CHAPTER FIVE: DISCUSSION

5. Discussion

5.1 Methodology

In general, the process of dental decay can be modeled in the laboratory using chemical or microbiological systems to produce early manifestation of caries, namely, the white spot lesion on smooth surfaces, caries on occlusal surfaces, secondary caries around restorations, root caries, and dentinal caries. In the mouth, this process takes much longer than in the laboratory models. The advantage of these models is that much can be learned about caries process involving a shorter period of time (Featherstone, 2004).

The chemical model was chosen because utilization of microbiological system in creating caries-like lesion on enamel *in vitro* may lead to early breakdown of enamel (Clarkson et al., 1984). Therefore, it will be difficult to prepare and hold thin specimen to evaluate enamel demineralization under polarized light microscopy.

Many chemicals model systems have been developed to simulate the caries process such as diphosphonate inhibitors system, buffer system and acid gel. These systems are limited to the study of demineralization of enamel. One of the systems which are most commonly used to simulate enamel demineralization is the acid gel technique as described by Wefel & Harless, (1984). This technique is shown to be efficient in producing caries-like lesion at rates comparable to those occurring *in-vivo*. It utilizes a gel medium with organic and inorganic elements that acts as a substitute for the plaque occurring in vivo (Kotsanos et al., 1989; Dionysopoulos & Kotsanos, 1998). Hals & Simonsen, (1972), Kidd, (1977) and Dionysopoulos et al., (1998) investigated and reported that caries-like lesions produced by the acid gel system was similar to that produced by clinical caries process. Furthermore, it is usually used to create secondary caries like-lesion around fluoride releasing materials by many workers (Gilmour &

Edmunds, 1998; Dionysopoulos et al., 1998; Pereira et al., 1998; Nury & Alev, 2002). It has an advantage of eliminating the external variables associated with formation of natural caries lesion i.e. substrate and microflora. The gel acts as a diffusion barrier for the dissolved minerals. The use of artificial caries systems greatly increases our knowledge of the demineralization process.

Acid gel in this study consisted of 0.1M lactic acid, 6% hydroxyethylcellulose and 0.1M sodium hydroxide (Tantibirojn, 2006). Acid gel has been used to produce demineralization on enamel (Wefel & Harless, 1984; Kidd et al., 1984; Tantiborjn et al., 1997), and secondary caries-like lesion adjacent to restoration (Kidd, 1977; Dionysopoulos et al., 1998; Nury & Alev, 2002). In addition, it is reported that different concentration and exposure time do not change the main characteristic of the lesion (Groeneveld & Arends, 1975). Based on the literature, immersion period varies from three days to six weeks. However, in this study the immersion period of four weeks is chosen based on preliminary work carried out prior to a full scale study.

Ericsson in 1949 stated that in patients with low salivary concentrations of calcium and phosphate, the critical pH for enamel dissolution was 6.5, whereas, in those with high salivary calcium and phosphate concentrations, it was 5.5. Barron et al., (2003) reported that, the drop in pH below 5.5 would dissolve the crystals of hydroxyapatite in enamel. The pH values for enamel demineralization that has been used in demineralization or remineralization studies were 3.75 (Westerman et al., 2004), 5.1 (Tantibirojn et al., 1997), and 4.5 (Wefel & Harless, 1984). The pH of acid gel in this study was kept at 5.3 so that it matches the pH of the qat extract which were 5.3 and 5.34 for 10% and 20% qat extract respectively.

The adverse effects of qat on hard and soft structure have been investigated (Al-Sharabi, 2002). However the effect of qat chewing on enamel demineralization was not. Therefore, this study is considered as the first attempt to investigate the demineralization potential of qat on smooth enamel surface and at restoration interface. Young fresh qat leaves are chewed and formed into a bolus and held in the lower buccal pouch unilaterally for three hours or longer. In order to simulate this clinical situation fresh qat leaves were ground and soaked in deionized water and stored in an incubator at 37ºC for four hours. The obtained qat extract were not boiled as that could lead to degradation of some elements such as tannins (Al-Hebshi, 2006). Fresh qat leaves were used in this study as some substances may be labile and thus degrade when qat leaves were being dried (Al-Hebshi, 2005). Qat extract were changed every two days because the solution becomes cloudy after three to four days as it was observed in the preliminary work. However, no attempt was made to investigate this phenomenon.
A further study may have to be carried out to ascertain this observation.

Extracted premolars from young patients (14-19 years old) were used in this study to ensure that the test and control specimens were from similar age group (Kidd et al., 1984). Furthermore, the morphology of the caries-like lesion produced on premolars was reported to be more uniform (Boyle et al., 1998). All the premolars were extracted for orthodontic purposes and were stored in 0.5% chloramine for a week at room temperature, 23ºC. Chloramine (0.1-1%) was one of the most common disinfecting agents used for disinfecting extracted teeth (Munksgaard & Irie, 1988). Other disinfecting agents that have been used were 10% formaline (Dionsysopolus et al., 1998) and 0.1% thymol (Gilmour et al., 1990). After a week, the teeth were stored in distilled water at 4°C until ready to be prepared. All the teeth were prepared within six month post extraction.

It should be noted that the margins for the cavity preparation was etched but not beveled. This was done to standardize cavity preparation as bevel technique might vary. Furthermore, the placement of bevels in Class V cavities does not significantly reduce microleakage around composite restoration as described by Santini et al., (2004).

A nanohybrid composite, Grandio (Voco, Germany) was chosen to be the filling material for the prepared Class V cavities in this study. The manufacturer claimed that this composite has filler size of less than 10 nm (0.01 μm) thus providing better aesthetics, strength, durability and reduced shrinkage and with improved adaptation to cavity walls. Nanocomposites shows to have high translucency, high polish and their polish retention is similar to those of microfilled composites while maintaining physical properties and wear resistance equivalent to those of several hybrid composites (Mitra et al., 2003). In this study Solobond M (VOCO,Germany) was used as Manhart et al., (2001) showed that it was comparable to others and exhibited the least microleakage at restoration interface.

The chosen imbibition media in this study prior to polarized light microscopy examination was water. Imbibition of specimens section in other solution such as, quinoline causes collapse of demineralized hard tissues through dehydration, make accurate lesion depth measurements more difficult (Gilmour et al., 1997).

Polarized light microscopy was used in this study to examine the depth of caries-like lesions, as it is the most frequently used technique to determine the extension of demineralization zones (Darling, 1956; Hals & Laegreid, 1974; Kidd, 1977; Wefel & Harless, 1984; Gilmour & Edmundss, 1998; Periera et al., 1998; Nury & Alev, 2002). Polarized light microscopy is capable of highlighting even the early stages of the

demineralization process. The technique further enables quantitative measurements of the extent or size of the lesion (Wandera, 1998). The measurements that were carried out in this study were according to Swift, (1989). The irregular nature of advancing edge of caries-like lesions made it necessary for the depth of the lesion to be derived from the mean of a series of measurements at approximately 0.2mm interval.

5.2 Demineralization of Smooth Enamel Surface

The demineralization of enamel was produced after four weeks of immersion in acid gel and 10% & 20% qat extract. On gross examination, white spot lesion was clearly observed on enamel surface due to acid gel. The enamel lesions appeared dull and rough. This feature of enamel demineralization was observed in this study were in agreement with those reported previously showing that smooth enamel surface exhibited extensive signs of dissolution when exposed to acid attack *in vitro* (Tantbirojn et al., 1997 and Kidd et al.,1984) for periods between four and ten weeks. Discoloration on smooth enamel surface due to qat extract was clearly observed. The enamel surface appeared dull and rough. It was reported that the appearance and texture of the enamel surface can often give clues of caries activity. A dull and rough appearance often suggests that the lesion is in an active state of demineralization (Nyvad et al., 1999).

5.2.1 Histopathology of smooth enamel surface demineralization.

Caries-like lesion in enamel under polarized light microscopy appeared as a dark area after being imbibed in water (Wefel, 2006). The water molecules enter the pores in the tissues and since the refractive index of water is different to that of enamel, this area appears dark in a way described as (positive birefringence). In this study only body lesion zone is observed on smooth enamel surface of acid gel group. The body lesion in enamel appeared dark under polarized light microscopy. However, Hals & Narnaes,

(1971); Wefel & Harless, (1984); Kidd et al., (1984); Holmen et al., (1985) described two zones i.e. surface zone and body lesion zone when specimens were imbibed in water. The absence of surface zone in this study was in agreement with previous finding by Westerman et al., (2004). The formation of surface zone has been attributed to the presence of a layer of plaque over the lesion during caries process (Kidd & Joyston-Bechal, 1997). It was suggested that the plaque acts as a diffusion barrier, trapping calcium, phosphate and fluoride ions released by subsurface dissolution or from saturated solution in plaque are reprecipitated onto the enamel surface. This suggestion implies that the surface zone of enamel caries may in part be a manifestation of remineralization (Kidd & Joyston-Bechal, 1997) and this area usually appear translucent under polarized light and it has been described as negative birefringence. The absence of negative birefringence in this study may indicate that no manifestations of remineralization occurred. Such a finding was expected since in this study there was no remineralization process unlike oral environment, thus limiting the clinical correlation. It was reported that when pH drops in the plaque fluid to the range 5.5-4.5 in the oral environment, hydroxyapatite will be dissolved (Dawes, 2003). Thylstrup & Fejerskov (1994) stated that the fluoride within the enamel will lead to the simultaneous formation of flourapatite in the outer layer of the demineralized area. Thus, reducing the loss of dental minerals due to the increased fluoride levels in the outer layers of caries lesions.

It was also observed that the smooth enamel surface overlying the lesions from acid gel and qat extract had irregular surface that suggests an early surface enamel loss as it was described by Westerman et al, (2004). On the other hand, if the lesion was allowed to progress, the surface zone would eventually breakdown and a cavity would be formed as described by Kidd and Joyston-Bechal (1997).

Histologically, specimens immersed in 10% and 20% qat extract showed the same features as those immersed in acid gel. However, the advancing front of enamel demineralization in the acid gel was irregular and commonly reaching up to the enamel-dentine junction. However, the advancing front in 10% and 20% qat extract groups was more uniformed and less aggressive.

The result of this study demonstrated that qat caused significantly low enamel demineralization on smooth enamel surface compared to acid gel. The mean depth of demineralization in the acid gel group was 311.23µm (±71.07). Specimens immersed in qat extract also showed caries-like lesion, with mean depth of demineralization 146.54µm (±33.76) and 153.89µm (±44.68) for the 10% and 20% qat groups respectively. However, the depth of enamel demineralization was not significantly affected by concentration of qat extract. It should also be noted that even though two different concentrations i.e. 10% and 20% qat extract were investigated, the pH of both qat preparations was almost similar, 5.3 and 5.34 respectively. This is probably due to the deionized water added to the ground qat leaves to form 10% and 20% qat solution. The deionized water used in this study has a pH of 7. It is likely that freshly ground qat leaves may have lower pH. However, further work should be carried out to ascertain this assumption.

The demineralization depth for 10% and 20% qat extract was significantly lower than acid gel group despite the similar pH and immersion period. The difference in the extent of demineralization may be due to chemical components present in the qat extract. Al-Hebshi et al, (2005) suggested that tannin in qat may contribute to it being anticariogenic.

This suggestion was based on their work on the ability of qat in inhibiting synthesis of water-soluble and water-insoluble glucans which were important for *Streptococcus mut*ans attachment.

Kempler et al., (1977) and Rosen et al., (1984) have shown that tannin in tea extract can inhibit caries formation in animals. It was later reported that 1% tannin acid when combined with 2% sodium fluoride (Ta-F) were able to increase the acid resistance of enamel (Yu et al., 1995). They demonstrated that Ta-F were able to inhibit calcium release in to acid solution of 98%. Tannin concentration in qat ranges from 3.5-9.7g/100g of qat leaves (Dhaifala & Santavy, 2004). Qat was also shown to contain fluoride in low levels (Hill & Gibson, 1987). Hattab (1999) found that qat leaves contained negligible amount of fluoride (0.93 part/million fluoride). However, Gibbs et al., (1995) suggested that even at very low concentrations of less than 0.05 parts per million, fluoride has been shown to promote the formation of apatite and enhance the remineralization of teeth.

Fluoride was reported to promote the formation of apatite and enhance the remineralization of teeth. Mineral would recrystallize in partially demineralized enamel when fluoride, calcium and phosphate ions were present in adequate proportions. The newly formed mineral contained hydroxyapatite and fluoroapatite, both of which are less soluble than the original carbonated calcium hydroxyapatite (Kautsky & Featherstone, 1993). That might explain the slow progress of demineralization in the qat groups. However, with longer immersion period, demineralization might continue and further studies will have to be carried out to ascertain this finding. On the other hand, it has been reported that lactic acid dissociates more readily than the other acids, producing hydrogen ions that rapidly lower the pH of acid gel (Featherstone &

Mellberg, 1981). As the pH is lowered, an acid diffuses rapidly into the underlying enamel.

Hydrogen ions dissociate as the acid diffuse into the enamel (Featherstone & Mellberg, 1981). The hydrogen ions readily dissolve the mineral, freeing calcium and phosphate into solution, which can diffuse out of the tooth. This may explain the extent of demineralization on smooth enamel surface due to acid gel compared to the qat extract.

The findings of this study suggest that the acidity of qat may be detrimental to the enamel surface. Although one may argue that in the oral environment, the buffering capacity of saliva may increase the pH of qat. Jensen (1986) reported that neutralization of plaque acids by the alkaline buffer system in saliva may take as long as two or more hours. The likelihood of enamel demineralization occurring within a short period of time is not known. It would be interesting to investigate the effect of qat on enamel demineralization with shorter exposure time.

Al-Sharabi (2002) showed there was a positive association between qat chewing and xerostomia. He also indicated that there was an increase in the prevalence of cervical caries, attrition and staining. This observation can be attributed to the amphetamine-like effect as qat has similar chemical structure to amphetamine (Al-Hebshi & Skaug, 2005b). Amphetamine users are shown to have rampant caries and this has been attributed to their reduced salivary flow (Klasser & Epstein, 2005). As a result, the lack of salivary flow may cause the rate of enamel demineralization to increase in an acidic environment (Garg & Malo, 1997). This acidic pH may be further enhanced as ascorbic acid content of qat can be considered to be high, where a 100g of fresh leaves contains 325 mg of ascorbic acid (Hattab, 1999; Al-Hebshi et al., 2005) and Giunta, (1983) had shown that chewable 60mg vitamin C tablets may cause decrease in pH because of its

acidity, which contributes to enamel demineralization. He found that chewing vitamin C tablets deposits a layer of ground vitamin C particles on the enamel, which insulates it from the buffering action of the saliva. It should be recognized that his finding was based on a report case (Giunta, 1983).

The most important factor in pathogenesis of dental caries is the capacity of certain oral bacteria to produce lactic acid from dietary carbohydrates. That is especially in gaps and crevices, where pH is low (Göhring et al., 2004). At the so-called critical pH value (pH 5.5), significant amounts of dental hard tissues can dissolve. In addition, extrinsic (dietary) and intrinsic (gastric) acid attack may act as an erosive agent and cause mineral loss during dissolution of dental hard tissues (Kelleher & Bishop, 1999). However, it should be recognized that in *vitro* conditions demineralization study differ from *in vivo* situations as there is no protective salivary pellicle (Zahradnik et al., 1978). Any procedure that removes or reduces the thickness of the pellicle might compromise its protective properties and accelerate the erosion process. Procedures such as tooth brushing with abrasive dentifrice products, professional cleaning with prophylaxis paste, and tooth whitening will remove the pellicle and may render teeth more susceptible to demineralization (Zero, 1996). Qat is usually chewed for more than four hours daily and this may lead to the removal of the protective salivary pellicle through abrasive processes. The time required for the pellicle to reform and provides optimal protection is still a subject of debate. It was reported that the time may vary from as little as 3 minutes (Hannigue et al., 2004) to as long as 7 days (Meurman & Frank, 1991).

The prolonged qat chewing may also cause excessive occlusal loading. It was found that enamel dissolution is significantly increased in sites subjected to cyclic tensile

loading *in vitro* (Palamara et al., 2001). However, further investigation need to be carried out to explain this phenomenon.

5.3 Demineralization at restoration interface

In this study the lesion appearance at restoration interface for all groups under polarized light microscopy showed two parts, an outer lesion and wall lesion. The wall lesion is formed as a consequence of microleakage of acidic products along the restoration interface as described by Hals & Narnaes (1971) and Kidd (1977).

5.3.1 Histopathology of demineralization at restoration interface

Under polarized light the lesion at restoration interface of acid gel group showed positive birefringence which is represented as brownish dark zone for outer surface lesion and dark wall lesion in agreement with previous studies (Kidd, 1977; Gilmour & Edmunds 1998). 10% and 20% qat extract groups exhibited the same feature.

The shape of the outer lesion in all groups and its relationship to the wall lesion was influenced by the direction of the enamel prisms. The outer surface lesion at coronal aspect follows the direction of the enamel prisms towards the cavity wall as described by Kidd in 1977. The angulation of the prisms appeared to be important. When the prisms are perpendicular to the surface, etching is improved hence increased demineralization (Gilmour & Edmunds, 1998). It was also stated that at the cervical area, the lesion was directed away from the cavity wall. However in this study, this was not observed as it was parallel to the cavity wall, as the cervical cavity margin was placed 2mm away from the CEJ.

In this study, the wall lesions appeared as a dark line extension along the restoration interface. This dark line has been described as ribbon-like extension by Gilmour & Edmunds, (1998). Ribbon-like extension was also observed in most of specimens from all groups. Its formation has been attributed to the limited diffusion of hydrogen ions into microspace at the restoration interface (Gilmour & Edmunds, 1998). It was also reported that this dark line may be indicative of comparatively slow rate of demineralization being limited by the presence of the restoration (Kidd, 1977).

However wall lesion at coronal part of restoration appeared in 30% of specimens in all groups as a downturn of the outer lesion (Figure 4.9) as a consequence of change in angulations of enamel prisms toward the cavity margin. This finding was in agreement with Gilmour & Edmunds (1998) and Kidd (1977).

The polymerization contraction causes the composite to shrink. In *in vitro*, the initial gap around composite resin restoration varies between 10 to 30 µm (Tjan et al., 1992). This initial gap formation could be the passageway for the acid to attack the walls of cavity around restoration. Although better materials have been developed in recent years, microleakage still occurs between the tooth and the restorative materials (Manhart et al., 2002). Furthermore, contraction of restorative material away from the cavity wall will form microgap, followed by leakage. That is considered to be the most important factor that leads to eventual development of secondary caries at tooth restoration interface (Litkowski et al., 1991). Possible deleterious effects at restoration interface such as, expansion and contraction of restoration by thermal cycling have not been tested. In spite of these limitations, this study provides valuable information regarding the demineralization effect of qat on restoration interface.

It has been found that composite restoration is prone to develop secondary caries if a gap exists between restoration and tooth (Dijkman and Arends, 1992). Although microleakage around composite is common, it may be reduced by using the acid etching technique (Gilmour et al., 1993).

It was also observed in acid gel group, 40% of specimens showed ” V” shape notch at enamel restoration interface in the cervical margin. The feature of “V” notch at cavosurafce margin was described in several previous studies (Clarkson et al., 1984; Purton & Rodda, 1988; Dionysopoulous et al., 1994). A possible explanation for this phenomenon could be the manifestation of shrinkage and dissolution of the minerals crystallite followed by the collapse of hard tissue due to aggressive acid attack (Purton and Rodda, 1988). Pereira et al., (1998) attributed that observation to the dehydration of tooth hard tissue during sample preparation for polarized light study, material loss or contracting away from cavosurafce margin during tooth sectioning resulting in opening of the restoration interface. However, the most suitable explanation for this feature observed in this study is the aggressive attack of acid gel, as specimens exposed to 10% and 20% qat extract did not exhibit this feature. It is likely that acid gel cause softening of the composite and demineralization of enamel at the restoration margin. During sample grinding and polishing for polarized light microscopy, the softened composite and demineralized enamel at the margin restoration collapsed, leading to the V notch configuration at the restoration margin.

The mean depth of outer surface enamel lesion, at coronal aspect of restoration was significantly different between acid gel (256.28µm ± 142) and qat extract, 10% and 20% at 77.24µm ± 47.46, 104.87µm ± 58.43 respectively. However, there was no significant difference between 10% and 20% qat extract, although the mean outer surface lesion depth for specimens exposed to 20% qat extract were deeper than for

10% qat extract. There was also significant difference in the cervical outer lesion depth between acid gel and qat extract (10%, 20%) groups. The cervical outer lesion depth for acid gel was 253.93µm ± 98.07 and 99.70µm ± 72.70, 116.81µm ± 58.20 for 10% and 20% qat extract respectively. However, there was no significant difference between coronal and cervical outer lesion depth in 10% and 20% qat extract groups. This observation further substantiates the reliability of results of the smooth enamel surface lesion. Enamel thickness does not seem to have an effect on the depth of demineralization when specimens were immersed for four weeks. Although cavity margin position was found to play a significant role in the formation of wall lesion, this was not elucidated in the results of this study. The location of the cervical margin that was prepared 2mm from the CEJ seemed to be that of the enamel thickness which is sufficient to achieve high quality bonding. While in other studies of microleakage most of the cervical margin was prepared 0.5-1mm away from CEJ. The problem of polymerization shrinkage of composites in thin enamel at cervical cavity margin as described by Kidd, (1990) was not a problem in this study.

The coronal wall lesion depth for acid gel was 193.61µm ± 120.50 and 116.42µm ± 117.50, 72.44µm ± 65.61 for 10% and 20% qat extract respectively. The mean depth of the coronal wall lesion between acid gel group and 20% qat extract was statistically significant. Similarly, the mean depth of the cervical wall lesion between acid gel group (251.07 µm ± 102.56) and 20% qat extract (111.58 µm ± 81.68) was statistically significant.

Two- way ANOVA revealed that there was no significant difference between the wall lesions at the coronal and the cervical area. The observed partial eta squared was low (.06) indicating a small effect size. That might be due to the sample size which is not

large enough for this aspect of the study. The depth of wall lesion seemed to be demineralizing agent/solution dependent i.e. acid gel caused deeper wall lesions compared to qat extract but not restoration location margin.

Despite the pH and immersion period being the same, the coronal and cervical wall lesion depth due to acid gel and 10% qat extract was significantly higher compared to 20% qat extract. A possible explanation is that microleakage and formation of cavity wall lesions would have taken place to a greater extent with more diluted solutions. Further work should be carried out in order to substantiate this explanation.

The demineralization at cavity wall at coronal and cervical part of restoration due to qat may lead to microleakage and eventually secondary caries may be formed. Even though the number of samples with no wall lesion was higher for samples exposed to 10% qat extract than for acid gel, the depth of wall lesion in acid gel group was not significantly different from the 10% qat extract group for both coronal and cervical aspect of restoration. However, the lesion depth was significantly lower in the 20% qat extract group compared to 10% qat extract.

Although, the scope of this study was not to investigate leakage, yet the acid ions of qat was speculated to have infiltrated through restoration interface. Due to that they become responsible for the formation of wall lesion observed in this study that will eventually lead to secondary caries.

Therefore, it may be possible to use 10% qat extract to investigate the formation of secondary caries. The advantage of using qat is that they may be correlated directly to the formation of secondary caries and may be more clinically relevant compared to dye penetration for microleakage studies.