

CHAPTER II

LITERATURE REVIEW

Clinically, marginal deterioration of composite resin restorations remains problematic and has been a major reason that dramatically shortens lifetime of adhesive restorations. In order to predict the clinical performance of these adhesive systems, laboratory tests (tensile, shear bond strength and microleakage tests) are commonly performed.

Contemporary adhesive systems currently have been classified according to their mode of clinical application and mechanism of adhesion (De Munck *et al.*, 2005b; Van Meerbeek *et al.*, 1998a). Bonding to tooth tissue can be achieved through "etch-and-rinse," "self-etching" or "glass-ionomer" approaches. Bond strength testing and measurement of marginal sealing effectiveness (the two most commonly employed methodologies to determine "bonding effectiveness" in the laboratory) are evaluated upon their value and relevance in predicting clinical performance.

An overview of currently available adhesive systems was provided and a categorization of these adhesives according to their clinical applications and their mechanisms of adhesion was proposed. Basic mechanism of bonding to enamel and dentin is essentially an exchange process involving replacement of minerals, removed by acidic conditioning from the dental hard tissue, by resin monomers, which upon setting become micro-mechanically interlocked in the created porosities (Van Meerbeek *et al.*, 2003). Based upon the underlying adhesion strategy, three mechanisms of adhesion are currently in use with modern adhesive systems (Fig. 1).

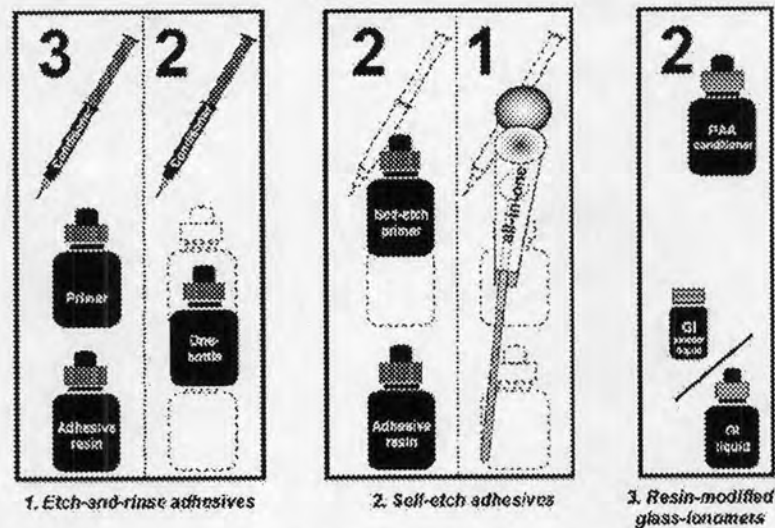


Figure 1 Classification of contemporary adhesives according to adhesion strategy and number of clinical application steps. Gi = glass ionomer; PAA = polyalkenoic acid (Van Meerbeek *et al.*, 2001, 2003).

Etch-and-Rinse Adhesives

This approach completely removes smear layer using acidic conditioners that are simultaneously applied to enamel and dentine, termed a total-etch or etch-and-rinse technique. It is hypothesized that conditioning with acidic or chelating agents demineralizes dentin surface to a certain depth, leaving behind a collagen-rich meshwork. Hydrophilic monomers alter the collagen-fiber arrangement in a way that facilitates penetration of adhesive resin, resulting in a mechanical, intermingled link between collagen and adhesive resin.

Their mechanism is principally based on the combined effect of hybridization and formation of resin tags (Nakabayashi *et al.*, 1982). Etch-and-rinse adhesives involve a separate etch-and-rinse phase. In their most common configuration, an acid (mostly 30-40% phosphoric acid) is applied on enamel and dentin simultaneously for 15-30 seconds and rinsed off. This conditioning step is followed by a priming step and application of the adhesive resin, resulting in a three-step application procedure. Because of this relatively complicated, technique sensitive and time-consuming three-step clinical approach, most recent innovations in adhesive technology are directed toward a simplified application technique by combining the primer and the adhesive resin into one component, resulting in two-step etch-and-rinse system.

Self-Etching Adhesives

An alternative approach is based on the use of non-rinse acidic monomers that simultaneously condition and prime tooth structure, the so called 'self-etching' adhesives. The most widely used self-etching adhesive systems involve two application steps: the conditioning of dentin and enamel with a self-etching primer, followed by the application of an adhesive resin. Nowadays, one-component self-etching adhesives are increasingly introduced to the market. Using a simplified application procedure, smear layer dissolving adhesives provide slightly acidic primers, or so-called self-etching primers, that partially demineralize smear layer and underlying dentine surface without removing the dissolved smear layer remnants and unplugging tubule orifices. Apart from simplification, the rationale behind this system is to superficially demineralize dentin and simultaneously penetrate it with monomers, which can be polymerized *in situ*. The application of recent adhesive systems induces structural changes in dentin surface morphology creating a retentive interface, called the resin-dentin interdiffusion zone, between dentin layer and composite filling material. This resin-dentin interdiffusion zone offers bonding sites for copolymerization with the resin composite, and concurrently may have protective potential for the pulp tissues (Van Meerbeek *et al.*, 1992).

Categorized according to the acidity, there are basically two types of 'self-etch' adhesives: 'mild' and 'strong' (Van Meerbeek *et al.*, 2001). 'Strong' self-etch adhesives have a very low pH (< 1) and exhibit a bonding mechanism and interfacial ultra-morphology in dentin resembling to that produced by etch-and-rinse adhesives. 'Mild' self-etch adhesives (pH of around 2) dissolve the dentin surface only partially, so that a substantial number of hydroxyapatite crystals remain within the hybrid layer. Specific carboxyl or phosphate groups of functional monomers interact with hydroxyapatite creates the hypothetical chemical bond with this residual crystal (Yoshida *et al.*, 2004). The two-fold bonding mechanism (*i.e.*, micro-mechanical and chemical bonding) has been claimed to be advantageous in terms of restoration durability. It has a micro-mechanical bonding component that may in particular provide resistance to abrupt debonding stress. The chemical interaction may result in bonds that better resist hydrolytic break-down, thus, keep the restoration margins sealed for a longer period (Sano *et al.*, 1999).

Glass Ionomers and Glass-ionomer Adhesives

Glass-ionomers are still considered the only materials that truly self-adhere to tooth tissue (Yoshida *et al.*, 2000). A short polyalkenoic acid pre-treatment cleans the tooth surface, removes the smear layer and exposes surface collagen fibrils to a depth of 0.5 μm ; glass-ionomer components interdiffuse and establish a micromechanical bond following the principle of hybridization in addition to chemical bond established by interaction between the carboxyl groups and mineral ions. The additional chemical adhesion with dentinal substrate may be beneficial in terms of resistance to hydrolytic degradation. However, basic difference between resin based self-etch approach and glass-ionomer systems is that glass ionomers self-etch through the use of a relatively high molecular weight (from 8,000 to 15,000) polycarboxyl-base polymer, which limits their infiltration capacity, therefore, only shallow hybrid layers are formed.

Advantage of self-etching approach

With the relatively aggressive etch-and-rinse technique, dentin may also be demineralized to a depth that might be inaccessible to complete resin impregnation. Voids of impregnated collagen fibrils have been demonstrated at the base of the hybrid layer. More signs of incomplete resin penetration were observed as a microporous dentin zone present at the base of the hybrid layer in the absence of gap formation. This special type of microleakage has been termed nanoleakage (Sano *et al.*, 1995). The residual water had effect on dentin bond strength and hybrid formation of two-step etch-and-rinse adhesive, because of excess of water on dentin surface, separation between bonding resin and resin-reinforced dentin occurred. In contrast to desiccated dentin, dehydration causes collapse of the exposed collagen scaffold that probably impedes penetration of bonding resins (Gwinnett, 1994). Thus, from the clinical standpoint, wet bonding is believed to be very technique sensitive method (Nakaoki *et al.*, 2002). Because of this relatively complicated, technique sensitive and time-consuming three-step clinical approach, most recent innovations in adhesive technology are directed toward a simplified application technique by combining the primer and the adhesive resin, resulting in simplified adhesive systems.

Self-etch adhesives have been introduced to solve difficulties commonly associated with the clinical application of etch-and-rinse adhesives. The concept of self-etching adhesives is based on the use of polymerizable acidic monomers that simultaneously condition and prime dentin and enamel. Self-etching primers eliminate the technique-sensitive rinsing step to remove phosphoric acid from enamel and dentin, therefore, exhibiting a low technique sensitivity with regard to the conditions of dentin surface (dry, wet, moist), resulting in a very low level of post-operative sensitivity. It is obvious that low risk of postoperative sensitivity has to do with the fact that dentin decalcification and penetration of dentin by the acidic and co-monomers occur up to the same depth (Sano *et al.*, 1995).

Regarding user-friendliness and technique sensitivity, the self-etching approach seems clinically most promising because it eliminates the rinsing phase, which not only lessens the clinical application time, but also significantly reduces the technique-sensitivity or the risk of making errors during application. The most widely used self-etching adhesive systems involve two application step, conditioning of dentin and enamel with a self-etching primer, followed by application of an adhesive resin. Nowadays, one-component self-etching adhesives are increasingly introduced to the market. Combination of etchant, primer and adhesive resin into an all-in-one adhesive has been proposed to be advantageous in that it may reduce the application time as well as errors that may occur during each bonding step.

Substrate difference: enamel vs. dentin

The primary component of mature enamel is 95-98% hydroxyapatite. The remainder consists of 4 wt% water and 1 wt% organic material. The major inorganic fraction exists in the form of submicron crystallites, preferentially oriented in three dimensions, in which the spread and contiguous relationship of the crystallites contribute to the microscopic unit, called the rod or prism. Enamel is almost homogenous in structure and composition, irrespective of its depth and location, except for some aprismatic (prismless) enamel at the outer surface, in which the crystallites run parallel to each other and perpendicular to the surface (Nanci, 1985). The existence of a surface aprismatic layer in both deciduous and permanent teeth has been well

documented in the literature. Such layer has been reported to be less conductive in bonding via acid conditioning comparison to prismatic enamel (Van Meerbeek *et al.*, 2001).

Dentin is a vital hydrated composite material with structural components and properties that vary with locations. Furthermore, dentin is modified by physiological aging and disease processes to create different qualities of dentin (Fig.2). Tubule number is lowest at the DEJ and highest at the predentin surface at the junction to the pulp chamber. The tubule lumen is lined by a highly mineralized cuff of peritubular dentin, containing mostly apatite crystals with little organic matrix. The tubules are separated by intertubular dentin composed of a matrix of type I collagen reinforced by apatite. The amount of intertubular dentin varies with locations. The apatite crystals are much smaller than the apatite found in enamel and contain 4-5% carbonate as compared to hydroxyapatite.

DeminerIALIZED dentin must be sufficiently hydrated to maintain the integrity of collagen network, which provides porosities for mechanical penetration of resin, but it should not be overwet, because there may be dilution or deterioration of the monomer, reduction in final degree of cure, and formation of water-containing defects within the adhesive layer (Tay *et al.*, 1997). Dentin's intrinsic wetness increases with depth, and moisture is frequently a barrier to good bonding (Grayson *et al.*, 1997). Generally bond strengths are higher in superficial than deep dentin (Nakamichi *et al.*, 1983). This probably reflects the difference in amount of solid dentin available for bonding, as well as differences in moisture content.

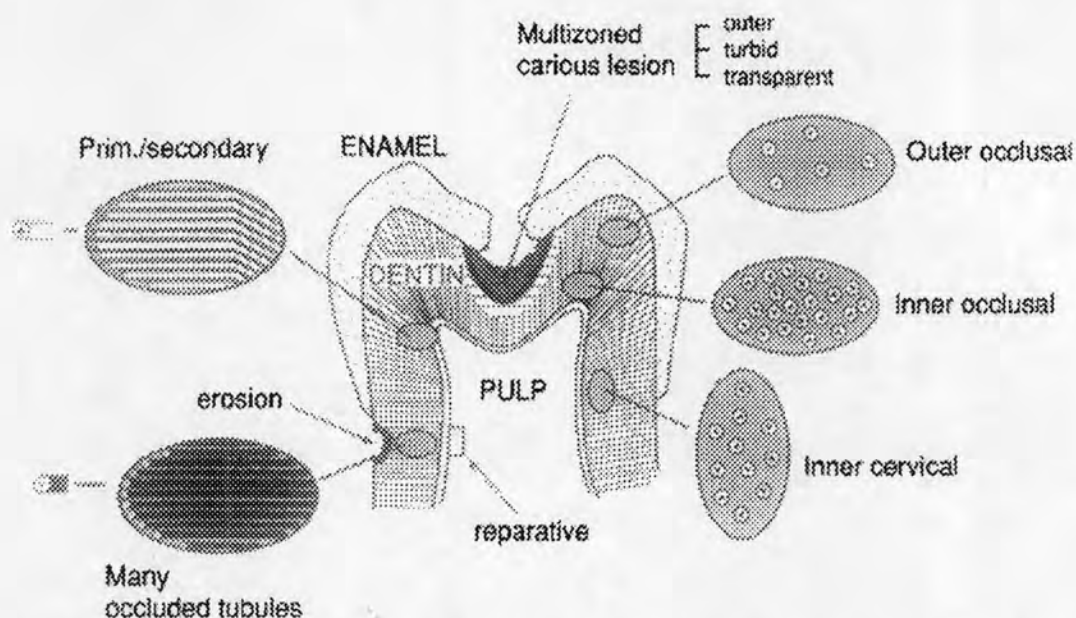


Figure 2 Schematic diagram of dentin structural variations with intratooth location, formation of secondary dentin and several altered forms of dentin due to occlusal caries and cervical sclerosis (Grayson *et al.*, 1997).

Performance of adhesives through different tooth substrate

Self-etching adhesive could produce different etching pattern on unground enamel depending on their respective aggressiveness. Etching pattern on intact enamel depends on acidic dissociation constants (pKa values) of their acidic monomers in the adhesive (Pashley and Tay, 2001). The etch-and-rinse adhesives provided statistically higher microtensile bond strength than any of the other adhesives regardless of the enamel preparation (Goracci *et al.*, 2004). Commercial self-etching adhesives performed better on prepared enamel than on unprepared enamel (Perdigao and Geraldini, 2003). Enamel bond strength of mild self-etching adhesive increased when enamel was roughened, comparable to strong self-etching adhesive.

The etch-and-rinse adhesive presented the thickest hybrid layer and was the only adhesive to produce resin tag in high density and uniform distribution along the whole dentin surface, independent of the smear layer thickness. The thick smear layer was not totally removed by the mild self-etching primer. Resin tags varied in density and shape among the self-etching adhesives. Thicker hybrid layers were observed for the strong self-etching and etch-and-rinse adhesives (Kenshima *et al.*, 2006).

Significance of enamel bond

Up until recently, it has been advocated that bonding procedures on enamel provide reliable and relatively stable bonds, based mainly on homogeneous characteristics of the substrate (Nakabayashi and Pashley, 1998). However, it is important to consider that over the last four decades, clinical stability of bonds to enamel has been, almost exclusively, related to the use of hydrophobic resin (Di Hipolito *et al.*, 2005). In clinical situation, one can rely on durable dentin bonding using three-step or two-step etch-and-rinse adhesives if all cavity margins are located in enamel. For cavities with margins ending in dentin, three-step etch-and-rinse adhesives are preferred. The resin-dentin bond formed by etch-and-rinse adhesives is prone to water degradation, the two-step etch-and-rinse adhesives are more susceptible to water degradation than three-step etch-and-rinse adhesives; and a surrounding resin enamel bond protects the resin-dentin interface against water degradation (De Munck *et al.*, 2003b). The 6-month stability of bond strength values in specimens with enamel border can be attributed to the protective role of the surrounding resin–enamel bond against degradation (Hashimoto *et al.*, 2002). The enamel borders may work as retarding obstacle to water diffusion and, therefore, water needs to cross a longer diffusion path into the indirect-exposure specimens. The peripheral enamel margin helps prevent deleterious effects because enamel is an intrinsically dry substrate, and an insignificant amount of water is present in the body of enamel itself. This prevents water movement within the monomers when the adhesive is light-cured. It was already demonstrated that residual water or solvents entrapped in between monomer moieties compromises the polymerization of the resin, thus reducing its physical properties and rendering the bond weak and less stable over time (Gamborgi *et al.*, 2007). More predictable long-term performance can be obtained in composite restoration as long as there is an enamel border around the cavity margins.

Bond strength results of self-etching adhesive

When composite polymerization shrinkage is restricted by adhesion to the cavity walls, stresses build up at the bonded interface. Under such condition, mechanical properties of adhesive resin would play an important role to withstand the stresses

(Takahashi *et al.*, 2002). The effect of shrinkage stress can be determined through microleakage and microtensile bond strength testing. The higher the bond strength mediated by an adhesive, the smaller the marginal gaps formed by a composite resin in a dentine cavity treated with adhesives has been shown. However, results of bond strength tests did not always positively correlate with laboratory tests that evaluated the marginal seal of restorations such as microleakage or gap analysis (Cenci *et al.*, 2005).

For self etching adhesives, the ability to remove dentin or enamel smear layer and the formation of an etching pattern on enamel are major requirements for reliable bonding. The etching action of self etching systems is due to monomers present in the solution, which are made acidic by the fixation of one or more acid groups (carboxylic or phosphate). The acidic group is there to etch enamel and dentin, while the methacrylate is available to copolymerize with bonding agent and composite resin (Moszner *et al.*, 2005). Ultramorphological characterization showed that interfacial morphology and the pH of the self-etch primer/adhesive are strongly associated (Tay and Pashley, 2001).

“Strong” self-etching adhesives usually have a pH of 1 or below. At enamel, the resulting acid-etch pattern resembles to that of a phosphoric acid treatment. At dentin, collagens are exposed and nearly all hydroxapatites are dissolved, resulting in relatively deep dentin hybridization several micrometers thick. This suggests that resin-dentin interfaces similar to that of phosphoric acid etching could be produced without an additional rinsing step (Tay and Pashley, 2001), and is comparable to the etching effect of phosphoric acid on intact unground enamel (Perdigao *et al.*, 1997).

“Mild” self-etching adhesives have a pH around 2 or higher, and demineralize superficial dentin only to a depth of 1 μm , keeping residual hydroxyapatite attached to collagen. The preservation of hydroxyapatite within the submicron hybrid layer may serve as a receptor for additional chemical bonding (Nakabayashi and Saimi, 1996; Inoue *et al.*, 2000). The challenge is to have a functional monomer that interacts with hydroxyapatite, so that the resulting calciumcarboxylate or calcium-phosphate bonds are long-term stable within a hydrophilic environment (Yoshida *et al.*, 2004).

Thermocycling did not enhance chemical or mechanical degradation of the bonds produced by a two-step self-etching and a three-step etch-and-rinse adhesive to dentin (De Munck *et al.*, 2005a). The all-in one self-etching adhesive tested was not able

to withstand polymerization shrinkage stress, nor thermocycling, when applied in class I cavities. The lower bonding effectiveness of one-step self-etching adhesives should be attributed in part to phase separation between adhesive ingredients. One-step self-etching adhesives are more commonly associated with lower bonding effectiveness to both enamel/dentin compared to the multi-step version. Since such adhesives theoretically combine the 3 functions of three-step adhesives-etching, priming, and bonding-both hydrophilic and hydrophobic monomers are blended, with a relatively high concentration of solvent required to keep them in solution and being acidic (Pashley *et al.*, 2002). In this 'difficult' mixture, water is essential as an ionization medium to enable self-etching activity to occur. Due to their high hydrophilicity, one-step self-etching adhesives behave as semi-permeable membranes, allowing fluids to pass through and consequently seriously jeopardizing bond durability (Tay *et al.*, 2002).

Clinical studies of the mild two-step self-etch adhesive Clearfil SE showed excellent 3 year and 5 year clinical services (Peumans *et al.*, 2005b; Peumans *et al.*, 2007). The excellent retention rate of Clearfil SE should probably be ascribed to its two fold bonding mechanism. First, micromechanical interlocking created by partial demineralization and infiltration of dentin with monomers leads to a shallow submicron hybrid layer and hybridized smear plugs. Secondly, chemical interaction of the functional monomer, 10-methacryloxydecyl dihydrogen phosphate (10-MDP), present in Clearfil SE, with residual hydroxyapatite (remaining around the exposed collagen fibrils within the hybrid layer) must also play a significant role. This chemical interaction is expected to contribute to the adhesive potential of the self-etch adhesive to both enamel and dentin, as both contain hydroxyapatite (Yoshida *et al.*, 2004).

Etching pattern of enamel using a mild self-etching adhesive appears to be less retentive than that produced by phosphoric acid etching (Di Hipolito *et al.*, 2005; Perdigao and Geraldeli, 2003). In spite of this less-pronounced etch pattern, relatively high initial bond strengths to instrumented enamel were noted *in vitro* (Van Landuyt *et al.*, 2006a). In addition, *in vitro* durability studies mentioned a significant decrease in enamel bond strengths for mild two-step self-etching adhesives after thermo-mechanical fatigue loading, and water storage (Miyazaki *et al.*, 2000; Wang *et al.*, 2004).

Durability of adhesion using contemporary adhesive systems

Both *in vitro* and *in vivo* studies show favorable bonding efficacy for the two-step self-etching adhesives (Akimoto *et al.*, 2007; De Munck *et al.*, 2005c; Peumans *et al.*, 2007). Although high early bond strengths of current adhesive systems to dentin have been reported, the durability of adhesive bond is still one of the areas of current interest in adhesive dentistry (Koshiro *et al.*, 2004). A reduction of bond strength and morphological changes in the resin–dentin bond result from esterization in the resin in hybrid layer. Degradation of resin–dentin interface progresses by diffusion of water through the so-called nanoleakage pathway (Tanaka *et al.*, 1999).

Comparison of retention of class V adhesive restorations, as a measure to determine clinical bonding effectiveness of adhesives, revealed that glass-ionomers most effectively and durably bond to tooth tissues. Three-step etch-and-rinse adhesives and two-step self-etch adhesives showed clinically reliable and predictably good clinical performances. The clinical effectiveness of two-step etch-and-rinse adhesives was less favorable, while an inefficient clinical performance was noted for the one-step self-etch adhesives (Peumans *et al.*, 2005a).

The three-step application procedure with a low technique-sensitive application of, respectively, etchant, primer, and adhesive, the apparent favorable composition with regard to hybridization efficiency (Van Meerbeek *et al.*, 1996), the particle-filled adhesive providing elastic shock-absorbing potential (Van Meerbeek *et al.*, 1993), the formation of a separate coupling resin layer, and the lower hydrophilicity of the cured resin as compared with the two-step version may have resulted in low sensitivity to water degradation. The ethanol based adhesives resulted in higher bond strength than acetone-based adhesive, and bonding to moist dentin resulted in higher bond strength (Cardoso Pde *et al.*, 2005). A comparison of contemporary adhesives revealed that three-step, ethanol-water-based etch-and-rinse adhesives remained the 'gold standard' in terms of durability. Any kind of simplification in the clinical application procedure resulted in loss of bonding effectiveness. Only the two-step self-etching adhesives approached closely the gold standard, and may have some additional clinical benefits (De Munck *et al.*, 2005b).

To keep the exposed collagen scaffold penetrable to resin, it has been recommended that the conditioned dentin surface be maintained in a visibly moist condition, a clinical technique commonly referred to as wet bonding (Tay *et al.*, 1996). The amount of water provided with the hydrophilic primer solution of either of the OptiBond system (Kerr, USA) or Scotchbond Multi-Purpose system (3M ESPE, USA) was adequate to re-hydrate and re-expand the gently air-dried and collapsed collagen network. When the adhesives were dry-bonded, no ultrastructural evidence of collapsed demineralized collagen, incompletely or not at all infiltrated by resin, could be detected (Van Meerbeek *et al.*, 1998b). In addition, when the two adhesives were bonded to wet dentin, no signs of overwetting phenomena existed.

Bond strength of Scotchbond Multi-purpose to dentin was lower when compared to Optibond FL, which could be partly due to the incorporation of a high molecular-weight polyalkenoic acid copolymer into the adhesive system. Previously, phase separation was shown to occur with the copolymer being filtered out by the collagen network and deposited as a distinct gel layer on the exposed collagen network (Van Meerbeek *et al.*, 1996), the gel hindered adequate resin-interdiffusion, by which the hybrid layer would be constituted of collagen infiltrated by the low molecular-weight 2-hydroxyethylmethacrylate (HEMA) that was polymerized to linear poly-HEMA chains, and residual water (solvent) was insufficiently removed. In addition, analysis of the failure planes showed abundant unprotected collagen fibrils.

Modified application to improve bonding effectiveness

Because clinical approach of three-step etch-and-rinse adhesive is relatively complicated, technique sensitive and time-consuming; most recent innovations in adhesive technology are directed toward a simplified application technique resulting in simplified adhesive systems. Bonding effectiveness of self-etching adhesives has been improved; however, bonding to enamel and dentin was less effective than their multi-step versions (De Munck *et al.*, 2005b; Peumans *et al.*, 2005a).

Water is present as an essential component in self-etching adhesive to enable ionization of the acidic monomers and demineralization of dental hard tissues. Because aggressive self-etching adhesives are very acidic; concentrations of ionic and acidic

resin monomers have been increased, the adhesive layer after solvent evaporation can be quite thin, suggesting that polymerization may be inadequate due to oxygen inhibition. One-step self-etching adhesives are commonly associated with lower bonding effectiveness, which must be attributed in part to the dissolution of hydrophilic and hydrophobic monomers in a relatively highly concentrated solvent (Van Meerbeek *et al.*, 2005b).

Bonding of unfilled all-in-one adhesive to dentine may be improved by application of a second adhesive layer after light-curing the first layer to ensure that the exposed dentine surface and dentinal tubules are coated with adequately polymerized adhesive (Pashley *et al.*, 2002). Application of at least two coats of unfilled adhesive is generally advised. To minimize thick oxygen inhibited layers generated by single-step self-etching adhesive, a second adhesive coat comprising more hydrophobic resins has been recommended following the application of these simplified adhesives, in order to reduce their permeability as well as absorbing the uncured acidic monomers into the overlying adhesive layer where they would co-polymerize with the more hydrophobic resin monomers (King *et al.*, 2005; Loguercio *et al.*, 2006; Pashley *et al.*, 2002).

One-step adhesives bond less effectively to enamel/dentin than do their multi-step versions. Bonding effectiveness to dentin was determined using a microtensile bond strength protocol (De Munck *et al.*, 2005b). The lower bond strength of the one-step adhesives was associated with light-microscopic observation of multiple droplets that disappeared slowly (Tay *et al.*, 2002). The prompt disappearance of droplets upon application of a small amount of HEMA (2-hydroxyethyl methacrylate) or a HEMA-containing bonding agent, as well as the absence of droplets at the interface of all HEMA-containing adhesives, strongly suggests that the adhesive monomers separate from water upon evaporation of ethanol/acetone. Upon polymerization, the droplets become entrapped within the adhesive, potentially jeopardizing bond durability. This can be avoided by strong air-drying of the adhesive, thereby removing interfacial water and thus improving bonding effectiveness (Van Landuyt *et al.*, 2005). Adding a preceding etching step is beneficial for enamel, but should be avoided for dentin as this will decrease bond strengths, and may even jeopardize the bonding durability (Van Landuyt *et al.*, 2006b). The latter must be attributed to hampered resin infiltration of the

one-step self-etching adhesive within the relatively deeply exposed collagen fibril network. Recently, a randomized controlled clinical trial did not reveal any difference in clinical behavior, when Clearfil SE Bond was applied following manufacturer's instructions compared to when enamel was selectively acid-etched with phosphoric acid prior to the application of Clearfil SE Bond. Although, in general, no difference in clinical performance was recorded when Clearfil SE was applied following either of the experimental protocols, more marginal defects at the enamel side were noticed when enamel was not beforehand etched with phosphoric acid. However, these defects were small and of clinically negligible relevance (Peumans *et al.*, 2007; Van Meerbeek *et al.*, 2005a).

Thermocycling as a means to artificial aging

A widely used artificial aging method is thermocycling. Exposure of restorations in extracted teeth to cyclic thermal fluctuations to simulate one of the many factors in the oral environment has been common in many tracer penetration, marginal gap and bond strength laboratory tests. The ISO TR 11450 standard (1994) indicates that a thermocycling regimen comprised of 500 cycles in water between 5 ± 1 and $55\pm 1^\circ\text{C}$ is an appropriate artificial aging test. A literature review concluded that 10,000 cycles corresponds approximately to 1 year of *in vivo* functioning, rendering 500 cycles, as proposed by the ISO standard, as being very minimal in mimicking long-term bonding effectiveness (Gale and Darvell, 1999). Thermocycling simulates the introduction of hot and cold extremes in the oral cavity and shows the relationship of the linear coefficient of thermal expansion between tooth and restorative material. Thermal cycling stresses the bond between resin and the tooth, and, depending on the adhesive system, may affect bond strength (Davidson *et al.*, 1993). Hot water may accelerate hydrolysis of interface components, and subsequent uptake of water and extraction of breakdown products of poorly polymerized resin oligomers (Hashimoto *et al.*, 2000; Miyazaki *et al.*, 1998). Due to the higher thermal contraction/expansion coefficient of the restorative material (as compared to that of tooth tissue) repetitive contraction/expansion stresses are generated at the tooth-biomaterial interface. This may result in cracks that propagate along bonded interfaces, and once a gap is created, changing gap

dimensions can cause inflow and outflow of pathogenic fluids, a process known as 'percolation' (Gale and Darvell, 1999).

Thermocycling did not enhance chemical or mechanical degradation of the bonds produced by a two-step self-etching and a three-step etch-and-rinse adhesive to dentin (Shirai *et al.*, 2005). The one-step self-etching adhesive was not able to withstand polymerization shrinkage stress, nor thermocycling, when applied in class I cavities (De Munck *et al.*, 2005b). Thermocycling resulted in combined contraction/expansion stress and accelerated chemical degradation. However, the relative contribution of each is strongly dependent on the specific test set-up and the adhesive used.

Most *in vitro* testing of bonding systems is performed using specimens made in a mold with a low configuration (C) factor (ratio of bonded/unbonded surfaces), whereas clinically the C-factor is usually much greater. An increase in C-factor could increase shrinkage stress at the adhesive interface, thus, impairing the sealing ability (Davidson and Feilzer, 1997). Thermocycling up to 5,000 cycles had a very significant negative effect on bond strength in human dentin when a high C factor testing design was used (Price *et al.*, 2003). The conventional class V cavity presents a great challenge to the adhesive systems used due to the high C-factor (Santini *et al.*, 2004).

Studies regarding microleakage test

Primary objective of a dental restoration is to create a perfect seal, preventing leakage of contaminants contained in the oral environment. Microleakage is defined as the clinically undetectable passage of bacteria, fluids, molecules, or ions between a cavity wall and the restorative material applied to it (Kidd, 1976). Microleakage is not uniform along the circumference of a restorative margin (Tay *et al.*, 1995). Microleakage studies provide adequate screening methods, possibly determining clinical success and longevity of adhesive systems. *In vitro* microleakage tests are numerous and diverse methods have been used to demonstrate the leakage of restorative materials. Many techniques have been devised to test the cavity sealing properties of restorations both *in vivo* and *in vitro*. *In vitro* studies include the use of dyes, chemical tracers, radioactive isotopes, air pressure, bacterial studies, neutron activation analysis, scanning electron microscopy and electrochemical studies (Taylor and Lynch, 1992).

Chemical tracers used in leakage studies

The use of non-radioactive chemical tracers is distinct from the use of dyes in that these tracers rely upon the reaction between one and more of the chemicals used. A 50% silver nitrate solution is commonly used to immerse the *in vitro* model, which later is reacted with a photographic developing solution, such as benzene 1,4-diol (hydroquinone), (Douglas *et al.*, 1989). This technique gives a discrete, high-contrast marking of the restoration/dentin interface. Chemical tracer study, however, had similar problems to that of the dye-leakage study, especially for interpretation of the results (Amarante de Camargo *et al.*, 2006).

The silver nitrate method of measuring microleakage is an acceptable technique and considered to be a severe test, because silver ion is extremely small (0.059 nm in diameter) when compared to the size of a typical bacterium (0.5-1.0 μm), thus, being more penetrative (Youngson *et al.*, 1998). However, as the organic developer molecule has a larger diameter, it is the penetration of this chemical which is being observed. It is, therefore, unlikely that observations are of the full extent of silver ion penetration (Douglas *et al.*, 1989).

Among the different methods, a dye penetration measurement on sections of restored teeth is the most common technique for evaluating microleakage of tooth-restoration interface. One of the major drawbacks of this method is the two-dimensional measurement of three-dimensional phenomenon. Microleakage is not uniform along the interface and three-dimension evaluation seems to indicate more severe leakage than two-dimensional assessments (Gwinnett *et al.*, 1995). A suitable alternative to cross-section could be a clearing technique: a three dimensional portrait of leakage pathways was illustrated using photographic and computer digitization (Tay *et al.*, 1995). Nevertheless, while some authors find this technique simple and inexpensive, others consider this method as time-consuming and difficult to quantify (Hilton, 2002).

The problem with most microleakage studies was that they were evaluated subjectively, the assessment of the restoration as a whole was difficult, because most sectioning was done on class V restorations in the sagittal plane. Investigators have taken single section from the mid point of class V restoration without evidence that such sections involved the best position to evaluate leakage (Gwinnett *et al.*, 1995). Dye

penetration study had been criticized on the ground that assessment demands destruction of the specimen, thus preventing continuous monitoring of the microleakage over a period of time (Manhart *et al.*, 2001).

Microleakage may be more extreme at the end surfaces and these end surfaces should be scored, so that an accurate microleakage value could be assigned to composite restorations. Increased microleakage at the end surface may have resulted from polymerization shrinkage of resin composite (Mixson *et al.*, 1991).

The results of these *in vitro* studies of dye penetration must be considered as comparisons and not as absolute conclusions. A single section seems to be insufficient to reliably detect the maximum tracer penetration at the tooth restoration interface. Three sections may avoid underestimating of *in vitro* microleakage (Raskin *et al.*, 2003). The tests using fewer than three cutting sites would underestimate tracer depth, and may not represent the actual microleakage. Maximum dye penetration measured on each tooth, which complies with the aim of the *in vitro* evaluation of sealing efficiency allowing powerful statistical analysis of results, seems to be the best evaluation criterion (Dejou *et al.*, 1996).