An *in vitro* Study

Testing the Abililty of Intermediary Sealants

to

Prevent Enamel Decalcification

Around Bonded Orthodontic Attachments

By

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INTRODUCTION

Since the advent of fixed orthodontic appliances, clinically observable demineralization of enamel (opaque whitened areas), often referred to as decalcification, has been accepted as one of the hazards of fixed appliance therapy (Noyes, 1937; Mizrahi, 1982, Mitchell, 1992). Esthetic and restorative problems related to decalcification can in some cases outweigh the benefits of orthodontic treatment (Ogaard, 1989; Zachrisson, 1978).

Professionally administered oral hygiene instruction and preventive fluoride programs (office applied or self administered rinses) have been recommended to reduce decalcification (Noyes, 1937; Artun and Brobakken, 1986; ; Ogaard, 1989; Geiger et al., 1992).

Significant demineralization around bonded orthodontic brackets has been reported for patients who were provided hygiene instruction and instructed to brush regularly (O'Reilly and Featherstone, 1987; Ogaard et al., 1992). The apparent ineffectiveness of plaque control programs is likely related to compliance. Professional oral hygiene instruction for the duration of treatment has also been criticized as being too labor intensive and costly (Mitchell, 1991).

Professional office fluoride programs also require extra time and expense. Noting only a 13% compliance rate, Geiger et al. (1992) identified patient cooperation as a significant problem in self-administered fluoride rinse programs. Ideally, measures to inhibit decalcification should operate independent of patient cooperation.

Fluoride releasing adhesives and sealants for bracket bonding, have recently attracted considerable interest (Sonis and Snell, 1989; Mitchell, 1992; Ogaard, 1992). Advantages offered by these materials include slow release of low levels of fluoride and site specificity that is not dependent on patient compliance (Ogaard, 1992). Light cured fluoride releasing composites and sealants also offer the added advantage of additional working time for bracket positioning and removal of excess materials.

LITERATURE REVIEW

Decalcification and Fixed Appliances

The visual appearance of white spots is caused by an optica phenomenon due to subsurface tissue loss (decalcification) and is exaggerated by drying (Gorlic, 1982). The acidogenic, or chemoparasitic theory of cariogenesis involving the interaction of plaque, carbohydrates, and tooth structure has been accepted as fac (Menaker, 1980). Investigators agree that white spots represent areas of decalcification and are the precursors or the early lesions of ename caries resulting from prolonged retention of bacterial plaque on ename (Darling, 1956; Zachrisson and Zachrisson, 1971; Mizrahi, 1982).

There is no doubt that fixed orthodontic appliances reduce the efficacy of oral hygiene procedures (Geiger, 1992), and studies document increased plaque accumulation around cemented bands and bonded brackets.

In an in vivo comparison of banded and bonded teeth, Ciancio et al (1985) observed a predisposition to plaque accumulation on tooth surfaces around bonded attachments. Although bands had more plaque at the gingival margin, bonded brackets and their associated tooth surfaces had more plaque overall.

Using a scanning electron microscope (SEM), Gwinnett and Ceen (1979) reported an increase in dental plaque volume on the resin surfaces adjacent to bonded attachments and at the junction of the bonding resin and the etched enamel surfaces. One of the most common sites of accumulation appeared to be at the resin-enamel junction just peripheral and gingival to the bracket base.

Ballenseifen and Madonia (1970) reported that the presence of intraoral orthodontic appliances leads to unfavorable environmenta changes. Changes were characterized by a drop in pH, and an increase in carbohydrate, streptococci, and lactobacilli. As well as an increase in the total amount of plaque, a greater concentration of bacteria and carbohydrate were found in each mg of plaque.

Zachrisson and Zachrisson (1971) reported an almost linear correlation between plaque accumulation around fixed orthodontic attachments and the development of carious lesions in orthodontic patients.

Gorelic et al. (1982) studied the incidence and severity of white spot lesions in 121 patients after a full term of orthodontic treatment. Post-treatment white spots were found in 10% of treated teeth compared to 3.6% in a sample from 50 untreated patients. After treatment, 50% of the patients experienced an increase in white spots. Due to the cross-sectional nature of the study strict comparisons were not made. The investigators suggested that the obvious potential for iatrogenic damage during orthodontic treatment implied a need for preventive programs using fluoride.

Artun and Brobakken (1986) and Ogaard (1989) reported an increase in white spot lesions of 14% and 10%, respectively, over nontreated controls. Although compliance was not assessed, patients were provided fluoride rinses in both studies.

Demineralization around orthodontic appliances has been demonstrated to progress at a fairly rapid rate. A study by Gatz and Featherstone (1985) has shown that measurable demineralization (up to 25%) can occur adjacent to orthodontic brackets after one month in vivo.

Bonding

Buonocore (1955) introduced the use of acid solutions to etch or "condition" enamel prior to placement of acrylic restorations to enhance the edge adaptation.

Studies have shown that etching with acid solution produces distinct changes in the enamel surface (Silverstone, 1974). In addition to removing a thin layer of enamel with its associated cuticle, the acid solution renders the remaining enamel surface porous. Resin applied to the etched surface penetrates its pores and bonds mechanically with the enamel. Successful bonding results in resin tags within enamel which are responsible for the interlocking.

Using Buonocore's methods, Newman (1960) was the first to report direct bonding of orthodontic brackets to enamel surfaces. Initially, orthodontic bonding systems failed to provide adequate bond strength for clinical application and it wasn't until the early 1970s that materials strong enough for routine clinical use became available (Craig, 1993; Proffit, 1993). At that time, the most successful systems were stabilized bisphenol-A glycidyl dimethacrylate (bis GMA) resins. Initially, the systems were self-curing (chemically cured) with two pastes that polymerized several minutes after mixing. Polymerization was driven by initiators and accelerators incorporated into the component pastes. To enhance physical characteristics, fillers (silica, glass, or colloidal silica) were added to the resins to produce composite resins.

In 1972, ultraviolet light-cured, bis-GMA resins for orthodontic bonding were introduced (Cohl et al., 1972). In addition to allowing increased working time, these resins offered command set capabilities and attained full cure after about one minute of ultraviolet light exposure (Pollack and Lewis, 1981).

Hazards related to exposure to ultraviolet radiation (Birdsell et al., 1977), led to the development of visible light-cured resins in the late 1970s. A visible light-cured resin for orthodontic bonding was first reported in 1979 (Tavas and Watts, 1979). Polymerization of these resins is initiated by exposure to visible light (450-470 nm). Used for direct bonding, light cured adhesives are cured under metal brackets by translumination through tooth structure (Tavas and Watts,1979).

Over the years bonding increased in popularity, and by 1979, 93% of the orthodontists responding to a survey by Gorelick (1979) used bonding in their practices.

Resin Sealants

In 1965, Gwinnet and Buonocore introduced pit and fissure sealants as a means of preventing occlusal caries. The technique involved placement of lightly filled acrylic resin into acid-etched occlusal pits and fissures. Early test results indicated that as long as the resin stayed in place, it was 100% effective in the prevention of pit and fissure caries.

Buonocore et al. (1968) described an ideal sealant as having wetability for enamel surfaces and high surface tension to enhance filling of the capillary surfaces. After placement the sealant should polymerize into a tough, impermeable, abrasion and bacterial resistan layer.

Resin sealants can be used as an intermediary between bracke adhesives and etched enamel in orthodontic bonding. A liquid sealan (usually the monomer of the adhesive) is applied to etched enamel prio to placement of the attachment coated with adhesive. Most of the commonly used sealants are bis-GMA based and polymerization can be initiated chemically or by visible light. Hypothetically, intermediary sealants provide four basic functions: protection of the enamel enhancement of bond strength, reduction of interfacial permeability between the enamel and bonding resin, and facilitation of debonding with less damage to enamel (Zachrisson, 1978; Gwinnett, 1982; Wang and Tarng, 1992).

Although it has been demonstrated that certain resin systems require an intermediary layer of sealant to prevent marginal leakage (Hembree and Andrews, 1976), the value of sealants in orthodontic bonding has been questioned.

Sealants were originally thought to enhance bond strength by penetrating farther into etched enamel than higher viscosity resins used in bracket adhesives and restorative resins (Dogon, 1976) Pahleven et al. (1976) showed that resin sealers and composite resins penetrated etched enamel to the same extent. Other evidence also indicates that intermediary sealants do not necessarily increase bond strengths of composites to etched enamel (Mitchem and Turner, 1974).

In a recent study by Wang and Tarng (1992) using a two paste (self-curing) orthodontic bonding system (Concise), sealants were found to have no effect on bond strength. It was suggested that the sealant may provide extra protection for the enamel during debonding Of the attachments bonded with composite alone, 4% exhibited enamel detachment during debonding. Enamel detachment did not occur during debonding of attachments bonded with an intermediary sealant. It wa concluded that sealants are probably not necessary for orthodonti bonding.

Silverstone (1975) demonstrated that pit and fissure sealant can impart acid resistance to enamel that persists even after th surface sealant is abraded away. Sealants placed in vivo were cut ou of extracted teeth. After exposure to lactic acid, only the enamel originally subjacent the sealant (~30 μm in depth) was left intact and unaffected. Adjacent unsealed enamel, and enamel beneath the sealed surface were demineralized. Resin tags of sealant remaining in the enamel surface were thought to be responsible for the acid resistance. Although the tags were presumed to be present in the resistant layers, microscopic evidence was not presented.

Microsolubility studies by Silverstone (1977) on sealed and abraded enamel support his theories regarding sealants and acid solubility. Using the acid etch technique, ultraviolet light-cured pit and fissure sealant was applied to extracted teeth. The sealant was then ground off with a diamond wheel. The abraded samples along with adjacent areas of sound enamel were etched repeatedly with acetic acid (pH 3.0). In terms of depth of decalcification, the untreated enamel was almost twice as soluble as the sealed and abraded enamel. In another part of the study, Silverstone (1977) showed that etched and unsealed enamel has increased solubility for at least 24 hours in saliva before it remineralizes. Silverstone suggested that this could mean that a patient is at greater risk of decalcification if etched enamel peripheral to bonded attachments is not sealed with resin.

Davidson (1980) has also demonstrated the acid resistance of sealed and abraded enamel. Using his in vitro abrasion data it was calculated that a well sealed enamel layer can function cariostatically for at least two years after the bulk of a pit and fissure sealant is worn away.

In a clinical study of a self-curing sealant (Concise) for orthodontic use, Zachrisson (1977) reported a "striking reduction" in the incidence of white spot lesions on teeth coated with sealant prior to bracket placement. Teeth without sealant had increased demineralization along the enamel-adhesive border, under loosened brackets, and along the gingival margin. Precoating etched enamel with sealant, especially along the gingival margin was recommended.

Applying sealant for orthodontic bonding presents special problems as the material is not confined anatomically as it is in occlusal pits and fissures. Zachrisson (1979) reported that self-curing sealants failed to provide a thin surface film on smooth enamel adjacent to bonded brackets. Nonpolymerization of the sealant due to oxygen inhibition, and flow of the resin before it cured, were cited as reasons for the inadequacy. The inhibiting effect of oxygen is based on the formation of copolymers between monomers and oxygen in preference to polymerization of methacrylate polymers. To avoid excess oxygen entrapment, Zachrisson cautioned against overmixing of sealants. He also suggested that air inhibition could be further avoided by conducting inert gas over the surface of a polymerizing resin. Sealants used in the study had marked flow properties and sealant accumulated in the gingival and distal interproximal areas as well as islands of increased thickness in other areas. In these locations, the resin was apparently thick enough to avoid air inhibition. Zachrisson recommended that bonding sealants be of sufficient viscosity to prevent drift before polymerization.

Ceen and Gwinnet (1980) bonded 60 extracted teeth with metal brackets using five chemically cured sealants and one U.V. light cured sealant. Scanning and light microscopy were used to map sealant distribution and to measure sealant thickness at the periphery of the bracket. Results showed a wide range of thickness from 0 to 228 μm with considerable interproduct and intraproduct variation. The authors felt that while the enamel was clinically covered with resin initially in all teeth, oxygen inhibition of polymerization in some products involved almost the full thickness of the resin film. Subsequent washing of the samples prior to microscopic examination removed this inhibited film. The U.V. light cured product (Nuvaseal, L.D.Caulk) provided the best coverage and thickness (30-228 μm) with all treated areas of enamel having some demonstrable thickness of sealant. The chemically cured sealants tested often failed to provide a film after the air inhibited layer was removed. Three of the chemically cured sealants (Bondmor, General Orthodontic Laboratories; Concise, 3M Corporation; Endur, American Ormco) produced thinner layers of polymerized sealant (Range = 0 - 126 mm). Two of the chemically cured sealants tested (Interlock, Rocky Mountain Orthodontics; 1:1, TP Orthodontics) failed to produce any measurable sealant coverage. A relationship between viscosity and film thickness was also noted in which the sealants which were more viscous produced thicker films o polymerized sealant.

In another study Ceen and Gwinnett (1981) exposed 30 extractec teeth to an artificial caries environment for 96 hours after bondin brackets with 5 different resins and sealants. Four chemically curec systems (Concise, 3M Corporation; Endur, American Ormco; Interlock Rocky Mountain Orthodontics; Solo Tach, Caulk Corporation) and one U.V light cured system (Nuva Tach, Caulk Corporation) were compared. The artificial caries medium consisted of a sodium lactate buffer ir hydroxy-ethyl cellulose at pH 4.5. After exposure to the medium the teeth were embedded and sectioned longitudinally. The sections were examined by polarization microscopy, SEM, and contac microradiography. Teeth that were coated with chemically catalyzec sealants consistently failed to prevent white spot formation adjacen to the bracket. The investigators also reported that repeatec applications of some chemically cured sealants did not result in any accrual of resin to produce thicker layers. In one sample presented five repeated applications failed to produce any significant increases in resin thickness leaving areas of exposed enamel as islands withir the sealant. Ceen and Gwinnett concluded that the chemically curec sealants used at that time did not provide adequate protection o smooth surfaces adjacent to bonded brackets.

Light-cured bis-GMA sealants are reported to have characteristics that make them better suited for orthodontic application. In a scanning microscope (SEM) study Joseph et al.(1992 compared the relative abilities of self-cured and visible light-curec bis-GMA sealants to seal buccal enamel of 24 teeth in vitro. The investigators made no attempt to simulate intraoral conditions. Afte sealant application and polymerization the teeth were just swabbec with alcohol before sputter coating and SEM examination. Only the light-cured products demonstrated formation of a protective film witl its associated resin tags. Photomicrographs presented with the repor showed a polymerized layer of sealant 130 μm deep for one brand o light cured sealant (Transbond, 3M Unitek). Teeth with self-curec sealants showed an almost total absence of a cured sealant layer o tags. The investigators related the differences between the tw products to polymerization inhibition associated with high concentrations of oxygen that were incorporated into the self cure sealants during mixing. It was proposed that light-cured sealants depend only on white light in the 460 nanometer range to initiate the polymerization process. This allows the resin to polymerize in thin layers as oxygen is not incorporated like it is during mixing of chemically cured sealants. Although an oxygen inhibited layer was also observed with the light cured products examined in the study, it was not quantified. The investigators reported that this unpolymerized layer was easily removed with alcohol.

Not all studies of visible light cured sealants have reported favorable results and there are likely significant differences among the many different brands available today. Banks and Richmond (1994) compared the effectiveness of two new enamel sealant systems in preventing enamel decalcification in eighty patients undergoing fixed appliance therapy. Forty patients were treated with a chemically cured filled sealant (Maximum Cure, Reliance) prior to bond placement, and forty patients were treated with a visible light cured sealant (Transbond, Unitek/ 3M Corporation) prior to bond placement. Alternate teeth without sealant were used as controls. After completion of orthodontic therapy and appliance removal the teeth were scored using an enamel decalcification index. Seventy five percent of the patients had some decalcification. The viscous chemically cured sealant reduced decalcification 13% over controls ($P < 0.01$). Compared to controls, the visible light cured sealant had no significant effect on decalcification.

Joseph et al. (1994) compared the ability of a chemically cured (Concise, 3M) and a visible light cured sealant (Transbond, Unitek corporation) to seal enamel in 24 extracted teeth. Teeth were coated with one of the two products prior to orthodontic attachment placement and isolated cross sections were examined in a SEM. Teeth coated with the light activated Transbond exhibited a sealant layer surrounding the brackets and covering the buccal enamel. Indirect bonding using the chemically cured sealant with custom copings to limit exposure to air was found to produce sealant layers in the order of 60 μm around the bracket. When used for indirect bonding without a coping or for direct bonding, Concise failed to form a polymerized layer of sealant adjacent the bracket. In those samples with sealant coverage, resin tags in the order of 20μm were demonstrated in sections decalcified with 5% hydrochloric acid.

Fluoride and Caries

The cariostatic effect of fluoride is related to inhibition of demineralization at crystal surfaces, and by enhancement of remineralization of calcium and phosphate in a form more resistant to acid attack (Silverstone, 1988).

Contrary to earlier beliefs, investigators now believe that fluoride present in solution or as soluble precipitates, may play a more important role in inhibiting acid dissolution than fluoroapatite (Cate and Duijesters, 1983; Nelson and Featherstone,1982). Recent evidence has led Silverstone (1988) to recommend frequent, low concentration, applications of fluoride as opposed to intermittent, concentrated, applications designed to incorporate fluoride into apatite.

Studies by Cate and Duijesters (1983) suggest that precipitation of calcium fluoride may be more effective than fluoroapatite at blocking diffusion pathways in cariogenesis. Formation of intraoral calcium fluoride has been demonstrated after exposure to fluoride from mouth rinses and tooth pastes (Gerould, 1945; Leach 1959).

In vitro, the initial remineralization of softened enamel or white spot lesions is increased when fluoride is added to remineralizing media (Koulourides et. al.,1961). Remineralization of carious lesions is more complete when fluoride concentrations are kept low (Silverstone 1981), and some studies have shown that levels of fluoride as low as 0.1 ppm can promote crystal growth (Brown, 1974; Amjad and Nancollas, 1979).

High concentrations of fluoride (e.g. topical professional applications) can cause mineralization of surface enamel that may actually delay remineralization of the body of a lesion by impeding the passage of fluoride and minerals to deeper levels of lesions (Silverstone,1988).

Fluoride Releasing Resins

The anticariogenic effect of silicate and glass ionomer cements is well documented (Phillips and Swartz, 1957; Sadowsky et al., 1981; Brandau et al., 1984). The basic mechanism involved is that fluoride leached from the cement by oral fluids reacts with adjacent tooth structure to limit decalcification and/or promote remineralization.

In an effort to duplicate the caries resistance of silicate and glass ionomer cements, fluoride was added to dental resin systems. Phillips and Swartz (1957) were the first to publish a study of fluoride containing resins. Three experimental resins with different fluoride concentrations (5% NaF, 2% NaF, and 2% SnF) and one commercial product (Fluoron) were tested. Although not as effective as silicates, the new resins reduced acid solubility when applied to powdered and intact enamel in vitro. More chemical and histological research was recommended before the materials could be recommended for dental use.

Swartz et al. (1976) examined fluoride-supplemented pit and fissure sealants in vitro. Sodium fluoride of various concentrations was added to cyanoacrylate and bis-GMA sealants. The sealants were applied to acid etched labial enamel on extracted incisor teeth. After storage in 37° C water for two weeks, the superficial sealant was removed by abrasion. The teeth were then exposed to an acid solution (pH 4.0). Compared to control values, the sealants to which 2% - 5% NaF was added produced a substantial increase in enamel fluoride content with an associated reduction in acid solubility.

In the early 1980s anion-exchanging resins were introduced for use as sealants, orthodontic adhesives, and restorative materials (Turpin-Mair et al., 1982; Rawls and Zimmerman, 1983). Fluoride salts covalently bonded to resin polymers were designed to release fluoride by ion exchange rather than by dissolution from filler particles. Fluoride-releasing anion-exchanging resins are reported to act as barriers to demineralization and promoters of remineralization (Rawls and Zimmerman, 1983).

Light cured fluoride releasing composite resins for orthodontic bonding have been studied extensively in vitro (Temin and Csuros, 1988 Cheung et al., 1989, ; Swift, 1989; Underwood et al., 1989; Chan et al.

1990; Joseph et al., 1990; Ogaard et al., 1992) One of these bonding systems (Fluorever) was based on an anion exchanging resin and included a fluoride releasing sealant.

The cariostatic effect of fluoride releasing resins has been questioned on the basis that they only release small amounts of fluoride (Bishara et al. 1991; Swift, 1989). When immersed in deionized water for two weeks, one fluoride containing adhesive (Fluorever) released about half as much fluoride as glass ionomer cement (Swift, 1988).

Temin and Czuros (1988) reported continuous, low levels of in vitro fluoride release in water from a anion-exchanging composite resin (Flouorever) for four years in vitro. It was estimated that unsealed composite could continue to release low levels of fluoride (1.4 $\mu g/cm^2$/day) for another twenty years. Initial comparisons with silicate and glass ionomers showed that the composite released fluoride at rates lower than silicate cement but comparable to glass ionomer cement. Fluoride release was moderately reduced when the composite was coated with a nonfluoride sealant. Behavior of the composite sealed with a fluoride releasing sealant was not investigated.

Recent evidence provided by Ogaard (1992) suggests that the fluoride release rates in water may not be relevant to release rates in vivo. An experimental fluoride-containing orthodontic adhesive was found to release fluoride in distilled water but not in neutral pH saliva. When the salivary pH was lowered to 4.0, fluoride was released in amounts comparable to those in water. This would suggest that the resin may selectively release fluoride when the ambient pH is lowered as is seen in carious attack.

Sonis and Snell (1989) examined the Fluorever orthodontic bonding system in vivo using twenty-two patients. In clinical trials averaging 25 months, 206 teeth coated with <u>fluoride releasing sealant</u> and bonded with the fluoride-releasing composite, showed no decalcification of facial surfaces. An equal number of control brackets bonded with a conventional (nonfluoride releasing) light cured adhesive and intermediary sealant, had an overall decalcification rate of 12.6%.

Underwood et al. (1989) clinically tested an anion-exchanging orthodontic adhesive bonded without intermediary sealant. After 60 days in vivo, demineralization did occurr with the new adhesive, but compared to controls a 93% reduction of early demineralization was noted.

Ogaard (1992) investigated an experimental, visible light-cured, orthodontic adhesive (Orthodontic Cement VP 162) containing a fluoride-releasing agent in a dispersed filler phase. The new material was compared to a nonfluoride adhesive for four weeks in vivo. Both materials were bonded without intermediary sealant. Compared to non-fluoride controls, the new adhesive reduced lesion depths by about 48%.

Using the same adhesive as Ogaard (1992), Eliades et al. (1992) used combined wavelength-energy dispersive electron probe microanalysis to examine enamel fluoride uptake. Reportedly, this technique could detect fluoride levels in enamel as low as 0.15% by weight (1500 ppm). Cylindrical molds of the new adhesive and a nonfluoride adhesive were bonded in vivo. In each of the two series half of the teeth had adhesive bonded directly to enamel, and half had an intermediary fluoride-free sealant applied to the etched enamel prior to adhesive placement. After 9 months the teeth were extracted and sectioned for microanalysis. Confirming a report by Jorgensen and Shimokobe (1975), it was noted that none of the filler particles penetrated the resin tags. Fluoride concentrations in the outer 50 µm of enamel for the new adhesive were 2648 +/- 605 ppm for the new adhesive alone, and 2109 +/- 496 ppm for the new adhesive with intermediary sealant. Values for the controls were slightly lower (2250ppm +/- 553 ppm without intermediary sealant and 2216 +/- 595 ppm with sealant). There was no statistically significant difference ($p < 0.05$) between any of the fluoride levels reported.

RATIONALE

The problem of decalcification around fixed orthodontic appliances is well recognized in the orthodontic profession.

Present day investigators (Wang and Tarng, 1991) question the value of using intermediary sealants because they do not enhance bond strength.

Resin tags have been demonstrated with a SEM after mechanical removal of light-cured sealants (Joseph et al., 1992). Reports by Silverstone (1975, 1977) suggest that residual resin tags from sealants may protect enamel during acid attack.

Fluoride releasing orthodontic adhesives have shown promise in reducing decalcification around bonded orthodontic attachments (Sonis and Snell, 1989; Underwood et al., 1989; Ogaard et al., 1992). Of the few clinical trials designed to investigate the ability of fluoride-releasing orthodontic adhesives to inhibit decalcification in vivo, the best results have been achieved by Sonis and Snell (1989) using a fluoride containing intermediary sealant.

If the action of low levels of fluoride in solution is one of the major mechanisms for its' cariostatic effect, the addition of low concentrations of fluoride to sealants may provide added protection against decalcification.

Since it is believed that filler particles do not penetrate resin tags, (Jorgensen and Shimokobe,1975), fluoride releasing resins with polymer-bound fluoride may provide a source of fluoride when the surface film of resin is abraded away. Evidence presented by Ogaard (1992) suggests that fluoride present in residual resin tags may be selectively released in an acidic environment. As yet, there are no studies comparing the abilities of fluoride releasing and conventional intermediary sealants to prevent decalcification when used in conjunction with fluoride releasing adhesives.

Microprobe analysis will be used to determine the levels of fluoride released from the fluoride releasing sealant into enamel.

Exposing the sealants to thermocycling and toothbrush abrasion followed by acid attack, should provide a realistic indication of the their clinical durability and long-term anticariogenic potential in vivo.

MATERIALS and METHODS

I. In Vitro Acid Challenge

Thirty sound, caries and restoration-free, human premolar teeth extracted for orthodontic purposes were cleaned of debris and stored in distilled water with 0.1% thymol crystals. The teeth were randomly divided into three equal groups of ten (two experimental groups and one control group).

The buccal enamel surfaces of all teeth (20 experimental teeth and 10 control teeth) were cleaned with flour of pumice using a slow-speed dental handpiece with a rubber prophylaxis cup. After rinsing with distilled water the teeth were dried with compressed air. The entire buccal surfaces of all teeth were etched with 37% phosphoric acid for 30 seconds, rinsed with copious amounts of water for twenty seconds and thoroughly dried with compressed air. Experimental teeth in the NS treatment group were coated with a conventional light cured sealant (Delton, 3M Company) and cured with a Visilux II light (3M Company) for 20 seconds. In the same manner teeth in the FS experimental group were coated with a light cured, fluoride releasing sealant (Light-Bond, Reliance, Chicago, Illinois). To provide complete coverage at least one brush load or more of sealant was applied to each experimental tooth and the sealant was immediately cured to prevent prepolymerization drift. In both experimental groups acid etch treatment and sealant application included the entire buccal enamel surface. Control teeth were etched in the same manner as experimental teeth but were not coated with sealant prior to attachment bonding.

A lingual button orthodontic attachment (3.5 mm in diameter, lingual curved base; GAC International, Central Islip. N.Y.) was bonded to the center of the buccal surface of all the teeth using a light cured, fluoride releasing adhesive (Light-Bond, Reliance, Chicago, Illinois). Excess adhesive was removed and the resin was polymerized for 40 seconds using a Ortholux XT visible light curing unit (3M). As per the manufacturer's instructions, the light was directed at the gingival and occlusal aspects of the attachment for twenty seconds each.

All teeth were thermocycled 4°C to 55°C for 1200 cycles with a 25 second dwell time followed by 12,000 toothbrush strokes on a tooth brushing machine to simulate 1 year of tooth brushing (see fig.1). During tooth brushing teeth were bathed in a solution of distilled water (~37° C) and fluoride containing toothpaste (Crest, Proctor and Gamble). Using a modification of the protocol developed by Ceen and Gwinnett (1981), the teeth were then subjected to an artificial caries medium of 2% gelatin and hydrochloric acid (pH 4) at 37°C. After 96 hours of exposure to the artificial caries media the teeth were removed, rinsed with distilled water and stored in a humidor.

Teeth were removed from the humidor rinsed with distilled water and air dried prior to decalcification assessment. To aid in defining the extent of decalcification, the teeth were coated with ink (Sharpie, permanent marker, Sanford) and swabbed with a cotton tipped applicator soaked in acetone. Decalcified areas absorbed the ink and remained darkly stained after swabbing with acetone. Polymerized bis-GMA resin is insoluable in acetone, and the acetone removed the ink from areas coated with polymerized sealant without disrupting the sealant layer.

The area to be studied was defined using an adhesive ring reinforcer (6.35 mm in diameter). The reinforcer was placed on a movable stage which was placed in contact with the buccal surface of the tooth so that the bonded attachment was visually centered in the area of study with the adhesive ring tangent to the buccal tooth surface. As noted above, the adhesive used to bond the attachments was designed by its' manufacturer to release fluoride. Hypothetically, enamel close to the fluoride releasing adhesive could have been exposed to higher concentrations of fluoride than areas farther away from it. Centering the attachment in the field of study should have standardized the effect of any possible fluoride gradient may have had on enamel in the area of study. Baumrind (1971) has noted that visual estimation of the center of a structure as is done with some cephalometric landmarks is generally good.

Decalcification was recorded using a JAVA video imaging software (Jundel Scientific, San Rafael, A.). The image of the tooth was projected onto a monitor by a video camera attached to a stereo microscope (fig. 2). The areas of decalcification were traced on the monitor and totaled for each sample. The total area of decalcification was measured twice and the two values averaged.

Error of Measurement

Since the video image was a two dimensional representation of the buccal tooth surfaces, estimating surface area with the video imaging system could result in errors of underestimation when convex surfaces are studied. Random designation of teeth to one of the three groups studied should have minimized any bias resulting from this error.

For each sample the total area of decalcification was calculated twice. The standard error of the measure (SEM) calculated using the formula SEM = $d^2 / 2N$, (where d = the difference between the two measurements and N = sample size) was 0.06 mm^2.

The group means and standard deviations were calculated using Kwikstat computer software. The findings were compared using a Student's t-test. A p value of less than 0.05 was considered statistically significant.

II. SEM INVESTIGATION

For qualitative investigations, two additional teeth in each group (FS, NS and control) were used. The teeth received the same treatments as the groups in the acid challenge portion of the experiment but were not coated with ink to quantify decalcification.

Surface Analysis

In the acid challenge portion of the experiment areas of apparent decalcification (i.e. incomplete sealant coverage) were noticed prior to placing the samples in the artificial caries medium. In order to ascertain the fate of the sealant with each treatment, polysiloxane impressions of the samples were taken after thermocycling, tooth brushing and acid challenge. The impressions were poured up with epoxy resin and allowed to set for 24 hours. Epoxy models of the teeth were sputter coated (~ 40 nm gold and palladium) and examined in a scanning electron microscope (SEM) (JEOL, JSM-7T330A) at 10 kv accelerating voltage. After exposure to the artificial caries media, the teeth were also sputtercoated and examined in the SEM.

Resin Tags

One tooth from each of the NS and FS treatment groups was embedded in epoxy resin, sectioned longitudinally through the buccal surface and polished using silicon carbide papers to 600 grit, 1000 silicon carbide paste, and 5.0 μm aluminum oxide paste. Sections were designed to cross decalcified areas as well as areas where the sealant was intact so that direct comparisons of the two could be made within the same section. The sections were then etched with 5% hydrochloric acid for 3 minutes, washed with distilled water for 30 seconds and air dried. After sputter coating (~40 nanometers of gold and palladium) the sections were examined under the SEM (JEOL, JSM-7T330A) at 10 kv accelerating voltage.

Microprobe Analysis

Combined wavelength-energy dispersive electron microprobe analysis (Eliades, 1992) was performed on a sample from each experimental group and a control tooth to measure the fluoride content of the enamel. The teeth were sectioned longitudinally and polished using the abrasive sequence mentioned above. Using a JEOL computerized microprobe (JEOL electron microprobe, model 6400) at 10 kv and 10 nanoamps, 100 second exposures were recorded at various areas in the enamel subjacent to the adhesive and sealants. A sample of 3.7% fluoroapatite was used for calibration.

RESULTS

I. ACID CHALLENGE

The buccal and lingual surfaces of the control teeth were uniformly demineralized. With the exception of one tooth (NS 6) all the teeth in the groups coated with sealant showed some degree of decalcification. A summary of the results for the experimental teeth is provided in Tables I and II. The total area under study (22.24 mm^2) was calculated by subtracting the total surface area of the attachment (3.5 mm diameter) from the total surface area outlined by the reinforcement ring (6.35 mm in diameter). In the NS group the total decalcification ranged from 0.00 mm^2 to 3.46 mm^2 (15%) with a mean of 1.46 mm^2 (6.6%). In the FS group total decalcification ranged from 0.56 mm^2 (2.5%) to 4.47 mm^2 (20%) with a mean of 2.49 mm^2. The p value provided by the t test was 0.087 indicating that the differences between the two sealant groups were not statistically significant at the 0.05 level of confidence.

II. SEM INVESTIGATION

Surface Analysis

SEM analysis of the epoxy replicas revealed that after thermocycling there were etched areas devoid of sealant. These deficient areas persisted in the same basic size and pattern after tooth brushing and acid challenge. SEM photomicrographs (fig.3-5) demonstrate this repeated pattern as it occurred on a NS sample.

To investigate the possibility that the etched enamel surfaces were never completely covered with sealant, 3 additional teeth were etched and coated with sealant and swabbed with alcohol. Air drying of the teeth showed frosty appearing areas that lacked the typical gloss associated with sealant coverage. Application of ink followed by acetone swabbing resulted in staining of the frosted areas.

Resin Tags

Etching with 5% hydrochloric acid exposed resin tags beneath all areas covered with sealant in both the NS and FS groups (fig. 6, 7). On average the tags appeared to be about 10 μm in length with both sealants. In areas without sealant coverage no resin tags were apparent. The relationship between resin tags and sealant is nicely illustrated when areas covered with sealant bordered areas devoid of sealant (fig. 8). In addition to a lack of resin tags, areas without sealant coverage were decalcified to a depth of approximately 20-25 μm.

Sealant when present was usually the order of about 10-15 μm in thickness. Areas of sealant beneath bracket adhesive were in general the same thickness (10-15 μm) as sealant present on exposed enamel surfaces (fig. 9).

Microprobe Analysis

The microprobe employed for fluoride assay could reliably detect fluoride levels in excess of 0.1 - 0.3% by weight. Microprobe analysis of the NS, FS and control teeth failed to detect any fluoride above the these levels in enamel subjacent to fluoride releasing sealant, fluoride releasing adhesive and non fluoride releasing sealant. Fluoride could not be detected in any portion of the samples including the fluoride releasing sealant and adhesive.

Samples of the fluoride releasing sealant (Light Bond, lot 09262) and composite (Light Bond, lot 079203, lot 12853) were placed on mylar strips and polymerized according to the manufacturers specifications. Microanalysis of these fresh samples also indicated that if fluoride was present it was at a concentration of less than 0.3%

DISCUSSION

The protocol used here was a modification of that used by Ceen and Gwinnett (1981). Although it resulted in universal decalcification of the buccal and lingual surfaces of the control teeth, the decalcification was not marked. To obtain accurate tracings with the JAVA imaging program it was necessary to stain decalcified areas with ink to enhance the video image.

As evidenced by the uniform decalcification of control teeth, the fluoride-releasing adhesive was not effective at reducing decalcification in any areas peripheral to it. These observations are in agreement with the opinions of some investigators (Bishara et al., 1991; Swift, 1989) who felt that the low levels of fluoride released from fluoride-releasing resins may make them less effective than silicates and glass ionomer cements in preventing decalcification.

The results of this study indicate that it is incorrect to assume that light cured sealants provide a uniform protective film. With the exception of one tooth (NS 6), the conventional and fluoride releasing sealants used here failed to provide complete protection when applied to etched enamel. Observations similar to these were reported in a study of chemically cured sealants performed by Ceen and Gwinnett (1981). Using similar experimental conditions these investigators observed white spot formation adjacent brackets bonded to teeth coated with chemically cured sealant.

Banks and Richmond (1994) also reported that at least one unfilled visible light cured sealant (Transbond) may not be effective in preventing enamel decalcification in vivo.

In contrast to the findings of this study, Joseph et al. (1992,1994) reported complete sealant coverage with a visible light cured sealant (Transbond) and with a chemically cured (auto cure) sealant (Concise) on teeth covered with a coping. These studies involved small samples without any treatments to simulate intraoral insults, therefore their relevance in terms of long term clinical serviceability is questionable.

Although the difference between the two groups of teeth treated with sealants was not significant at the chosen level of confidence (p <

0.05), on average the teeth treated with fluoride releasing sealant had greater than 40% more decalcification than those coated with conventional sealant. The differences between the two groups may have proved significant with a larger sample size.

The similar patterns of decalcification (fig. 3 - 5) noted after the thermocycling, tooth brushing, and exposure to artificial caries medium would suggest that some of the decalcified areas present after acid attack were also present after thermocycling. It is possible that a significant amount of the decalcified areas present after exposure to the artificial caries medium were not initally covered with sealant. Since polysiloxane impressions and epoxy replicas were not made immediately after sealant application (i.e. before thermocycling) this remains a matter of speculation, but the presence of etched unsealed stainable enamel observed on the 3 teeth stained after sealant application supports this contention.

The relative effects of thermocycling, tooth brushing and acid attack on the sealants studied is unknown. Final SEM micrographs (fig. 9) indicate the thickness of sealant under the bonded attachments was about the same thickness (~10 μm) as sealant in the areas exposed to tooth brushing. Since the sealants were polymerized before the attachments were bonded, sealant abrasion from tooth brushing is likely minimal. Decalcification of exposed enamel would be expected to reduce sealant coverage by undermining sealant in adjacent sealed areas.

The duration of protection of sealant is said to be related to the thickness and distribution of sealant (Ceen and Gwinnett, 1981). Although complete coverage by the sealant is desirable to maximize its' protective function to date there is no accepted ideal or minimum thickness of sealant necessary to provide a protective film. The sealant layers observed here (from 0 to 10-15 μm) were in the lower ranges of values reported by Ceen and Gwinnett (1980) for chemically cured resins. Joseph et al. (1992) found that Transcend light-cured resin produced intact sealant layers in excess of 120 μm. Although Delton light cured sealant was used in the same study, no values or micrographs were provided by Joseph et. al. so that direct comparison of their results to those reported here is not possible.

Explanations for initial incomplete sealant coverage as well as the relatively thin sealant layers are largely a matter of speculation and include factors such as: inadequate etching of the enamel, pooling of the sealant prior to polymerization, polymerization inhibition related to oxygen levels, and insufficient sealant application.

It is unrealistic to expect the enamel layers of teeth taken from different individuals to react uniformly to etching procedures and individual variations in enamel structure and composition could account for some of the deficiencies observed here.

Compared to chemically cured sealants, the command set capability of light cured sealants should reduce flow and pooling prior to polymerization. A certain amount of prepolymerization redistribution due to the topography of the enamel surface and gravity likely still occurs with light cured sealants.

Oxygen inhibition of polymerization has been noted with visible light-cured sealants (Joseph et al. 1992,1994) and it is reasonable to assume that oxygen inhibition affected the amount of sealant coverage observed in this study. The exact role of oxygen inhibition in determining the amount of sealant coverage with light cured sealants is unknown. As has been noted with chemically cured sealants (Ceen and Gwinnett 1980), there is likely much variation between the various products available.

The possibility exists that inadequate amounts of sealant were applied to the etched enamel surfaces. Sealant was brushed on to the etched enamel surfaces according to the manufacturers instructions and was deemed more than adequate. Application of excessive amounts of sealant would result in flow and pooling in vitro. Ceen and Gwinnett (1980) have noted that successive coats of sealant do not result in increased sealant coverage and areas that were initially uncovered generally remain so. Sealant thicknesses in the order of 200 µm are not desirable as they could alter expression of the appliance prescription especially if the sealant is not evenly distributed.

The areas devoid of sealant did not show any evidence of resin tags. It appears that rather than wearing away, the sealant was likely never present in some of these areas. Areas devoid of sealant showed extensive decalcification (20-25µm) after application of 5%

hydochloric acid (fig. 8). This degradation is the result of the three separate acid applications (i.e. initial etch with phosphoric acid, artificial caries environment and hyrdrochloric acid to demonstrate resin tags). The increased susceptibility of etched, unsealed enamel to further decalcification was also noted by Silverstone (1977). In vitro remineralization is reported to reduce this rate within 24 hours, but it still remains higher than that of normal enamel (Silverstone, 1977).

Davidson (1980) and Silverstone (1977) have suggested that sealants may reduce enamel solubility after the surface layer is abraded away. The results presented here do not support this hypothesis as it applies to sealants used in orthodontic bonding as abrasion does not appear to be significant. Since the model used here to simulate the abrasive effects of tooth brushing did not have an archwire it would tend to over-estimate toothbrush abrasion on the mesial and distal aspects of the buccal surfaces. In vivo it is conceivable that excessively coarse diets could increase sealant abrasion in areas occlusal to orthodontic attachments.

Given the inability of light cured sealants to completely cover areas of etched enamel, the addition of fluoride to sealants might provide protection to adjacent unsealed areas of enamel. If fluoride was present in fluoride releasing sealant and adhesive tested in this study, it was not present in concentrations high enough to reduce decalcification under the conditions of the study.

In initial communications with an employee of the company (Reliance Orthodontic Products Inc.) that produces the fluoride releasing sealant, I was told that it contained 2.5% anionic fluoride by weight. After the initial microprobe tests failed to detect fluoride levels above 0.1 - 0.3% by weight in subjacent enamel, sealant, and adhesive, the company was informed of our results. In subsequent communication with directors of the company I was informed that the sealant (Light Bond) contained a hydrogen fluoride concentration of 0.15% by weight and the adhesive (Light Bond) contained a combined hydrogen fluoride and sodium fluoride concentration of 0.29% by weight. Subsequent analysis of a second set of samples from Reliance failed to demonstrate any fluoride above the 0.1 - 0.3% range in either the sealant or adhesive.

Recently, Alwi and Creanor (1994) had an abstract published concerning a study of Light Bond and another product by Reliance (Rely-A-Bond) using a small in vivo sample. Although values were not presented, compared to controls, statistically significant less demineralization and more salivary fluoride were reported with the two products.

Although some research (Silverstone, 1981,1988; Cate and Duijesters, 1983; Brown, 1974) suggests that levels as low as 0.1 ppm can promote remineralization, there is no generally accepted or prescribed concentration of fluoride that has been demonstrated to inhibit decalcification and/or promote enamel remineralization.

One possible limitation of this in vitro experiment is that unlike saliva, the artificial caries medium did not contain calcium which may be necessary for low levels of fluoride to exert an inhibitory effect on decalcification and promote remineralization (Cate and Duijesters, 1983).

Clinical Significance

Clinically it is impossible to etch only those areas that will be covered by orthodontic attachments and extension of etched enamel beyond the area of attachment bonding is unavoidable. The results presented here indicate that while sealants may not provide complete protection for etched enamel, they can help protect those etched areas not covered by the bonded attachment.

Due to the incomplete and unpredictable nature of sealant coverage, additional etching of enamel beyond the areas of orthodontic attachment bonding to protect adjacent enamel is a questionable practice. Etching of peripheral areas may actually place them at greater risk for future decalcification. The already increased potential for demineralization around orthodontic brackets may be enhanced by the reduced resistance of etched enamel to decalcification as well as increased plaque accumulation on the roughened etched enamel.

Given the evidence to support the role of fluoride in the prevention of decalcification, it is logical to assume that all things being equal, a fluoride releasing sealant should help to reduce decalcification around orthodontic attachments. The results of this study indicate that factors (i.e. fluoride concentration and availability, and the ability of the sealant to adequately polymerize) other than manufacturers claims must be considered in selecting a sealant.

Future Study

Given some of the recent theories regarding the anticariogenic mechanism of fluoride (Silverstone, 1988; Cate and Dejuisters, 1983), in vitro testing of a fluoride releasing sealant should be performed in artificial saliva to allow interaction between fluoride and dissolved minerals (i.e. calcium).

To accurately assess the effect of fluoride addition to sealants, only products with demonstrable levels of fluoride should be tested.

It is possible that some of the deficiencies reported here may be due to variations in enamel etch patterns. The role of the acid etch pattern in sealant coverage could be assessed using epoxy replicas of etched teeth for SEM examination of the etch pattern. The replicas could then be compared with their respective teeth after sealant application to correlate absence of sealant coverage with variations in the etch pattern.

The ideal way to test the anticariogenic effect of the fluoride releasing sealant is to perform randomized clinical trials with experimental and control teeth in each patient.

More study needs to be done on the role of oxygen inhibition of polymerization of light-cured sealants. As has been noted with chemically cured sealants there is likely considerable variation between different products.

SUMMARY AND CONCLUSIONS

The purpose of this study was to compare the relative ability of conventional and fluoride releasing intermediary sealants to prevent white spot formation around orthodontic attachments bonded with fluoride releasing adhesive.

In an in vitro study 30 extracted premolars were randomly separated into 3 equal groups. Ten teeth were etched and sealed with conventional light cured sealant and 10 teeth were etched and sealed with light cured, fluoride releasing sealant prior to attachment bonding. All 3 groups had orthodontic attachments bonded with fluoride releasing adhesive and were subjected to thermocycling (4^{o} C - 55^{o} C for 1200 cycles; 25 second dwell time), toothbrushing (12,000 strokes), and artificial caries medium (2% gelatin; pH 4.0). Decalcification was quantified using video imaging software (JAVA) and the results were assessed with a t test.

As an adjunct to the artificial caries test, impressions were taken of representatives of each group after each of the three treatments. Epoxy replicas poured from the impressions were sputter coated and analyzed in a SEM.

Longitudinal sections of representatives from each of the three groups were polished and etched with hydrochloric acid to demonstrate resin tag formation.

Combined wavelength energy dispersive electron probe microanalysis was used to assay fluoride levels in the enamel beneath the adhesive and sealant.

The following conclusions were drawn:

1. The fluoride releasing adhesive tested did not reduce decalcification of enamel around bonded orthodontic attachments.

2. Compared to controls, both the sealants markedly reduced overall decalcification but failed to provide complete enamel coverage and protection.

3. Decalcification of teeth treated with conventional sealant ranged from 0.0% - 20.5% of the area studied with mean of 6.6% Decalcification of teeth treated with the fluoride releasing sealant ranged from 2.5% - 20% of the area studied with a mean of 11.1%. On

average the teeth coated with fluoride releasing sealant had 40% more decalcification than those coated with conventional sealant. A Students t test used to evaluate differences between the two groups of sealant treated teeth provided a p value of 0.087. The difference between the two groups may have proved significant at the level of confidence chosen for the study ($P < 0.05$) with a larger sample.

4. There were areas of etched enamel that were not initially covered with sealant. The relative importance of these uncovered areas is unknown but they may represent a significant proportion of those areas that were demineralized after exposure to the artificial caries medium.

5. Etching of areas peripheral to orthodontic attachments may place those areas at greater risk of decalcification as etched areas devoid of sealant appeared highly susceptible to acid attack.

6. Sealant when present was usually about 10 μm thick and was abraded minimally by approximately 1 year of simulated tooth brushing.

7. Since resin tags were present only in areas covered with sealant, the study did not confirm the hypothesis that resin tags reduce decalcification after surface sealant is worn away.

8. Combined wavelength-energy dispersive microprobe analysis failed to detect fluoride above a range of 0.1 - 0.3% in the enamel of teeth coated with the fluoride releasing sealant or in the fluoride releasing sealant and adhesive. The levels of fluoride reported by the manufacturer to be present in the fluoride releasing sealant and adhesive were at the limit of detectability for the microprobe analysis. If fluoride was present in either of the components, it was not present in concentrations high enough to reduce decalcification under the conditions of this study.

APPENDIX

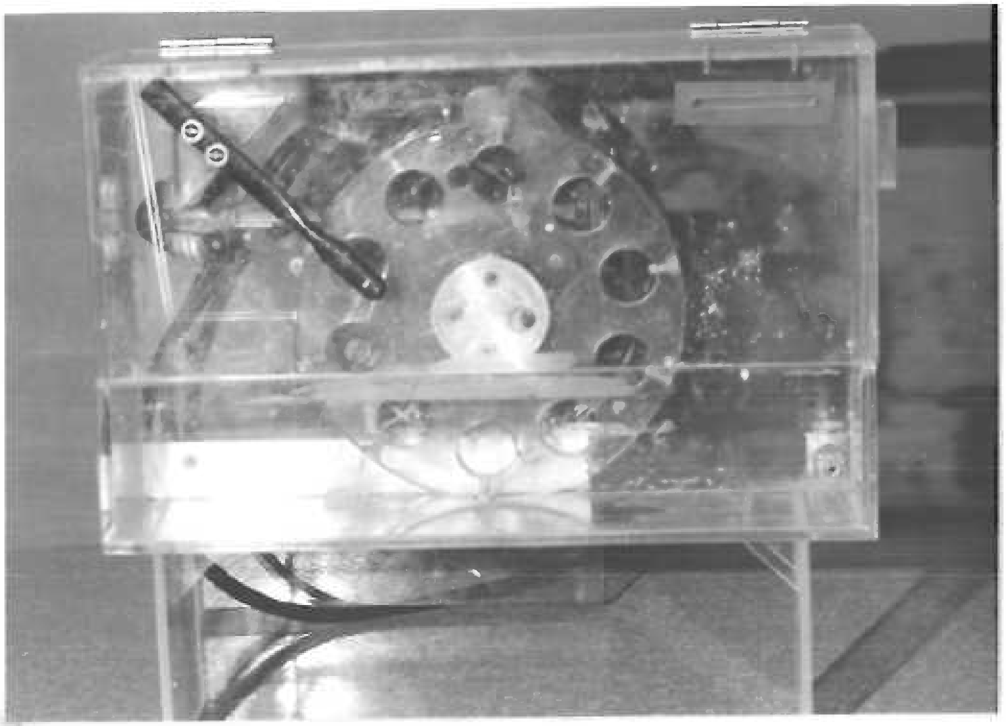

Fig.1 Tooth brushing machine.

Fig.2 Stereo microscope with attached videocamera that was used to image the experimental teeth.

TABLE I. Total area of enamel decalcification of teeth pre-coated with fluoride releasing sealant (FS group) and teeth pre-coated with conventional nonfluoride releasing sealant (NS group) after 96 hours of exposure to artificial caries environment.*#

Tooth.	M 1 ($mm.^2$)	M 2 ($mm.^2$)	Mean ($mm.^2$)	Percent Decal.
FS 1	1.7535	1.7438	1.7486	7.9%
FS 2	2.2025	2.3083	2.2554	10.1%
FS 3	3.5536	3.4408	3.4972	15.7%
FS 4	4.1643	4.3619	4.2626	19.2%
FS 5	3.1483	3.1896	3.1689	14.2%
FS 6	2.2694	2.3660	2.3177	10.4%
FS 7	0.5316	0.5893	0.5604	2.5%
FS 8	4.6296	4.4712	4.5504	20.5%
FS 9	2.1188	2.2716	2.1952	9.9%
FS 10	0.4509	0.4522	0.4515	2.0%
NS 1	3.5127	3.4642	3.4884	15.7%
NS 2	0.8468	0.8333	0.8400	3.8%
NS 3	0.4771	0.4830	0.4800	2.1%
NS 4	1.6451	1.6925	1.6688	7.5%
NS 5	1.1326	1.1625	1.1475	5.1%
NS 6	0.0000	0.0000	0.0000	0.0%
NS 7	0.7444	0.6983	0.7213	3.2%
NS 8	3.4001	3.3004	3.3502	15.1%
NS 9	1.4612	1.4319	1.4465	6.5%
NS 10	1.5846	1.4886	1.5366	6.9%

*Standard Error of the Measure = .06 $mm.^2$

Percent decalcification was calculated for a total study area of 22.24 mm^2.

TABLE II. Group Means and Standard Deviations **

Group n=10	Mean $mm.^2$	S.D. $mm.^2$	Decal. Range
FS	2.496	1.392	2.0 - 20.5%
NS	1.464	1.462	0.0 -15.7%

**There was no statistical difference between the FS and NS results ($P < 0.05$).

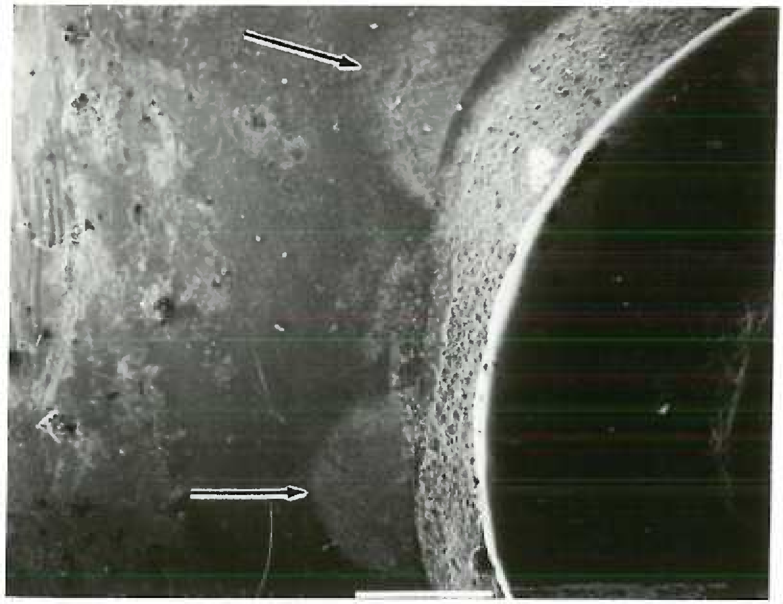

Fig. 3. A view of the buccal surface of an epoxy replica of a NS tooth after thermocycling. N
the two semilunar shaped areas devoid of sealant. (35 X magnification. Bar = 500 µm)

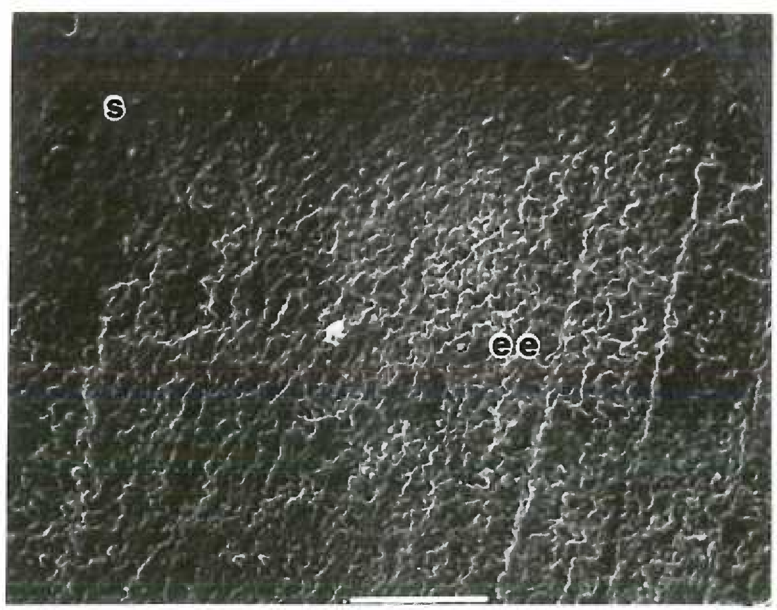

Fig. 4. A higher magnification of the boundry of one of the semilunar areas noted in Fig.
Roughened etched enamel (ee) along the lower portion of the micrograph borders the sea
portion (s) in the upper area. (1000 X magnification. Bar = 50 µm)

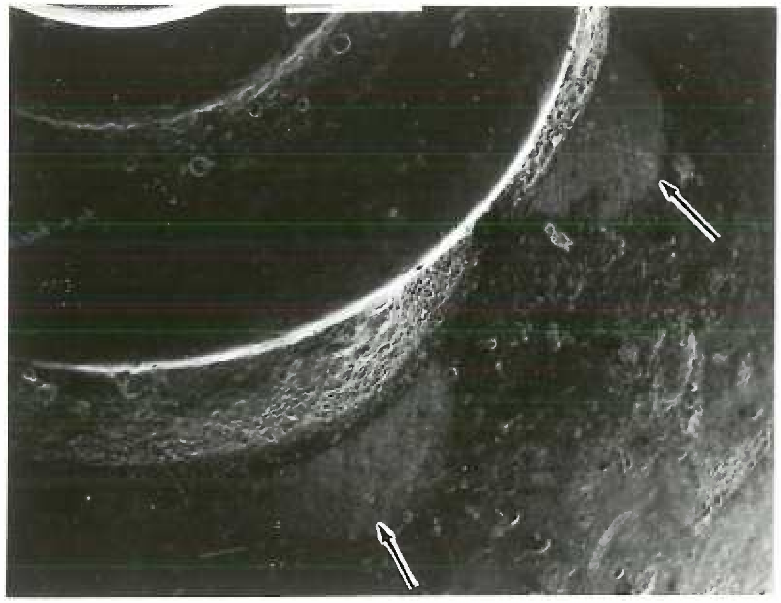

Fig. 5. Attachment-enamel boundry of the area shown in fig. 1 after exposure to artific caries medium. Note the similarly appearing semilunar decalcified areas. (35 X magnificati Bar = 500 µm)

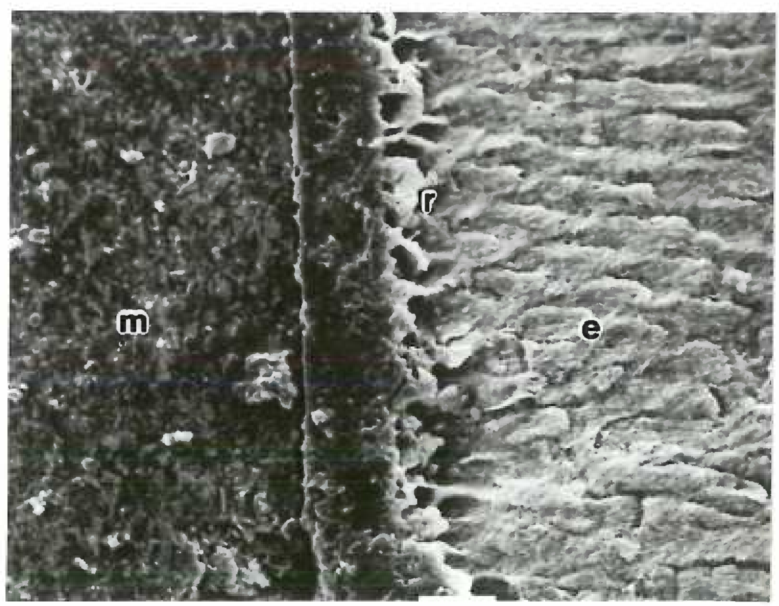

Fig. 6. The resin-enamel junction of a longitudinally sectioned NS tooth treated v hydrochloric acid showing (from left to right) embedding medium (m), sealant (s) with re tags (r), and enamel (e). (1000 X magnification. Bar = 10 µm)

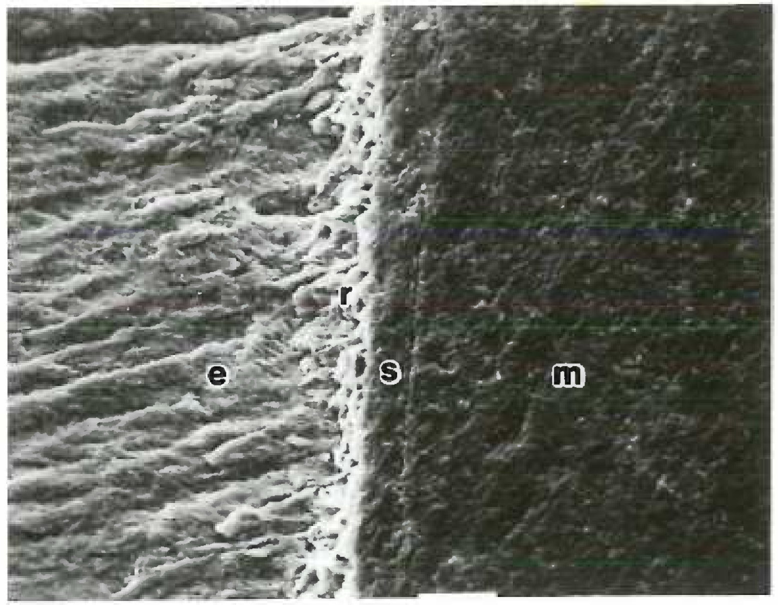

Fig. 7. Sealant-enamel junction of a longitudinal section of a FS tooth etched with hydrochl
acid showing (from left to right) enamel (e), sealant (s) with resin tags (r), and embedd
medium (m). Sealant resin tags are similar in appearance to those noted for the NS sam
(1000 X magnification. Bar = 10 μm).

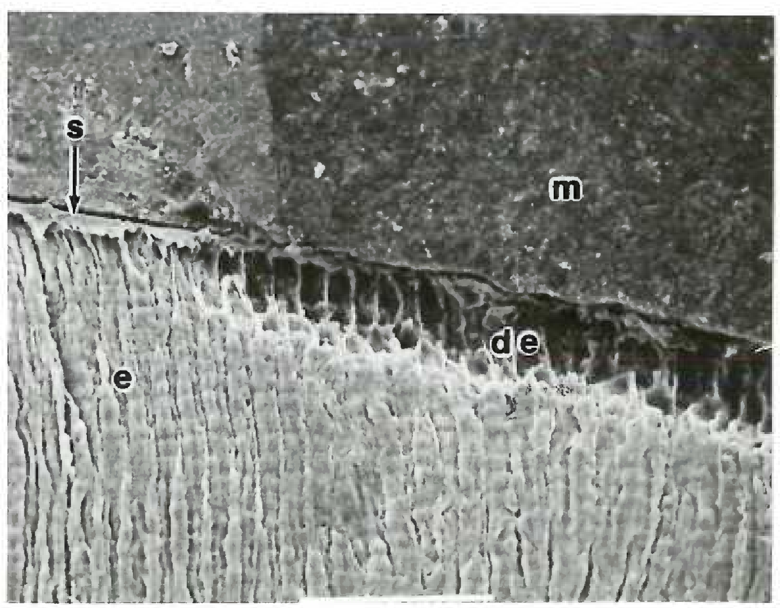

Fig. 8. Longitudinal section of a NS tooth showing (from left to right) enamel (e) covered
sealant (s), adjacent an area of unsealed decalcified enamel (de). (750 X manification. Ba
50 μm.)

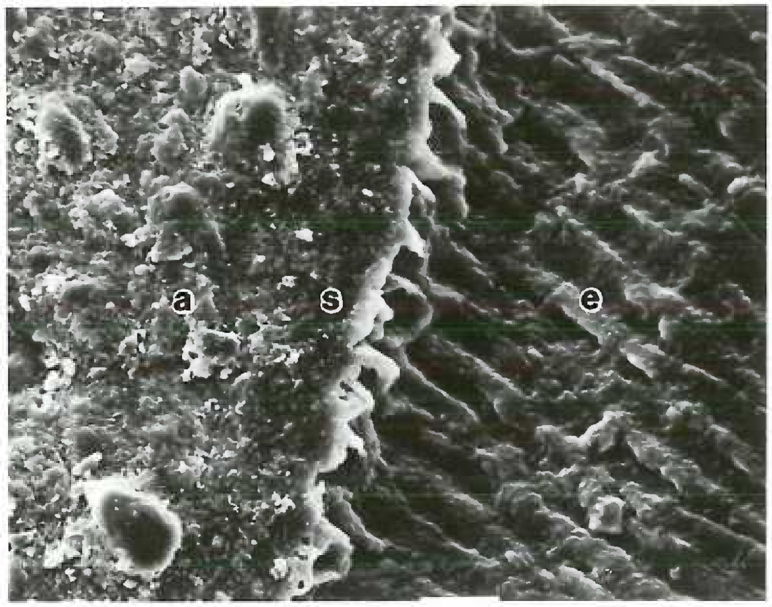

Fig. 9. Adhesive-sealant interface in a sectioned tooth (NS) etched with hydrochloric a showing (from left to right) composite adhesive(a) with filler particles, sealant (s) with re tags, and enamel (e). (1000 X magnification. Bar = 10 μm.)

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