, dye can be used as a stringent test of marginal microleakage (Arcoria et al, 1990).

In a review of literature by Alani and Toh, 1997, they mentioned that the concentration of methylene blue used in microleakage studies usually ranged from 0.5% to 10%. In this present study, 2% methylene blue was used, as it has been found suitable for the purpose of determining microleakage of the Class V composite restoration. It also provides a simple, relatively cheap, qualitative and comparable method in assessing marginal microleakage.

The disadvantages of the dye penetration technique are it required sectioning of the specimen and evaluation is done in two dimensions. If the leakage has penetrated deeply into a region that are not exposed by the section, then the leakage results would be misleadingly low. Several studies found increased in microleakage when the number of slices increased. Gwinnett et al (1995) used a serial grinding method that exposed the surface to be evaluated in 80 micrometer increments. They found that the median leakage detected was twice than when slice through the midline of a buccal Class V restoration's. Williams et al (2002) compared the methods of measuring dye penetration in restoration microleakage studies concluded that analyzing the whole wall method was superior to the slice method and the used of two slices rather than one did not statistically improve the ability to detect microleakage.

Youngson in 1992 studied microleakage three-dimensionally using serial sections of a restored tooth, allowing the measurement of areas of microleakage and volumes of dye penetration. He used transparencies that were projected onto perspex sheet and scribed the outline of the tooth with the area of dye penetration. The area of dye leakage was filled with self adhesive acrylic film and a scale model of each tooth was then constructed. The models were photographed using transmitted light. He analyzed the transparencies with image analysis apparatus. He found that the pattern of dye penetration into the dentine was extremely complex, and the pattern varies as the midline of the tooth approached. However the dye tends to follow the general pathway of the dentinal tubules towards the pulp.

In 1999, Youngson et al, continued to assess microleakage but using different method. They studied microleakage of three bonding systems using a fluid filtration and clearing technique followed by perfusion with silver nitrate and scoring penetration. They found that there was no statistically significant correlation between the fluid filtration measurements and silver nitrate penetration measured by scoring system. However fluid filtration was more technique sensitive.

Even though many papers documented the inherent inaccuracy of the slice method, studies continue to report leakage based on this method (Abdalla & Davidson, 2000; Santini et al, 2000; Kuramoto et al, 2000; Ferrari et al, 2000).

5.1.7 Test condition

When conducting an *in-vitro* study, specimens are preferably exposed to conditions similar to the oral environment (Palmer et al,1992).This is especially so for the studies involving the evaluation of sealing properties of composite material, where the coefficient of thermal expansion is significantly different from the tooth structure (Nelson et al,1952). Teeth that were restored with composite material, and thermocycled immediately, showed a higher dye penetration as compared to those that were stored in water before cycling. This finding was attributed to the water sorption potential of composite resins. It was thus recommended that microleakage test on composites restoration should be carried out only after 24 hours of specimens storage to permit water sorption of resin to occur first (Alani & Toh,1997).In this present study, the specimens were kept in distilled water for one week at 37 ^{o}C before thermocycling process was carried out.

The temperatures used for *in-vitro* thermocycling have ranged from 0^{o}C to 68^{o}C (Shortall, 1982).Harper et al (1980) suggested that the temperature variation in the mouth was quite small. In this present study temperatures used for the cold and hot bath were 5 ^{o}C and 55 ^{o}C respectively. These temperatures were selected as they have been used by many researchers in their studies (Grieve et al, 1993). In this study, 500 cycles was used as recommended by the ISO/TR11405:1994(E).The numbers of temperature cycles employed has ranged from 1 to 2500. It was reported that microleakage increased with an increased number of cycles when resin restorative materials were used (Peterson et al, 1966).However Mandras et al (1991) have shown that the differences in microleakage of composites thermocycled at 250 and 1000 cycles were not significant. Youngson et al (1999) in his composite study concluded that thermocycling of the restoration with clinically realistic temperatures and durations had no statistically significant effect on the measured microleakage.

The time used for the alternate immersion of specimen in hot and cold solution has ranged between 10 seconds to 120 seconds (Alani & Toh, 1997).Rossomando and Wendt (1995) found that the extent of the leakage increased with the increase in dwell time for amalgam restorations, although there were no significant differences in dye penetration for thermocycled composite restorations. In the present study, 15 seconds immersion time in hot and cold bath with 5 seconds turn over were chosen because cycling regimes using a short dwell time is more realistic clinically (Causton et al,1984).

5.1.8 Different pH immersion groups

When conducting an *in-vitro* study, specimens are preferably exposed to conditions similar to the oral environment (Palmer et al, 1992**).** The pH of oral saliva ranged at 5.2-6.2 in unstimulated (resting) condition and 6.5-8.0 in stimulated condition (Jenkins, 1978). The acidity or alkalinity of fluid in the oral cavity as measured by pH varies from around pH 4 to pH 8.5, whilst the intake of acid fruit juices or alkaline medicament can extend this range from pH 2 to pH 11 (Mc Cabe & Walls, 1998).Based on these situations, specimens in the present study were grouped into pH range 2.5 to 11.5. pH 7.0 was chosen as the controlled group because of its natural condition, and usually used as medium in dental materials studies. Most of soft drinks and fruit juices have pH ranged between 2.5 to 4.5.For example Cola and orange drinks have pH 2.5 and 2.9 (Stephan,1966), Pepsi, Pepsi T and 100 Plus have pH 2.95, 3.30 and 3.74 (Balasingam et al, 2003).Orange slices, lemon slices, apple and grapes have pH 3.4, 2.5, 4.3 and 3.4. pH 5.5 is known as critical pH where demineralization of enamel occurred in the mouth (Stephan,1966).

Most of the microleakage studies, did not relate the effect of pH and microleakage. Studies had been done in relation of pH to hardness and surface changes. Arias et al (2004) used 2% methylene blue at pH 7.0 with 4 hours immersion time in their microleakage study of three adhesive systems. Tahir et al (2005) in their study of effects of pH on the microhardness of resin-based restorative materials found that, the highest microhardness value was observed when the materials were conditioned for one week in a medium with neutral pH 7.0 (controlled group), and all the materials were degraded after conditioning in acids. In their study, they used five difference pH, which were pH 3, 4, 5, 6 and 7. The critical pH at which degradation occurs varies. The composite (Esthet X,Dentsply-De Trey, Konstanz) showed a high percentage reduction of microhardness in acids at pH 3, whereas compomer (Dyract Extra, Dentsply-De Trey, Konstanz) and giomer (Beautiful, Dentsply-De Trey, Konstanz) exhibited marked degradation at pH 5.

Citric acid was chosen in this present study because it is one of the most common acids found in fruit juices and drinks and is often added as a preservative to drinks and food stuff. Citric acid is also commonly used *in vitro* studies (Barbour et al 2003; Eisenburger et al, 2003).

5.1.9 pH and time of immersion

In present study, 10 minutes immersion time in respective pH group was chosen because it is a very realistic time which provides interaction to occur between restoration material, tooth structure and pH medium. The clearance of diet from the oral cavity and buffering capacity of the saliva is different among individual. Bashir et al (1995) in their research concluded that the clearance of citric acid from saliva rinse was very rapid during the first minute, eliminating more than 90% of the ingested acid. However they found, that the clearance pattern was a highly individual property that was not significantly dependent on the unstimulated salivary flow rate or the residual volume of saliva after swallowing. Several researchers found that pH in the oral cavity can be buffered within less than 10 minutes in most people. According to Stephan (1944), in our saliva, pH of the plaque would reduced after taking carbohydrate or sugary food, but started to increase after 10 minutes due to buffering effect and it will return to the normal value after 40 minutes (Figure 2.1). Millward et al (1997) reported that the pH fell rapidly within 1st minute after ingestion of the citric acid solution, but then recovered quite rapidly, reaching pH 5.5 in less than 5 minutes. In 2000, Moazzez et al, studied the oral pH and drinking habit during ingestion of a carbonated drink found that buffering capacity of the saliva for erosion group and control group ranged between 4-6.5 minutes.

Changes of the restorative materials after immersion into the solution also can be seen in less than 10 minutes. Maupome et al (1998) in their study of enamel microhardness after exposure to eroding immersion in a cola drink found that the greatest erosive effect occurred right after the first immersion which was five minutes. Balasingam et al (2003) found that enamel microhardness was most reduced in Pepsi (pH 2.95), followed by Pepsi T (pH 3.30) and 100 Plus (pH 3.74) from baseline up to 8 minutes. At the end of 10 minutes, Pepsi still showed the lowest enamel microhardness.

5.1.10 Evaluation of microlekage

The evaluation criteria used in this study are commonly used by researchers in microleakage studies of dental restorative materials (Going, 1972; Tjan & Tan, 1991) where score 0 indicated no leakage, and score 1, 2, 3 indicated the different extent of microleakage. This scoring system allowed the evaluation of microleakage to be done at various levels and severity, and not only present or absence of the leakage. This is different to some studies that used ‘ 0, 1, 2, 3, 4’ and ‘1, 2, 3, 4’ which score 0 or 1 represent no leakage followed by different levels of dye penetration within the restoration walls ,and highest score for the dye that penetrate beyond the axial wall (McComb et al,1990; Aranha & Pimenta,2004). Another scoring system were described by Glyn Jones et al (1979).They used seven levels, four levels for penetration within the cavity wall and three levels for penetration beyond the axial wall. It was complex and confusing because evaluation on occlusal and cervical was not separated.

5.1.11 SEM evaluation

The main advantage of using SEM method to evaluate microleakage is that it provides direct visual observation of the adaptation of restorative materials to the cavity margins (Boyde & Knight, 1969), but the disadvantages are the potential to introduce error and artifacts related to drying, cracking and sectioning (Kidd, 1976a).However many researches continued to use this method (Soares et al, 2005, Davila et al, 1986, 1988; Van Dijken & Horsted, 1989). In this study the main objective of using SEM was to compare and analyze the severity of microleakage that scored 3 in the most acidic, neutral and the most alkaline solution.

5.2 Results

One main factor that caused microleakage at the interface of tooth and material was dimensional changes. The dimensional changes in materials may be due to polymerization shrinkage, thermal contraction, water absorption and the mechanical stress produced (Staninec et al, 1986).

In this study, all groups including the controlled group showed leakage at occlusal and cervical margin. In all groups leakage occurred more at the cervical margin. Out of 70 specimens that were studied, cervical leakage occurred in 50 (71.4%) specimens, whereas at the occlusal margin only 40 (57.2%) specimens exhibited leakage. This finding concurred with a lot of studies of composite microleakage (Charlton & Moore,1992).It has been suggested that the difference in microleakage pattern at the occlusal and cervical margins of composite restoration was possibly due to different coefficients of thermal expansion for enamel, dentine and composite. The coefficient of thermal expansion of the tooth differs widely from the composite (Jensen & Chan, 1985). Different enamel thickness may also be a contributing factor to the differences seen in microleakage pattern occlusally and cervically. A scanning electron microscopic study by Gwinnett (1967) revealed that the cervical enamel of human teeth possesses an irregular prismatic pattern.

Specimens that were immersed in pH 2.5 exhibited more leakage if compared to the other pH groups. 80% of it showed leakage at the occlusal margin with 60% were score 3. Whereas at the cervical margin, 90% of the specimens showed leakage with 60% were also score 3. This situation occurred because interaction of the pH medium with composite restoration and enamel structure.

The influences of low pH on composite properties may be explained by the hydrolysis of ester groups present in the resin matrix. In particular, the hydrolysis of ester bonds leads to the formation of free carboxylic acid groups that could lower the pH inside the polymeric matrix. According to Gopferich (1996) pH affects reaction rates through catalysis. Asmussen (1984) and Chadwick et al (1990) demonstrated a decrease in hardness value for composite when it was soaked in acidic solution. Maupome et al (1998) in their study of enamel microhardness after exposure to eroding immersion in a cola drink (pH 2.6) found that the greatest erosive effect occurred right after the first immersion which was five minutes and showed statistically significant differences between day 0 and 1.

Specimens in alkaline groups showed 90% (pH 11.5) and 70% (pH 8.5) leakage at cervical margin. At occlusal margin, leakage occurred in 50% of the specimens for both groups. This might be due to difference action of the alkaline to the difference enamel thickness and also interaction with composite restoration.

Hosoda et al (1987) studied the surface structural changes of composite resin under alkaline conditions. The degradation process was evaluated after immersion in 0.1N NaOH solution for one week. They found structural changes in the subsurface damage layer of the resins. Crack formation between filler particles and resin matrix, partial dissolution and exfoliation of filler particles and swelling of resin matrix were observed, with varying degrees depending upon the individual resin. Kondo et al, 1989 evaluated strength of composite resins in different aqueous environments (deionized water, coke, orange juice and 1.0M KOH alkaline solution) at three different temperatures (37, 55 & 70^{o}C).The 24 hours compressive strength of all composites stored in 1.0M KOH at 37^{o}C decreased by 40-50% in each of the 24 hours compressive strength tests when compared to composites stored in deionized water at 37^{o}C.

In this present study, all three specimens viewed under SEM showed larger gap at the cervical margins. This result was expected as from dye penetration evaluation, microleakage was found higher at the cervical margins. Gap between the restorations and the cavities walls might be due to polymerization shrinkage of the composite or due to the technique used while packing the composite into the cavities. The skill when handling the material is a critical factor on determining the extent of leakage (Trowbridge, 1987). There is possibility that dye penetration (score 3) occurred due to poor adaptation of restorative material to the cavities walls totally, and not because of interaction in the pH solution. In this study, the technique used has been standardized as only one operator performed the research.

Under higher magnification (500X), enamel and composite texture of the specimen in pH 2.5 showed more irregularities if compared to specimen in pH 7 and 11.5. The changes might be due to erosion of the solution to the surfaces. This finding was similar to the study by Maupome et al (1998) and Abu Bakr et al (2000). Maupome et al (1998) found that the greatest erosive effect of enamel occurred after five minutes. Abu Bakr et al (2000) found SEM changes in surfaces texture of materials immersed in low pH soft drinks after 60 days. SEM revealed the surface of resin composite was slightly affected; whereas the surfaces of compomer and resin modified glass ionomer cement (RMGIC) showed a rough surface with voids and protruding glass particles.

5.3 Limitations of the study

In this study, human premolar tooth that was extracted for orthodontic reason was selected, but as mentioned by Young (1975) the composition of human teeth is known to be highly variable. This factor more or less affected the interactions of the specimens to the difference pH medium and might influence the result.

Rossomando and Wendt (1995) found that leakage might also be influenced by thermocycling process. Thermocycling process might lead to more leakage under certain conditions, like increase in dwell time and number of cycles. However this is not similar to the findings by Wendt et al (1992) which found that there was no increase on dye penetration of samples that were thermocycled.

Evaluation of microleakage with dye penetration using image analyzer was not a difficult process. However two specimens exhibited unusual pattern of dye penetration. The dye was visible at the axial wall of the restoration but no dye was seen at occlusal or gingival margin. Gonzales (1992) suggested two possible reasons, that either the dye illumination was not adequate or diffusion of the tracer from another plane of the tooth to the axial wall as a cause of this leakage pattern. In this present study, microleakage in cases like these were evaluated as score 3 because no dye was seen to penetrate the apical seal.