EFFECT OF GAP GEOMETRY ON SECONDARY CARIES IN VITRO

by

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Dedication

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INTRODUCTION

Dental caries is a process resulting from the microbial deposits covering the tooth surfaces.[1] It is a dynamic process formed from the interaction of many factors, including acid attack from micro-organisms, protective ions from the oral environment and buffering capacity and mechanical clearance of saliva. Although during recent years a decline in the incidence of dental caries has been observed for most industrialized countries,[2-4] this disease remains a significant concern.[5] Approximately 60 percent of all tooth surfaces that are restored each year are replacements of existing restorations.[6] Causes of these restoration failures include fractures, marginal breakdown, pulpal problems, periodontal disease and secondary caries.[7]

Based on multiple reports, the most common reason given for restoration replacement is secondary caries, regardless of the type of the restorative material.[7-14] Secondary caries can be defined as the carious lesion that occurs near existing restorations.[1, 14] Secondary caries remains an important unresolved issue.[7, 15]

It is well established that secondary caries lesions involve two parts; an outer lesion and a wall lesion (Figure 1).[13, 16-18] The outer lesion occurs due to the presence of plaque at the restoration margin. The development of the wall lesion is dependent on the size of the gap at the tooth-restoration interface. The size of this gap has been found to have an effect on the development of secondary caries lesions.[19-22]

The presence of this microspace has been considered as a potential predictor for subsequent caries development underneath restorations. However, this topic is still debated by many researchers regarding its validity. Several investigations have suggested a positive correlation between gap size and secondary caries.[16, 19-23] On the other hand, a number of studies have reported no such association.[24-26] Gaps at the

margins of restorations allow bacterial invasion and plaque accumulation causing further demineralization along the restored cavity wall.[23] Evidence from several studies has supported the presence of microspaces at the tooth-restoration interface after placing any restoration. This suggests that restorative materials do not completely eliminate microleakage.[27-33] It was reported that the initial gap around light-activated materials could range from 5 to 100 µm.[34-37] The inherent polymerization shrinkage of composites can be considered as the main reason for the formation of these gaps.[38]

Secondary caries has been reported by many papers to take place in areas of plaque stagnation most likely in the gingival wall of class II and class V cavities.[1, 14] These areas are in most cases difficult to visualize.

According to Bernardo et al.,[39] the risk of secondary caries was 3.5 times greater in resin-matrix composites than amalgam restorations with an annual failure rate of up to 2.83 percent for amalgam restorations and up to 9.43 percent for composite restorations. Still, composite placement is tricky and technique sensitive[40] especially in posterior areas of the oral cavity with limited visibility and accessibility.

Despite this, the use of composite restoration is increasing.[40] Many practitioners are shifting from using amalgams to using the more esthetic resin composites. Although the properties and the handling parameters of resin-matrix composites has improved over the years, composite resin still poses a problem in restoring posterior teeth especially in stress bearing areas.

Presence of gaps along resin-matrix composite interface is an implication of the technique-sensitivity of placing this type of material. Along with the gap size, fluoride was reported to have an effect on secondary caries development. Fluoride released from restorative materials within 200-µm gaps was shown to play a role in

the prevention of secondary caries.[34, 41] In a recent study by Cenci et al.,[23] fluoride present within the gaps overcame the effect of gap size. Also it was suggested that fluoride from other sources such as dentifrices could be effective in inhibiting secondary caries.[37, 42]

It can be assumed from a clinical perspective that gaps developing around restorations are non-uniform. Since, most of the time, the superficial part of the tooth-restoration interface is readily accessible to the practitioner to verify the absence of any discrepancy. On the other hand, there is no way for the dentist to check the inner part of the cavity for any voids unless he/she takes a radiograph; which is not a regular procedure after finishing the restoration. Furthermore, sometimes the restoration will superimpose the area of the void preventing the practitioner from observing it.

Most of the reports mentioned in the literature that focused on investigating the effect of these gaps on secondary caries development assumed that these gaps are uniform. Furthermore, most of these efforts did not mention the possibility that these interfaces could be of different widths along the cavity wall or did not utilize any methodology that could establish this. Although the mechanism of secondary caries development around uniform gaps has been discussed to a great deal in the literature, none of the previous reports attempted to study non-uniform gaps.

It could be assumed that gaps that develop near restorations are not uniform. For example, faulty restoration placement with lack of proper adaptation of resin-matrix composite to the inner part of the cavity may leave some areas not completely filled. This could be seen in the proximal cavities in posterior part of the oral cavity where the dentin part of the gingival wall of a class II cavity might fail to be bonded. This will leave a defect under the clinically acceptable margin. Kakaboura and

colleagues[43] evaluated the three-dimensional marginal adaptation of light-cured composites to dentin. They found an increase in gap sizes and porosities at the bottom sites of dentin cavities. These discrepancies resulted from a combination of polymerization shrinkage and weak bonding to dentin.

Another reason for developing voids underneath resin-matrix composite restorations is the presence of too much residual water left from the etching and washing procedures.[44] The presence of too much water can create small blister-like spaces or voids that are trapped along the surface of the hybrid layer.[45] Furthermore, excess solvent that can remain on the primed dentin may prevent complete adaptation of resin and can result in voids at the dentin-resin interface.[46]

Debonding of light-cured composite from dentin cavity walls as a result of polymerization shrinkage has been previously identified.[47, 48] Contrary to the success achieved with bonding to enamel,[49] bonding to dentin has been less predictable because of the organic composition of dentin along with its wet tubular structure.[50] Although recent developments in dentin adhesives have made recently placed resin composites nearly free of microleakage with bond strengths approaching those of enamel bonding,[51-54] the effect of thermocycling and water sorption on dentin bond strength has been found to be deleterious.[55] It was found by Okuda and colleagues that the strength of dentin bonds dramatically decreased and nanoleakage increased gradually when specimens were subjected to periods of storage in water.[56, 57]

According to these data, debonding of resin-matrix composites from dentin due to thermocycling, water sorption or polymerization shrinkage is more likely to occur than failure of the enamel bond. This could leave the inner part of the cavity empty in areas such as gingival walls of class II restorations.

Based on this information, it can be proposed that gaps developing near restorations tend to be non-uniform in configuration; with larger gaps developing near the base or the dentinal part of the cavity. The effect of this geometric variability has not been considered in the published literature. It is possible that secondary caries lesions progress in a different manner next to these non-uniform gaps. In addition to that, the mechanism of secondary caries development may differ from those described in the literature,[16, 22] since caries dynamics are likely to change at different gap widths.

In summary, although the relationship between gap size and the rate of secondary caries development has been studied previously,[19-22] the effect of gap geometry on the rate of development of secondary caries has not been studied previously.

The purpose of this study was the investigation of the effect of the size of the space between the restoration and the dentinal wall of the tooth on the development of secondary caries lesions (especially the wall lesion).

The study was based on the hypothesis that the bigger the size of the space between the restoration and dentinal wall of the tooth, the bigger the size of the secondary carious lesion. The null hypothesis tested was that the size of the space between the restoration and dentinal wall of the tooth will not have an effect on the development of secondary caries.

LITERATURE REVIEW

DENTAL CARIES

Dental caries is a transmissible bacterial disease process caused by acids from bacterial metabolism diffusing into enamel and dentin and dissolving the mineral.[58] The term "caries" can be used to refer to both the caries process and the caries lesion that forms as a result of that process.[59] The caries process is initiated in the biofilm or dental plaque.[60] Biofilms form on any solid surface exposed to appropriate amounts of water and nutrient. However, surfaces of the dental tissues are first coated by a salivary pellicle to which the microbial cells attach.[58, 59] These bacteria are essential to the disease process and include mutans streptococci and the lactobacilli species which are able to produce organic acids during the metabolism of fermentable carbohydrates.[61-63] Furthermore, a biofilm is formed from the bacterial aggregation along with the exopolymer matrix that they secrete. The biofilm tends to form and mature in certain locations on the tooth, notably the occlusal surface, especially during eruption, the approximal surface cervical to the contact point, and along the gingival margin. The bacteria in the biofilm are always metabolically active, causing fluctuations in pH.[59] These fluctuations may cause loss of minerals from the tooth when the pH drops or gain of minerals when the pH increases.[60] The acids produced by the bacteria as by-products have been shown to readily dissolve the minerals of enamel and dentin.[64, 65]

The cumulative result of these demineralization and remineralization cycles may be a net loss of minerals, leading to dissolution of the dental hard tissues and the formation of a caries lesion.[58, 59] Remineralization is the body’s natural repair process for subsurface non-cavitated carious lesions.[66] However, if the rate of demineralization is higher than the rate of the reparative process, cavitations in the

tooth structure will result.[58] At this stage, restorative treatment is needed to restore the integrity of the tooth surface allowing the patient to maintain the area clean.[1]

RESTORATION FAILURES

Failure of dental restorations is a major concern in dental practice. It has been estimated that dentists spend between 60 to 75 percent of their working time replacing failed restorations,[1, 39] which causes high personal and social costs.[67]

In a study conducted in Portugal, the annual failure rate ranged from 0.16 to 2.83 percent for amalgam restorations and from 0.94 to 9.43 percent for resin-matrix composite restorations over a period of seven years. The survival rate documented in the same study for amalgam and resin-matrix composite restorations was 94.4 percent and 85.5 percent, respectively.[39] Surveys conducted in Denmark reported that replacement of failed restorations accounted for around 61 percent of all amalgam restorations and 62 percent of resin-matrix composite restorations. In these surveys, the age of amalgam restorations ranged from 0 to 46 years compared to an age range of 0 to 19 years for resin-matrix composite restorations.[68, 69] A similar percentage of amalgam replacements was reported in Finland.[70] In Australia, out of 2716 restorations placed in private practices, 46 percent were new restorations while 54 percent were replacement restorations. The main reason for these restoration replacements was secondary caries for all materials (amalgam, resin-matrix composite and glass ionomer).[71] Mjör and Toffenetti[72] reported the portion of restoration replacements in Iceland being 47 percent of all restoration placed with secondary caries as the main reason for the retreatment of these fillings.

A survey conducted in Brazil aimed to report the reasons for placing and replacing of direct restorative materials. Of all the restorations placed, approximately

40 percent were first-time placements, while 60 percent were replacements. For first-time placements, the main reason for placement was primary caries. For amalgam restorations, the main reason for replacement was to obtain improved esthetic appearance with a tooth-colored material while the main reason for replacing resin-matrix composite restorations was the diagnosis of secondary caries. The longevity reported for amalgams, resin-matrix composites and glass ionomers were 13.6, 7.1 and 5.7 years, respectively.[11]

In most of the cited studies, survival of amalgam restorations was greater than that of resin-matrix composite restorations. However, in a study by Opdam and coworkers, better survival rate in composite than amalgam restorations was reported.[12] Nordbø and his colleagues reported that the 10-year failure rate of resin-matrix composite restorations was 30 percent in their clinical trial that was aimed to study the performance of saucer-shaped class II composite resin-matrix restorations.[73] In studies on the longevity of resin-matrix composites in posterior teeth restoration, survival rates between 55% and 95% during an observation period of 5 years have been documented.[74] The difference between the longevity of resin-matrix composite and amalgam could be due to differences in material properties such surface roughness or antibacterial effect or technique sensitivity of resin-matrix composite.[75] Svanberg et al.[76] found more caries-related microorganisms on resin-matrix composite than on amalgam restorations.

An effort by Raskin[77] and his coworkers to evaluate the clinical performance of posterior resin-matrix composites after 10-year showed that the failure rate of composite ranged from 40 to 50 percent. However, the main reason reported for this high rate of failures was not recurrent caries or fractures; but loss of occlusal anatomy and proximal contacts. This is the different from other reports that considered

secondary caries as the main reason for restoration failures regardless of the type of the restorative material.[7-12, 14] A review of prospective studies on the clinical performance of posterior resin-matrix composites published between 1996 and 2002 was published in 2003. Authors found that during the first five years after placement, the main reason for failures was restoration fracture followed by secondary caries. However, after five years of service, the main reason for failure and replacement of resin-matrix composite restorations was secondary caries. They also reported that the primary reasons for resin-matrix composite failure were secondary caries, restoration fracture, and marginal defects. The effect of isolation method and the professional status of the operator were not found to be significant.[78]

SECONDARY CARIES

Secondary caries or recurrent caries can be defined as a caries lesion that develops adjacent to a restoration.[79] It might be considered as a primary lesion around restorations.[9, 59] Histological studies describe the secondary caries lesion as having two parts: an outer lesion formed due to acid attack on the outer surface of the tooth next to the filling and a wall lesion which is assumed to develop there by diffusion of hydrogen ions through tooth-restoration interface.[16, 17] This would suggest that demineralization could develop adjacent to the margin of a restoration that is clinically intact but allowing leakage.[80] It was suggested that dental caries create the wall lesions by the action of hydrogen ions directly on the cavity walls.[17] These ions evolving from within the tooth-restoration interface directly attack enamel and dentin leading to their demineralization.[81]

Recent studies suggest that the development of outer lesions might be more important than the development of wall lesions.[14] Results from a survey conducted by

Mjør and Qvist showed that combined wall lesions and caries underneath restorations were found much more frequently if the pre-operative diagnosis was a marginal defect with caries than if no caries was detected clinically. Furthermore, cavity wall lesions as diagnosed after the removal of restorations were uncommon whenever a marginal defect was diagnosed without caries. This suggests that the outer lesion constitutes the most important part of secondary caries.[82] However, the use of the outer lesion as a predictor for wall lesion progression presents significant limitations. An in vitro study by Grossman and Matejka concluded that although the presence of an outer lesion strongly indicated the presence of a wall lesion, the absence of an outer lesion did not mean the lack of a wall lesion and that the outer lesion was unreliable to predict the extent of the wall lesion underneath.[83] Wall lesions are the result of microleakage as believed by some researchers.[84, 85] However, not all restorations suffering from microleakage develop secondary caries.

Secondary caries is the most common cause cited by practitioners for restoration replacement.[7-11, 14, 72, 86-88] It is well documented that all types of restorative materials including amalgam, resin-matrix composite and glass ionomer have been replaced due to the diagnosis of secondary caries. Friedl and his coworkers investigated the reasons for placement and replacement of amalgam and resin-matrix composite restorations in Germany. They found that approximately half the treatment provided was replacing old restorations and the most frequent cause for these replacements was secondary caries.[89, 90] A report based on a survey of general practitioners in Sweden on the reason for restoration replacement there, indicated that almost half of the glass ionomer cements were being replaced because of secondary caries.[82]

DIAGNOSIS OF SECONDARY CARIES

Secondary caries is difficult to diagnose,[91] and thus practitioners are inconsistent and inaccurate in their diagnosis.[92] Clinical diagnosis of this disease is often obscured by other types of restoration failures such as marginal defects, ditches and discoloration.[9] It known that not all dentists make the same decision regarding the need for treatment and treatment choices. In a clinical study conducted by Ermiş and Aydin,[93] the decision to replace class I amalgam restorations showed only moderate level of agreement between participants in identification of secondary caries requiring treatment (kappa value=0.58).

Secondary caries is rare on occlusal surfaces possibly because the margin of the restoration is cleansable.[1] The main locations for secondary caries development are the areas of plaque stagnation, such as the cervical margins of restorations.[14] Secondary caries is more common at the gingival locations regardless of restoration type or restorative material used.[94] Nordbø and colleagues reported the main location of secondary caries in their clinical trial to be the gingival margin of class II cavities.[73] A survey of 261 class II amalgam restorations and 102 resin-matrix composite restorations examined by general dentists in order to evaluate the frequency of secondary caries at various anatomical positions found that recurrent caries occurred more frequently at the cervical and proximal margins than at the incisal or occlusal locations.[95] In another study by Jokstad and Mjör,[96] secondary caries was recorded on the proximal surfaces of class II amalgam restorations and was mainly associated with gingival areas. In resin-matrix composite restorations, secondary caries was observed before the restoration was removed was usually located gingivally, regardless of the type of the cavity preparation, according to Mjör and Qvist.[82]

The likelihood of secondary caries was suggested to increase with the increase of marginal deterioration in caries active populations.[97] Several investigations studied marginal defects as the determining factor for secondary caries development. A cross-sectional clinical study by Goldberg et al.[21] found that marginal integrity had a significant effect on the likelihood of occlusal and smooth surfaces being affected by secondary caries. Another clinical study investigating the level of bacteria under the margins of amalgam restorations showed that samples associated with wide ditches (> 0.4 mm) yielded significantly more bacterial levels. However, there was no difference between the infection levels underneath intact restoration and those with ditches narrower than 0.4 mm.[98] An in vitro study by Kidd and O'Hara to examine the caries status of amalgam restoration associated with both defective and sound margins showed a low prevalence of caries lesions in the outer regions of enamel. However, lesions were present in enamel adjacent to the cavity wall in 54 percent of the specimens, whether the margin was defective or not.[24] It was suggested that a defective margin alone should not be an indication for replacement. Similar results were reported by Pimenta et al.[25] The presence of a narrow ditch at the margin of a restoration should not trigger operative intervention.[99]

Another complicating factor in the diagnosis of secondary caries lesions is the presence of marginal discoloration. A study of staining around resin-matrix composite restorations reported that outer and wall lesions were more prevalent adjacent to stained margins compared to non-stained margins, based on histological data.[100] Other studies found that grey discoloration might be a useful aid in detecting secondary caries around occlusal amalgam restorations. However, the same could not be found in the interproximal amalgam restorations.[101, 102] A clinical effort by Foster[103] investigating the consistency and color of dentin underneath the defective amalgam

restoration scheduled for replacement showed that discoloration at the restoration margin was not associated with the consistency of the underlying dentin. The author also reported that external stains could be taken up by caries-affected dentin causing discoloration around restorations and led practitioners to suspect recurrent caries. The same conclusion was stated by Kidd et al.[104]

GAPS ADJACENT TO RESTORATIVE MATERIALS

Several studies have supported the presence of microspaces after the placement of any restorative material.[27, 28, 30-33] A study by Brännstrom et al.[27] was conducted to study the initial gap around resin-matrix composite restorations in vitro. Resin containing a fluorescent additive was applied to all margins and passively drawn into the gap. The width of the contraction gaps found to vary from 3.5 to 16 µm. In a similar study by Bergvall and colleagues, contraction gaps varied from 2 to 20 µm with wider gaps observed at the floor of dentin cavities.[105] In a study of cervical gap formation in class II resin-matrix composite restorations, average gaps of around 35 µm were demonstrated.[106] It was suggested that the use of a bonding agent significantly reduced dentin marginal gaps. Results from Idriss et al.[107] supported this where contraction gaps of 2 to 4 µm width were recorded. However, a more recent study reported that, even with use of the newer bonding agent systems, 40 µm-gaps can still form.[32] Although water sorption of these materials may reduce the size of the gap, results from studies suggested hygroscopic expansion after storing the material in water reduced the size of the gap but did not close it completely.[31, 108] Glass ionomer cements and indirect restorations were also found to demonstrate marginal gap formation. Studies of glass ionomer cements showed that gaps ranging from 3 to 29 µm occurred inside the dentin cavity.[30] A study of resin-modified glass ionomer

showed contraction gaps at the interface occurring 15 minutes after dehydration.[109] This could be explained by the setting reaction, according to Chutinan et al.[110] Gaps ranging from 20 to 92 µm around crowns[111, 112] were recorded and gaps up to 182 µm adjacent to ceramic and composite veneers were found in marginal gap integrity studies.[113]

RELATIONSHIP BETWEEN GAP SIZE AND SECONDARY CARIES

Early studies by Jørgensen and Wakumoto suggested that a minimal marginal defect of 50 µm is needed for the production of secondary caries, which can be detected by routine clinical examination.[20] Adjacent to indirect restorations, Jahangiri et al. used gaps of 30 µm width to act a theoretical acceptable gap discrepancy and as cut off point in evaluating the sensitivity and specificity of clinical evaluation of cast restoration margins.[114] Other studies have reported 30 µm to be a clinically acceptable gap discrepency.[115-117] Hodges et al. performed an investigation to study the relationship between gap width and secondary caries development adjacent to occlusal margins of amalgam restorations. The authors reported a difference of 187 µm between mean gap widths at sites with recurrent caries and sound locations, with gaps at the secondary caries sites being wider.[19]

Thomas and colleagues[75] reported, based on their in situ study, that the presence of the gap is necessary but not a sufficient condition for the formation of wall lesions adjacent to restorations. They also concluded that secondary outer lesions appeared and progressed as primary lesions. Papagiannoulis et al.[118] reported no lesion development at gap-free regions adjacent to glass ionomer and resin-matrix composite restorations in vivo and lesions did not develop in all gap locations, 75.5 percent of glass ionomer and 62.5 percent of resin-matrix composite developed

lesions. According to them, the reason for that was that the initiation of secondary caries is not directly related to the marginal defect, but requires plaque accumulation with cariogenic potential and that marginal sealing has a critical role in restoration integrity. The authors also found that all lesions were located at the vicinity of the gap entrance.[118] This may have been due to the calcification effect that occurs due to bacterial colonization at the highly plaque retentive areas such as the inner parts of the gap. The explanation to this phenomenon could be that deeper areas of the gap have little effect of the salivary pellicle proteins that have an inhibiting effect on calcium phosphate precipitation. However, this mechanism does not occur at the entrance of the interface where the abrasive forces due to mastication and tooth brushing frequently renew the absorbed salivary proteins, exposing enamel margins to the continuously changing intraoral conditions.[119] Another report states that different mechanisms may account for the differences reported in plaque composition and cariogenicity between deep fissures and smooth surfaces or fissure orifices, the latter being more virulent.[120]

Totiam et al. found a positive correlation between the size of the gap adjacent to resin-matrix composite and secondary caries wall lesion. These authors also found a significant increase in the size of wall lesion when a 500 µm difference was present between the study groups.[16] In a study conducted by Dérand et al., the relationship between secondary caries and gap formed around amalgam restoration in vitro was investigated. Specimens were incubated with glucose or sucrose bacterial broth with *Streptococcus mutans*. In the presence of sucrose, dentin caries was found in all groups where a 30 µm or wider gap was present. With the presence of glucose, dentin caries was detected only in specimens with a 60 and 80 µm gaps. Caries lesions were detected on the outer surface of enamel on all specimens.[22] On the other hand

Rezwani-Kaminski et al., failed to observe an association between gap size and secondary caries development.[26]

In conclusion, conflicting data can be found in the literature regarding the effect of the gap size on the development of secondary caries lesions. While some authors believe there is a positive correlation,[16, 19-21, 23] others did not observe such a correlation.[24-26] More studies are needed to verify the presence or absence of this association.

BACTERIA AND SECONDARY CARIES

After the restorative material is placed in the mouth, it is immediately covered with a salivary pellicle, which spreads rapidly into surface irregularities and into these microspaces.[81] The oral bacteria are able to adhere to this salivary pellicle and multiply invading the microspace.[16] Many studies have demonstrated the presence of bacteria in gaps between restoration and tooth structure.[13, 121-124] Provided with suitable conditions and time, these bacteria would potentially demineralize the tooth structure along the cavity wall.[16]

Many studies demonstrated the presence of bacteria in gaps between restorations and tooth structure.[13, 121-124] A study by Varpio et al.[125] investigated marginal adaptation and bacterial penetration in primary molars filled with class II resin-matrix composite that had been in service for three years. While gaps were recorded in 42 percent of the specimens, bacteria were observed underneath the filling in 75 percent of the specimens and inside the dentinal tubules in 61 percent of them. They concluded that bacterial leakage was associated with marginal defects. A secondary caries study in dentin showed that there was a considerable variation in numbers and types of microorganisms next to recurrent lesions.[126] However, other

studies reported mutans streptococci and lactobacilli as the causative organisms of primary as well as secondary caries lesion development.[13, 122, 123, 127]

An in vitro study by González-Cabezas and colleagues,[13] using immunofluorescent techniques and confocal laser scanning microscopy (CLSM) for identification of bacteria in secondary caries lesions, showed that mutans streptococci were detected in approximately 89 percent of samples analyzed. The same group of authors studied the distribution of the three suspected cariogenic groups of bacteria, namely mutans streptococci, *Actinomyces naeslundii* and lactobacilli, in secondary caries lesions around amalgam restorations and found that the three bacterial groups were widely present and concluded that these bacterial may have an important role in the development of secondary caries.[122] In 2002, they also found that the same group of microorganisms in secondary caries lesions around tooth-colored restorations.[123] Cenci et al. found that there was no significant difference between finished or polished restorations in relation to the presence of white spots.[8] This suggests the lack of association between surface roughness and bacterial adhesion.[128, 129]

The introduction of self-etching primer and self-etching adhesive system to simplify the bonding procedure is accompanied with partial removal of the smear layer that acts as a layer of bonding substrate.[55, 130] This implies that residual bacteria in the cavity may remain at the tooth-restoration interface and dentinal tubules, increasing the risk of secondary caries.[81, 130] Microgaps at the tooth-restoration interface can rapidly be filled with tissue fluid from the freshly cut dentinal tubules or from saliva and provide space and nutrients for bacterial growth.[81] Bacteria in the smear layer is less likely to survive with good adaptation of the restoration to the cavity wall because the nutrition to this bacteria is inhibited from the oral cavity.[131] However, bacteria can still survive in the smear layer despite the surface sealing

according to Brannstrom and Nyborg.[132] An in vitro study by Živković et al.[133] was conducted to assess the quality of the marginal seals of seven restorative materials by means of bacterial penetration using histological bacterial staining. It was found that the use of a restorative material did not entirely eliminate microleakage.

The size of gap interfaces could have an effect on the metabolism of the bacteria inhabiting it. With small gap sizes, the diffusion of carbohydrates to the deeper areas of the tooth/restoration interface maybe hindered. This can reduce the bacterial activity and results in smaller wall lesions. Conversely, the presence of larger gaps may facilitate the diffusion of nutrients to the deeper areas, making the bacterial biofilm more active, eventually leading to wall lesions of larger sizes.[16]

VOIDS UNDERNEATH RESIN-MATRIX COMPOSITE RESTORATIONS

The bond strength of dentin adhesive resins is greatest when the surface of the dentin is slightly moist.[134-136] However, if the dentin surface is too wet the adhesive resin does not fully penetrate the dentinal tubules or the demineralized dentin.[45, 137, 138] Sources of wetness include fluid from the dentin tubules, hydrophilic primers, sulcular fluid, humidity of the mouth, and residual water left from the etching and washing process.[44] The presence of too much water can create small, isolated, blister-like spaces or voids that are trapped along the surface of the hybrid layer.[45] The voids are partially filled with extraneous resin globules dispersed within an amorphous matrix and are often continuous with incompletely sealed tubular orifices.[44] Excess solvent remaining on a primed dentin surface will prevent complete adaptation of bonding resin and may result in non-attachment or voids at the resin–dentin interface.[46, 139]

If the demineralized dentin at the gingival location of class II preparations is too wet and not fully hybridized, it can become vulnerable to hydrolytic breakdown and penetration by bacterial enzymes.[138] This could lead to compromising the integrity and strength of the dentin-adhesive bond and ultimately the restoration.[140, 141] Gaps between the resin-matrix composite and tooth also increase the risk for restorative failure and gingival margin of restorations appear to be especially likely to have such gaps.[142-144]

The anatomy of the tooth at the gingival wall may account for the vulnerability of restorations to gaps.[44] The density of dentin tubules 1.0 mm above the CEJ at the gingival cavity wall is 49% greater than at the axial wall.[145] After dentin is acid etched, the surface area of exposed tubules can increase from 1% to 13% at the DEJ and from 22% to 34% close to the pulp.[44] As the number of tubules increases, the pulp can force moisture in the form of intrinsic pulpal fluid to increase the moisture on the dentin surface. This excess moisture can reduce the adaptability of the restorative material to the prepared surface.[44]

Voids in restorations have an impact on the performance of adhesive materials. In a study to analyze the voids in class II resin-matrix composite restorations conducted by Purk et al., they found more voids through the adhesive at the gingival wall of the cavity than the axial wall.[44] These voids significantly affected the microtensile bond strength of the resin and were found to have more impact than the condition or the location of the restoration. The *in vivo* gingival group of their study had the lowest bond strength and the highest percentage of voids. This indicates that less predictable bonds occur during clinical procedures than what is observed by laboratory studies. Voids throughout the adhesive layer were found to be approximately 5–40 μm diameter blister-like spaces and resulted in stress

concentration and incomplete adhesion to dentin. Others reported more voids at the gingival wall occurring under in vivo conditions from moisture contamination due to hydrostatic pulpal pressure, dentinal fluid flow after removal of the smear layer, working in an environment where isolation of the tooth is difficult, pooling of solvents and adhesives at the corners of the preparation box, increased permeability of dentin at the gingival wall or probably the most important is the inability of air drying to remove excess water at this location.[138, 145-149] When contaminated with water, bonding resins that contain HEMA and Bis-GMA can undergo phase separation that can reduce the conversion level of the adhesive system by 50%. As little as 9% volume of water added to a HEMA/Bis-GMA bonding resin under wet conditions can weaken the resin by 64% as reported by Paul et al.[146] The study by Kakaboura et al.[43] further highlights these findings. They reported larger gaps and more porosity in dentinal cavities restored with light-cured resin-matrix composites. This was due to polymerization shrinkage from underlying underexposed areas and weak bonding effect due to dentin wetting by unreacted resin monomers before setting.

In an attempt to study the contraction gaps and marginal adaptation of microhybrid resin-matrix composites, Kakaboura and colleagues conducted a study where cervical cavities were prepared in human extracted molars and filled these cavities with microhybrid resin-matrix composites without any adhesive cavity pretreatment. After that, the specimens were imaged by computerized X-ray microtomography. Authors found significant difference between middle and bottom parts of the cavity; with larger gaps and more porosities occurring at the bottom of the cavity. The investigators explained that by a weak bonding effect due to dentin wetting by unreacted resin monomers before setting and by shrinkage compensation from underlying underexposed areas.[43]

Bergvall and colleagues[105] studies the initial gap developed around large resin-matrix composite restorations. They reported that the contraction gaps at the floor of the cavity varied between 2 and 20 µm and was usually around 9 µm. The gap was narrower along the lateral wall varying from less than 1 to 10 µm. In a similar attempt by Brännstrom et al.,[27] authors reported lack of adaptation of resin-matrix composite to the floor of large cavities due to entrapped air or fluid, from dentin or residual water, blocking the gap. Another reported reason was the polymerization of resin-matrix composite too rapidly.

RESIN-MATRIX COMPOSITE AND SECONDARY CARIES

In recent years, the use of resin-based composites for restoration of posterior permanent teeth has increased significantly, despite the fact that they are more technique-sensitive to place and more costly.[40] There is some evidence that the longevity of resin-matrix composite restorations is less than that of amalgam restorations in similar conditions.[94, 150] Bernardo and colleagues reported that the main reason for restoration failures was secondary caries which accounted for 87.6 percent and 66.7 percent of the failures that occurred in amalgam and resin-matrix composite restorations, respectively. They also reported that the risk of secondary caries in resin-matrix composite restorations was 3.5 times greater than in amalgam restoration.[39]

One study that compared the longevity of amalgam and resin-matrix composite restorations in teenagers and adults found out that the longevity of the restorations placed in teenagers was five to six years shorter than that of restorations placed in adults.[151] Poor oral hygiene habits, as presented in teenagers, may have been responsible for the elevated rates of secondary caries in the more susceptible resin-matrix composite restorations.[39] Braga and collaborators reported the main reason for

replacing resin-matrix composite restorations in Brazil was secondary caries while the main reason for replacing the more durable amalgam restorations was to achieve better esthetics.[11] Secondary caries and poor appearance accounted for equal proportions of composite restoration failures according to Wilson et al.[6]

BONDING TO ENAMEL AND DENTIN

In 1955, Buonocore[49] introduced the acid-etch technique. He used phosphoric acid to roughen enamel surfaces in order to create micromechanical retention with resin-matrix composites through the formation of resin tags that fill the microporosities produced by the acid.[152, 153] Since then, bonding to enamel has been considered predictable, long lasting and successful.[54, 55, 152] When resin-based composite is placed on properly etched enamel, regardless of how long the resin-matrix comspoite has been bonded to the tooth, it is nearly impossible to remove the resin from the surface of the tooth without utilizing rotary instruments.[54]

Bonding to dentin has had a different and less successful history.[54] The main reason for the less predictable dentin bonding is the difference in its composition. Dentin is a less mineralized tissue containing more organic materials than enamel. Furthermore, the moist tubular ultrastructure of dentin leads to the formation of a smear layer when dentin is instrumented.[50, 153, 154] These characteristics were found to impair the bonding capacity of resin materials to this substrate[38] and make adhesion to dentin more complex than adhesion to enamel.[55]

With the use of early hydrophobic resins, acid treatment of dentin did not produce bond strengths similar to those achieved when bonding to enamel surfaces.[155] Later, adhesive systems incorporating acid-etching and hydrophilic monomers were

developed.[152] Enamel and dentin acid-etching could be done simultaneously and there was an increase in the adhesive bond strength.[152, 156]

Current dentin adhesives employ two different means to achieve the goal of micromechanical retention between resin-matrix composite and dentin. The first method removes the smear layer completely and demineralizes the subsurface intact dentin via etching with acids. Following rinsing, a multi-step application of a primer and an adhesive, or a simplified self-priming adhesive is applied to the conditioned substrate to complete the bonding protocol.[55]

The second method uses the smear layer as a bonding substrate. There are two types of simplified adhesives that are applied to the smear layer. One is a self-etching primer that includes two steps: the primer is applied without rinsing then a layer of adhesive resin is applied. The other type is more simplified, one-step self-etching adhesive that includes a single application to the tooth.[55]

High early bond strengths of current adhesive systems to dentin have been reported[157] with bond strengths approaching those of enamel bonding.[52-54, 158] However, the durability of these adhesive bonds is still one of the areas of current interest in adhesive dentistry.[55] Thermal cycling simulates the introduction of hot and cold extremes in the oral cavity and shows the relationship of the linear coefficient of thermal expansion between tooth and restorative material. Thermal cycling stresses the bond between resin and the tooth and may affect bond strength.[159, 160] Davidson et al.[161] examined the durability of the shear bond strength of adhesive systems to human dentin by thermocycling the specimens up to 300 cycles. They observed a significant decrease in bond strength after thermal cycling depending on the adhesive system tested. Price et al.[162] also reported thermal cycling up to 5000 cycles had a very

significant negative effect on bond strength in human dentin when a high C factor testing design was used.

During thermal cycling the specimens are subjected to thermal changes and also to additional exposure to water.[55] Thermal stresses generate mechanical stresses by differences in the coefficient of thermal expansion and can result in bond failure at the tooth-restorative interface.[163] However, a major cause for the reduction in bond strengths is believed to be the possible effect of hydrolysis at the interfaces of the bonding resin and hybrid layer,[55] which might be enhanced by the thermocycling process. Burrow et al.[164] reported that the bonded resin absorbs a significant amount of water which may adversely affect the longevity of restorations. Furthermore, cured single-step adhesives may act as semipermeable membranes allowing water diffusion from the bonded hydrated dentin to the intermixed zone between the adhesive and the resin-matrix composite, as reported by Tay et al.[165]

Permeability of single-step adhesives to water may hasten the rate of water sorption and leaching of resin components,[166] challenging the durability of resin-dentin bonds produced by these adhesives.[55] This explains why bond strength to dentin decreased on aging of self-etching adhesives. Other researchers have reported the bond strengths in dentin dramatically decreased[56] and leakage was gradually increased at the dentin interfaces.[57]

EFFECT OF ANATOMIC VARIATIONS ON DENTIN BONDING

Differences in anatomic structures in different parts of the tooth can have an effect on resin-matrix composite bonding. Research shows that resin bonded under in vitro conditions in class V lesions to parallel-oriented tubules located in the occlusal part of the tooth had higher microtensile bond strengths than resin bonded to

perpendicularly oriented tubules in the gingival locations.[167, 168] Tubule direction may also determine the intrinsic wetness of the surface.[168] The gingival wall of a class II cavity contains predominantly perpendicular oriented tubules.[169] Cutting and etching tubules oriented in a perpendicular direction, such as in gingival walls, might result in a continuous reservoir of fluid that is fed by capillary action resulting in a wetter surface. On the other hand, cutting and etching tubules in a parallel direction might result in a less fluid oozing from the dentinal tubules without a continuous feed. These differences in the anatomy of the two locations may account for the larger number of debondings at gingival sites than at axial sites.[44]

Voids along the tooth–restoration interface resulting from excessive moisture can result in fluid and bacterial movement through the interface that can be experienced by the patient as post-operative sensitivity and recurrent caries.[170, 171] Narrow gaps, crevices, ditches, and microleakage do not lead to secondary caries, but wide voids may.[14]

Clinicians interested in enhancing bond strength must control the amount of wetness on the dentin surface. In order for a good bond to develop between the dentin and adhesive, all excess moisture on the dentin surface should be replaced by monomers in the primer and adhesives during the bonding procedure.[44] It is very difficult for an operator to determine if the dentin surface is ‘too wet’ or ‘too dry’. Rinsing and drying steps are difficult to standardize under clinical conditions.[137, 148] Ethanol or acetone adhesives that displace water and are more volatile might behave better in a wet environment at the gingival wall of class II preparations. Reducing the amount of time dentin is etched might also reduce the amount of wetness from the dentinal tubes.[44]

As mentioned earlier, in vitro conditions may over-estimate bond strengths in in vivo conditions. Greater effort should be directed to in vivo approaches to bond strength testing in order to determine the optimal etchants and etching times for conditioners used on dentin.[172, 173] Reducing the number of voids at the adhesive dentin interface will improve the tensile bond of the adhesive to the dentin and improve clinical outcomes for patients.[44]

The occurrence of pathophysiological alterations can affect the bond strength to dentin.[55] Variations such as sclerotic dentin and hypermineralization associated with erosion or abrasion lesions have been reported to cause complication when dentin adhesion is attempted.[50, 174]

POLYMERIZATION SHRINKAGE OF RESIN-MATRIX COMPOSITES

Marginal debonding of light-cured resin-matrix composites from dentin cavity walls due to setting shrinkage has long been identified as a major polymerization defect of great clinical implications.[47, 48] Many efforts have been undertaken to reduce the effect of this phenomenon and consequently minimizing the interfacial gaps formed. These include the introduction of low-shrinking monomers,[175, 176] increase in the filler volume loading and development of new types of filler, use of incremental placement techniques, development of irradiation modes that provide increase plastic flow during pre-gel polymerization state and implementation of flexible cavity liners to counteract the composite shrinkage stresses.[177-180] However, despite all these advances, production of gaps around resin-matrix composites remains a major clinical dilemma.[43]

The bond strengths obtained in dentin is not always strong enough to counteract the stresses developed during polymerization shrinkage which affect the

sealability. Cavities with high C-factor, which is the ratio between bonded and unbounded surfaces, could increase the shrinkage stress at the adhesive interface, consequently, impairing the sealing ability.[181] Amaral and colleagues[180] attempted to study the effect of polymerization shrinkage of resin-matrix composite restorations on microleakage and gap sizes formed. They utilized vertical slot cavities prepared in bovine teeth that were filled with resin and then thermocycled. They concluded that thermocycling significantly increased the gap formation, but, they did not observe a correlation between microleakage and gap formation.

MICROLEAKAGE

Secondary caries formation depends on the interaction of the sealing stability of the restoration and the microenvironmental conditions of the oral cavity. The lack of marginal integrity in either enamel or dentin induced by polymerization shrinkage of resin-based materials should be counteracted.[182]

Microleakage is the penetration of fluids, bacteria, toxins, ions and other molecules that can be observed at the tooth-restoration interface.[81] During the past few years, considerable efforts have been focused on the development of new adhesives to prevent secondary caries and restoration failures.[183-185] Marginal breakdown and microleakage due to polymerization shrinkage of resin-matrix composite occur if the bonding system is not able to prevent postcuring composite shrinkage.[184, 186] Since the micromechanical adhesion to dentin takes place with the formation of the hybrid layer,[165] the bonding stability is greatly dependant on the hybrid layer integrity and ability to resist demineralization of the marginal dentin and gap formation.[187]

One of the most common techniques employed to evaluate microleakage is by using the dye method. In this technique migration of a dye along the tooth-restoration interface is evaluated.[153] Although this method is considered fast and economic, the subjectivity in reading the specimens has been noted as a shortcoming related to this methodology.[188] Araujo[153] and colleagues used this technique to evaluate the microleakage of seven adhesive systems on enamel and dentin. They detected higher leakage in dentin when compared to enamel with all the adhesive systems tested. According to them the reason for that was that bonding to enamel is stronger and more stable than the bonding to dentin[38] due to the difference in composition between these two substrates.[154]

In a recent study to evaluate the in situ influence of microleakage and surface roughness on caries formation around composite restorations, the investigators concluded that the presence of microleakage at the adhesive interface did not affect significantly the enamel demineralization.[8] Cenci and colleagues conducted a randomized, double-blind in situ study to investigate the association between microleakage and secondary caries. Results from their study reinforced the concept of lack of association between microleakage and caries adjacent to restorations.[37] Although microleakage is still considered a potential etiological factor for secondary caries,[15] most of the studies suggesting this were performed in vitro.[67]

In summary, the inherent polymerization shrinkage of resin-matrix composites can produce gaps between tooth-restoration interfaces.[38] Microleakage has been strongly associated to marginal gaps[189] and in vitro studies have associated the presence of secondary caries with microleakage.[15] However, clinical findings have not supported this association.[9]

STUDY DESIGN

A secondary caries bacterial model developed by Totiam et al.[16] was used to study the effect of the space between the restoration and the dentinal wall of the tooth on secondary caries development. The steps of the study are shown in Figure 2.

Four groups of specimens were used (Figure 3). Each group consisted of ten specimens. The first group had a uniform gap size of 30±10 μm throughout both enamel and dentin. This group acted as a lower limit for caries development (negative control). The second group had a 30±10 μm enamel gap size with a 530±10 μm dentinal gap. Group three had a 525±20 μm gap in both enamel and dentin. The final group had a 525±20 μm and a 1025±20 μm gap in enamel and dentin, respectively (Table I).

The lower limit used for gaps in the study was 30 μm since it has been shown that secondary caries wall lesion development is minimal at gaps of this range.[16, 114] The 500 μm gaps used in groups two through four represent clinically unacceptable restoration margins that would require operative intervention.

The within-group standard deviation estimate for the wall lesion area used in the sample size calculations was estimated to be 5000 μm^2 based on the study by Totiam et al.[16] With a sample size of 10 specimens per group, the study was expected to have 80% power to detect a difference of 8632 μm^2 between any two groups, assuming two-sided significance tests at an overall significance level of 5%.

The groups were sterilized and then incubated in the microbial caries model referenced above with *Streptococcus mutans* TH16 in 1% sucrose tryptic soy broth for 1 h, four times daily, and with a buffer solution for the rest of the day for a period of 8 days. After the incubation period, lesions size was measured quantitatively using Confocal Laser Scanning Microscopy (CLSM).

MATERIALS AND METHODS

TOOTH SPECIMEN PREPARATION

Specimens were obtained from extracted human molars stored in 0.1% Thymol solution (IRB#0306-64). Selected teeth were examined for any signs of caries, cracks or other defects. Teeth with any defects were eliminated. Selected teeth were cut in half in a bucco-lingual direction using a high speed cutting machine (Lil' Trimmer, Lapcraft, Inc., Powell, OH). Proximal surfaces were used to obtain the specimens (Figure 4). Cut teeth were mounted on plastic plates using sticky wax with the CEJ parallel to the surface of the plastic plate. Two parallel saw blades (Isomet, Buehler, Lake Bluff, IL) were used to cut the specimens, 1mm above and 3mm below the CEJ (Figure 5).

A final specimen block with 4 × 4 × 2.5 mm dimensions and a thickness of 2.5 mm was obtained (Figure 6) with an enamel thickness on the simulated cavity wall in the range of 300–500 μm. Specimens were then mounted on plastic blocks using sticky wax and the simulated cavity wall surface was ground using 500-grit, 1200-grit, 2400-grit and 4000-grit sand papers followed by polishing with 1 μm diamond abrasive paste on a polishing cloth (Figure 7). Both grinding and polishing were done using a rotary polishing machine (RotoPol 31/RotoForce 4, Struers Inc., Westlake, OH). The enamel thickness on the simulated cavity wall was measured using a stereomicrospe (Nikon Stereocopic Zoom Microscope SMZ1500, Nikon Digital DXM1200F camera, Japan) and analyzed with imaging software (Nikon ACT-1 version 2.63 software, Japan)

Specimens designated for groups 2 and 4 were further modified to achieve the non-uniform geometry (Figure 8). Specimens were mounted on plastic rods using sticky wax. An abrasive disc was used to mill the specimens utilizing a slow speed

milling machine (Custom designed by Stellar Systems, Inc., USA) (Figure 9). The edge of the disc was aligned with the enamel margin on the cavity wall. Then, the disc was moved horizontally until the dentino-enamel junction (DEJ) was visible (this corresponded to 300-500 μm of horizontal movement depending on the enamel thickness of the specimen). The disc was further moved horizontally until the distance from the edge of the disc to the DEJ was the same as the distance from the DEJ to the outer edge of the enamel. This procedure was conducted with the help of a digital camera (Panasonic CCTV camera, model WV-CP284, Japan) that magnified the region of interest. Then, the milling machine was turned on and the disc was gradually lowered until it touched the specimen (verified by the digital camera). Once the disc came into contact with the specimen surface, the micrometer of the machine was set to zero and gradual milling was performed until the indicator reached the 500 μm mark. Occasionally, the disc was further lowered to the 510 or 520 μm marks to compensate for the resistance encountered during the milling process.

This newly obtained surface “step” was examined under the stereomicroscope to ensure that the distance from the DEJ to the edge of the step was within the acceptable range of 300-500 μm and the distance from the outer edge of enamel to the edge of the step was within the 600-1000 μm range. The step was, then, polished using fine and ultrafine discs (Sof-Lex 3M ESPE, Dental Products, St. Paul, MN). The cavity surfaces of all the specimens were polished with ultrafine Sof-Lex discs. All specimens were washed thoroughly with deionized water.

RESIN-MATRIX COMPOSITE SPECIMEN PREPARATION

Resin-matrix composite specimens were made by plugging 2 increments of resin-matrix composite (Point4, Kerr, CA, USA) into a custom-made silicone mold

made from vinyl polysiloxane impression material. Each increment was light cured for 40 seconds. Before curing the second increment, a mylar strip and a glass slide were placed on the resin-matrix composite. Resin-matrix composite bars with 11 × 4 × 2.5 mm dimensions were produced. Each bar was further sectioned to obtain two 4-mm-wide blocks corresponding to the size of the tooth specimens (Figure 10).

PROFILOMETRIC ANALYSIS

Since the creation of the modified wall was a modification of the original model, profilometric analysis was conducted to assess the effectiveness of the polishing technique utilizing Sof-Lex discs on the different surfaces of the specimen.

Five random specimens involving a dentin step were analyzed using an optical profilometer (Proscan 2000; Scantron, Venture Way, Taunton, UK). On each specimen, three areas were examined. A 200 µm×200 µm area was analyzed on the enamel and 500 µm×500 µm areas were analyzed on the dentin and the dentin step areas. Images were analyzed using dedicated software (proscan 2000; Scantron). The results of the scan were interpreted as the average of R_a values in the X and the Y direction of the five specimens.

MODEL ASSEMBLY

Tooth and resin-matrix composite specimens were mounted on a custom made specimen stage that allows for the creation of different gap sizes between tooth and resin specimens (Figure 11). Gaps were created by inserting shim stocks of different thickness at the tooth/resin interfaces. A 25 µm thick shim stock (Tri-Mat, Matrix Strip Kit, ¼ inch, Pascal Co. Inc., Bellevue, WA, USA) was used in groups 1 and 3 and a 500 µm shim stock was used in groups 2 and 4. Cyanoacrylate cement was used

to fix the specimens on the stage after being aligned to the stage interface by the use of a reference square block.

After the glue was dry, the sliding stages were then tightly closed to the fixed one by turning an attached screw. The resin-matrix composite specimens were mounted next to the tooth specimen separated by the specific metal strip creating the desired gap size. Digital images of the gap were taken under a microscope (Leco LM247AT, Hitachi CCD camera, model XP-M1AN, Japan) and analyzed (Confident software 2.5.2, USA) to verify that gap widths were within the expected gap size. Specimens with gaps outside this range were excluded from the experiment.

STERILIZATION

The sliding stage of each model was opened until a 2-mm gap size between tooth and resin-matrix composite specimens was achieved. Then, models were attached to Petri dishes (100×25 mm) (Extra-deep Petri dishes, 100x25 mm, Fisher catalog #08-757-11, USA) (Figure 12). The 40 specimens were assigned to 3 Petri dishes. The dishes were exposed to ethylene oxide gas for 1 hour, in moist condition, at 55°C (8XL sterilizer/aerator, 3M Health Care, USA). After aeration for 12 hours, in the same moisture and temperature conditions, specimens were soaked in sterile deionized water for 1 hour at room temperature.

DEMINERALIZATION AND REMINERALIZATION

The samples were inoculated with 50 µl of a fresh overnight culture of *Streptococcus mutans* (TH16) in tryptic soy broth with 1% sucrose (TSBS, Bacto, Dickinson and Company, Sparks, MD, USA) at an absorbance of 0.536 at 540 ηm (Spectronic 20D+, TheroSpectronic, USA) and incubated in a 5% CO_2 environment for 2 hours at 37°C. After this period, the sliding stages were closed to the designated

gap size under a laminar flow hood. The stages were incubated daily with 50ml mineral wash buffer solution without fluoride (pH=7.71) for 20 h/day and TSBS (pH=7.55) for 4 h/day (1-hour incubations, 4 times/day) in a 37°C environment.

After 8 days, the TSBS was discarded; the stages were rinsed with deionized water, cleaned with an ultrasoft brush (449 Ultra Soft, Butler G-U-M, John O. Butler Company, Chicago, IL, USA) and re-rinsed with deionized water. The tooth specimens were then removed from the models and gently cleaned with cotton pellets and deionized water.

CONFOCAL LASER SCANNING MICROSCOPE ANALYSIS

The tooth specimens were glued on plastic rods before being covered with an acrylic resin (SNAP, Parkell, Farmingdale, NY, USA) (Figure 13). The specimens were cut in half using a hard tissue microtome (Silverstone-Taylor Hard Tissue Microtome, Series 1000 Deluxe, USA). Specimen halves were stained overnight with a 0.1 mM solution of rhodamine B (Aldrich Chem. Co., Milw., WI, USA) (Figure 14). The dentin part of the specimens was immediately analyzed after taking the specimens out of the dye with a confocal microscope (Zeiss LSM 150, Carl Zeiss Inc.) using a helium/neon laser with 543-ηm excitation wavelength, a 25 μm confocal slit, and a long pass barrier filter between 565-m and 615-m (Table II). The specimens were allowed to air-dry for 1 h before the enamel part was analyzed. Digital images were taken and analyzed (Metamorph software, 5.0.1, Universal Image Corp., West Chester, PA) for lesion severity. Different parts of the lesion were measured as lesion area in four locations as follows (Figure 15 and Figure 16):

1- Enamel Outer Lesion (EOL): the first 250 μm on the enamel surface starting at the enamel border.

2- Enamel Wall Lesion (EWL): between the inner border of the outer lesion and the DEJ.

3- Dentin Wall Lesion-A (DWL-A): the first 150 µm next to the DEJ.

4- Dentin Wall Lesion-B (DWL-B): 150 µm measured at a depth of 750 µm from the DEJ.

The value for each specimen was recorded as the average of the sectioned halves. Analysis of lesion areas in these four locations and comparison between the groups were done.

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) was used to test for differences between the groups for enamel outer lesion (EOL) area, enamel wall lesion (EWL) area, dentin wall lesion area next to the DEJ (DWL-A), and dentin wall lesion area 750 µm from the DEJ (DWL-B). Pair-wise comparisons between the groups were performed using Tukey's method to control the overall significance level at 5% for each outcome.

RESULTS

Results of different group comparisons are illustrated in Figures 17 to Figure 21. Groups did not have significantly different EOL area (p=0.41). Although the overall test for any difference between groups for enamel wall lesion area was significant (p=0.0185), none of the individual group comparisons were significant However, there was a clear numerical trend towards higher EWL area for Group 4 than for Groups 1 and 2.

Group 3 had significantly higher DWL-A and DWL-B area than Groups 1 and 2, and significantly higher DWL-B area than Group 4. Group 4 had significantly higher DWL-A and a numerical trend towards higher DWL-B than Group 1.

Results from the profilometry scan are provided in Table III. Although, some decrease in the surface roughness of the dentin surface was observed, little difference was noticed on the values of enamel and dentin step areas. Furthermore, It was observed that the average R_a value of the step area (R_a=2.47) was approximately 8 times the value of the dentin area polished with the Struers machine (R_a=0.3).

TABLES AND FIGURES

TABLE I

Summary of gap sizes used in the four groups of the study

Group	Enamel Gap Size (μm)	Dentin Gap Size (μm)
1	30±10	30±10
2	30±10	530±10
3	525±20	525±20
4	525±20	1025±20

TABLE II

The settings used for the confocal laser scanning microscope analysis of the specimens

Parameter	Enamel	Dentin
Laser Wavelength	543 ηm at 20%	
Pass Barrier Filter	565-615-ηm	
Pin Hole	160	158
Detection Gain	308	576
Amplification Offset	- 0.1	0.017
Amplification Gain	1.2	1

TABLE III

Summary of the profilometric analysis of five random specimens with step surface fabricated

Surface	Prepolish R_a Average	Postpolish R_a Average
Enamel	0.36	0.35
Dentin	0.42	0.30
Step	2.66	2.47

R_a was calculated as the average of the values in the X and Y directions.

TABLE IV

Summary of the results obtained by confocal laser scanning microscopy for the four secondary lesion areas

	Group	N	Mean	SD
Enamel Outer Lesion	1	10	8497	2319
	2	9[a]	8808	3877
	3	10	9833	4798
	4	9[a]	11888	6802
Enamel Wall Lesion	1	10	8765	4045
	2	9[a]	8570	4347
	3	10	14446	8823
	4	10	15577	4970
Dentin Wall Lesion A	1	10	4247	4239
	2	10	6950	4893
	3	10	12574	1571
	4	10	10495	4205
Dentin Wall Lesion B	1	10	1090	2329
	2	10	2703	2966
	3	10	11859	6166
	4	10	6015	4371

[a] A specimen could not be analyzed due to damage occurred during sectioning.

TABLE V

Results of the statistical analyses of the between-group comparisons, showing the p-value of each comparison

Comparison	Enamel Outer Lesion	Enamel Wall Lesion	Dentin Wall Lesion A		Dentin Wall Lesion B	
1 vs 2	0.9989	0.9999	0.4283		0.8282	
1 vs 3	0.9190	0.1574	0.0002 [a]	1 < 3	0.0000 [a]	1 < 3
1 vs 4	0.4054	0.0652 [b]	0.0058 [a]	1 < 4	0.0607 [b]	
2 vs 3	0.9638	0.1530	0.0148 [a]	2 < 3	0.0001 [a]	2 < 3
2 vs 4	0.5110	0.0648 [b]	0.2024		0.3119	
3 vs 4	0.7755	0.9733	0.6429		0.0190 [a]	3 > 4

[a] indicates statistical significance ($p \leq 0.05$).
[b] indicates marginal statistical significance.

FIGURE 1. A diagram showing the parts of the secondary caries lesion and their association with the gap at the tooth-restoration interface.

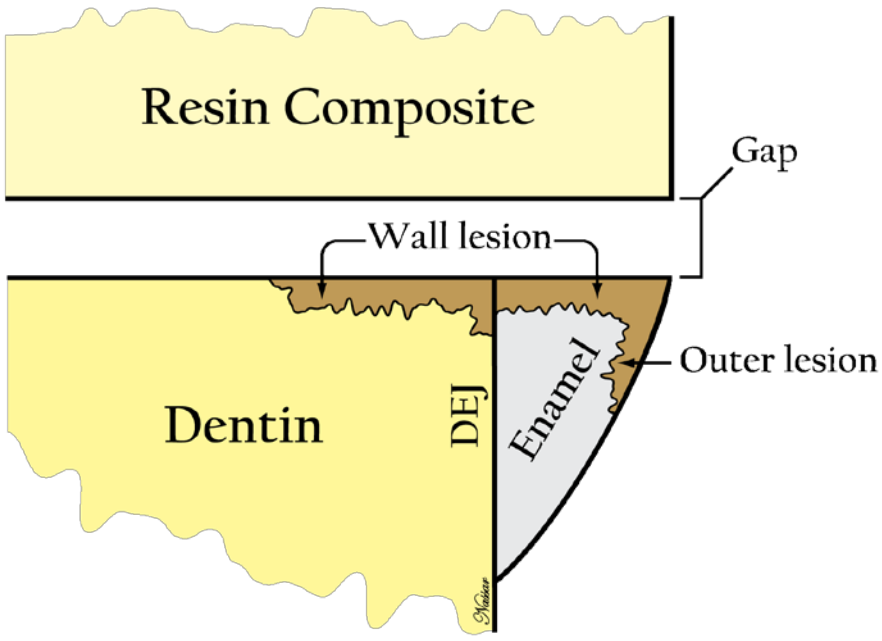


FIGURE 2. A flowchart showing the steps of the study

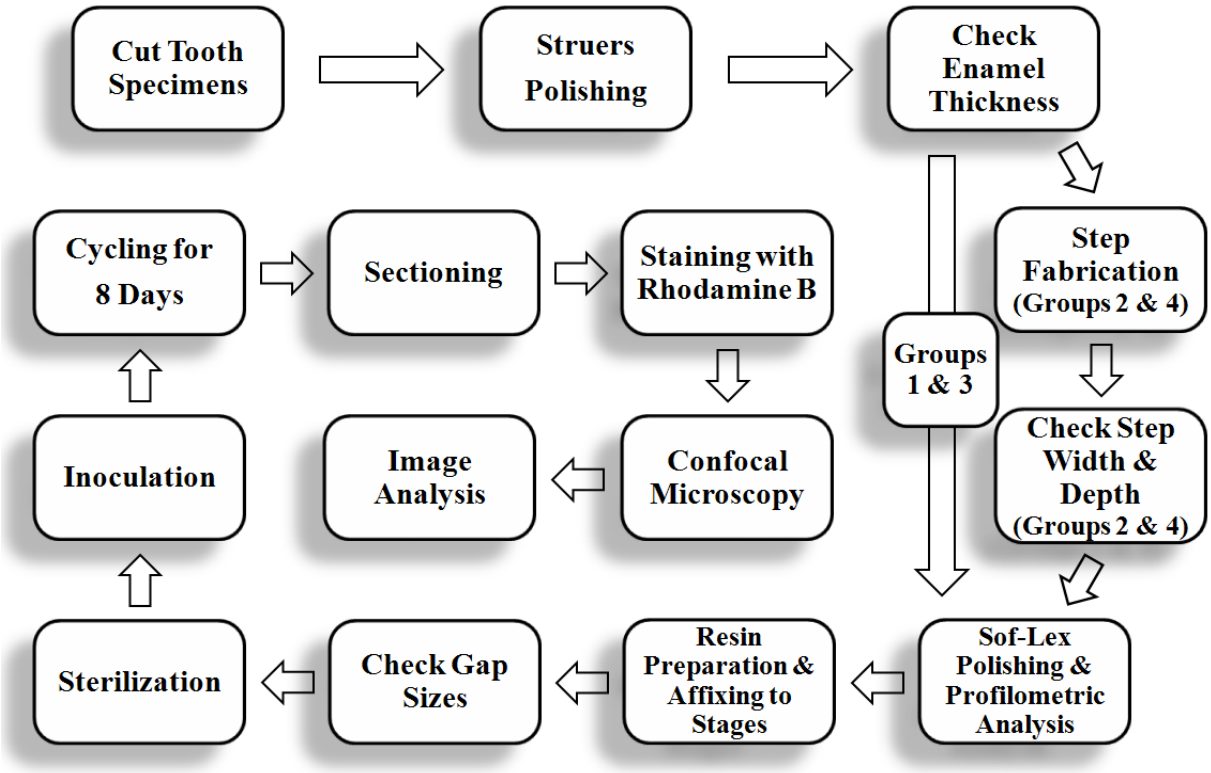


FIGURE 3. A diagram showing the four groups used in the study; group 1 (small gap and no step), group 2 (small gap with step), group 3 (large gap and no step) and group 4 (large gap with step).

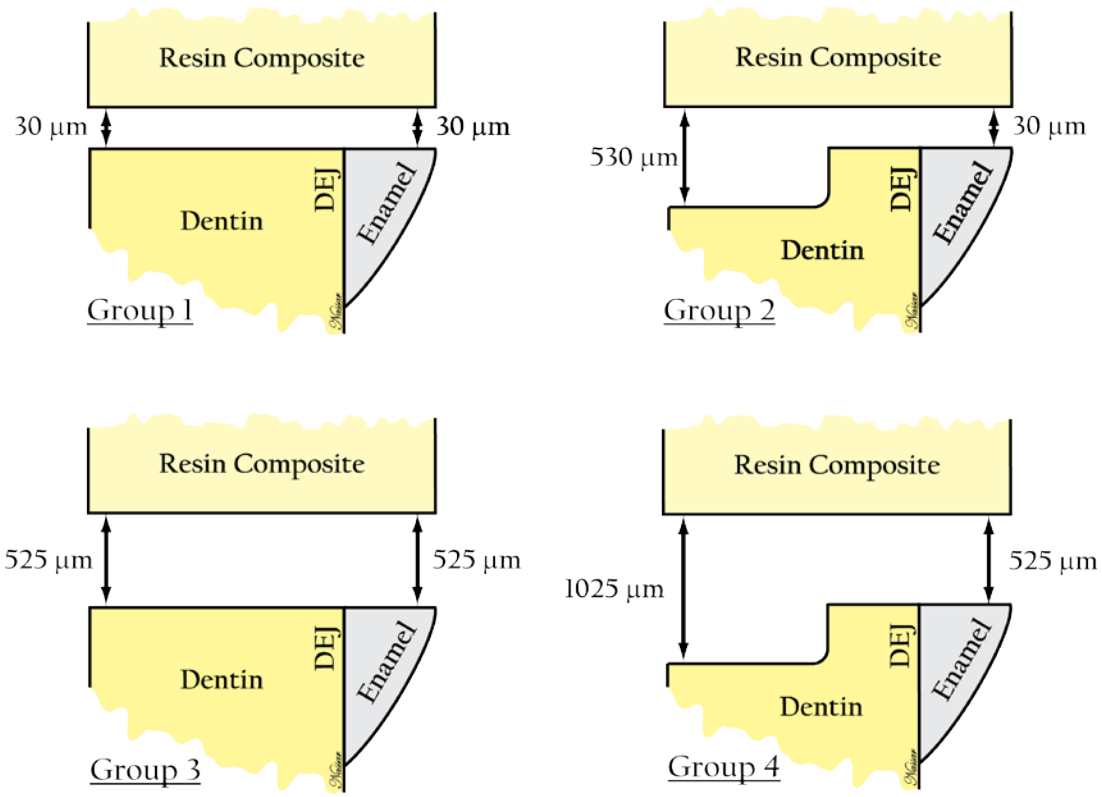


FIGURE 4. A diagram showing the location from which the specimens were obtained. Proximal surfaces of human molars were utilized. Horizontal cuts were made 1 mm occlusal and 3 mm cervical to the DEJ in order to control for enamel thickness.

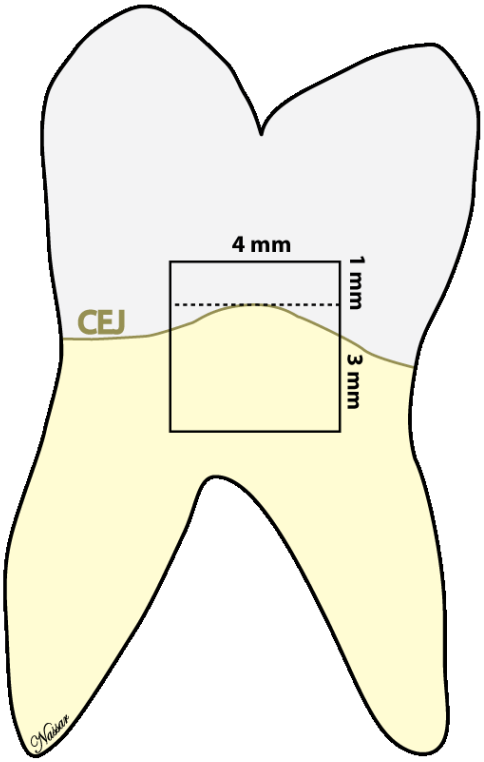
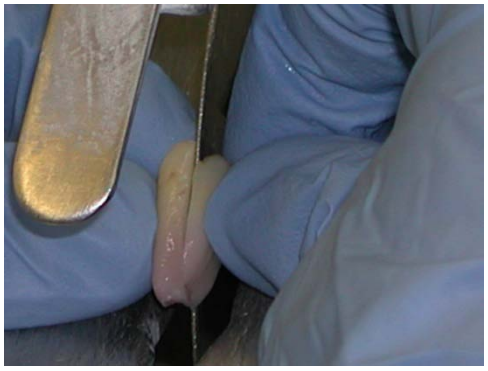
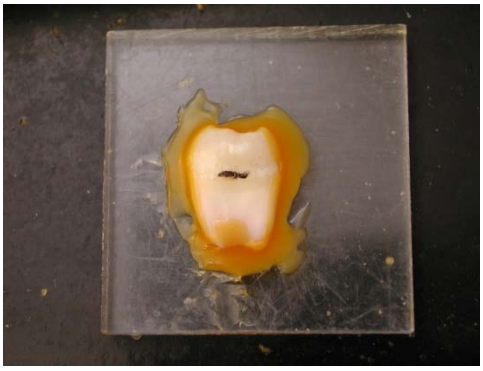


FIGURE 5. Photographs of the procedure for obtaining specimens. Human molars were cut in half using a high speed cutting machine (a), teeth halves were mounted on plastic plates using sticky wax with the CEJ parallel to the surface of the block (b), two parallel saw blades were used to cut the specimens (c), 4×4 mm specimens were obtained (d).

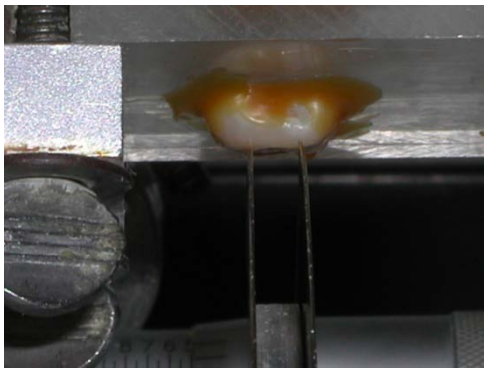
(a)

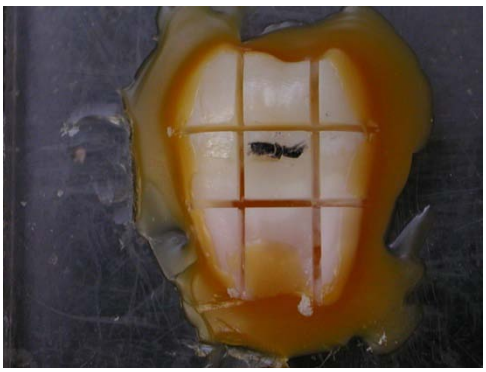




(b)

(c)





(d)

FIGURE 6. Diagrams of the final specimen block. Side view (left), direct view of tooth specimen wall (right).

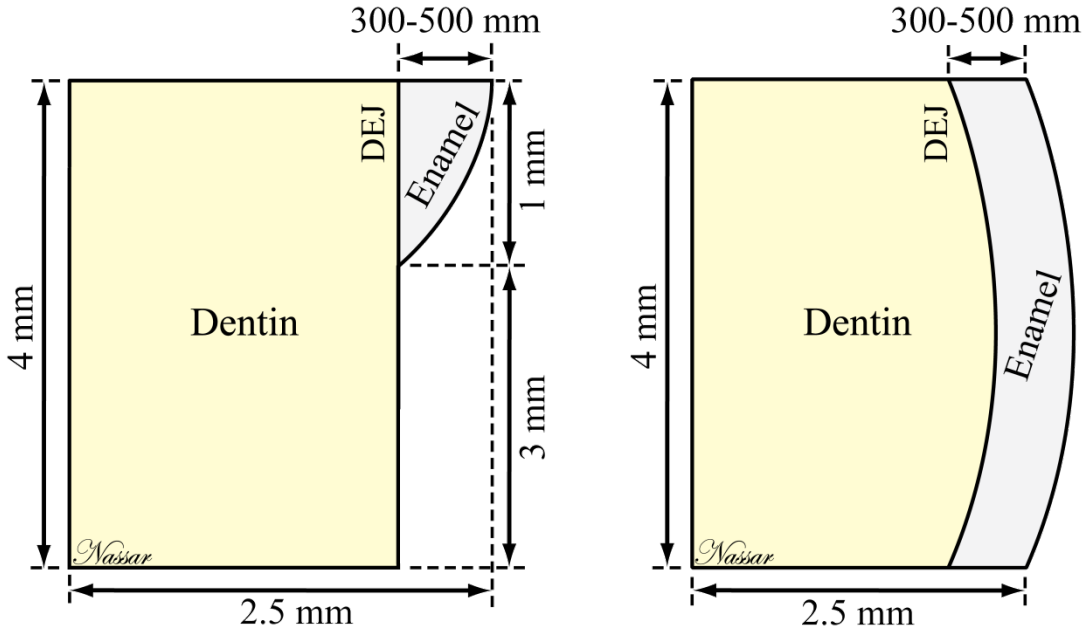


FIGURE 7. Photographs of polishing the simulated cavity wall of the specimens. Specimens were mounted on plastic blocks using sticky wax (a) and then ground and polished on a rotary machine (b).

(a)

(b)

FIGURE 8. Diagrams of specimens used in groups 2 and 4 after step fabrication, showing the location of the step area. Side view (left) and direct view of tooth specimen wall (right).

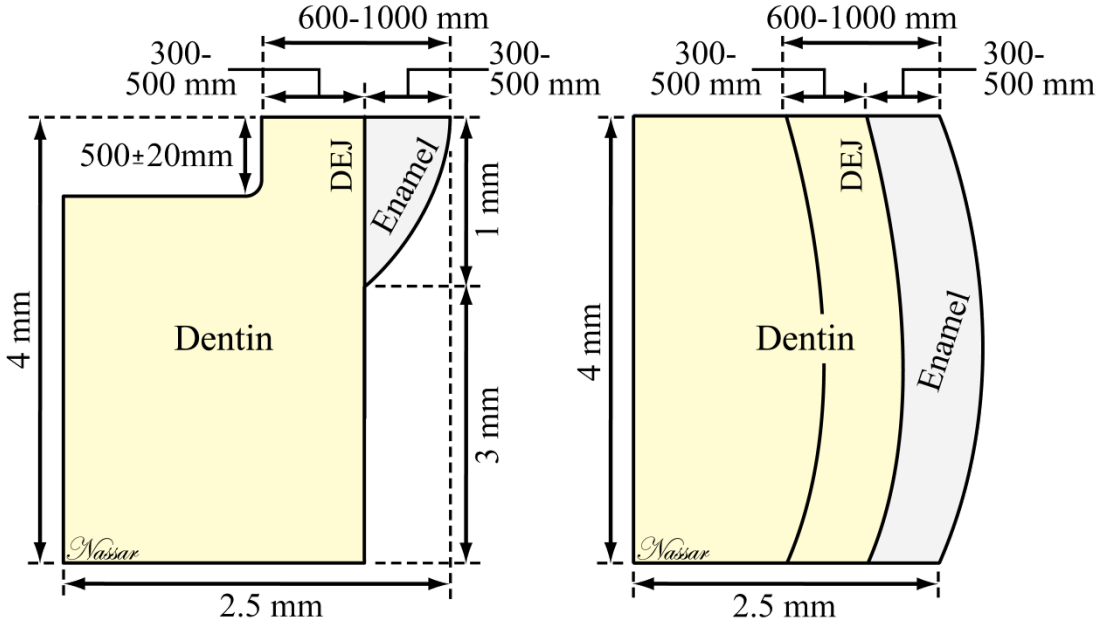


FIGURE 9. Diagrams of the steps for fabricating the additional wall space (“step”) for specimens in groups 2 and 4. The edge of the abrasive disc was aligned with the enamel margin (a), the disc was moved horizontally until the DEJ was visualized (b), the disc was further moved horizontally in an amount similar to the amount achieved in the previous step (c), lastly, the machine was turned on and the disc was lowered until the desired depth of 500 µm was reached (d).

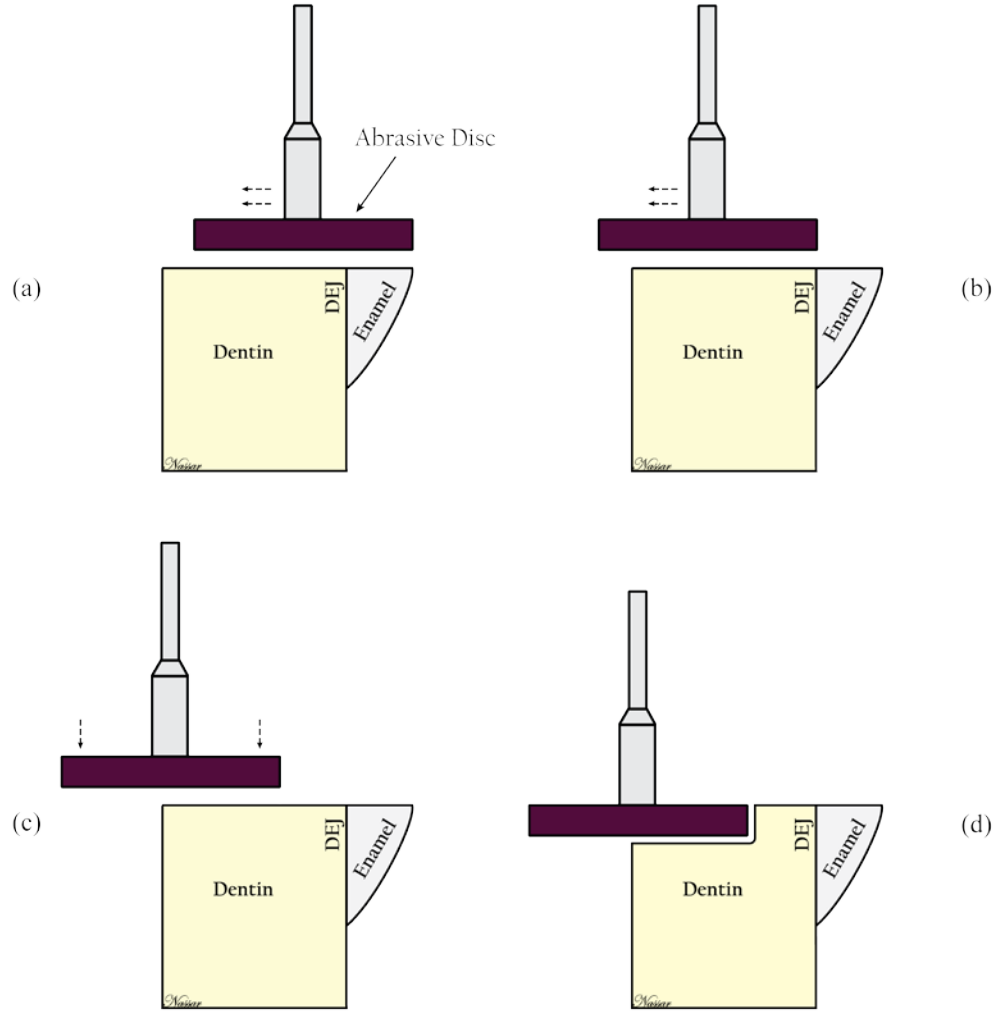
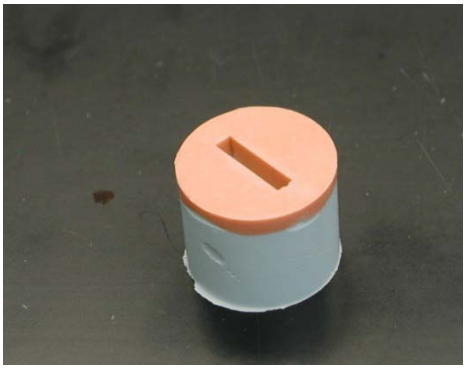
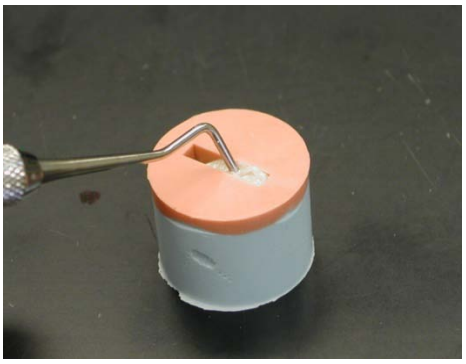


FIGURE 10. Photographs of resin-matrix composite preparation procedure. Vinyl polysiloxane mold (a), resin-matrix composite condensed into the mold (b), complete filling of the mold (c), a mylar strip and a glass slide placed (d), light curing (e), removal of the resin (f), removal of 11×4×2.5 mm bars from the mold (g), two 4 mm-wide resin blocks were obtained after sectioning of each bar (h).

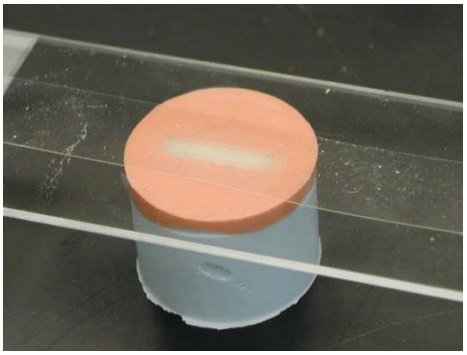
(a)





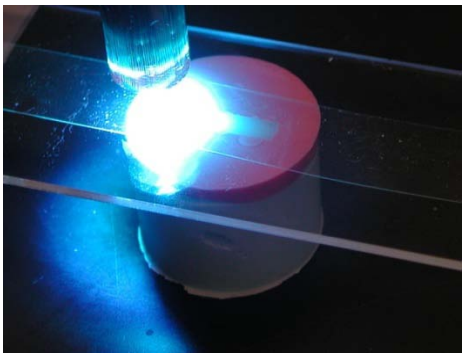
(b)

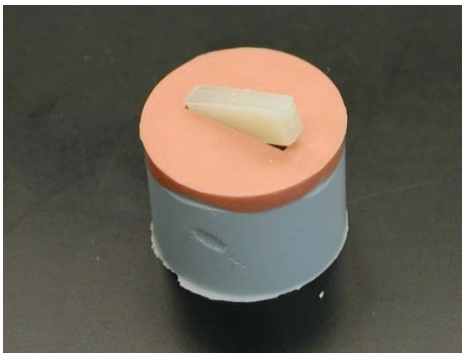
(c)



(d)

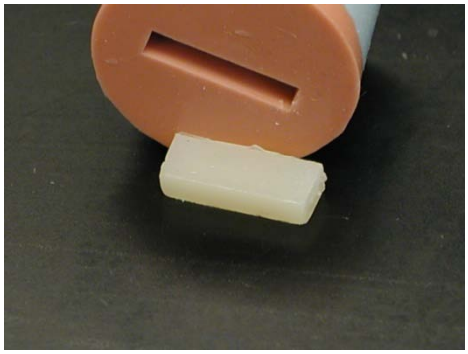
(e)

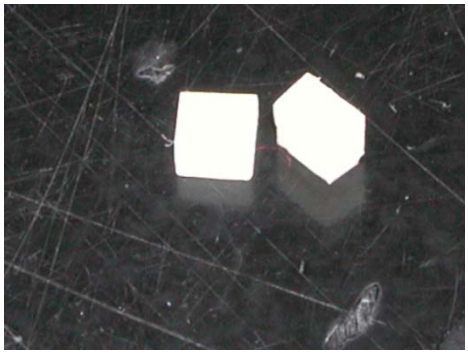




(f)

(g)

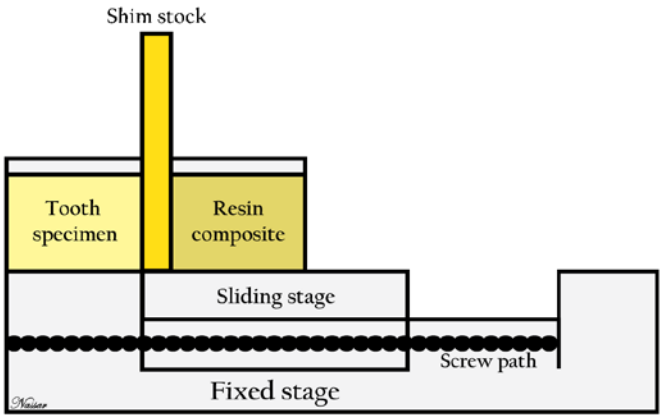




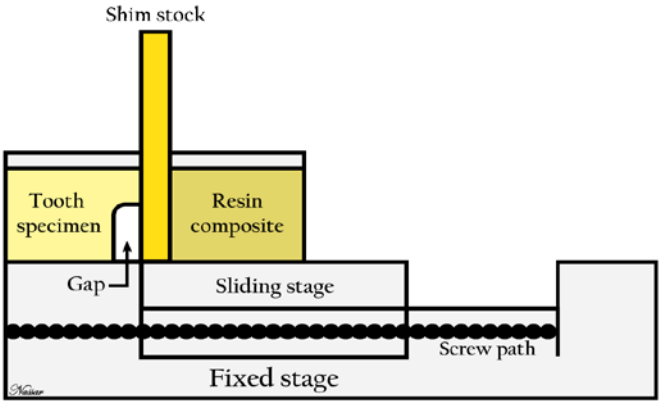
(h)

FIGURE 11. The custom-made stages used in the study. A diagram of stages used in groups 1 and 3 (a), a diagram of stages used for groups 2 and 4 (b) and a photograph of a stage after affixing the tooth and resin-matrix composite specimens (c).

(a)



(b)



(c)

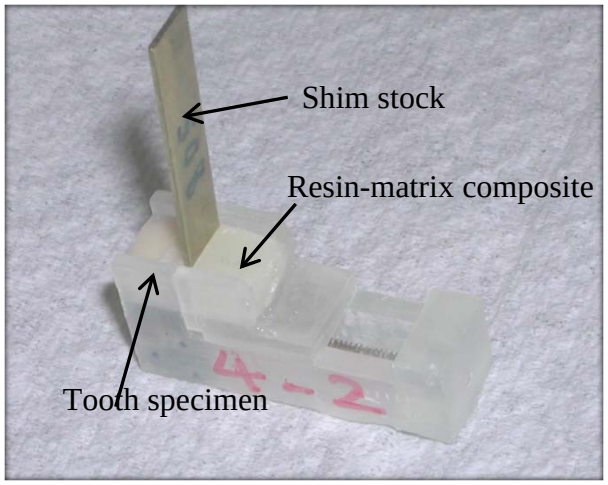


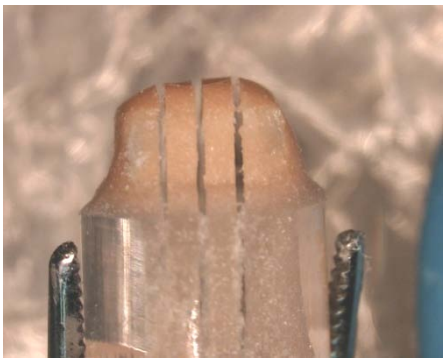
FIGURE 12. A photograph of stages glued to Petri dishes before sterilization.

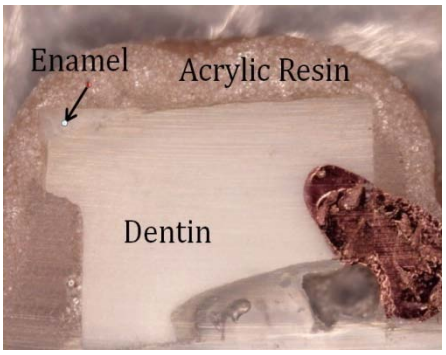
FIGURE 13. Photographs of cutting the specimens in the microtome. Specimens were glued on plastic rods (a), covered with acrylic resin (b), and then sectioned with a hard tissue microtome (c). Figure (d) shows a specimen's section.

(a)

(b)

(c)





(d)

FIGURE 14. A photograph of the specimen's sections in Rhodamine B solution in preparation for confocal laser scanning microscopy.

FIGURE 15. A diagram of the different areas of the lesion that were analyzed. Enamel outer lesion (the first 250 µm on the enamel surface starting at the enamel border), Enamel wall lesion (between the inner border of the outer lesion and the DEJ), Dentin wall lesion-A (first 150 µm next to the DEJ) and Dentin wall lesion-B (150 µm measured at a depth of 750 µm from the DEJ). Note that Dentin wall lesion-B for groups 1 and 3 is represented by the solid line. Whereas, Dentin wall lesion-B for groups 2 and 4 is represented by the dashed line.

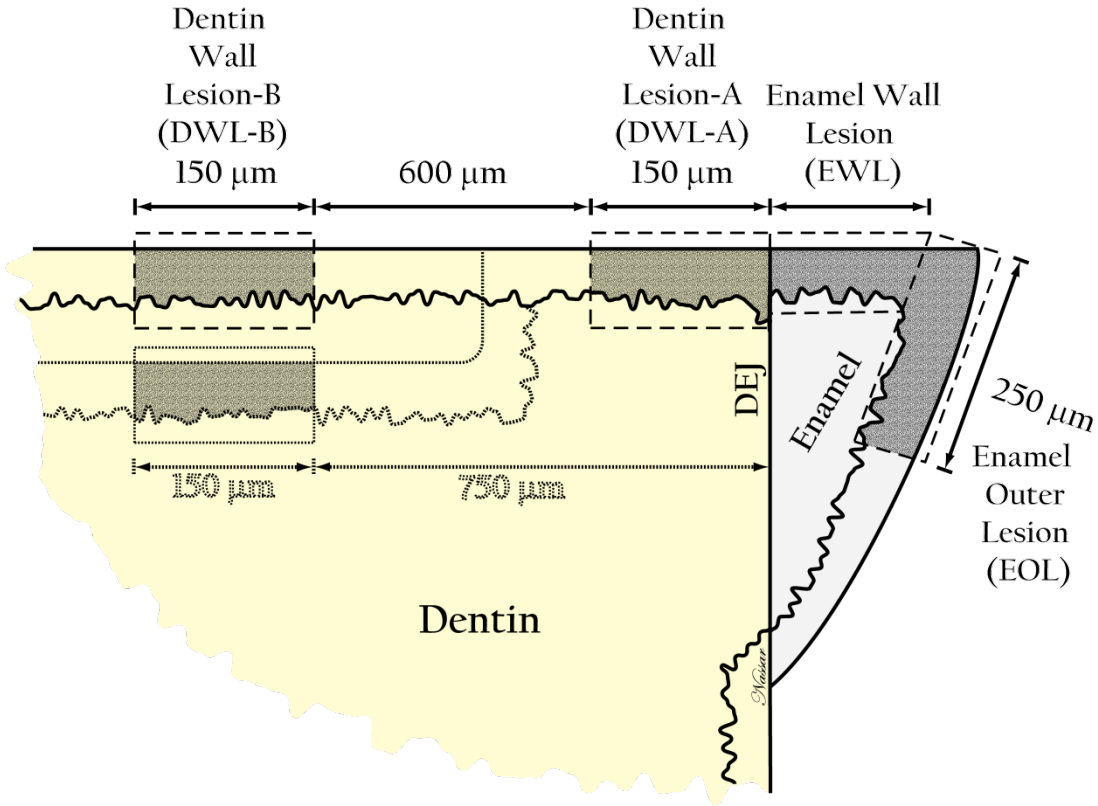
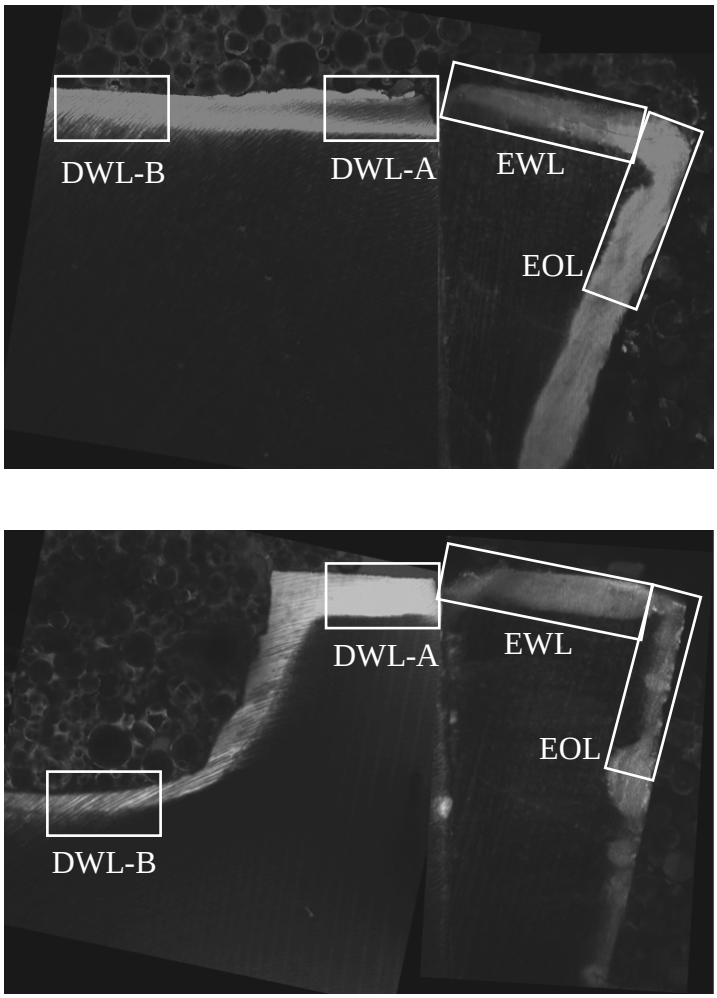


FIGURE 16. Digital composites of multiple confocal micrographs illustrating the four areas that were analyzed. Top view showing a specimen from group 3 and bottom view showing a specimen from group 4.



EOL: enamel outer lesion area, EWL: enamel wall lesion area, DWL-A: dentin wall lesion area next to the DEJ and DWL-B: dentin wall lesion area at 750 µm from the DEJ.

FIGURE 17. A graph showing the mean enamel outer lesion area among the four groups. Error bars represent ± 2 SE. There was no significant difference among the groups ($p>0.05$).

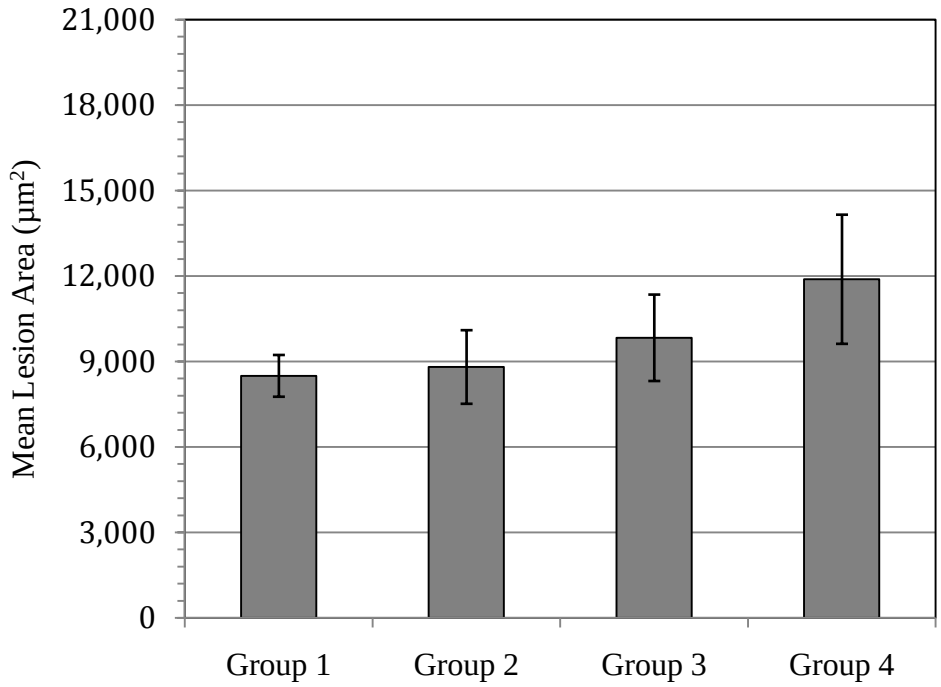
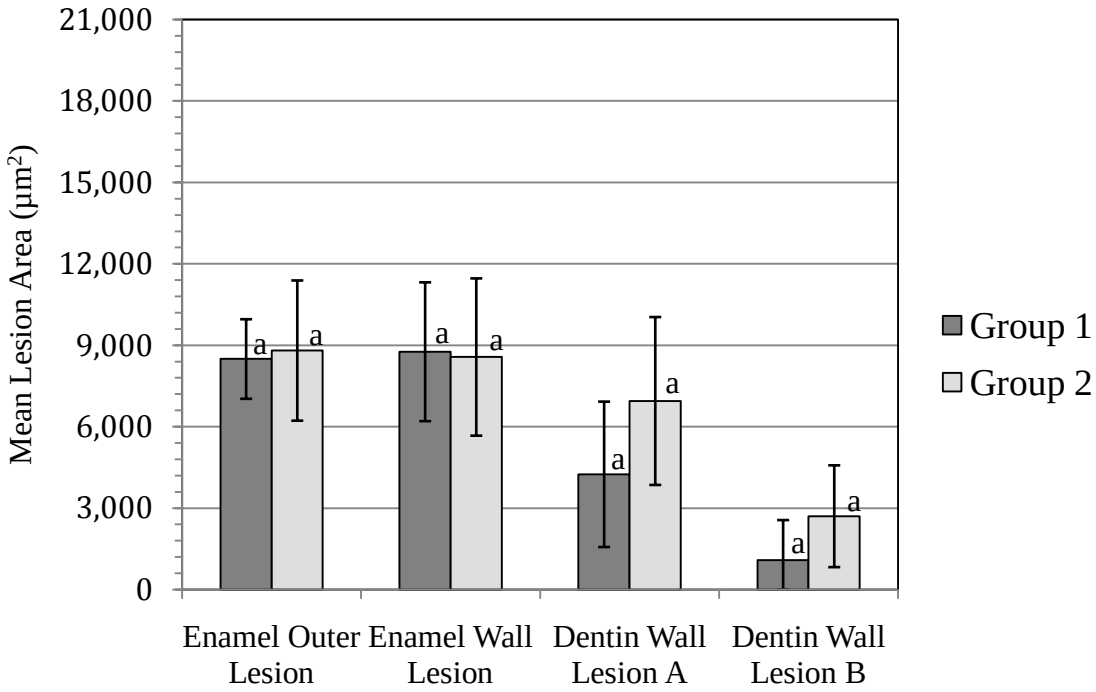
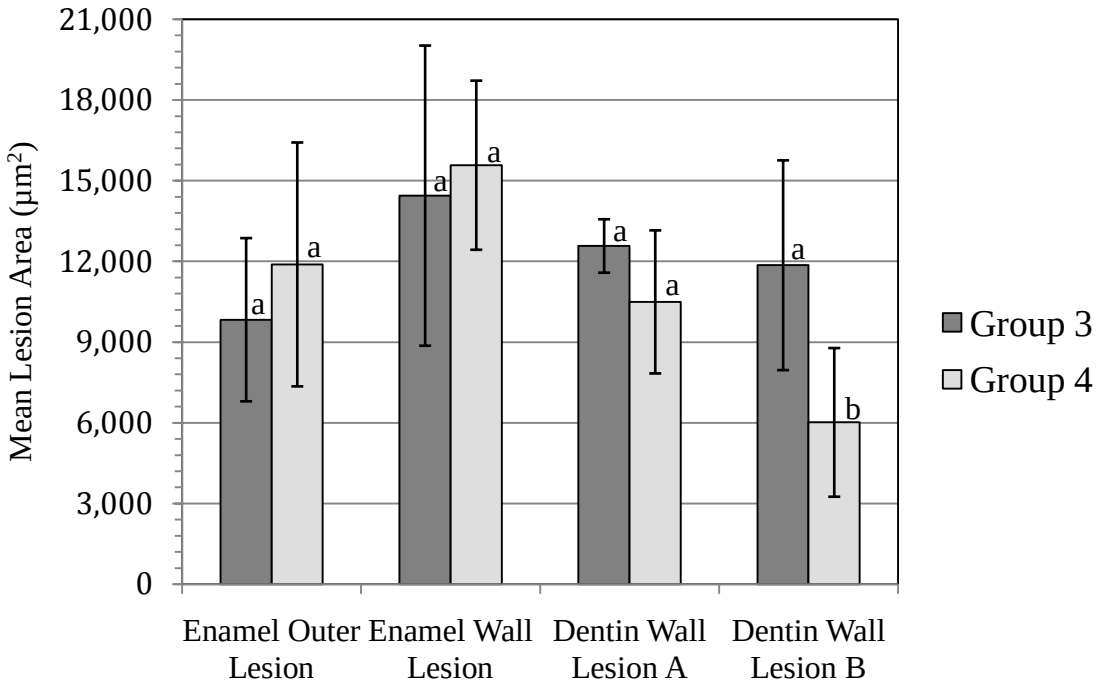


FIGURE 18. A graph showing mean lesion areas between groups 1 and 2. Error bars represent ± 2 SE.



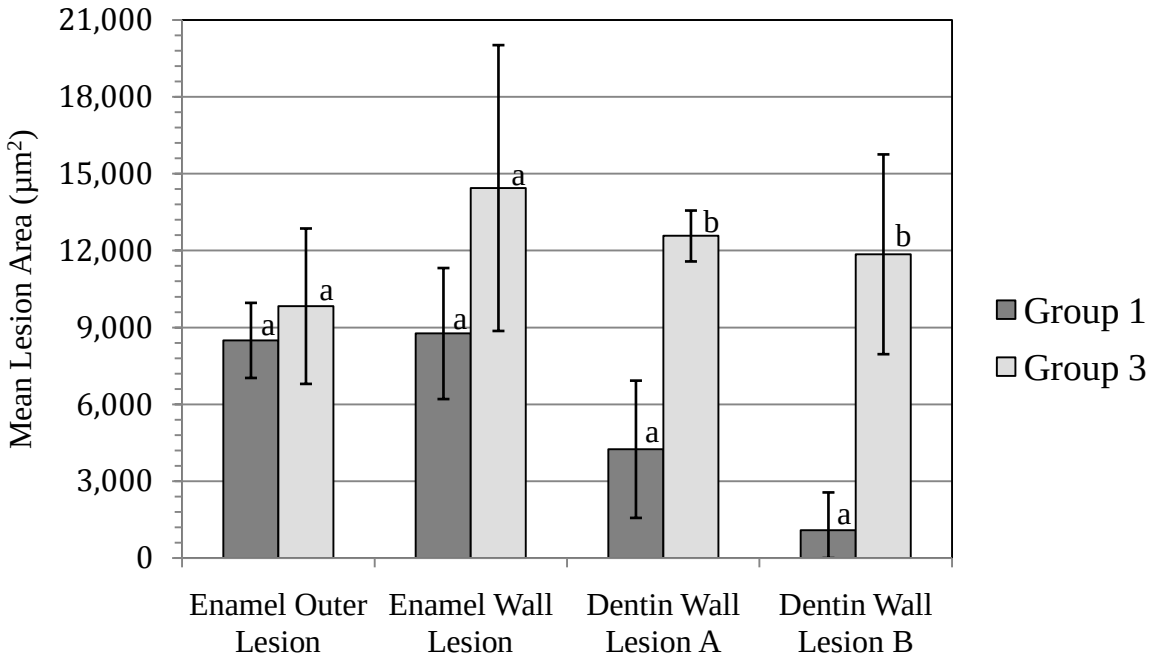
Groups with different letters were significantly different ($p \leq 0.05$).

FIGURE 19. A graph showing mean lesion areas between groups 3 and 4. Error bars represent ± 2 SE.



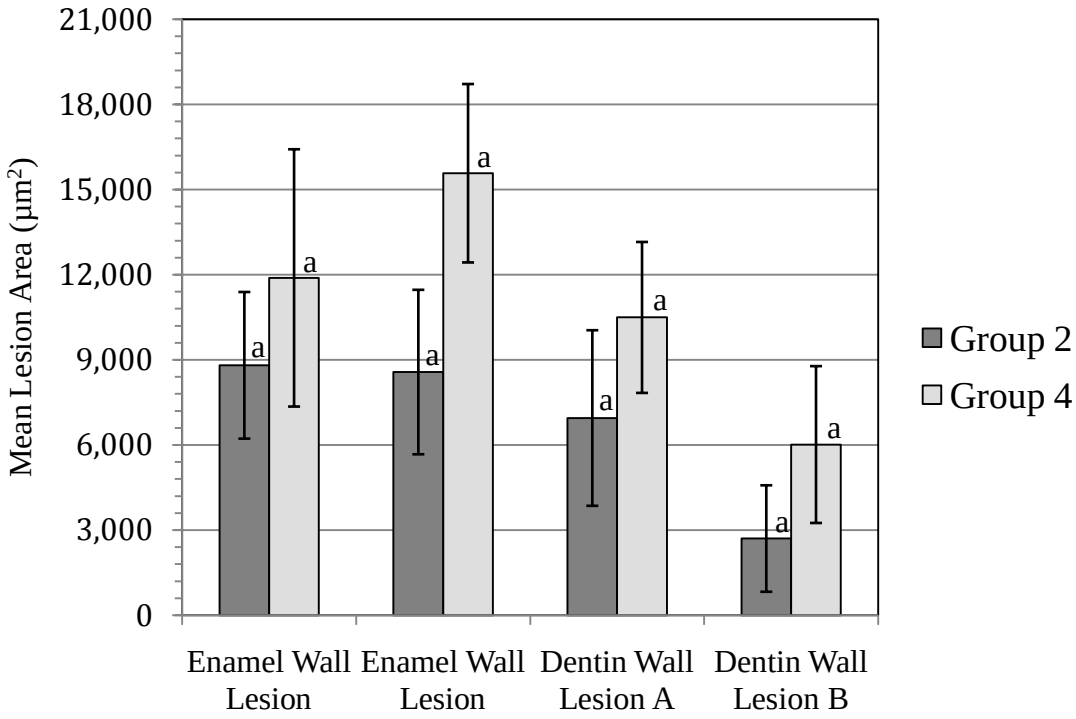
Groups with different letters were significantly different ($p \leq 0.05$).

FIGURE 20. A graph showing mean lesion areas between groups 1 and 3. Error bars represent ± 2 SE.



Groups with different letters were significantly different ($p \leq 0.05$).

FIGURE 21. A graph showing mean lesion areas between groups 2 and 4. Error bars represent ± 2 SE.



Groups with different letters were significantly different ($p \leq 0.05$).

DISCUSSION

This investigation was based on the hypothesis that the bigger the size of the space between the restoration and dentinal wall of the tooth, the bigger the size of the secondary carious lesion. The rationale for this hypothesis was that bigger gaps would provide more space for more microorganism colonization and better access to nutrients leading to the creation of larger wall lesions. Results from this investigation support the above-mentioned proposed hypothesis in case of uniform gaps, but do not support it if non-uniform geometry of gaps is present.

To create a standardized distance between tooth structure and resin-matrix composite, both specimens were prepared separately and then assembled without using a dentin bonding agent. The gap was opened before inoculation and was left open for 2 hours of initial incubation to ensure that the bacteria had penetrated; simulating what may result from long term exposure in the oral cavity.

Since the tooth specimen is the structure that was analyzed for lesion severity, it was more convenient to fabricate the step in the tooth specimen rather than the resin-matrix composite block. This allowed for more control of the position of the step in relation to the DEJ and provided better orientation during analysis of the different regions of the specimen.

Resin-matrix composite was used in this study for several reasons. In recent years, the use of resin-based composites for the restoration of posterior permanent teeth has increased significantly.[40] Furthermore, some practitioners have shifted into amalgam-free practices, where resin-matrix composite might be the only material used to restore posterior teeth.[190] Polymerization shrinkage of resin-matrix composite is still a concern especially in the light-cured formulations. This setting contraction is believed to form gaps that could lead to microleakage and in some cases secondary

caries, though this has not been proven to date. Another reason was the high percentage of composite failures compared to other restorative materials reported in the literature.[39, 150, 191] In addition to that, the original model developed by Totiam et al.[16] used resin-matrix composite and the model was shown to develop reproducible wall lesions adjacent to such material. If changing the restorative material in the model is considered, additional investigation of this "new" configuration should be carried out before attempting to study the effect of the gap geometry.

The 30 μm enamel gaps that were present in groups 1 and 2 was chosen since it has been reported that secondary caries wall lesion development is minimal at gaps of this range.[16, 114] The 500 μm gaps in group two through four were used because it represented clinically unacceptable restoration that would require operative intervention. Totiam et al.[16] found a clear increase in secondary caries development when a difference of 500 μm was present between groups. The values of the dentin gap size in groups 2 and 4 were merely the sum of the enamel gap size and the depth of the step in each group.

Our findings are in agreement with previous in vitro studies demonstrating that secondary caries lesions develop next to marginal gaps.[22, 118] However, the main focus in our study was the effect of the presence of a defect underneath the resin that provided a larger space at the deeper regions of the gap.

Provided in Table V are the group comparisons done and their outcome. Comparison between group-1 (small gap and no step) and group-2 (small gap and a step) was done. Similarly, comparison between group-3 (large gap and no step) and group-4 (large gap and a step) was done. In both these comparisons, the main interest was to determine the effect of the presence of the gap at the inner portion of the

simulated cavity wall. In addition to that, comparison between group-1 and 3 was done to determine the effect of increasing the gap size.

Although comparison between group 2 and 4 was done, caution was taken in drawing conclusions based on this comparison. The reason of that was the presence of two variables. The two groups were different in terms of enamel gap size (30 μm vs. 525 μm) and dentin gap size (530 μm vs. 1025 μm).

Between-group comparison regarding EOL did not yield statistical significance (p value=0.41), as shown in Figure XVI. That was expected since the conditions of the three Petri dishes were similar and they all were subjected to the cycling processes in the same manner. It should be noted that each Petri dish contained specimens from each group. It appears that larger gaps in groups 3 and 4 had little effect on EOL, even though a small numerical trend seems to be consistently present. The same findings were documented by Totiam et al.,[16] where deeper lesions were observed adjacent to larger gaps but without significant difference.

Groups 1 and 2 were compared mainly to evaluate the effect of the presence of a defect under a clinically acceptable margin. Results suggest that the presence of the step does not affect the development of wall lesions. We speculate the reason for this lack of impact is that the size of the interface entrance, which was the same in both groups and was relatively small, was the dominating factor affecting the dynamics of caries development. The reason for that could be that within smaller gaps, the minerals dissolved from tooth structure due to the acid attack would remain and supersaturate the gap. This would lead to remineralizing the area limiting the size and progression of wall lesions.[16] Jorgensen and Wakumoto[20] reported no caries in gaps smaller than 35-50 μm suggesting that secondary caries lesions do not tend to develop around smaller gaps. Lagerweij et al.[192] attempted to study the caries susceptibility

related to dentinal grooves of different widths. They reported smaller lesions in association with narrower grooves. This could be the same dynamics that yielded differences between groups 1 and 3, as will be discussed later.

Comparison between groups 3 and 4 was done to evaluate the presence of the defect adjacent to larger gaps. No differences were observed between the groups except in the size of wall lesions at the deeper areas of dentin (DWL-B) where larger lesions occurred in group 3 compared to group 4. Although we were expecting that larger defects would accumulate more plaque leading to more demineralization, the presence of the step itself at the deeper areas of the simulated cavity could have led to conditions where the diffusion of nutrients might have been impaired by the distance that these substances had to travel to reach the inner part of the cavity. The same thing could be said about the demineralization products that had to travel the same distance to reach the outside of the interface. According to Rølla and colleagues,[119] in areas with high plaque retentive potential, like the inner part of the gap, long-term bacterial colonization may induce a calcification effect by eliminating the inhibition action of pellicle proteins on calcium phosphate precipitation. This does not occur at the entrance of the gap interface where the abrasive forces of mastication and tooth brushing frequently renew the absorbed pellicle proteins, thus, exposing enamel margins to the continuously changing oral conditions. Another explanation was reported by Jenkins,[193] proposed a difference in plaque composition and cariogenicity at different depth levels in the fissures, the plaque at the deepest level being the most aggressive. Combination of these factors could have led to creating remineralizing conditions at the step area where smaller wall lesions were developed.

In comparing groups 1 and 3, no differences were found at EWL. However, both DWL-A and -B were larger in group 3 than group 1. These results are in

agreement with previous reports that found a positive correlation between the size of the gap and the size of the wall lesion developed around it. As discussed earlier, the smaller gaps are likely to provide a better environment for remineralization than larger gaps. Larger gaps provided more bacterial colonization and better access to nutrients that led to larger wall lesions.[16]

The difference in surfaces roughness between the area of DWL-B between the groups with the step and the groups without it could have an effect on the results. The difference in surface roughness between enamel, dentin and the step area may have affected the bacterial adhesion to these substrates. However, previous reports have shown no association between surface roughness values and bacterial adhesion on restorative materials.[128, 129] Furthermore, the effect of surface roughness on fluid penetration inside the gap cannot be eliminated. Diffusion of fluids throughout the gap interface is likely to occur due to capillary action. It can be assumed that the capillary action can be affected by the geometry of the vessel that the fluids are passing through. Even more, the surface morphology of the walls of this vessel may affect the diffusion speed. Surface roughness can affect the surface tension of the fluids. The increase in surface roughness of the step area may have led to the increase in the surface tension of the fluid inside the gap. This could have led to decrease in the diffusion speed of the fluids which consequently led to less supply of nutrients to the bacteria in the deep parts of the step. The larger DWL-B in group 3 than group 4 could have been caused by this proposed phenomenon.

Regarding profilometric analysis it can be concluded that Sof-Lex discs can be used to polish dentin surfaces since a decrease in R_a values between prepolishing and postpolishing scans was observed. However, other means should be used to polish enamel surfaces and dentin surfaces with very high R_a values since little change was

observed when polishing these surfaces with Sof-Lex discs. Of special note is that the proposed polishing technique for the step area should inflect little effect on the depth of the step region in order to maintain the depth within the acceptable range.

Although this investigation involves simulating a clinical condition, apparent differences between this in vitro model and the oral cavity are present. Among these differences are the dissimilar incubation conditions from what is found in the oral cavity. Furthermore, the composition of the buffer solution utilized is different from saliva constitution. Another importance limitation is the difference in surface roughness between the dentin part and the step area of the specimens in groups 2 and 4. Although, to our knowledge, this is the first time that such model configuration was used, standardizing the conditions of areas of interest should be considered. Future studies investigating gap geometry effect should develop a polishing technique that is effective for all areas of the specimen with little effect on the depth of the step region. Still, results from this investigation could help establish future models to study secondary caries in relation to non-uniform gaps.

In conclusion, this investigation provided important information regarding the effect of gap geometry on secondary caries development adjacent to resin-matrix composites. Results from this study show that the presence of additional space at the dentin wall area did not affect secondary caries development as long as the enamel gap was small. However, with larger enamel gaps, the presence of the additional gap space at the dentinal wall led to the development of smaller dentinal wall lesions at the deeper parts of the simulated cavity. Also, in uniform gaps, the size of the interface was positively correlated with size of the dentinal wall lesions.

SUMMARY AND CONCLUSIONS

Prevalence of primary caries has decreased in many countries during the last few decades. Despite this, the disease is still a major problem especially in certain groups of the population. Management of dental caries in the form of restorations is not always ideal since it has been found that secondary caries is associated with a high percentage of restoration failures.

Histopathologically, secondary caries consists of two regions, an outer lesion and a wall lesion. It has been suggested that the development of the wall lesion in associated with the size of the microspace at the tooth-restoration interface. Data from the literature support the common presence of these gaps regardless of the restorative material used. Although not completely verified, there seems to be an association between secondary caries and gap size.

In this study, we studied the mechanism of secondary caries development around non-uniform gaps under controlled conditions. Although many models and techniques have been used to study secondary caries, none of these have been used to investigate the mechanism of caries formation around a non-uniform geometry of the tooth-restoration interface.

Tooth-resin-matrix composite specimens were mounted on custom-made gap-model stages. Specimens were divided into four groups (n=10). Group 1 had a uniform gap size of 30 μm throughout both enamel and dentin. Group 2 had 30 μm enamel gap size with 530 μm dentinal gap. Group 3 had 525 μm gaps in both enamel and dentin. Group 4 had 525 μm and 1025 μm gap in enamel and dentin, respectively. Specimens were attached to plastic Petri plates, gas-sterilized and then incubated in a microbial caries model with *S. mutans* TH16 in (1% sucrose tryptic soy broth for 1 h,

4 times/day, and with a buffer solution for the rest of the day). After 8 days of incubation, tooth specimens were sectioned and stained with a rhodamine B solution.

Digital images were taken under a confocal microscope and analyzed for lesion size at the enamel outer lesion (EOL), enamel wall lesion (EWL), dentin wall lesion next to the DEJ (DWL-A) and dentin wall lesion at 750µm from the DEJ (DWL-B).

In summary, results from this study show that the presence of additional space at the dentin wall area did not affect secondary caries development as long as the enamel gap was small. However, with enamel gaps of ≈500 µm, the presence of the additional gap space at the dentinal wall led to the development of smaller dentinal wall lesions at the deeper parts of the simulated cavity. Also, in uniform gaps, the size of the interface was positively correlated with size of the dentinal wall lesions, as it has been shown in previous studies. The methodology used in this project can act as a foundation for development of techniques to study secondary caries around non-uniform gaps which resemble the clinical situation more closely.

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APPENDICES

APPENDIX A – CONFOCAL LASER SCANNING ANALYSIS RAW DATA

Group	Specimen	Enamel Outer Lesion	Enamel Wall Lesion	Dentin Wall Lesion-1 (next to DEJ)	Dentin Wall Lesion-2 (750µ from DEJ)
1	1	12331.32	9054.7	13431.95	188.7635
	2	6822.24	17858.2	7648.63	6637.93
	3	10557.395	5894.77	815.992	0
	4	8504.755	7871.58	264.566	0
	5	8070.75	8091.56	6956.005	0
	6	5514.27	11603.7	1966.405	0
	7	11076.1	10325.5	5402.795	0
	8	9529.955	4069.56	175.3865	0
	9	6166.4	4024.97	1474.4355	4072.53
	10	6392.685	8855.53	4337.1	0
2	1	2043.695	881.39	1527.94	6342.15
	2	12061.535	10815.97	4475.325	0
	3	8605.83	15778.8	7290.43	7092.7695
	4	8101.965	6669.145	7375.15	0
	5	13067.8	8834.715	3145.07	408.7395
	6	7068.965	4927.17	4185.49	2382.58
	7	N/A	N/A	19148.335	3423.008
	8	4285.82	8799.05	6165.28	0
	9	11250.3	7696.195	9757.725	6594.815
	10	12783.87	12724.415	6429.845	783.294

(continued)

(continued)

APPENDIX A – CONFOCAL LASER SCANNING ANALYSIS RAW DATA

3	1	4109.325	6884.66	13131.7	16278.25
	2	5321.045	12651.6	10720.87	8851.06
	3	8947.695	10871	12876	0
	4	9225.63	32401.9	13460.15	15231.9
	5	11985.7	1762.78	10648.05	17803.2
	6	9124.555	11744.9	10749.105	11689.97
	7	8880.81	11471.5	13109.4	9901.9
	8	21278.25	25585.6	12191.605	10052
	9	12547.55	16403.1	13154	7101.644
	10	6909.94	14686.4	15703	21682.5
4	1	20981	19259.8	3427.465	117.4195
	2	5350.775	12028.815	9919.72	4848.395
	3	19524.38	14381.7	12046.7	7146.255
	4	10781.825	9650.725	16853.45	5298.7535
	5	12262.2	20289.85	13540.45	7265.16
	6	1965.66	20811.5	15315.1	13653.37
	7	N/A	8683.12	9348.975	0
	8	7731.87	13466.1	11417.95	8527.05
	9	19613.55	14137.94	6759.81	2721.46
	10	8784.205	23055.9	6319.855	10572.25

APPENDIX B – CONFOCAL LASER SCANNING ANALYSIS RAW DATA (BY PETRI DISH)

PETRI DISH#1

Group	Specimen	Enamel Outer Lesion	Enamel Wall Lesion	Dentin Wall Lesion-1 (next to DEJ)	Dentin Wall Lesion-2 (750 µm from DEJ)
1	1	12331.3	9054.7	13431.95	188.7635
	4	8504.76	7871.58	264.566	0
	7	11076.1	10325.5	5402.795	0
	10	6392.69	8855.53	4337.1	0
2	1	2043.7	881.39	1527.94	6342.15
	4	8101.97	6669.15	7375.15	0
	6	7068.97	4927.17	4185.49	2382.58
3	1	4109.33	6884.66	13131.7	16278.25
	4	9225.63	32401.9	13460.15	15231.9
	7	8880.81	11471.5	13109.4	9901.9
4	10	8784.21	23055.9	6319.855	10572.25
	3	19524.4	14381.7	12046.7	7146.255
	6	1965.66	20811.5	15315.1	13653.37

PETRI DISH#2

Group	Specimen	Enamel Outer Lesion	Enamel Wall Lesion	Dentin Wall Lesion-1 (next to DEJ)	Dentin Wall Lesion-2 (750 µm from DEJ)
1	2	6822.24	17858.2	7648.63	6637.93
	5	8070.75	8091.56	6956.005	0
	8	9529.96	4069.56	175.3865	0
2	2	12061.5	10816	4475.325	0
	5	13067.8	8834.72	3145.07	408.7395
	7	N/A	N/A	19148.335	3423.008
	9	11250.3	7696.2	9757.725	6594.815
3	2	5321.05	12651.6	10720.87	8851.06
	5	11985.7	1762.78	10648.05	17803.2
	8	21278.3	25585.6	12191.605	10052
4	1	20981	19259.8	3427.465	117.4195
	4	10781.8	9650.73	16853.45	5298.7535
	7	N/A	8683.12	9348.975	0

PETRI DISH#3

Group	Specimen	Enamel Outer Lesion	Enamel Wall Lesion	Dentin Wall Lesion-1 (next to DEJ)	Dentin Wall Lesion-2 (750 µm from DEJ)
1	3	10557.4	5894.77	815.992	0
	6	5514.27	11603.7	1966.405	0
	9	6166.4	4024.97	1474.4355	4072.53
2	3	8605.83	15778.8	7290.43	7092.7695
	10	12783.9	12724.4	6429.845	783.294
	8	4285.82	8799.05	6165.28	0
3	3	8947.7	10871	12876	0
	6	9124.56	11744.9	10749.105	11689.97
	9	12547.6	16403.1	13154	7101.644
	10	6909.94	14686.4	15703	21682.5
4	2	5350.78	12028.8	9919.72	4848.395
	5	12262.2	20289.9	13540.45	7265.16
	8	7731.87	13466.1	11417.95	8527.05

APPENDIX C – PROFILOMETRIC ANALYSIS RAW DATA

Specimen	Surface	Prepolishing			Postpolishing		
		R_a (X)	R_a (Y)	Average	R_a (X)	R_a (Y)	Average
1	Enamel	0.29	0.17	0.23	0.31	0.24	0.27
	Dentin	0.29	0.26	0.28	0.19	0.21	0.20
	Step	3.36	1.99	2.67	3.17	2.16	2.66
2	Enamel	0.92	0.23	0.57	0.45	0.23	0.34
	Dentin	0.48	0.36	0.42	0.17	0.17	0.17
	Step	2.90	2.42	2.66	3.15	2.56	2.85
3	Enamel	0.56	0.64	0.60	0.40	0.25	0.32
	Dentin	0.59	0.40	0.49	0.30	0.42	0.36
	Step	3.22	2.03	2.63	2.91	2.11	2.51
4	Enamel	0.21	0.17	0.19	0.49	0.33	0.41
	Dentin	0.55	0.35	0.45	0.51	0.34	0.42
	Step	2.82	2.49	2.65	2.08	1.40	1.74
5	Enamel	0.21	0.20	0.21	0.50	0.26	0.38
	Dentin	0.48	0.41	0.44	0.47	0.27	0.37
	Step	2.87	2.50	2.69	2.778	2.56	2.56

APPENDIX D – PROFILOMETRIC ANALYSIS AVERAGES

Surface	Prepolishing Average	Postpolishing Average
Enamel	0.36	0.35
Dentin	0.42	0.30
Step	2.66	2.47

APPENDIX E – DETAILED STATISTICAL MEASURES FOR THE FOUR SECONDARY LESION AREAS MEASURED BY CONFOCAL LASER SCANNING MICROSCOPY.

	Group	N	Mean	SD	SE	Min.	Max.
Enamel Outer Lesion	1	10	8497	2319	733	5514	8497
	2	9[a]	8808	3877	1292	2044	8808
	3	10	9833	4798	1517	4109	21278
	4	9[a]	11888	6802	2267	1966	20981
Enamel Wall Lesion	1	10	8765	4045	1279	4025	17858
	2	9[a]	8570	4347	1449	881	15779
	3	10	14446	8823	2790	1763	32402
	4	10	15577	4970	1572	8683	23056
Dentin Wall Lesion A	1	10	4247	4239	1340	175	13432
	2	10	6950	4893	1547	1528	19148
	3	10	12574	1571	497	10648	15703
	4	10	10495	4205	1330	3427	16853
Dentin Wall Lesion B	1	10	1090	2329	736	0	6638
	2	10	2703	2966	938	0	7093
	3	10	11859	6166	1950	0	21683
	4	10	6015	4371	1382	0	13653

ABSTRACT

Objective: To investigate the effect of the size of the space between the restoration and the dentinal wall of the tooth (i.e. the dentinal portion of the gap) on the development of secondary caries. **Methods:** Tooth-resin-matrix composite specimens were mounted on custom-made gap-model stages. Specimens were divided into four groups (n=10). Group 1 had a uniform gap size of 30μm throughout both enamel and dentin. Group 2 had a 30μm enamel gap size with a 530μm dentinal gap. Group 3 had 525μm gaps in both enamel and dentin. Group 4 had 525μm and 1025μm gaps in enamel and dentin, respectively. Specimens were attached to plastic Petri plates, gas-sterilized and then incubated in a microbial caries model with *S. mutans* TH16 in (1% sucrose tryptic soy broth for 1 h, 4 times/day, and with a buffer solution for the rest of the day). After 8 days of incubation, tooth specimens were sectioned and stained with a rhodamine B solution. Digital images were taken under a confocal microscope and analyzed for lesion size at the enamel outer lesion (EOL), enamel wall lesion (EWL), dentin wall lesion next to the DEJ (DWL-A) and dentin wall lesion at 750μm from the DEJ (DWL-B). **Results:** No difference in EOL size was found between the groups. DWL-A and -B were larger in group 3 than groups 1and 2. Larger DWL-B was found in group 3 than group 4. Group 4 had marginally significant larger EWL than groups 1 and 2 ($p=0.0652$ and $p=0.0648$, respectively). Also, group 4 had marginally significant ($p=0.0607$) larger DWL-B than group 1. **Conclusions:** Based on the results of this study, it can be concluded that the presence of additional space at the dentinal wall area did not affect secondary caries development as long as the enamel gap was small. However, with enamel gaps of ≈500 μm, the presence of the additional gap space at the dentinal wall led to the development of smaller dentinal wall lesions at the deeper parts of the simulated cavity. Also, in uniform gaps, the size of the interface was positively correlated with size of the dentinal wall lesions.

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