Durability and Aging of Dental Fissure Sealants

By

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Abstract

The purpose of this study was to evaluate the physical properties of dental sealant resins under aging conditions to determine the critical factors controlling functional property loss with time. The effect of processing on the chemical and thermal properties of several different sealant formulations was evaluated. These processing parameters included the blue light exposure time for each light-curing system and time after illumination.

The results indicate that the level of cure for each system was incomplete at the end of all processing procedures. Heating as well as further aging of the cured resin advanced the cure. Additionally, one of our goal was to model the curing characteristics of the sealant as a function of depth in the fissure to evaluate the thickness influence on the sealant mechanical properties. The strength and stiffness of the light-cured sealant varied as a function of depth in the fissure. This results in a gradient of deformation which could cause early fracture of the resin upon chewing. Incomplete resin conversion is important since dental adhesives have been shown to be leached by saliva and the elution products have been recently shown to be potentially estrogenic. Samples of differently processed commercial sealants were immersed in an ethanol/water solution and extractions were analyzed by HPLC. An inverse correlation between the degree of cure and the % of elution as well as high level of extraction was found.

An *in vivo* study has been performed on fifteen pigs. The purpose of the experiment is to obtain data on *in vivo* sealing ability of the sealant. Low sealant retention rates have been found but interesting observations of sealed fissures were made and the pig can be considered like an acceptable model.

Acknowledgments

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1. Introduction

A major problem in restorative dentistry is the lack of adhesion of dental materials to mineralized tissues. As a result, microleakage of saliva and bacteria occurs at the tooth/dental material interface. This results in staining, breakdown at the margins of the restorations, secondary caries at the interface and postoperative sensitivity.

Enamel pit and fissure sealants are commonly used to seal defect sites that collect bacterial plaque and lead to early formation of caries. The success of these resins is related to the strong adhesion of the methacrylate resin to the enamel surface and to the hardness of the polymerized resin.

The success of pit and fissure sealants in preventing caries beneath sealed surfaces has been well documented (Buonocore, 1971 [1]; Williams, Price and Winter, 1978[2]; Going et al., 1978[3]). Meticulous application procedures have resulted in high retention rates in recent clinical trials (Stephen et al., 1978[4], 1981 a[5], b[6]) and high in vitro bond strengths, approaching the tensile strength of dental enamel (Thomson et al., 1980[7]).

While these results might imply that there may be little scope for improving adhesion still further, economic considerations now suggest there are two other areas where improvements may reduce the real cost of fissure sealing. One improvement would be to minimize the time for sealant application and another would be to decrease the frequency of re-sealing. Less frequent resealing would reduce patient exposure to monomer ingestion which is a valid concern since some recent work has shown that pit and fissure sealants might be estrogenic (Oleas, 1996[8]). Reduction in monomer ingestion should reduce potential estrogenic effects.

Our overall aim was to understand the level of sensitivity of pit and fissure sealants to variations in the preparation process and to determine the link between conversion and

their performance. This evaluation is composed of four major parts: (1) determining the effect of processing variables on the sealant conversion (2) evaluating the influence of processing on the mechanical behavior of the sealant and (3) estimating the amount of unpolymerized resin leached by saliva as a function of sealant processing (4) evaluating the performance of the sealants in a clinical study to obtain data on *in vivo* sealant fracture patterns.

The first part of this effort has been achieved by polymerizing light cure commercial sealants for different light exposure times and by comparing the level of cure of the same sealant by thermal and infrared evaluation. The second part of this work consisted in creating experimental sealants cured under windows of Bis-GMA and testing the specimens in tension. The third part of the study evaluated the elution of commercial light cure sealants and self cure sealants by High Performance Liquid Chromatography (HPLC). The last part of the study consisted in a clinical study, during which pigs were treated with pit and fissure sealants.

2. Literature review

2.1 Pit and fissure sealants

2.1.1 What are pit and fissures ?

Pits and fissures in the occlusal surfaces of both deciduous and permanent teeth are particularly susceptible to decay. Figure 2.1 and Figure 2.2 show the structure and complexity of the occlusal surface of a tooth. The pits and fissures are enamel faults that result from noncoalescence of enamel during tooth formation, see Figure 2.3. This lack of enamel coalescence may extend to the dento-enamel junction. The susceptibility of occlusal pits and fissures to caries is related to the physical size, shape and location of the pit or fissure, which can provide shelter for organisms and obstruct oral hygiene procedures. The unusual anatomy of the pit and fissure causes such sites to exhibit a high incidence of dental caries. Unfortunately, this anatomy also causes difficulty in diagnosing the early stages of dental caries. Figure 2.4 and Figure 2.5 show cross-sectional views of typical fissure morphology.

The conventional approach to prevent pit and fissure caries is a restorative procedure, prophylactic odontomy, during which occlusal fissures are cut away and filled with dental amalgam. A "nondestructive" approach to prevent pit and fissure caries was evaluated in preliminary clinical trials in 1965 using pit and fissure sealants. The purpose of the sealant was to penetrate all cracks, pits and fissures on the occlusal surfaces of teeth to seal off these susceptible areas from the microbiological environment[9].

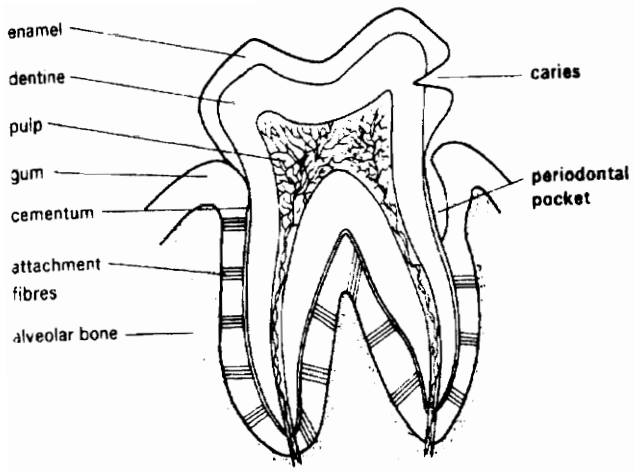


Figure 2.1: Cross-section of a tooth

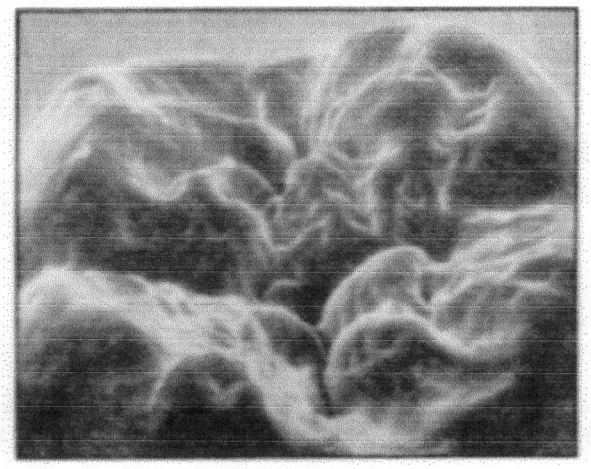
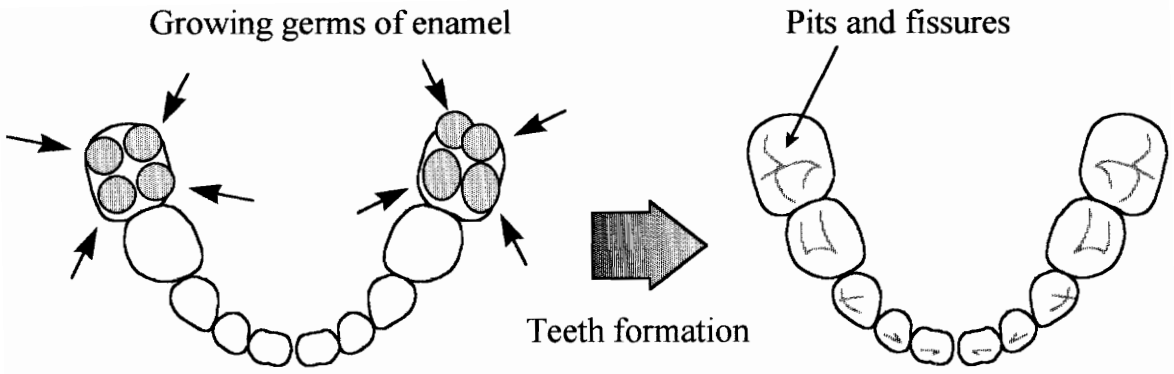


Figure 2.2: Complexity of the occlusal surface of a tooth



As the enamel germinal centers coalesce, defects are created at the boundaries. These flaws are called pits and fissures

Figure 2.3: Formation of pits and fissures

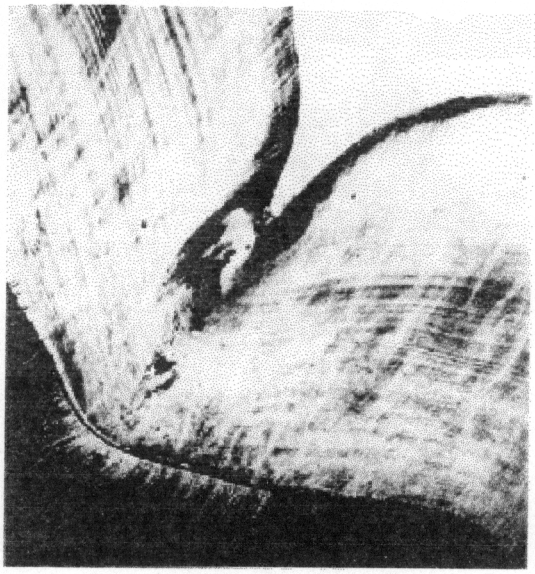


Figure 2.4: Fissure with a narrow V configuration

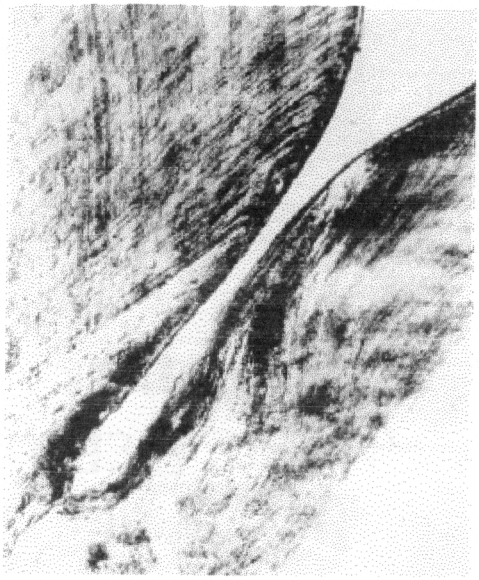


Figure 2.5: Fissure with a constricted form and a bulbous base

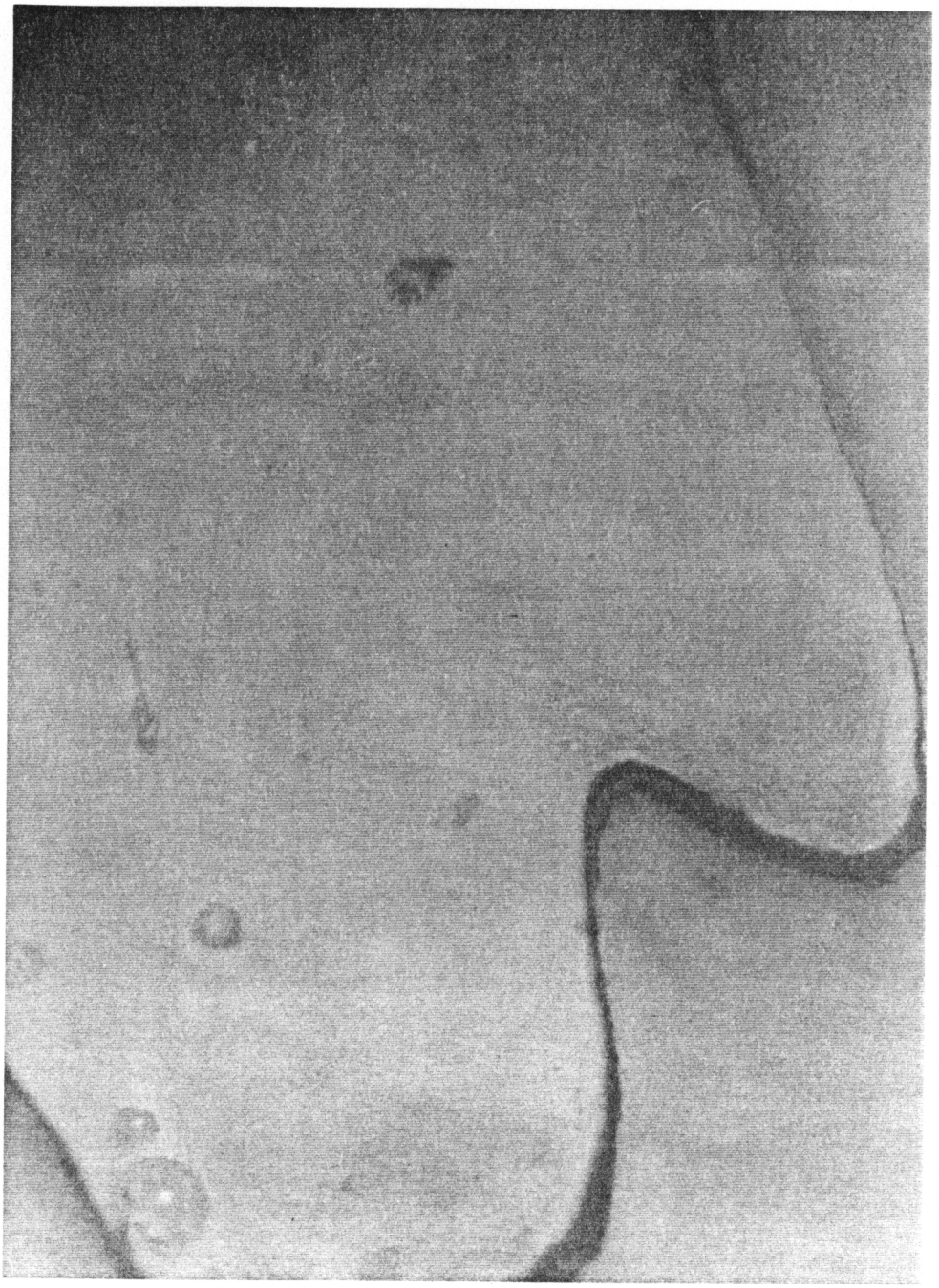


Figure 2.6: Example of a fissure filled with sealant

Periodic reapplications of sealant are often needed due to wear and fracture of the polymer that diminishes its efficiency. This also necessitates reexposing the patient to potentially harmful monomer if reapplication is required. Therefore, there is a major trade off between sealing capacity and durability of the sealant material, acid damage to the enamel or monomer ingestion.

The success of pit and fissure sealants in preventing caries beneath sealed surfaces has been well documented (Buonocore, 1971[1]; Williams, Price and Winter, 1978 [2]; Going et al., 1978[3]). Meticulous application procedures have resulted in high retention rates in recent clinical trials (Stephen et al., 1978[4], 1981 a[5], b[6]) and high *in vitro* bond strengths, approaching the tensile strength of dental enamel (Thomson et al., 1980[7]).

While these results might imply that there may be little scope for improving adhesion still further, economic considerations now suggest there are two other areas where improvements may reduce the real cost of fissure sealing. First, one improvement might be considered by minimizing the time for sealant application without adversely affecting retention and by reducing the frequency of re-sealing. This second consideration would be to further reduce the exposure of the patient to monomer ingestion and acid damage to enamel.

2.1.2 Composition of pit and fissure sealants

Dental sealants contain the following components: monomers which polymerize to form a supporting polymeric matrix, inorganic or organic reinforcing filler particles; chemicals which initiate and promote the polymerization reaction; and coupling agents which enhance the adhesion of the fillers to the coupling matrix.

The first commercial sealants were based on cyanocrylates [10]. Now, the base monomers used to form the polymeric matrix are dimethacrylates such as Bis-glycidyl dimethacrylates (Bis-GMA,) or urethane dimethacrylate (UDMA) [11] Bis-GMA is

considered by its originator to be a hybrid between a methacrylate and an epoxy resin (Bowen, 1982). These base monomers have molecular weights of 500-1000 g/mole, and some, such as Bis-GMA, are very viscous and need substantial dilution to yield fluid viscosities that allow flow into the crevices of the pits and fissures. Bis-GMA and its copolymers copolymerize with lower molecular weight diluents (100-300 g/mole) by a free radical addition involving substantial cross-linking. A typical diluent monomer is triethyleneglycol dimethacrylate (TEGDMA). Commercially available sealants differ in their content of inert fillers, and can either be clear, tinted, or opaque. Another difference between sealants is in the manner in which polymerization is initiated. Initial sealant formulations were activated with an ultraviolet light source. Second generation sealants were autopolymerized and set upon mixing with a chemical catalyst-accelerator system. The third generation sealants now are photoinitiated with visible light. Another innovation is the creation of fluoride-containing sealants which are thought to harden the enamel and act as fluoride releasing agents suppressing microbiological attachment. A study [12] comparing the effectiveness of an autopolymerized sealant with a light-polymerized fissure sealants found that thirty one months after placement of a sealant, no significant difference was seen in the clinical performance and retention between the two sealants.

Since light cure sealants are the newest type of product created, longer-term studies are still needed to evaluate the durability and sealing efficiency of these products, especially considering the sensitivity of the procedure. Strang *et al.* [13] found that a curing time of more than 60s was needed to ensure complete setting of some visible light initiated sealants, which is longer than is generally recommended in practice. Also Chosak and Eidelman [14] found that sealant penetration into etched enamel, as measured by the length of polymerized tags, was shorter when the time interval between sealant placement and exposure to the light source decreased. In this study, the light exposure time will be a variable, but the working time will be kept as constant as possible.

2.1.2.1 Polymerization

Bis-GMA monomers (see Figure 2.7) can undergo free radical addition polymerization to give a rigid cross-linked polymer. A free radical addition reaction occurs between two molecules (either the same or dissimilar) to give a larger molecule without the elimination of a smaller molecule such as water. The polymerization of the monomers to form the polymeric matrix is accomplished with the aid of chemicals such as benzoyl or lauryl peroxide, various tertiary amines, and camphorquinone. The free radical polymerization follows the three following stages

2.1.2.1.1 Polymerization reaction stages

See Figure 2.8.

- ***Activation and initiation:*** The polymerization requires the presence of free radicals which are very reactive chemical species having an odd (unpaired) electron. Such free radicals are formed, by example, in the decomposition of a peroxide. This decomposition can be achieved either by (a) ultra-violet light or visible-light radiation, (b) other chemicals such as tertiary amines.

- ***Propagation:*** A radical can react with a monomer unit, producing a two unit chain radical. Propagation is the growth of these chains by successive addition of monomer units to the radical end of the chain. This leads to the formation of a polymer chain.

- ***Termination:*** This occurs when two free radicals react to form a stable molecule.

This monomer is referred to as Bis-GMA according to US Patent 3,066,112

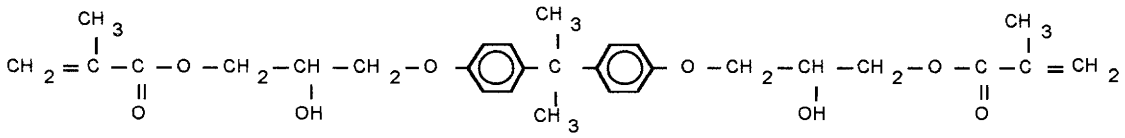


Figure 2.7: Bis GMA

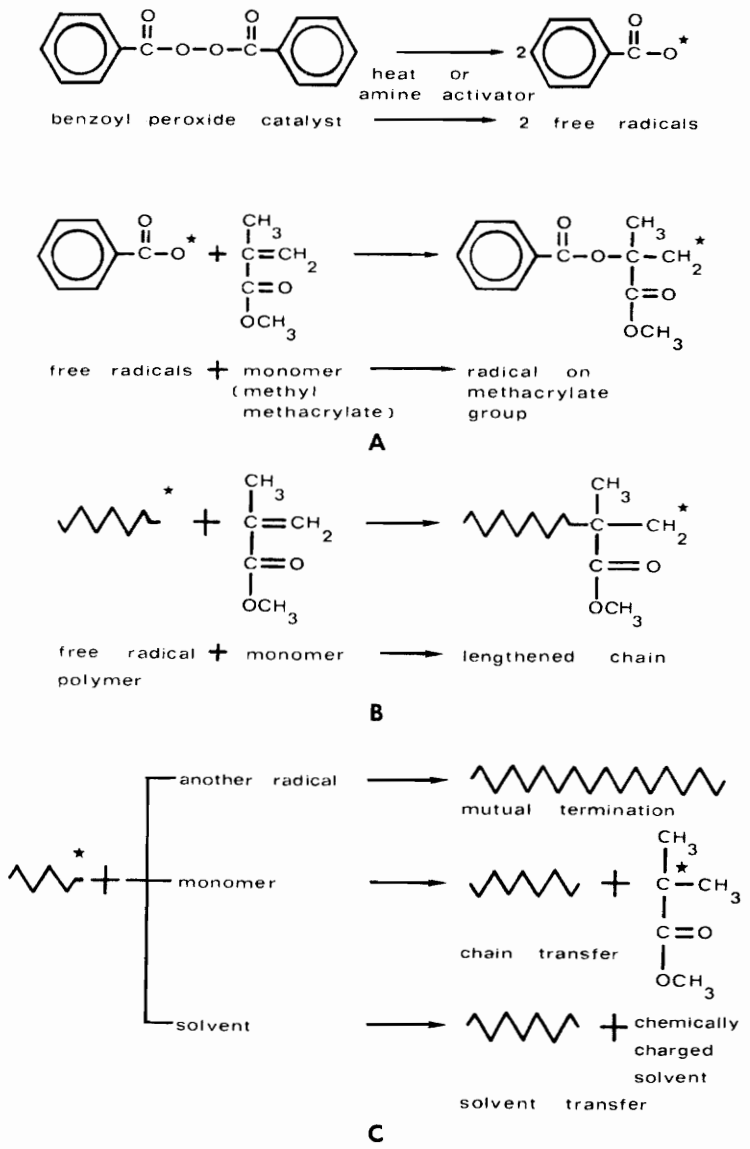


Figure 7–2 Polymerization steps for acrylic resins. (A) Formation of free radicals (initiation). (B) Propagation or growth of chain. (C) Termination or end of reaction by three possible steps: mutual termination, chain transfer, and solvent transfer.

Figure 2.8 : Polymerization steps for acrylic resins

2.1.3 Incomplete Conversion of Reactive Groups Due to Vitrification

When densely crosslinked networks are prepared by low-temperature polymerization, incomplete conversion of the reactive groups is often observed [15]. The cross-linking reaction produces a gel structure which severely reduces molecular mobility and greatly slows the rate of polymerization. Therefore the final network structure contains between 25% and 50% of unreacted methacrylate groups. Most of the unreacted carbon-carbon double bonds are on molecules which have reacted at one end and are thus bound to the polymer and are not free to elute. These species are referred to as pendant methacrylate groups. Additionally the polymer also contains a small proportion of residual monomer.

2.1.4 Mechanical properties as a function of sealant conversion

Sealant conversion can vary significantly in dimethacrylate sealant materials. Variations in both sealant composition and light exposure can alter the degree of crosslinking in photo-initiated systems. The presence of monomers in the cured sealant can be problematic for several reasons. The monomer can alter the mechanical properties of the sealant, decreasing the sealant's ability to withstand the stresses induced upon mastication. Residual monomer also poses a toxicological concern as well, because the monomer can leach out of the sealant and be ingested.

Achieving adequate cure of the sealant is important to ensure optimum mechanical properties of the pit and fissure sealant. Studies [16, 17] have shown that a hard top surface was not an indication of polymerization throughout the depth of the restoration. It has been pointed out that incomplete curing of the bulk of the restoration is analogous to a restoration composed of different materials[17]. This could lead to a pattern of deformation responsible for early fracture and leakage of the restorations. Also an insufficient degree of conversion may result in inadequate wear resistance and adhesion to tooth tissues. Therefore, inadequate cure depth may impair the clinical longevity of

dental restorations [18]. Most of the preceding observations were made for composite resins, but, since dental composite restorations have a Bis-GMA matrix, these considerations should also be true for pit and fissure sealants.

The deformation mechanisms of the sealant may also be influenced by the degree of conversion of the resin. Certain polymers, particularly thermoplastics in the glassy state are capable of undergoing a localized form of plastic deformation known as *crazing* [19]. Crazes appear as small crack-like entities which are usually initiated on the specimen surface and are oriented perpendicular to the tensile axis. Crazes are not true cracks, although the cracks leading to failure usually nucleate within pre-existing crazes. The craze is roughly 50% polymer and 50% void. The craze is constituted of cylindrical fibrils of highly oriented polymers.

Correlation has been found [20] between the type and the size of the crazes and their mechanical properties, particularly fracture toughness and elongation at break. The introduction of crosslinking increases the brittleness of polymers. This is explained by the fact that an increasing degree of crosslinking transforms a linear chain structure into a three-dimensional network that reduces chain mobility, reduces plastic deformation and makes the samples more brittle. With an increasing level of crosslinking the deformation mode may change from craze to craze/shear and to shear deformation only [21].

2.1.5 Leaching of unpolymerized resin

Since a significant amount of residual monomer or short chain polymers remain unbound in the cured sealant, leaching of unpolymerized resin from pit and fissure sealants may be possible.

Leaching of components from dental materials has a potential impact on both the stability and the biocompatibility of the material. The latter is of more concern. Components may be eluted into salivary fluids and brought into contact with mucosal

tissues. In addition, for dental composites, components may be extracted into dentine where they can diffuse toward the pulp. The realization that a large proportion of polymerizable groups in dental composites did not react during curing led many researchers to study the elution of these unbound molecules and other constituents of composites into various media. Several studies [22,23] have been realized for dental composite materials and orthodontic materials.

The results of these studies suggest that elution of leachable components from dental materials is rapid, with the majority of the components being leached during the first hours. It has been verified that virtually all the components in dental composites may be leached into solution [24]. Different techniques of investigation have been used to estimate quantitatively and qualitatively the nature of the components leached from dental materials.

Braden and Pearson [25] used infra-red (IR) spectroscopy to analyze the eluate from Bis-GMA based and urethane-based composites which had been soaked in water for 6 months. At approximately the same time, Inoue and Hayashi [26] (1982) used High Performance Liquid Chromatography (HPLC) to verify that Bis-GMA and other monomers were eluted from different dental composites. The use of UV spectroscopy to monitor the loss of residual base has been reported in 1982, by Thomson, Miller and Bowles[23]. More recently, Tanaka *et al.* (1991) [27] has utilized gas chromatography and mass spectroscopy (GC-MS) to identify large amounts of TEGDMA and smaller amounts of Bis-GMA unreacted from dental composites.

Fillers and polymerization promoters/inhibitors have also been detected in the eluted components, but unreacted monomers make up the largest fraction of the species extracted from dental materials so most of the studies are centered around them.

These studies have also shown that the extent and rate of elution of components from dental materials is dependent upon several factors. The quantity of leachables has been

correlated to the degree of cure of the polymer network. The composition and solubility characteristics of the extraction solvent influence the kinetics and mechanism of elution.

Since several factors can influence the level of components extracted from dental materials, the choice of different experimental parameters is important. For example, the level of extractable components will vary if the sample is immediately submerged in the solvent or if time elapses before the illuminated samples are exposed to the solvent. In fact, the polymerization reaction continues for hours after light-curing; it is possible that higher levels of extraction could be found if the samples were already submerged in the solvent.

The preceding statement is related to the fact that the amount of leachable components depends directly on the level of resin conversion. The greater the extent of the polymerization reaction, the fewer residual monomer are available to be leached. Results from a study from Rueggeberg & Craig (1989) [28], show an excellent inverse correlation between the degree of cure and % of elution.

Another factor relating to elution is the chemistry of the solvent. Cross-linked dimethacrylate resins are virtually insoluble but are capable of swelling in good solvents. Wu & McKinney [29] determined that ethanol solutions had good solubility parameters to dimethacrylate polymers and were clinically relevant solutions for dentistry. In particular, solutions containing 75% ethanol/ 25% water (weight %) were the most suitable solvents for dental polymers. The oral cavity probably presents an environment somewhere between the more aggressive solvents and water.

Studies [30, 31] have also shown that approximately 5-10% of the unbound monomer elutes into an aqueous solution. In other words, less than 10% of the available methacrylate groups are extractable in water. Only a small portion of the unreacted carbon-carbon double bonds in the set dental composite are present in residual monomer or elutable oligomers. The amount is less than predicted, because 90% of the unreacted

methacrylate groups are found on pendant molecules bound to the polymer network and are not available to be leached as residual monomers. Therefore, although the degree of cure in dental composites is low by comparison to polymethyl-methacrylate, the biological effect of these unreacted methacrylate groups may be decreased by the fact that they remain bound within the composite.

2.2 Adhesion to enamel

2.2.1 Physical and mechanical properties of enamel:

Enamel covers the anatomical crown of the tooth, and it is the hardest substance in the tooth and in the body. It consists mostly of inorganic components (97%), apatite in its hydroxy, fluoro, or carbonate forms. [32]. The mineral phase has an apatite structure, being principally hydroxyapatite, of empirical formula $Ca_{10}(PO_4)_6(OH_2)_2$. It is thickest over the cusps and thinnest at the base of pits and fissures.

The intact tooth surface is generally described as relatively inert and of low surface energy. It was found [33], that the surface free energy of human polished enamel is 88 ± 9 $erg.cm^{-2}$, which is of the same order of magnitude as obtained for hydroxyapatite. The health of the environment is dependent upon this low surface energy state which helps to minimize the accumulation of microorganisms upon its surface [34]. However the enamel is covered by organic substrates, the principal of which is the pellicle, composed of selectively absorbed salivary proteins. In addition, pellicle is preferentially colonized by microorganisms which constitute an adherent plaque. Occlusal surfaces show a relatively consistent pattern of plaque accumulation. Most of this plaque remains undisturbed during oral hygiene procedures. This stagnation is probably encouraged by the complexity of the pits and fissures themselves.

The occlusal enamel surface is physically and chemically complex. It is principally organic, contaminated, rough and unless modified it exists as a poor bonding substrate.

2.2.2 Principles of adhesion

Adhesion is the force which causes unlike molecules to attach to each other. Adhesion may involve attractions that are essentially strong physical forces acting between the molecules, or these attractions may be of a chemical nature. A chemical bond between the adhesive and the adherend is referred as a primary bond. These forces are quite strong and are the most desirable for adhesion. The most common type of adhesion involving physical or secondary bonds are known as Van der Waals forces. For a strong adhesive bond to be created, extensive interfacial contact between the substrate and the adhesive is essential (see Figure 2.9). Incomplete contact will produce interfacial defects such as voids that can act as stress concentrators and lower the adhesive bond strength. These defects can arise because most real surfaces are not perfectly smooth. Tooth surfaces are no exception and manifest numerous topographical imperfections. In order to achieve this contact, the adhesive must be able to flow over (i.e., to wet) the surface of the adherend. The wetting characteristics of an adhesive are generally determined by contact angle measurements. The reactivity of surface (i.e., *surface energy*), the surface roughness, and the cleanliness of the surface are factors which strongly influence wetting.

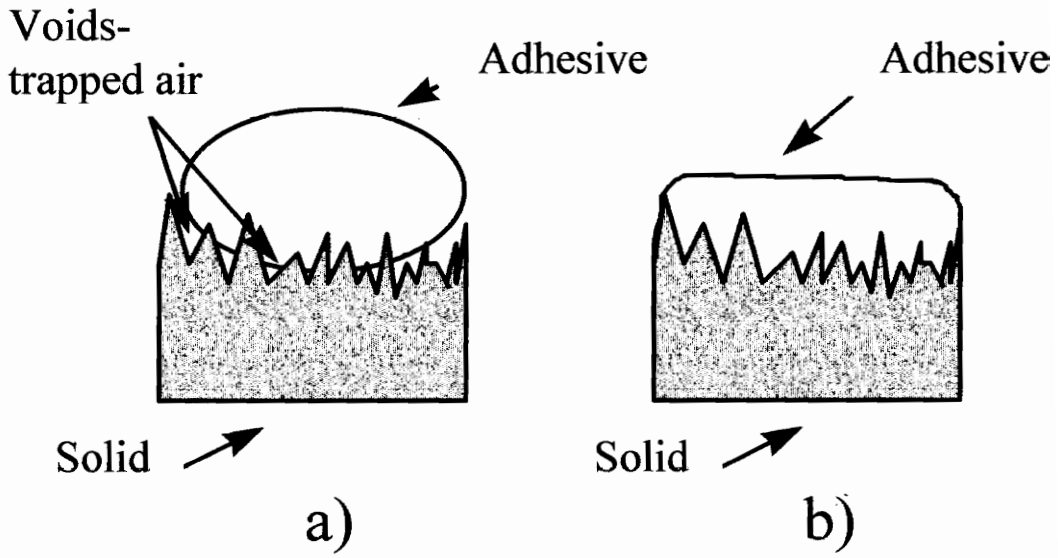


Figure 2.9: Schematics of an adhesive on a solid surface illustrating

a) poor interfacial contact

b) extensive interfacial contact

In applying these considerations to dental structures, it appears that few of the conditions required for strong adhesion are present in the oral cavity. Since enamel and dentine are inhomogeneous in a tooth and their components are partly organic and partly inorganic [32], chemical attachment leading to strong adhesion may occur on isolated sites, but will not be uniform over the entire surface. Also, the adhesive may not flow easily into surface imperfections such as tubules, pits, and fissures. Furthermore, these imperfections act as stress concentrators. When the adhesive is subjected to thermal stresses or mechanical stresses induced upon mastication, the localized high stress could fracture the adhesive at these high stress locations. Another factor affecting adhesion is the presence of water from saliva. A microscopic layer of water is almost always present on the tooth surface and cannot be removed by dehydration without injuring the pulp or the tooth structure. This film prevents the adhesive from intimately contacting the tooth. Even if an enamel bond can be created, the joint generally deteriorates with exposure to saliva, as the hydrophilic character responsible for the adhesive properties of a material also makes the adhesive-substrate interface susceptible to hydrophylic attack. Since the most important factor in joint durability is the environmental stability of the adhesive-substrate interface, the joint durability will be influenced by the diffusion of water through the polymer. A recent study [35] has pointed out that variation in tensile bond strength occurs with changes in temperature. So, thermal gradients in the oral cavity, due to food, are also an issue for the durability of dental adhesives.

Since the natural state of enamel is not favorable to adhesion, etching with various acids is generally used to overcome organic contamination and to produce a surface texture that can mechanically interlock with even poorly adherent restorative materials [36]. Buonocore (1955), [37] was the first to demonstrate the effectiveness of acid as an enamel cleaning agent. Acid treatment usually removes a few microns of enamel from the surface and exposes the basic prismatic structure (see Figure 2.10). The retention of a

sealant in a fissure is the result of mechanical bonding caused by penetration of the sealant into the fissures and the etched areas of enamel to form tags. Acid etching of the enamel surface improves the retention of the sealant by cleaning the area to be sealed, improving the wettability of the enamel, increasing the surface area and forming spaces into which the sealant can penetrate to form tags.

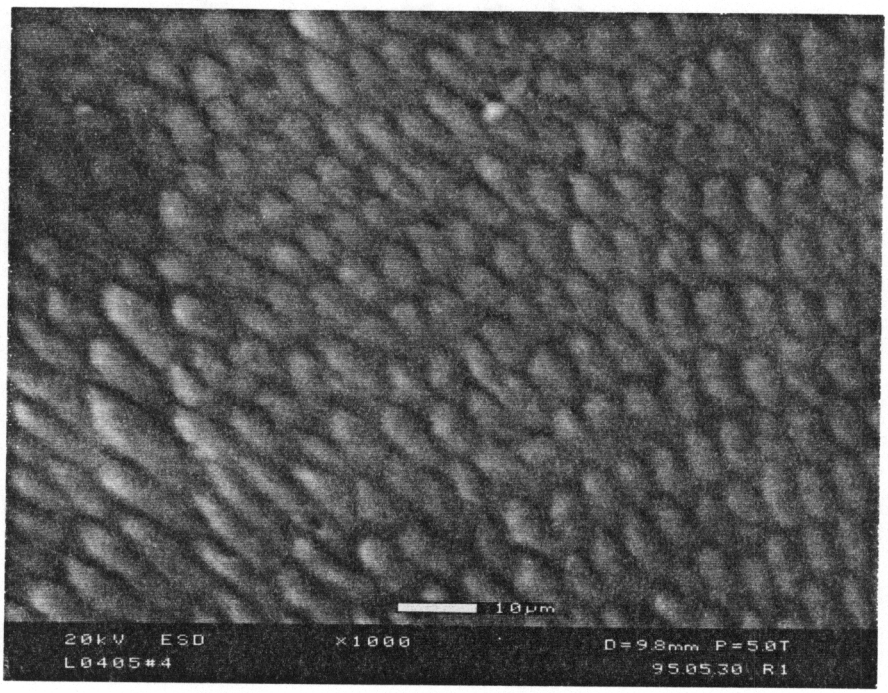


Figure 2.10: Scanning electronic microscopy of acid-etched enamel. Formation of pores

2.2.3 *In vitro* evaluation of adhesion:

2.2.3.1 General presentation of adhesive bond testing

Before selecting and using any test method, it is very important to identify the characteristics of both the adhesive and the substrate. This will include the mechanical and chemical characteristics of the materials, the pretreatment of the substrate, as well as the service environment of the adhesive.

Factors that affect the strength of adhesive joints are listed in table 1. Some typical joints [38] used in testing are presented in Figure 2.11.

Table 2.1: Factors affecting the strength of adhesive joints

Table 1: Factors affecting the strength of adhesive joints	
Joint design	• Geometrical configuration, bondline thickness
Adherends	• Susceptibility to deterioration, linear coefficient of thermal expansion and permeability, nature of adhesive and substrate, mechanical properties
Adherend surface	• Surface chemistry, cleanliness, surface topography
Nature of primer	• Viscosity, chemical composition, mechanical properties
Nature of adhesive	• Viscosity, chemical composition, reactivity, mechanical properties, linear coefficient of thermal expansion, permeability
Bonding conditions	• Temperature of substrate, ambient temperature, humidity, working time, airborne contamination, applied pressure
Internal stress	• Cure shrinkage, environmental conditions
Service conditions	• Stress, moisture, temperature
Testing conditions	• Strain rate, cyclic frequency, temperature

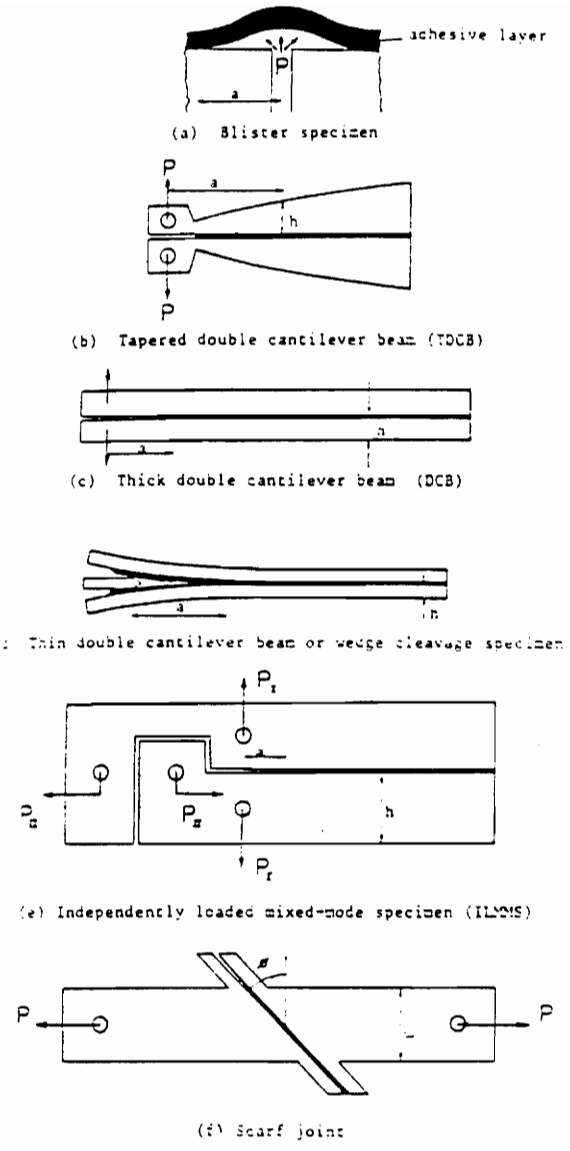


Figure 2.11: Different bond strength tests

2.2.3.2 ***General presentation of adhesive bond testing for dental adhesives***

As mentioned earlier, to test the strength of an adhesive system it is necessary to have a good knowledge of the characteristics of both the adhesive and the substrate. Unfortunately, the mechanical properties of enamel, dentine and dental adhesives or sealants are not well documented. In addition, the *in vitro* testing of adhesion is quite often carried out on bovine teeth as opposed to human teeth. Nakamichi et al. [39] investigated the validity of using bovine teeth and found that the adhesive strength to enamel showed no statistically significant difference between bovine teeth and human teeth. An additional problem comes from the variations in the quality of enamel within a sample. Very often, the enamel needs to be polished to obtain a flat surface to apply the adhesive, and, since the quality of the enamel and its water content change from the occlusal surface to the roots, it is very difficult to obtain big enough specimens of constant quality. Different bonding variables are presented in Table 2.2, the variables were found for adhesion to dentine [40], but most of the variables are also true for adhesion to enamel.

Table 2.2: Bonding to dentine variables

A. Substrate		B. Etching	
1.	Human or bovine dentin?	1.	Etch or no etch?
2.	Superficial, middle or deep dentin?	2.	What type of etchant?
3.	Occlusal, proximal, buccal?	3.	How much etchant? Renewed?
4.	Third molars or incisors?	4,	How big?
5.	Sanded surfaces? Grit? Method? Al_2O_3, SiC, 320, 600, 800 or 1000 grit.	5.	Passive or active?
6.	Dental burs? Diamond, carbide?	6.	Rinse? How long?
	Low-speed vs. high-speed, air-water?	7.	Dry ? How long?
7.	Reuse of teeth?	8.	Rewet? How much?
8.	Mount in plastic, stone, etc.?		
C. Priming		**D. Bonding**	
1.	Cover entire surface or apply within matrix	1.	How much adhesive for how long?
2.	How much primer?	2.	Spread with air? How thin, how dry?
3.	Passive or active? How long?	3.	What diameter bonding area?
4.	Wash or evaporate? How long?	4.	Pack with pressure or no pressure?
5.	Light-cure or not?	5.	Pulpal fluid/pressure or not?
6.	Wet vs. dry? How wet, how dry?	6.	Light curing. How much light? How long?
E. Storage		**F. Testing**	
1.	Water, isotonic saline, etc.?	1.	Shear vs. tensile
2.	Room temperature or 37cC?	2.	Stress rate
3.	100% RH or water?	3.	Immediate vs. 24 hr. vs. months.
4.	Preservatives? sodium azide, thymol, chloramine?	4.	Express dentin bonds in MPa or as % of enamel bond strength ?
5.	Pulpal pressure or not, magnitude? Composition of fluid?	5.	Microleakage vs. bond strength
6.	Time. 24 hr.? months? years?	6.	Gap size vs. bond strength

7.	Thermal stress? Temperatures, dwell time, number of cycles?	7.	Regional bond strengths vs. center
8.	Tooth flexure (load) tests. Magnitude, number of cycles?	8.	Gingival floor of Class V vs. occlusal floor of Class I cavities
		9.	Configuration factors. Flat surfaces vs. 3-D cavities.

For dental materials, maybe because of the limited specimen size, these typical adhesion tests are not very widely used. The most common one is a shear test applied to a system very similar to the butt joint specimen[41, 42] (see Figure 2.3). Human or bovine teeth are ground to obtain a flat surface. The tooth is then fixtured in the testing jig and the adhesive is applied on the flat surface. A composite resin cylinder is then applied to the adhesive and a shear load is applied. In an *in vivo* situation, however, bonding systems will be more likely challenged by repeated applications of stress which are below the maximum stress they could withstand [43]. Therefore, fatigue test results would be more adequate predictors of the *in vivo* performance of a dental bonding system.

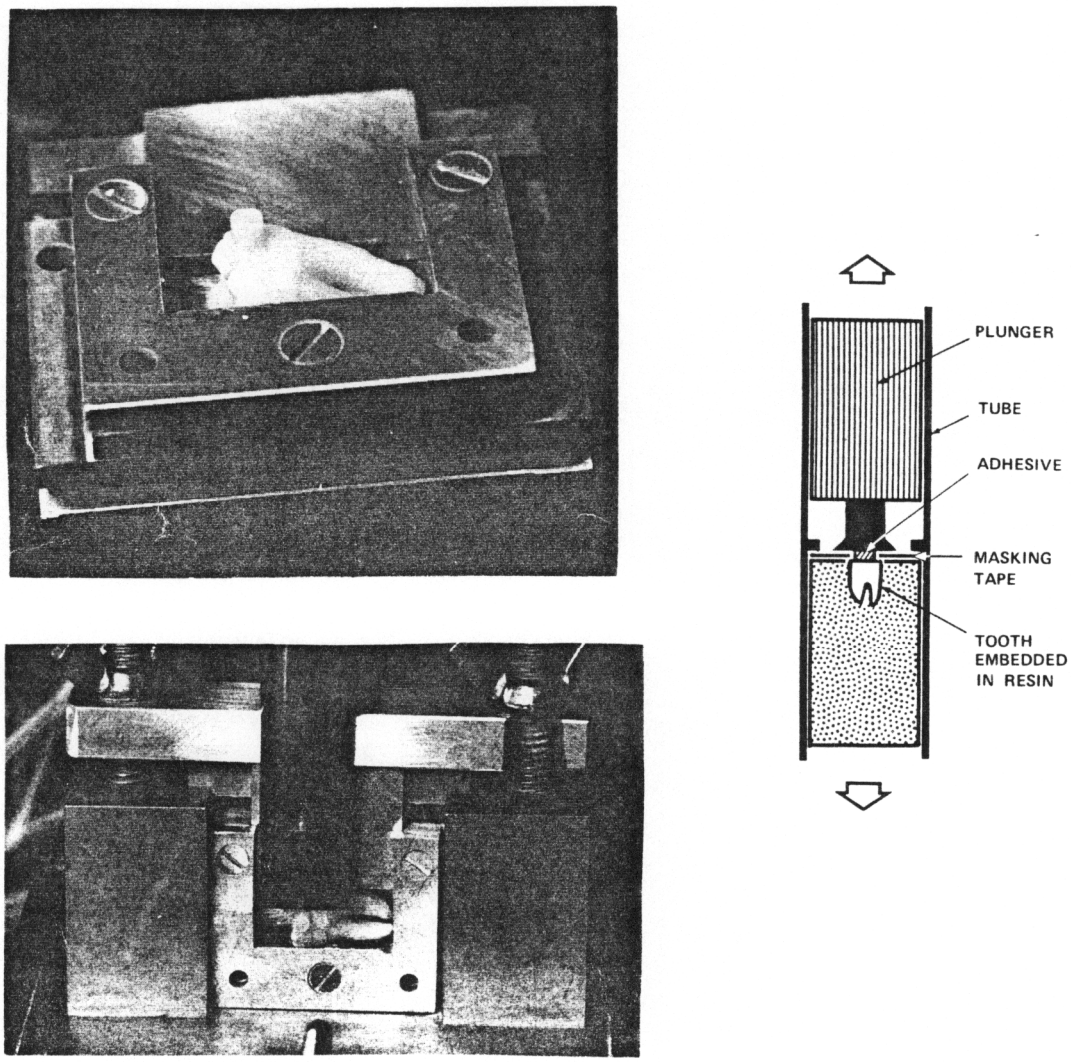


Figure 2.3: A typical bond strength test of dental adhesives

2.2.3.3 Discussion

Although much information can be obtained from laboratory tests, there is still no substitute for *in vivo* studies of the efficacy of the bond integrity service life [44]. Strength of a material fabricated under laboratory conditions may be of limited value in predicting *in vivo* characteristics of the same material fabricated by a dentist in the mouth of a patient, because the *in vivo* conditions cannot be duplicated reliably. This problem occurs in many biomaterial problems and is increased when the prediction of the *in vivo* bond strength between dissimilar materials is considered. So one can wonder how *in vitro* shear and tensile bond strength data aid a clinician in selecting a bonding system.

When possible, it is desirable that test conditions should simulate the service environment as closely as possible. For dental materials the service environments contains oral fluids, which consist largely of water, but also in a variety of solutes dissolved in it. As we mentioned earlier, bonded joints generally deteriorate with exposure to service environment containing water. It has been demonstrated that the effect of pressure on the pulpal fluid in the dentinal tubules, *in vivo*, also influences bond strength [45]. Especially in the case of adhesion to dentine, *in vitro* adhesion tests should evaluate extreme ranges of tooth structure (deep and superficial) and wetness (dry and moist) as well as different temperature gradients to screen the efficacy of new bonding systems. Also, it should be kept in mind that bond strength tests are useful for determining idealized performance, but that other factors such as mechanical properties and shrinkage of the composite used, non-ideal tooth structure, and moisture contribute to variations in clinical success of bonded composites. Thus, bond strength tests may provide useful information on technique changes but the actual numbers have little meaning. Dental material scientists presently have available a number of *in vitro* tests that measure mechanical properties and allow ranking of products and techniques.

Unfortunately, the results often do not seem to correlate directly with clinical findings. This review has highlighted the need for a better understanding of the methods available for measuring the adhesive strength of dental materials and the fact that one must be very careful on how the results found will be related to a clinical situation.

2.3 In vivo evaluation of sealant retention and fracture patterns

2.3.1 Clinical evaluation

The retention of pit and fissure sealants has been evaluated clinically. The sealing ability of the sealant has also been observed in vitro. however, there is very little data on the in vivo leakage pattern and sealant retention in vivo. Newburn et al [46] investigated caries in rat teeth sealed with commercial pit and fissure sealants. They observed a greater loss of the sealant than on human beings. They pointed out that chewing patterns and tooth morphology was not a suitable model for use in evaluating sealants. Wilkins et al [47] tested pit and fissure sealants in the monkey, since it was suggested that the subhuman primates would provide a more suitable animal model. The use of the monkey for evaluating the sealing properties of the pit and fissures presented a number of problems : (a) the conditions to which the sealants are subjected are more rigorous in a monkey than in a man; (b) the degree of occlusal abrasion was greater than what is generally observed for a man, this can explain the loss of material from the fact that some of the sealant had been applied to exposed dentine; (c) the sealants were more difficult to apply in the animal than in the man; (d) since the teeth are smaller, the pits and fissures are smaller and the reduced surface area provides less retention.

Nevertheless, it was concluded that very pertinent observations can be made from an *in vivo* evaluation. All these observations were very useful to create our animal study to establish a good correlation between the behavior of the sealants in the animal and in the man.

The *in vivo* study is being conducted to determine the *in vivo* leakage pattern and retention of differently processed sealant. Fifteen pigs were treated with commercial pit and fissure sealants; different types of initial polymerization were used. The decision to work with pigs was taken for several reasons : (a) Young animals could be obtained easily from the college of agriculture since they have been previously used for nutrition studies, (b) they could be sheltered at Virginia Tech swine farm, (c) pigs present very similar tooth morphology as humans (d) Bovine teeth, which are the most commonly used for dental evaluations presented very bad occlusal surfaces as shown Figure 2.12 and Figure 2.12.

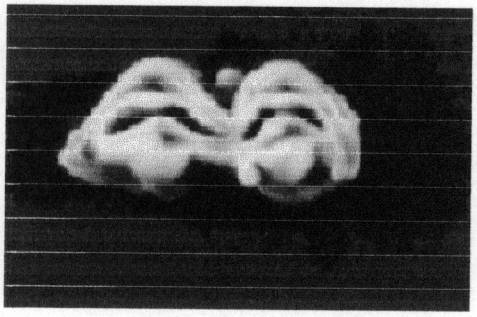


Figure 2.12: Occlusal surface of a bovine tooth

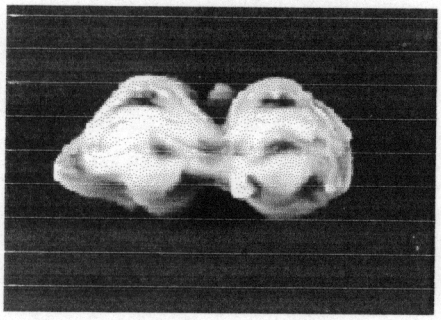


Figure 2.13: Occlusal surface of a sealed bovine tooth

2.3.2 Different modes of failure and related fracture patterns

An adhesive joint can fail in three different modes, each mode corresponding to a different level of bond strength between the adhesive and the substrate. By the use of fractography, which is the analysis of fracture surface, we can determine which mode of failure occurred. The first observation tool should be the eye, followed by a low-power microscope, then a scanning electron microscope (SEM) should be used. Visual observation is required to identify the mode of failure, but the resolution of the SEM is required to identify the mode of failure which is often microscopic in details [40]. These different modes of failure are illustrated in Figure 2.14.

- **Adhesive failure:** The failure occurs at the adhesive-adherend interface. It is a result of poor adhesion. The surface of the adherend appears intact and free of adhesive.

- **Cohesive failure in the adhesive**: The failure occurs within the adhesive layer. It is the result of a weakness in the adhesive. During the analysis of the fractured surfaces, adhesive will be found on the substrate

- **Cohesive failure in the adherend**: The failure occurs within the adherend. It is the results of a weakness in the adherend. The adherend will appear broken, pieces of adherend may even appear on the adhesive.

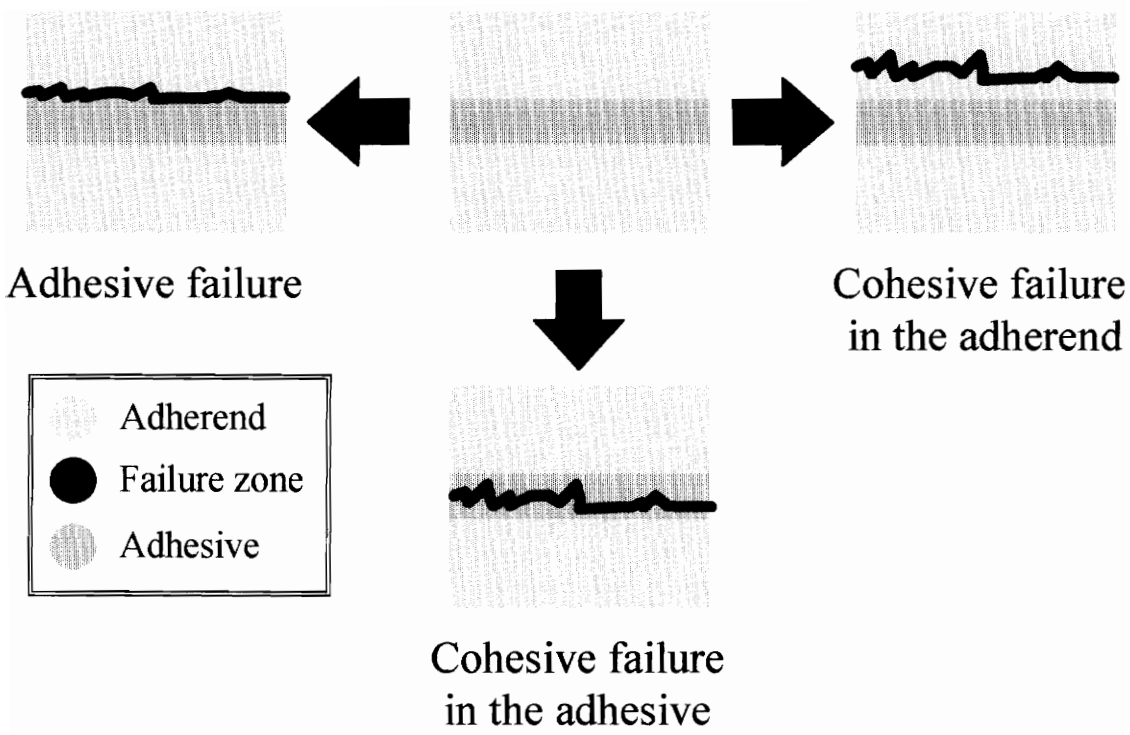


Figure 2.14: Three different modes of bond failure

3. Experimental procedure

In vitro experiments

3.1 Material

3.1.1 Commercial products

The commercial pit and fissure sealants used during this study were supplied by 3M Co. and Dentsply, Co. We wanted to study a self-cure sealant and a light-cure sealant to allow us to comparisons during the experimentation. The difference between a clear and an opaque sealant was also evaluated during the *in vivo* evaluation of the materials.

Three different commercial sealants have been polymerized conventionally:

- Delton Clear Self Cure Sealant, Dentsply Co.
- Concise White Self Cure Sealant, 3M Co.
- Concise Light Cure Sealant, 3M Co.

A Patterson blue light curing unit was utilized for the polymerization of the light cure sealant.

The self cure version of each sealant is composed of two parts: a base and an initiator. According to the manufacturer, these resins should be mixed in equal quantities. But to vary the degree of initial polymerization, three different mixing ratios were investigated. Three different times of light exposure, one below and one above the recommended time, were utilized for the same reason for the light cure version of the sealant.

Table 3.1 lists the different mixing ratios and light exposure times evaluated during the study.

Table 3.1: Different processing variables used during the study

	Mixing ratio	Exposure time
Above clinical value	M1: 1 drop base/2 drops initiator	t1: 20s
Clinical value	M2: 1 drop base/1 drop initiator	t2: 10s
Below clinical value	M3: 2 drops base/1 drop initiator	t3: 60s

The samples were polymerized on glass slides. A layer of approximately 15 to 20 mg of monomer was deposited over 2 to 3 cm^2. The polymerization of the sealant was then performed by exposing the monomers to blue light or by mixing for the self cure sealant. After polymerization, the samples were peeled off the glass. Samples of relatively uniform thickness have been obtained with this method. These samples have been prepared for differential scanning calorimetry analyses, Fourier transform infrared spectroscopy and high performance liquid chromatography evaluations.

3.1.2 Experimental products

Since pit and fissure sealants are expensive and large amounts of polymer were needed for the mechanical study, an experimental sealant has been also synthesized [48]. It is composed of approximately two thirds of Bis-GMA resin, one third of MMA (to lower the viscosity of the sealant), 2% of camphorquinone as a blue light absorber and 2 % of dymethyl p. toluidine as an initiator (used in the polymerization of methyl methacrylate and aromatic dimethacrylates.). The composition of the experimental sealant is detailed in Table 3.2.

Table 3.2: Composition of the experimental sealant

Name of the component	Weight %	Weight
Bis GMA resin[1]	58 % weight	250 mg
MMA[2]	38 % weight	75 mg
Camphorquinone[2]	2% weight	4.25 mg
Dymethyl p. toluidine[2]	2% weight	3.25 mg

1: Nupol

2: Aldrich Chemicals

The formulations were mixed and carefully cast in a steel mold between two sheets of CTFE (Allied Signal Inc., known by the tradename Aclar). The thickness was controlled to a nominal average of 0.4 mm with a doctor blade. The system was then illuminated with the blue light unit for two minutes. The samples were exposed to light on only one side, cf. Figure 3.1.

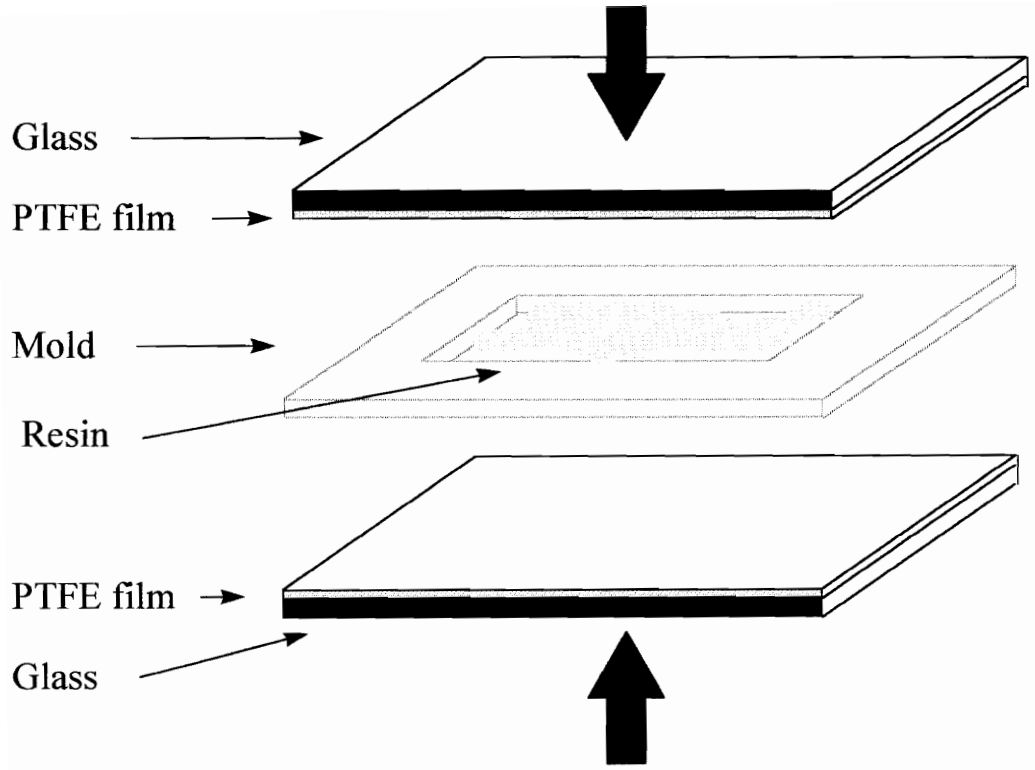


Figure 3.1: Preparation of the experimental samples

Since dental fissures can be very deep (4 mm long) and narrow (0.2 mm diameter), the curing characteristics of the light-cure sealant may be different between the tip and the base of the fissure. Adequate curing is important to ensure optimum mechanical properties of the sealant. If incomplete curing occurs, the sealant would be analogous to a sealant composed of several different materials with different values of elastic Moduli [49]. This gradient pattern of properties may create a gradient of deformations in the sealant and may reduce the longevity of the sealant. To evaluate the influence of thickness on the sealant properties, samples corresponding to different depth in the fissure were created. Figure 3.2 shows the model used to characterize the variations of the sealant as a function of depth in the fissure. The degree of cure, or degree of conversion (DC) of carbon-carbon double bonds, is altered by curing the sealant through windows of varying thickness of previously cured composite in order to reduce the light intensity and thus DC in a systematic manner. The system used to model the behavior of the sealant as a function of depth in the fissure is shown in Figure 3.3. Windows of cured Bis-GMA were created by following the method described previously. The thickness of the window was carefully leveled with a doctor blade. These windows were then placed between the blue light source and the sample. After polymerization of the sealant, dog bone shape specimens, cf. Figure 3.4, were created using a polymer cutting dye.

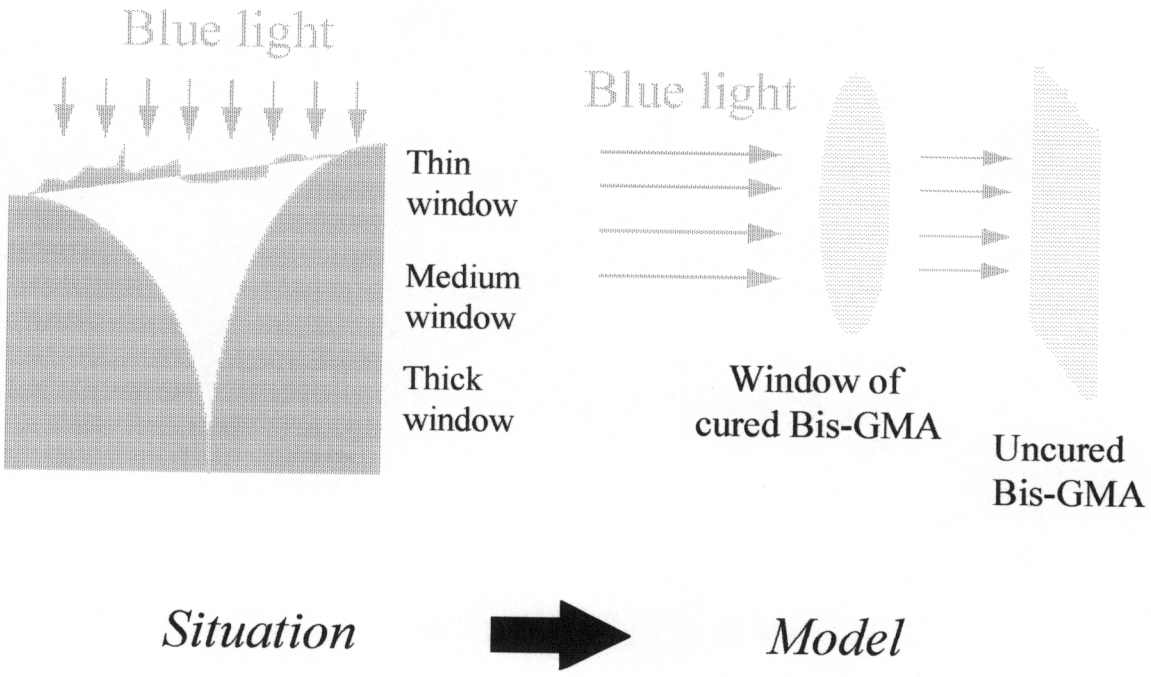


Figure 3.2: Modelization of the variations of the sealant characteristics as a function of depth in the fissure

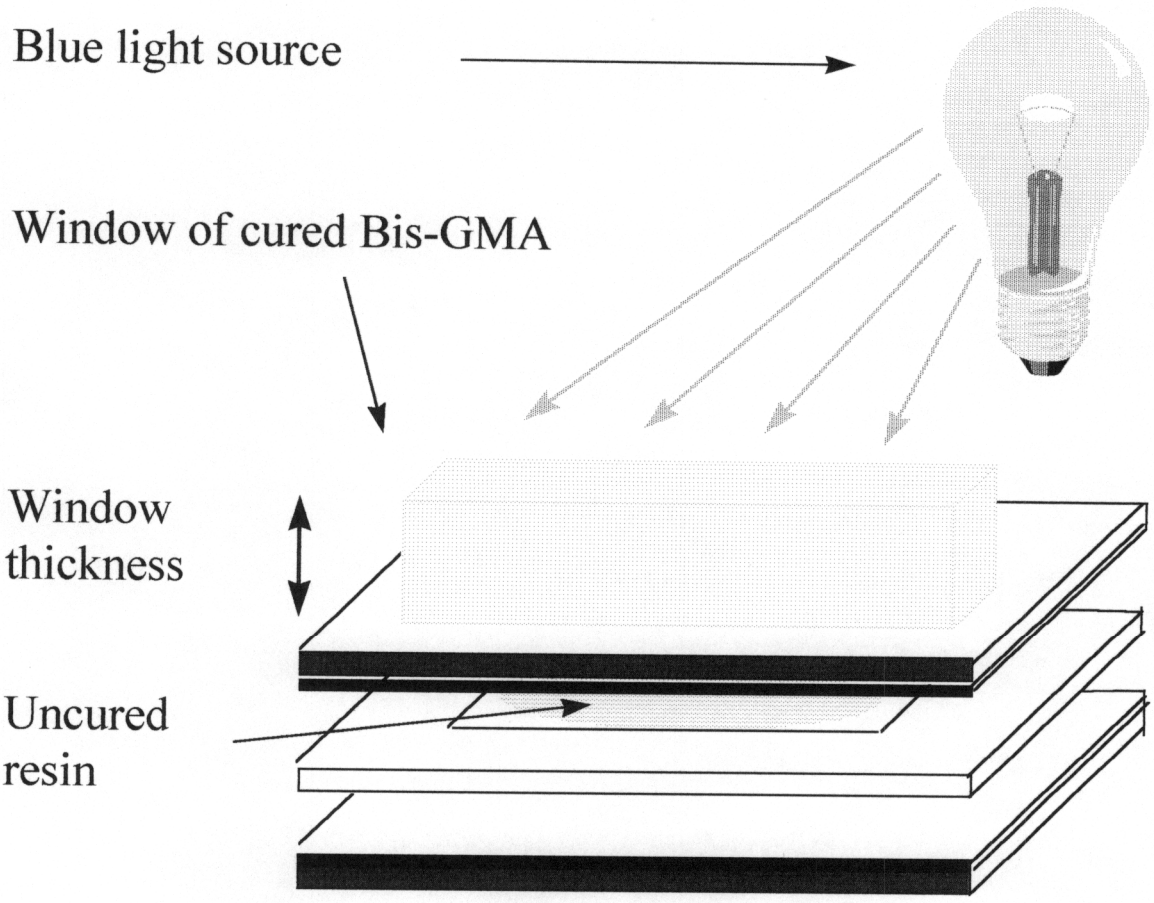


Figure 3.3: Preparation of the samples for the tensile test.
Evaluation of the properties of the sealant as a function of depth in the fissure

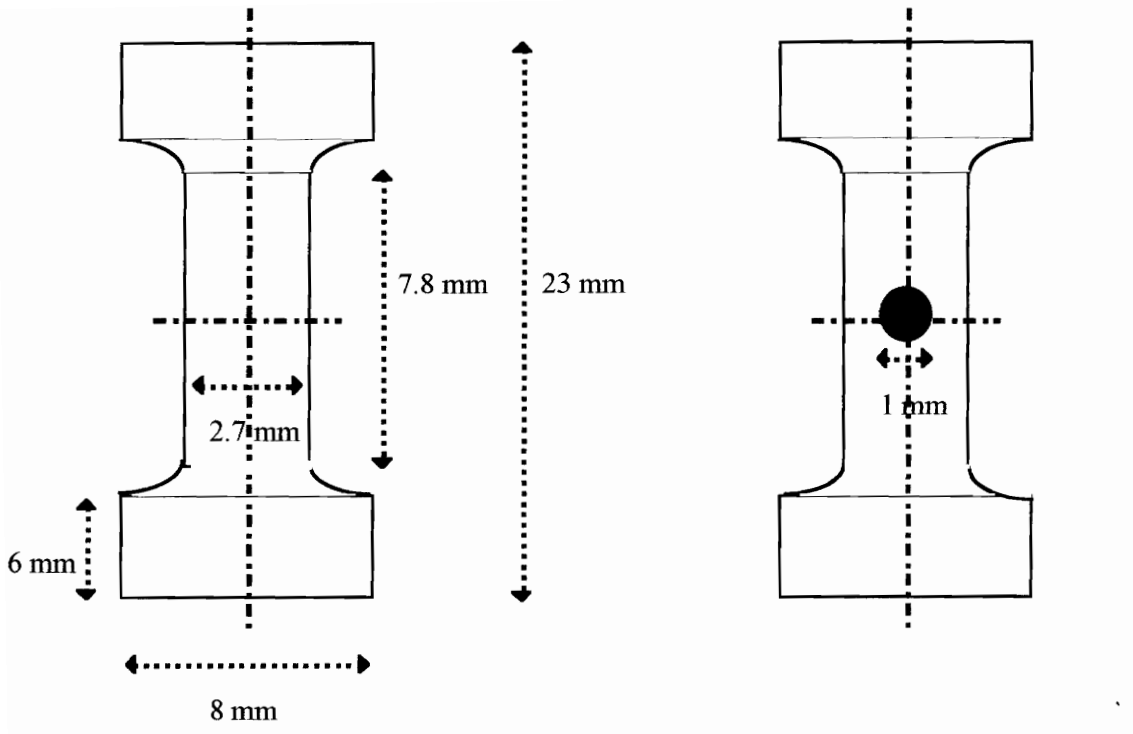


Figure 3.4: Schematic illustration of the dog bone tensile test specimens

3.2 Characterization of the sealants

3.2.1 Differential scanning calorimetry (DSC)

In some recent studies [50], thermal changes occurring during the setting of restorative materials have been measured accurately using differential scanning calorimetry. Differential Scanning calorimetry (DSC) analyses were performed to determine differences in the glass transition temperature (Tg) between samples polymerized under different conditions. The aim of these experiments was to determine the influence of the polymerization method on the degree of crosslinking of the sealant [51]. Samples were ramped at 10°C/min. between -50°C and 150°C in a Mettler TA 3000 (DSC). We obtained a plot of rate of heat output against temperature, an example of a DSC curve is shown in Figure 3.5. The temperature scale of the instrument was calibrated using chemically pure simples of indium and zinc. Only two types of sealants were employed during this evaluation : Delton self cure sealant and Concise white light cure sealant. Each material was mixed or light cured according to the chosen processing variables. The polymers were then sectioned and placed in a pre-weighed aluminum sample pan. The samples weighed between 8 to 16 mg. The pan containing the samples was then transferred to the DSC. Originally, our purpose was to prepare the samples in less than one minute after the time of mixing or light curing. This time was considered to be clinically realistic. But, unfortunately, the preparation of the pans could not be done in less than 10 min, and since the polymerization continues after the reaction [22],it is possible that higher levels of conversion occurred within the resin. Additionally, some specimens were analyzed also two days after being polymerized to estimate the influence of post cure aging on the degree of polymerization.

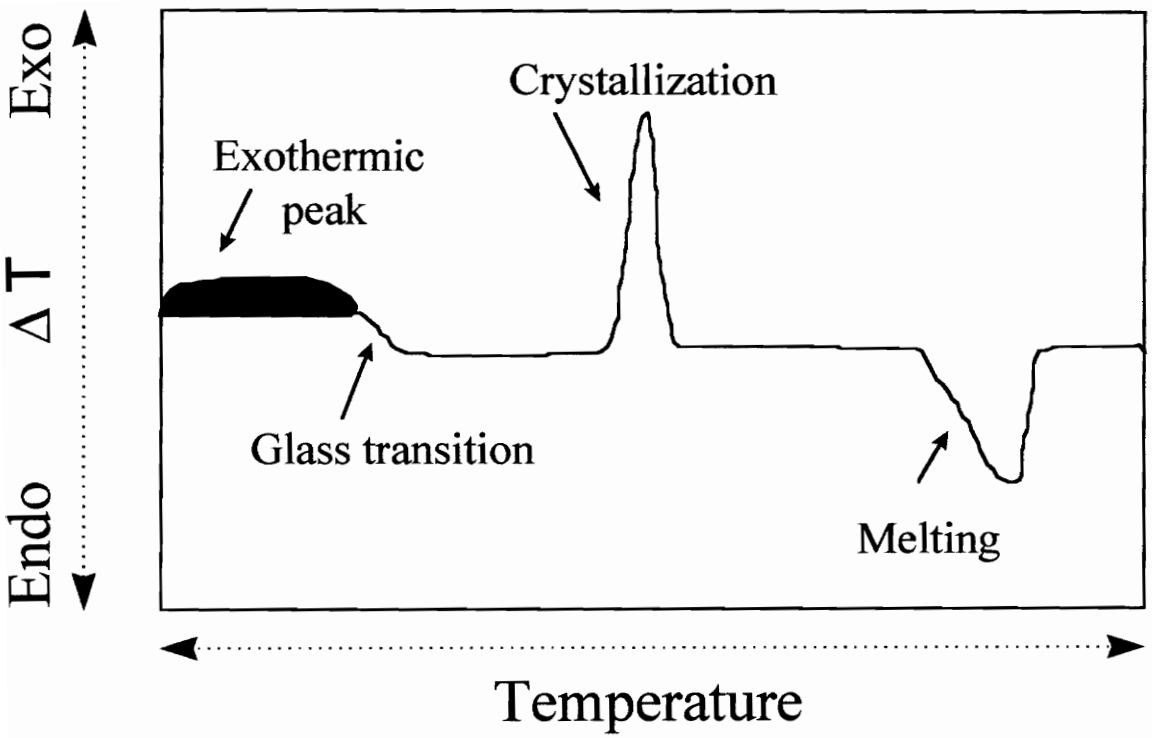


Figure 3.5: Example of a DSC curve

3.2.2 Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was used to track changes in the degree of conversion with processing variables, using a Nicolet 7505X FTIR with a TGS detector. The sample spectra as well as the background spectra for the IR chamber were collected for each sample, 400 scans per sample were taken. Each film's transmission spectrum was ratioed to the background. The spectra was collected with a Nicolet computer system and was in the form of absorbance versus frequency (cm^{-1}). This was then converted to GRAMS software for analysis.

Figure 3.6 shows a typical FTIR curve. For the light cure commercial sealant, a thin layer of unpolymerized resin was applied on the surface of a KBr disk and analyzed in the transmission mode. Further, a new film of sealant was created, light illuminated for two seconds and examined. The same sample was light exposed for two more seconds and then analyzed. The procedure was repeated several times but with increasing intervals of exposure time.

For the self cure sealant, a similar procedure could not be followed. Five samples were made for each of the three different mixing ratios and tested. This method was used previously with the self cure version of the sealant but was abandoned because of the difference in the samples which created too much data scatter. The influence of aging on the degree of polymerization of the resin was also examined. Several samples were created and analyzed, shielded from visible light for a month and then analyzed a second time.

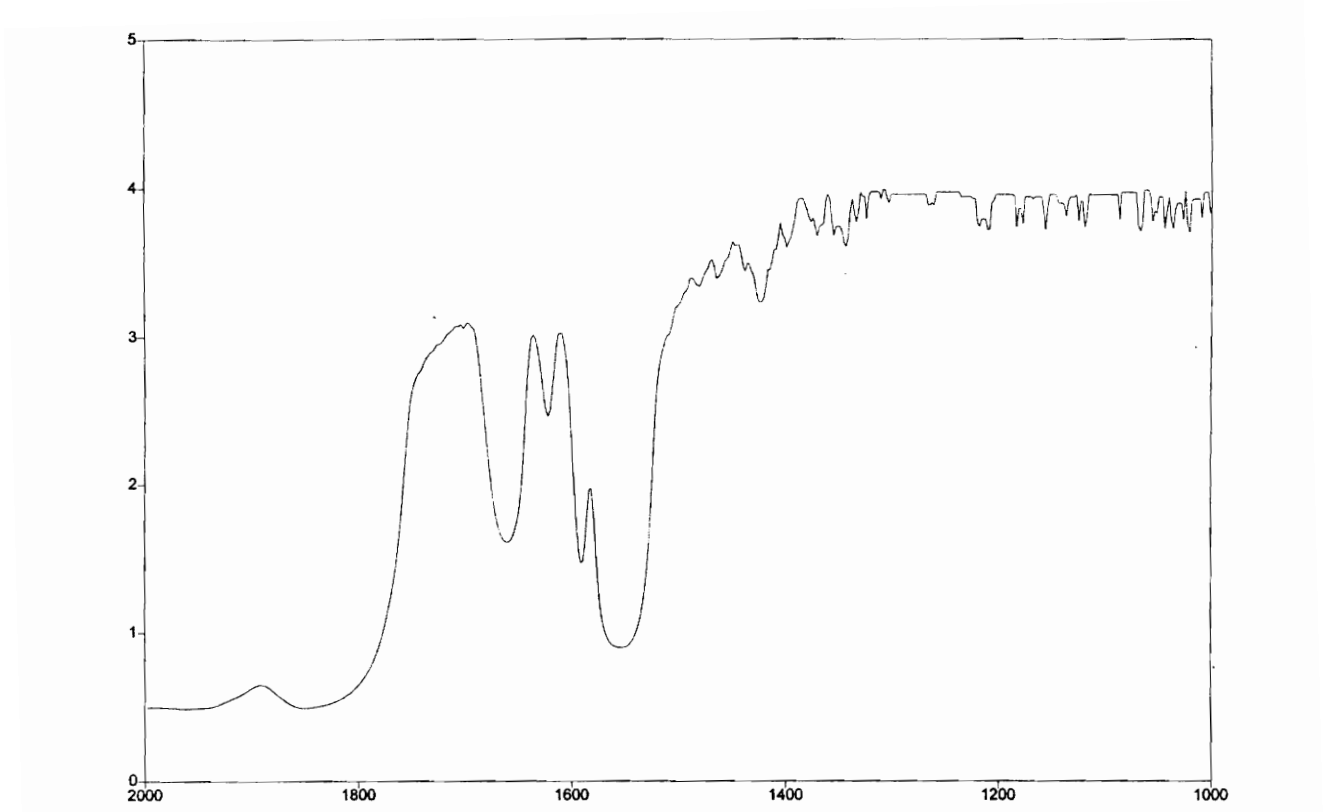


Figure 3.6: Example of a FTIR curve

3.2.3 High performance liquid chromatography (HPLC)

There have been many investigations on the adverse reactions of residual monomer released from methacrylate-based restorative materials [52, 53, 54, 55, 56]. Various biocompatibility aspects of orthodontic adhesive resins and dental composites, have been examined and both *in vitro* and *in vivo* reactions have been reported[23]. The quantity and composition of the eluted substances are key factors for the study of the toxic potential of dental resins. Since no study on the leaching of unpolymerized resin from pit and fissure sealants has been reported in the literature, we decided to perform this evaluation. Compared to most restorative materials, bigger amounts of polymer are put in the patient's mouth. The level of leachable compounds being proportional to the amount of polymer and to the surface area in contact with the saliva, the amount of residual monomer release may be more significant. The influence of the initial polymerization process of the resin on the leaching of monomers has also been studied. (see Figure 3.7).

High performance liquid chromatography has been employed to quantify the post-polymerization residual monomer eluted from Bis-GMA/ TEGDMA based chemically-cured and light-cured pit and fissure sealants. Two types of commercial pit and fissure were used: a visible light cured (Concise, 3M Co.) and a two-part chemically cured (Delton, Dentsply Co.). The samples were prepared on glass slides. Three illumination times (10, 20 and 60s) and three different mixing ratios (M1, M2, and M3) were used as processing variables.

Four minutes after setting, the samples were weighed and each specimen was immersed in 5ml of agitated 75% ethanol/ 25% (weight %) water solutions maintained at room temperature. The choice of the ethanol/water (75:25) solution as an immersion medium was based on the potential of this solution to increase the rate of monomer release. After

fifteen hours of exposure the specimens were removed and the extract analyzed for the quantitative determination of eluted monomers.

The analyses were then performed by high performance liquid chromatography. The system is a Varian Vista 5500 equipped with a packed silica column. The analyses were achieved under the following conditions: methanol/water (80:20) mobile phase, isocratic elution mode; 1 ml/min flow rate; detection at 254 nm; 10 μL sampling loop, 25°C temperature.

The column was calibrated with known concentrations of triethylene glycol dimethacrylate (TEGDMA) (Aldrich Chemicals Co.,) and bisphenol A-Glycidyl dimethacrylate (Nupol, Cook Composite Polymers). Solutions of each monomer at concentrations of 2.5%, 1%, 0.5%, 0.25%, 0.1%, 0.05% and 0.025% (weight %) were created. The HPLC analyses were performed five times for each concentration (n=5). The linear fittings of Bis-GMA and TEGDMA calibration curves were used to calculate the concentration of the monomers in the extract. A representative HPLC run of unpolymerized TEGDMA and BisGMA is shown respectively in Figure 3.8 and Figure 3.9. As one can see, the TEGDMA is passed through the column at 4.32 min and the Bis-GMA is passed at 10.75 min. This suggests that there is no overlap between these two compounds using this column and these processing parameters.

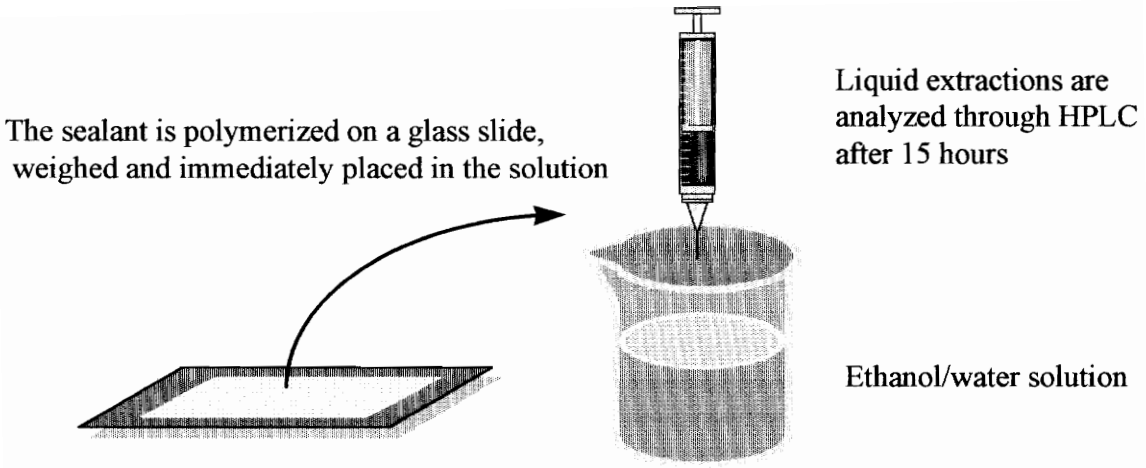


Figure 3.7: Apparatus for the evaluation of the amount of leached monomers from pit and fissure sealants.

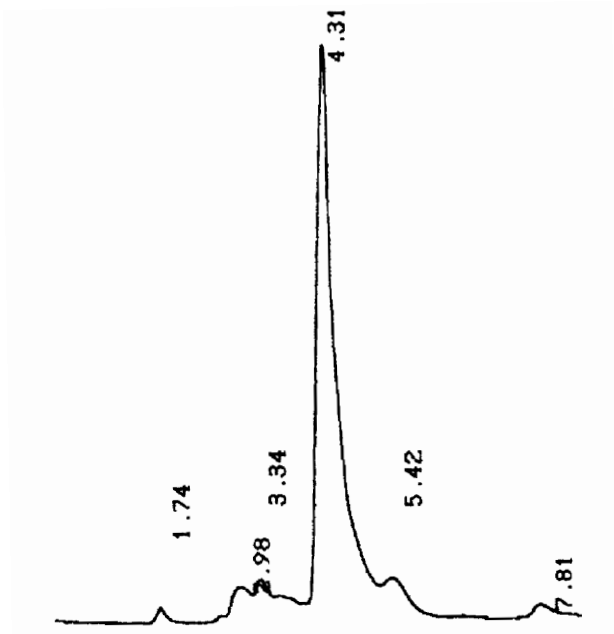


Figure 3.8: HPLC analysis of TEGDMA, RT: 4.32 min.

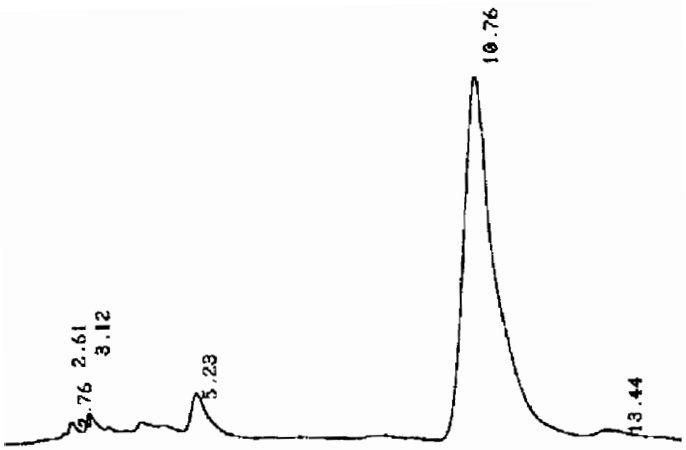


Figure 3.9: HPLC analysis of Bis-GMA, R.T.: 10.75 min.

3.3 Mechanical properties of the sealant as a function of depth

3.3.1 Tensile testing

Tensile tests were performed on dog bone shape specimens of experimental sealant. The samples were tested in tension using a Polymer Laboratories miniature materials tester (minimat). A constant deformation rate of 0.1 mm/min was used for each test in order to allow a comparison of the mechanical properties of the sealant. The very small thickness of the samples required the placement of sandpaper between the sealant and the clamps to prevent slippage of the samples during the test. It soon appeared that the interval of time between the creation of the samples and their analysis strongly influenced the results. Therefore specimens from the same batch of material were tested at constant intervals of time to enable reasonable comparisons between the results and to quantify the influence of aging on the mechanical properties of the sealant. Three different blue light exposure conditions, consisting of placing different windows of sealant between the light source and the polymer, were studied. From each plate of sealant, five to six dog bone specimens were created. The number of samples and their conditions of manufacturing and testing are detailed in the following tables.

Table 3.3 : Creation and testing of the samples

Curing conditions: window thickness	Number of plates created	Number of samples tested per plate
Thickness: 0 mm	5	5 to 6
Thickness: 0.85 mm	5	5 to 6
Thickness: 1.70 mm	5	5 to 6

The samples were tested at constant intervals of time after polymerization.

Table 3.4: Number of samples for each aging time and conditions of cure

Aging time (min.)	15	45	75	105	135	165
No window	5	5	4	5	5	2
1 window	5	5	4	4	4	2
2 windows	5	5	5	5	5	3

A total of approximately 80 samples were mechanically tested in tension to measure the effect of curing on the mechanical properties of the sealant. These different conditions of cure may have occurred between the surface and the tip of the fissure as a result of depth effect.

3.3.2 Fracture analysis

The goal of this experiment was to observe micro-mechanical deformation mechanisms as a function of degree of polymerization. Dog bone specimens were created with the experimental sealant. An attempt to create a hole in the center of the sample by using a Dremel *tool* with a small engraving cutter bit was performed without success: cracks propagated through the material. So, using an Exacto Knife with a No. 11 Fine Point Blade, holes having an average of 1 mm in diameter were created (see Figure 3.4). During sample fabrication, we found that the brittle behavior of the samples didn't enable us to create notched samples. Attempts to produce notches led to uncontrolled cracking around the notch area or complete fracture of the sample. These defects were supposed to create centrally-located stress concentration regions. Since sample deformation would be expected to occur in regions at the tip of the notch, the presence of defined notches would have helped in the formation and identification of tensile deformations mechanisms. However, as shown by Figure 3.10 , from Smith , a very good observation of craze formation can be made in the vicinity of a small circular hole punched in the center of samples when the latter are pulled in tension. The specimens were stressed in tension until failure or, in some cases, up to a constant level of deformation. The fractured specimens were observed under an SEM and an optical microscope.

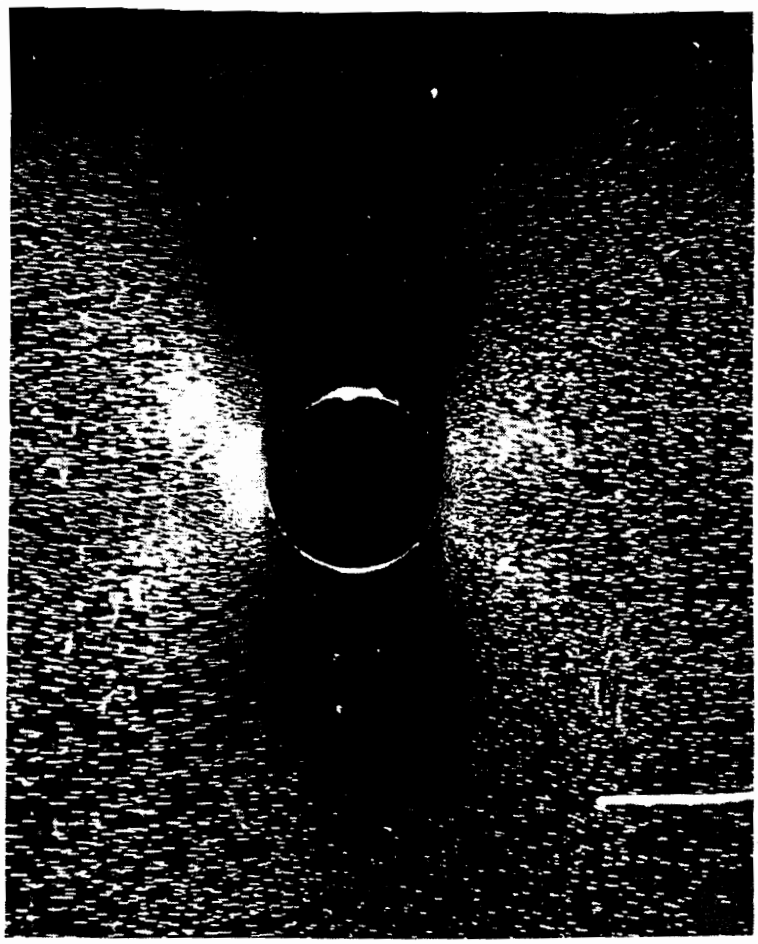


Figure 3.10: Craze formation in the vicinity of a hole in a strip of PMMA loaded in tension (Result obtained by L.S.A Smith), [57]

3.3.2.1 Optical Microscopy

Micrographs showing the fracture surface of the stressed samples were taken using an Olympus BHSM optical microscope equipped with an Olympus 35 mm camera and crossed polarizers.

3.3.2.2 Scanning Electronic Microscopy

An International Scientific Incorporate, model SX40, with a voltage of 20 kV, Scanning Electronic Microscope was used to analyze the broken samples. The specimens were sputtered with gold to eliminate sample charging during the SEM observations.

3.3.3 Compressive testing

The behavior of the sealant under compressive stress was studied since the material is mainly stressed in compression under chewing forces.

3.4 In vivo experiments

3.4.1 Clinical procedure

The experiment was conducted on August 9 and 10, 1996 with the participation of Dr. H. Veit, professor at the VA/MD Regional College of Veterinary Medicine, Dr. L. Kyle, dentist in private practice in Blacksburg, Mr. Tony Pease a veterinarian medical student and Ms. Megan a staff research technician at the Veterinary School. The pigs were obtained from the Virginia Tech College of Agriculture.

The animals were all males and their weights varied between 95 to 115 lb. The pigs were approximately 3 months old. One of the requirements was to work with young animals since already damaged teeth can not be sealed. The teeth appeared to be relatively free of caries and the molars were developed enough to present deep occlusal surfaces. After being anesthetized, the animals were taken into the operating room where the dental work was performed by Dr. L. Kyle.

Only the back two molars of each mouth quadrant received sealant. For each animal, the treatments were applied randomly on the four quadrants (see Figure 3.11). Three different commercial sealants were used and some quadrants were left untreated as controls. The treatments were performed according to Table 3.6 and Figure 3.12.

Table 3.5: Processing variables

	Mixing ratio	Exposure time
Above clinical value	M1: 1 drop base/2 drops initiator	t1: 20s
Clinical value	M2: 1 drop base/1 drop initiator	t2: 10s
Below clinical value	M3: 2 drops base/1 drop initiator	t3: 60s

Table 3.6 : Organization of the treatments in the mouth

Treatment	Processing variables	# quadrants treated
Delton, *Clear, Self Cure, Dentsply*	M1	8
	M2	8
	M3	8
Concise, *White, Light Cure, 3M*	t1	8
	t2	8
	t3	8
Delton, *Clear, Light Cure, Dentsply*	t1	2
Concise, *White, Self Cure, 3M*	M1	2
Control	N.A.	8

The detail of the treatments and the identification of the samples is more fully detailed in appendix , table 5.

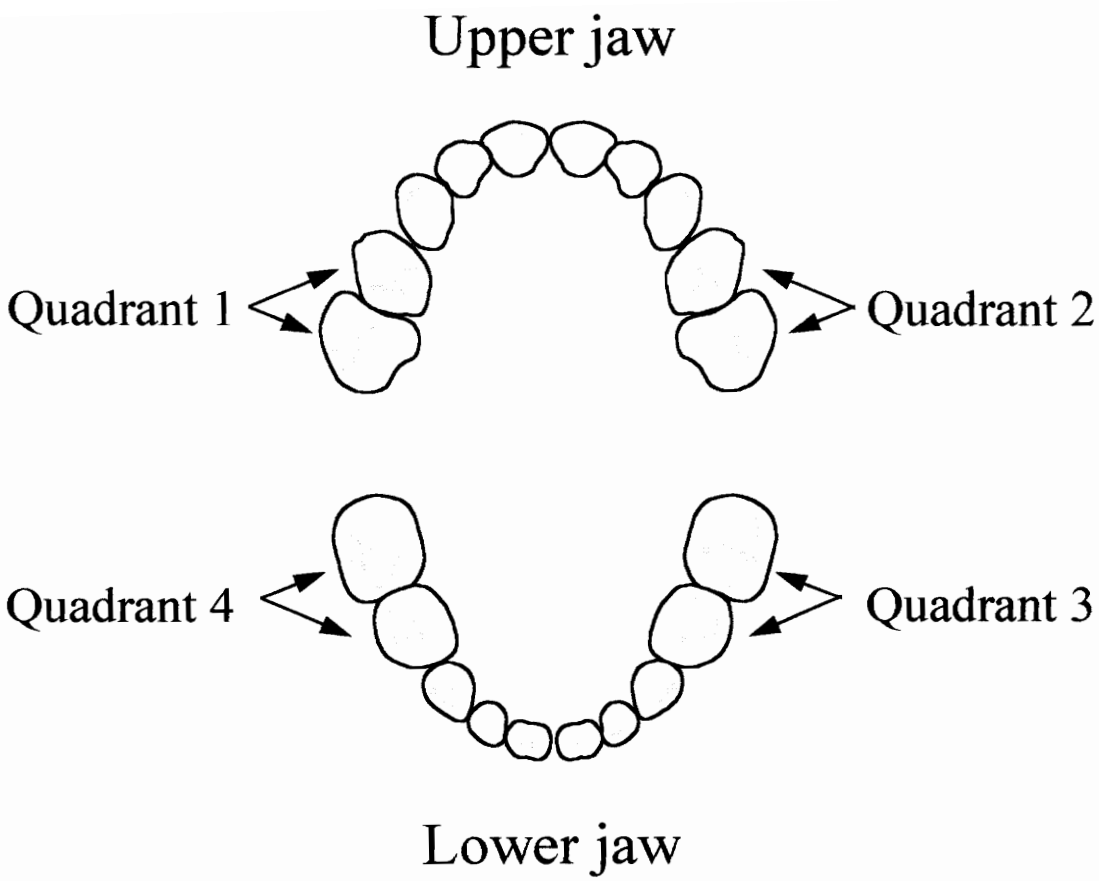


Figure 3.11: definition of the quadrants treated in the mouth during the in vivo evaluation

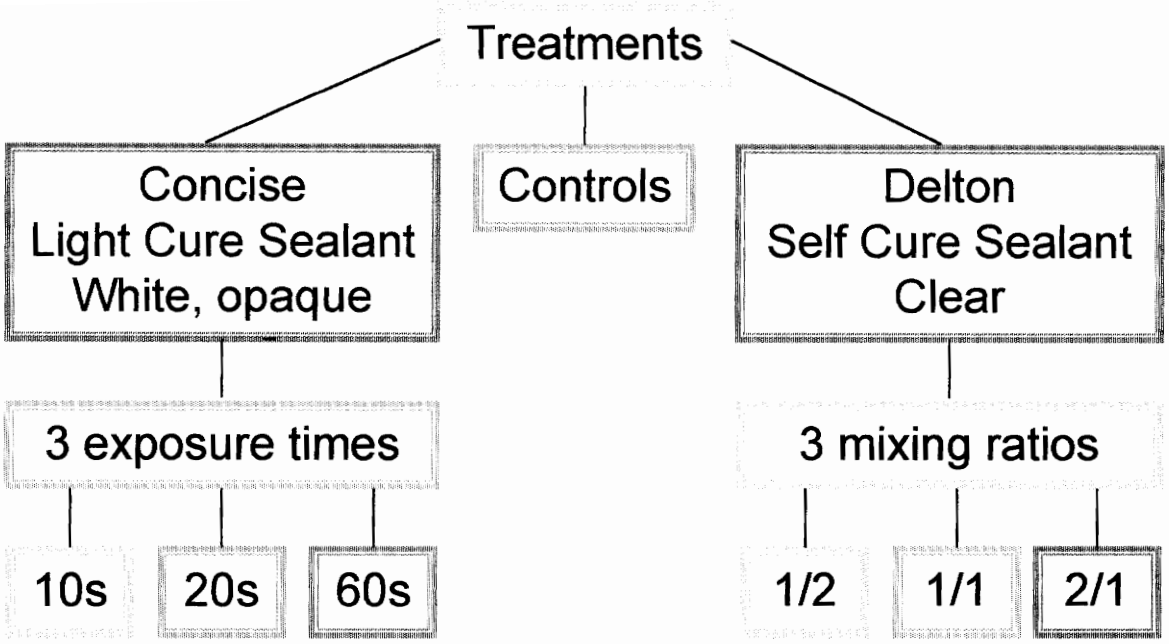


Figure 3.12: Different treatments used during the in vivo evaluation of the sealants

Manipulation of the sealant :

The technique for handling pit and fissure sealants involves seven basic steps that were followed sequentially [58]. These steps include cleaning and etching the surfaces, washing and drying these areas, mixing the sealant reaction products for the self cure version, applying the sealant to the pit and fissure, polymerizing and finishing.

Each quadrant of teeth was isolated with some cotton rolls and the two back molars were cleaned with pumice for approximately 30 seconds. The teeth were then rinsed with water and dried (see Figure 3.13). Although pumice cleaning is the cleaning procedure recommended by dentists, the crevices did not appear to be perfectly cleaned. The etching gel was applied on the teeth for approximately 10 seconds (see Figure 3.14). The cleaning or etching solutions, which are also known as preconditioning solutions, are generally 37 % or 50% solutions of phosphoric acid in water. The teeth were rinsed with water and carefully dried. Care was taken to avoid saliva contact with enamel after this step. Moisture contamination can be a cause for failure of pit and fissure sealants [59].

For the light cure sealant, the sealant was applied half way up the cuspal slopes using a disposable brush tip. The sealant was then cured for a certain exposure time using the blue light illumination unit (Figure 3.15). The light exit window was placed 1-2 mm from the surface. When set, the sealant was hard and formed an opaque film. For the self cure version of sealant, the base and the initiator were mixed for 5 to 10 seconds and applied on the occlusal surface. Although care was taken to obtain a relatively constant mixing and working time, some differences were still observed. The time between the beginning of the mixing of the products and the end of the polymerization was approximately 1 min. It was noticed that the polymerization was faster than in clinical conditions since the temperature of the animals was slightly higher than the temperature of humans.

The animals were sheltered at the Virginia Tech Swine Center for three months before being slaughtered. The stresses applied to the sealant were higher in the porcine subjects than in the mouth of a normal human patient since pigs chew harder food and do not follow any rule of hygiene. The sealant was considered to be placed under "accelerated aging conditions".

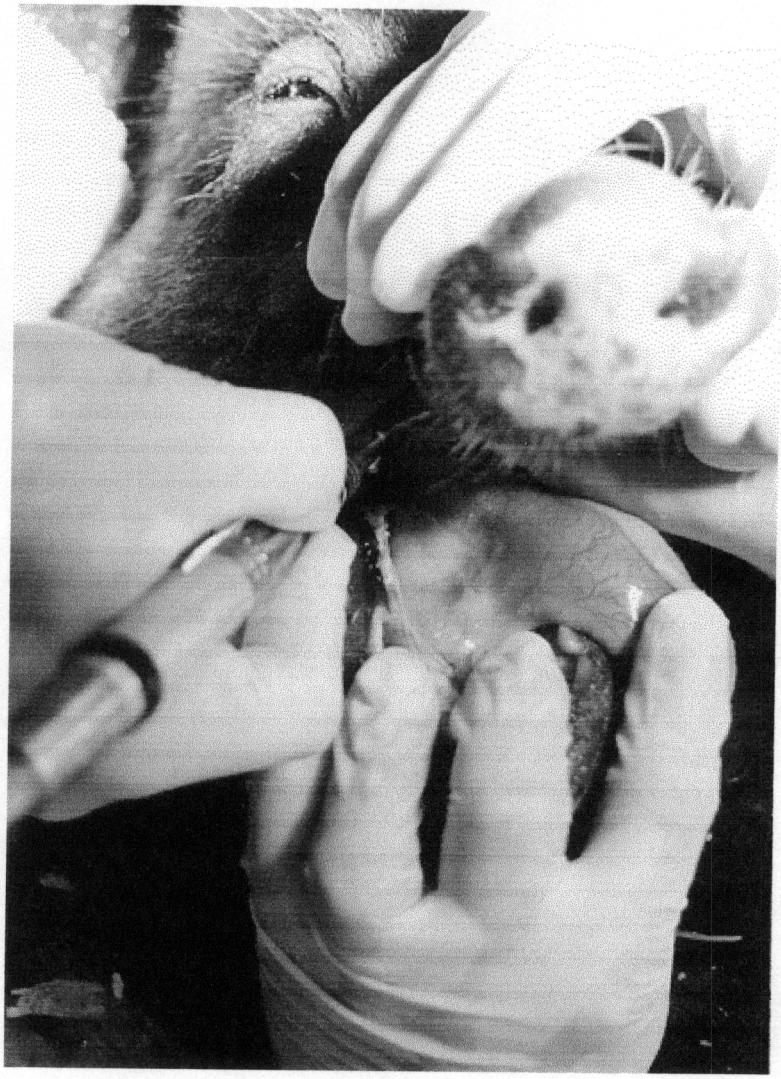


Figure 3.13: Cleaning of the teeth prior to the application of the sealants

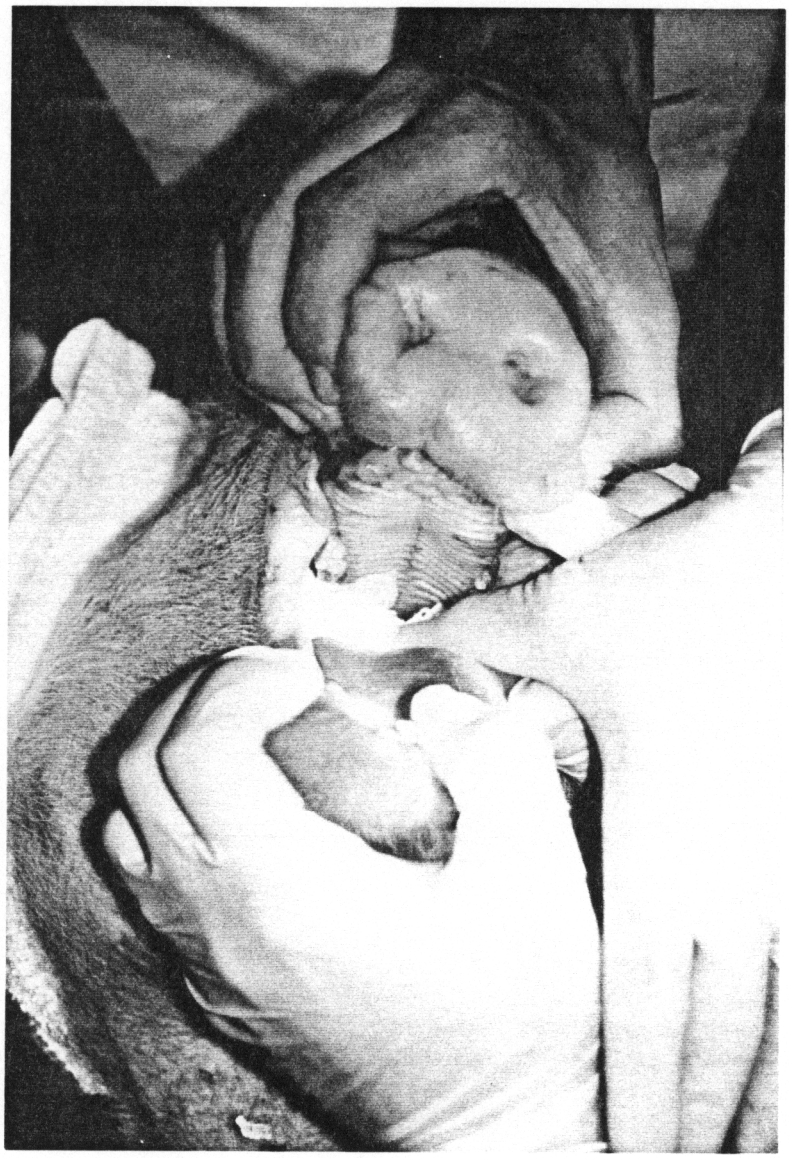


Figure 3.14: Etching of the teeth for conditioning the enamel

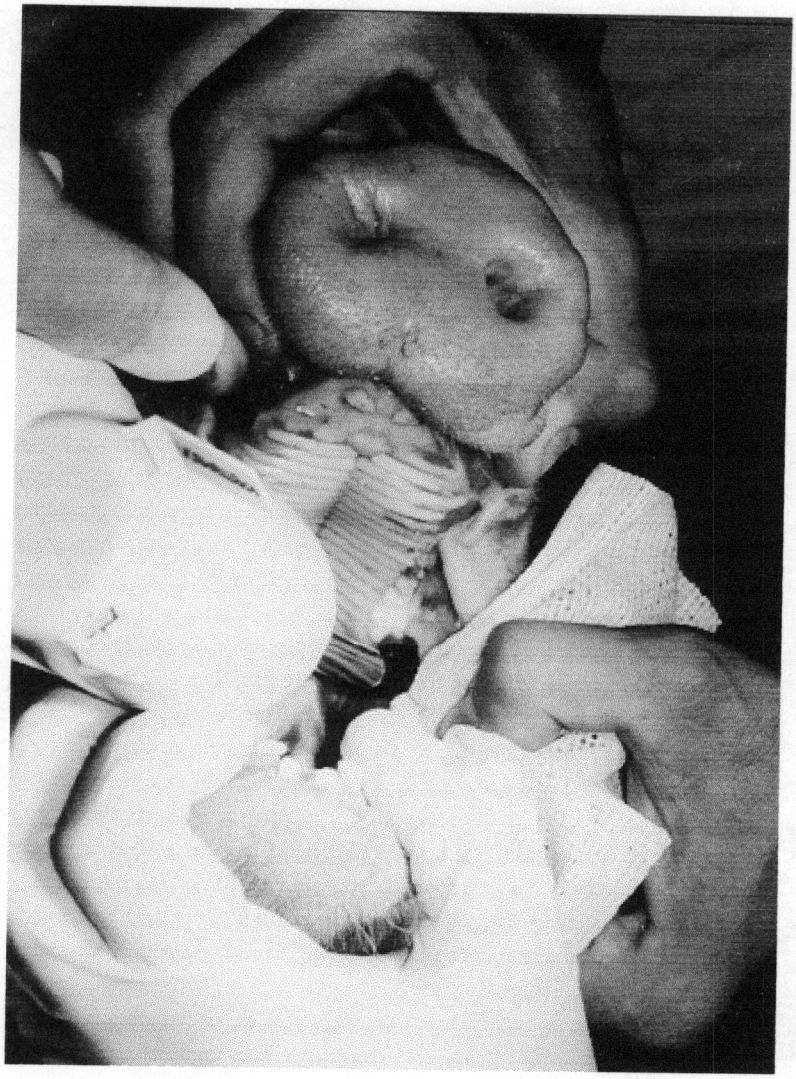


Figure 3.15: Polymerization of the sealant by exposure to a blue light source

3.4.2 Characterization

After three months, the animals were sacrified for food purpose and the teeth were extracted and stored in water for two months.

Different characterization techniques were performed on the teeth as shown in Figure 3.16.

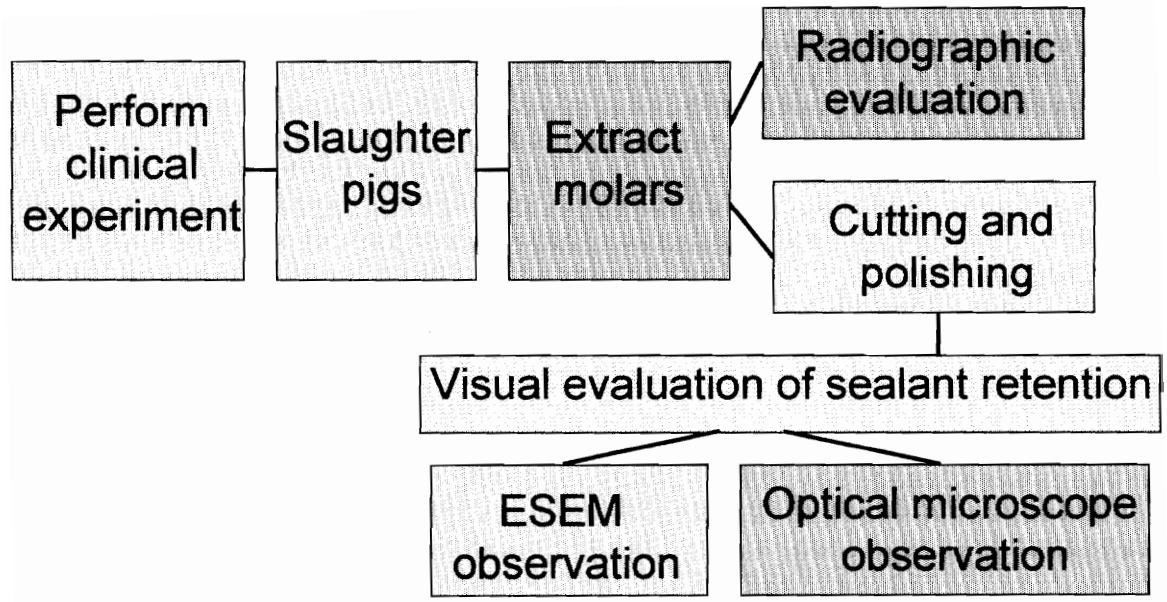


Figure 3.16: Different characterization techniques performed on the teeth

3.4.2.1 X ray evaluation

Radiographic evaluation of some of the teeth was performed to estimate leakage and loss of the sealant in a non destructive fashion. A good image quality was found for a beam of 80 Kvp and an exposure time of 18s. Representative samples were placed in an X ray sensitive solution prior to being analyzed. The penetrant composition is detailed in Table 3.7.

Table 3.7 : Composition of the penetrant

Components	Amount
Zinc iodide	60 g
Alcohol	10 ml
photo flo	10 ml
water	10 ml

3.4.2.2 Visual evaluation of the sealant retention

The level of sealant retention has been evaluated visually for each tooth. Three different levels of retention have been defined. These levels of retention are detailed in Table 3.8.

Table 3.8: Levels of sealant retention

Retention	Visual evaluation
1	No trace of sealant can be found on the tooth
2	Traces of left over sealant are found but can't prevent the tooth from decay
3	The retention of the sealant is complete

3.4.2.3 Optical Microscopy

Micrographs showing the sealant left over on the teeth were taken using an Olympus BHSM optical microscope equipped with an Olympus 35 mm camera.

Prior to these observations, the teeth were cut and polished according to the following procedure. After a visual observation of the teeth, the samples were sectioned through the most significant fissure or sealed part if left over sealant was found. The cutting was realized with a diamond saw under cutting oil. The samples were then carefully polished with a 1200 grade polishing paper followed by cloth polishing with a 5 and 1 μm alumina solution.

They were then inspected under an optical microscope to determine the presence of any residual sealant and sealant penetration into the fissure.

3.4.2.4 Environmental Scanning Electron Microscopy

The samples were evaluated using an Electroscan^{TM} Environmental Scanning Electron Microscope (ESEM). An operating environment of 5 Torr of water vapor allowed the non-conducting sealant-enamel samples to be examined without the application of a conductive coating.

The samples were then observed under the SEM to establish if cracks at the interface between the enamel and the sealant develop in a particular way. For some specimens, we wondered whether the sealant has been broken during the *in vivo* study or during the cutting of the teeth. For further studies a marker should be used prior to cutting and polishing.

4. Results :

4.1 Characterization of the sealants :

4.1.1 Experimental sealant processing:

The samples were polymerized successfully and a good processing scheme was found. However, the mixing method still needs to be improved since many bubbles and defects were found in the samples. We identified bubbles and thickness variations in the samples as well as incompletely dissolved traces of camphorquinone on occasion.

4.1.2 DSC analyses :

Differential scanning calorimetry (DSC) was performed on the commercial pit and fissure sealants to evaluate the influence of processing on the degree of conversion of the sealant. The glass transition temperature, Tg, is the temperature at which the transition from the glass to the rubber like state occurs. Since this temperature is a function of the molecular motion, when the degree of conversion increases in a polymer, its crosslink density increases, the molecular motion in the polymer decreases and the T_g rises. The T_g increases linearly with the number of crosslinks [60]. The value of the T_g gives an indication of the degree of conversion of the samples.

Unfortunately, most of the generated thermographs contained a lot of scatter and the value of the T_g could not be determined accurately. This can be explained by the difference in the size of the segment of the polymer network. This difficulty in determining the value of the T_g may also be due to the presence of a residual exotherm which might mask the T_g.

No significant difference was observed between sealants processed under different conditions (see Figure 4.1). However, for all the differently processed sealants, there is a

residual exothermic peak at approximately 60 °C. When the samples were cooled and analyzed a second time (see Figure 4.1), this peak disappeared : a transformation having occurred during the first analysis. Thus level of conversion was thermally increased during the run in the DSC. A similar observation has been made between samples that were polymerized and immediately analyzed and samples that were aged for two days at room temperature (Figure 4.2).

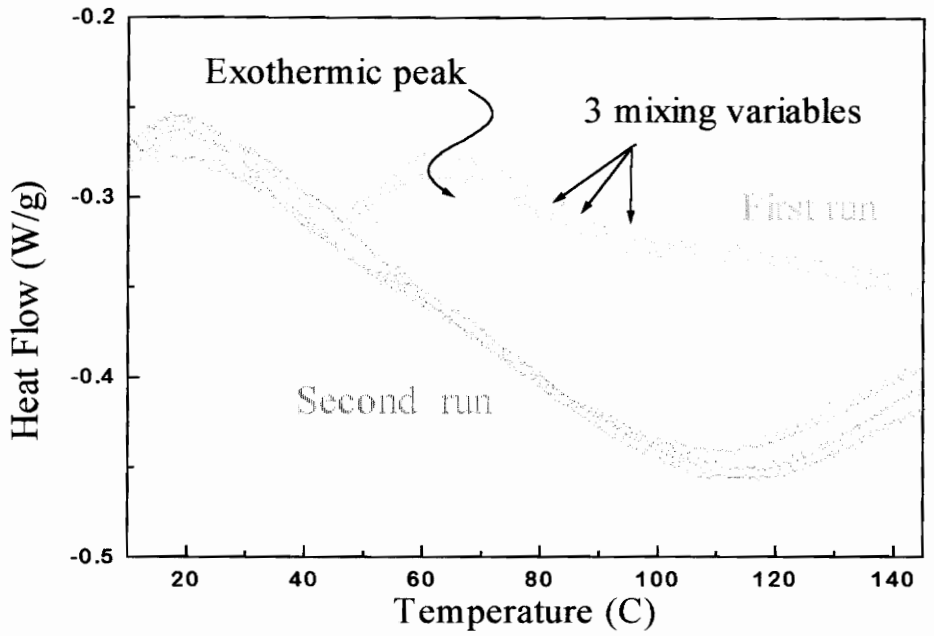


Figure 4.1: DSC curves of three differently processed self cure sealant. The samples are run a second time : the exothermic peak has disappeared upon curing in the DSC cell. An advancement in the cure of the material has occurred during the analysis.

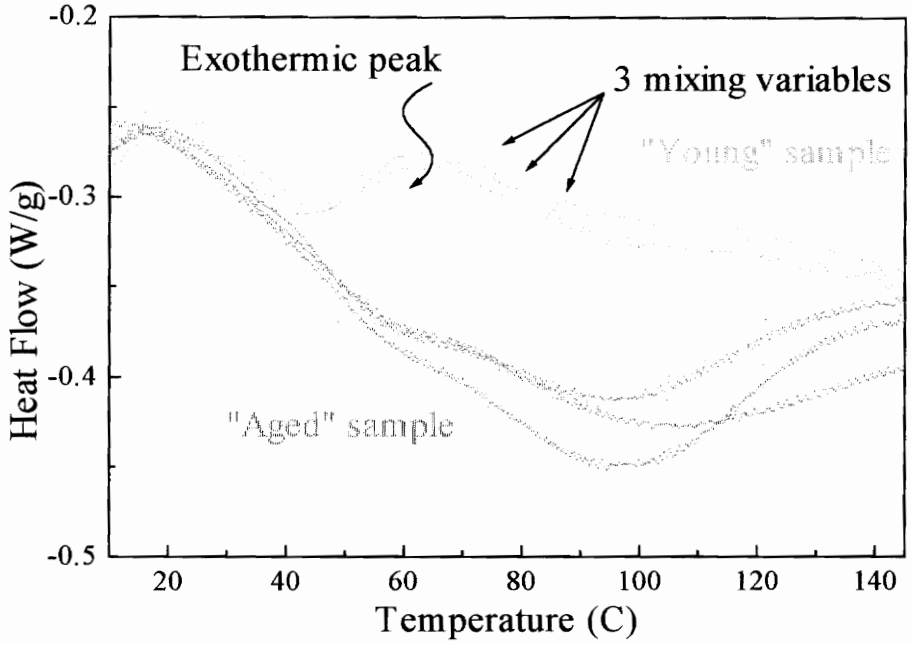


Figure 4.2: DSC curves of three differently processed self cure sealant. The degree of cure is advanced upon aging.

4.1.3 Fourier transform infrared spectroscopy (FTIR)

Since the results obtained from the DSC analysis did not allow a quantitative estimation of the degree of polymerization, an infrared evaluation of the sealants was also required. The degree of cure was measured from the absorbance ratio of the C=C stretch of the methacrylate group (at 1636 cm-1) to the reference aromatic ring peak (at 1607cm-1) in the cured and uncured materials [61, 62]. The values of the peaks heights are listed in table 1, in the appendix.

For the light cured specimens, an increase in the degree of conversion of the methacrylate group due to increased exposure time was observed by FTIR. After 20 s of illumination, the degree of conversion plateaus and can not increase upon further exposure to blue light (see Figure 4.3). The degree of conversion reached an upper limit for relatively short illumination times for the samples. Relatively thin samples were used for the analysis compared to the sealant thickness used in dentistry. Longer illumination times may be required to reach the same degree of conversion under clinical conditions. The level of conversion may reach its upper limit for longer exposure times than 10s.

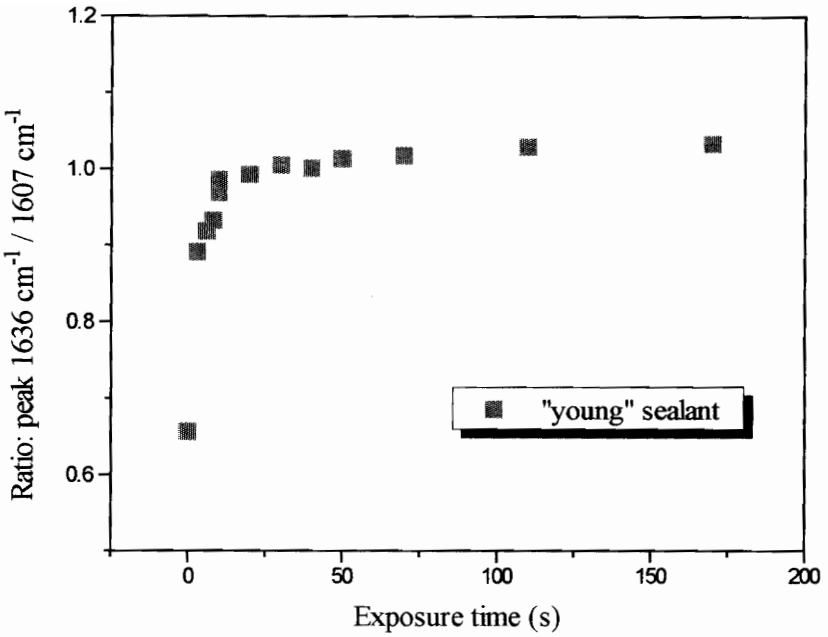


Figure 4.3: Fourier transform infrared spectroscopy of light cure sealant. Degree of conversion according to the blue light exposure time.

For the self cure sealant, only three different mixing ratios were studied during the evaluation. No significant difference was found in the degree of conversion of the three different products (see Table 4.1 and Figure 4.4). It seems that a plateau in the degree of conversion of the sealant was achieved. The difficulty in making a standard specimen and in maintaining a constant working time for the preparation of the specimens may have led to some scatter. The chemical cure might be more predictable in terms of consistent conversion.

Table 4.1: Results from the Fourier transform infrared spectroscopy for the self cure sealant

Mixing variables	Ratio of the peaks 1636cm^{-1} / 1607cm^{-1} Average	Ratio of the peaks 1636cm^{-1} / 1607cm^{-1} stdev
M1= mix 1/2	0.993	0.001
M2= mix 1/1	1.005	0.006
M3= mix 2/1	1.009	0.010

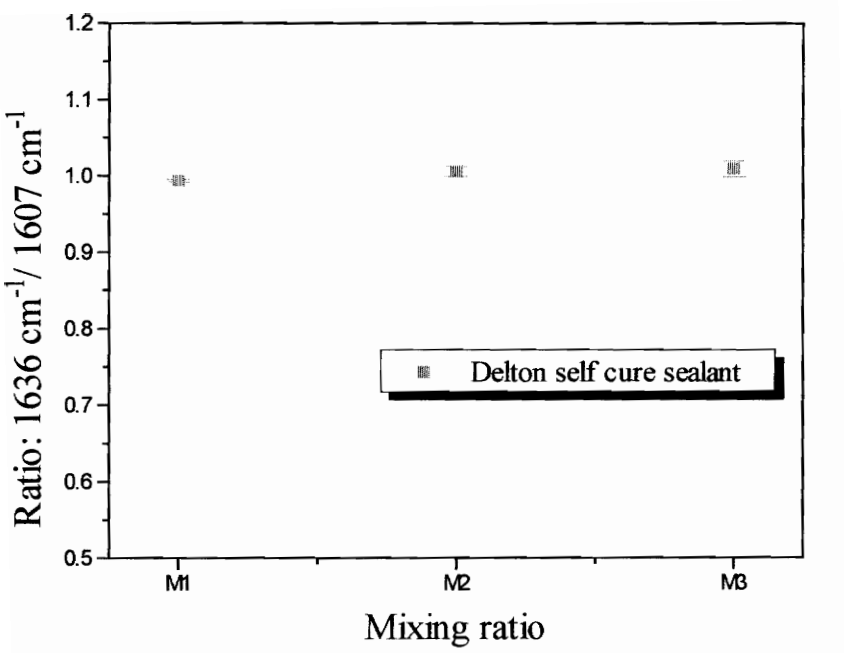


Figure 4.4: Fourier transform infrared spectroscopy of the self cure sealant. Degree of conversion according to the mixing ratio.

The same type of procedure, which consisted of creating many samples with different illumination times, was used for the characterization of aging of the light cure sealant. Different films were created and analyzed, but the difficulty in creating a standard specimen may have led to some of the data scatter. The results are presented in Table 4.2 and Figure 4.5.

The samples which were tested after two hours of aging showed an increase in the degree of conversion of the resin.

Table 4.2: Fourier transform infrared spectroscopy of aged and unaged light cure sealants

Light Exposure time (s)	*Unaged* Ratio of the peaks 1636cm^{-1}/ 1607cm^{-1} Av	*Unaged* Ratio of the peaks 1636cm^{-1}/ 1607cm^{-1} stdev	*Aged 2 hrs* Ratio of the peaks 1636cm^{-1}/ 1607cm^{-1} Av	*Aged 2 hrs* Ratio of the peaks 1636cm^{-1}/ 1607 cm^{-1} study
0	0.577	0.026	0.000	0.000
10	0.978	0.011	1.007	0.007
20	0.999	0.008	1.024	0.020
40	1.006	0.020	1.011	0.018
60	1.000	0.014	1.033	0.013

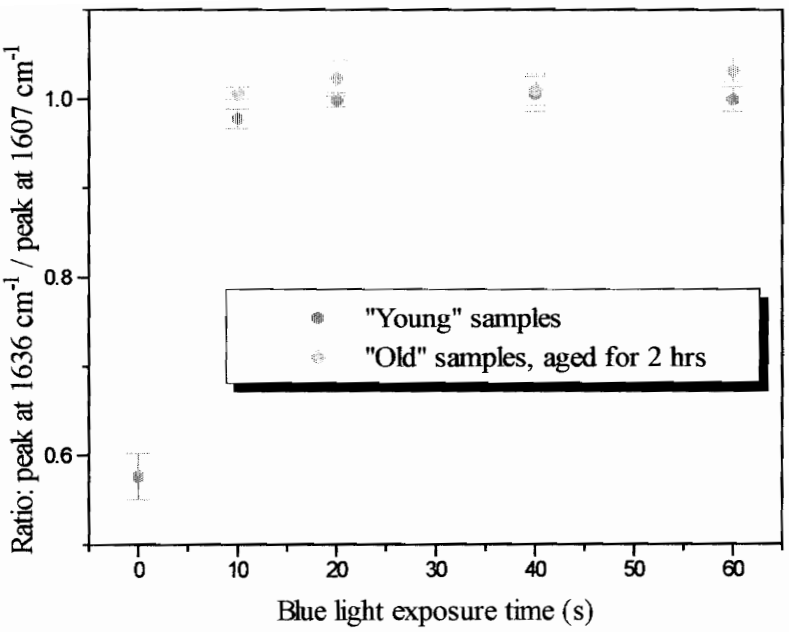


Figure 4.5: Fourier transform infrared spectroscopy of unaged and aged light cure sealants

4.2 Mechanical analyses :

4.2.1 Tensile testing :

As discussed before, the experimental sealant samples that were made contained defects, such as air bubbles and variable thickness. This could lead to more potential for non uniform deformation and produce more scatter in the tensile tests results as shown by the error bars in Figure 4.6 and Figure 4.7. Different mechanical properties were measured and listed in table 2 in appendix. A stiffening of the sealant with increasing time after illumination and decreasing window thickness was generally observed. Also, as the stiffness of each sample increased, they embrittled. Figure 4.8 and Figure 4.9 show the stress and the strain at failure respectively, a decreasing window thickness increases conversion and embrittles the sealant. The stress at failure increases with aging time. Short aging of the sealant strongly influences the properties of the sealant. For example, the stiffness is at least doubled after two hours of aging. The effect of aging on the properties of the sealant is not negligible. The thickness of the window also influences the properties of the sealant: the properties of pit and fissure sealants may vary along the fissure and may be responsible for the sealant early fracture and leakage.

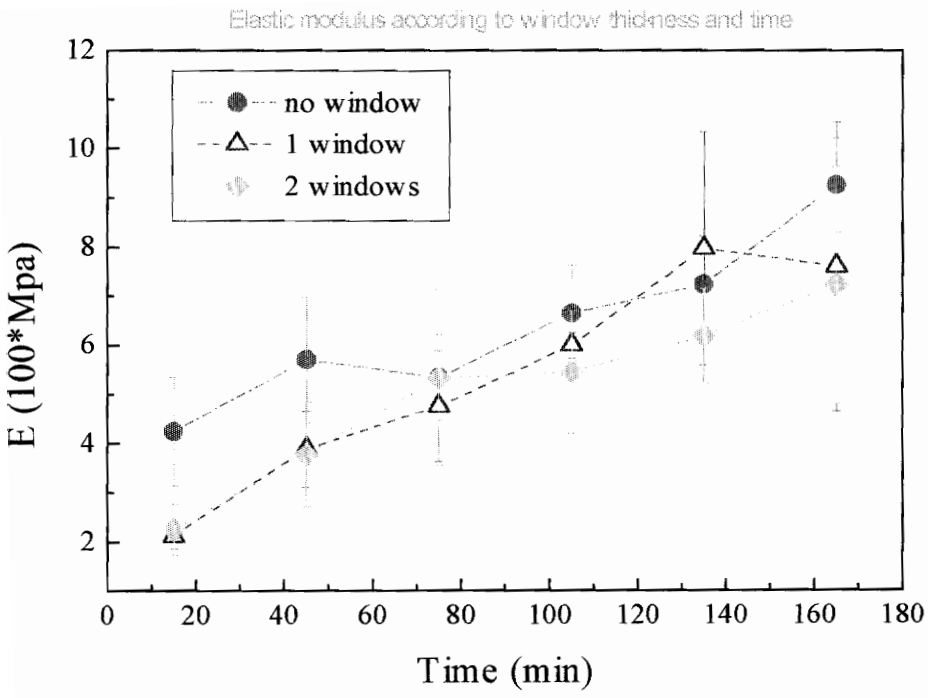


Figure 4.6: Elastic modulus according to the window thickness and aging time

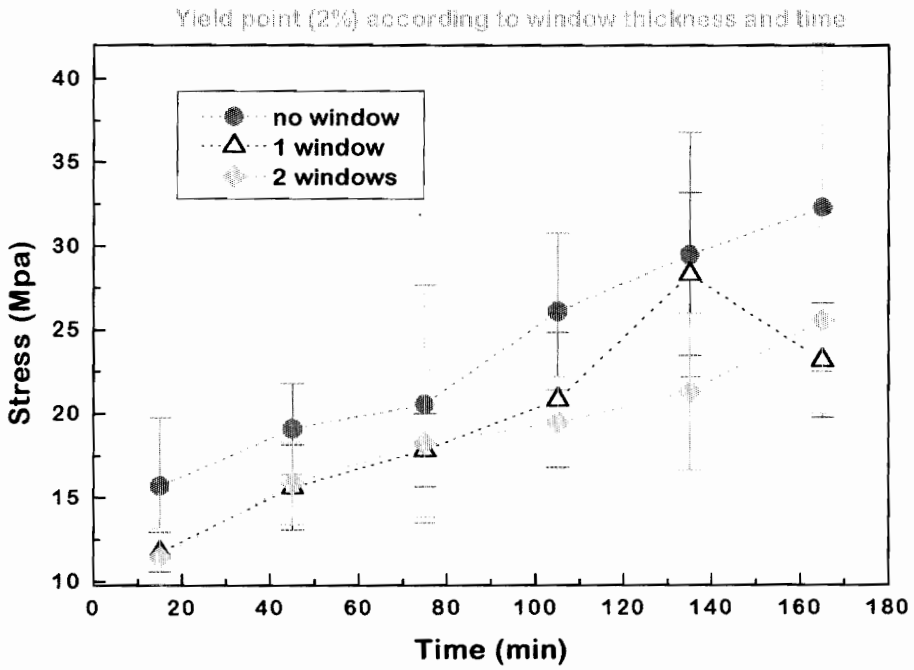


Figure 4.7: Stress at 2% deformation according to the window thickness and aging time

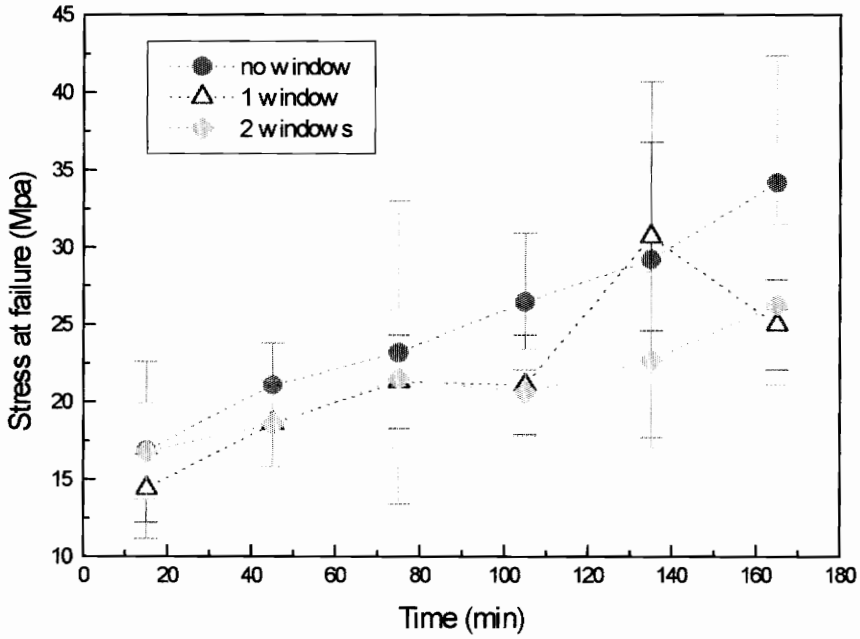


Figure 4.8: Stress at failure according to the window thickness and aging time

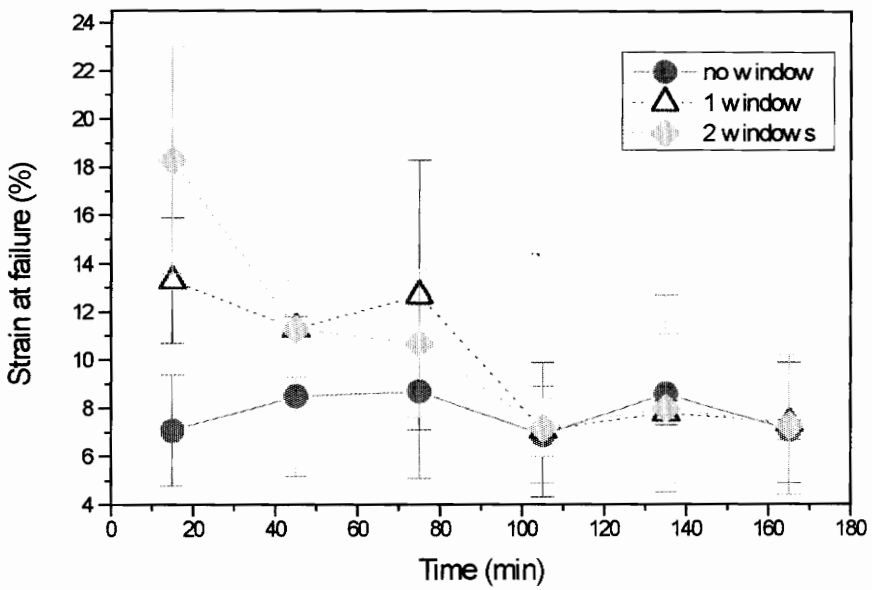


Figure 4.9: Strain at failure according to the window thickness and aging time

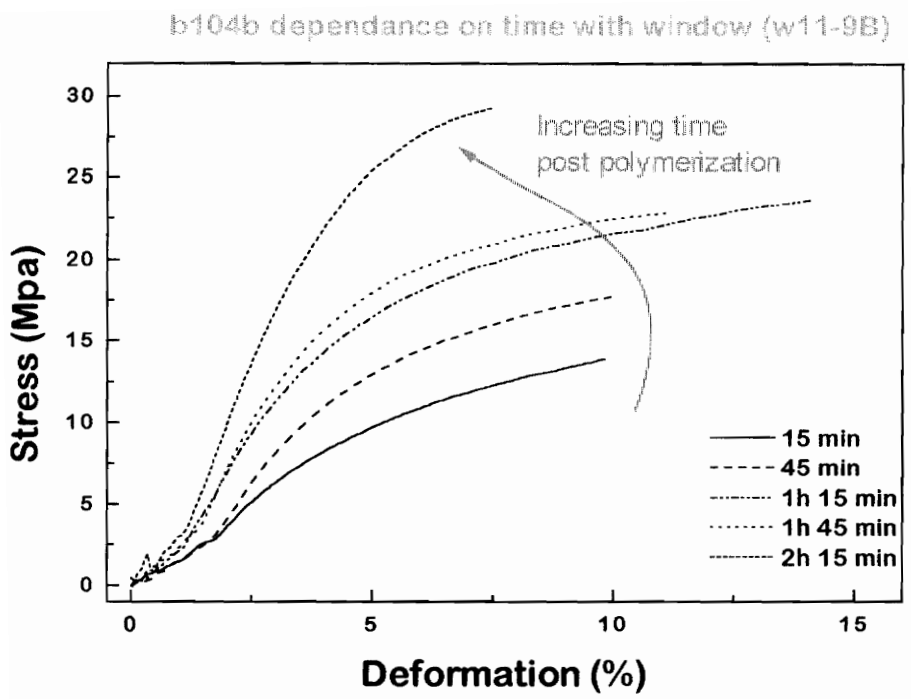


Figure 4.10: Example of stress/strain curve of a sealant cured under one window.

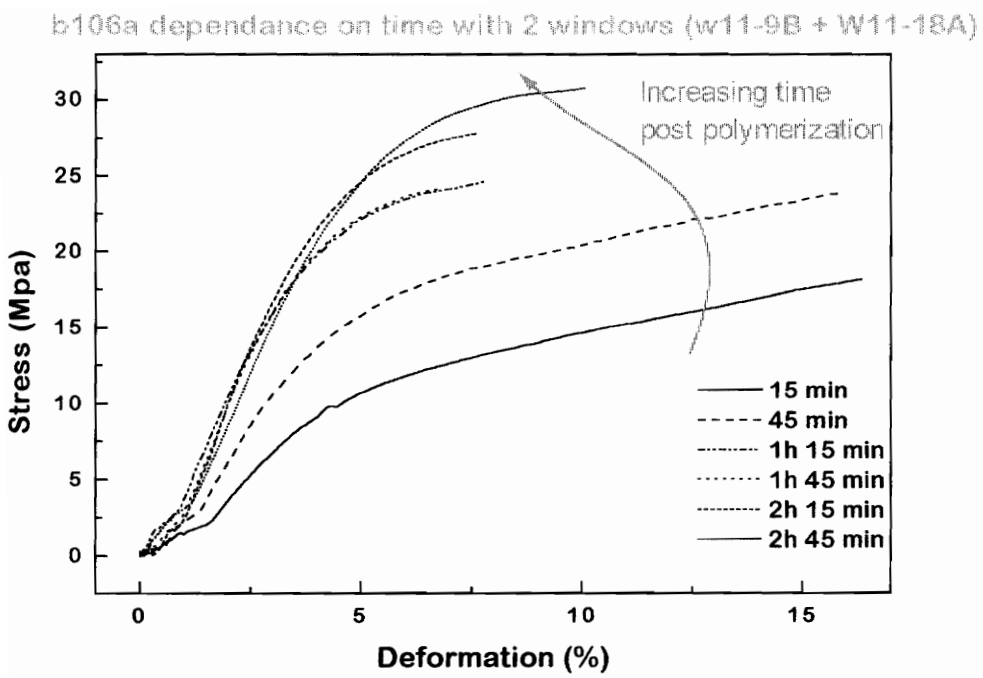


Figure 4.11: Example of stress/strain curve for a sealant cured under two windows

4.2.2 Fracture analyses :

SEM and optical microscope observations were performed on the fractured samples failed during the tensile tests. As it is the case for metals, the fracture surface of polymers reflects both the microstructure and the deformation mechanisms of the materials. According to the model of crack advance in association with craze matter presented in Figure 4.12, we tried to identify signs of crazing on the fracture sealant specimens. Since the crack grows along the crack-matrix boundary interface, the fracture surface should contain pieces of craze attached to the surface fractures [63]. From the SEM observations of our specimens, no indication of a crazing behavior was found. The fracture patterns, see Figure 4.13, were related more to a brittle type of failure and no sign of craze matter was found. Even for the samples presenting the lower degree of conversion, no evidence of crazing was found. No more evidence of crazing was found from the optical microscope observation of the failed samples. As indicated in the optical micrograph (see Figure 4.14) the fracture lines are more representative of a brittle type of failure. Some samples present bigger amounts of ductility as shown in Figure 4.15. The fissure are curved and seem blunted at the end. The sealant seem to have developed some level of plastic deformation. But even for this case, no evidence of crazing has been seen.

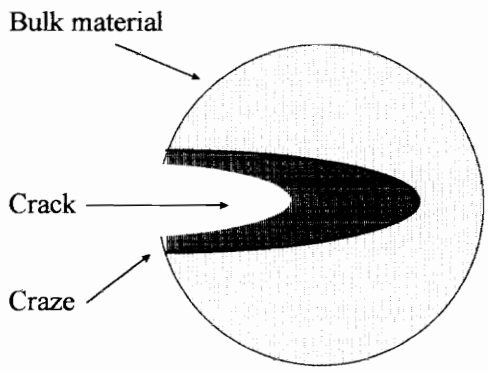


Figure 4.12: Crack propagation in a craze

Signs of craze matter should appear on the broken surfaces

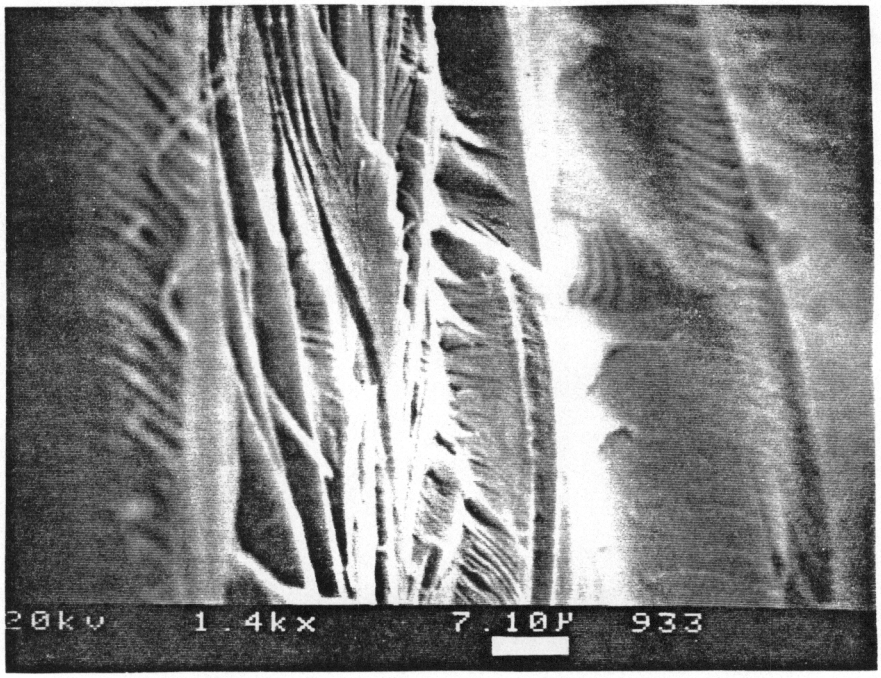


Figure 4.13: SEM picture of fractured samples

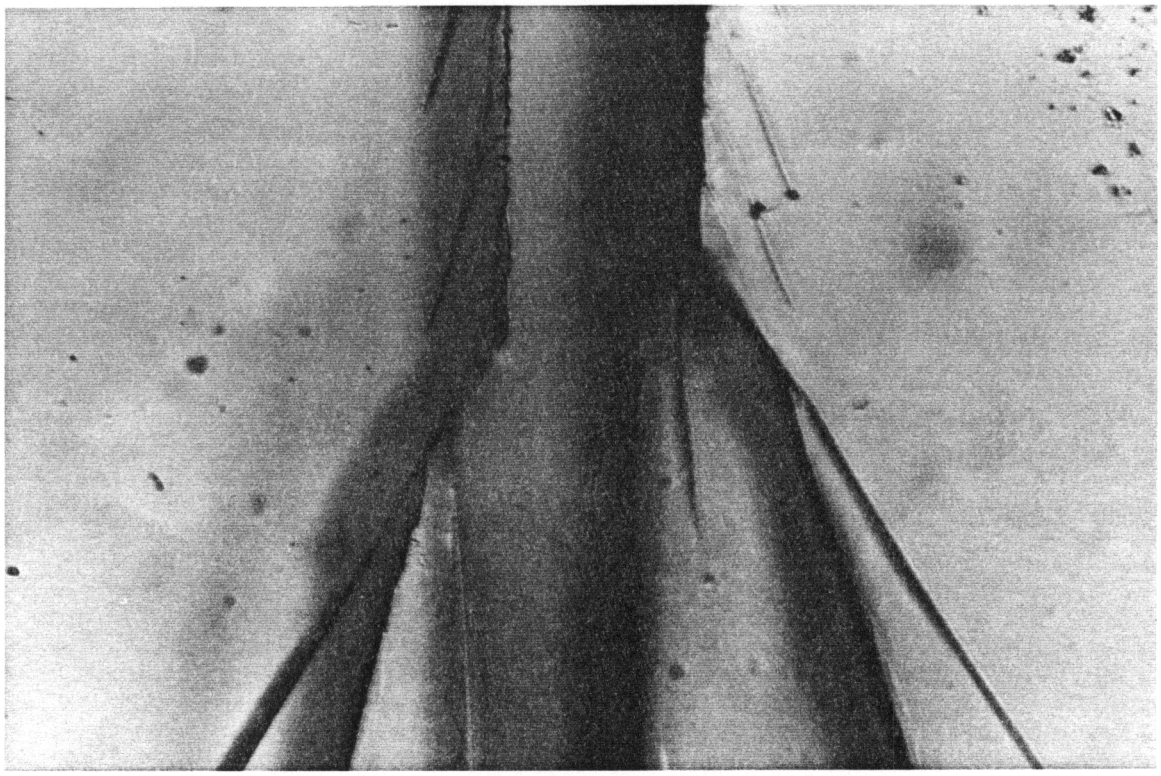


Figure 4.14: Micrograph of a failed sample.

Fissures are irradiating from both sides of the failed sealant

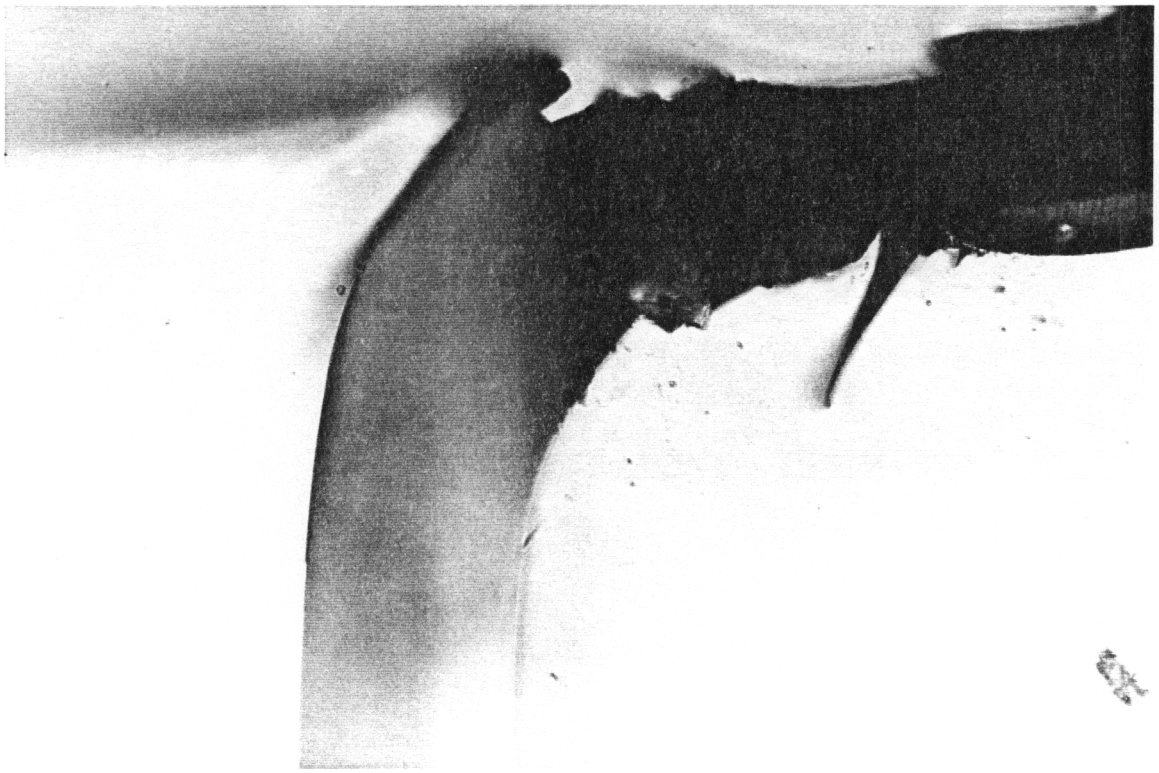


Figure 4.15: Micrograph of a failed sample.

The fissures are curved and appear blunted at the end.

4.2.3 Leaching of unpolymerized resin from pit and fissure sealants

The first step to measure the amount of leachable species from pit and fissure sealant was to create calibration curves for both TEGDMA and Bis-GMA. These calibration curves are presented on Figure 4.16 for TEGDMA and Figure 4.17 for BisGMA. The values used for the curves are shown in table 3 and table 4, appendix.

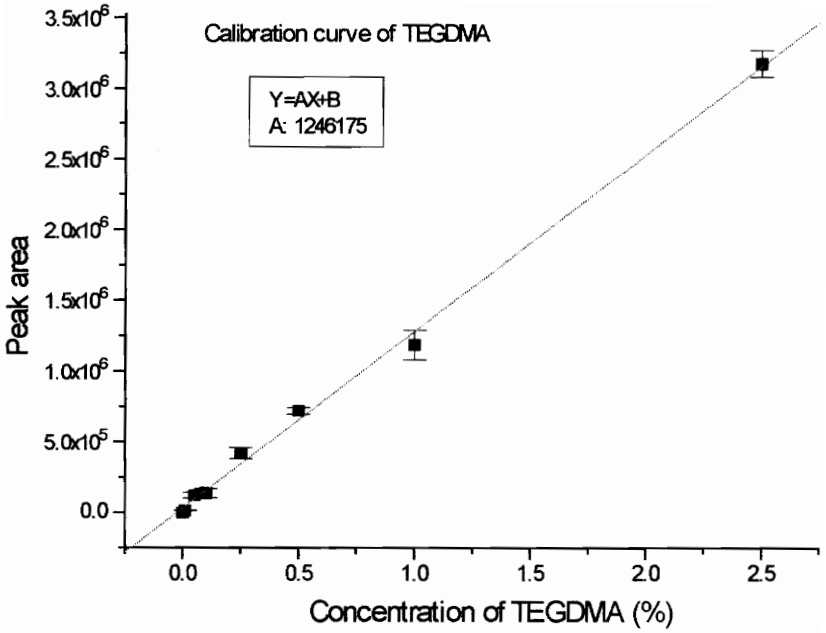


Figure 4.16: Calibration curve of TEGDMA

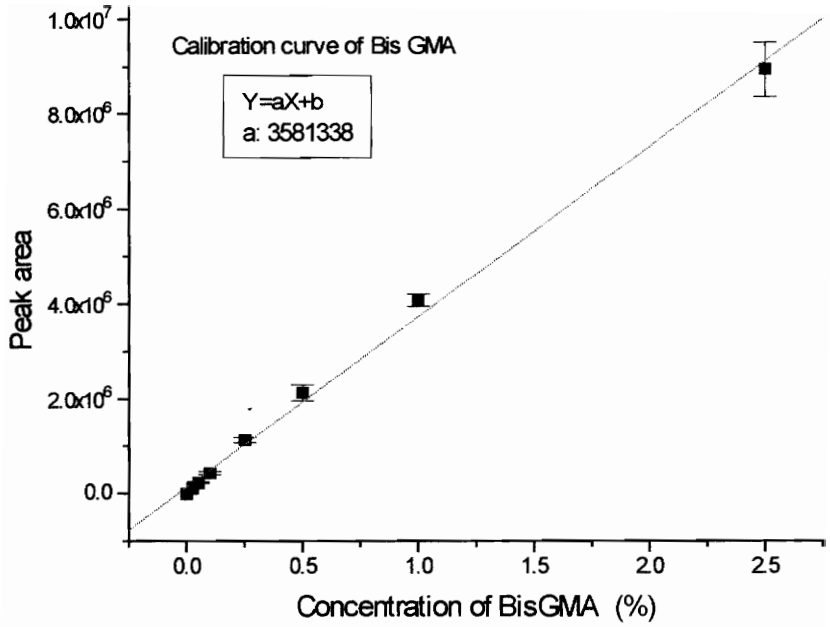


Figure 4.17: Calibration curve of BisGMA

Self cure sealant:

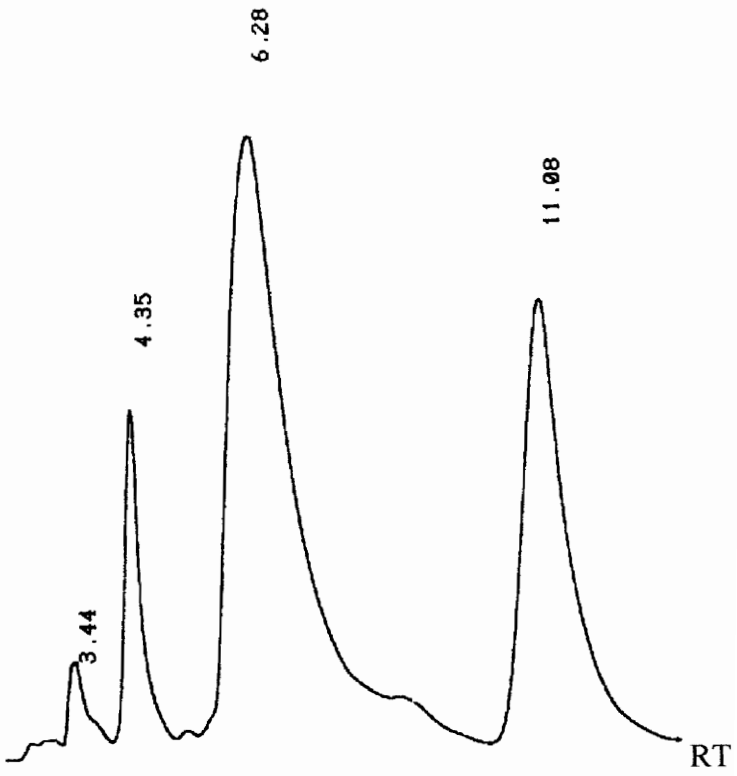


Figure 4.18: Output curve of a self cure sealant

On this curve we can see two major peaks which appear at 4.3 and 11 min, these peaks correspond respectively to TEGDMA and BisGMA. A third peak has been found for all the self cure sealants at 6.3 min. The compound corresponding to this retention time has not been yet identified. It could be an another constituent of the sealant like Benzoyl peroxide, MMA or other chemicals.

Table 4.3:Different processing variables used during the study

	Mixing ratio
Above clinical value	M1: 1 drop base/2 drops initiator
Clinical value	M2: 1 drop base/1 drop initiator
Below clinical value	M3: 2 drops base/1 drop initiator

Table 4.4: Liquid chromatography evaluation of the amount of TEGDMA released from differently processed self cure sealant after immersion in an ethanol water solution.

	Average peak area RT= 4.3	STDEV peak area RT=4.3	Average Weight % of tegdma in the solution	S.TDEV Weight % of tegdma in the solution	Av. Wt % of tegdma released from the sealant (1)	Stdev. Wt % of tegdma released from the sealant
M1	296089	99919	0.226 %	0.051	9.04	2.04
M2	337759	65259	0.198 %	0.075	7.92	3.00
M3	480718	90139	0.334 %	0.067	13.36	2.68

Equation 1 :

Weight % of TEGDMA in the solution = X

We have 4 g of ethanol/water solution

*Weight = X*4/100*

Weight % released from the sample. Each sample weights 100mg.

*Weight % = (X*4/100)*100/0.1 = X*40*

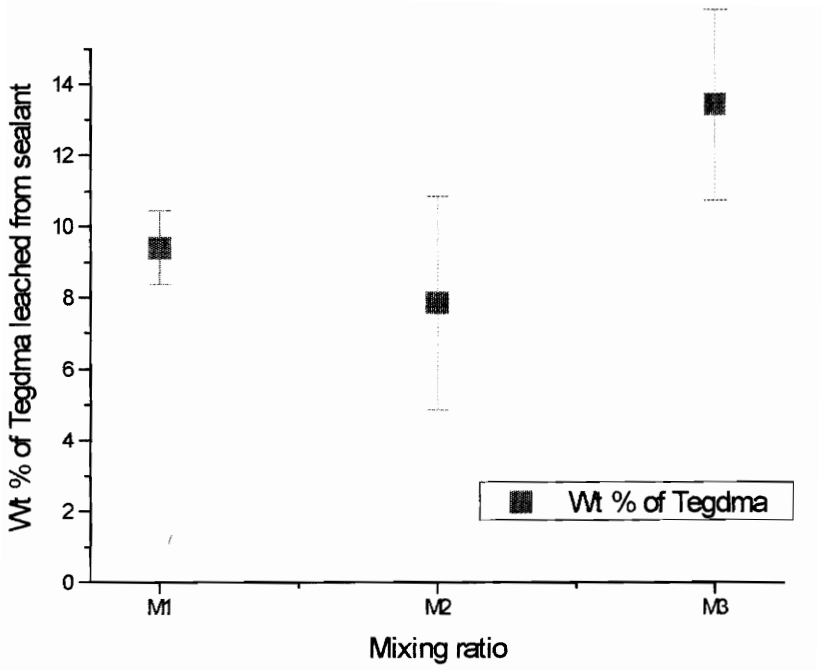


Figure 4.19: Delton self cure sealant

Concentration of TEGDMA leached according to different mixing ratios

The amount of TEGDMA leached from the sealant is high. Up to 13% in weight of the original resin has been leached in the solution. It also appears that the mixing ratio slightly affects the amount of TEGDMA elution. The lower amount of monomer found in the solution corresponds to sealants processed according to the manufacturer recommendation. The chemicals must be provided in a more stochiometric ratio to insure proper conversion.

Table 4.5: Liquid chromatography evaluation of the amount of BISGMA released from differently processed self cure sealant after immersion in an ethanol water solution.

	Average peak area RT= 10.9	STDEV peak area RT= 10.9	Average Weight % of BisGMA in the solution	S TDEV Weight % of BISGMA in the solution	Av Wt % of BisGMA released from the sealant (L)	Stdev W of BisG releas from t sealar
M1	779767	296073	0.174	0.065	6.96	2.60
M2	647525	120299	0.144	0.027	5.76	1.08
M3	963454	139024	0.214	0.031	8.56	1.24

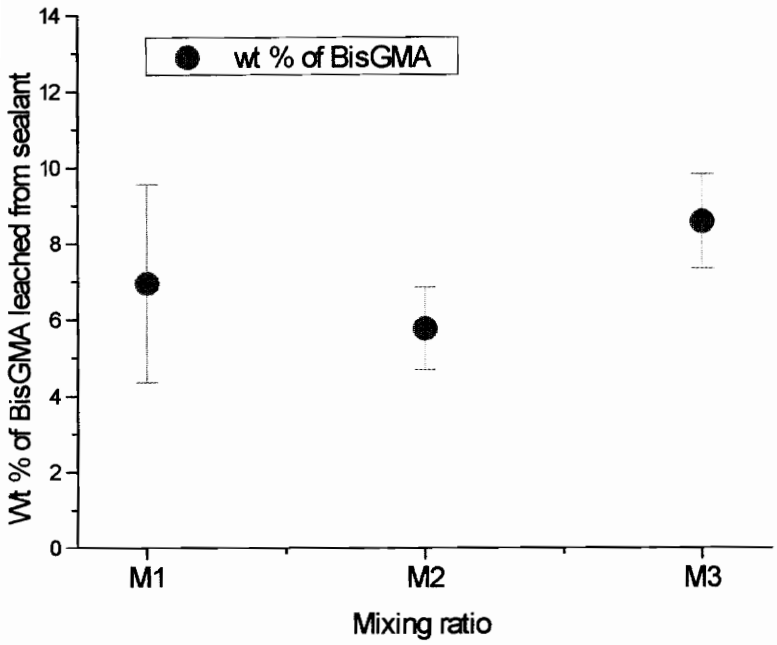


Figure 4.20: Delton self cure sealant

Concentration of BisGMA leached according to different mixing ratios

The amount of leached BisGMA is lower than TEGDMA, but it is still considered as a high level of monomer release. As for TEGDMA, the better degree of sealant polymerization (lower level of leached monomers) is obtained for the recommended mixing ratio.

Light cure sealant:

A representative curve for the light cure sealant corresponding to a 60s exposure time is shown in Figure 4.21.

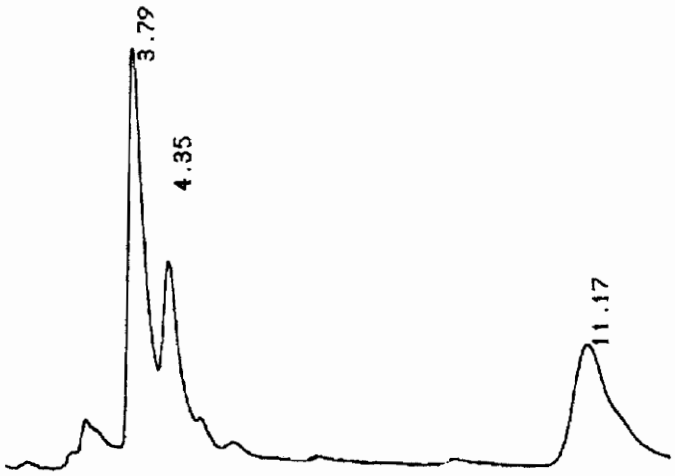


Figure 4.21: HPLC output of a light cure sample (Concise, 3M) cured for 60s

On this curve two major peaks are observed at 4.3 and 11 min, these peaks correspond respectively to TEGDMA and BisGMA. A third peak has been found for all the specimens at 3.8 min. The compound corresponding to this retention time has not been yet identified.

Table 4.6: Liquid chromatography evaluation of the amount of TEGDMA released from differently processed light cure sealant after immersion in an ethanol water solution.

	Average peak area RT= 4.3	STDEV peak area RT= 4.3	Average Weight % of tegdma in the solution	S TDEV Weight % of tegdma in the solution	Av Wt % of tegdma released from the sealant (1)	Stdev W of tegd release from t sealan
t_1=10s	780672	15724	0.174	0.003	6.96	0.12
t_2=20s	501018	71777	0.113	0.017	4.52	0.68
t_3=60s	220059	152805	0.050	0.035	2.00	1.40

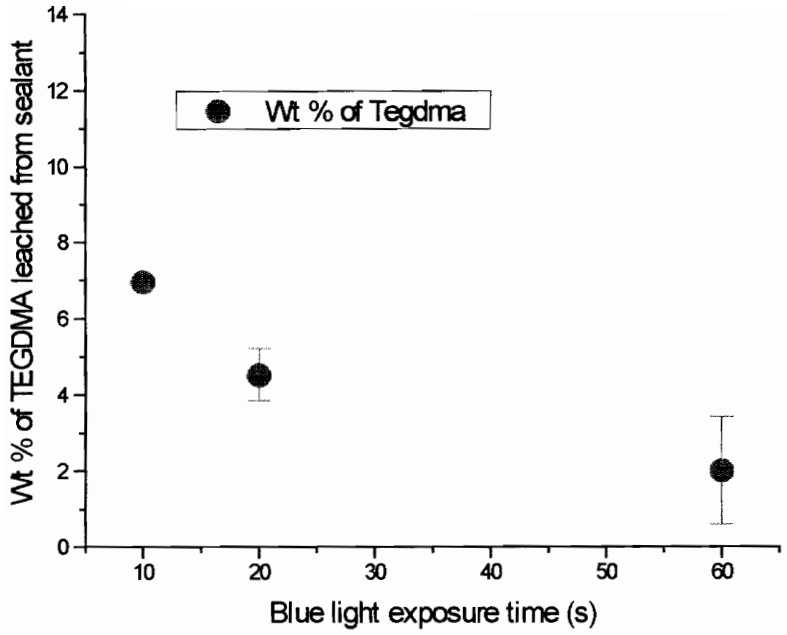


Figure 4.22: Concise light cure sealant

Concentration of BisGMA leached according to different mixing ratios

Table 4.7: Liquid chromatography evaluation of the amount of BISGMA released from differently processed light cure sealant after immersion in an ethanol water solution.

	Average peak area RT= 10.9	STDEV peak area RT= 10.9	Average Weight % of BisGMA in the solution	STDEV Weight % of BisGMA in the solution	Av Wt % of BisGMA released from the sealant (1)	Stdev W of BisG release from t sealan
10s	1200155	74183	0.268	0.017	10.72	0.68
20s	566042	85378	0.128	0.020	5.12	0.80
60s	223865	150760	0.049	0.032	1.96	1.28

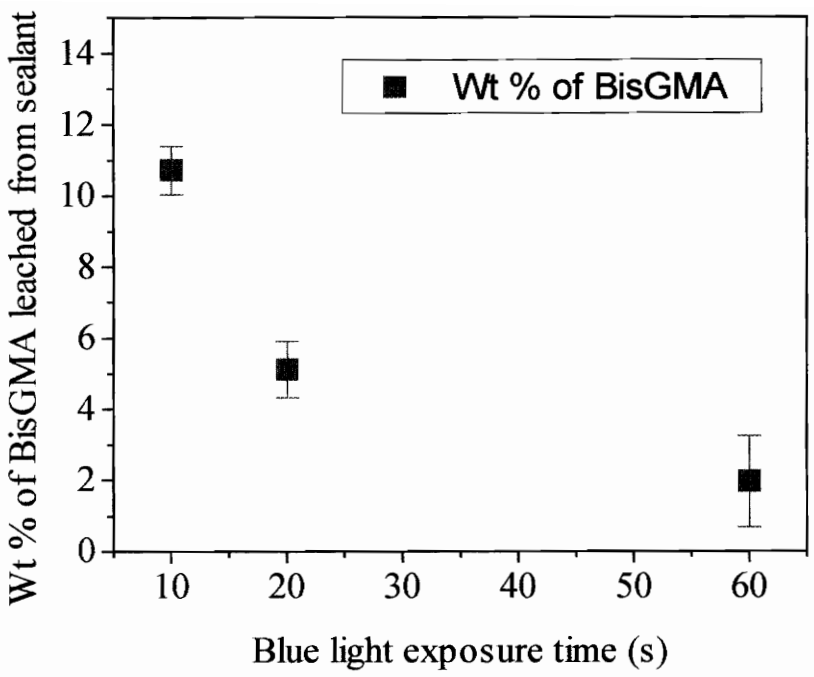


Figure 4.23: Concise light cure sealant

Concentration of BisGMA leached according to different mixing ratios

The level of leached TEGDMA and BisGMA decreases with increasing light exposure time. The level of monomer released is slightly higher for BisGMA than it is for TEGDMA.

For both types of sealant, the high level of monomer leached in solution may be explain by the fact that the sealants were nearly immediately polymerized and put into solution. Also a layer of liquid appeared on the sealant, clinically, the sealants should be rinsed after polymerization but we didn't. If this layer were unpolymerized resin, this would increase the level of monomer in solution. We wanted to evaluate the worst scenario.

4.3 In vivo experiment :

4.3.1 X Ray evaluation of the sealant microleakage

After three months, the animals were killed and the treated teeth stored in water.

Radiographic evaluation of some of the teeth was performed to estimate leakage and loss of the sealant in a non destructive fashion. Some of the samples were placed in an X ray sensitive solution prior to being analyzed. No additional information was obtained by using the penetrant solution. As shown in (Figure 4.24), the structure of the teeth appears clearly on the radiographs, but no notable sign of sealant or leakage can be seen. The radiographic evaluation of the teeth was not performed on other samples since no real information concerning the presence of the sealant has been found. This evaluation was of a good clinical relevance, but we didn't get an indication concerning microleakage of the sealant.

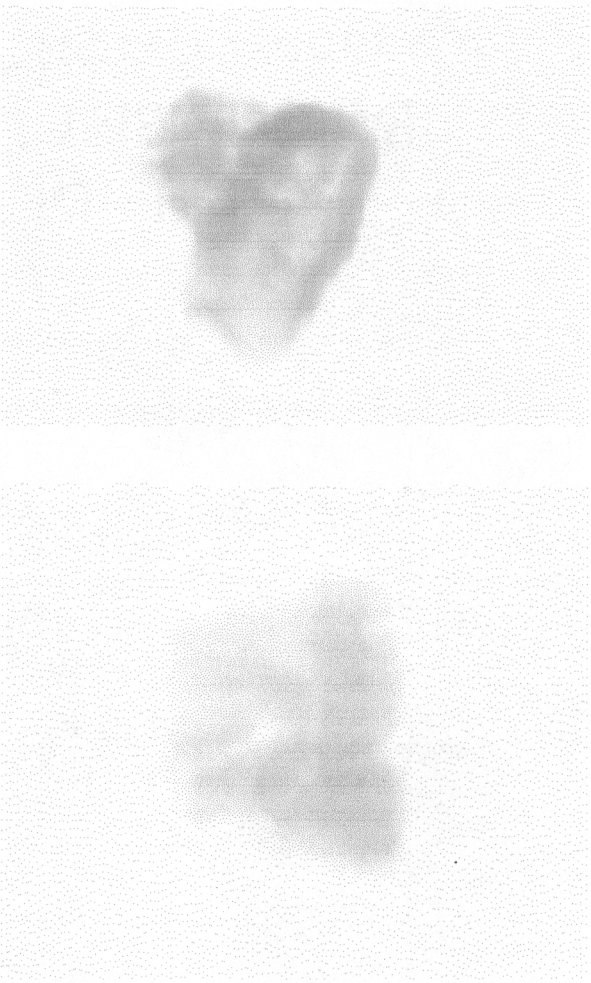


Figure 4.24: X ray images of teeth

4.3.2 Visual evaluation of the sealant retention

The first observation was that the level of sealant retention was very low compared to clinical retention values. A difference in the retention level was also observed between teeth of different sizes. Since the morphology of the tooth apparently influenced the level of retention of pit and fissure sealant, during the visual evaluation, we indicated if the tooth was "big", or "small", corresponding to the first and second molar. The complete results from the visual retention are shown in table 5 in appendix. A three level analysis for visual observation was used. Each number corresponds to a different level of residual sealant on each tooth. Table 4.9 and Table 4.10 show the number of teeth presenting each level of retention for the different treatments.

Figure 4.25 and Figure 4.26 show the average degree of retention found for each of the treatment.

Table 4.8: Levels of sealant retention

Retention	Visual evaluation
1	No trace of sealant can be found on the tooth
2	Traces of left over sealant are found but can't prevent the tooth from decay
3	The retention of the sealant is complete

Table 4.9: Visual evaluation of the retention of the self cure sealant

Tooth characteristics	Big			Small		
Level of retention	1	2	3	1	2	3
Process: M1	8	1	0	1	5	4
Process: M2	4	2	1	0	3	4
Process: M3	7	1	0	1	1	6

Table 4.10: Visual evaluation of the retention of the self cure sealant

Tooth characteristics	Big			Small		
Level of retention	1	2	3	1	2	3
Process: t1	7	2	1	2	4	4
Process: t2	4	3	0	3	4	1
Process: t3	6	0	2	1	2	5

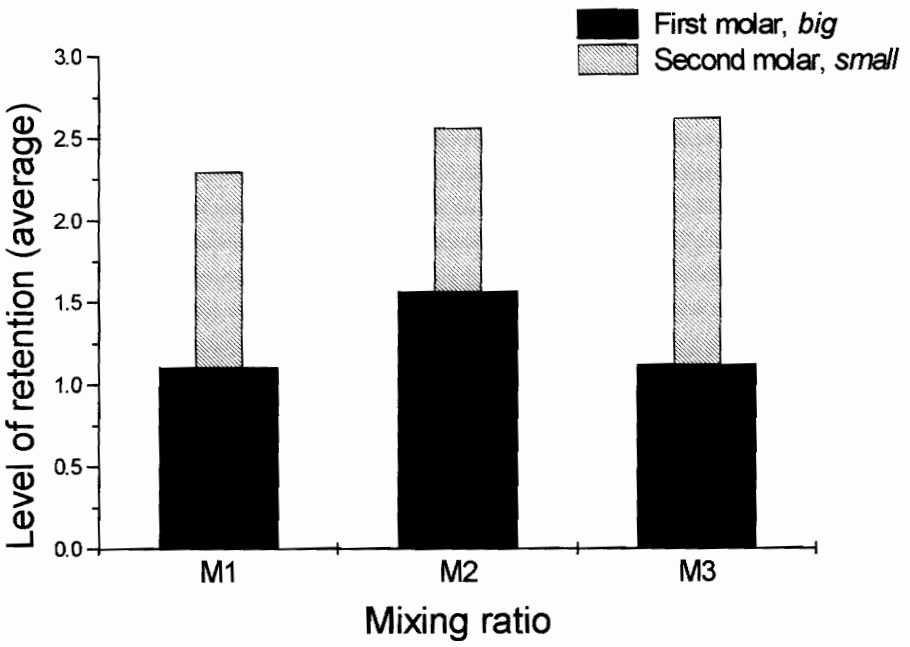


Figure 4.25: Degree of retention (average) according to the mixing ratio for the self cure sealant

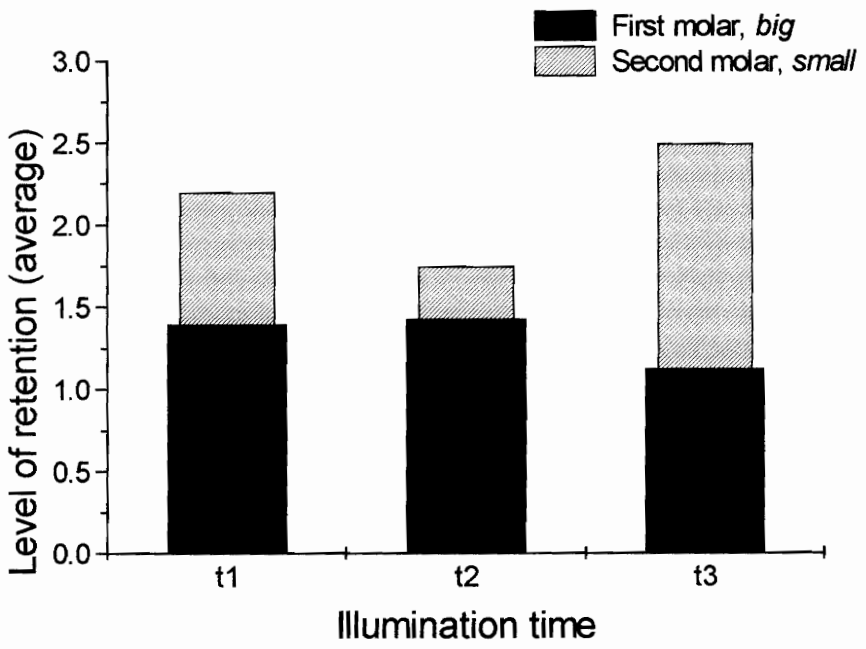


Figure 4.26: Degree of retention (average) according to the exposure time for the light cure sealant (Concise, 3M)

4.3.3 Optical Microscopy

The teeth were then inspected under an optical microscope to determine the presence of any residual sealant and sealant penetration into the fissure.

Different geometric factors for the fissure, amount of left over nutrients, degree of sealant filling, and signs of fracture and defects in the sealant have been observed. These are detailed below.

1) Is there sign of sealant in the fissure ?

2) Shape of the fissure:

    - V shape
    - U shape
    - Narrow shape
    - Wide fissure
    - Very wide (nearly no curvature)
    - Bulbous end
    - Two fissures or more sealed by the same piece of sealant

3) Signs of sealant fracture

4) Signs of bubbles

5) Nutrients trapped

    - At the tip of the fissure
    - In the middle/top of the layer
    - Thick sealant/enamel interface

The following micrographs give an indication on the diversity of the fissure shape and examples of different situations encountered.

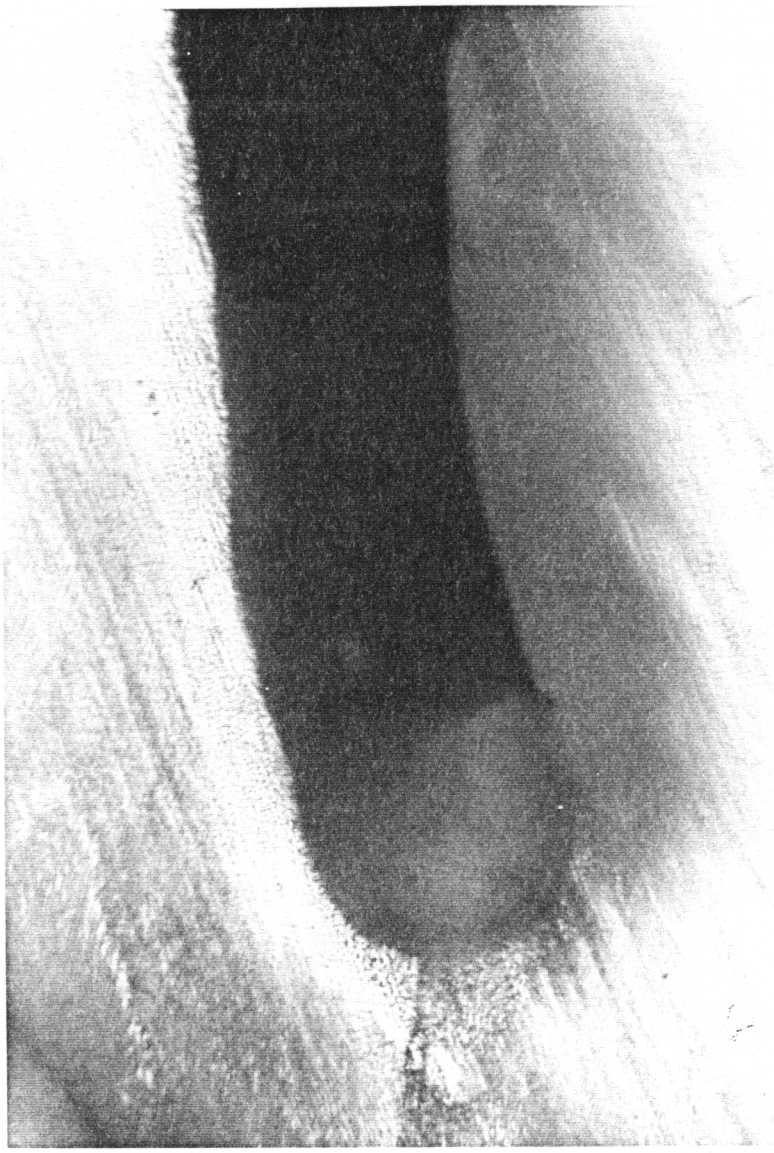


Figure 4.27: Empty fissure, narrow shape

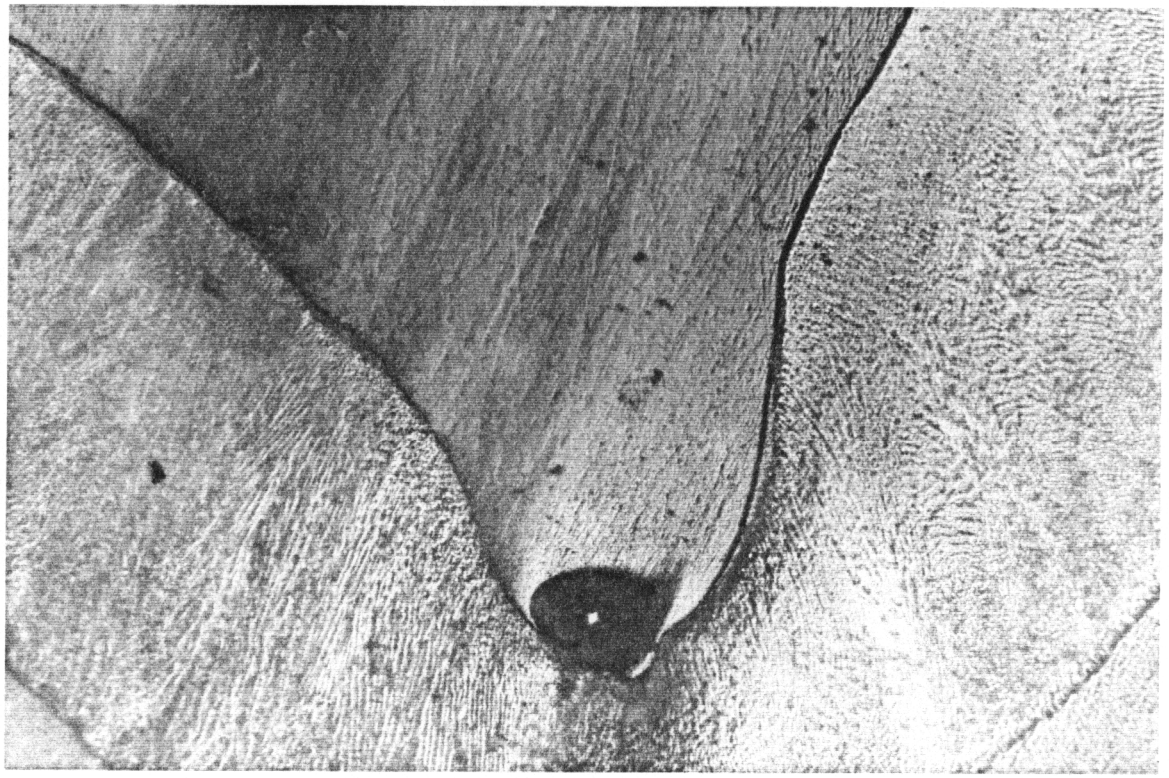


Figure 4.28: U shape fissure, contact interfacial contact between the sealant and the fissure

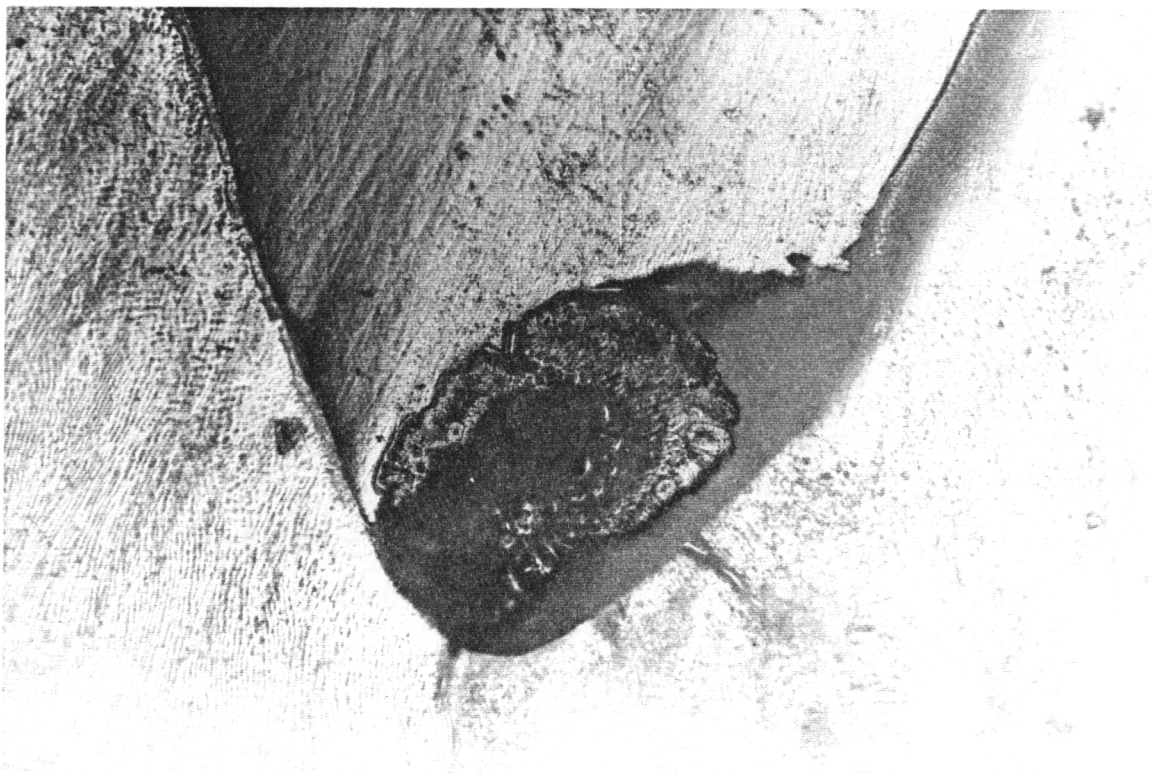


Figure 4.29: U shape wide fissure, nutrients trapped at the and are obstructing the fissure.

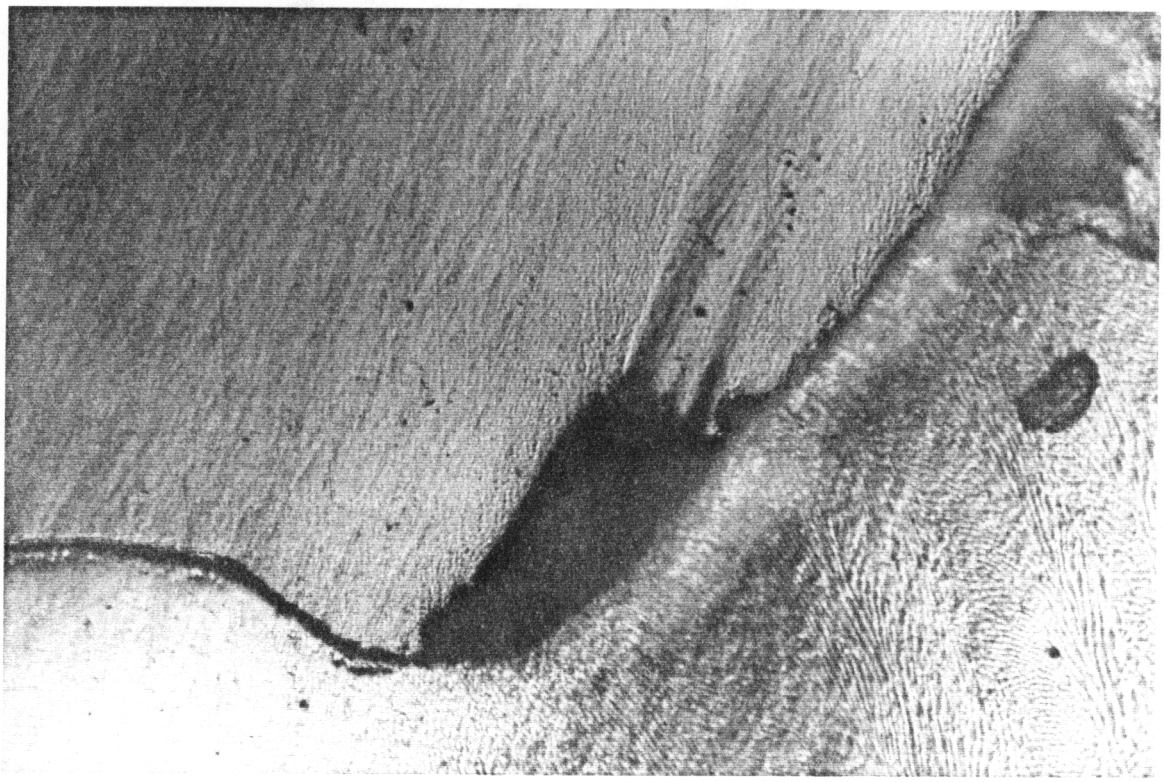


Figure 4.30: Filled fissure, very narrow tip. Thick sealant/enamel interface.

The sealant was too viscous to flow all the way down the fissure.

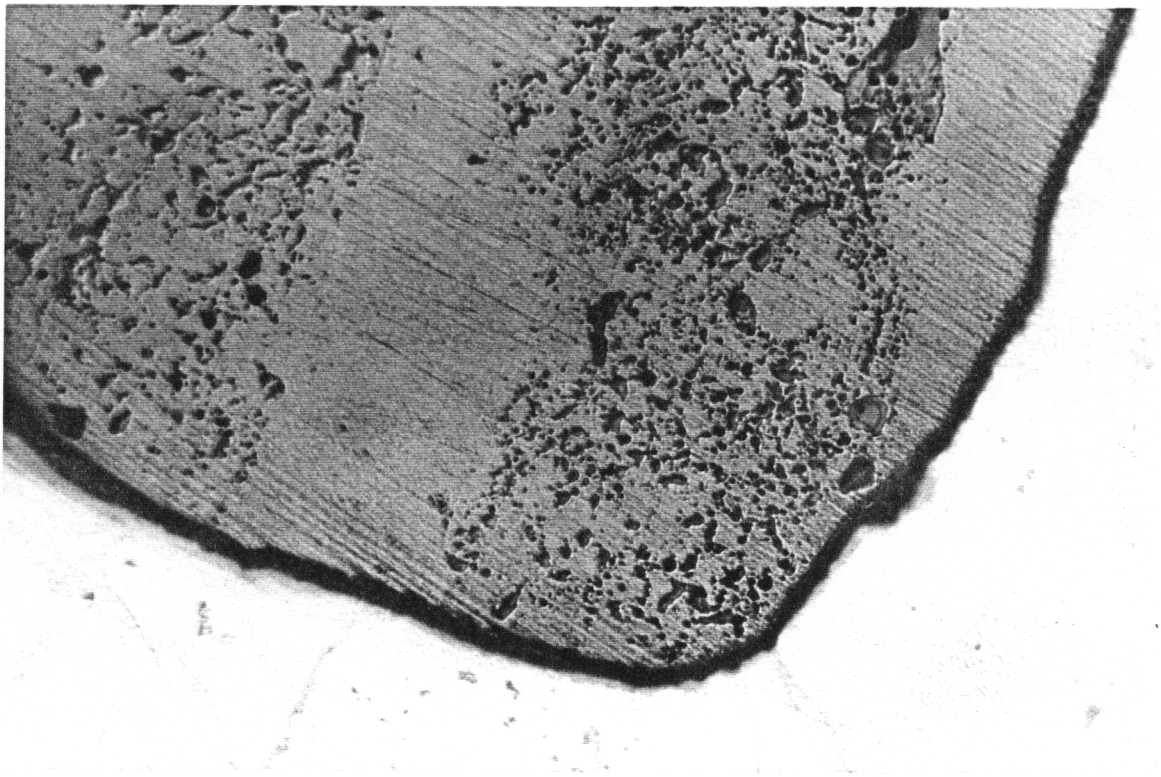


Figure 4.31: Wide V shape fissure. Thick sealant/enamel interface at the bottom of the fissure. Signs of enamel fracture

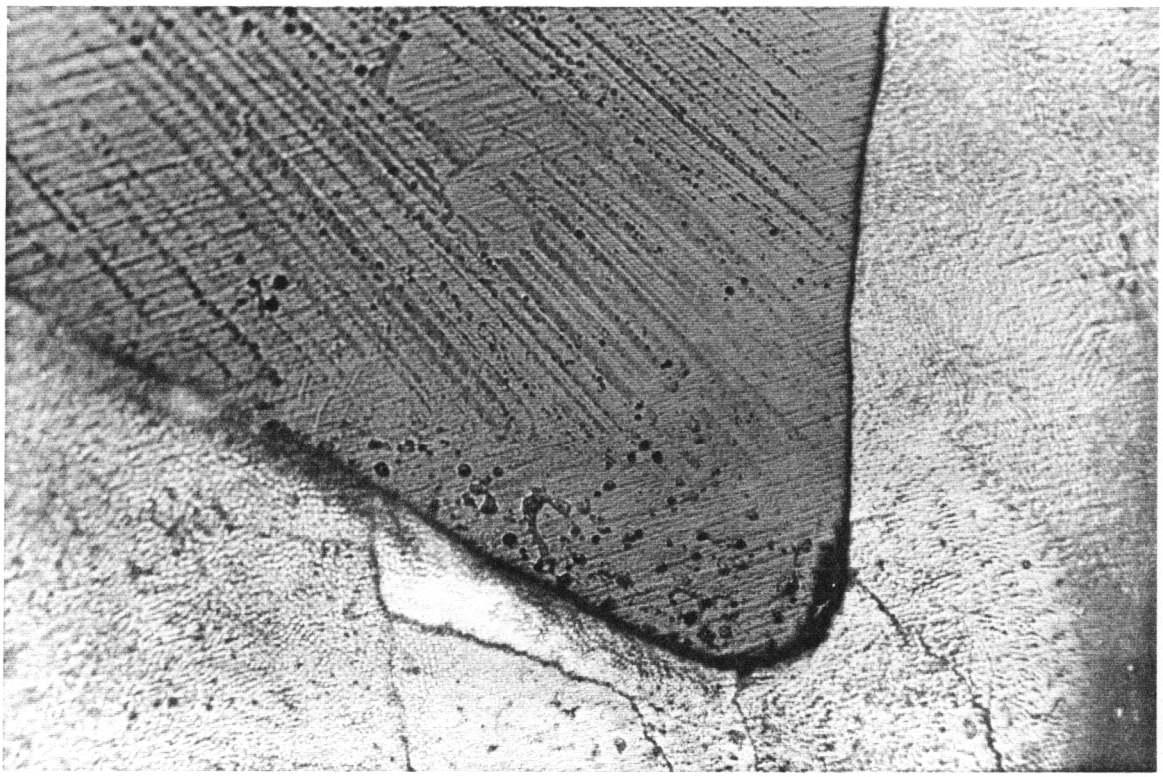


Figure 4.32: Very wide fissure. Thick sealant/enamel interface.

Little fissures in the enamel

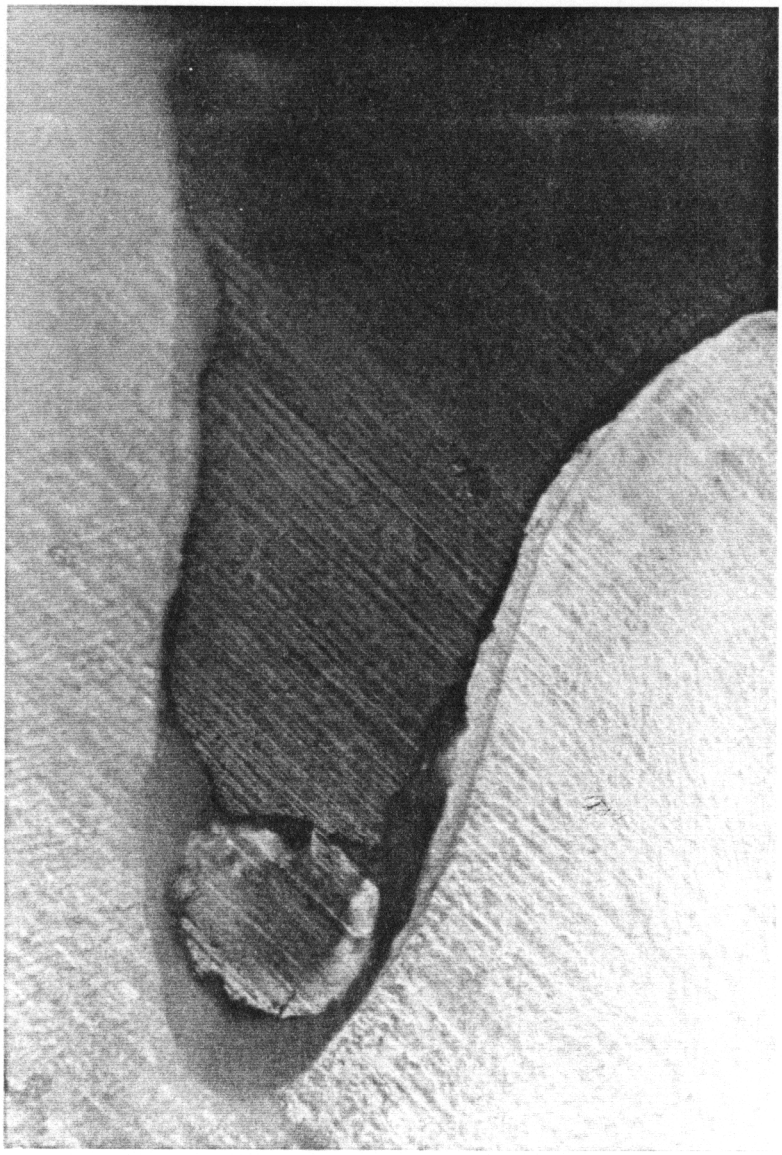


Figure 4.33: Narrow fissure. Small broken piece of sealant at the tip.

Poor interfacial contact in some places maybe due to a too high viscosity of the sealant.

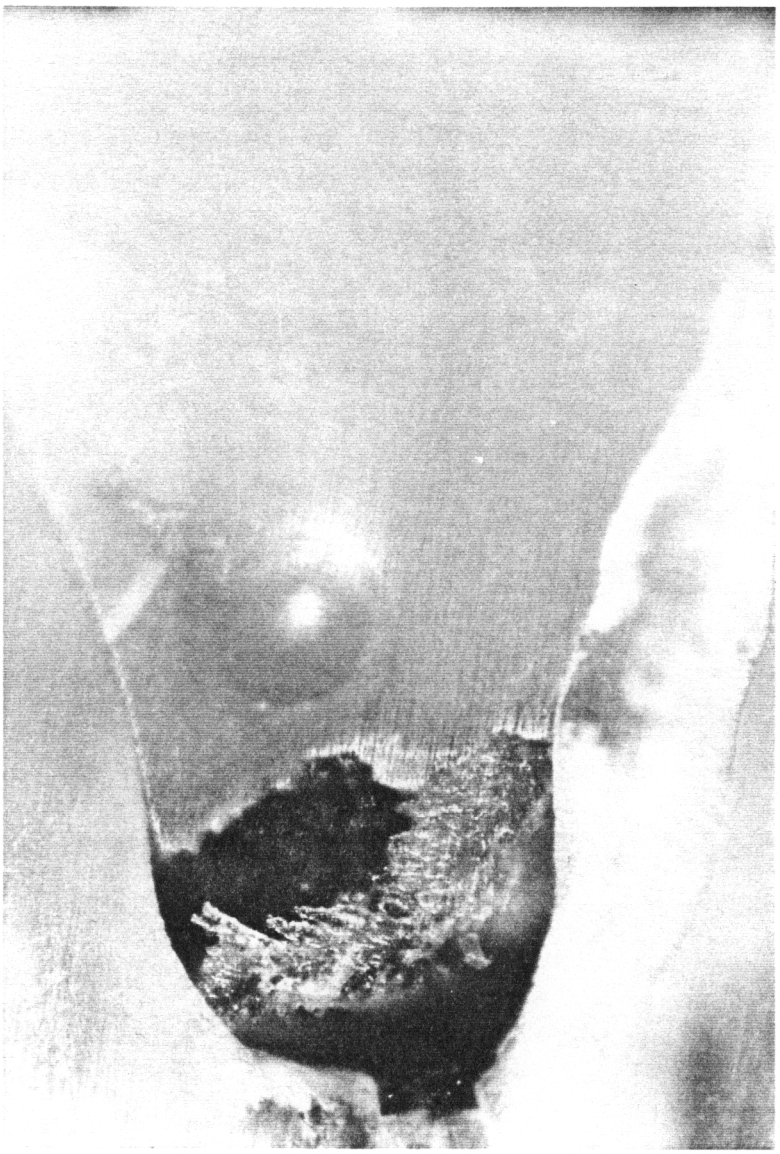


Figure 4.34: U shape fissure, nutrients trapped at the bottom preventing the sealant from completing filling the fissure. Very thin sealant/enamel interface

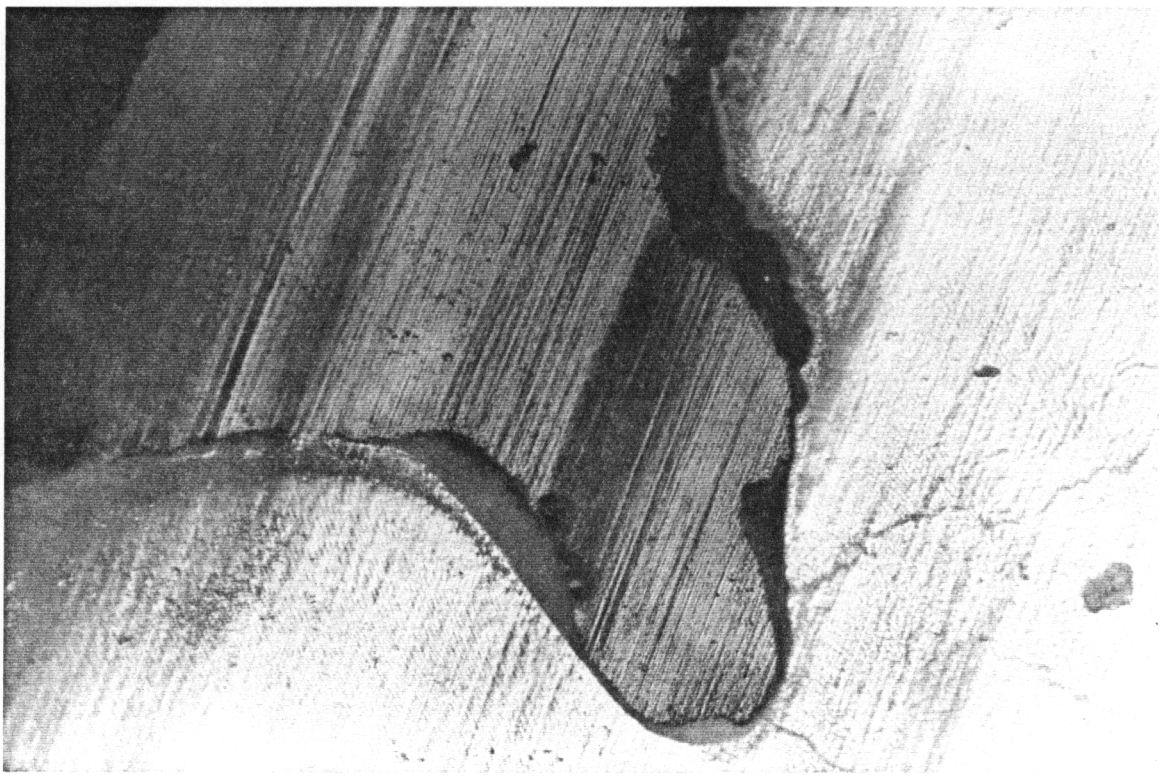


Figure 4.35: Unusual fissure shape. Poor interfacial contact on many places, maybe due to a poor wetting of the sealant. On the top right side of the sealant/enamel interface, nutrients seem prevent adhesion to occur. Small fissures appear in the enamel.

These micrographs give us a good indication on the diversity of the different situations encountered. Very wide fissures as well as very narrow fissures have been found. The level of penetration of the sealant in the fissure seem related to the viscosity of the sealant or sometimes to the presence of nutrients obstructing the fissure. Also a very thick and dark interface has been observed on many pictures. It would be interesting to analyze the exact composition of this interface which might be a weak layer between the adhesive and the substrate and which would further reduce the bond strength. It is very likely that this interface is composed of nutrients and microorganisms. The cleaning procedure, which consisted in pumice cleaning followed by acid etching, didn't succeed in withdrawing all the pelicle. Fissures in the enamel were observed in several teeth.

4.3.4 Environmental Scanning Electronic Microscopy

The samples were observed under the SEM to establish whether cracks at the interface between the enamel and the sealant develop in a particular way. For some specimens, we can wonder whether the sealant has been broken during the *in vivo* study or during the cutting of the teeth. Even if the sectioning of the samples has been performed with care, it is difficult to claim that no fracture pattern that we observed was unrelated to sample preparation.

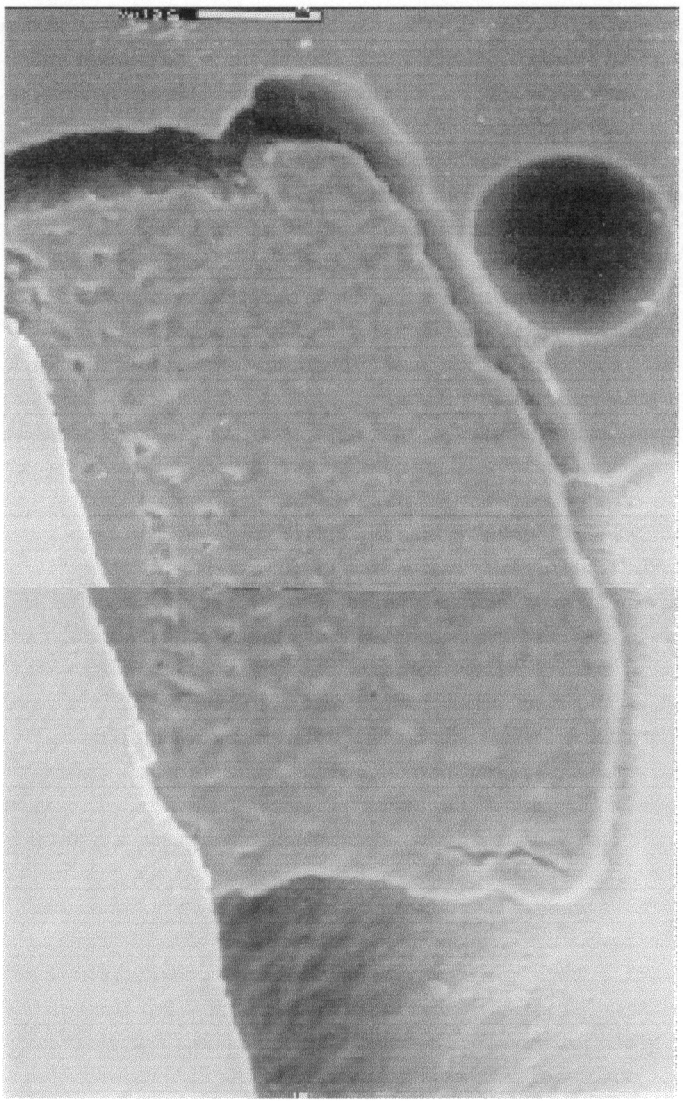


Figure 4.36: Fissure partially filled with sealant. Magnitude X 350.

The sealant is broken in the middle, near a big bubble.

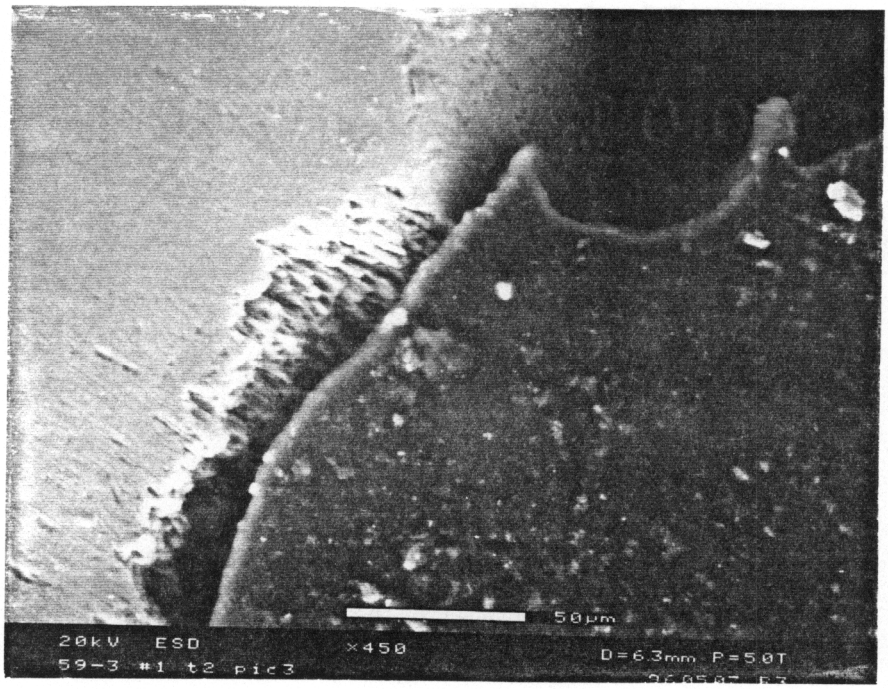


Figure 4.37: Interface sealant enamel at the end of the sealant. A really poor contact is observed between the sealant and enamel. Enamel appears like "etched", or even dissolved.

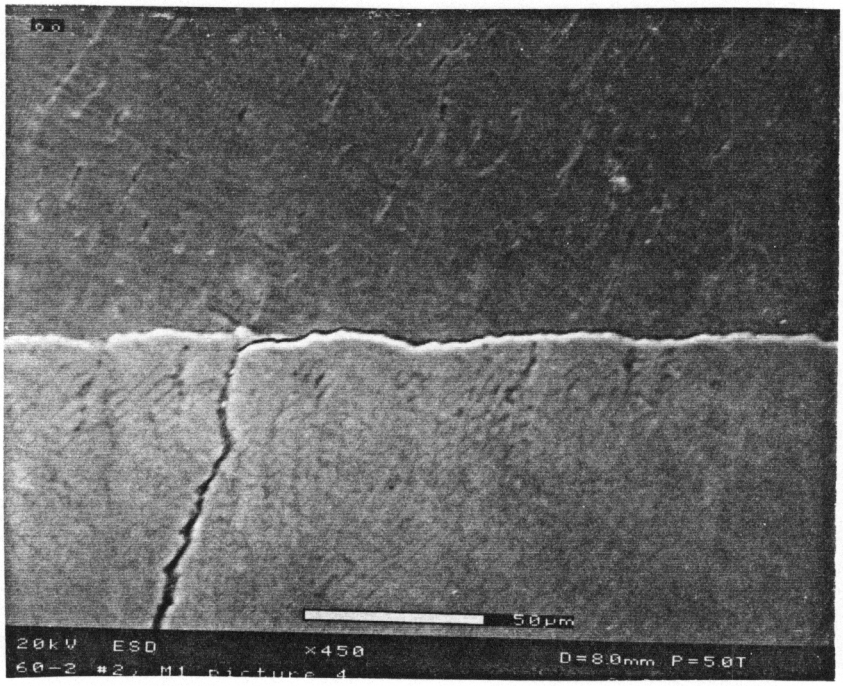


Figure 4.38: Fissure developing along the enamel/sealant interface.

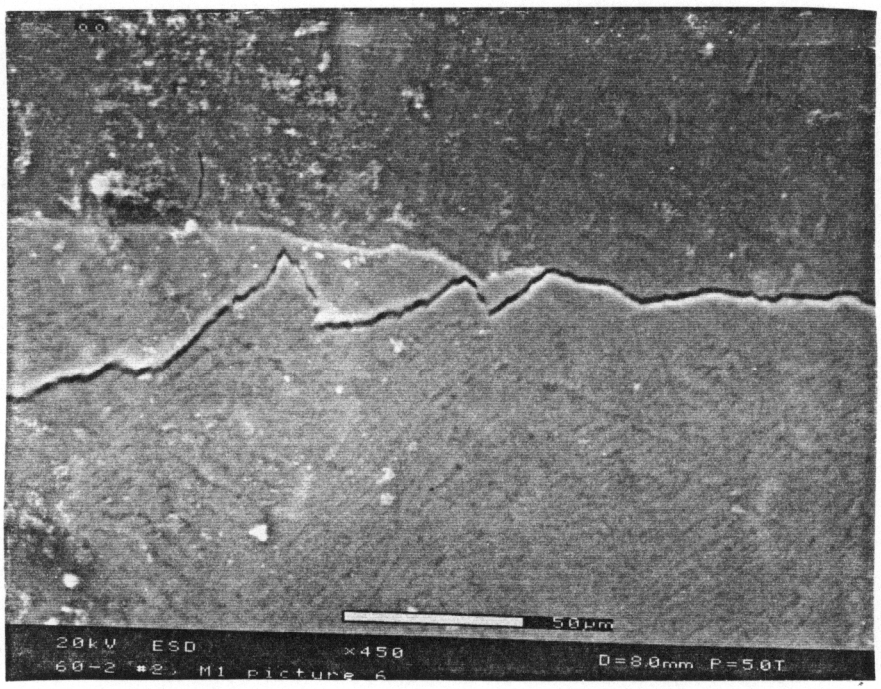


Figure 4.39: Fissure developing along the enamel/sealant interface

Our observation didn't really enable us to conclude on a predominant mode of failure between the sealant and the enable. More observations are still needed. Also, some of the fissures, as shown in Figure 4.38 and Figure 4.39, look like failure at the interface enamel/dentine. These failures might have even occurred during the ESEM evaluation. In fact the sealant appear really dry after observation. The ESEM might have dehydrated the sample and caused fractures in the enamel. Future observations should be performed with an SEM to see if the failure at the enamel/dentine still occurred.

5. Discussion

5.1 Characterization of the sealants

5.1.1 Experimental sealant processing

A good sealant processing scheme was found which enabled us to create, in a reproducible way, samples of relatively uniform thickness. However, some defects, like air bubbles and incompletely dissolved camphorquinone were found, these flaws may explain some of the scatter found in the mechanical test data. Also, this experimental sealant can only be considered as a model to obtain qualitative data on the behavior of pit and fissure sealants. In fact, the composition of commercial sealants varies significantly from the experimental sealant. Commercial sealants can be filled, opaque or clear, self cured or light cured, and they may also contain different types of additives. A self cure experimental sealant should also be created to estimate the influence of the mixing ratio on the mechanical behavior of the sealant.

5.1.2 DSC analyses

It was concluded that the level of cure for each system is incomplete upon processing and that no important variation can be identified between the degree of cure of sealants processed under different conditions. Further aging of the cured resin advances the cure. DSC also showed that thermal processing advances the resin cure. One potential explanation for these observations is that upon some level of cure, the growing free radicals are pinned and the molecular mobility is too low to propagate the chain length. The reaction rate is very low, and since this is a kinetic process, polymerization will continue to advance with time or upon heating. These observations are in accord with Kloosterboer 14. When densely crosslinked polymers are prepared by low-temperature

polymerization, incomplete conversion of the reactive groups is often observed. The reduced mobility caused by the increasing viscosity and crosslinking reduces the rate of polymerization which becomes limited by diffusion. This may explain why the rate slows but still continues after processing. Free monomers can still diffuse and the degree of conversion will be increased with time. It is interesting to know that the properties of the sealant evolve during a long time after application. The response of the polymer to the forces induced upon chewing will be different immediately upon application and a few days after.

5.1.3 Fourier transform infrared spectroscopy (FTIR)

The conclusions made earlier about the incomplete conversion of the reactive groups due to vitrification are validated with the infrared evaluation of the sealant: the conversion of the sealant is incomplete upon processing and is increased upon aging. Similar results indicating an incomplete conversion of BisGMA composite resin have been found by Chunk et al [64]. The infrared spectra of seven available posterior composite resins have been analyzed. The degree of conversion ranged from 43.5 to 73.8 %, also indicating unreacted resin.

For the light cure sealant, a plateau in the degree of conversion is reached after a ten seconds of illumination time and can't be increased upon further blue light exposure. This indicates two things : a) a minimum light exposure time exists for optimum cure of the sealant, b) further blue light exposure doesn't improve the level of conversion.

The different mixing variables don't significantly affect the level of sealant conversion. A slightly higher degree of crosslinking has been found for the manufacturer's mixing recommendations. An increase in the degree of conversion upon aging has also been found for the self cure sealant.

Better stability and long term durability of the sealant might be obtained for more crosslinked resins. It has been pointed out [31] that incomplete polymerization of light cured Bis-GMA resins could result in increased solubility and sorption due to incomplete conversion of the resin in addition to softened mechanical properties. Higher levels of conversion could be obtained with an elevation of the reaction temperature or by exposing the sealant to increased temperature after application. Since in the DSC output, the exotherm peak corresponding to an increase of the resin conversion occurs around 60°C, exposing the sealant to a heat source in this range of temperature would increase the level of conversion of the resin. In a clinical perspective, this might be realistic, since this range of temperature should not affect the tooth and gum.

5.2 Mechanical analyses

5.2.1 Tensile testing

A good scheme was found to model the mechanical behavior of the sealant as a function of depth. From the evaluation, a strong correlation exists between the depth and the mechanical properties of the sealant. The sealant is tougher and more brittle on the surface than at the tip of the enamel fissure. Upon chewing the sealant will deform in a non uniform way and this may create stress concentrations responsible for the failure of the adhesive. Our results are different from Chunk et al [65], who evaluated a correlation between the degree of conversion, the filler concentration and the mechanical properties of composite resins. No correlation was found between the degree of conversion and any of the mechanical properties of the composite resin tested. They concluded that the filler concentration plays a prominent role in determining the properties of composite resins. Some of commercial pit and fissure sealants are filled and the unfilled experimental polymer that we used for the tensile tests does not reflect the exact mechanical characteristics of all the other sealants. Other studies have been conducted on composite

resin and relate variations in the degree of conversion according to depth in the sealant. Rueggeberg et al [66] indicated that the most significant factor influencing resin composite polymerization is the thickness of the resin. Both the duration of exposure and the light intensity demonstrated high and equal importance on conversion. They also found that the filler content had minimal influence. Different results were found by Rueggeberg et al [67] in an other study, they evaluated different factors affecting the cure at depth within light-activated composites. They determined that the most influential factors were changing as a function of depth. On the surface, they found that the order of influential factors were filler type and exposure duration, at 1mm the order of influential factors were the exposure duration, the filler type and the source intensity. At depth 2 mm and more only the source intensity and duration factor affected the conversion. Nomoto et al [68], found that the depth of cure for light-cured composite resins could be expressed as a logarithmic function of the total amount of exposure. From these observations, they found the attenuation coefficient and the critical amount of exposure capable of initiating polymerization at each depth in a composite.

5.2.2 Fracture analyses

No sign of crazing has been found on both the samples with or without a hole. The processing variables didn't seem to affect the failure mode of the sealant. This could be explained by the fact that an increase in the degree of crosslinking might have changed the deformation mode [69]. In fact a study of the effect of crosslinking on deformation modes in PS has been reported by Henkee and Kramer [70]. An increased crosslink density creates additional restraints which influences micromechanical deformation mechanisms. When the level of crosslinking increases, the deformation mode changes from craze to craze/shear and to shear deformation only. It has also been found that at very high level of crosslinking density, cracks propagated in a catastrophic manner at low strains, as at very low crosslink densities, crazes acting as crack nucleation sites.

Observation of the tensile test samples (no hole):

From our results we can make two hypotheses: a) The level of crosslinking was too high for each sample to enable any craze formation b) The crazes exist but were not observed on the micrographs.

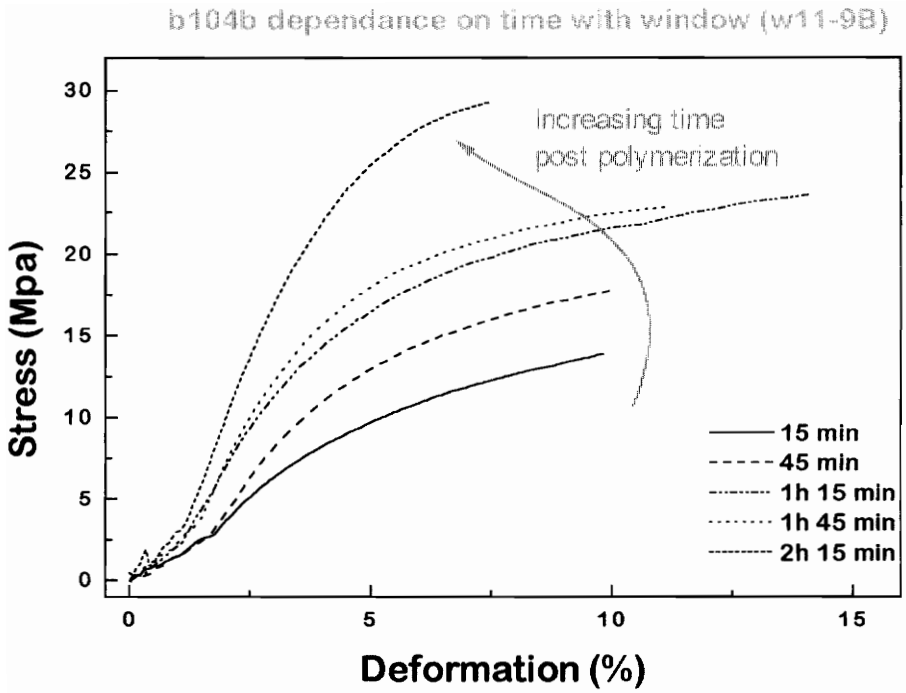


Figure 5.1: Stress - strain for samples polymerized under a window of 1mm thick

According to figure 1-1, the first hypothesis doesn't seem relevant. In fact, since large differences between differently processed sample stress/strain curves have been observed, we presumed that the specimens might experience different modes of failure according to the level of conversion. Another explanation for the lack of difference observed between the differently failed specimens may be due to the defects present in the sample. The samples might not be consistent enough to present reproducible fracture characteristics.

For the notched samples we expected to see crazes in the vicinity of the hole, perpendicular to the stress direction. This was not the case. During the sample preparation, our difficulty to create a hole gave us an indication on the high level of brittleness of the samples. The holes presented some imperfections which may have been the site of early fracture initiation. Because of these irregularities, fracture of the specimens may have occurred earlier in the deformation process before crazing.

5.2.3 Leaching of unpolymerized resin from pit and fissure sealants

The criteria for selection of an ethanol/water solution was based on the potential of this solution to increase the rate of monomer release and to simulate extreme conditions in the oral cavity. As precise simulation of the oral environment is almost impossible, this solution was used to evaluate the maximum potential of monomer leaching.

From our evaluation we obtained really interesting results. A really good reproducibility in the results as well as a very reasonable data scatter was found. Our results matched the results from Rueggeberg & Craig . They found an excellent inverse correlation between the degree of cure and the % of elution. A decrease of monomer release with an increase in blue light exposure time has been observed for the light cure sealant. For the self cure sealant, the lowest elution has been found for the mixing ratio recommended by

the manufacturer, this mixing ratio corresponds to the highest level of conversion according to infrared spectroscopy evaluations.

From the literature review, it appeared, that for dental composite resins, 25 to 50% of the methacrylate groups remained unreacted upon processing. From this amount, it appeared, that less than 10% of the available methacrylate groups are extracted in solution. So an amount of 5-10% of the unbound monomer was found to elute into solution. Slightly higher results were found during our evaluation. Up to 13% in weight of TEGDMA and 10% in weight of Bis-GMA were eluted for the self cure sealant. Lower amounts of TEGDMA around 7%, even for the shortest illumination times, were found for the self cure sealant. But higher amounts of Bis-GMA (11% for the shortest illumination time) were found. These results are slightly higher than for composite resins, these higher levels of elution relate to the presence of fillers in the dental resins.

A study from Oleas et al[8] has pointed out the possible estrogenicity of dental sealants and much research is currently ongoing to estimate the danger of dental sealants (Hileman, 1997[71]). During an *in vivo* study, Oleas et al. [8] found that saliva samples collected from patients who have been treated with dental sealants were estrogenic. Bisphenol-A was identified in the saliva. But Bisphenol-A is not an active ingredient in dental sealants. In fact, many commercial sealants have been analyzed by HPLC and no trace of Bisphenol A has been found (Hileman, 1997[71]). This should not be appear as a contradiction, since the enzymatic activity of saliva combined with mechanical damage leading to wear and erosion may have contributed to degradation of Bis-GMA and the release of Bisphenol-A. A study from Freund et al. (1990 [72]) has pointed out a significant decrease in the hardness of the composite restoration upon exposure to saliva. Bisphenol-A, which does not appear in the analysis of solution during an *in vitro* study, may in fact be released in the human saliva. More *in vivo* studies are needed to evaluate the level of Bisphenol-A release and its potential danger. The impact of the monomer elution detected is unclear so far, and quantitative determination of the threshold value

associated with possible side effects is also needed. Additionally, the properties of the polymer will be affected by the elution process. The weight loss observed should certainly affect the polymer properties and sealant durability. From a clinical perspective, we should advise a very meticulous cleaning and rinsing of the pit and fissure sealant surface upon polymerization. In fact, a layer of liquid is always present on the polymer surface upon curing, the exact composition of this layer should be evaluated, it is very likely that this liquid is mainly composed of monomers. The removal of this layer would further reduce the amount of monomer elution in saliva.

5.3 In vivo experiment

5.3.1 X Ray evaluation of the sealant microleakage

No real information was found on the leakage of pit and fissure sealants using this technique. This method of observation seemed to be most clinically relevant since nearly similar X ray radiographs are used by dentists.

5.3.2 Visual evaluation of the sealant retention

Generally, the level of retention of the sealant appeared to be much lower than in any clinical evaluation. Also the tooth shape appeared to directly control the level of sealant retention. Most first molars (Figure 5.2) didn't present any sign of residual sealant whereas larger amounts of sealant were found on the second molars. The first molars were nearly two times bigger than the second molars. The low level of sealant retention may be due to important stresses applied on these particular teeth. The second molars may have been less exposed to chewing forces and wear. The second molar morphology was the more alike human tooth morphology. Also, it was interesting to notice that most of the time, when sealant was observed on the teeth, large pieces were found. This last

observation would lead us to think that the loss of sealant was more related to a loss of adhesion or a fracture of the sealant than to a wear mechanism.

The level of retention of the sealant didn't appear to be directly linked to the degree of conversion of the sealant. In fact, for the self cure system, the lowest level of retention is found for an exposure time of 20 s, which is not the lowest or the highest level of conversion evaluated. Other factors seem to more directly control the level of sealant retention. In fact, we have to keep in mind that we are working with a living system with a very high number of variables. As mentioned by Kenneth et al [73], the prediction of clinical performance based on material characteristics is a really difficult challenge. Clinical failures can fall into one or more of the following categories a) material deficiencies, b) patient factors, and c) clinician factors. We wanted to estimate the material deficiencies according to the processing variables, but a poor oral hygiene prior to sealant application, caries risk susceptibility, excessive bite force of the animal model, might have more controlled the level of sealant retention. In fact the use of the pig for evaluating the sealing properties of pit and fissure sealants presented a number of problems. Without a doubt, the conditions to which the sealants are subjected are more rigorous in the pig than in the man. Also, the sealant might have been more difficult to apply in the animal than in the man. We could not accurately check for occlusal interference on the newly applied sealant and in some instances, excessive amounts of sealant may have been applied to the animal teeth, increasing the possibility that the resin would be subjected to masticatory stresses. Lower sealant retention rates were found compared to those found in human studies. But this corresponds to other retention work found in another *in vivo* evaluation realized on monkeys [47]. The conditions were more rigorous than in a man and the teeth were too small. In this last concern, the pig was a better model.

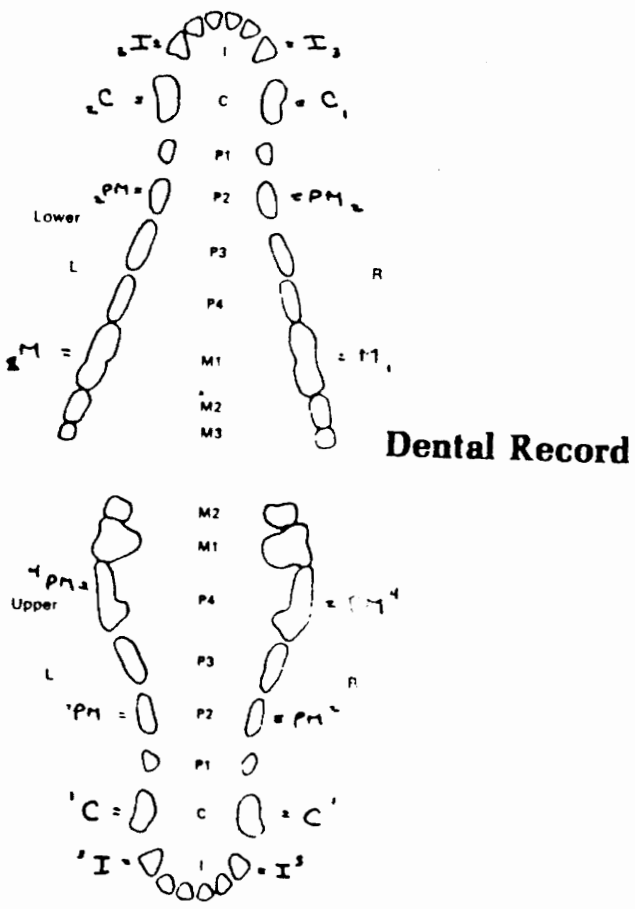


Figure 5.2: Nomenclature system and relative size of the teeth of a porcine jaw

5.3.3 Optical Microscopy

Interesting observations on the diversity of the size and shape of pit and fissures have been made. Because of this variety in the samples, no quantitative results could be found. However, it was quite instructive to observe that so many different types of fissures could be found.

Not many samples presenting obvious signs of sealant fracture were found. It appeared that if failure, it must have affected the entire sealant. That would lead us to think that an interfacial mode of failure may be dominant. This would be the sign of a relatively weak bond strength between the adhesive and the substrate. Also a thick and dark layer was found on many pictures on the enamel/sealant interface. No data on the composition of this layer has been found so far, but it is very likely that it is a weak layer lowering the bond strength between the enamel and the sealant. Further evaluations of the nature of this layer are needed.

The cleaning procedure also needs to be improved. In fact large amounts of nutrients were found in the fissure, some pieces were clearly obstructing the fissures. A better cleaning of the fissures could improve the level of sealant retention. A vibration etching technique was developed by Tadakoro et al [74]. The fissures are simultaneously cleansed and acid-etched using a fissure needle mounted on an electromagnetic vibrator. This technique might improve the level of sealant retention.

Besides, the quality of the teeth, as indicated by small fissures in the enamel, may have been affected during the extraction, storage or more likely during the sectioning and polishing operations.

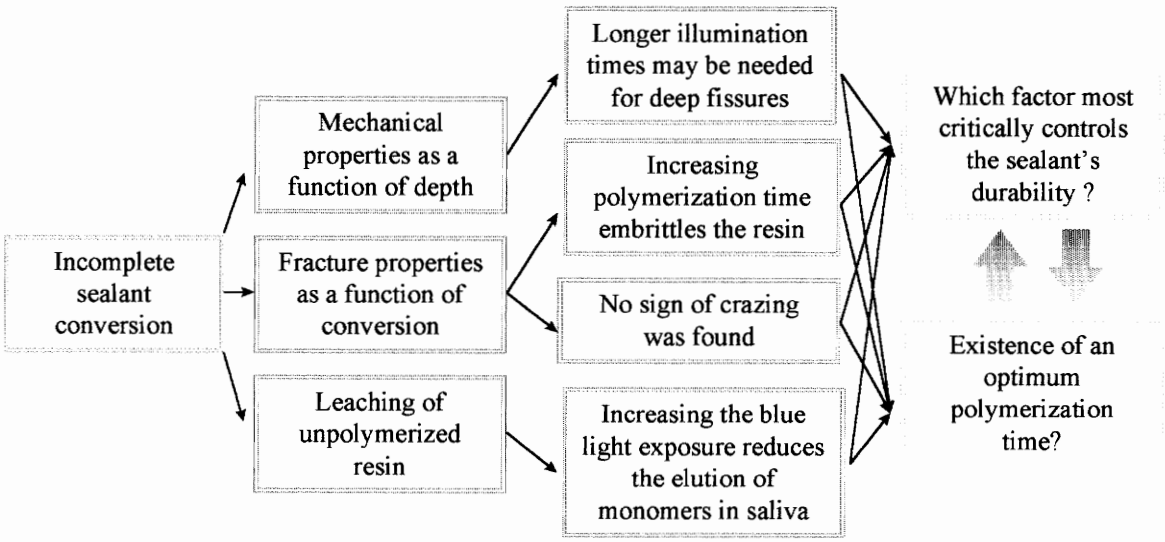
5.3.4 Environmental Scanning Electronic Microscopy

Not enough work has been found done to enable us to conclude on a preferential mode of failure of the sealant. As mentioned in the optical microscope observations, fissures were found in the enamel and at the interface enamel/dentine. Also the teeth might have been dehydrated in the ESEM, increasing the potential for fissure formation.

6. Conclusions

The conclusions from this study can presented according to the following diagrams, the first concerning sealant conversion and the second, the usefulness of the pig *in vivo* model.

Sealant conversion:



A From the DSC and Infrared spectroscopy it appeared that the level of conversion of the resin was incomplete upon processing. No processing variable led to a complete conversion of the resin. Aging and thermal processing increased the degree of cure

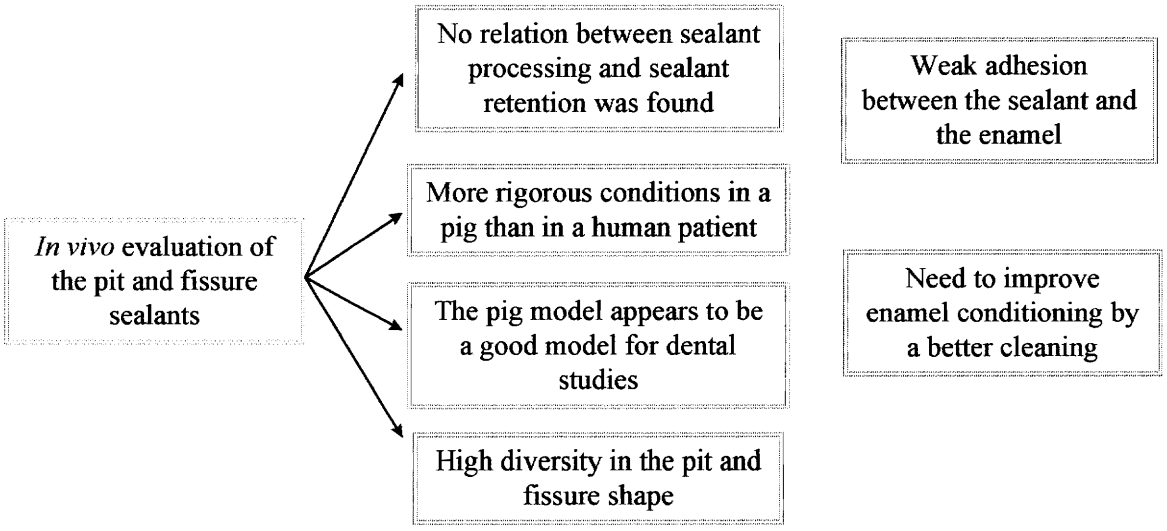
B The influence of this incomplete resin conversion on the mechanical properties, fracture properties, and level of monomer release in saliva was evaluated.

C It has been shown that the level of resin conversion varied as a function of depth in the fissure, creating variations in the mechanical response of the polymer. A more uniform sealant conversion along the pits and fissures should improve the sealant retention. An attempt was made to estimate the micromechanical deformations in the polymer as a function of processing. Most of the samples failed in a brittle fashion and a few presented signs of plastic deformation. No

sign of crazing was found. A lower level of conversion will increase the ductility of the polymer, and may reduce the level of catastrophic failure. Pit and fissure sealants were found to be leachable when interacting with saliva. An inverse correlation between the degree of cure and the percentage of elution was found. Higher levels of conversion will be safer for the patient.

D An optimum degree of conversion needs to be found to lower the level of sealant elution and homogenize the sealant within a fissure. Also this level should not be too high since the sealant is already brittle and might fail catastrophically upon chewing. The addition of aldehyde to the resin has been reported to significantly increase the degree of conversion of the resin [75].

***In vivo* model**



A The *in vivo* evaluation was performed to obtain data on the failure patterns and level of sealant retention of differently processed resin.

B The level of retention appeared to be lower than during clinical trials. This can be explained by the more rigorous conditions encountered by the sealant in a pig's mouth. This also explains why no correlation has been found between the level of sealant retention and its processing conditions. Other factors, like the tooth shape and function have been more determinant for the sealant retention. Also it was very interesting to observe the large diversity of pit and fissures. That is why a living model, because of its natural diversity is really difficult to reproduce *in vitro*.

C The bond between the enamel and the sealant appeared to be relatively weak. Since nutrients and a thick interface between the sealant and the enamel has been found on several samples, further improving the tooth hygiene prior to sealant application should increase the level of sealant retention.

7. Future work

7.1 Estimation of the rate of conversion as a function of depth

A better model of the mechanical behavior of the sealant as a function of depth in the fissure should be realized. More different depths should be evaluated.

7.2 SEM observations of the teeth from the in vivo experiment

More observation of failed samples might be useful to obtain further information of the sealant predominant failure mode.

7.3 Adhesion testing

7.3.1 Contact angle measurement

These measurements will be conducted primarily with the self cure sealants. In fact, the mixing ratio of the two components does not have a noticeable effect on the degree of conversion. However, since the setting time of the sealant changes with the mixing ratio, the viscosity is also affected.

7.3.2 Bond strength testing

Some research reviews[44] have pointed out the difficulty in relating the in vitro performance of a dental material to its clinical performance. However, in vitro testing of the bond strength of the sealant to the enamel will help to identify the failure mechanism of the system and relate it to in vivo experiments.

Shear bond testing, inspired by previous studies [41] will be done on bovine enamel. The enamel surface of two teeth will be flattened and acid etched for 20s. The quality of the enamel will then be analyzed by SEM to ensure that the quality of the enamel has not been changed by grinding. One of the teeth will be placed in a jig, the sealant will then be applied and the second tooth will be placed on top. The jig will be placed in a vertical position on the Instron machine. The crosshead will then apply a shear stress on the second tooth. Because of the variation between enamel texture, problems of reproducibility may occur and the testing procedure may be subjected to changes.

8. VITA

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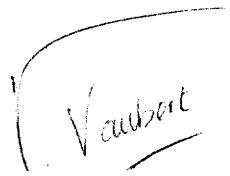

9. References

[1] Buonocore, M.G., "Caries prevention in pits and fissures sealed with an adhesive resin polymerized by ultra-violet light: a two year study of a single adhesive application." *Journal of the American Dental Association*, 82, 1090-1093, 1971

[2] Williams B., Price R & Winter G. B., "In vitro studies of physical factors affecting adhesion of fissure sealant to enamel." *Proceeding of the International Symposium on the Acid Etch Technique*, p. 50 North Central Publishing Co., U.S.A.

[3] Going, R.E, Loesche, W.J., Grainger, D.A. & Syed, S.; "The viability of micro-organisms in carious lesions five years after covering with a fissure sealant." *Journal of the American Dental Association*, 97, 455-62, 1978.

[4] Stephen, K.W., Kirkwood, M., Young K.C., Gillepsie, F.C., MacFadyen, E.E. & Campbell, D.; "Fissure sealing of first permanent molars. An improved technique applied by a dental auxiliary." *British Dental Journal*, 144, 7-10, 1978

[5] Stephen, K.W., Kirkwood, M, Main, C., Gillepsie, F.C & Campbell, D.; "A clinical comparison of two filled fissure sealants after one year." *British Dental Journal*, 150, 282-84, (1981 a)

[6] Stephen, K.W., Kirkwood, M., Campbell, D., Young K.C & Boyle, P.; "Fissure sealing with Nuva-seal and Alphaseal: two year data." *Journal of Dentistry*, 9, 53 (1981 b)

[7] Thomson, J.L., Main, C., Gillepsie, F.C. & Stephen, K.W.; "Effects of reduced etch time on adhesion, and tensile strength studies on fissure sealants using new methods." Presented at 27th meeting of British Division I.A.D.R., Shefield. Abstract No. 62. *Journal of Dental Research*, 59, Special Issue D part 1.

[8] Oleas, N.; Pulgar, R.; Pérez, P.; Olea-Serrano, F.; Rivas, A.; Novillo-Fertrell, A.; Pedraza, V.; Soto, A.; Sonnenschein, C. (1996) : Estrogenicity of resin-based composites and sealants used in dentistry, *Environ Health Perspect*, 104: 298:305.

[9] Craig, R.G. "Restorative dental materials", 6th edition, edited by R. G. Craig, Publisher St. Louis : Mosby, 1980

[10] Ripa, L.W. "Sealants Revisted: An update of the effectiveness of pit-and-fissure Sealants", *Caries Research* 27 (suppl 1):77-82; 1993

[11] Ruyter, I.E. & Oysaed, H., "Composites for use in posterior teeth: composition and conversion.", *Journal of Biomedical Materials Research*, 21, 11-23, 1987

[12] Houpt, M., Fucks,A., Shapira, J., Chosack, A., Eidelman, E, "Autopolymerized versus light-polymerized fissure sealant", *Journal of the American Dental Association*, 115, 55-56; 1987

[13] Strang, L., Cummings, A., Stephen, K.W., "Laboratory evaluation of new fissure sealants : Microhardness and setting times", *Caries Research* 18:179-180; 1984

[14] Chosak, A., Eidelman, E. "Effect of time from application until exposure to light on the tag lengths of a visible light-polymerized sealant", *Dental Materials*, 4: 302-306; 1988

[15] Kloosterboer, J.G., "Network formation by Chain Crosslinking Photopolymerization and its Applications in Electronics", *Advances in Polymer Science*, Springer-Verlag Berlin Heidelberg New-York London Paris Tokyo, 84, 4-56, 1988.

[16] De Lange, C., Baush, J.L & Davidson, C.L., "The curing pattern of photo-initiated dental composites." *Journal of Oral Rehabilitation*, 66, 727, 1980

[17] Pilo,R., Cardash, H.S, "Post-irradiation polymerization of different anterior and posterior light-activated composites. *Dental Materials*, 8, 299-304, 1992

[18] Lundin, S.A. & Koch, G., " Cure profiles of visible-light-cured class II composite restorations in vivo and in vitro." *Dental materials*, 8, 7-9, 1992

[19] Young, R.J. and P.A Lovell, "Introduction to polymers, second edition", Chapman & Hall, 367-371, 1991

[20] Michler, G.H., "Correlation between craze formation and mechanical behaviour of amorphous polymers." *Journal of Materials Science*, 25, 2321-34, 1990

[21] Sauer, J.A., Hara, M., "Effect of molecular variables on crazing and fatigue of polymers", *Advances in Polymer Science, Crazing in Polymers, vol.2*, 91/92, 68-115, 1990

[22] Ferracane J.L, "Elution of leachable components from composites". *Journal of oral Rehabilitation*, 21, 441-452, 1994

[23] Thompson, L.R., Miller, E.G., Bowels, WH. "Leaching of unpolymerized materials from orthodontic bonding resins.", *Journal of Dental Research*; 61, 989-92, 1982.

[24] Braden, M., Causton, B.E & Clarke R.L, "Diffusion in water of composite filling materials." *Journal of Dental Research*, 55, 730-32, 1976

[25] Braden, M. and Pearson, G.J., "Analysis of aqueous extract from filled resins." *Journal of Dentistry*, 9, 141-43, 1981

[26] Inoue, K., Hayashi, I., "Residual monomer (Bis-GMA) of composite resins." *Journal of Oral Rehabilitation*, 9, 493-97, 1982

[27] Tanaka, K., Taira, M., Shintani, H., Wakasa, K. & Yamaki, M. "Residual monomers (TEGDMA and Bis-GMA) of a set visible-light-cured dental composite resin when immersed in water." *Journal of Oral Rehabilitation*, 18, 353-62, 1991

[28] Rueggeberg, F.A & Craig, R.G. "Correlation of parameters used to estimate monomer conversion in a light-cured composite." *Journal of Dental Research*, 67, 932-37, 1989

[29] Wu, W. & McKinney, J.E. "Influences of chemical on wear of dental composites. *Journal of Dental Research*, 61, 1180-83, 1982

[30] Ferracane, J.L. & Condon, J.R. "Post-cure heat treatments for composites: properties and fractography." *Dental Materials*, 8, 290, 1992

[31] Pearson, G.J & Longman, C.M., "Water sorption and solubility of resin-based materials following inadequate polymerization by a visible-light curing system." *Journal of Oral Rehabilitation*, 16, 57-61, 1989

[32] Gwinett, A.J., "Structure and composition of enamel", *Operative Dentistry*, 5, 10-17, 1992

[33] Bussher, H.J., de Jong H.P., Van Pelt A.W.J. and Arends J., "The surface free energy of human dental enamel", *Biomat., Med. Dev., Art. Org.*, 12 (1&2), 37-49, 1984

[34] Biocompatibility of Dental Materials, Volume II, 16-46

[35] Burrow M.F., Taniguchi Y., Nikaido T., Satoh M., Inai N., Tagami J. and Takatsu T., "Influence of temperature and relative humidity on *early bond strength to dentine"* Journal *of Dentistry,* 23, 41-45, 1995

[36] Soetopo, Beech, D.R., Hardwick J.L., "Mechanism of adhesion of polymers to acid-etched enamel, effect of acid concentration and washing on bond strength.", *Journal of Oral Rehabilitation*, 5, 69-80, 1978

[37] Buonocore, M.G., "A simple method of increasing of acrylic filling materials to enamel surfaces." *Journal of Dental Research*, 50, 1517

[38] Engineered Materials Handbook, Volume 3 : Adhesives and Sealants, ASM International

[39] Nakamichi, L., Iwaru, W. and Fusayama, T., *Journal of Dental Research.* 62, 1076, 1983

[40] Pashley, D.H., Sano, H., Ciuchi, B., Yoshiyama, M., Carvalho, R.M., "Adhesion testing of dentin bonding agents: A review.", *Dental Materials*, 11, 117-125, 1995

[41] Titley K.C., Torneck C.D., Smith D.C. and Adibfar A., "Adhesion of composite resin to bleached and unbleached bovine enamel", *Journal of Dental Research* , 67, 12, 1523-1528, December 1988

[42] Tokuji H., Retief D.H., Russell C.M and Dennis F.R., "Shear bond strength and quantitative microleakage of a multipurpose dental adhesive system resin bonded to dentine.", *Journal of Prosthetic Dentistry.* 73, 432-438, 1995

[43] Ruse N.D., Shew R. and Feduik D., "*In vitro* fatigue testing of a dental bonding system on enamel", *Journal of biomedical Materials Research,* 29, 411-415, 1995

[44] Powers, J. M. " Conference Reflections : Lifetime prediction of dental materials- an engineering approach", *Journal of Oral Rehabilitation,* 22, 491-492; 1995

[45] Prati C., Pashley D.H. and Montanari G., "Hydrostatic intrapulpal pressure and bond strength of bonding systems.", *Dental Materials* 7, 54-58, 1991

[46] Newburn, E., Plasschaert, A.J.M, and König, K.G., "Progress of caries in fissures of rat molars treated with occlusal sealants", *Journal of the American Dental Association*, 89, 121-126, 1974

[47] Wilkins, J.S., Swartz, M.L., Philips, R.W., "Testing of pit and fissure sealants in the monkey", *Journal of. Prosthetic Dent*istry, 37, 666-673, 1977

[48] Cook, W.D, "Photopolymerization kinetics of dimethacrylates using the camphorquinone/amine initiator system.", *Polymer* 33, 600-609; 1992

[49] Shortall, A.C, Wilson, H.J. and Harrington, E., "Depth of cure of radiation activated composite restoratives", *Journal of Oral Rehabilitation* 22: 337-342; 1995

[50] McAbe, P.F., Wilson, H.J., "The use of differential scanning calorimetry for the evaluation of dental materials", *Journal of Oral Rehabilitation*, 7, 103-110, 1980

[51] Ban, S., Takahashi, Y., Tanase, H. and Hasegawa, J., "Heat Curing Behavior of Light-cured Composite Resins investigated by dynamic differential scanning calorimetry.", *Dental Materials Journal*, 9, 153-162; 1990

[52] Fujisawa, S., Imai, Y., Kajima, K., Masuhara, E. "Studies of the haemolitic activity of Bisphenol Adiglicidl methacrylate (Bis-GMA)" *Journal of Dental Research*; 57, 98-102, 1978

[53] Fujisama, S., Mashuhara, E. "Binding of methyl methacrylate to bovine serum albumin." *Journal of Dental Research*, 59, 2056-61, 1980

[54] Harsanyi, B.B., Angelopoulos, A.P., Gourley, J.M. "Subcutaneous tissue response to composite resin in dogs." Oral Surg. *Oral Med Oral Pathol*, 37, 308-19, 1974

[55] Hume, W. R, Hood, AM. "Cytotoxicity comparison between composite resins containing di-, tri-, or tetra-ethyleneglycol dimethacrylate." , *Journal of Dental Research*, (Special Issue), 70, 423, 1991

[56] Imai, Y., Watanabe, A., Chang, P.I., Mashuhara, E. "Evaluation of the biologic effects of dental materials using a new cell culture technique." *Journal of Dental Research*; 61, 1024-7, 1982.

[57] Ward, I.M, Hadley D.W., "An Introduction to the Mechanical properties of solid polymers", *John Wiley & Sons, Chichester* - New York - Brisbane - Toronto - Singapore, 1993

[58] Instructions from the manufacturer.(3M, Inc. and Dentsply, Inc.)

[59] Craig, R.G., O'Brien, W.J., Powers, J.M., "Dental materials, properties and manipulation", second edition, (C.V. Mosby Co., St. Louis), 34, 1979

[60] Cowie, J.M.G, "Polymers: Chemistry and Physics of Modern Materials", second edition, Published by Blackie Academic & Professional, London. Glasgow. New York. Tokyo. Melbourne. Madras, 1991

[61] Cook, W.D. and Johannson, M. "The influence of postcuring on the fracture properties of photo-cured dimethacrylate based dental composite resin.", *Journal of Biomedical Materials Research*, 21, 979-989; 1987

[62] Cook, W.D. and Moopnar, M. "Influence of chemical structure on the fracture behaviour of dimethacrylate composite resins", *Biomaterials*, 11, 272-276; 1990

[63] Hertzberg, R.W., "Deformation and fracture mechanics of engineering materials", Second edition, John Wiley & Sons, New York - Chichester - Brisbane - Toronto - Singapore, 259-262, 1989

[64] Chunk K, Greener E.H., "Degree of conversion of seven visible-light cured posterior composites.", *Journal of Oral Rehabilitation (England)*, 15, 6, 550-560, 1988

[65] Chung, K.H., Greener, E.H., "Correlation between degree of conversion, filler concentration and mechanical properties of posterior composite resins.", *Journal of Oral Rehabilitation (England)*, 17, 487-94, 1990

[66] Rueggeberg, F.A., Caughman, W.F., Curtis, J.W.Jr, Davis, H.C., "A predictive model for the polymerization of photo-activated resin composites.", *International Journal of Prosthodontics (United States)*, 7, 159-66, 1994

[67] Rueggeberg, F.A., Caughman, W.F., Curtis J.W.Jr, Davis, H.C., "Factors affecting cure at depths within light-activated resin composites.", *American Journal of Dentistry (United States)*, 6, 91-5, 1993

[68] Nomoto, R., Uchida, K., Hirasawa, T., "Effect of light intensity on polymerization of light-cured composite resins.", *Dental Materials Journal (Japan)*, 13, 198-205, 1994

[69] Sauer, J.A., Hara, M., "Effect of molecular variables on crazing and fatigue of polymers", *Advances in Polymer Science, Crazing in Polymers*, 2, 71-115, 1990

[70] Henkee C.S., Kramer E.J., *Polymer*, 23, 1183, 1984

[71] Hileman, B. (1997) : Bisphenol A : "Regulatory, Scientific Puzzle. Concerns about health effects of estrogen mimic trigger a flurry of intense research", *Chem Eng News*, March 24, 37:39.

[72] Freund, M.; and Munksgaard, E. (1990): "Enzymatic degradation of BisGMA/TEGDMA-polymers causing decreased microhardness and greater wear *in vivo*", *Scand J Dent Res*, 98: 351-5.

[73] Anusavice, K.J., Glasspole, E.A., "Introduction to invited papers presented at the 1994 ADM annual meeting, Southampton, Bermuda", *Dental Materials*, 11, 102, 1995

[74] Tadakoro, Y., Iwaku, M., Fusayama, T., "A laboratory report on vibration etching for fissure sealants", *Journal of Dental Research*, 61, 780-785, 1982

[75] Peutzfeldt, A., "Quantity of remaining double bonds of propanal-containing resin", *Journal of Dental Research*, 70, 1657-62, 1994